IT TAKES A VILLAGE: THE EXPANDING MULTI-DISCIPLINARY APPROACH TO BRAIN METASTASIS

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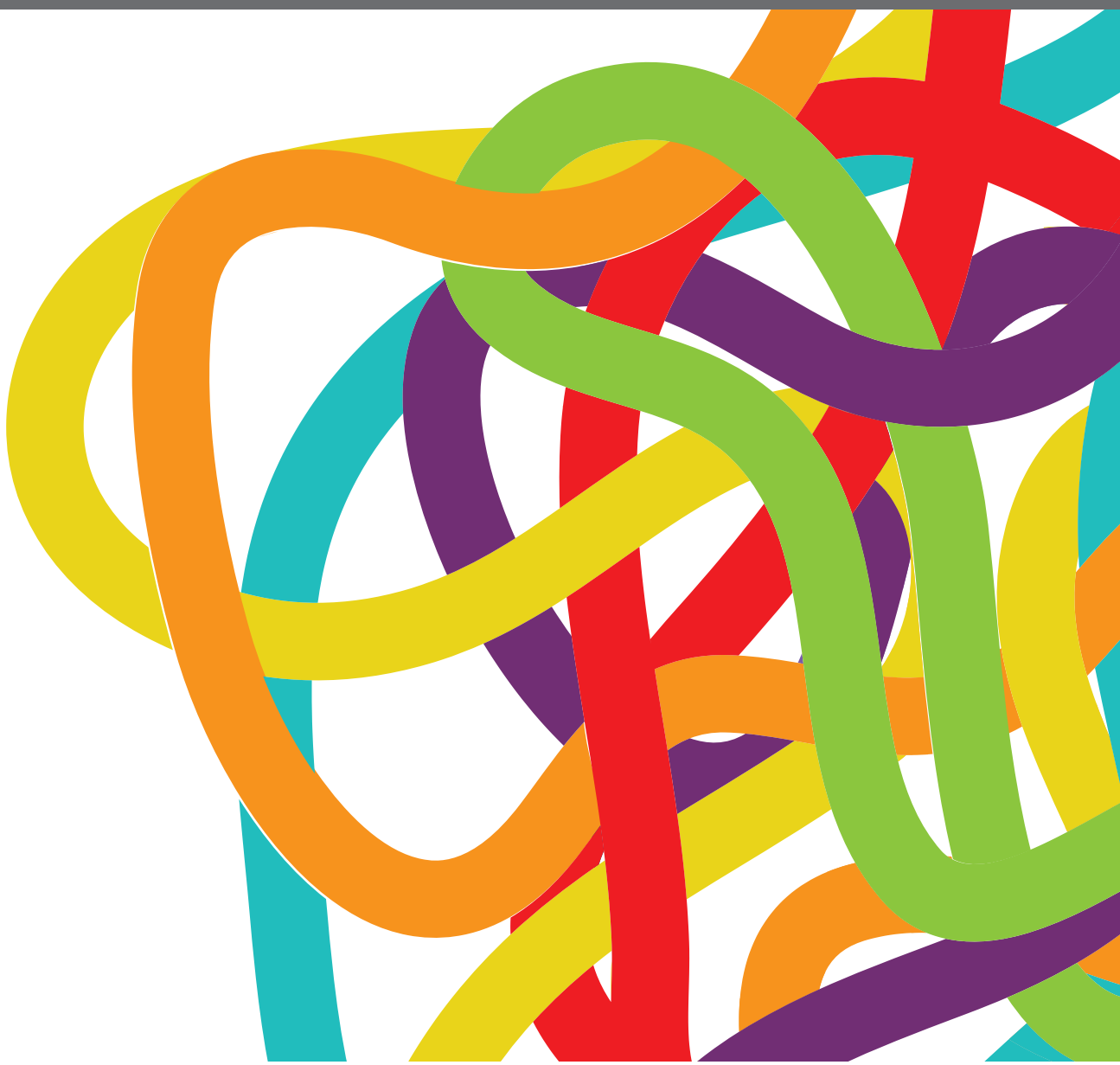

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IT TAKES A VILLAGE: THE EXPANDING MULTI-DISCIPLINARY APPROACH TO BRAIN METASTASIS

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Editorial: It takes a village: The expanding multi-disciplinary approach to brain metastasis

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Editorial on the Research Topic
It takes a village: The expanding multi-disciplinary approach to brain metastasis

Perspective

Brain metastases (BrM) represent the most common adult intracranial malignancy and continue to herald relatively poor survival. Approximately 180,000 to 216,000 of the 1.44 million cancer patients in the United States will develop BrM each year (1, 2). Ultimately, 20 to 40% of patients with solid cancers will develop BrM over the course of their advanced disease (1, 3). The risk of BrM varies considerably between primary cancer types, although the most common sources consist of lung (50-60%), breast (15-20%), and melanoma (5-10%), followed by renal cell, colorectal, pancreas, and urologic/gynecologic cancers (4–6). This incidence is approximately 20-fold higher than glioblastoma, the most common primary brain cancer, and nearly 3-fold higher than the incidence of all primary brain tumors combined (7, 8). The prevalence of BrM has continued to increase as improvements in cancer screening methods and extracranial systemic treatments, including immunotherapy, have evolved. Thus, patients are increasingly surviving longer such that later disease sequelae, including intracranial progression, are more common occurrences (9, 10). As a result, BrM have now evolved into a leading cause of both morbidity and mortality across many types of advanced cancer.

Despite the increasing demand, few BrM-specific therapies exist, and integrated programmatic, collaborative approaches toward BrM research have been virtually absent. Multi-disciplinary efforts to devise and discern novel therapies are desperately needed. Such efforts will require the inputs of key contributing subspecialties, which include (but are not limited to) medical or pediatric oncology, neuro-oncology, neurosurgery, radiation oncology, neuroimaging, neuropathology, and palliative care. The last few years has seen the emergence of coordinated "brain metastasis clinics" at a handful of medical centers, offering patients access to varying modes of multi-disciplinary care, BrM-focused clinical trials, and more advanced treatment recommendations from cooperative tumor boards. For true progress in clinical care to be made in the imminent future, however, these team-based approaches to clinical care will need to evolve from exception to norm.

Akin to what has been seen with clinical care, much of the research performed to date on BrM has been performed in silos, focusing on a single disease histology (i.e., melanoma), or a single phase or facet of tumor progression (i.e., immune evasion, microenvironment, or tumor cell signaling). As a result, there has been a failure to capitalize on successes or knowledge gains that can: 1) inform across disease groups; 2) link scientific approaches, such as genomics, immunology, and cell signaling; 3) overcome central nervous system (CNS)-imposed treatment barriers; and 4) determine overlapping networks common to brain metastatic progression in disease-agnostic fashion. Furthermore, breaking down silos in the research arena can at times be more challenging than doing so in the clinical realm, as recognizing and incentivizing fruitful research collaboration can provide unique challenges. Granting agencies have yet to foster team science aimed at BrM to the extent that they have for primary cancers or brain tumors, an important short-coming when one considers the greater dependence on multi-disciplinary therapeutic approaches that characterizes BrM. Research "requests for applications" (RFAs) aimed at identifying and bringing together scientists whose work may be wittingly or unwittingly applicable to BrM must be brought forward, and multidisciplinary conferences focused on the same likewise further emerge. The recent development of a focused annual meeting on brain metastasis by the Society of Neuro-oncology and the American Society of Clinical Oncology represents a notable victory for recognition and highlights an appropriate future direction for the field.

In the same vein, this collection of articles (see below summary) represents the editors' efforts to call attention to the key quandaries facing those tackling BrM from both a clinical and research perspective, as well as to consolidate those quandaries into one approachable resource. Identifying, agreeing upon, and properly focusing attention on these issues will be an important first step for the field. While historical questions have been of the genre of i.e. increasing drug access across the blood-brain barrier (BBB), newer questions will perhaps focus instead on molecular differences across primary tumor and metastasis, personalized approaches, CNS-specific immune evasion, and mechanisms of leptomeningeal spread, to name a few. The goal of this issue is to stimulate discourse, foster collaboration, and shed light on a growing population of patients whose needs are currently outstripping our provided options.

Summary of articles

A number of articles in this collection offer varying and thoughtful angles on the modern evolving approaches toward the diagnosis, management, and study of BrM (Brenner and Patel, Kirkpatrick, Ene and Ferguson, Sarmey et al). Others aim to interpret recent clinical trial results (Tan et al, Taslimi et al); tackle brain-specific concerns within the tumor microenvironment (Toh et al, Heet et al); or highlight the increasing use of detailed molecular and immunogenomic profiling for the purpose of creating personalized and targeted therapeutics (Shen et al, Routh et al). Finally, as our capacity grows for improving survival amongst patients with BrM, so must we develop new focus on issues of survivorship. We thus also include articles that address delayed treatment effects such as radiation necrosis (Park et al), as well quality of life more broadly (Wu et al).

Author contributions

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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ORIGINAL RESEARCH
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Peritumoral Brain Edema in Metastases May Be Related to Glymphatic Dysfunction

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Objectives: The proliferation of microvessels with increased permeability is thought to be the cause of peritumoral brain edema (PTBE) in metastases. The contribution of the glymphatic system to the formation of PTBE in brain metastases remains unexplored. We aimed to investigate if the PTBE volume of brain metastases is related to glymphatic dysfunction.

Materials and Methods: A total of 56 patients with brain metastases who had preoperative dynamic susceptibility contrast-enhanced perfusion-weighted imaging for calculation of tumor cerebral blood volume (CBV) and diffusion tensor imaging for calculations of tumor apparent diffusion coefficient (ADC), tumor fractional anisotropy (FA), and analysis along perivascular space (ALPS) index were analyzed. The volumes of PTBE, whole tumor, enhancing tumor, and necrotic and hemorrhagic portions were manually measured. Additional information collected for each patient included age, sex, primary cancer, metastasis location and number, and the presence of concurrent infratentorial tumors. Linear regression analyses were performed to identify factors associated with PTBE volume.

Results: Among 56 patients, 45 had solitary metastasis, 24 had right cerebral metastasis, 21 had left cerebral metastasis, 11 had bilateral cerebral metastases, and 11 had concurrent infratentorial metastases. On univariable linear regression analysis, PTBE volume correlated with whole tumor volume (β = -0.348, P = 0.009), hemorrhagic portion volume (β = -0.327, P = 0.014), tumor ADC (β = 0.530, P <.001), and ALPS index (β = -0.750, P <.001). The associations of PTBE volume with age, sex, tumor location, number of tumors, concurrent infratentorial tumor, enhancing tumor volume, necrotic portion volume, tumor FA, and tumor CBV were not significant. On multivariable linear regression analysis, tumor ADC (β = 0.303; P = 0.004) and ALPS index (β = -0.624; P < 0.001) were the two independent factors associated with PTBE volume.

Conclusion: Metastases with higher tumor ADC and lower ALPS index were associated with larger peritumoral brain edema volumes. The higher tumor ADC may be related to increased periarterial water influx into the tumor interstitium, while the lower ALPS index may indicate insufficient fluid clearance. The changes in both tumor ADC and ALPS index

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may imply glymphatic dysfunction, which is, at least, partially responsible for peritumoral brain edema formation.

Keywords: brain metastasis, peritumoral brain edema, glymphatic system, apparent diffusion coefficient, cerebral blood volume, dynamic susceptibility contrast-enhanced perfusion-weighted imaging, diffusion tensor imaging, ALPS (analysis along perivascular space) index

INTRODUCTION

Metastases are the most frequent brain tumor in adults (1). Most metastases are associated with peritumoral brain edema (PTBE), which increases intracranial pressure and causes neurological deficits (2). The pathogenesis of PTBE in brain metastases remains unclear and is traditionally thought to represent the net transport of fluid from the intravascular compartment into the brain interstitium due to the proliferation of microvessels which have defects in their inter-endothelial tight junctions (3). This theory, however, does not explain the formation of PTBE in low-grade gliomas with intact tight junctions (4, 5) and meningiomas which are extra-axial and have no direct contact with the brain interstitium.

The glymphatic system has been recently recognized as a pathway for waste clearance and maintaining fluid balance in the brain parenchymal interstitium (6). This highly organized fluid transport system involves cerebrospinal fluid (CSF) inflow along the perivascular spaces of the penetrating arteries and transfer into the brain interstitium under the influence of the aquaporin 4 (AQP4) water channels. With its solute, the CSF–interstitial fluid is then directed towards the venous perivascular spaces, thereafter leaving the brain parenchyma. In rodent experiments, the formation of PTBE has been related to glymphatic dysfunction, including reduced CSF efflux (7) and glymphatic pathway downstream remodeling (8). However, being limited by the invasiveness of current evaluation tools (*e.g.*, intrathecal contrast medium injection) (9–11), these findings regarding the glymphatic system of animals are not yet confirmed in humans.

Advanced MR imaging techniques, such as dynamic susceptibility contrast-enhanced (DSC) perfusion-weighted imaging (PWI) (12–14) and diffusion tensor imaging (DTI) (12, 15), offer an opportunity for the noninvasive assessment of fluid dynamics in the tumor intravascular compartment, tumor interstitium, and glymphatic system. DSC-PWI dynamically measures T2*-weighted signal intensity loss related to intravascular gadolinium concentration, from which relative cerebral blood volume (CBV) can be computed for the measurement of intravascular fluid volume that is related to microvascular proliferation in tumors (16–19). Apparent diffusion coefficient (ADC) and fractional anisotropy (FA) are quantitative metrics derived from DTI for water diffusivity measurement. In addition to tumor cellularity (20) and fluid viscosity (21), they may also reflect the volume and flow directionality of the tumor interstitial fluid (12). The ALPS index, recently proposed by Taoka et al., is another quantitative diffusion metric derived from DTI (22). It estimates the diffusivity along the perivascular spaces of medullary veins and has been used as a noninvasive quantitative marker to assess human glymphatic activity in clinical conditions including Alzheimer's disease (22, 23), normal pressure hydrocephalus (24, 25), Parkinson disease (26, 27), age-related iron deposition (28), diabetic cognitive impairment (29), and meningioma-associated brain edema (30).

To the best our knowledge, the associations of PTBE volume with tumor diffusion and perfusion properties and glymphatic function in patients with brain metastasis remained unexplored. In this study, we took advantage of these advanced MR techniques to evaluate the changes in fluid dynamics associated with brain metastases. We hypothesized that the PTBE of metastases is associated with fluid dynamics in the tumor intravascular compartment, tumor interstitium, and glymphatic system as evidenced by alterations of tumor CBV, tumor ADC, tumor FA, and ALPS index.

MATERIALS AND METHODS

Study Subjects

Approval for reviewing the clinical data of the patients and the preoperative MRI studies was obtained from our institutional review board. Between 2006 and 2018, a total of 74 consecutive patients with subsequent histopathological diagnosis of brain metastasis underwent preoperative MRI using a dedicated tumor protocol which included DSC-PWI and DTI at our institution. These patients were initially screened for eligibility to enter prospective glioblastoma studies but were later excluded due to a pathologic diagnosis of brain metastasis. A total of 18 patients were excluded due to motion artifacts (n = 2), purely hemorrhagic tumors (n = 14), and tumors limited to the infratentorial compartment (n = 2). Patients with partial hemorrhagic tumors were included if their enhancing tumor portions were not obscured by susceptibility artifacts.

Thus, a total of 56 patients (30 women, 26 men; mean age, 56.9 ± 11.6 years; age range, 34–77 years) were analyzed. No patients had begun corticosteroid treatment, diuretic therapy, radiation therapy, and chemotherapy or had a previous brain surgery at the time of their MRI studies. An overview of the characteristics of the patients is found in **Table 1**.

Clinical and Imaging Information

The medical records of patients and MRI studies were retrospectively reviewed to collect clinical and imaging information, including sex, age, primary cancer, cerebral hemisphere involved (right, left, or bilateral cerebral hemispheres), tumor location, number of tumors (solitary or

TABLE 1 | Patient characteristics.

Characteristics	Number of patients
Number of patients	56
Mean age ± SD (years)	56.9 ± 11.6
Sex	
Woman	30
Man	26
Cerebral hemisphere involved	
Right	24
Left	21
Bilateral	11
Tumor location	
Frontal	24
Parietal	12
Occipital	9
Temporal	7
Deep gray nucleus	4
Number of tumors	
Solitary	45
Multiple	11
Concurrent infratentorial tumors	
No	45
Yes	11
Primary cancer	
Lung	32
Breast	7
Genitourinary system	6
Gastrointestinal tract	4
Liver	2
Head and neck	1
Unknown primary	4

Except where indicated, data are numbers of patients.
SD, standard deviation.

multiple), and concurrent infratentorial tumors (yes or no). A histopathologic diagnosis was made by a board-certified neuropathologist with 20 years of experience.

MRI

All MRI studies were performed using a 3-T unit (Magnetom Tim Trio, Siemens, Erlangen, Germany) with a 12-channel phased-array head coil. All examinations included T2-weighted, DSC-PWI, DTI, and T1-weighted sequences acquired in the transverse plane before and after administration of the gadolinium contrast medium. DTI was performed using single-shot echo-planar imaging with the following parameters: TR ms/TE ms, 5,800/83; diffusion gradient encoding in 20 directions; b = 0, 1000 s/mm^2; field of view (FOV), 256 × 256 mm; matrix size, 128 × 128; section thickness, 2 mm; and number of signals acquired, four. A total of 50–60 sections without intersection gap were used to cover the cerebral hemispheres, brainstem, and upper cerebellum. Generalized autocalibrating partially parallel acquisitions with the reduction factor set at 2 were used during DTI acquisitions.

DSC-PWI was obtained with a T2*-weighted gradient-echo EPI sequence during a bolus injection of a standard dose (0.1 mmol/kg) of intravenous gadopentetate dimeglumine (Magnevist; Schering, Berlin, Germany). The injection rate was 4 ml/s for all patients and was immediately followed by a bolus injection of saline (total of 20 ml at the same rate). The DSC-PWI sequence parameters included the following: TR/TE, 1,640/40 ms; flip angle, 90°; FOV, 230 × 230 mm; section thickness, 4 mm; 20 sections and acquisition time of 1 min and 28 sec. A total of 50 measurements were acquired, allowing at least five measurements before bolus arrival. No contrast agent was administered before DSC-PWI. Contrast-enhanced T1-weighted images (TR/TE, 2,000/2.63 ms; section thickness, 1 mm; TI, 900 ms; acquisition matrix, 224 × 256, and FOV, 224 × 256 mm) were acquired after completion of the DTI and DSC-PWI sequences.

Image Postprocessing and Analysis

The software nordic Image Control and Evaluation, version 2 (Nordic Imaging Lab, Bergen, Norway), was used for all volume measurements and for processing of perfusion and diffusion tensor data. All images were coregistered based on a 3D non-rigid transformation and mutual information. The adequacy of registration was visually assessed, and manual adjustments were performed by changing the transformation parameters of translation, rotation, and/or scaling as necessary. The ALPS index was measured with 3D Slicer, version 4.10.2 (http://www.slicer.org). Two neuroradiologists (with 16 and 6 years of experience, respectively) independently performed all measurements. If the tumors were found in both hemispheres, only those in the hemisphere with a larger PTBE volume were selected for measurements of volume, perfusion, and diffusion metrics. If multiple tumors or PTBE areas were present, all were included as long as their sizes were larger than 1 × 1 cm^2.

Measurements of Volume of PTBE, Whole Tumor, Enhancing Tumor Portions, Necrotic Portions, and Hemorrhagic Portions

One polygonal region of interest (ROI) was first placed on each T2-weighted image to include the entire PTBE and tumor, followed by another ROI drawn to include the entire tumor on each contrast-enhanced T1-weighted image. Subtracting the second ROI from the first ROI yielded the isolated PTBE area. If necrotic and hemorrhagic portions were present, they were measured by placing the ROIs on contrast-enhanced T1- and T2-weighted images, respectively, with reference to precontrast T1-weighted and SWI images. Subtracting ROIs of necrotic and hemorrhagic portions from tumor ROI yielded the enhancing tumor area. The slice volume of each ROI was computed by multiplying the area by slice distance (slice thickness + slice gap). The total volumes of PTBE, whole tumor, enhancing tumor portion, necrotic portion, and hemorrhagic portion were calculated by summing up all slice volumes. An example of ROI segmentation is shown in **Figure 1**.

Measurements of ADC, FA, and CBV of Enhancing Tumor Portions

Diffusion-weighted images were co-registered to the non-diffusion weighted (b = 0) images to minimize artifacts induced by eddy current and subject motion. The ADC and FA were calculated from diffusion tensor data using standard algorithms (12, 15). The CBV for each voxel was estimated by

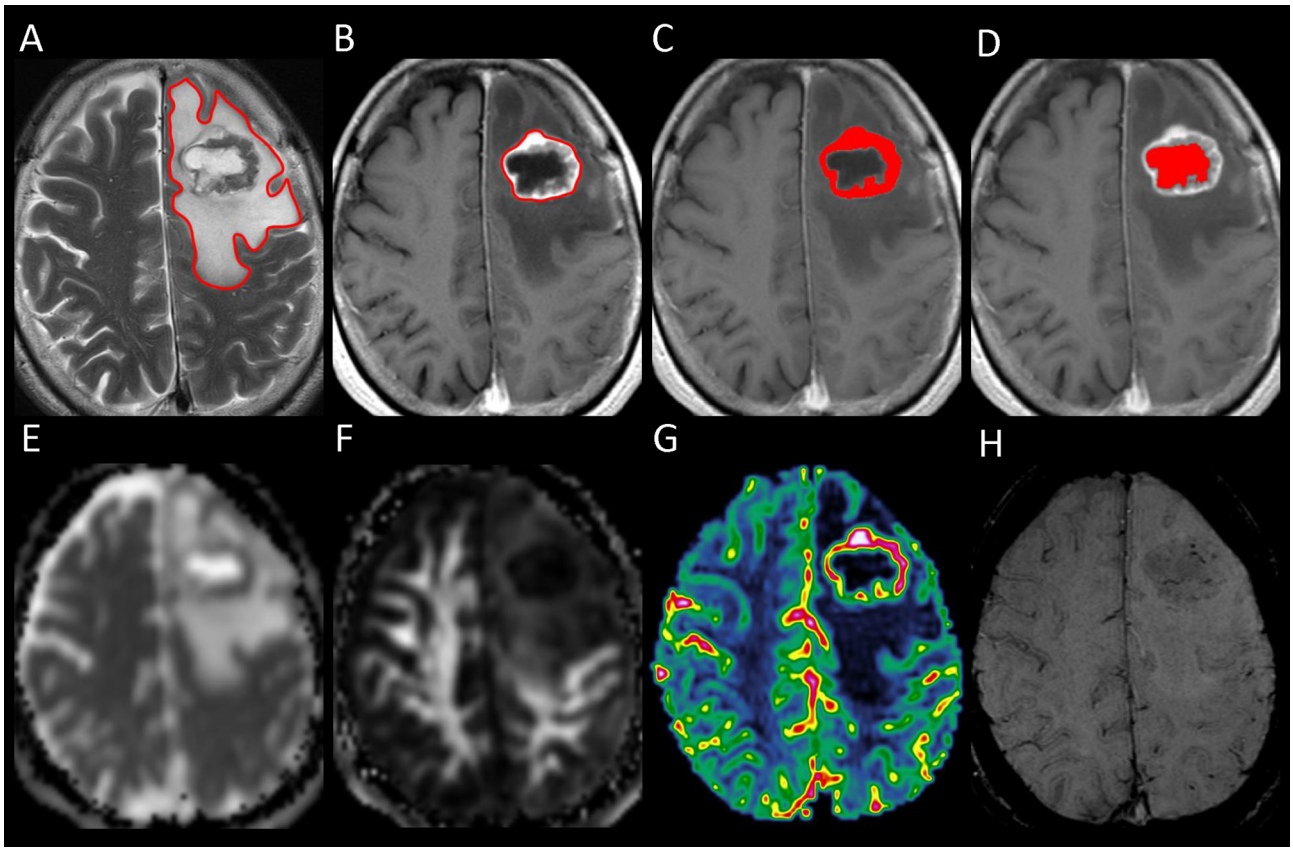

FIGURE 1 | Example of how regions of interest (ROIs) were segmented in a left frontal metastatic brain tumor. Transverse T2-weighted image **(A)** shows a manually drawn polygonal ROI that includes the entire peritumoral brain edema and the whole tumor. On contrast-enhanced T1-weighted images **(B–D)**, the ROIs of whole tumor **(B)**, enhancing tumor **(C)**, and necrotic portion **(D)** are shown. The ROI of the enhancing tumor **(C)** is used to measure the tumor apparent diffusion coefficient (ADC), tumor fractional anisotropy (FA), and tumor cerebral blood volume (CBV) by overlaying the ROI on the corresponding ADC **(E)**, FA **(F),** and CBV **(G)** maps. On susceptibility-weighted image **(H)**, there is no hemorrhagic tumor portion.

integrating the relaxivity–time curve converted from the dynamic signal intensity curve. Contrast leakage correction was performed as it has been shown to improve tumor grading by using a technique outlined by Boxerman et al. (13, 14).

The ADC, FA, and CBV of enhancing tumor were measured by using the ROIs transformed from contrast-enhanced T1-weighted space. The mean ADC, FA, and CBV values of the whole enhancing tumor volume were calculated by averaging the values of all slices, with the enhancing tumor volume of each slice taken into account. Before all quantitative comparisons, the mean CBV values were normalized and expressed as ratios. The ratios were calculated by dividing the mean values of the whole tumor by the values obtained from a circular ROI (size range, 50–100 mm^2) placed in the contralateral normal-appearing white matter.

Measurement of ALPS Index

DTI-ALPS method (22) was used to evaluate the glymphatic function. This method evaluates the diffusivity along the perivascular space on a transverse slice at the level of the lateral ventricle body. The medullary veins, accompanied by their perivascular spaces, run perpendicular to the ventricular walls at the level of the lateral ventricular bodies in a right–left or left–right direction (*i.e.*, *x*-axis in the image coordinates). In this level, the corticofugal corona radiata projection fibers run in the craniocaudal direction (*i.e.*, *z*-axis in the image coordinates) adjacent to the lateral ventricles. The superior longitudinal fascicle, which represents the association fibers, runs in the anterior–posterior direction (*i.e.*, *y*-axis in the image coordinates) and is located lateral to the corona radiata. As the perivascular space is nearly perpendicular to both the projection fibers and association fibers, the major difference between the *x*-axis diffusivity in both fibers (D_{xproj} and D_{xassoc} for *x*-axis diffusivity in projection fiber and association fiber, respectively) and the diffusivity that is perpendicular to the *x*-axis and to the direction of fiber tracts (*y*-axis for projection fiber, where diffusivity is denoted as D_{yproj}; *z*-axis for association fiber, where diffusivity is denoted as D_{zassoc}) is the existence of the perivascular space. To quantify glymphatic activity, the ALPS index is defined as follows:

$$\text{ALPS index} = \frac{\text{mean}(D_{\text{xproj}}, D_{\text{xassoc}})}{\text{mean}(D_{\text{yproj}}, D_{\text{zassoc}})} \quad (1)$$

Diffusion metric images were generated by using 3D Slicer, version 4.10.2 (http://www.slicer.org). The ROIs of projection (mean size, 35 ± 19 mm^2) and association fibers (mean size, 30 ± 18 mm^2) were drawn on a slice at the level of the lateral ventricular body based on a directionality encoded map. The ALPS index was computed according to the equation above (1). The concept of DTI-ALPS method and an example of ROI placement for ALPS index measurement are shown in **Figure 2**.

Statistical Analysis

A commercially available statistical software package (SPSS 22; IBM, Armonk, New York) was used for analysis, and *P*-values <0.05 were considered to indicate a statistical significance. Continuous variables are denoted as mean ± standard deviation unless otherwise noted. The Kolmogorov–Smirnov test was used to assess the normality of continuous variables and guide the selection of a parametric or nonparametric test for the comparison of variables. Variance inflation factors were used to detect multicollinearity.

The interobserver variability in the measurements of volumes, ADC, FA, CBV, and ALPS index was assessed by intraclass correlation coefficients (ICCs) with 95% confidence intervals based on an absolute-agreement, two-way, random-effects model. The final values of all measurements were obtained by taking the mean of the independent measurements of two observers.

The associations of PTBE volume with age, sex, tumor location, cerebral hemisphere involved, number of tumors, concurrent infratentorial tumors, primary cancer (lung cancer *vs.* others), whole tumor volume, enhancing tumor volume,

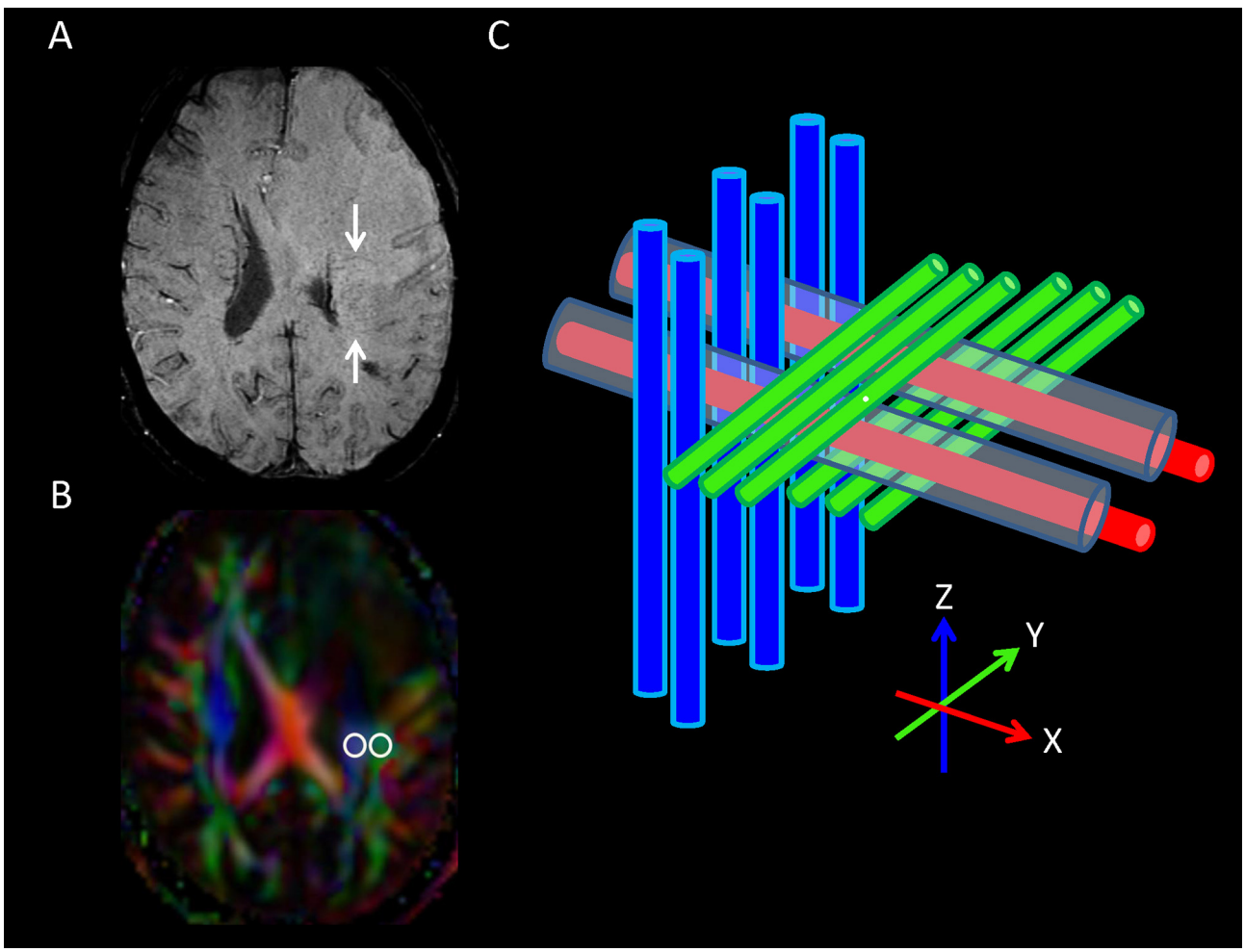

FIGURE 2 | **(A)** On transverse susceptibility-weighted image, the deep medullary veins (arrows) run in the right–left direction (x-axis) at the level of the lateral ventricle body. **(B)** Directionally encoded color map illustrates the regions of interest of projection (blue area) and association (green area) fibers in the left periventricular region for calculation of analysis along the perivascular space ALPS index. **(C)** Schematic diagram presenting the relationship between the directions of the perivascular spaces (gray cylinders) surrounding the deep medullary veins (red cylinders; x-axis), the projection fibers (blue cylinders; z-axis), and the association fibers (green cylinders; y-axis). Note that the direction of the perivascular spaces is perpendicular to both the projection and association fibers.

necrotic portion volume, hemorrhagic portion volume, tumor ADC, tumor FA, tumor CBV, and ALPS index were first analyzed with univariable linear regression. All variables were entered as potential covariates in the stepwise multivariable linear regression analysis to identify independent factors associated with PTBE volume.

RESULTS

Among 56 patients, 45 had solitary metastasis, 24 had right cerebral metastases, 21 had left cerebral metastases, 11 had bilateral cerebral metastases, and 11 had concurrent infratentorial metastases. The locations of the metastatic tumors were frontal (n = 24), parietal (n = 12), occipital (n = 9), temporal (n = 7), and deep gray nucleus (n = 4). The primary sites of tumors were the lung (n = 32), breast (n = 7), genitourinary (n = 6), gastrointestinal (n = 4), liver (n = 2), head and neck (n = 1), and unknown (n = 4) primaries. **Table 1** depicts the clinical characteristics of 56 patients.

There were excellent interobserver agreements (ICC = 0.084–0.998, $P < 0.001$) in the measurements of PTBE volumes, whole tumor volumes, enhancing tumor volumes, necrotic portion volumes, hemorrhagic portions volumes, tumor ADC, tumor FA, tumor CBV, and ALPS index. The mean volumes (cm^3) of PTBE, whole tumor, enhancing tumor, necrotic portion, and hemorrhagic portion were 87.67 ± 45.77, 24.95 ± 21.56, 17.80 ± 16.34, 2.53 ± 5.74, and 4.61 ± 11.37, respectively. The mean tumor ADC (10^{-6} mm^2/s), tumor FA, tumor CBV, and ALPS index were 1091.8 ± 195.2, 0.08 ± 0.03, 7.57 ± 5.28, and 1.226 ± 0.176, respectively. **Table 2** summarizes all measurements.

On univariable linear regression analysis, the PTBE volume correlated with whole tumor volume (β = -0.348, P = 0.009), hemorrhagic portion volume (β = -0.327, P = 0.014), tumor ADC (β = 0.530, $P < 0.001$), and ALPS index (β = -0.750, $P < 0.001$). The associations of PTBE volume with necrotic portion volume (β = -0.263, P = 0.050) and tumor CBV (β = 0.255, P = 0.057) were marginally significant. **Figure 3** shows the correlations of PTBE volume with factors that were significant or approaching significance on univariable linear regression. No correlations were found between PTBE volume and age, sex, tumor location, number of tumors, enhancing tumor volume, concurrent infratentorial tumor, and tumor FA.

TABLE 2 | Results of volume, ADC, FA, CBV, and ALPS index measurements.

Measurement	Mean ± SD	Range
Peritumoral edema volume (cm^3)	87.67 ± 45.77	0–178.09
Whole tumor volume (cm^3)	24.95 ± 21.56	2.64–85.22
Enhancing tumor volume (cm^3)	17.80 ± 16.34	1.15–72.11
Necrotic portion volume (cm^3)	2.53 ± 5.74	0–36.72
Hemorrhagic portion volume (cm^3)	4.61 ± 11.37	0–49.40
Tumor ADC ± SD (10^{-6} mm^2/s)	1,091.8 ± 195.2	763.0–1,606.9
Tumor FA ± SD	0.08 ± 0.03	0.04–0.19
Tumor CBV ratio ± SD	7.57 ± 5.28	0.28–21.64
Mean ALPS index ± SD	1.226 ± 0.176	0.910–1.612

ADC, apparent diffusion coefficient; ALPS, analysis along the perivascular space; CBV, cerebral blood volume; FA, fractional anisotropy; SD, standard deviation.

On stepwise multiple linear regression analysis, tumor ADC (β = 0.303; P = 0.004) and ALPS index (β = -0.624; $P < 0.001$) were the two independent factors associated with PTBE volume. The results of univariable and multivariable linear regression analyses of factors associated with PTBE volume are summarized in **Table 3**. Examples of brain metastases with small and large volumes of PTBE are shown in **Figure 4**.

DISCUSSION

Our study showed that the PTBE volume of metastases correlated positively with tumor ADC and inversely with ALPS index. Metastases with a larger volume of PTBE had higher ADC and lower ALPS index. These findings suggest that the PTBE of metastases may be related to intratumoral water diffusivity and glymphatic dysfunction. In contrast, changes of tumor intravascular fluid volume may not contribute to PTBE formation as the tumor CBV was not correlated with the PTBE volume.

Metastatic brain tumors are known to have a disrupted inter-endothelial tight junction (31) due to the downregulation of tight junction components, including claudin-1, claudin-5, and occludin (32, 33). With the increased microvascular permeability, intravascular fluid has been considered as the water source of PTBE. Tumor CBV is a surrogate marker of angiogenesis as it correlates with microvascular proliferation (16–19) and the expression of endothelial growth factor (34, 35). In our study, the association between tumor CBV and PTBE volume was not significant, suggesting that the proliferation of microvessels, and thus the increased intravascular fluid volume, is not related to PTBE formation. This finding agrees with the results of a previous study in which no correlations were present between the PTBE volume of brain metastases and microvessel density as determined by anti-CD34 staining (36). Based on these findings, we speculate that intravascular fluid may not be the water source of PTBE.

Systemic steroids are the mainstay of treatment for PTBE (37) and may result in rapid edema reduction and symptom relief through the restoration of tight junctions and reduction of capillary permeability by binding to glucocorticoid receptors (38). This effect, however, is typically transient and diminishes within weeks or months (39), suggesting that PTBE formation may be related to causes other than the disruption of tight junctions. A recent study shows that the blood–brain barrier is more complex than anticipated (40). Changes in the supporting structures of the blood–brain barrier, such as astrocyte, pericytes, and microglial cells, may also be associated with influx of fluid into the brain interstitium. The astrocyte covering of brain microvessels seems to be rate limiting to water movement (40), and it is suggested that water channels AQP4 located on astrocytic foot processes may play a significant role in PTBE formation. A strong correlation between PTBE and upregulated astrocyte AQP4 expression in human astrocytomas and metastatic adenocarcinomas suggests that increased AQP4 expression may be essential to the pathogenesis of PTBE (41). Since AQP4 water channels are part of the glymphatic system, we also postulate the possibility that PTBE in metastases may be related to glymphatic dysfunction with an increased periarterial influx of CSF into the tumor interstitium. While the

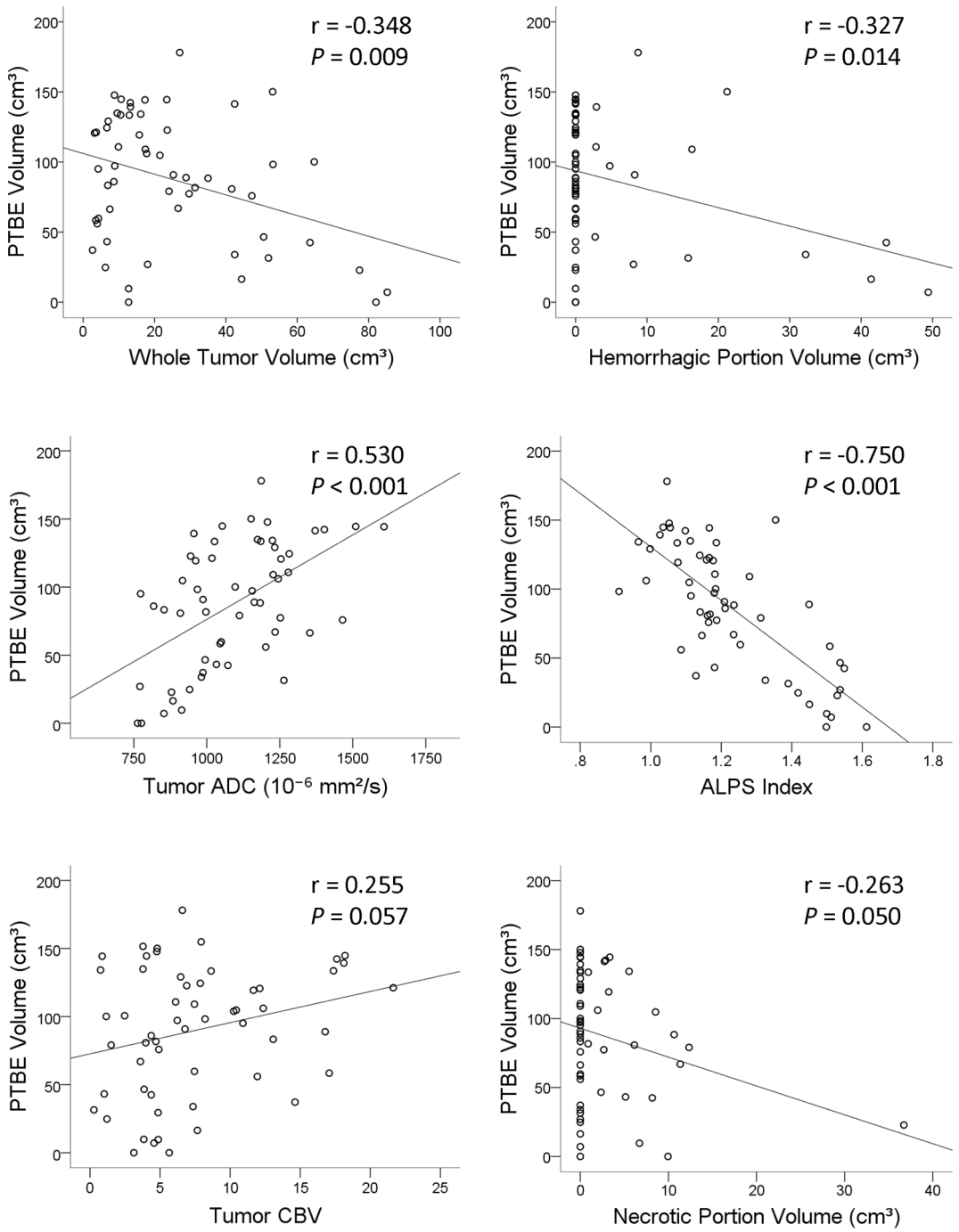

FIGURE 3 | Scatterplots with regression line showing the correlations of the peritumoral brain edema volume of metastatic brain tumors with whole tumor volume, hemorrhagic portion volume, tumor apparent diffusion coefficient, analysis along the perivascular space index, tumor cerebral blood volume, and necrotic portion volume.

current understanding of the mechanism of PTBE was developed prior to the discovery of the glymphatic system, incorporating the role of the glymphatic system into the current theory of PTBE formation may help in the development of effective treatments for reducing PTBE.

Although ADC has been considered as a marker of tumor cellularity, the correlations between the two were inconsistent (42). As ADC measures extracellular water diffusivity and the quantity of mobile water molecules, it may also reflect fluid volume in the tumor interstitium. In our study, tumors with

TABLE 3 | Univariable and multivariable linear regression analyses of factors associated with peritumoral brain edema (PTBE) volume.

Characteristics	PTBE volume							
	Univariable linear regression				Multivariable linear regression			
	B	SE	β	*P*-value	B	SE	β	*P*-value
Age	0.665	0.527	0.169	0.213				
Sex	4.426	12.363	0.049	0.722				
Cerebral hemisphere involved	9.237	8.071	0.154	0.257				
Tumor location	1.558	4.935	0.043	0.754				
Number of tumors	-3.372	15.529	-0.033	0.811				
Concurrent infratentorial tumor	-2.308	15.534	-0.020	0.882				
Primary cancer	13.052	12.347	0.142	0.295				
Whole tumor volume	-0.739	0.271	-0.348	0.009*				
Enhancing tumor volume	-0.389	0.377	-0.139	0.307				
Necrotic portion volume	-2.093	1.046	-0.263	0.050				
Hemorrhagic portion volume	-1.316	0.517	-0.327	0.014*				
Tumor ADC	1.242	0.271	0.530	<.001*	0.723	0.236	0.303	0.004
Tumor FA	-160.961	201.864	-0.108	0.429				
Tumor CBV	2.290	1.180	0.255	0.057				
ALPS Index	-193.578	23.482	-0.750	<.001*	-167.747	26.493	-0.624	<.001

ADC, apparent diffusion coefficient; ALPS, analysis along the perivascular space; CBV, cerebral blood volume; FA, fractional anisotropy; SD, standard deviation; B, unstandardized coefficient; β, standardized coefficient; SE, standard error.
**P-value <0.05.*

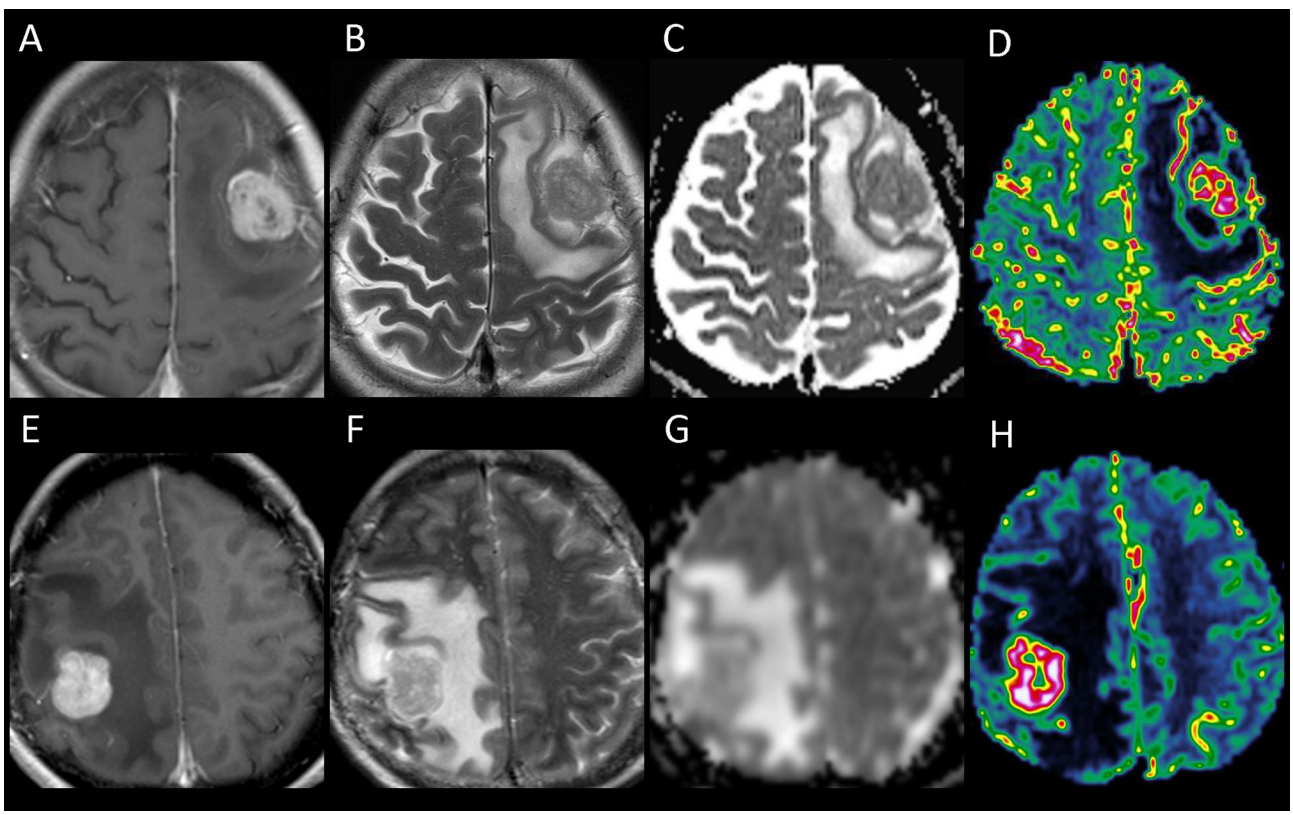

FIGURE 4 | The upper panel shows the contrast-enhanced T1-weighted **(A)**, T2-weighted **(B)**, apparent diffusion coefficient (ADC) **(C)**, and cerebral blood volume (CBV) **(D)** images of a left frontal metastasis with peritumoral brain edema (PTBE) of 85.98 cm^3, ADC value of 817.9 × 10^{-6} mm^2/s, and relative CBV of 4.37. The lower panel **(E–H)** shows the corresponding images from a right parietal metastasis with PTBE of 133.63 cm^3, ADC value of 1,185 × 10^{-6} mm^2/s, and relative CBV of 17.37. The ADC and CBV of the right parietal metastasis with a larger PTBE volume are higher than those of the left frontal metastasis which has a smaller PTBE volume.

higher ADC were associated with a larger PTBE volume. We hypothesize that the higher tumor ADC may reflect water increase in the tumor interstitium as a consequence of AQP4-mediated fluid influx. On the other hand, a correlation between AQP4 expression and ADC changes was observed in meningiomas (43), rat models of ischemia (44), hydrocephalus (45), and AQP4-knockdown brain (46). These results suggest that the ADC values correlate with AQP4 expression under certain pathological conditions, and ADC may be a surrogate marker of AQP4 expression. Based on this, we suggest that, similarly, the ADC of metastatic tumors may also reflect AQP4 expression.

In the glymphatic pathway, a periarterial influx of CSF is balanced by the perivenous efflux of interstitial fluid (47) under normal physiological conditions. The growth of metastases may disrupt this balance and result in the accumulation of interstitial fluid, *i.e.*, PTBE. The inverse relationship between the ALPS index and the PTBE volume in our study suggests that a higher glymphatic function may facilitate interstitial fluid clearance and reduce or even prevent PTBE. As stated above, insufficient glymphatic function for interstitial fluid clearance may contribute to PTBE formation. A similar inverse relationship was observed in meningiomas, and glymphatic dysfunction was proposed to be the cause of PTBE (30).

In mice harboring gliomas and melanomas, glymphatic function is increased to reduce PTBE by remodeling of meningeal lymphatic vessels (MLVs), which are downstream of the glymphatic system (8). In mice with defective MLVs, impaired drainage of brain parenchymal interstitial fluid aggravates PTBE. We speculate that the brain metastases with higher ALPS index in our study may have greater remodeling of MLVs, which facilitates the efflux of interstitial fluid from the brain parenchyma. Alternatively, metastases with a higher ALPS index may have more glymphatic reserve capacity, which serves to relieve PTBE. To the best of our knowledge, no human studies have reported the inverse relationship between PTBE volume of metastases and interstitial fluid clearance in glymphatic system.

There are limitations to our study. First, the diffusion signal measured in clinical settings reflects overall changes in water mobility associated with many processes occurring at scales much smaller than typical MRI voxels. Therefore, we cannot definitely state that the ALPS index is a measure of glymphatic function, and the changes of tumor ADC were due to the expression of AQP4 and fluid volume increase in the tumor interstitium. The validation of ALPS index as a quantitative tool for measurement of glymphatic function is currently impeded by the invasiveness of evaluation (*e.g.*, intrathecal contrast medium injection). Despite that, the potential of ALPS index to identify altered glymphatic function has been demonstrated in many neurological conditions. Second, our study is a snapshot in time and does not include longitudinal data on the temporal changes of ALPS index and PTBE volume following treatment. These pieces of information would be helpful to further establish the role of the glymphatic system in PTBE formation.

CONCLUSIONS

In conclusion, metastases with higher tumor ADC and lower ALPS index were associated with larger peritumoral brain edema volumes. The higher tumor ADC may be related to increased periarterial water influx into the tumor interstitium, while the lower ALPS index may indicate insufficient fluid clearance. The changes in both tumor ADC and ALPS index may imply glymphatic dysfunction, which is, at least, partially responsible for peritumoral brain edema formation.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Chang Gung Medical Foundation Institutional Review Board. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

CT, TS, and MC contributed to conception and design of the study. CT and TS contributed to the acquisition and analysis of data. CT and MC contributed to the drafting of the text and the preparation of the figures. The first draft of the manuscript was written by CT. All authors read and approved the final manuscript.

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Integrated Transcriptome Analysis Reveals the Impact of Photodynamic Therapy on Cerebrovascular Endothelial Cells

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Blood vessels in the brain tissue form a compact vessel structure and play an essential role in maintaining the homeostasis of the neurovascular system. The low dosage of photodynamic intervention (PDT) significantly affects the expression of cellular biomarkers. To understand the impact of photodynamic interventions on cerebrovascular endothelial cells, we evaluated the dosage-dependent impact of porfimer sodium-mediated PDT on B.END3 cells using flow cytometer, comet assay, RNA sequencing, and bioinformatics analysis. To examine whether PDT can induce disorder of intracellular organelles, we did not observe any significance damage of DNA and cellular skeleton. Moreover, expression levels of cellular transporters-related genes were significantly altered, implying the drawbacks of PDT on cerebrovascular functions. To address the potential molecular mechanisms of these phenotypes, RNA sequencing and bioinformatics analysis were employed to identify critical genes and pathways among these processes. The gene ontology (GO) analysis and protein-protein interaction (PPI) identified 15 hub genes, highly associated with cellular mitosis process (*CDK1*, *CDC20*, *MCM5*, *MCM7*, *MCM4*, *CCNA2*, *AURKB*, *KIF2C*, *ESPL1*, *BUB1B*) and DNA replication (*POLE2*, *PLOE*, *CDC45*, *CDC6*). Gene set enrichment analysis (GSEA) reveals that *TNF-α/NF-κB* and *KRAS* pathways may play a critical role in regulating expression levels of transporter-related genes. To further perform qRT-PCR assays, we find that *TNF-α/NF-κB* and *KRAS* pathways were substantially up-regulated, consistent with GSEA analysis. The current findings suggested that a low dosage of PDT intervention may be detrimental to the homeostasis of blood-brain barrier (BBB) by inducing the inflammatory response and affecting the expression of surface biomarkers.

Keywords: photodynamic therapy, RNA seq, blood-brain barrier, gliomas, endothelial dysfunction

INTRODUCTION

Blood circulation system plays a critical role in maintaining the homeostasis physiology and supplying the essential nutrients to the targeted organisms or tissue (1). In the nervous system, the blood-brain barrier (BBB) was composed of endothelial cells and other types of cells, for example pericytes and neuron end-foot, to form the compact vascular system (2, 3). The compact BBB can blockade most of the unnecessary molecules to be diffused to the nervous system, and positively pump harmful metabolism waste into blood circulation (4). However, dysfunction or impairment of BBB involves or promotes some pathological processes, such as promoting neurodegenerative inflammation in the brain (5, 6), breakdown of BBB in stroke (7), and psychosis due to BBB disorder (8). Moreover, cerebrovascular BBB could be stimulated by various exogenous factors, such as vascular endothelial growth factor (VEGF) (9, 10), to promote angiogenesis and support the growth of brain tumor (11) during tumorigenesis. These results indicated that any unexpected interventions might lead to a severe stimulus that would disrupt the homeostasis of the neuron-blood system. Thus, exploring the stimulus response of vasculature after any therapeutic interventions, including the damage and biological impact on the biological functions, is imperative.

Photodynamic therapy (PDT) has been widely utilized in the glioblastoma multiforme (GBM) therapy, including the interstitial PDT (iPDT) and post-PDT (12). PDT requires three essential components: oxygen, radiation light, and photosensitizers (13). The intrinsic mechanism of PDT is the interaction between cellular components and reactive oxygen species (ROS), causing damage of cellular components and leading to cell apoptosis (14). After receiving PDT intervention, the overall survival (OS) of GBM patients can be prolonged from 15 months to 27 months (15). The potential reasons for improving the OS of GBM patients may be attributed to increased permeability of chemotherapeutic drugs (16) across the BBB. These results from clinical reports implied that the PDT intervention might strongly stimulate the BBB endothelial cells for specific responses. To further identify and explore the pivotal changes in endothelial cells after PDT intervention may provide more necessary information to guide the clinical application of PDT on GBM therapy.

The interventional effects of PDT on the endothelial cells are decided by types of photosensitizers and specific cell lines (17, 18). Hitherto, many types of photosensitizers have been clinically approved for disease therapy; for example, 5-aminolevulinic acid (5-ALA) (19), hematoporphyrin derivative (HPD) (20), and porfimer sodium (21, 22). Hemoporfin-mediated photodynamic therapy induces cellular autophagy to prevent cellular apoptosis (23). Aloe-emodin-mediated PDT activates the MAPK signaling pathway on HUVECs to inhibit angiogenesis and cell metastasis (24); verteporfin-mediated PDT promotes the expression of vascular endothelial growth factor (VEGF), VEGF receptor (VEGFR)-3, and pigment epithelium-derived factor (PEDF) (25); a low dose of photofrin-mediated PDT increased the expression of VEGF and promoted endothelial cell proliferation in normal brain (26). However, the effect of PDT on the cerebrovascular endothelial cells, especially at transcriptomic levels, might improve a emerging scope for evaluating the impact of PDT on the nervous system.

Herein, we explored the biological impact of the photodynamic intervention on endothelial cells, including apoptosis, DNA damage, cellular skeleton, and expression levels of critical transporter-related genes. Then, we utilized RNA-seq to analyze the biological impact of porfimer sodium-driven photodynamic intervention on the cerebrovascular endothelial cells of the mouse (B.END3 cells). We identified 187 and 2976 differentially expressed gene (DEG) depending on two different interventional dosages, respectively. Bioinformatics analysis using gene ontology (GO), gene set enrichment analysis (GSEA), and KEGG gene sets identified critical pathways that were confirmed by quantitative real-time PCR. Thus, the current study provides additional information about the transcriptome changes in the cerebrovascular endothelial cells during the PDT process and the scope to further evaluate the impact of the photodynamic intervention on BBB homeostasis.

METHODS

Cell Culture

Rat endothelial B.END3 cells were obtained from the Cell Bank of the Chinese Academy of Sciences and cultured in DMEM medium (SH30022.01B, Gibco, USA) with 10% fetal bovine serum (FBS; SH30084.03, HyClone, USA) and 100 U/mL penicillin-streptomycin (Gibco, Cat. 15140122, USA) under 5% CO_2 at 37°C. When the B.END3 cells formed the monolayer, the photosensitizer Porfimer sodium (300 μg/mL) was added to the medium and incubated for 90 min. Then, the B.END3 cells received the laser exposure (635 nm with 100 mW/cm^2) and the light doses at 10 J/cm^2 and 20 J/cm^2, respectively.

RNA Sequencing

After PDT intervention, the cells were lysed, collected, and stored at -80°C until delivered to the Tianjin Novogene Bioinformatic Technology Co., Ltd for further analysis. The whole-genome transcriptome profiling was examined by RNA sequencing process: sequencing on the Illumina Hiseq2500 using 150 bp paired-end reads (6.0 G of throughput). The RNA sequencing data have been deposited to NCBI GEO database (GEO accession cat. GSE172198).

Total RNA was extracted from the cell lysis samples using Total RNA Extraction Kit (R1200/100T, Solarbio Life Sciences, China) and then reverse-transcribed into cDNA using the PrimeScript™ RT MasterMix (Perfect Real Time) (TaKaRa, Japan). The cDNA was used for qPCR using TB Green® Premix Ex Taq™ (Tli RNaseH Plus) (TaKaRa) with gene-specific primers, and the data were normalized against *β-actin* as the control. PCR primers are listed in **Table S1**.

Bioinformatics Analysis

Before further analysis, the RNA data were aligned against the mouse genome (GRCm39, GenBank assembly accession: GCA_000001635.9) and deposited in NCBI GEO database. The GO function analysis was performed using g:profilter website

(https://biit.cs.ut.ee/gprofiler/gost), in which the "ordered query" was selected and other parameters were set as default. The protein-protein interaction (PPI) network was assessed using the STRING website (https://string-db.org/), while the minimum required interaction score was set as the highest confidence (0.900) and kmeans clustering was set as 5. The PPI network was re-generated using Cytoscape (version 3.6.0) with a circular layout. GSEA was performed using GSEA software (v4.1.0) with the molecular signature database obtained from the GSEA website (http://www.gsea-msigdb.org/gsea/msigdb/index.jsp); number of permutations was set as 10000; no collapse was aligned to gene symbols; permutation type was set as gene set, and other parameters were set as default. The function-related information of above-mentioned genes in this study was obtained from the GENE section of NCBI (https://www.ncbi.nlm.nih.gov/). Transcription factors of DEGs were obtained from TRRUST database (https://www.grnpedia.org/trrust/).

All the statistical results and figures were generated using GraphPad_Prism 5.0, and the Venn diagram was obtained from the Van der Peer Lab bioinformatics website (http://bioinformatics.psb.ugent.be/webtools/Venn/). For GSEA analysis, the significance of enriched pathways was set as |Normal Enrichment Score|>1.0 and NORM p-value < 0.05 and FDR q-value <0.05. For other analyses, p-value < 0.05 was considered statistically significant.

Comet Assay

After PDT intervention, 1×10^5 cells were digested, purified, and mixed with 30 μL of low-melting-point agarose (LMPA, 1% DMEM solution). Then, this cell solution was dropped on the glass slide to form a thin film and cooled for 10 min using ice to allow solidification. Then, an additional 75 μL of LMPA (1% DMEM solution) was dropped on this glass slice as the top layer, and the process was repeated. These samples were dipped in the lysis solution (containing 10 mM Tris-HCl, 2.5 M NaCl, 100 mM Na_2EDTA, 1% Triton X-100) overnight. The DNA sample was unwound for 20 min in the alkaline electrophoresis solution and electrophoresis performed for 20 min (voltage 1 V/cm and current 300 mA). Finally, these samples were stained using ethidium bromide (EB; 100 μL, 20 μg/mL). The images of DNA damage were obtained under Zeiss 880 confocal microscopy.

Immunofluorescence

For p53 translocation assay and actin staining assay, B.END3 cells were seeded on glass coverslips and then fixed in 4% paraformaldehyde at room temperature for 10 min. The preparation protocol was performed using standard processes described previously (27, 28). p53 (MA5-12557), Alexa Fluor™ 647 (Invitrogen™, A20186), and Alexa Fluor™ 488 Phalloidin (Invitrogen™, A12379) were purchased from ThermoFisher Scientific.

Statistical Analysis

Statistical examination and image preparation of assays were performed using GraphPad Prism 5.0 software (GraphPad Software Inc.). Student's *t*-test was performed: *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.0001$; ***ns***, no significance.

RESULTS AND DISCUSSION

Biological Impact of the Photodynamic Intervention on Endothelial Cells

The therapeutic products of PDT are reactive oxygen species (ROS) (29) that can strongly interact with cellular components and affect down-stream gene expression. However, the biological impacts of PDT through ROS are complex. Herein, we firstly examined the impact of PDT-generated ROS on cellular apoptosis, which is a major reason to cause tumor cell death. As shown in **Figure 1A**, we observed that the apoptotic percentage of B.END3 cells after receiving PDT is only 1.49%, similar with control (2.04%) or single-factor interventional group (1.81% and 3.11%, respectively). This result implied that low dosage PDT could not induce cellular apoptosis and corroborate safety on BBB endothelial cells.

Next, we investigated the effects of PDT on DNA and cellular skeleton. To explore the effect of PDT, we performed the comet assay to examine whether PDT intervention caused DNA damage. As shown in **Figure 1B**, fluorescence tails of DNA after PDT did not display any significant change at 1.5 h and 3.0 h in B.END3 cells, because the length of fluorescence tail is the critical index for evaluating DNA damage. These results implied that PDT at 20 J/cm^2 laser dose and 300 μg/mL porfimer sodium could not directly damage the DNA in cerebrovascular endothelial cells.

The cellular skeleton plays an essential role in supporting cellular structure and further affecting the cellular processes (30). Herein, we investigated whether PDT can affect the structure of B.END3 cellular skeletons by immunostaining method (31) as shown in **Figure 1C**. After intervention, we cannot observe any morphological difference between intervention-treated group and control groups (including sham and single-factor intervention), although the fluorescent intensity of cellular boundary was stronger than that of other groups. These results could be attributed to the cellular stimulus for ROS and may affect the cell migration, requiring further investigation.

The biological integrity of BBB is decided by the compact cell stack and the specific expression of surface biomarkers, i.e. molecular transporters (4). These surface transporters can selectively pump the necessary nutrients into brain parenchyma and the harmful components out of the nervous system (32). To evaluate the impact of PDT on BBB cellular transporters, we quantitatively measured the mRNA expression levels of critical transporters compared to the no intervention group. As shown in **Figure 1D**, we observed that *SLC2A3* (GLUT-3), *SLC35F6*, *ABCC1* (MRP1), *SLC22A18* (efflux transporter-like protein), and *SLC17A5* (acidic Sugar Transporter) are significantly inhibited, while *ABCC4* (MRP4) is significantly up-regulated. The down-regulation of *SLC2A3* and *SLC17A5* implied that PDT might affect the cellular uptake of glucose into brain parenchyma tissue, owing to that these genes are highly associated with glucose uptake (33, 34). *ABCC1*, *ABCC4*, and *SLC22A18* participate in the drug resistance pathway (35). The down-regulation of *ABCC1* and *SLC22A18* implied that PDT improves the BBB uptake of chemotherapeutic drugs, while significant up-regulation of

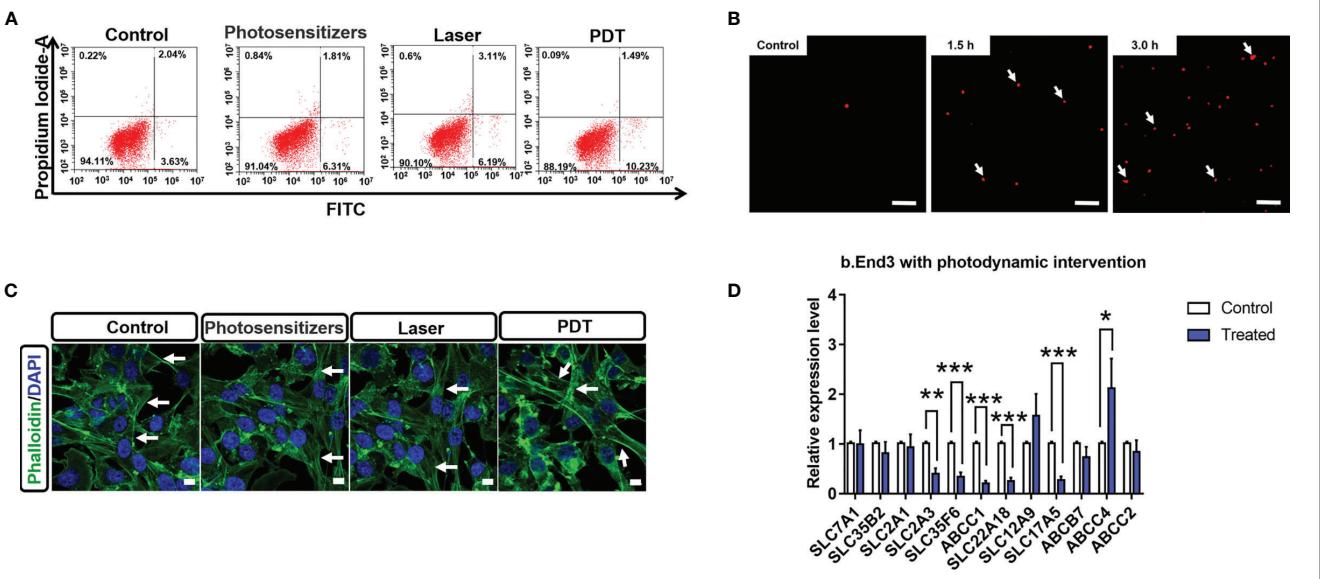

FIGURE 1 | **(A)** Percentage of apoptotic cells stained by Annexin V-FITC and propidium iodide after PDT intervention at 24 h **(B)** Comet assays of B.END3 cells with PDT intervention at 1.5 h and 3.0 h compared to the control group. Nucleus is stained by ethidium bromide. The scale bar is 100 μm. **(C)** Immunostaining for cellular skeletons on control, photosensitizer-treated group, only laser-treated group, and PDT-treated group. Nucleus is stained by DAPI. The scale bar is 50 μm. **(D)** Quantification for mRNA levels of BBB critical biomarkers in B.End3 cells after photodynamic intervention (dose 20 J/cm^2) (n = 3/group). *p<0.05; **p<0.01; ***p<0.001.

ABCC4 implied that PDT might blockade the pumping of therapeutic drugs out of nervous system. The comprehensive impacts of PDT on BBB functions and therapeutic drugs are unclear and should be well determined in the future.

Identification of DEGs

In order to explore the molecular mechanisms of PDT intervention on the cerebrovascular endothelial cells, we performed an RNA sequencing assay to identify the DEGs in the B.END3 cell line. The schematic illustration of the experimental protocol is illustrated in **Figures 2A, B**. END3 cells are seeded dish to form the monolayer and then incubated with photosensitizer (300 μg/mL) for 90 min. Then, the cells were exposed to a 635 nm laser with light doses of 10 J/cm^2 and 20 J/cm^2, respectively. RNA-seq assays were employed to identify the genetic profiles after RNA extraction from these cell samples, and the differentially expressed genes were obtained by the comparative transcriptome analysis between PDT-intervention samples and control samples.

Firstly, principal component analysis (PCA) was conducted to analyze the quality of RNA sequencing. As shown in **Figure 2B**, the PCA score of all samples based on the PKFM values of the gene can be divided into three independent subgroups: Ctrl, Ep1, and Ep2, and confirmed that these RNA sequencing data can be employed in the following assays. The relative expression levels of these genes were plotted as a Volcano map (**Figures 2C, D**), and DEGs were highlighted with blue and red colors, respectively. The threshold value considered as the significant difference was set as p-adj <0.05 and $|\log_2(\text{foldchange})|>0.6$. Herein, we identified 187 and 2976 DEGs in Ep1 and Ep2 groups compared to the control group, respectively. The Venn overlapping diagrams of DEGs in both groups are shown in **Figure 2E**, and 103 joint genes were observed in both groups. Moreover, the significant increase in the DEGs from 187 to 2976 implied that the higher dose of PDT might activate several pathways or biological annotations for PDT stimulus response.

PPI of DEGs

To elucidate the stimulus response and identify the hub genes of endothelial cells after PDT treatment, PPI analysis of DEGs was employed (threshold value of significant difference: p-adj <0.05 and $|\log_2(\text{foldchange}|>1.0$). Herein, all the DEGs were uploaded to the STRING website, and 267 interaction nodes were obtained for further analysis. As shown in **Figure 3A**, we found that three major gene clusters were identified, which can be attributed to DNA repair and cell cycle based on GO analysis. In the largest gene cluster, top-ranked 15 genes (**Figure 3B**) were identified based on the degree nodes and relative biological functions were presented in **Table 1**. A total of 14 enriched genes could be attributed to the cellular mitosis process (*CDK1*, *CDC20*, *MCM5*, *MCM7*, *MCM4*, *CCNA2*, *AURKB*, *KIF2C*, *ESPL1*, *BUB1B*) and DNA replication (*POLE2*, *PLOE*, *CDC45*, *CDC6*).

For cellular mitosis process-related hub genes, these genes can be attributed to E2F targeted genes and cell cycle pathways. To our knowledge, PDT can up-regulated intracellular ROS levels and induced oxidative stress to further regulate down-stream pathways (36). Higher levels of ROS can activate canonical MAPK pathway, and further regulate E2F-mediated gene transcription by p38/COX2/TGFβ/Rb pathway (37). Enrichment results implied that PDT process may participate into regulation of E2F-mediated gene transcription and further regulate expression of down-stream genes. For these genes, *CDK1* is significantly up-regulated after the H_2O_2-induced oxidative stress by inactivating the

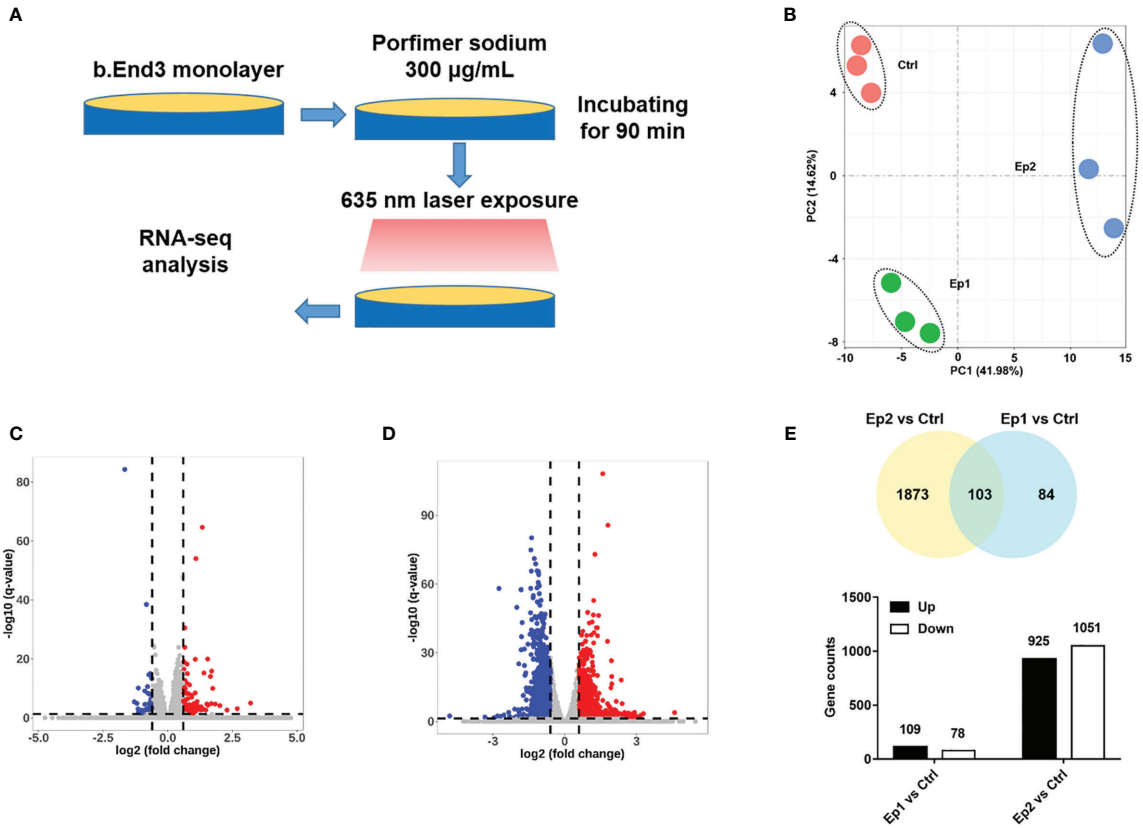

FIGURE 2 | **(A)** Schematic illustration of RNA-sequencing protocol in this investigation. **(B)** PCA analysis of RNA-seq samples based on the genetic components. **(C, D)** Volcano plot of expressed genes based on the intervention dosage. **(E)** Statistical results of different genes (p-adj <0.05 and $|\log_2 FoldChange|>0.6$).

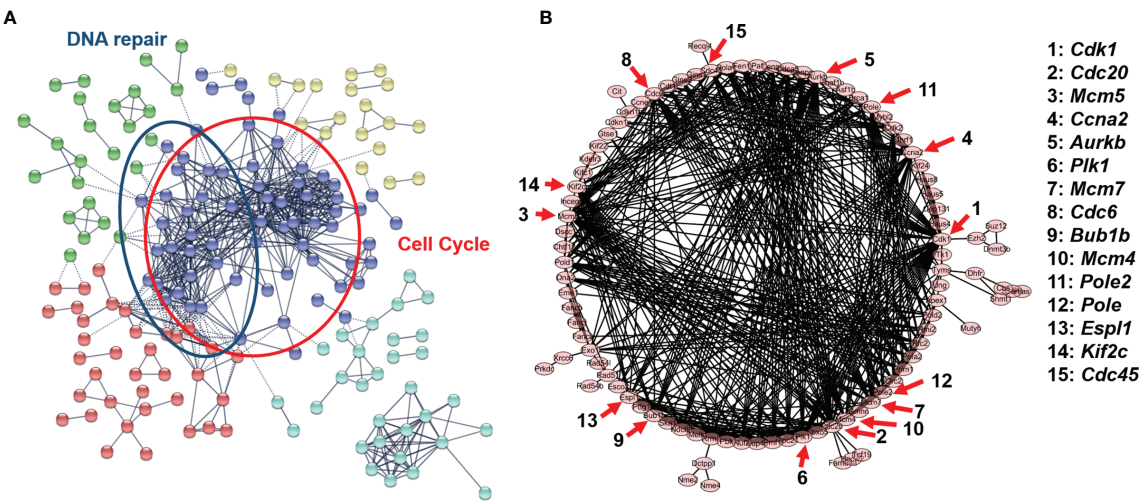

FIGURE 3 | **(A)** Protein-protein interaction network of B.END3 cells with PDT intervention (p-adj<0.05 and $|\log_2 FoldChange|>1.0$). **(B)** Sub-network of cell cycle-related genes and 15-top genes.

TABLE 1 | Biological functions of hub genes in cell cycle-related gene network.

Gene	Biological Function	Gene	Biological Function
CDK1 (DOWN)	Essential for G1/S and G2/M phase transitions of eukaryotic cell cycle	BUB1B (DOWN)	Play a role in the inhibition of the anaphase-promoting complex/cyclosome (APC/C), delaying the onset of anaphase and ensuring proper chromosome segregation
CDC20 (DOWN)	Required for two microtubule-dependent processes, nuclear movement prior to anaphase and chromosome separation	MCM4 (UP)	Essential for the initiation of eukaryotic genome replication
MCM5 (DOWN)	Upregulated in the transition from G0 to G1/S phase of the cell cycle and may actively participate in cell cycle regulation	POLE2 (UP)	Involved in DNA repair and replication
CCNA2 (DOWN)	This protein binds and activates cyclin-dependent kinase 2 and promotes transition through G1/S and G2/M	POLE (DWON)	Involved in DNA repair and chromosomal DNA replication
AURKB (DOWN)	These kinases participate in the regulation of alignment and segregation of chromosomes during mitosis and meiosis by association with microtubules	ESPL1 (DWON)	Stable cohesion between sister chromatids before anaphase and their timely separation during anaphase are critical for chromosome inheritance
PLK1 (DOWN)	Ser/Thr protein kinase and depletion of this protein in cancer cells dramatically inhibited cell proliferation and induced apoptosis	KIF2C (DOWN)	Functions as a microtubule-dependent molecular motor
MCM7 (DOWN)	Essential for the initiation of eukaryotic genome replication	CDC45 (UP)	Essential protein required for the initiation of DNA replication
CDC6 (DOWN)	Essential for the initiation of DNA replication		

DOWN, labeled gene is down-regulated; UP, labeled gene is up-regulated.

PI3K/AKT signaling (38); *CCNA2, CDC45*, and *MCM4* are downstream genes of cyclin-dependent kinase inhibitor p16 in D-galactose-induced aging in mice (39); *BUB1B* is involved in cell division and induces the vulnerability for oxidative stress (40); *PLK1*, as the serine/threonine-protein kinase gene, also plays a major role in chromosomal instability (41) and cell cycle progression (42). These hub genes indicated that low-dose PDT intervention induces may participate into regulation of cell cycle-related pathways.

Impact of Photodynamic Intervention on Transcription Factors

Before further functional analysis, we explored the transcription factors of the DEGs using TRRUST database. As shown in **Figure 4A**, the top 3 transcription factors of these DEGs are E2F1, TP53, and SP1. By overlapping gene sets based on transcription factors, we can find that several genes are identified as shown in **Figure 4B**. TP53 is one of the critical transcription factors to regulate the expression of ATP binding cassette (ABC) transporter-related genes and further modulate cerebrovascular functions (43). To corroborate the effect of PDT on p53 activity, an immunostaining assay was performed in B.END3 cell line after PDT. As shown in **Figure 4C**, fluorescent staining shows that ROS can significantly promote the nuclear translocation of p53 protein to the nucleus, implying that TP53 plays an essential role in affecting the gene expression of DEGs.

Functional Analysis of DEGs

To explore the effect of PDT on biological processes, gene ontology (GO) analysis of DEGs was employed. GO analysis of DEGs can provide the scope of molecular mechanism affected by external stimulus, especially for identifying specific pathway to explain how to affect molecular network. Among these GO analysis tools, GO:profiler is a robust tool for functional enrichment analysis using DEGs (44). Herein, we employed go: profiler for GO analysis and obtained the most affected annotations of DEGs in various groups.

For DEGs in Ep1 group compared to control group, the top 15 enriched annotations are listed in **Figure S1**, wherein the cutoff of FDR q-value was set as 0.05. Among these annotations, cellular response-related, endogenous stimulus-related, and vasculature development-related annotations (labeled by red square) are highlighted. These annotations could be attributed to the chemical stimulus, which might originate from ROS stimulus by photodynamic photosensitizer. Among these annotations, oxidative stress might be the major pathway for PDT response due to higher levels of intracellular ROS. To the best of our knowledge, oxidative stress is highly related to vascular diseases (45); for example, participating in nitric oxide pathway in atherosclerosis pathogenesis (46) and inducing inflammation in aging (47). These enriched annotations confirmed the potential damage of PDT intervention on the vessels.

When the laser dosage of PDT intervention was increased to 20 J/cm^2, number of differentially expressed genes were increased to 2976, and these genes could be divided into up- and down-regulated groups (**Figures 5A, B**), which would be utilized for further GO analysis. For up-regulated DEGs, the 15 top enriched GO annotations were annotated in **Figure 5A**, and many annotations about cellular biological functions were enriched. For example, actin cytoskeleton and cell migration-related annotations (i.e., positive regulation of cell migration, cell motility, and location). The migration or motility-related annotations are highly associated with cell skeleton that is regulated by assembly and disassembly of actin filaments (48). These enriched annotations are consistent with the immunostaining data (**Figure 1C**). Moreover, high migration of endothelial cells promotes angiogenesis in tumor tissue (49, 50) and participates in the vascularization process (51). These enriched annotations of up-regulated DEGs implied that PDT intervention might affect the biological functions of endothelial cell skeleton that need to be determined in the future.

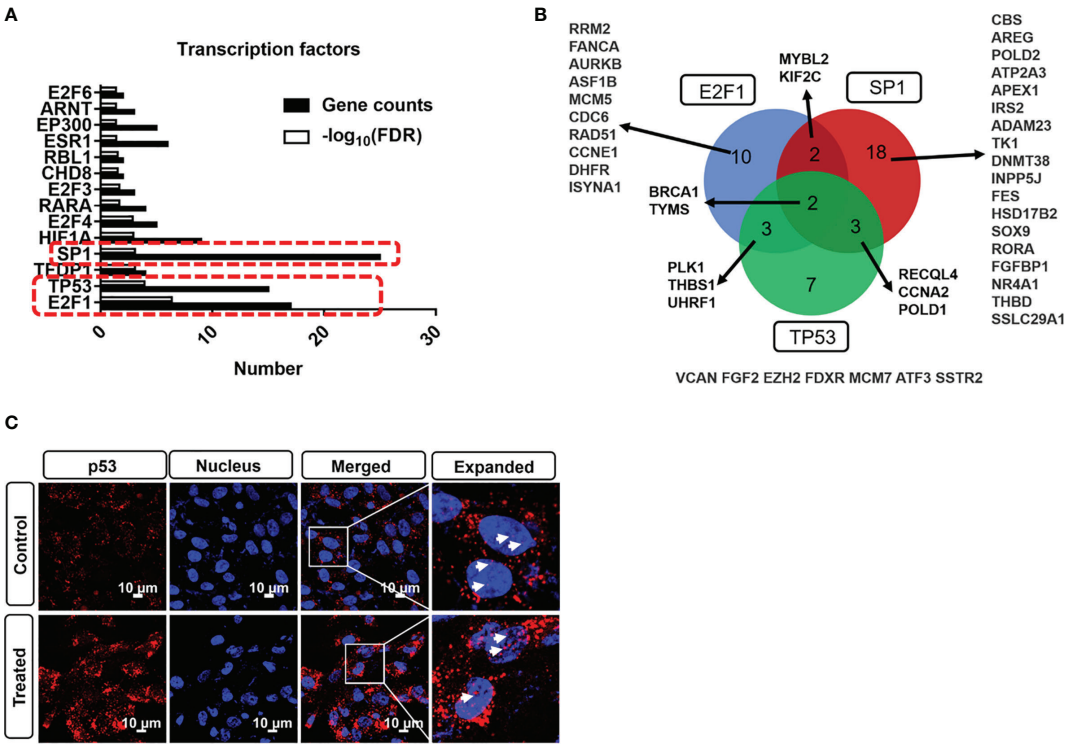

FIGURE 4 | **(A)** Transcription factor profile of DEGs with photodynamic intervention (dose 20 J/cm^2) using TRRUST database. **(B)** DEGs overlap among E2F1, SP1, and TP53 transcription factors. **(C)** Immunostaining of p53 nuclear translation after photodynamic intervention (20 J/cm^2). Scale bar is 10 μm.

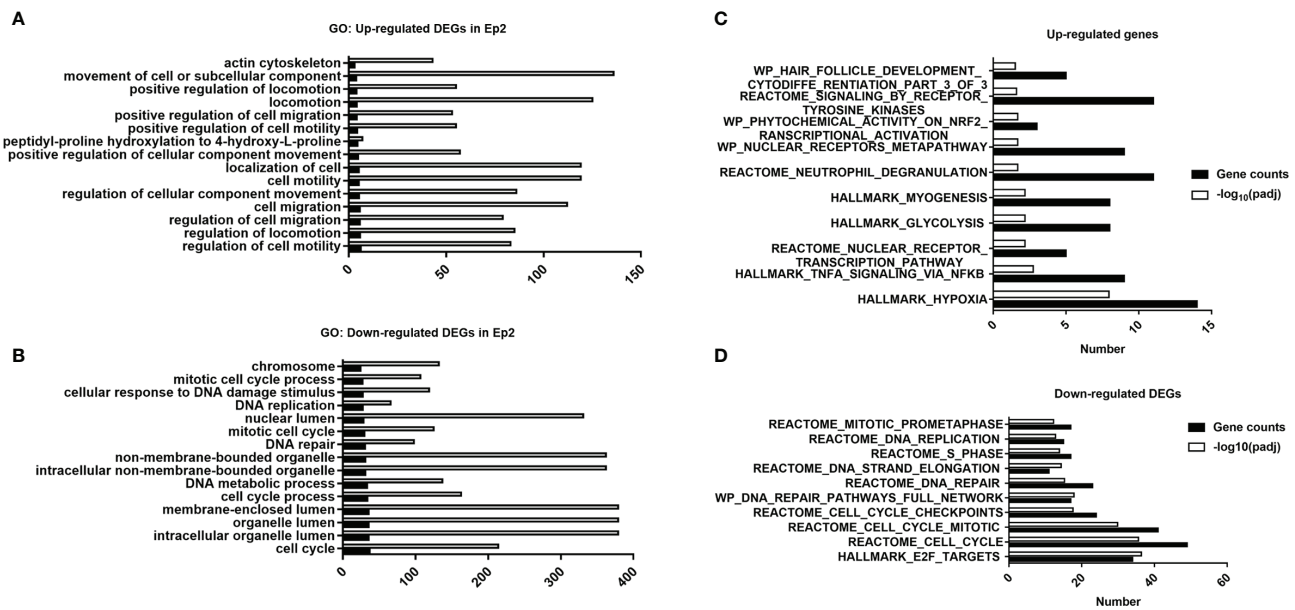

FIGURE 5 | Functional analysis of DEGs in various groups. **(A)** Top 15 annotations of DEGs in the Ep1 group. **(B)** Top 15 annotations of up-regulated DEGs in the Ep2 group. **(C)** Top 15 annotations of down-regulated DEGs in the Ep2 group. **(C)** Computed overlaps between the up-regulated DEGs and MSigDB gene sets. **(D)** Computed overlaps between down-regulated DEGs and MSigDB gene sets. The threshold value considered as the significant difference is $p < 0.05$ and FDR q-value < 0.05.

For down-regulated DEGs, cell cycle-related annotations (for instance, mitotic cell cycle process, DNA replication, and DNA repair) can be obtained as shown in **Figure 5B**. The down-regulation of these biological pathways indicated that the cell cycle of endothelial cells may be suppressed and cause damage to cellular mitosis process. Moreover, the enrichment of chromosome, DNA repair, and DNA metabolic process confirmed that the PDT process damaged the DNA. The suppression of organelle-related annotations (non-membrane-bounded organelle, lumen-related annotations) indicated that PDT might damage nucleoplasm. However, we did not observe any significant impact of PDT on cell apoptosis (**Figure 1A**). All the enriched GO terms are related to the translation process and cell cycle. As reported previously, photodynamic therapy induces cellular autophagy to prevent cellular apoptosis (23). These findings implied that PDT might activate cellular autophagy against the exogenous stimulus.

GSEA Analysis

GO and PPI analysis of DEGs could predict the potential impact on the cellular biological processes. However, it is difficult to identify the attribution of PDT to specific pathways. Conversely, GSEA, which ranked all genes based on the expression level, can be employed to evaluate roles of DEGs on targeted pathways (52). Herein, we employed GSEA to identify critical pathways affected by PDT, including Hallmark, KEGG, Wikipathways, and PID pathway gene sets.

Before the GSEA scoring analysis, we firstly analyzed the overlaps between DEGs and pathway gene sets, which can be divided into up- and down-regulated DEGs, and top 10 ranked pathways are listed in **Figures 5C, D**. For up-regulated DEGs, the top 10 ranked pathways were Hallmark_hypoxia, Hallmark_TNFA_signaling_*via*_NFKB, Reactome_nuclear_receptor_trascritpion_pathway, Hallmark_glycolysis, Hallmark_myogenesis, Reactome_neutrophil_degranulation, WP_nuclear_receptor_metapathway, WP_phyochemical_activity_on_nrf2_transcription_activation, Reactome_signaling_by_receptor_tyrosine_kinases, and WP_hair_follicle_development_cytodiffe_rentiation_part_3_of_3. For down-regulated DEGs, top 10 ranked pathways were Hall_E2F_targets, Reactme_cell_cycle, Reactome_cll_cycle_mitotic, Reactome_cell_cycle_checkpoints, WP_DNA_repair_pathways_full_network, Reactome_DNA_repair, Reactome_DNA_strand_elongation, Reactome_S_phase, Reactome_DNA_replication and Reactome_mitotic_prometaphase. These results suggested that major pathways affected by PDT may focus on inflammation response and cell cycle regulation, which is consistent with GO analysis.

To address the status of critical pathways after PDT treatment, GSEA plots were performed using Hallmark, KEGG, Wikipathways, and PID pathway gene sets (Normalized enriched score, |NES|>1.0 and NOM p-value < 0.05 and FDR q-value <0.05). However, we did not obtain any GSEA terms in Ep1 group compared to control group. Subsequently, only GSEA results of Ep2 group compared to control group were analyzed. As shown in **Figure 6**, only 8 pathways are significantly up-regulated (NES>1.0): coagulation, complement, UV response, inflammatory response, protein secretion, hypoxia, KRAS, and TNFA signaling *via* NFKB. The activation of *KRAS signaling* in endothelial cells induces ERK activity and promotes the expression of angiogenesis and notch signaling, which enhances the cell migration (53). *Coagulation* term means regulation of the blood coagulation system, which is

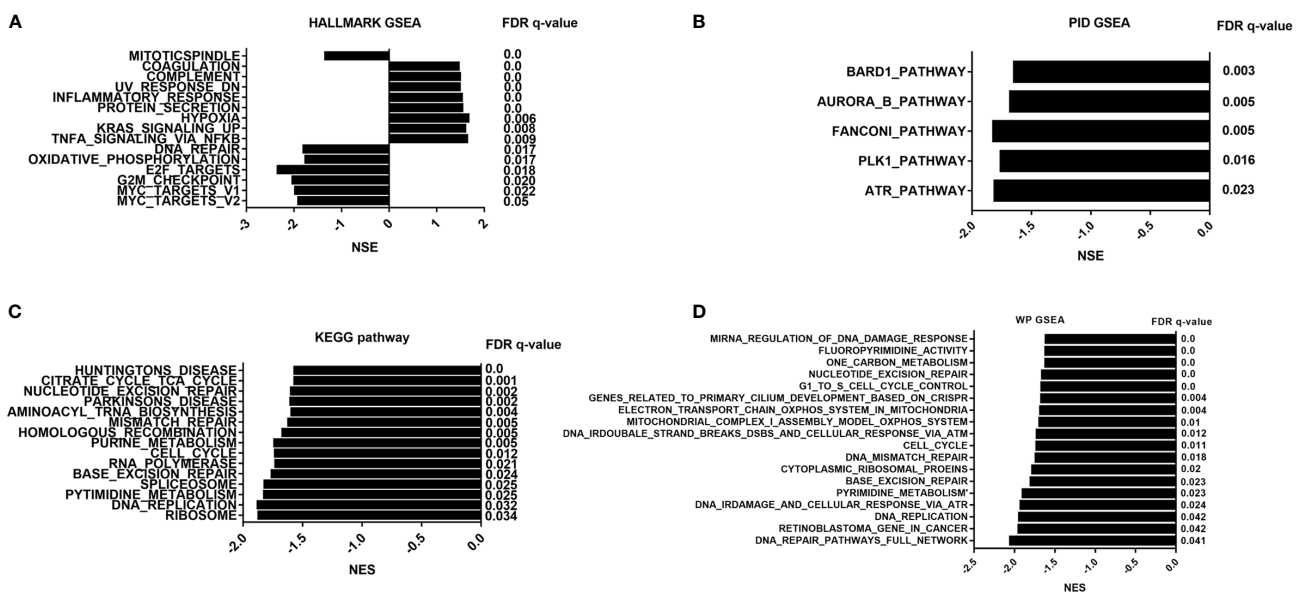

FIGURE 6 | GSEA pathway analysis of B.END3 cells treated with PDT intervention. **(A)** Hallmark gene set analysis; **(B)** PID pathway gene set analysis; **(C)** KEGG pathway gene set analysis; **(D)** WikiPathways gene set analysis. The threshold value considered as the significant difference is P < 0.05, FDR q-value < 0.05, and |NES|>1.0.

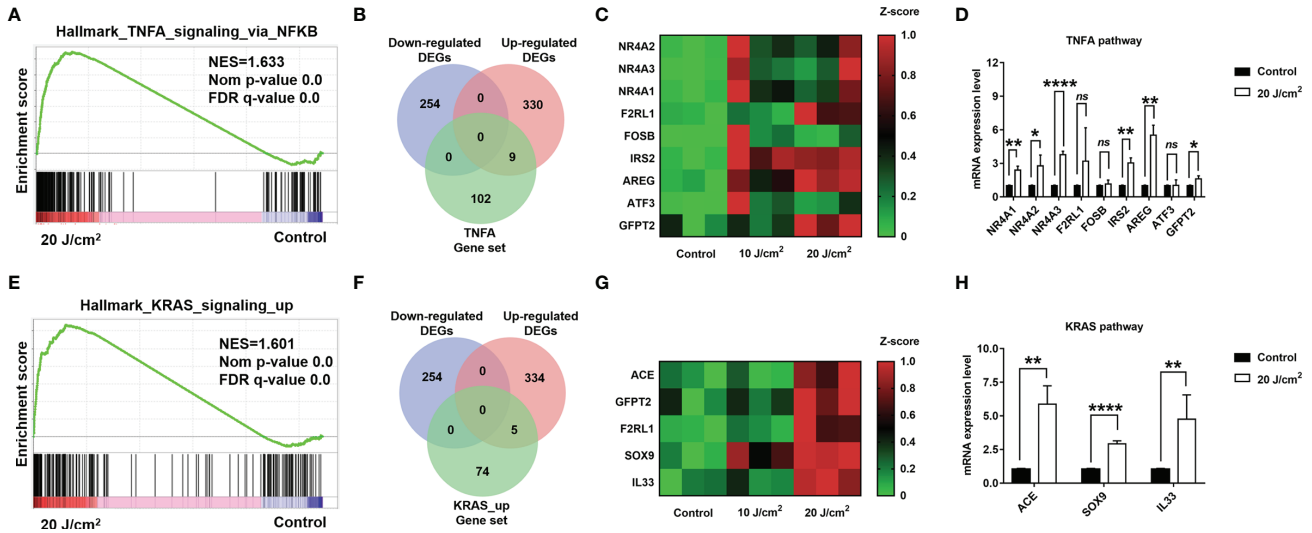

FIGURE 7 | **(A)** Enrichment score profile of enriched pathway, TNFA_signaling_*via*_NFKB. **(B)** Venn diagram of overlapped genes among DEGs and leading edge gene set. **(C)** Heatmap of overlapped gene expression level. **(D)** Quantification for mRNA levels of DEGs in TNF-α/NF-κB pathway (dose 20 J/cm^2). **(E)** Enrichment score profile of enriched pathway, KRAS_signaling_up. **(F)** Venn diagram of overlapped genes among DEGs and leading-edge gene set. **(G)** Heatmap of overlapped gene expression level. **(H)** Quantification for mRNA levels of DEGs in KRAS_signaling_up pathway (dose 20 J/cm^2). *p<0.05; **p<0.01; ****p<0.0001; ns, no significance.

also related to platelets (54). *Hypoxia* is always up-regulated and under low oxygen conditions. *Complement*, *inflammatory response*, and *TNFA signaling via NF-κB* is the major immunological response for the exogenous stimulus that is related to the vasculature disease (55). As above-mentioned assays, expression levels of several transporter-related genes were significantly down-regulated, except *ABCC4*. As previous reported, inflammation response can suppress the expression of ABC-related transporters through affecting Toll-like receptors (56). Moreover, inflammation-related stimulus can promote the expression of MRP4 (*ABCC4*) through ROS/NF-κB pathway (57). Moreover, NES value of 48 GSEA terms was less than -1.0, which implied the down-regulation of these pathways, and these annotations were highly associated with cell cycle process. As previous reported, ROS can regulate cell cycle by p38/ERK MAPK (58) or Cdc25C activity (59). As a result, these results indicated that PDT intervention can affect BBB function through inflammatory response, i.e. *TNFA signaling via NF-κB*.

To further determine whether PDT treatment can up-regulate *TNFA signaling via NF-κB* and *KRAS* pathways, we utilized qRT-PCR to examine expression levels of critical genes in these pathways. Firstly, we identified the critical genes by overlapping DEGs and pathway gene sets, i.e. *TNF-α signaling via NF-κB* and *KRAS* signaling pathways (**Figures 7A–H**), and these critical genes included *NR4A1, NR4A2, NR4A3, F2RL1, FOSB, IRS2, AREG, ATF3, GFPT2*, *ACE*, *SOX9*, and *IL33*. The qRT-PCR assays confirmed the up-regulation of *NR4A1, NR4A2, NR4A3, IRS2, AREG, GFPT2, ACE, SOX9*, and *IL33*, respectively. These results demonstrated that *TNF-α/NF-κB* and *KRAS* pathways were substantially up-regulated after PDT intervention.

CONCLUSION

In summary, we built one approach of RNA sequencing to well-understand the effect of photodynamic intervention in cerebrovascular endothelial cells at cellular transcriptome level. These results provide essential information to elucidate the stimulus response of endothelial cells receiving PDT intervention, which might be associated with affecting the expression of BBB endothelial transporters by activating inflammatory response pathways and cell cycle-related pathways. This stimulus response is crucial for the normal cerebrovascular endothelial cells and to maintain BBB homeostasis. Thus, we speculated that the current study could guide the clinical application of PDT in nervous system diseases and further decrease the drawback of PDT intervention on nervous functions.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: https://www.ncbi.nlm.nih.gov/, GSE172198.

AUTHOR CONTRIBUTIONS

YKH and TXL: Conceptualization, Methodology, Funding acquisition, Supervision. YYH: Investigation, Conceptualization, Methodology, Funding acquisition, Writing – original draft preparation, Resources. LD and HW:

Investigation, Writing – original draft preparation, Writing – review & editing. SC and TYL: Methodology, Writing – review & editing, Resources.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2021.731414/full#supplementary-material

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SYSTEMATIC REVIEW
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Comparative Efficacy of Systemic Agents for Brain Metastases From Non-Small-Cell Lung Cancer With an EGFR Mutation/ALK Rearrangement: A Systematic Review and Network Meta-Analysis

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Background: Brain metastases (BM) from non-small-cell lung cancer (NSCLC) are frequent and carry significant morbidity, and current management options include varying local and systemic therapies. Here, we performed a systematic review and network meta-analysis to determine the ideal treatment regimen for NSCLC BMs with targetable EGFR-mutations/ALK-rearrangements.

Methods: We searched MEDLINE, EMBASE, Web of Science, ClinicalTrials.gov, CENTRAL and references of key studies for randomized controlled trials (RCTs) published from inception until June 2020. Comparative RCTs including ≥10 patients were selected. We used a frequentist random-effects model for network meta-analysis (NMA) and assessed the certainty of evidence using the GRADE approach. Our primary outcome of interest was intracranial progression-free survival (iPFS).

Results: We included 24 studies representing 19 trials with 1623 total patients. Targeted tyrosine kinase inhibitors (TKIs) significantly improved iPFS, with second-and third-generation TKIs showing the greatest benefit (HR=0.25, 95%CI 0.15-0.40). Overall PFS was also improved compared to conventional chemotherapy (HR=0.47, 95%CI 0.36-0.61). In EGFR-mutant patients, osimertinib showed the greatest benefit in iPFS (HR=0.32, 95%CI 0.15-0.69) compared to conventional chemotherapy, while gefitinib + chemotherapy showed the greatest overall PFS benefit (HR=0.26, 95%CI 0.10-0.70). All ALKi improved overall PFS compared to conventional chemotherapy, with alectinib having the greatest benefit (HR=0.13, 95%CI 0.07-0.24).

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Conclusions: In patients with NSCLC BMs and EGFR/ALK mutations, targeted TKIs improve intracranial and overall PFS compared to conventional modalities such as chemotherapy, with greater efficacy seen using newer generations of TKIs. This data is important for treatment selection and patient counseling, and highlights areas for future RCT research.

Systematic Review Registration: https://www.crd.york.ac.uk/prospero/display_record.php?RecordID=179060.

Keywords: targeted therapy, brain metastases, non-small cell lung cancer, neuro-oncology, EGFR inhibitors, ALK inhibitors

INTRODUCTION

Non-small cell lung cancer (NSCLC) is one of the most common and lethal cancer subtypes, with 25-30% of patients developing brain metastases (BMs) over the course of their disease (1). While surgery and radiation-based therapies have been the mainstay of management for local disease control in the brain (2–5), the emergence of targeted therapeutics based on the molecular features of tumors – such as tyrosine kinase inhibitors (TKIs) - have expanded our therapeutic armamentarium. Whereas traditional chemotherapeutic regimens have had limited efficacy against BMs (6), partly perhaps due to the inability to cross the blood-brain barrier (BBB), TKIs have shown significant promise in the management of people with NSCLC BM harboring targetable mutations in several clinical trials (3, 4, 7–9). In particular, newer generations of TKI have been developed to improve BBB penetrance and overcome resistance that has developed to earlier generations, improving their efficacy.

Despite convincing randomized controlled trial (RCT) data, however, to date there has been no comprehensive pooled analysis of the efficacy of the various generations of TKIs in comparison to traditional therapies for BMs, including systemic chemotherapy combined with other local therapies. The emergence of newer generations of TKIs, their individual side effect profiles, and their potentially prohibitive cost, necessitates assessment of their comparative efficacy in order to provide physicians with clinically relevant data that can aid decision-making and provide comprehensive patient counseling. However, head-to-head comparisons in the setting of an RCT are limited.

A network meta-analysis (NMA) allows for comparisons of multiple interventions, particularly when direct comparisons between interventions may be lacking (10). As such, we performed a systematic review and NMA to compare the efficacy of the various targeted therapies, compared with conventional chemotherapy and radiotherapy as a reference, in patients with EGFR mutated or ALK rearranged NSCLC BMs.

METHODS

This study was performed based on a predefined protocol and in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) Extension statement for reporting on network meta-analyses. This study is registered with the International Prospective Register of Systematic Reviews (PROSPERO), ID CRD42020179060.

Search Strategy

We searched MEDLINE, Embase, Cochrane Controlled Register of Trials (CENTRAL), and Web of Science from inception until June 2020 for RCTs. We also searched the grey literature including ClinicalTrials.gov, as well as references of included papers and past review articles. We utilized filters to select for RCTs and human studies wherever possible. We did not restrict results by language. Search terms included "brain metastases", "immunotherapy", "targeted therapy", "surgery, "radiosurgery", and "chemotherapy." A full set of search terms and strategies for each database can be found in Supplement A.

Study Selection and Eligibility Criteria

All studies were screened independently and in duplicate by KB, JD, YE, and WH. Our study was designed using the PICOS method, as outlined in detail in the following sections. Our population included all adults with NSCLC with either an activating EGFR or ALK mutation, with one or more BM confirmed *via* imaging (CT/MRI). We included all RCTs independent of language with ≥10 patients, that compared at least two independent treatment regimens for EGFR mutant or ALK rearranged NSCLC and reported data on patients with BMs. Foreign language studies were translated to English.

Data Extraction and Quality Assessment

Data were extracted independently and in duplicate, using a standardized form. We sought to contact primary authors for missing data where possible. Pre-specified variables of interest included design-related variables, phase, eligibility criteria, intervention arms and descriptions, performance status (KPS or ECOG), duration of treatment and follow-up, and patient demographics (age [median, range], sex).

Our primary outcome was intracranial progression free survival (iPFS), with secondary outcomes including overall PFS, overall survival (OS), intracranial time to progression (iTTP, defined as the time from randomization to disease progression in the brain), and adverse reactions. Many NSCLC clinical trials have excluded patients with BMs or the main outcomes of interest have not included the response of BMs to

therapy. Furthermore, most individuals with metastatic disease succumb to their systemic tumor burden. Therefore, we selected iPFS as the primary outcome in order to focus on the efficacy of any given treatment on the burden of intracranial disease, without confounding from the primary cancer. We only included studies that reported a comparative hazard ratio (HR) between arms for each outcome; the raw median survival times were not used in the analysis.

We performed quality assessment of the included studies using the Cochrane Risk of Bias 2.0 tool (11). Two analysts completed risk of bias assessment in duplicate, and disagreements were resolved *via* consensus. We used CiNEMA, a novel GRADE-based method for assessing confidence in results when multiple interventions are compared, to assess the overall certainty of evidence associated with each analysis (12, 13).

Data Synthesis and Statistical Analysis

A fixed effects or random effect meta-analysis was planned to compare the overall effect of targeted therapy with conventional chemotherapeutic agents for primary and secondary outcomes. We then performed a planned subgroup analysis for EGFR mutated and ALK re-arranged patients. For each outcome, we used HR and calculated the corresponding standard error (SE) for all analyses. In each subgroup, to compare different treatments, we used a frequentist NMA. This approach synthesizes metrics of both direct and indirect comparisons to refine and generate estimates of all possible pair-wise comparisons within a network. When both direct and indirect evidence of a comparison between treatment modalities were available, we first tested the null hypothesis that direct and indirect estimates were similar when enough information was available. When the null hypothesis was not rejected, the treatment effect was synthesized together to yield a network treatment effect. We then used the Rücker & Schwarzer method to rank treatments (14). We combined similar treatments into single nodes where necessary to complete the analysis. In particular, we combined most traditional chemotherapeutic regimens into a single node for most analyses, as various combination approaches have been shown to be similarly efficacious to traditional monotherapy in large trials (15, 16). Where necessary, we grouped EGFR inhibitors (EGFRi) by generation, with first generation defined as gefitinib, erlotinib, and icotinib, second generation as afatinib, and third generation as osimertinib. We also grouped ALK inhibitors (ALKi) similarly, with first generation as crizotinib, and second generation as ceritinib, alectinib, and brigatinib.

We assessed heterogeneity using Cochran's Q statistics or the Chi square test in the case of pairwise meta-analysis. A P value of 0.1 was considered significant heterogeneity. In case of heterogeneity between studies a random effects model was used, otherwise a fixed effects model was used. A two-way P value of less than 0.05 was considered statistically significant. R software version 3.6.3 was used for all analyses.

RESULTS

Search Results and Study Characteristics

Twenty-four studies were included representing 19 unique trials, with 1623 patients total (**Figure 1**). All trials included patients with favorable performance status (ECOG 0-2 or KPS>70) (7–9, 17–33). Nine trials included patients with EGFR mutations, and 10 included patients with ALK rearrangements.

Importantly, most trials that reported outcome data on BMs as a subgroup analysis of all-comer NSCLC patients excluded BMs that were symptomatic or required urgent treatment, meaning many of these patients may have been previously treated with modalities such as surgery or radiation. This was true for all included studies except for Yang 2017 (7). Baseline characteristics and extracted data from included trials are shown in **Tables 1**, **2**.

Efficacy

The efficacy analysis was done using several individual networks, as there was insufficient overlap between all 19 trials to produce a single coherent network graph for each outcome. In addition, not every trial reported all of our outcomes of interest, and analysis of each outcome was done with the available data. Therefore, each efficacy analysis below includes a subset of the nineteen total trials. Supplement E contains league tables showing the results of all pairwise comparisons for each analysis.

Pooled Analyses of EGFRi or ALKi Versus Conventional Chemotherapy for NSCLC Patients With Brain Metastases

iPFS

This analysis included 5 studies, 400 patients with targeted therapy and 114 with conventional chemotherapy. Two focused on patients with ALK re-arrangements and 3 on EGFR mutated patients[7,9(p3),17–19]. We grouped all first-generation targeted therapies together and compared against newer targeted therapies (such as second and third generation). This was done as several individual trials compared first-generation TKIs with second/third generation TKIs, but did not compare different first-generation TKIs against each other. All conventional chemotherapy arms were also grouped together, and we included one study with WBRT added to chemotherapy in the chemotherapy arm (**Figure 2A**) (7). As treatment arms were grouped together, a random effects model was used despite non-significant Q statistic (Q=2.95, df=3, P value=0.39). Both direct and indirect estimates from the model were in agreement (**Supplement C**, **Figure S1**). Targeted therapies were superior to conventional chemotherapy in improving iPFS (**Figure 2A**). Moreover, newer generations TKIs showed greater benefit compared to first generation TKIs (HR=0.39, 95%CI 0.26-0.58), and ranked first in improving iPFS (P-score=1.0) (**Supplement C**, **Figure S2**). The overall certainty of evidence was moderate to high (**Supplement D**, **Table S3**).

Overall PFS

Here, we included nine studies with patients harboring either EGFR mutations or ALK rearrangements (n=419 TKI, n=312 conventional chemotherapy) and reporting overall PFS (7, 20, 21, 23, 26, 28–30). This was a traditional pairwise meta-analysis (**Figure 2B**). TKIs significantly improved overall PFS compared to conventional chemotherapy (X^2 = 16.76, df=8, p=0.03; HR=0.47, 95%CI 0.36-0.61). The overall certainty of evidence was high (**Supplement D**, **Table S4**).

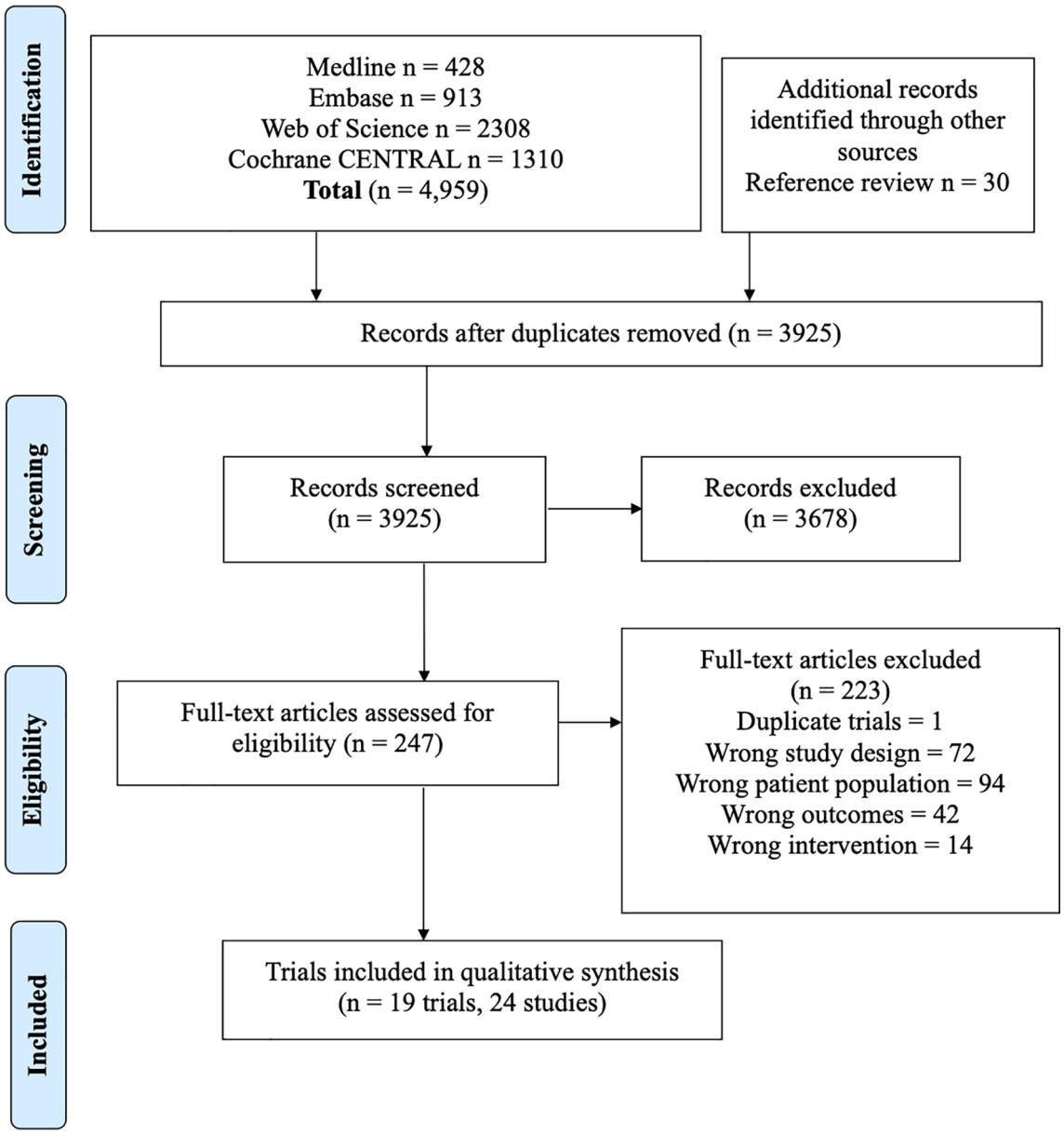

FIGURE 1 | PRISMA flowchart outlining study screening process, with reasons for exclusion at full-text screening stage outlined.

Overall Survival

Seven studies were included with 572 total patients (n=376 TKIs, n=146 chemotherapy, n=50 TKI + chemotherapy, with 6 studies focusing on patients with EGFR mutations and one on patients with ALK re-arrangements) (7, 19, 23, 30–32). First generation TKIs were grouped together, and studies combining first generation TKIs with chemotherapy were treated as a separate node. Newer TKIs (second or third generation) were grouped (**Figure 2C**). Both direct and indirect estimates from the model were in agreement (**Supplement C**, **Figure S3**).

Among included treatments, first generation TKI (gefitinib) plus chemotherapy ranked first in improving overall survival (P score=0.91) and showed a trend toward significance (HR=0.72, 95%CI 0.40-1.27) (**Figure 2C**) (**Supplement C**, **Figure S4**). TKIs alone did not improve overall survival compared to platinum-based chemotherapy alone. The overall certainty of evidence was moderate for all comparisons (**Supplement D**, **Table S5**).

Subgroup Analyses: EGFR Mutant NSCLC With BM

For this set of analyses, we included studies that only enrolled patients with EGFR mutated NSCLC. All first generation EGFRis (gefitinib, erlotinib, icotinib) were grouped.

TABLE 1 | Summary demographics and characteristics of included trials.

Study ID	Trial Design	Patient Population	Arm	Category of Intervention	N BM patients	N women (%)	Median age, years (range)	Previous BM treatments
Camidge et al. (17) (ALTA-1L, NCT0273750)	Phase III, Open-Label, Multicentre, international	ALK-rearranged NSCLC Asymptomatic, stable BMs only	Arm A: Brigatinib	TKI (ALK Gen 3 + EGFR Gen 3)	40	69 (50%), full cohort	58 (27-86), full cohort	Brain radiotherapy, n=18
			Arm B: Crizotinib	TKI (ALK Gen 1)	41	81 (59%), full cohort	60 (29-89) full cohort	Brain radiotherapy, n=19
Hida et al. (18) (J-ALEX, JapicCTI-132316)	Phase III, Open-Label, Multicentre, Japanese centres only	ALK-rearranged NSCLC Asymptomatic, stable BMs only	Arm A: Alectinib	TKI (ALK Gen 2)	14	62 (60%), full cohort	61 (27-85), full cohort	Brain radiotherapy, n=6/16
			Arm B: Crizotinib	TKI (ALK Gen 1)	29	63 (61%), full cohort	59.5 (25-84), full cohort	Brain radiotherapy, n=16/31
Yang (7) (BRAIN, NCT01724801)	Phase III, Open-Label, Multicentre, Chinese centres only	EGFR-mutated NSCLC	Arm A: Icotinib	TKI (EGFR Gen 1)	85	53 (62%)	57 (51-64)	No prior TKI or WBRT
			Arm B: WBRT + Platinum-based Chemotherapy	WBRT + Traditional Chemotherapy	73	41 (56%)	58 (48-63)	
Wu et al. (9) (AURA3, NCT02151981)	Phase III, Open-Label, Multicentre, international	EGFR-mutated NSCLC Stable, asymptomatic BMs only Leptomeningeal metastases excluded	Arm A: Osimertinib	TKI (EGFR Gen 3)	75	41 (55%)	58 (34-82)	Brain radiotherapy, n=28
			Arm B: Platinum-based Chemotherapy	Traditional Chemotherapy	41	29 (71%)	59 (20-79)	Brain radiotherapy, n=20
Soria et al. (19) (FLAURA, NCT02296125)	Phase III, Double-Blind, Multicentre, International	EGFR-mutated NSCLC Stable BMs only	Arm A: Osimertinib	TKI (EGFR Gen 3)	53	178 (63.8%), full cohort	64 (26-85), full cohort	No prior treatment for advanced disease, no prior treatment with TKI
			Arm B: Standard EGFR-TKI (Gefitinib or Erlotinib)	TKI (EGFR Gen 1)	63	172 (62%), full cohort	64 (35-93), full cohort	
Novello et al. (21) (ALUR, NCT02604342)	Phase III, Open-Label, Multicentre, international	ALK-rearranged NSCLC All patients had two lines of previous systemic therapy, including 1 line of previous Crizotinib therapy. Asymptomatic BMs OR symptomatic BMs and ineligible for radiotherapy only.	Arm A: Alectinib	TKI (ALK Gen 2)	47	31 (43.1%), full cohort	55.5 (21-82), full cohort	WBRT (n=23), SRS (n=2), other (n=3). All patients had previous crizotinib therapy
			Arm B: Chemotherapy (Pemetrexed OR Docetaxel)	Traditional Chemotherapy	26	18 (51.4%), full cohort	59 (37-80), full cohort	WBRT (n=9), SRS (n=5), other (n=2). All patients had previous crizotinib therapy
Peters et al. (22) (ALEX, NCT02075840)	Phase III, Open-Label, Multicentre, international	ALK-rearranged NSCLC Leptomeningeal metastases excluded Asymptomatic BMs only	Arm A: Crizotinib	TKI (ALK Gen 1)	58	87 (58%), full cohort	54 (18-91), full cohort	Surgery (n=1), SRS(n=4), WBRT (n=16), other (n=1)
			Arm B: Alectinib	TKI (ALK Gen 2)	64	84 (55%), full cohort	58 (25-88), full cohort	Surgery (n=1), SRS (n=5), WBRT (n=17), other (n=4)
Solomon et al. (23–25) (PROFILE 1014, NCT01154140)	Phase III, Open-Label, Multicentre, international	ALK-rearranged NSCLC Stable and previously treated BMs only	Arm A: Crizotinib	TKI (ALK Gen 1)	39	19 (49%)	48 (29-70)	No prior systemic treatment of advanced disease

(Continued)

TABLE 1 | Continued

Study ID	Trial Design	Patient Population	Arm	Category of Intervention	N BM patients	N women (%)	Median age, years (range)	Previous BM treatments
			Arm B: Platinum-based Chemotherapy	Traditional Chemotherapy	40	31 (78%)	51 (25-76)	
Wu et al. (26) (PROFILE 1029, NCT01639001)	Phase III, Open-Label, Multicentre, Chinese centres only	ALK-rearranged NSCLC Stable and previously treated BMs only	Arm A: Crizotinib	TKI (ALK Gen 1)	21	54 (51.9%), full cohort	48 (24-67), full cohort	No previous systemic therapy for advanced disease
			Arm B: Platinum-based Chemotherapy	Traditional Chemotherapy	32	60 (58.3%), full cohort	50 (23-69), full cohort	
Zhou et al. (27) (ALESIA, NCT02838420)	Phase III, Open-Label, Multicentre, international	ALK-rearranged NSCLC All symptomatic BMs had to be previously treated with radiotherapy	Arm A: Alectinib	TKI (ALK Gen 2)	44	61, full cohort	51 (43-59), full cohort	Brain radiotherapy (n=8)
			Arm B: Crizotinib	TKI (ALK Gen 1)	23	28, full cohort	49 (41-59), full cohort	Brain radiotherapy (n=5)
Shaw et al. (28) (NCT00932893)	Phase II, Open-Label, Multicentre, International	ALK-rearranged NSCLC, all patients had previous 1 line of platinum-based therapy. Asymptomatic BMs only.	Arm A: Crizotinib	TKI (ALK Gen 1)	60	98 (56.64%), full cohort	51 (22-81), full cohort	Progression after 1 platinum-based chemotherapy regimen
			Arm B: Chemotherapy (Pemetrexed or Docetaxel)	Traditional Chemotherapy	60	96 (55.17%), full cohort	49 (24-85), full cohort	
Shaw et al. (29) (ASCEND-5, NCT01828112)	Phase III, Open-Label, Multicentre, International	ALK-rearranged NSCLC, all patients had previous platinum-based chemotherapy and crizotinib. Asymptomatic BMs only.	Arm A: Ceritinib	TKI (ALK Gen 2)	60	68 (59%), full cohort	54, full cohort	Progression after prior treatment on crizotinib + chemotherapy
			Arm B: Chemotherapy (Pemetrexed or Docetaxel)	Traditional Chemotherapy	59	61 (53%), full cohort	54 (47-64), full cohort	
Soria et al. (20) (ASCEND-4, NCT01828099)	Phase III, Open-Label, Multicentre, International	ALK-rearranged NSCLC, Stable and asymptomatic BMs only.	Arm A: Ceritinib	TKI (ALK Gen 2)	59	102 (54%), full cohort	55 (22-81), full cohort	Brain radiotherapy (n=24). Adjuvant or neoadjuvant chemo (n=10)
			Arm B: Platinum-based Chemotherapy	Traditional Chemotherapy	62	114 (62%), full cohort	54 (22-80), full cohort	Brain radiotherapy (n=26). Adjuvant or neoadjuvant chemo (n=9)
Schuler et al. (30) (LUX-Lung 3, NCT00949650)	Phase III, Open-Label, Multicentre, international	EGFR-mutated NSCLC, no prior treatment for NSCLC, no prior TKI. Stable, asymptomatic BMs only.	Arm A: Afatinib	TKI (EGFR Gen 2)	20	14 (70%)	60.5 (37-71)	WBRT (n=7)
			Arm B: Platinum-based Chemotherapy (Cisplatin/ Pemetrexed)	Traditional Chemotherapy	15	12 (80%)	63 (31-74)	WBRT (n=5)
Schuler et al. (30) (LUX-Lung 6, NCT01121393)	Phase III, Open-Label, Multicentre, international (Asia only)	EGFR-mutated NSCLC, no prior treatment for NSCLC, no prior TKI. Stable, asymptomatic BMs only.	Arm A: Afatinib	TKI (EGFR Gen 2)	28	19 (67.9%)	53.5 (30-78)	WBRT (n=6).
			Arm B: Platinum-based Chemotherapy (Cisplatin/ Gemcitabine)	Traditional Chemotherapy	18	12 (66.7%)	55 (35-70)	WBRT (n=6)

(Continued)

TABLE 1 | Continued

Study ID	Trial Design	Patient Population	Arm	Category of Intervention	N BM patients	N women (%)	Median age, years (range)	Previous BM treatments
Park et al. (31) (LUX-Lung 7, NCT01466660.)	Phase IIB, Open-Label, Multicentre, international	EGFR-mutated NSCLC, no prior treatment for NSCLC, no prior TKI. Stable, asymptomatic BMs only.	Arm A: Afatinib	TKI (EGFR Gen 2)	26	91, full cohort	63 (30-86), full cohort	NR
			Arm B: Gefitinib	TKI (EGFR Gen 1)	24	106, full cohort	63 (32-89), full cohort	NR
Hosomi et al. (32) (NEJ009, UMIN000006340)	Phase III, Open-Label, Multicentre, Japanese centres only	EGFR-mutated NSCLC, Asymptomatic BMs only	Arm A: Gefinitib	TKI (EGFR Gen 1)	38	108, full cohort	Mean 64 (SD 8.4), full cohort	Brain radiation (n=15)
			Arm B: Gefitinib + Platinum-based Chemotherapy	TKI (EGFR Gen 1) + Traditional Chemotherapy	50	114, full cohort	Mean 64.8 (SD 7.8), full cohort	Brain radiation (n=17)
Saito et al. (33) (NEJ026, UMIN000017069)	Phase III, Open-Label, Multicentre, international	EGFR-mutated NSCLC, Asymptomatic BMs only	Arm A: Erlotinib + Bevacizumab	TKI (EGFR Gen 1) + Traditional Chemotherapy (VEGFi)	36	71 (63%), full cohort	67 (61-73), full cohort	Patients could not have received previous chemotherapy other than adjuvant chemotherapy
			Arm B: Erlotinib alone	TKI (EGFR Gen 1)	36	73 (65%), full cohort	68 (62-73), full cohort	
Noronha et al. (8) (CTRI/2016/08/007149)	Phase III, Open-Label, Single-centre, India	EGFR-mutated NSCLC	Arm A: Gefinitib	TKI (EGFR Gen 1)	34	83 (47%), full cohort	56 (27-78), full cohort	WBRT (n=31)
			Arm B: Gefitinib + Platinum-based Chemotherapy	TKI Gen 1 + Traditional Chemotherapy	30	86 (49%), full cohort	54 (27-75), full cohort	WBRT (n=22)

iPFS

Three studies with 4 distinct arms of treatment were included in this analysis, with 390 total patients (7, 9, 19). Treatment arms included platinum-based chemotherapy, WBRT plus platinum-based chemotherapy, icotinib (first generation EGFRi), and osimertinib (third generation EGFRi) (**Figure 3A**). Osimertinib significantly improved iPFS (HR=0.32, 95%CI 0.15-0.69) compared to platinum-based chemotherapy alone and ranked first among treatment arms for improving iPFS (P score=0.99) (**Supplement C**, **Figure S5**). Using a first-generation EGFRi or adding WBRT to platinum-based chemotherapy did not improve iPFS (**Figure 3A**). The overall certainty of evidence was low (**Supplement D**, **Table S6**).

Overall PFS

Eight different studies were included in this subgroup with 629 total patients (7, 8, 19, 30–33). As a result, seven distinct treatment arms were compared (**Figure 3B**). A fixed effects model was used (Q=1.59, df=2, P value=0.45).

First generation EGFRi (gefitinib) plus platinum-based chemotherapy (P score=0.94) ranked first followed by osimertinib alone (P score=0.84) and afatinib alone (P score=0.57) in improving overall PFS (**Supplement C**, **Figure S6**). WBRT with chemotherapy or first generation EGFRi alone did not improve overall PFS compared to platinum-based chemotherapy alone (**Figure 3B**). Afatinib alone (HR=0.51, 95%CI 0.27-0.95), osimertinib alone (HR=0.31, 95%CI 0.12-0.86) and gefitinib plus platinum-based chemotherapy (HR=0.26, 95%CI 0.10-0.70) improved overall PFS compared to platinum-based chemotherapy alone. The overall certainty of evidence was low (**Supplement D**, **Table S7**).

Overall Survival

Six studies were included (493 patients) (7, 19, 30–32). All first-generation EGFRi were grouped together for this analysis, resulting in 6 distinct treatment arms (**Figure 3C**). All the included treatment arms showed similar efficacy as platinum-based chemotherapy and did not significantly increase OS (**Figure 3C**). The overall certainty of evidence was low (**Supplement D**, **Table S8**).

Subgroup Analyses: ALK Rearranged NSCLC Patients With BM

For these analyses, we compared ALKi with chemotherapy. All conventional chemotherapy arms were entered under the same node (Chemotherapy) in the network.

iPFS

Two trials (124 patients) with a total of three arms comparing generations of ALKi were included (**Figure 4A**) (17, 18). Alectinib (second generation TKI) showed a trend toward

TABLE 2 | Extracted outcome data from each study.

Study ID	Treatment Arm	Overall Survival	Overall PFS (Definition)	Overall PFS (HR)	Intracranial PFS (Definition)	Intracranial PFS (HR)	Intracranial Time to Progression (Definition)	Intracranial TTP (HR)
Camidge et al. (17) (ALTA-1L, NCT0273750)	Arm A: Brigatinib	NR	NR	NR	Time from randomization to CNS disease progression based on RECIST v1.1 criteria, or death from any cause	0.27 (0.13-0.54)	NR	NR
	Arm B: Crizotinib					Reference		
Hida et al. (18) (J-ALEX, JapicCTI-132316)	Arm A: Alectinib	NR	NR	NR	Time to progression of BMs in patients with BMs at baseline, or death, progression based on RECIST v.1.1 criteria	0.16 (0.02-1.28)	NR	NR
	Arm B: Crizotinib					Reference		
Yang et al. (7) (BRAIN, NCT01724801)	Arm A: Icotinib	0.93 (0.6-1.44), p=0.734	NR	NR	Defined as the time from randomisation to progression of intracranial disease or death from any cause. BMs assessed *via* MRI every 6 weeks according to RECIST v1.1 criteria.	0.56 (0.36-0.90), p=0.014	Time from randomization to increase in symptoms from BMs or any symptoms of deterioration	0.75 (0.44-1.27), p=0.284
	Arm B: WBRT + Platinum-based Chemotherapy	Reference				Reference		Reference
Wu et al. (9) (AURA3, NCT02151981)	Arm A: Osimertinib	NR	NR	NR	Defined as time to intracranial progression or death from any cause. BMs assessed *via* CT or MRI according to RECIST v1.1 criteria.	0.32 (0.15-0.69), p=0.004	NR	NR
	Arm B: Platinum-based Chemotherapy					Reference		
Soria et al. (19) (FLAURA, NCT02296125)	Arm A: Osimertinib	0.83 (0.53-1.30)	Time to disease progression or death from any cause, assessed according to RECIST v.1.1 criteria. Tumors were imaged every 6 weeks until 18 months, then every 12 weeks until disease progression.	0.47 (0.30-0.74), p<0.001	Time to intracranial progression or death from any cause. BMs assessed *via* CT or MRI according to RECIST v1.1 criteria.	0.48 (0.26-0.86), p=0.014	NR	NR
	Arm B: Standard EGFR-TKI (Gefitinib or Erlotinib)	Reference		Reference		Reference		
Novello et al. (21) (ALUR, NCT02604342)	Arm A: Alectinib	NR	Time to disease progression or death from any cause, assessed every 6 weeks *via* CT or MRI using RECIST v1.1 criteria	0.12 (0.05-0.27), p<0.001	NR	NR	Time from randomization to radiographic brain tumour progression on MRI using RECIST criteria	0.16 (0.06-0.43)
	Arm B: Chemotherapy (Pemetrexed OR Docetaxel)			Reference				Reference
Peters et al. (22) (ALEX, NCT02075840)	Arm A: Crizotinib	NR	Time to disease progression or death from any cause. Progression assessed as per RECIST v1.1 criteria.	Reference	NR	NR	Time from randomization to radiographic tumour progression on MRI using RECIST v1.1 criteria. HR is cause-specific HR for CNS progression (excluding pts who had non-CNS progression OR death)	Reference
	Arm B: Alectinib			0.4 (0.25-0.64), p<0.0001				0.18 (0.09-0.36), p<0.0001
Solomon et al. (23–25) (PROFILE 1014, NCT01154140)	Arm A: Crizotinib	1.285 (0.716-2.306), p=0.3991	Time to disease progression or death from any cause. Progression assessed	0.4 (0.23-0.69), p<0.001	NR	NR	Intracranial time to tumor progression was defined as time from randomization to first	0.45 (0.19-1.07), p=0.063

(Continued)

TABLE 2 | Continued

Study ID	Treatment Arm	Overall Survival	Overall PFS (Definition)	Overall PFS (HR)	Intracranial PFS (Definition)	Intracranial PFS (HR)	Intracranial Time to Progression (Definition)	Intracranial TTP (HR)
	Arm B: Platinum-based Chemotherapy	Reference	as per RECIST v1.1 criteria.	Reference			documentation of objective intracranial progression according to RECIST v1.1 criteria	Reference
Wu et al. (26) (PROFILE 1029, NCT01639001)	Arm A: Crizotinib Arm B: Platinum-based Chemotherapy	NR	Time to progression of disease as defined by RECIST v1.1, including primary tumour, or death from any cause. Imaging was done every 6 weeks.	0.497 (0.26-0.95) Reference	NR	NR	The time from randomization to the first objective tumor progression considering only intracranial disease, according to RECIST v1.1 criteria.	0.67 (0.33-1.34), p=0.13 Reference
Zhou et al. (27) (ALESIA, NCT02838420)	Arm A: Alectinib Arm B: Crizotinib	NR	Time to progression of disease as defined by RECIST v1.1, including primary tumour, or death. Imaging done every 8 weeks.	0.11 (0.05-0.28) Reference	NR	NR	Progression due to newly developed CNS lesions or progression of pre-existing baseline CNS lesions per independent review committee assessment according to RECIST v1.1, imaging done every 8 weeks *via* brain MRI. Competing risk analysis done for HR (cause-specific HR for CNS progression without previous systemic progression reported)	0.14 (0.06-0.3), p<0.0001 Reference
Shaw et al. (28) (NCT00932893)	Arm A: Crizotinib Arm B: Chemotherapy	NR	Time to progression of disease as defined by RECIST v1.1, including primary tumour, or death. Imaging done every 6 weeks.	0.67 (0.44-1.03) Reference	NR	NR	NR	NR
Shaw et al. (29) (ASCEND-5, NCT01828112)	Arm A: Ceritinib Arm B: Chemotherapy (Pemetrexed or Docetaxel)	NR	Time to progression of disease as defined by RECIST v1.1, including primary tumour, or death. Imaging done every 6 weeks until l8 months, then every 9 weeks thereafter.	0.5 (0.33-0.76) Reference	NR	NR	NR	NR
Soria et al. (20) (ASCEND-4, NCT01828099)	Arm A: Ceritinib Arm B: Platinum-based Chemotherapy	NR	Time to progression of disease as defined by RECIST v1.1, including primary tumour, or death. Imaging done every 6 weeks until 33 months, then every 9 weeks thereafter.	0.7 (0.44-1.12) Reference	NR	NR	NR	NR
Schuler et al. (30) (LUX-Lung 3, NCT00949650)	Arm A: Afatinib Arm B: Platinum-based Chemotherapy (Cisplatin/ Pemetrexed)	1.15 (0.49-2.67), p=0.752 Reference	Time to progression of disease as defined by RECIST v1.1, including primary tumour, or death. Imaging done every 6 weeks until 4 months, then every 12 weeks until progression.	0.54 (0.12-1.25), p=0.138 Reference	NR	NR	NR	NR
Schuler et al. (30) (LUX-Lung 6, NCT01121393)	Arm A: Afatinib	1.13 (0.56-2.26), p=0.732	Time to progression of disease as defined by RECIST v1.1, including primary tumour, or	0.47 (0.18-1.21), p=0.106	NR	NR	NR	NR

(Continued)

TABLE 2 | Continued

Study ID	Treatment Arm	Overall Survival	Overall PFS (Definition)	Overall PFS (HR)	Intracranial PFS (Definition)	Intracranial PFS (HR)	Intracranial Time to Progression (Definition)	Intracranial TTP (HR)
	Arm B: Platinum-based Chemotherapy (Cisplatin/ Gemcitabine)	Reference	death. Imaging done every 6 weeks until 4 months, then every 12 weeks until progression.	Reference				
Park et al. (31) (LUX-Lung 7, NCT01466660.)	Arm A: Afatinib	1.16 (0.61-2.21), p=0.21	Time from randomization to disease progression, pre RECIST v1.1	0.76 (0.41-1.44), p=0.93	NR≈	NR	NR	NR
	Arm B: Gefitinib	Reference	criteria, or death from any cause. Imaging done every 8 weeks until week 64 then every 12 weeks thereafter.	Reference				
Hosomi et al. (32) (NEJ009, UMIN000006340)	Arm A: Gefinitib	Reference	Time from randomization to disease progression,	Reference	NR	NR	NR	NR
	Arm B: Gefitinib + Platinum-based Chemotherapy	0.66 (0.4-1.07)	per RECIST v1.1, or death from any cause. Imaging done every 8 weeks until 12 months, then every 12 weeks thereafter	0.32 (0.19-0.53)				
Saito et al. (33) (NEJ026, UMIN000017069)	Arm A: Erlotinib + Bevacizumab	NR	Time from randomization to disease progression as per RECIST v1.1, or death from any cause. Imaging done every 6 weeks until 18 months, then every 12 weeks thereafter.	0.78 (0.42-1.43)	NR	NR	NR	NR
	Arm B: Erlotinib alone			Reference				
Noronha et al. (8) (CTRI/2016/08 /007149)	Arm A: Gefinitib	NR	Time from randomization to disease progression as	Reference	NR	NR	NR	NR
	Arm B: Gefitinib + Platinum-based Chemotherapy		per RECIST v1.1, or death from any cause. Imaging done every 9 weeks.	0.53 (0.29-0.98)				

improving the iPFS (HR=0.16, 95% CI 0.02-1.28) (**Figure 4A**). Alectinib (P score=0.81) ranked first followed by brigatinib (P score =0.65) in improving iPFS (**Supplement C**, **Figure S7**). Brigatinib was superior to crizotinib (first generation ALKi) in prolonging iPFS (HR=0.27, 95%CI 0.14-0.54). The overall certainty of evidence was low for these comparisons (**Supplement D**, **Table S9**).

Intracranial TTP

Five studies were included (394 patients) (21–23, 26, 27, 34). The three treatment arms in this subgroup were alectinib, crizotinib, and chemotherapy (**Figure 4B**). Alectinib ranked first for improving iTTP (P score=1) (**Supplement C**, **Figure S8**). Alectinib significantly improved iTTP compared to both crizotinib (HR=0.17, 95%CI 0.11-0.28) and chemotherapy (HR=0.11, 95%CI 0.06-0.20) (**Figure 4B**). Crizotinib showed a trend toward improved iTTP compared to chemotherapy (HR=0.64, 95%CI 0.39-1.04). The overall certainty of evidence was moderate to high (**Supplement D**, **Table S10**).

Overall PFS

Eight different studies were included (754 patients) (20–23, 26–29). There were four distinct treatment arms in this analysis (**Figure 4C**). All three targeted therapies improved overall PFS compared to conventional chemotherapy. Alectinib ranked first in improving overall PFS (P score=1) (**Supplement C**, **Figure S9**). The overall certainty of evidence was moderate to high (**Supplement C**, **Table S11**).

Quality Assessment

The quality assessment of included studies showed an overall low risk of bias in 13/19 trials and 6 trials with 'some concerns' overall. There were no studies with an overall high risk of bias. **Supplement B**, **Table S1** shows full RoB 2.0 results for all included studies.

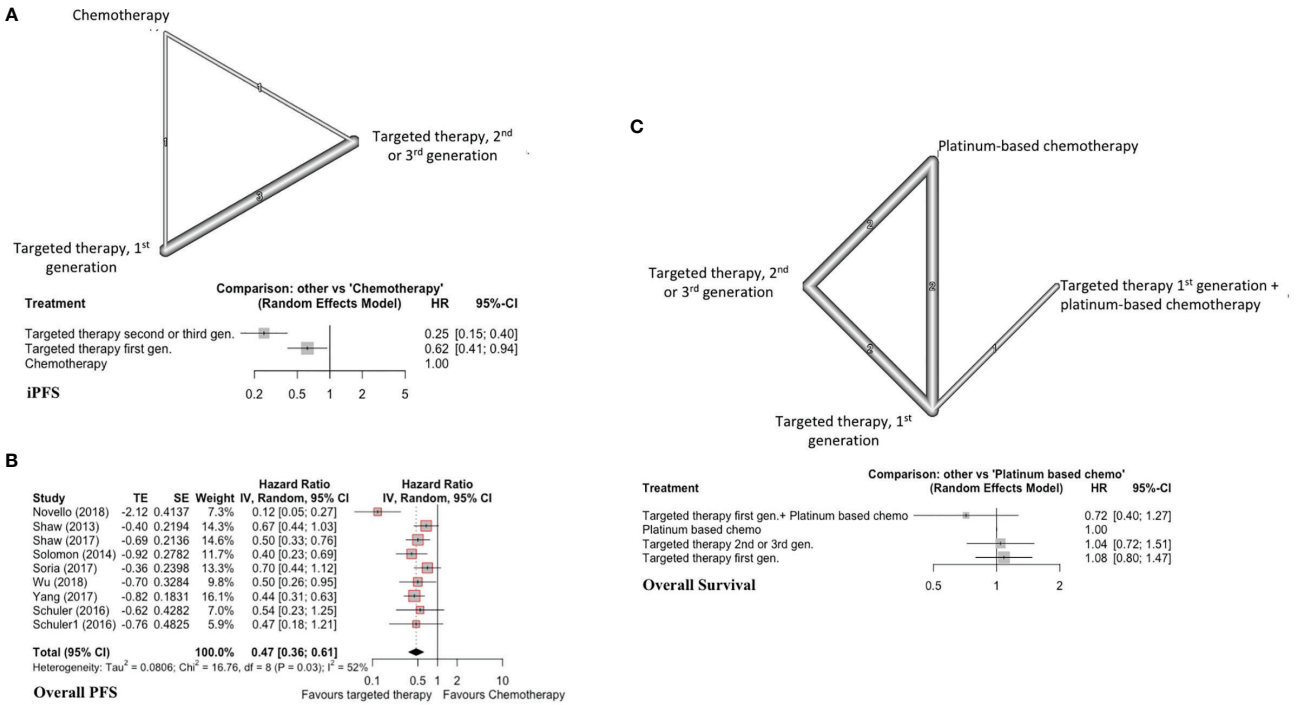

FIGURE 2 | **(A)** iPFS in EGFR-mutated/ALK-rearranged NSCLC. Upper panel - network graph with treatment nodes included in analysis. The number included in the link between treatments indicates the number of studies included in that direct comparison. Lower panel - forest plot showing comparison of included treatment arms in the network meta-analysis, with associated hazard ratios. The treatment with no shown CI was chosen as the reference study arm. **(B)** Forest plot of traditional pairwise meta-analysis comparing all targeted therapies versus traditional chemotherapy for overall PFS in EGFR-mutated/ALK-rearranged NSCLC. **(C)** OS in EGFR-mutated/ALK-rearranged NSCLC, with network graph (upper panel) and forest plot (lower panel).

Adverse Events

All studies reported adverse events, with traditional chemotherapy having similar incidence of grade 3/4 AEs across studies, and most targeted therapies with a similar safety profile. In studies directly comparing any EGFRi alone with EGFRi plus chemotherapy or chemotherapy alone, the EGFRi therapies had a lower incidence of Grade 3/4 AEs (7–9, 30, 32, 33). Among ALKi, alectinib showed a lower incidence of Grade 3/4 AEs than both chemotherapy and crizotinib in direct comparisons (18, 21, 22, 27). **Supplement B**, **Table S2** summarizes the incidence of grade 3/4 AEs across studies.

DISCUSSION

In this systematic review and NMA, we provide a quantitative comparison showing the superiority of TKIs against conventional chemotherapeutic agents in improving both iPFS and overall PFS in patients with NSCLC with BMs, with a moderate to high degree of certainty. This benefit was greater with newer generations of TKIs. The iPFS/overall PFS benefit with TKIs did not translate to a difference in OS compared to conventional chemotherapy, with or without WBRT. To the best of our knowledge, this is the first study to provide a comprehensive quantitative comparison based on RCT data of the efficacy of TKIs in patients with BMs from NSCLC and activating EGFR mutations or ALK rearrangements, which is an important subpopulation of patients with NSCLC. The use of a NMA allowed for comparisons between treatment arms that have never been directly assessed in existing trials, providing new quantitative insight into the comparative efficacy of these treatments, in addition to the already well-established qualitative superiority of these agents. Previous meta-analyses have demonstrated the efficacy of adding TKI therapy to traditional radiotherapy or chemotherapy approaches in EGFR-mutant patients, similar to our results in this analysis (35–38). However, a recent meta-analysis by Singh et al. found no PFS or OS benefit on addition of TKIs to RT in EGFR or ALK mutant patients (39). Importantly, this study and other past works have included numerous retrospective and non-randomized studies in their analysis, limiting the quality of evidence in each individual analysis. Our work differs from past meta-analyses in that it is the first comprehensive analysis based entirely on RCT data, thereby providing the highest level of evidence to inform future clinical decision-making in this population of patients. Our findings are also in keeping with the National Comprehensive Cancer Network Clinical Practice Guidelines in NSCLC, which recommend first-line TKIs in patients with metastatic disease and activating EGFR or ALK mutations (40).

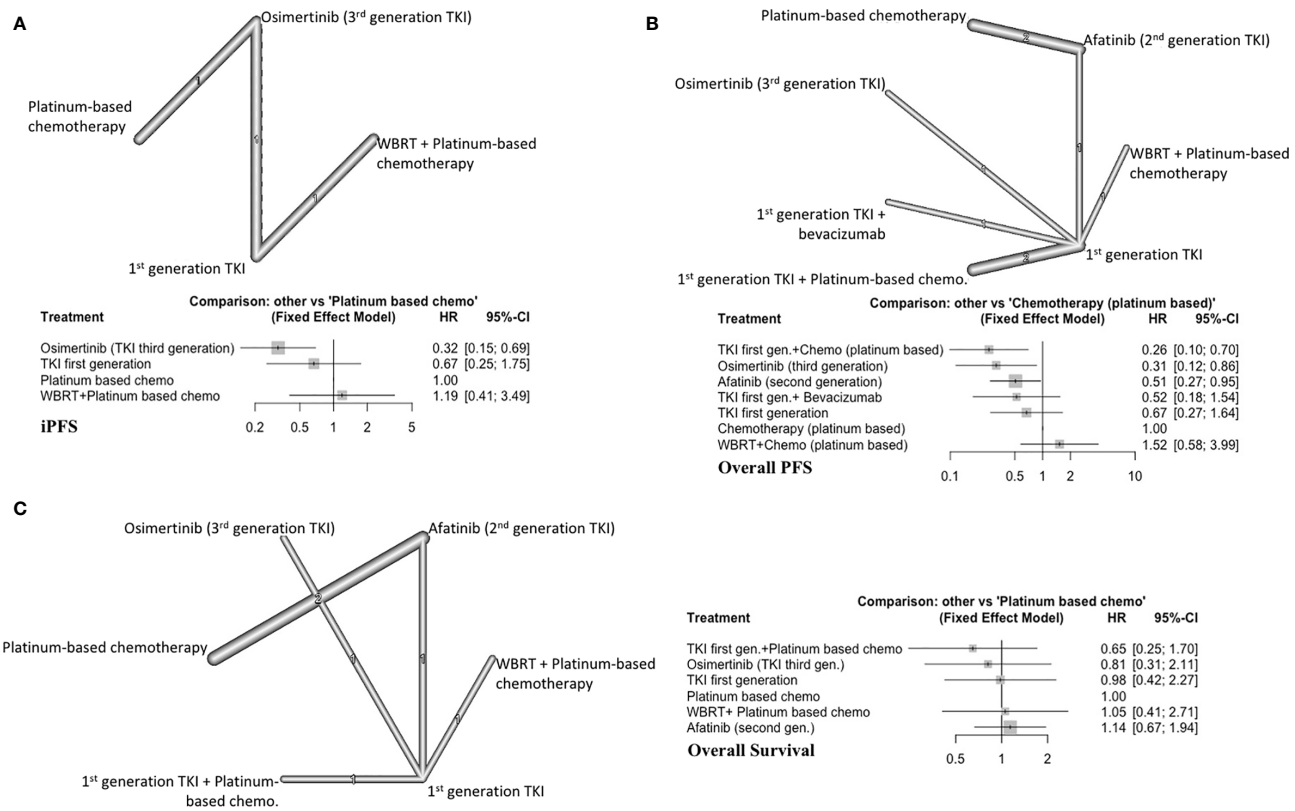

FIGURE 3 | **(A)** iPFS in EGFR-mutated NSCLC, with network graph (upper panel) and forest plot (lower panel), **(B)** Overall PFS in EGFR-mutated NSCLC, with network graph (upper panel) and forest plot (lower panel), **(C)** OS EGFR-mutated NSCLC, with network graph (left panel) and forest plot (right panel).

The improvement of iPFS we observed with newer generations of TKIs is likely in large part due to their proficiency in crossing the BBB, which not only enables targeting of bulk tumor but also micro-metastases (2, 4, 41–44). The current standard of care in NSCLC treatment in many center worldwide already focuses on use of TKIs rather than traditional chemotherapy wherever possible – however, we show significantly increased benefit with the use of newer generations of TKIs. The CNS penetrance of newer TKIs is particularly relevant as we have seen a recent paradigmatic shift in favor of SRS instead of WBRT in the local management of oligometastatic brain disease; while SRS is associated with a lower rate of long-term cognitive decline, the rate of distant BM recurrence is higher than with WBRT (45). Therefore, the use of CNS-penetrating TKIs may help reduce BM recurrence in patients receiving SRS instead of WBRT, or potentially allow select groups of patients to avoid these local treatments altogether. We were unable to find direct comparisons between SRS and TKIs, and indirect comparisons were not feasible. Assessing the efficacy of combinations of SRS and TKI as well as direct head-to-head comparisons of non-inferiority are important areas of future research.

The addition of WBRT to conventional chemotherapy did not improve overall PFS or OS in patients with EGFR mutated NSCLC with BMs. This reaffirms the notion that patients often succumb to their systemic disease and emphasizes the importance of cognitive preservation for as long as possible. Importantly, however, the lack of OS benefit with TKIs despite their intracranial efficacy may be partially explained by patient crossover to TKIs in individual trials after progression on ineffective chemotherapy, which may have confounded the results. This issue was observed in our analysis of overall PFS as well: gefitinib and chemotherapy led to an improvement of overall PFS compared to osimertinib, despite the latter having greater intracranial efficacy. This observation may be related to osimertinib being evaluated as a second-line agent whereas gefitinib and chemotherapy were studied as first-line therapy. Patients with BMs also represent those with more advanced disease, and may therefore be more likely to succumb to their disease independent of treatment. In addition, the combination of EGFR and ALK-positive patients in our analysis may have impacted OS results, since the prognosis of patients with these two activating mutations can differ significantly (23, 46–49).

Limitations

Using an NMA, we were able to compare the efficacy of different modalities of treatment, specifically, different generations of targeted therapies and conventional chemotherapy against each other in NSCLC with BMs. Conducting numerous RCTs to individually compare each of these treatment options is costly,

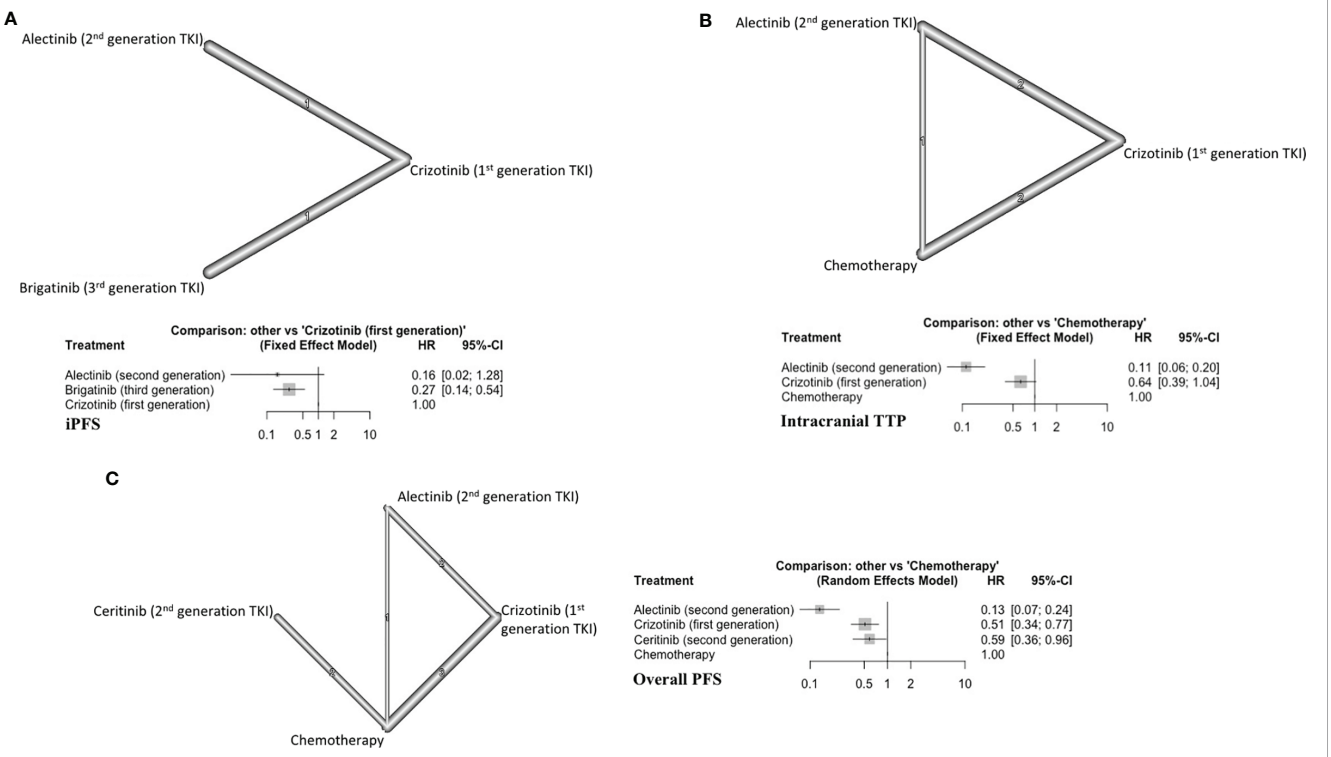

FIGURE 4 | **(A)** iPFS in ALK-rearranged NSCLC, with network (upper panel) and forest plot (lower panel), **(B)** Intracranial TTP in ALK-rearranged NSCLC (upper panel) and forest plot (lower panel), **(C)** Overall PFS in ALK-rearranged NSCLC (left panel) and forest plot (right panel).

not feasible, and in some cases unethical. To lower the internal bias, we only included RCTs. As a result, we did not include some other targetable genetic alterations in NSCLC such as *ROS1* translocations, *MET* exon-14-skipping mutations, or *RET* fusions. Further, we were unable to create a single network for each outcome due to several broken links between our included studies and limited outcome data. Therefore, our analysis was completed using several fragmented networks with a subset of studies in each network, limiting the power of each individual analysis. We also combined several treatment arms in order to obtain more robust comparisons; we grouped different generations of TKIs when possible and treated conventional chemotherapy as a single node wherever necessary. Any heterogeneity present within these individual classes may represent a source of confounding, as different chemotherapy regimens and TKIs may have varying efficacy. However, as shown in **Table 1**, the vast majority of the interventions classed as "traditional chemotherapy" used platinum-based doublet regimens or single-agent regimens with pemetrexed or docetaxel, which have been shown to have relatively comparable efficacy in the existing literature (15, 16, 50). In addition, the goal of our work was to perform a high-level class-based analysis of traditional chemotherapy approaches versus newer TKIs in BM patients with NSCLC. Combining classes of similar therapies is necessary to answer this specific question, despite differences in intra-class efficacy that may exist.

We also included several phase 2 trials, which might be at risk of small study bias (28, 31). Our analysis is also limited by the moderate or low certainty of evidence in some cases. Since many of our included studies excluded patients who had symptomatic or otherwise unstable BMs, the results of this work may also not be generalizable to patients suffering acute neurological decline from their BMs. Moreover, we included several studies that only enrolled patients who failed prior TKI or chemotherapy treatment; these patients may be distinct from chemotherapy-naïve patients and might have affected the result (20, 28, 29). Nonetheless, the inclusion of these patients reflects the real-world relevance of our results, as patients seen in everyday practice may often have had several rounds of therapy and stabilizing treatment prior to being considered for successive generations of targeted therapy.

Our study provides a comprehensive analysis of how the various interventions for NSCLC BMs with EGFR mutations/ALK rearrangements rank quantitatively in as close to a "real-world" setting as possible. Furthermore, although the cost-effectiveness of upfront next generation sequencing for known NSCLC mutations has been demonstrated, the cost-effectiveness of the respective generations of TKIs have been limited (51, 52). Our results provide valuable quantitative data on the comparative efficacy of TKIs in comparison to each other and chemotherapy, providing a basis for future work including cost-effectiveness analyses and RCTs focusing on BM patients in NSCLC.

CONCLUSIONS AND IMPLICATIONS FOR PRACTICE

In this work, we conducted a comprehensive systematic review and NMA on patients with either EGFR mutated or ALK rearranged NSCLC with BMs. TKIs showed improved intracranial and overall PFS compared to conventional modalities such as chemotherapy and WBRT, with greater benefit seen using newer generations of TKIs. The incidence of serious adverse events was also lower with most TKIs. Taken together, these results underscore the importance of genetic testing in defining targetable mutations in BMs from NSCLC, support the use of newer generations of TKIs, and point towards the need for the development of further precision therapies for the treatment of this set of tumours. We provide a quantitative basis for the design of future clinical trials evaluating the efficacy of these regimens on the specific cohort of BM patients with NSCLC. Further trials are necessary to establish the efficacy of these treatments in combination with other emerging agents and treatment approaches such as immunotherapy, surgery, and/or radiotherapy, thereby providing more definitive evidence for the management of BMs from NSCLC.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**. Further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

KB, ST, and AM developed the research question. JD, WH, YE, and KB completed data extraction and screening. KB, ST, and AM completed data analysis and wrote the manuscript. All authors contributed to the restructuring and editing of the manuscript. All authors contributed to the article and approved the submitted version.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2021.739765/full#supplementary-material

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Clinical Trial Eligibility Criteria and Recently Approved Cancer Therapies for Patients With Brain Metastases

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Brain metastases cause significant morbidity and mortality in patients with advanced cancer. In the era of precision oncology and immunotherapy, there are rapidly evolving systemic treatment options. These novel therapies may have variable intracranial efficacy, and patients with brain metastases remain a population of special interest. Typically, only patients with stable, asymptomatic and/or treated brain metastases are enrolled in clinical trials, or may be excluded altogether, particularly in the setting of leptomeningeal carcinomatosis. Consequently, this leads to significant concerns on the external validity of clinical trial evidence to real-world clinical practice. Here we describe the current trends in cancer clinical trial eligibility for patients with brain metastases in both early and late phase trials, with a focus on targeted and immunotherapies. We evaluate recent newly FDA approved therapies and the clinical trial evidence base leading to approval. This includes analysis of inclusion and exclusion criteria, requirements for baseline screening for brain metastases, surveillance cerebral imaging and incorporation of trial endpoints for patients with brain metastases. Finally, the use of alternative sources of data such as real-world evidence with registries and collaborative studies will be discussed.

Keywords: brain metastases, trial eligibility, intracranial efficacy, novel therapeutic agents, CNS metastases

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INTRODUCTION

Brain or central nervous system (CNS) metastases remain a significant cause of morbidity and mortality in patients with advanced cancers (1). The incidence of brain metastases may be increasing, in part due to greater detection through routine cerebral imaging and more effective systemic therapies allowing later manifestations of the disease to occur (2). Particularly in the era of precision oncology and immunotherapy, there are rapidly evolving systemic treatment options for many cancers. These novel therapies may have variable intracranial efficacy, and patients with brain metastases remain a population of special interest (3). Typically, only patients with stable, asymptomatic, and/or treated brain metastases are enrolled in clinical trials, or may be excluded altogether, particularly in the setting of leptomeningeal carcinomatosis. Consequently, this leads to

significant concerns on the external validity of clinical trial evidence to real-world clinical practice (4).

In this review, we describe the current trends in cancer clinical trial eligibility for patients with brain metastases in both early and late phase trials, with a focus on recently approved targeted and immunotherapies. The United States (US) Food and Drug Administration (FDA) approved therapies from 2018-2020 and the clinical trial evidence base leading to approval are evaluated. Key recommendations previously published by the American Society of Clinical Oncology (ASCO)–Friends of Cancer Research (FCR) Brain Metastases Working Group for the inclusion of patients with brain metastases in clinical trials to improve generalizability of trial evidence are considered (5). This includes an analysis of inclusion and exclusion criteria, requirements for baseline screening for brain metastases, surveillance cerebral imaging and incorporation of trial endpoints for patients with brain metastases. Finally, the use of alternative sources of evidence such as real-world evidence with registries and collaborative studies are discussed.

ANALYSIS OF RECENTLY FDA APPROVED CANCER THERAPIES

We conducted an analysis of newly FDA approved cancer therapies from 2018-2020 (6–8) as shown in **Supplementary Table 1**. For each cancer therapy, the registrational trial leading to regulatory approval was evaluated. The characteristics of the registrational trials are summarized in **Supplementary Table 2**. Trials conducted in the metastatic or late-stage cancer setting were initially assessed for eligibility for patients with brain metastases. Of 27 trials, 18 (67%) allowed enrollment of patients with stable and asymptomatic brain metastases (**Figure 1A**). Baseline screening for brain metastases with CT or MR imaging was required in 14/27 (52%) trials (**Figure 1B**). Surveillance cerebral imaging in patients without brain metastases at baseline was required in only 1/27 (4%) trials (**Figure 1C**). A prespecified trial endpoint evaluating patients with brain metastases was incorporated in 5/27 (19%) trials (**Figure 1D**).

ELIGIBILITY OF PATIENTS WITH BRAIN METASTASES

Patients with brain metastases have historically been excluded from clinical trials due to concerns relating to overall greater risks of toxicity and poorer survival outcomes. With improved local therapeutic options for brain metastases and greater survival outcomes, assessment of intracranial efficacy and toxicity is becoming ever more important. The potential lack of blood-brain barrier (BBB) penetration for novel therapies is also often cited as a rationale for exclusion. However, for established brain metastases, tumors compromise the integrity of the BBB acquiring neovasculature and a resulting blood-tumor barrier (9). Consequently, increasingly there are trends to include patients with stable, treated and/or asymptomatic brain metastases (10). This approach would improve the generalizability of trial results, particularly in cancer types with a high prevalence of brain metastases. Nevertheless, from our analysis (**Figure 1A**), we found that a significant proportion of recently approved cancer therapies still exclude patients with brain metastases from the initial registrational trials. This included trials in tumor types with a low overall prevalence of brain metastases, such as NAVIGATOR (avapritinib) (11) and INVICTUS (ripretinib) (12) for gastrointestinal stromal tumors (GIST) and Study EZH-202 (tazemetostat) (13) for epithelioid sarcomas. In addition, there were cases in which CNS disease may represent a distinct clinical entity such as CNS lymphoma (14, 15). However, even in cancers with a high prevalence of brain metastases, there were examples of trials which completely excluded patients with brain metastases. Notably this included small cell lung cancer (SCLC) with Study B-005 (lurbinectedin) (16), *EGFR* mutated non-small cell lung cancer (NSCLC) with ARCHER 1050 (dacomitinib) (17) and triple negative breast cancer (TNBC) with IMMU-132-01 (sacituzumab govitecan) (18). The lack of evidence for intracranial efficacy of agents that have received regulatory approval represents a significant limitation for these compounds (19, 20). Particularly for *EGFR* mutated NSCLC and TNBC where the prevalence of brain metastases may be as high as 32% and 46% respectively (21–23). Whilst patients with brain metastases may have been subsequently allowed in larger phase 3 trials such as for lurbinectedin (24) and sacituzumab govitecan (25), the initial exclusion also necessitated the initiation of further trials to generate data for this important patient subpopulation (26, 27). Furthermore, the rationale for excluding patients with brain metastases was not elaborated upon in the primary publications. The lack of efficacy and safety data from early phase trials may have been a contributory factor. However, lurbinectedin (28, 29) and dacomitinib (30) for example had allowed patients with non-progressive or treated/stable brain metastases in earlier trials.

BASELINE SCREENING FOR BRAIN METASTASES AND SURVEILLANCE CEREBRAL IMAGING

Screening for brain metastases at baseline is a common cause of screen failure, particularly in early phase clinical trials (31). Consequently, unless mandated this may lead to hesitancy from clinicians to perform cerebral imaging for risk of jeopardizing a patient's eligibility for trials (5). As trials increasingly allow patients with stable and treated brain metastases however, more completely characterizing the intracranial efficacy of novel therapies also becomes of heightened importance. From our analysis, nearly half (48%) of trials did not require mandatory cerebral imaging at baseline (**Figure 1B**), and only one (4%) trial required surveillance imaging for patients without brain metastases at baseline (**Figure 1C**). For many trials, cerebral

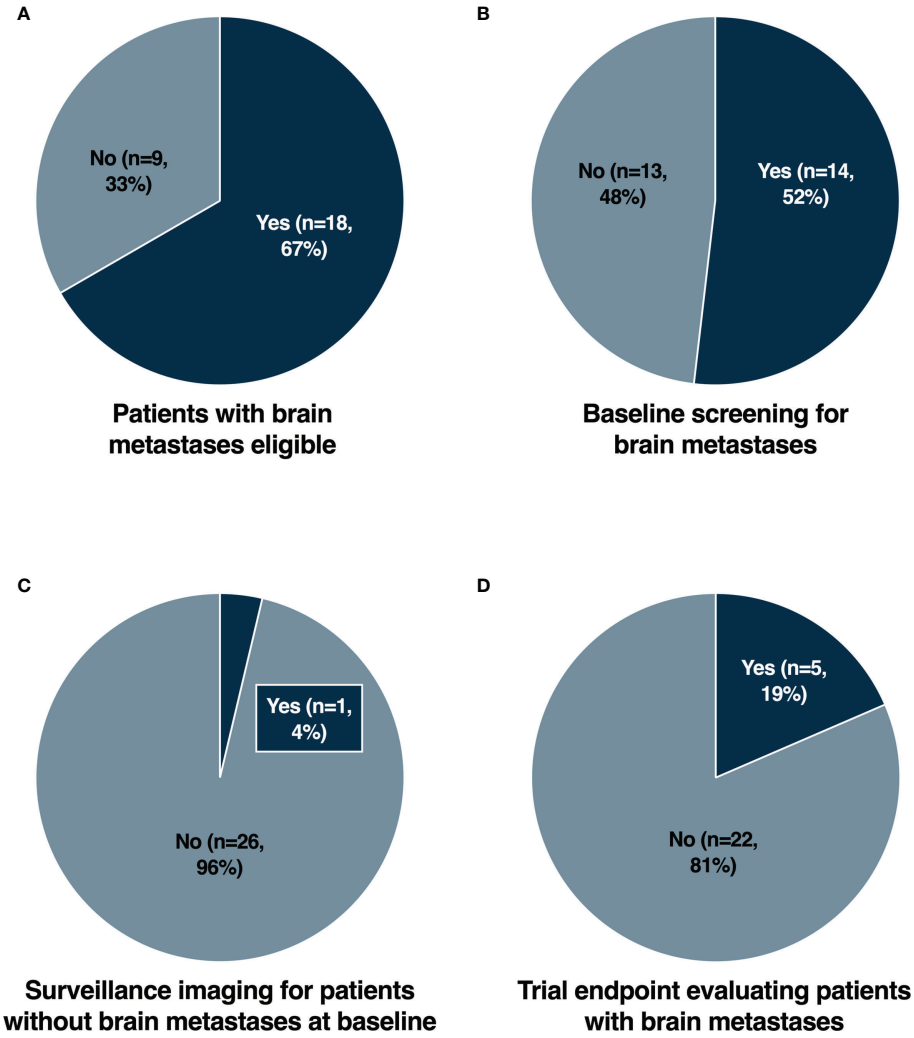

FIGURE 1 | Eligibility for patients with brain metastases **(A)**, baseline screening for brain metastases **(B)**, surveillance imaging for patients without brain metastases at baseline **(C)** and trial endpoint evaluating patients with brain metastases **(D)** in registrational trials for newly FDA approved cancer therapies from 2018-2020.

imaging during screening was at least required with known or suspected brain metastases. However, there is increasing evidence supporting routine standard of care screening for brain metastases in many cancers, such as advanced breast cancer, melanoma and NSCLC, both at diagnosis and after initiation of palliative systemic therapy (32). Despite this, there remained trials such as SOLAR-1 (alpelisib) (33), SOPHIA (margetuximab) (34), ARROW (pralsetinib) (35) and LIBRETTO-001 (selpercatinib) (36), that did not mandate baseline cerebral imaging. The NAVIGATOR trial (11), a phase 1 study of avapritinib which included a dose expansion cohort for *PDGFRA*-mutated GIST patients, was the only study with regular surveillance cerebral imaging. However, this was performed due to safety concerns regarding an increased risk of intracranial bleeding, rather than generating data elucidating intracranial efficacy. In addition to intracranial response rates, measures of intracranial efficacy such as time to CNS progression, are also increasingly reported. Therefore, the role for routine surveillance cerebral imaging may become important in cancers with a propensity for the development of brain metastases.

PROTOCOL SPECIFIC MANAGEMENT OF INTRACRANIAL PROGRESSION

The treatment paradigm for brain metastases includes a multimodality approach including surgery, radiation therapy and systemic therapy (3). Consequently, for patients that experience isolated intracranial progression, local therapeutic approaches whilst continuing systemic therapy beyond progression represents a commonly adopted clinical approach. For clinical trials, specific protocol guidance in such instances is crucial to safely and effectively treat progressive brain metastases whilst collecting adequate data for CNS outcomes. For example,

the COLUMBUS trial evaluating encorafenib plus binimetinib in patients with *BRAF* mutated melanoma (37), specified the potential for dosing beyond progression for new brain metastases treatable with stereotactic radiotherapy or surgery but not requiring whole brain radiotherapy. However, for a large majority of trials that allowed treatment beyond progression, there was no specific protocol guidance for the management of intracranial progression.

INCORPORATION OF TRIAL ENDPOINTS FOR PATIENTS WITH BRAIN METASTASES

With the improving intracranial efficacy of many novel targeted and immunotherapies compared with traditional chemotherapy, the prospective evaluation of CNS outcomes with prespecified endpoints is also becoming paramount. In our analysis, only a small number of registrational trials prespecified a trial endpoint evaluating patients with brain metastases (**Figure 1D**). This included Study B7461001 of lorlatinib for patients with *ALK* rearranged NSCLC (38), which included intracranial objective response rates as a co-primary endpoint. The remaining trials incorporated secondary endpoints assessing intracranial response and/or time to intracranial progression, including trials such as ARROW (pralsetinib) (35) and LIBRETTO-001 (selpercatinib) (36) for *RET* rearranged NSCLC, HER2CLIMB (tucatinib) (39) for *HER2* positive breast cancer and ALKA/STARTRK-1/STARTRK-2 (entrectinib) (40, 41) for *NTRK* rearranged solid tumors and *ROS1* rearranged NSCLC. *Post hoc* analyses describing outcomes for patients with brain metastases have subsequently been reported from many of the other registrational trials. However, without prospective plans to evaluate CNS response and progression, results may be more exploratory. For example, in the DESTINY-Breast01 trial of trastuzumab deruxtecan in *HER2* positive breast cancer (42), there was a cap of patients with brain metastases allowed for enrolment. Intracranial efficacy from this trial has been shown to be promising (43), however further prospective trials for patients with brain metastases have been initiated to better characterize the CNS activity (27).

ALTERNATIVE SOURCES OF DATA SUCH AS REAL-WORLD EVIDENCE

Despite increasing trends to include patients with brain metastases in clinical trials there remains subpopulations of patients that often remain excluded. This includes patients with leptomeningeal disease and symptomatic or active (new and/or progressive) brain metastases. Safety considerations or unsupportive pre-clinical evidence are potential reasons where exclusion from trials may still be appropriate (5). Therefore, there is heightened need for alternative sources of evidence in such populations, for which real-world evidence may provide an opportunity. Data sharing and collaboration through multi-center registries and trial networks are potential avenues to integrate supportive real-world evidence to clinical trial data (44). Particularly given the relative rarity of these subpopulations, pooled analyses may represent efficient methods of generating high quality prospective data. The Brain Metastases in Breast Cancer Network Germany is one such example (45), however future efforts need to be driven by both academia and industry. With rapid development of targeted and immunotherapies with unique mechanisms of action, greater reverse translation of our biological understanding of novel compounds from real-world evidence and clinical trial data to inform pre-clinical models and drug discovery pipelines is also paramount (46).

DISCUSSION

In our analysis, a significant proportion of registrational trials for new recently FDA approved cancer therapies allowed patients with brain metastases. However, there remained prominent examples of trials which excluded even stable or asymptomatic brain metastases. These trials may have been conducted in tumor types with extremely rare incidence of brain metastases, such as GIST (47). Nevertheless, the relatively rarity of brain metastases in these tumor types may not represent sufficient rationale for automatic exclusion from clinical trials – particularly when baseline screening for brain metastases is mandated. Whilst most trials also required baseline screening for brain metastases, only a minority of trials required surveillance CNS imaging for patients without baseline brain metastases, had protocol specified guidelines for intracranial progression, or incorporated a trial endpoint for patients with brain metastases. This highlights important areas in which we can improve our understanding of the intracranial activity of novel therapies from clinical trials (**Table 1**).

Recently, the FDA released specific guidance for industry, outlining recommendations for the inclusion of patients with brain metastases (48). It is emphasized that patients with active brain metastases or leptomeningeal disease should not be automatically excluded from trials. Eligibility in early drug development trials to inform criteria for later-phase trials, mitigation of uncertainties with separate subgroups within trials and the importance of CNS imaging at regular intervals are other key recommendations. Nevertheless, deeper considerations of risk benefit ratio with regards to cancer type, disease stage, known pre-clinical data and drug safety profile are all clearly influential in the development of clinical trial eligibility criteria and protocols. For novel therapies with unique mechanisms of action, such as newer immunotherapies, the potential for distinct toxicity profiles or adverse events due to CNS tumor inflammation and/or psuedoprogression is a relevant concern (49). However, from trials to date of immune checkpoint inhibitors in patients with brain metastases, the rates of toxicities and neurologic adverse events do not appear significantly different, and deaths due to neurologic complications remain rare (50). A greater molecular understanding of the development and progression of brain metastases within the unique brain microenvironment is also driving advances with more precise

TABLE 1 | Recommendations to improve the clinical trial eligibility criteria and evaluation of patients with brain metastases.

Recommendations	
Eligibility of patients with brain metastases	• Routine inclusion of patients with treated and/or stable brain or central nervous system (CNS) metastases • Strong consideration to include patients with active brain metastases (or enrolled as a defined subgroup) if there is sufficient scientific rationale for the investigational drug
Baseline screening for brain metastases and surveillance cerebral imaging	• Routine baseline screening for brain metastases if incidence of brain metastases is high and/or intracranial efficacy is a predefined endpoint • Regular surveillance CNS imaging if the risk of developing brain metastases is high and/or intracranial efficacy is a predefined endpoint
Protocol specific management of intracranial progression	• Prospective guidelines specifying management of new or progressive brain metastases, including the allowance for local therapies and continuation of investigational drug
Incorporation of trial endpoints for patients with brain metastases	• Incorporation of prespecified trial endpoints for CNS-related efficacy outcomes if incidence or risk of developing brain metastases is high and/or the investigational drug has strong scientific rationale for intracranial efficacy

approaches for local and systemic therapies (51). This has broader implications for the data generated from the inclusion of patients with brain metastases on trials evaluating novel compounds, where combination approaches may enhance the intracranial efficacy. Costs and impacts on trial efficiency however, remain other practical considerations (5). Regular surveillance CNS imaging for example, particularly with MRI may be burdensome for patients with tumor types with low prevalence of brain metastases. Our analysis must therefore also be viewed in the context of a markedly heterogenous collation of therapies and registrational trials. In addition, given the length of time required for drug development from early to late phase trials and regulatory approval, the trials in our analysis may not be wholly representative of more contemporary practices in trial protocol design. Ultimately however, the drug development landscape is rapidly evolving with an increasing incidence of accelerated approvals from early phase trials. Consequently, the critical evaluation of clinical trial evidence and its generalizability across the patient population is of heightened relevance.

With an increasing prevalence of patients with brain metastases, understanding the intracranial efficacy of novel therapies is crucial. Expanding the eligibility of patients with brain metastases in registrational trials, or the incorporation of procedures or endpoints in trial design will generate important high-quality data in this patient population with significant unmet need. This will enhance our ability to integrate systemic therapies in the multimodality treatment of patients with brain metastases.

AUTHORS CONTRIBUTIONS

AT and MK contributed to conception and design of the study. All authors contributed to manuscript preparation, read, and approved the submitted version.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2021.780379/full#supplementary-material

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Answering the Big Clinical Questions in Brain Metastasis Management

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Management of brain metastases is challenging, both because of the historically guarded prognosis and evolving, more efficacious treatment paradigms for metastatic cancer. This perspective addresses several of the important difficult questions that practitioners treating patients with brain tumors face in the clinic. Successfully answering these questions requires knowledge of the clinical evidence, thoughtful discussion of the patient's goals of care and collaboration in a multi-disciplinary setting.

Keywords: brain metastases, stereotactic radiosurgery, whole-brain radiotherapy, hippocampal avoidance, palliative care, leptomeningeal disease

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INTRODUCTION

As the articles in this special issue illustrate, the management of brain metastases has changed and continues to evolve. Advances in radiation therapy, surgery and, particularly, systemic treatment of metastatic cancer have improved prognosis and increased longevity, making the preservation of neurocognition and quality of life all the more important in patients with brain metastases. The choice of treatment for brain metastases – including early and timely access to palliative care – has become more complex and complicated. At the same time, the consequences of making the optimum management choice carry higher stakes for both the patient and practitioner. While there is no single correct approach, clearly the "best" decisions will come through attentiveness to the patient's goals of care and the input of multiple disciplines.

In this article, we share our perspective on some of the most common and important questions we encounter in the clinical management of brain metastases. The astute reader will notice that many of the "answers" to these questions, raise more questions than provide answers, and that there is no "magic eight ball" that provides a simple answer. However, a collegial effort on the part of the "village" - medical oncologists, radiation oncologists, surgeons, palliative care specialists, navigators and nurses - centered on addressing the patient's needs and based on evidence, will provide the best care and superior outcome for the patient presenting with brain metastases.

"MY PATIENT HAS A LARGE BRAIN METASTASIS – SHOULD I OFFER POST-OP, PRE-OP OR 'NO-OP' RADIATION THERAPY?"

Patients often present with large brain metastases that are producing or at impending risk of causing symptomatic mass effect on brain parenchyma, critical adjacent organs and the ventricular system. These patients are typically considered for surgical resection – particularly in the case of one or two brain lesions – for rapid relief and/or prevention of mass effect and obstructive hydrocephalus.

Radiation therapy is usually administered in combination following surgical resection of a brain metastasis, as either whole-brain radiation therapy (1) (WBRT) or stereotactic radiosurgery (2) (SRS) significantly and substantially reduce local recurrence compared to surgical resection alone. Post-operative SRS is frequently chosen over WBRT, given the reduced impact on neurocognition and the shorter time for recovery, which also permits more rapid initiation of systemic therapies (3).

However, even for modest-sized brain metastases, the resection cavity and resulting target volume for irradiation is often in excess of 3 cm (4), requiring a substantial dose reduction in order to administer single-fraction SRS safely (2). To overcome the limitations, radiation may be delivered over 3 to 5 fractions (hypofractionated SRS, HF-SRS), which appears to offer a better balance of treatment efficacy and toxicity (5, 6). A randomized trial of single-fraction versus hypofractionated SRS to the post-operative resection cavity is currently underway (ClinicalTrials.gov Identifier: NCT04114981.)

Pre-operative SRS is a potentially attractive alternative to post-operative SRS, as the target in the pre-operative setting is smaller and more regular with a more competent blood supply, presumably providing better oxygenation and, thus, increased radiosensitivity (7, 8). Moreover, cytoreduction of tumor by upfront SRS may reduce surgical tract contamination by viable cells during resection and consequently decrease the risk of recurrence and leptomeningeal disease (9, 10). Pre-operative SRS appears to offer very good local tumor control with minimal toxicity, and a randomized control trial of pre-operative versus post-operative SRS in brain metastases has been proposed and should provide needed information on comparative efficacy and safety of these two approaches. Note that timing of pre-operative SRS can be a challenge, particularly in the setting of symptomatic mass effect requiring immediate surgery, and the impact of up-front SRS on pathologic results is unclear.

If the patient is not surgical candidate, the treatment of large brain metastases with radiation therapy alone is an option. The need to balance toxicity with efficacy of treatment suggests that these patients may be best served by HF-SRS, as discussed above, rather than WBRT with its increased risk of neurocognitive deficits and prolonged recovery time or single-fraction SRS with greater risk of adverse-radiation effects at efficacious doses. Omitting resection in surgical candidates is more contentious. Some retrospective studies have shown substantially poorer local control for lesions exceeding 2cm diameter treated with single-fraction radiosurgery alone versus resection and radiosurgery (11), likely due to the combination of purposely reduced prescription dose for larger metastases (12) and higher tumor burden. However, other studies of single-fraction SRS using a small margin expansion about the target have not shown decreased efficacy for larger lesions (13) and studies of HF-SRS report high rates of local control for lesions >2cm diameter (6). Finally, a retrospective study of SRS alone versus resection followed by SRS found a significantly higher rate of nodular leptomeningeal disease in the surgery + SRS group versus those receiving SRS alone (21 vs 0%, P <.001) (14). This study suggests a potential advantage to avoiding surgery … or potentially utilizing pre-operative SRS.

In the absence of randomized trials of pre-op versus post-op versus no-op approaches – *which should include appropriate targeted and immunotherapy agents* – the optimal answer to the above question can best be achieved in a multi-disciplinary setting. Obviously, one needs to consider the patient's suitability for surgery and radiosurgery, the size, location, aggregate volume and number of lesions and the patient's performance status, disease burden and goals of care. In addition, the timing of and interaction with systemic treatments must be considered, as well as the appropriateness of any intervention in patients with poor prognosis and performance status (15). It is equally important to build a system ahead of time that can safely and adequately provide these options. For example, pre-operative SRS is only feasible when procedures for rapidly planning and delivering radiosurgery are in place, supported by robust QA processes and availability of appropriate equipment.

"WHY WOULD WE EVER GIVE WHOLE-BRAIN RADIATION THERAPY?"

WBRT was the mainstay for the treatment of patients with multiple brain metastases for many years, providing reasonable local and distant brain control. However, WBRT produces bothersome acute toxicities in almost all patients (fatigue, scalp irritation, alopecia) and multiple studies have shown that WBRT causes significant neurocognitive deterioration versus SRS alone (16–18). Consequently, SRS and, recently, some targeted and immune therapies are emerging as the dominant treatment modality for multiple brain metastases (19). For patients with a few (≤4) brain metastases, SRS alone or in combination with surgery to the dominant lesion is often the preferred treatment, a choice somewhat obliquely endorsed by ASTRO in its "2014 Choosing Wisely" list. The indication for SRS has expanded to include larger number of brain metastases, with 10 or fewer regarded by many practitioners, as appropriate for SRS alone, based on several clinical studies coupled with advances in treatment technology. JLGK0901, a prospective observational trial, evaluated outcomes patients with 2 to 10 brain metastases treated with a multicentric, single-fraction SRS technique. The study revealed no differences in survival, local recurrence, toxicity or neurocognition in patients treated to 2-4 versus 5-10 brain metastases (20, 21). Likewise, studies in patients with 4 – 10 brain metastases treated with single-isocenter, single-fraction or HF-SRS have revealed high levels of local control with minimal neurocognitive decline (22, 23). At the same time, improved planning and treatment techniques have significantly reduced the time to treat multiple brain lesions, and there is essentially no *technical* upper limit on the number of brain metastases that can be treated with a single-isocenter intensity-modulated radiosurgery technique.

However, the technical capability to radiosurgerize 30 brain lesions should not be equated with the *clinical* appropriateness of doing so. By intent, SRS delivers a minimal dose of ionizing radiation to non-target tissue, permitting untreated sub-clinical metastases to develop into visible lesions at later date. Consequently, multiple studies show that the incidence of development of new brain metastases is far higher without than with WBRT (17, 18, 24, 25). Data from the JLK trial appear to support the assumption that the risk of microscopic disease and post-SRS distant brain disease increase with a higher number of treated brain metastases. In addition, SRS clearly does not treat diffuse leptomeningeal disease (LMD), and post-operative SRS alone has been associated with increased risk of diffuse LMD, particularly when utilized in the posterior fossa. [note that nodular LMD is not equivalent to diffuse LMD, and SRS for the former often appears to be the preferred approach (26)]. Finally, it is important to recognize that none of the published trials of multiple brain metastases randomize patients to SRS versus WBRT, and we do not know if the outcome associated with one modality is truly superior to the other (see below.) In my opinion, the patient with 25 new, small brain metastases that have appeared on a short-interval brain MRI is unlikely to realize complete control of their intracranial disease with SRS alone and it would be misleading to suggest otherwise while downplaying the value of WBRT.

In response to the above question, when the patient has a high density of brain metastases, WBRT should be considered and may be the most appropriate option *if the patient has prospects of benefitting from treatment*. As the QUARTZ study showed (15), patients with poor performance status appear to fare no better (and perhaps worse) with WBRT versus best supportive care, and the approach of palliative SRS versus supportive care alone should be considered. In patients with multiple brain metastases and a reasonable expectation of benefit from control of brain disease by WBRT, the issue then becomes effective mitigation of neurocognitive decline. While memantine and hippocampal-avoidance WBRT (HA-WBRT) utilized separately, appear to offer some benefit in reducing the depth of neurocognitive decline, the combination of the two has been shown to significantly decrease neurocognitive deterioration (27, 28). 518 patients were randomly assigned to undergo HA-WBRT plus memantine versus conventional WBRT plus memantine. Across multiple domains, HA-WBRT plus memantine better preserves cognitive function and patient-reported symptoms. While patients with lesions within 5mm of the hippocampi and diffuse LMD were excluded from the trial, it appears that patients with as few as a single metastasis were eligible and no upper limit on either number or volume of lesions was applied. It would be quite interesting to see outcome from this trial analyzed based on a stratification by number/volume of brain lesions.

Given that memantine alone offers only partial neuroprotection and that substantial changes in non-hippocampal areas of the brain are observed post HA-WBRT (29), there is interest in utilizing agents that provide more complete global protection of the brain during WBRT. For example, a novel Mn-porphyrin superoxide dismutase mimetic, BMX-001, is undergoing a randomized phase trial in patients with 5 or more brain metastases receiving WBRT.

The above discussion reflects the Author's radiocentric experience in treating brain metastases. However, the prospect of deferring and potentially completely avoiding any radiotherapy to brain metastases is being entertained, as discussed elsewhere in this issue. For example, in non-small cell lung cancer metastatic to the brain, targeted agents with improved blood-brain barrier penetration, such as osimertinib, can effectively treat small brain metastases without brain irradiation (3, 30). However, in my experience many will eventually require brain radiotherapy. A large subset of patients brain metastases with melanoma respond quite well to dual checkpoint inhibitor immunotherapy. Tawbi et al (31) reported an "intracranial clinical benefit" (defined as the percentage of patients with complete response, partial response or stable disease at 6 months) of 57% in a cohort of 94 patients with brain metastases from melanoma treated with nivolumab + ipilimumab. The optimal combination and timing of radiation therapy, surgery and systemic treatments are poorly defined, and patients are best served by a multi-disciplinary, treatment-modality-agnostic discussion of their treatment options, preferably at a Tumor Board. It is essential that the proposed options be tailored to the patient's tumor, performance status, overall disease state and the recommendations be thoroughly and critically discussed with the patient and their family, including the role of palliative therapy.

"HOW WOULD YOU TREAT MY BRAIN METASTASES IF I WERE YOUR MOTHER?"

As I have gotten older, this question has changed from "… if I were your mother [or father]?" to "… if I were your sister [or brother]?" Many of my colleagues would say it be more appropriate to ask "… if I were your daughter [or son]?" I now recognize that this question opens the door to an opportunity to frame the patient's goals of care and to engage in a meaningful dialogue with the patient and their family. In a busy clinic, one's inclination is to give the rote answer, "I treat everyone equally. I am not your relative and it would not be appropriate for me to answer that question", moving on to a discussion of risks, benefits, side effects and logistics. However, by taking just a few more minutes at this critical point, a provider can truly help the patient chose an option best aligned with their goals of care.

If I have developed rapport with the patient, my first response is often, "well you don't know how I feel about my mother [or father], so you may want to be careful about any answer I would give you". This comment is surprisingly well received in most cases and is far more effective than a brief lecture on shared decision-making. Then, I typically follow-up with, "I would start by making sure I explained the different treatments to them [my parent] – as I've done with you – and by making sure that they *and I* understood how these options fit with their goals". Then, either I or the patient/family member will briefly recap the patient's goals and discuss how the management options fit with those goals. Throughout this dialogue, it is essential to repeat, acknowledge, clarify and rephrase what the patient is

telling you, making liberal use of phrases, such as, "Let me make sure I've got this right. You want to…"

Effective multi-disciplinary management of these patients requires that all team members share the summary of these discussions with one another, with a low threshold for referral to another specialty, as needed. In particular, one must be attentive to a need for improved symptom management and home health care, areas where a Palliative Care provider can offer exceptional support to the patient and their family.

DO I REALLY NEED TO TREAT THE PATIENT WITH SRS USING A RADIOSURGERY SYSTEM?

Yes. Safe and effective SRS of brain metastases requires more than a radiosurgery capable piece of equipment. Paraphrasing the guidelines for radiosurgery proposed by Barnett et al. (32), the key elements of a radiosurgery system include:

- A multidisciplinary team consisting of a neurosurgeon, radiation oncologist and radiation physicist, all trained in radiosurgery, in general, and the specific equipment, as well as a team of dedicated radiation therapists
- Sophisticated treatment planning based on high-resolution, high-fidelity imaging that yields highly conformal, precise and accurate dose delivery to the target with minimal irradiation of normal tissues
- A linear accelerator, particle therapy unit or radioactive isotope device, capable of delivering photon or particle radiation to a remote target with better than 1 mm accuracy and precision
- A combination of patient immobilization and on-machine image guidance that ensures that the target is localized with sub-mm/sub-degree accuracy in translational/rotational accuracy
- Robust, written and rigorous quality assurance procedures for every element of the process that ensures that every element of the system is correct during each and every procedure

If these requirements cannot be met locally, an alternative approach should be considered, including referral to a radiosurgery center, use of conventional radiotherapy and/or systemic treatment with proven efficacy in treating brain metastases, as appropriate.

CONCLUSION

Thoughtfully addressing the above questions with patients and their families in a multidisciplinary setting is a critical element in the treatment of brain metastases. Formulating and communicating evidence-based, specialty-agnostic recommendations with careful attention to the patient's needs and goals of care provides the patient with the basis to make optimal, personalized decisions on their care.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

The author confirms being the sole contributor of this work and has approved it for publication.

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A Need for More Molecular Profiling in Brain Metastases

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As local disease control improves, the public health impact of brain metastases (BrM) continues to grow. Molecular features are frequently different between primary and metastatic tumors as a result of clonal evolution during neoplasm migration, selective pressures imposed by systemic treatments, and differences in the local microenvironment. However, biomarker information in BrM is not routinely obtained despite emerging evidence of its clinical value. We review evidence of discordance in clinically actionable biomarkers between primary tumors, extracranial metastases, and BrM. Although BrM biopsy/resection imposes clinical risks, these risks must be weighed against the potential benefits of assessing biomarkers in BrM. First, new treatment targets unique to a patient's BrM may be identified. Second, as BrM may occur late in a patient's disease course, resistance to initial targeted therapies and/or loss of previously identified biomarkers can occur by the time of occult BrM, rendering initial and other targeted therapies ineffective. Thus, current biomarker data can inform real-time treatment options. Third, biomarker information in BrM may provide useful prognostic information for patients. Appreciating the importance of biomarker analyses in BrM tissue, including how it may identify specific drivers of BrM, is critical for the development of more effective treatment strategies to improve outcomes for this growing patient population.

Keywords: brain metastases, sequencing, biomarkers, neurosurgery, discordance

HIGHLIGHTS

- The genomic status of BrM can alter treatment plans for patients by providing new targetable options.
- Molecular profiling of BrM can indicate that a therapy is no longer effective for a patient.
- Biomarker information in BrM may provide useful prognostic information for patients.

INTRODUCTION

Far exceeding primary central nervous system (CNS) neoplasms in number, metastases to the brain pose a significant societal burden. Of an estimated 1.7 million new cancer diagnoses per year in the United States, approximately 6%–14% of these patients are expected to ultimately develop a metastasis to the brain (1–3). Brain metastases (BrM) most commonly arise in patients with primary lung, breast, and melanoma neoplasms but are also observed in patients with renal cell carcinoma, prostate cancer, colorectal cancer, and many other primary cancer histologies (4).

Patients with BrM face a dismal prognosis, with a median overall survival of <6 months regardless of primary cancer type based on historical data (5, 6). Clinically actionable molecular biomarkers, such as genetic alterations and aberrant gene expression, have been increasingly identified and translated into treatment options for cancer patients, with more specific emphasis placed on patients with BrM in recent years (7). Identifying accurate molecular biomarkers for BrM is crucial to developing more effective therapies and advancing personalized oncology care.

Modern management of BrM involves multidisciplinary consideration of surgery, radiation therapy, and systemic therapy options. Surgical resection of BrM provides a survival advantage for patients with a single metastasis (8). In modern practice, neurosurgical resection is considered for patients with a limited number of BrM, for larger metastases, for metastases that can be safely resected, when tissue is needed for diagnosis, and when debulking is needed to alleviate symptoms. Historically, patients were treated with whole-brain radiation therapy (WBRT) either alone or after surgical management given the ability for WBRT to extend intracranial progression-free survival (9). However, modern radiation treatments have shifted toward approaches that seek to mitigate the neurocognitive side effects of WBRT, such as hippocampal avoidant WBRT with memantine (10) or stereotactic radiosurgery (SRS) directed only at the BrM without WBRT. This is due to the ability for SRS to mitigate the neurocognitive side effects of WBRT, while providing comparable overall survival and local intracranial control outcomes (albeit at a cost of decreased distant intracranial control) (11). Increasingly, systemic therapies including chemotherapy, targeted therapies, and immunotherapies are applied for BrM patients. The identification of select BrM patients for whom surgery or radiotherapy can be deferred while the patients are treated with systemic therapies is a topic of investigation for many cancer subtypes.

When surgical management is a primary BrM treatment strategy, biomarker analyses of BrM tissues can offer additional clinical gains. Surgical intervention is often indicated for BrM that are >3 cm, situated in an accessible and/or superficial location, or causing mass effect on the brain (12). Currently, obtaining a tissue biopsy for the primary or sole indication of assessing biomarker information in BrM is not routinely performed due to associated clinical risks in a patient population with a relatively poor prognosis. Biopsies of BrM, including concurrent biopsies obtained during laser interstitial thermal therapy (LITT) (13), are routinely sent to pathology for diagnostic confirmation and/or differentiation from radiation necrosis. However, these biopsies are rarely sent for broad molecular profiling despite an overall increase in the use of commercial and in-house genomic and transcriptomic sequencing services as part of routine oncology care (14, 15).

It has been generally accepted that cancer progression involves somatic clonal evolution (16). Biomarkers identified from primary tumor resections are often assessed years prior to development of BrM and may not reflect emergence of resistance mechanisms that arise during the metastatic process and under treatment pressures. Molecular biomarkers presenting in distant metastases are frequently different from those initially presenting in primary sites. Studies demonstrate that biopsies of other extracranial metastatic sites also do not fully recapitulate the molecular features of BrM—due in part to clonal evolution during neoplasm migration and systemic treatment (16–19). Comparisons of the somatic landscape across visceral metastases may fail to take into account the unique requirements for BrM, such as enabling extravasation through non-fenestrated capillaries, hypoxia-induced neoangiogenesis, and adaptation to the CNS metabolic microenvironment (20). Newer and less invasive techniques for biomarker testing have emerged in recent years (e.g., liquid biopsies). In a recent study, next-generation sequencing of cell-free DNA (cfDNA) from cerebrospinal fluid was shown to be more sensitive than cytologic analysis for diagnosing leptomeningeal disease (21). In the future, cfDNA may be a beneficial tool to detect potential actionable biomarkers in BrM. The use of liquid biopsies to evaluate the response of metastatic tumors to treatment and to provide prognostic information still warrants future investigation (22).

Here, we review the discordance of clinically actionable biomarkers measured in BrM from lung cancer, breast cancer, and melanoma compared to primary sites and extracranial metastases. We discuss these emerging data within the framework of three principal motivations for increased molecular profiling in BrM. First, new treatment targets may be identified as unique actionable mutations emerge in BrM compared to the primary tumor or extracranial metastases. Second, BrM molecular profiling may identify biomarkers of resistance or loss of actionable alterations, thereby excluding ineffective therapies from a treatment plan. Third, new biomarker information in BrM could provide useful prognostic information to aid clinicians and patients in discussing expectations for care.

Obtaining genomic sequencing data on BrM will also help to identify novel drivers that may play a key role in promoting BrM. In a recent report where the authors performed whole-exome sequencing of brain metastases from lung adenocarcinomas (BM-LUAD) and primary lung adenocarcinomas using case–control analysis to identify genomic alterations that promote BrM, they identified three regions (*MYC*, *YAP1*, *MMP13*) that had significantly higher amplification frequencies and one region (*CDKN2A/B*) that had higher deletion frequencies in BM-LUAD as compared to primary lung adenocarcinoma (23). Additional

investigations will be needed to identify driver somatic alterations that promote brain metastases in other types of primary tumors.

While some clinicians may be daunted by the variety and complexity of biomarker testing options available, the impact of this hurdle is rapidly diminishing as "omics" data are increasingly incorporated into oncology practice. However, comprehensive molecular profiling of BrM tissues remains an underutilized option in most health systems, especially outside of dedicated multidisciplinary BrM services. The development of a common understanding among healthcare professionals of the importance of biomarker analysis in BrM will be critical for the development of more effective treatment strategies against BrM and the advancement of precision oncology approaches in this growing patient population.

IDENTIFICATION OF NEW, ACTIONABLE TARGETS

BrM tissue, obtained through either biopsies or surgical resection during standard care, can provide additional opportunities to identify new targets that were not present in primary tumors and that diverge from paired extracranial metastases. In seminal work on the molecular divergence of BrM, Brastianos et al. observed that more than half of BrM studied harbored at least one potentially actionable biomarker that was not present in the paired primary neoplasm (24). Their data from lung, breast, and renal cell cancers further demonstrated that these alterations were often unique to BrM when compared to lymph node and other extracranial metastases (24). These results have been supported by other recent analyses identifying potentially new and actionable biomarkers in BrM arising from non-small cell lung cancer (NSCLC), breast cancer, and melanoma, described below and summarized in **Table 1**.

Non-Small Cell Lung Cancer

Among the various biomarkers associated with lung cancer, genetic alterations in *epidermal growth factor receptor* (*EGFR*) are perhaps the most notable biomarker affecting the management of NSCLC patients with BrM. Previous reports have observed a discordance rate of *EGFR* mutation status between paired BrM and corresponding primary lung tumor samples from 19% to as high as 67% (25, 26), with BrM typically displaying a higher frequency of *EGFR* mutations than primary NSCLC tumors (79). Identification of *EGFR* mutations in BrM presents treatment opportunities, as studies suggest that first-generation EGFR tyrosine kinase inhibitors (TKIs), such as gefitinib and erlotinib, have CNS activity (80–83). It is important to note that patients with NSCLC BrM who received erlotinib or gefitinib plus radiotherapy or chemotherapy have exhibited significant intracranial responses and experienced longer progression-free survival (PFS) and median overall survival (OS) compared with patients who received erlotinib or gefitinib alone (27, 28). Similar considerations can be entertained for patients receiving the third-generation EGFR TKI osimertinib, which has emerged as an attractive first-line treatment for NSCLC and for NSCLC harboring *EGFR* Thr790Met (T790M) mutations (29).

Anaplastic lymphoma kinase (ALK) is another notable biomarker in the management of NSCLC patients with BrM (84, 85). The most prevalent *ALK* alteration involves the fusion of *ALK* with the *microtubule-associated protein-like 4 gene (EML4)*. The fusion event results in the autophosphorylation and constitutive activation of ALK kinase, which contributes to tumorigenesis and progression (86, 87). Current data suggest that the concordance for *ALK* gene fusion between the primary neoplasm site and the matched BrM appears high (33). Knowing the *ALK* mutation status in BrM is critical, as several drugs exhibiting CNS penetrance, in particular alectinib, brigatinib, and lorlatinib, have been approved by the FDA for the treatment of *ALK*-fusion-positive metastatic NSCLC (34, 35). Alectinib, brigatinib, and lorlatinib have all been demonstrated in clinical trials (Alex, ALTA-1L, and Crown) to have superior efficacy to crizotinib in the primary treatment of *ALK*-positive NSCLC (36–38). Intracranial response rates in these and other trials indicate that brigatinib and lorlatinib have significant efficacy against *ALK*-positive BrM (38, 39, 88), although the effectiveness of these agents on *ALK*-amplified BrM requires further investigation.

Many biomarkers demonstrate a significant rate of concordance between primary tumor sites and BrM. Nevertheless, routine molecular profiling of BrM will help identify possible new actionable biomarkers, especially when there are approved therapeutic options that exhibit good blood–brain barrier permeability, which were recently elegantly reviewed by Soffietti and colleagues (7). These targets include *ROS Proto-Oncogene 1 (ROS1)*, *MET Proto-Oncogene* (*MET) exon 14 skipping mutation*, *RET Proto-Oncogene* (*RET)*, *Neurotrophic Receptor Tyrosine Kinase* (*NTRK)*, *B-Raf Proto-Oncogene* (*BRAF)*, and *KRAS Proto-Oncogene* (*KRAS).* Both crizotinib and entrectinib, multi-targeted TKIs, are now U.S. Food and Drug Administration (FDA)-approved for treatment of NSCLC patients with *ROS1*-rearranged mutations (40, 43). However, as noted above, crizotinib has demonstrated limited intracranial efficacy in the clinic, while studies with entrectinib have reported intracranial response rates of up to 55% (89). Studies with lorlatinib and ceritinib in *ROS1*-positive NSCLC have also demonstrated high rates of intracranial response in patients with BrM (41, 88). In a Phase 2, open-label study, approximately 50% of NSCLC patients with *MET* exon 14 skipping mutations had some response to treatment with tepotinib and capmatinib (43, 44). Both capmatinib and tepotinib are FDA-approved for treatment of patients with *MET* exon 14 skipping mutant metastatic NSCLC, and recent studies report promising intracranial responses to both agents in patients with this mutation (44, 45). Selpercatinib and pralsetinib, two highly selective inhibitors of RET kinase, have been recently approved by the FDA for use in NSCLC patients with *RET* mutations (51, 52) and have both shown robust intracranial activity in patients with BrM (53, 54). *KRAS* is frequently altered in NSCLC, either through activating

TABLE 1 | Summary of therapeutic possibilities and prognostic information associated with biomarkers in brain metastases.

	Biomarkers (types)	Mechanisms of Actions	Discordance Rates Between BrM and Primary And Extracranial Neoplasm Sites	Therapeutic Options if Biomarkers Are Present In BrM	Alternative Therapeutic Options if Drug Resistance Has Occurred	Associated Prognostic Information
NSCLC	EGFR (mutation)	Receptor tyrosine kinase	19%–66.7% (25, 26)	TKIs: afatinib; erlotinib or gefitinib + radiotherapy or chemotherapy (27–29)	Osimertinib targeting EGFR T790M (30, 31)	↑ PFS in EGFR-mutant tumors treated with icotinib vs. uncommon EGFR mutations (32)
	ALK (rearrangement)	Receptor tyrosine kinase	ALK fusion: rare ALK amplification w/o fusion: 12.5% (33)	TKIs: ceritinib, alectinib, brigatinib, or lorlatinib (34–39)		
	ROS1 (rearrangement)	Receptor tyrosine kinase	ROS1 fusions enriched in BrM (26)	TKIs: entrectinib, lorlatinib, ceritinib (40, 41)		
	MET (mutation/ overexpression)	Receptor tyrosine kinase	Mutations and amplifications enriched in BrM (42)	TKIs: tepotinib, capmatinib (43–45)	Possibly contributing to EGFR treatment resistance; combination therapies under investigation (42, 46–50)	
	RET (mutation/ rearrangement)	Receptor tyrosine kinase		TKIs: selpercatinib, pralsetinib (51–54)		
	KRAS (overexpression/ mutation)	GTPase	13% (55)	TKIs: sotorasib (G12C) (56)		
Breast cancer	ER/PR (expression/ mutation)	Hormone receptor	ER: 13.6%–29.2% (57–59) PR: 4.2%–44.4%	Endocrine therapy: tamoxifen (57, 58)		
	HER2 (overexpression/ mutation)	Receptor tyrosine kinase	2.3%–23.8% (57–60)	Anti-HER2: trastuzumab, pertuzumab, lapatinib (14, 61) anti-AR: bicalutamide or enzalutamide (62, 63)		↑ OS likely attributed to treatment effects (59)
	PTEN (loss)	Regulation of PI3K/AKT/ mTOR pathway	Loss of PTEN is often seen in BrM, but is less commonly seen in extracranial sites (64–66)	PARP inhibitors: olaparib, veliparib (7, 67, 68)	Single-targeting therapies often found ineffective; combination therapies currently under investigation (e.g., HER3+PI3K or PI3K+mTOR) (69, 70)	↓ time to tumor recurrence in a distant site (63) ↓ OS in TNBC subtypes (71, 72)
	CDK pathway (mutation/loss)	Serine/threonine protein kinase; regulation of G1 checkpoint	Clinically actionable alterations in the CDK pathway genes in 28% of BrM not seen in primaries (24)	CDK4/6 inhibitors: abemaciclib, palbociclib, ribociclib (24, 73, 74)		
	RB1 (loss)	Regulation of G1 checkpoint	RB1 loss more commonly observed in BrM (24)		May contribute to CDK4/6 inhibitor treatment resistance (24, 73, 74)	
	HK2 (overexpression)	Glucose metabolism				↓ post-craniotomy survival in breast cancer patients w/ BrM (75)
Melanoma	BRAF (mutation)	Serine–threonine kinase	7% (76, 77)	TKIs: vemurafenib, dabrafenib (78)		

RTKis, receptor tyrosine kinase inhibitors; OS, overall survival; PFS, progression-free survival.

mutations or through amplification (55). The FDA approved in 2021 the first KRAS inhibitor, sotorasib, which specifically targets the G12C mutant form of *KRAS*, for metastatic NSCLC. Recent work in matched lung adenocarcinoma primary and BrM tissues reported that KRAS alterations were present in 13% of BrM tissues that were not present in the matched primary, with enrichment of G12C and G13C mutations (55). Given the potential intracranial efficacy of sotorasib (56) and ongoing trials to address this question, identification of a *KRAS* G12C mutation in a BrM may provide a potential new avenue for directed therapy in these patients.

Breast Cancer

Among a host of biomarkers important for the clinical management of breast cancer (BC), estrogen receptor (ER), progesterone receptor (PR), and HER2 are the most crucial. Hormone receptor (HR; ER or PR)-negative, HER2-positive, and triple-negative (TNBC; ER-, PR-, HER2-) statuses are associated

with increased risk for BCBrM (90). High discordance in these biomarkers exists between primary BC and BrM: ER: 13.6%–29.2%, PR: 4.2%–44.4%, and HER2: 2.3%–23.8% (57–59). In a recent large analysis, this discordance led to subtype switching between primary tumors and BrM in 22.8% of patients (91, 92). Furthermore, pathology and mRNA expression analyses have revealed a downregulation of ER (*ESR1*) and PR (*PGR*) gene expression and an upregulation of HER2 (*ERBB2*) gene expression in BrM, particularly in those arising from TNBC (19, 60, 91, 92).

Since HR and HER2 status are frequently used to determine eligibility for therapeutic options, it is important to analyze BrM tissues to obtain accurate biomarker information for appropriate treatment selection (58, 93). Importantly, most patients (63.6%) with biomarker discordance between the primary neoplasm and BrM also show discordance between extracranial metastases and BrM, with the primary and extracranial neoplasms typically being concordant (91, 92). Thus, different treatment options may have therapeutic activity in BrM that can currently only be identified by profiling BrM. For instance, anti-HER2 therapy (e.g., trastuzumab, pertuzumab, or lapatinib) can be used for HER2 amplification, which are frequently increased in BrM compared to primaries and extracranial metastases. Recently, newer HER2-targeted agents have shown an ability to reach BrM and generate intracranial responses (61). Excitingly, a recent exploratory analysis of 291 patients with BrM who were included in the HER2CLIMB randomized controlled trial demonstrated that the addition of tucatinib to trastuzumab and capecitabine doubled the intracranial response rate, highlighting a regimen that may be especially effective against HER2-positive BrM (14). Similarly, endocrine therapy (e.g., tamoxifen or aromatase inhibitors) can be applied to tumors with positive HR status (94, 95). While treatment options for TNBC BrM have historically been limited to chemotherapy, there is emerging evidence of effectiveness of androgen receptor (AR)-targeted therapies (e.g., bicalutamide or enzalutamide) in TNBC (62, 63).

Deletion of phosphatase and tensin homologue (*PTEN*) on chromosome 10 has been found in a significant portion of BCBrM (96). Furthermore, significantly decreased PTEN mRNA and protein expression has been observed in BCBrM compared to primary tumors (71, 97). Loss of PTEN may be a critical factor for BrM development, a possibility that is supported by research suggesting that the loss of PTEN is often exhibited in intracranial malignancies but less commonly in extracranial sites (64–66). Downregulation of PTEN expression has not been observed in bone metastases, suggesting that PTEN dysfunction may be uniquely supportive to metastatic growth in the brain microenvironment (97–99). PTEN antagonizes the phosphatidylinositol 3-kinase (PI3K)/AKT/mTOR pathway, with loss of PTEN resulting in aberrant activation of the pathway and enhanced tumor cell proliferation (100). Identifying PTEN loss in BrM opens the door to potential therapeutic modalities, including PI3K inhibition (7). It has also been suggested that loss of PTEN sensitizes malignant cells to polyadenosine diphosphate ribose polymerase (PARP) inhibition (67). Importantly, BrM profiling could potentially identify resistance mechanisms for PARP inhibitors to help rationally guide the selection of the next line of therapy. Additional therapeutic options targeting loss of PTEN in BrM require further investigation (68).

CDK4/6 inhibitors, including abemaciclib, palbociclib, and ribociclib, are another major class of treatment for BC metastases (7). Abemaciclib is the most brain permeable of the class and has been tested in a recent clinical trial of patients with HR-positive, HER2-negative BrM with promising results (101). Palbociclib has also demonstrated intracranial efficacy in patients with CDK pathway alterations and BrM in a basket trial, including in patients with BrM from breast cancer (102). However, clinical studies have linked homozygous retinoblastoma protein 1 (RB1) loss to resistance to CDK4/6 inhibitors (24, 73, 74). Homozygous RB1 loss has been observed more frequently in metastatic BC, especially BCBrM, as compared to primary tumor sites (24). RB1 mutations are linked to chromosomal rearrangements that subsequently disrupt genes that inhibit tumor growth and progression. Thus, molecular profiling of BCBrM may present additional treatment options, or may indicate potential resistance to additional options, for these patients.

Melanoma

BRAF is a gene that encodes the B-Raf protein, which is a serine–threonine kinase. Activating mutations in BRAF, the majority of which are $BRAF^{V600E}$, occur in approximately half of cutaneous melanomas (103). Previous studies have reported that up to 7% of *BRAF* mutations found in BrM are not found in primary melanoma sites (76, 77). Highly selective BRAF and MEK inhibitors (e.g., vemurafenib and dabrafenib) are now approved and demonstrate clinically meaningful activity in the brain (78). These results indicate that biopsies of BrM for subsequent BRAF analysis should be considered in select patients to guide treatment decisions.

Immune Checkpoint Blockade

The treatment of patients with a variety of solid tumors has benefitted from immune checkpoint blockade (ICB). While patients with intracranial metastases were historically excluded from systemic and immunotherapy trials, intracranial responses are increasingly observed following ICB, prompting newer interest in harnessing immunotherapy for these patients. In particular, agents targeting the programmed cell death-1 (PD-1)/programmed cell death ligand-1 (PD-L1) axis, as well as cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4), have been used clinically now across BrM from a number of primary disease indications and have been approved for use in melanoma and NSCLC (104, 105). Most famously, perhaps, dual checkpoint inhibitor therapy with ipilimumab and nivolumab demonstrated intracranial response rates of 52% in selected asymptomatic patients with active melanoma BrM (106). Overall survival (OS) in this study was 81.5% at 12 months, and median survival had not been reached at 30 months (106). Meanwhile, an early combined analysis of both lung and melanoma BrM patients from a further phase II study illustrated intracranial response rates to pembrolizumab (anti-PD-1) monotherapy of 33% and 22%, respectively, with nearly

identical extracranial response rates (107). This may shift the indication for ICB to up front rather than salvage therapy, as a number of these studies were conducted in patients receiving no prior therapy for their intracranial disease, and high concordances between intracranial and extracranial disease were typical.

Despite some notable successes, optimal biomarkers to guide therapeutic decision-making are lacking. Previous studies have reported that up to 50% of PD-1 expression that was found in BrM was not found in the primary melanoma site (108). This has prompted the search for additional predictive biomarkers for ICB, including tumor mutational burden (TMB). TMB, the total number of non-synonymous mutations in the coding regions of genes, has recently emerged as a potential biomarker to select patients for immunotherapy. The FDA granted accelerated approval to pembrolizumab (KEYTRUDA, Merck & Co., Inc.) for the treatment of adult and pediatric patients with unresectable or metastatic TMB-high (TMB-H) solid tumors that have progressed following prior treatment and who have no satisfactory alternative treatment options (109). Metastatic tumors have increased TMB at recurrence, and BrM is found to have the highest level of TMB among metastatic sites (110, 111). Given emerging evidence of response to ICB in intracranial tumors (112–114), specifically evaluating TMB as a predictive biomarker is a priority that will require increased molecular profiling or BrM.

Radiation Therapy Considerations

Radiation therapy has long been applied for BrM in a fashion that is largely agnostic to tumor histology. However, emerging evidence suggests that the genetic configuration of BrM could dramatically impact its response to radiation therapy. For instance, a recent pan-cancer analysis found that tumors containing pathologic genetic alterations in the apical DNA-damage response gene *Ataxia telangiectasia mutated (ATM)* have dramatically improved local control after radiation therapy compared to control tumors (incidence of irradiated tumor control 13% vs. 28% at 2 years) (115). This link between *ATM* pathogenic variants and radiosensitivity seems to extend to primary brain tumors (116). Thus, the mutational status of genes such as *ATM* may be one of several factors that, in the setting of a multidisciplinary BrM tumor board, could guide whether to approach a BrM with primary SRS, with surgery, or to reimage the brain following a trial of systemic therapy. Given the discordance between BrM and primary tumor genotype (24), sampling of the BrM *ATM* genotype would be expected to provide the most robust biomarker for radiosensitivity. Further validation of this finding and investigation of other genetic biomarkers that may be linked to radiosensitivity are warranted in the BrM setting.

IDENTIFICATION OF INEFFECTIVE TREATMENT STRATEGIES

Molecular profiling of BrM can help indicate whether certain targeted therapies are likely ineffective in this setting. First, resistance to molecularly targeted therapies can occur over the course of treatment and render therapies ineffective against late-stage disease, including BrM. Drug resistance can develop through multiple mechanisms, including but not limited to restoration/reactivation of downstream targets, activation of alternative signaling pathways, and mutations in the binding site of a targeted protein that alter binding of the drug (117). This therapeutic resistance may develop after initial treatments of the primary neoplasm and other metastatic sites. As a result, treatment for BrM based on tissue samples from the primary tumor or other metastatic sites alone may misinform clinical decision-making. Second, actionable targets that were once present in the primary and/or extracranial tumors may be lost in the BrM. Thus, continued treatment with the original matched targeted therapy would be ineffective in the BrM and subject the patient to unnecessary side effects and costs. In this section, we discuss mechanisms of drug resistance and loss of biomarkers in BrM from NSCLC, breast cancer, and melanoma and discuss how knowledge of BrM biomarkers can guide therapy away from ineffective therapies.

Non-Small Cell Lung Cancer

Although most NSCLC harboring an EGFR mutation are initially responsive to treatment with first-generation TKIs, the majority of patients develop drug resistance within 1–2 years (30). Approximately 60% of acquired resistance to early-generation TKIs is due to the acquiring of the EGFR T790M mutation (118). Tumors may also acquire resistance through activation of signaling molecules downstream of EGFR. Indeed, MET (N-methyl-N′-nitroso-guanidine human osteosarcoma transforming gene), a receptor tyrosine kinase that is considered an oncogenic driver in NSCLC (119–121), is suggested to be closely linked to the EGFR pathway (46–49) and its resistance to inhibitors (42, 50), and has been observed to have a higher rate of mutation in BrM versus primary NSCLCs (69, 70). Providers treating patients who progress after an EGFR TKI should consider molecular analyses of BrM tissue to confirm whether continued treatment with an EGFR inhibitor, or switching to a different TKI like the T790M mutant-specific, brain-penetrant inhibitor osimertinib (30, 31), will be effective this setting.

Breast Cancer

As discussed above, activation of the PI3K/AKT/mTOR pathway, such as through loss of PTEN, has been suggested to play a role in the mechanisms underlying poor responses to anti-HER2 therapy in BC metastases (122, 123) and has been found to be altered in more than half of BCBrM (122, 124). However, targeting a single biomarker of the PI3K/AKT/mTOR pathway (e.g., PI3K, HER2, or HER3) is often ineffective (125, 126). Combination therapies aimed against multiple molecular targets (e.g., HER3+PI3K or PI3K +mTOR) appear to be more efficacious against BCBrM than monotherapy in preclinical models (125, 126).

HR-positive BC has a lower frequency of metastasizing to the brain compared to other BC subtypes (127). However, in those patients that do develop BrM, their disease has frequently become resistant to hormone therapy at this late stage of the disease through acquisition of HR mutations (7). Furthermore, BCBrM also frequently demonstrates loss of ER and PR. Indeed, a recent analysis showed that 14.8% and 22.4% of BCBrM had

loss of ER and PR, respectively, contributing to the 22.8% of cases that had a subtype switch between primary or extracranial tumors and BrM (91, 92). Thus, hormone therapy may be ineffective in treating a significant portion of BrM given their frequent acquired resistance and/or loss of HR expression.

Melanoma

Melanoma patients often develop treatment resistance within 1 year of receiving BRAF/MEK-targeted therapy. Agents which target the BRAF/MEK pathway have shown meaningful clinical activity in patients with melanoma BrM, although resistance has been observed to develop within a shorter period of time (78). Several mechanisms for treatment resistance have been suggested, including receptor tyrosine kinase upregulation (e.g., PDGFRß, IGF1R), acquisition of MEK alterations, and activation of the RAS/RAF/MAPK pathway (128).

A recent report comparing melanoma BrM to matched primary and extracranial melanoma tumors demonstrated biomarker discordance between BrM and extracranial sites in 5/8 patients, including loss of mutant NRAS (111). Of note, 2 patients with multiple BrM also showed some differences in potentially actionable alterations between the individual BrM. While overall concordance with extracranial metastases is felt to be high with respect to driver mutations, studies have revealed important molecular differences in melanoma BrM, such as increased activation of the PI3K/AKT pathway (129).

PROGNOSTIC INFORMATION

Assessing the biomarker status of BrM is not only valuable for informing the treatment plan—both by adding new potential strategies and by ruling out ineffective ones—but can also provide prognostic information to improve patient and provider expectations for care. Prognostic information is particularly important to patients with BrM, as BrM symptoms are often associated with decreased functional status and severe reductions in quality of life.

Non-Small Cell Lung Cancer

Studies suggest that BrM with driver mutations, including EGFR and ALK, were associated with longer overall survival when treated with surgery, radiosurgery, and non-surgical interventions (5, 130–138). Specifically, Zhou et al. report that BrM patients with common EGFR mutations treated with icotinib exhibited a prolonged PFS compared to those with uncommon EGFR mutations (32). There is a solid body of evidence suggesting that significant survival increases are associated with NSCLC BrM with EGFR mutations compared to those without EGFR mutations (80, 131, 137–139). A recent meta-analysis of 18 studies supports this conclusion and posits that this is likely due to treatment sensitivities of the metastatic lesions (131).

Breast Cancer

A number of biomarkers hold prognostic value for BCBrM. Approximately 20%–25% of breast cancers have amplified HER2 status (140–142), which is associated with longer survival among BC patients with BrM (59). Clinical data suggest that increased survival associated with HER2 positivity is likely a reflection of treatment effects related to anti-HER2 therapy rather than a reflection of the HER2-associated biological composition of BrM (59).

As previously discussed, loss of PTEN may be a critical factor for BC metastases to develop in the brain parenchyma (64–66). Studies have shown that loss of PTEN was associated with decreased time to tumor recurrence in distant sites, including the brain, in BC metastases (71). Furthermore, loss of PTEN has been associated with worse overall survival in patients with TNBC (71, 72).

Hexokinase (HK2), which plays an essential role in glucose metabolism (143, 144), is overexpressed in BrM compared with primary breast tumors. Increased HK2 expression has been associated with decreased post-craniotomy survival in BC patients with BrM (75).

CHALLENGES AND FUTURE CONSIDERATIONS

Biomarker analyses of BrM offer potential clinical gains by identifying and/or eliminating candidate targeted therapies. Currently, clinicians do not always obtain biopsies or send resected BrM tissues for biomarker analyses, resulting in a missed opportunity to better inform patient care and potentially improve outcomes. Clinicians may also be daunted by the variety and complexity of biomarker testing options or not be aware of recent work in the genetics of BrM demonstrating biomarker discordance and sometimes unique genetic profile in these metastases. Furthermore, the application of targeted therapies to treatment of BrM is currently limited to those which can penetrate the blood–tumor/blood–brain barrier, providing an additional layer of complexity in screening potential therapeutic modalities. Providing clinicians access to biomarker testing, clearly summarized and annotated results, and to molecular tumor boards may help them to better appreciate the value and interpret results of biomarker profiling in BrM.

There are at least two potential reasons why clinicians may not seek to test BrM tissues for biomarkers despite the potential utility of this information. First, clinicians may not realize that biomarker analyses from BrM resections or biopsies can provide valuable information that is different from that obtained from the primary tumor or extracranial metastasis sites. Even when clinicians attempt to analyze BrM tissue for biomarkers, a large and growing number of complex testing options can present practical difficulties, particularly in resource-limited settings (145). Whole-transcriptome sequencing (WTS) and whole-exome sequencing (WES) platforms that are currently used for research purposes have recently become standard of care at many institutions and commercial providers. Pan-cancer whole-genome analyses of metastases have revealed therapy-associated mutations that contribute to drug resistance in individual patients (146–148). However, such analyses can be complex to interpret and utilize (149). Furthermore, practical considerations, such as which genetic

testing platforms are FDA approved, and whether genetic tests are covered by insurance, can make it difficult to recommend additional genomic profiling in BrM. The growing number of testing options, and practical considerations for each, makes it increasingly difficult for clinicians to order and select the most appropriate biomarker analyses. In the future, development of targeted panels for types of primary tumors that metastasize to the brain could be considered to augment accessibility of BrM biomarker analyses for clinicians.

The optimal use of targeted BrM therapies depends largely on the expertise of clinicians (150), many of whom have limited experience considering the efficacy of targeted therapies in crossing the blood–brain barrier (151). As a result, management of BrM often requires a multidisciplinary approach (12), with molecular tumor boards being a vital venue for discussion of treatment plans with input from multiple specialties (152). Access to molecular tumor boards would likely improve and increase the application of genomically guided cancer care for patients with BrM, including targeted clinical trial enrollment. Data suggest that less than half of all hospitals and only 5% of non-academic hospitals have access to molecular tumor boards (149). Clinicians at hospitals treating patients with BrM may face logistical obstacles in accessing molecular tumor boards, such as long distances to in-person meetings, low local patient volume, and limited personnel, although the recent global shift toward increasing comfort with web-based conferencing may serve to accelerate adoption of online multidisciplinary tumor boards. Organizing molecular tumor boards across multiple hospitals or hospital systems to provide clinicians access to relevant expertise is a logical and critical step forward in advancing use of molecular tumor boards across sites (149).

CONCLUSION

Targeted therapeutic strategies and prognostic stratifications for treatment of patients with BrM are increasingly common. Despite the fact that discordance often exists between BrM and both primary tumors and distant extracranial metastases, molecular profiling of resected BrM is not currently routine, and biopsies for the purpose of biomarker evaluation are rare. Biomarker information from BrM can identify new mutations with viable targeted therapies, eliminate agents from consideration when resistance or loss of actionable biomarkers has developed in the BrM, and improve prognostication. Clinicians may be initially dismayed by the variety and complexity of biomarker testing options, but this challenge can be overcome by (virtual) molecular-tumor boards to guide decision-making and advance personalized oncology care for patients with BrM.

AUTHOR CONTRIBUTIONS

Investigation: ES. Resources: KW, CRG. Data curation: ES, AV. Writing: ES, AV, MP, KB, RV, CB, BP, ZR, AS, JC, CA, PF, CRG, KW. Visualization: ES. Supervision: CRG, KW. Project administration: CRG, KW. All authors contributed to the article and approved the submitted version.

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Quality of Life and Role of Palliative and Supportive Care for Patients With Brain Metastases and Caregivers: A Review

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Brain metastases (BM) are the most commonly diagnosed secondary brain lesions in adults, influencing these patients' symptoms and treatment courses. With improvements in oncologic treatments, patients with BM are now living longer with their advanced cancers, and issues pertaining to quality of life become more pressing. The American Society of Clinical Oncology has recommended early implementation of palliative care for cancer patients, though incorporation and implementation of palliative and other supportive services in the setting of true multidisciplinary care requires additional attention and research for patients with intracranial metastases. We review the physical, cognitive, and psychosocial challenges patients with BM and their caregivers face during their cancer course as well as the current published research on quality of life metrics relating to this patient population and the diverse roles specialty palliative care, rehabilitation services, and other healthcare providers play in a comprehensive multidisciplinary care model.

Keywords: brain metastases, palliative care, supportive care, quality of life, advanced cancer, caregiver

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INTRODUCTION

Metastatic brain tumors are the most commonly diagnosed secondary brain lesions in adults, with an incidence of 8.3–14.3 per 100,000 people (1). Annually, ∼150,000–200,000 people are diagnosed with brain metastases (BM) in the United States alone (2). Lung cancer, breast cancer, and melanoma are the primary malignancies most likely to predispose to development of BM, which may encompass metastatic leptomeningeal disease as well.

Typically, development of BM indicates advanced cancer, and patients may be frail and chronically ill by the time they present for additional surgery, chemotherapy or radiation. Furthermore, metastatic brain tumors can additionally impact patients' neurological and cognitive function and their overall quality of life. Often, BM management regimens already involve specialists from disparate disciplines, as new treatment options, such as immunotherapy, emerge and gain traction (3). BM patients therefore benefit from coordinated care from a multidisciplinary team, consisting of their oncologists, radiation oncologists, surgeons, as well as providers from palliative care, social work, and therapy services if necessary to address needs in a holistic manner.

Outcomes from early implementation of palliative care (PC) in particular have been investigated in several studies, including randomized controlled trials, notably Temel et al. (4). The landmark study determined that patients with metastatic non-small cell lung cancer benefited from early PC with improvements in survival and quality of life. However, most advanced cancer patients do not necessarily receive early PC referral, as 48% of a cohort of BM patients received PC consultation with median timing of consultation to death of 1.6 months (5).

We present a comprehensive review of not only the challenges patients living with metastatic brain tumors and their caregivers face but also validated measures of quality of life before delving into a discussion of critical palliative and supportive care providers and resources that may enhance quality of life.

CHALLENGES OF LIVING WITH BRAIN METASTASES

Patients with metastatic brain tumors face unique challenges due to their disease. The treatments for BM also present potential short-term and long-term complications. Depending on their location, metastatic brain tumors lead to variable clinical presentations even while the primary cancer may be quiescent. Common symptoms include headaches (40–50% of patient presentations), seizures (15–20% of patient presentations), as well as different neurologic deficits, such as motor or language deficits (6). However, while the range of neurologic and cognitive symptoms may be large among metastatic brain tumor patients, the development of BM typically portends poor overall prognosis. For example, median survival for renal cell carcinoma patients with BM was 5 months, and the median survival for patients with solid BM from non-small cell lung cancer was 8.4 months (7, 8).

Fortunately, with treatment advances, certain BM patients can achieve good tumor control burden. Almost half of patients diagnosed with BM have a single, isolated intracranial metastasis at presentation, and they may undergo a variety of effective treatments, including surgery and stereotactic radiosurgery (9). Depending on the treatment regimen selected, patients with isolated BM have median survival ranging from 28.9 to 62.8 months (10). Combination therapies confer benefits to quality of life for this patient population as well; 88% of those who underwent surgery and whole brain radiation therapy (WBRT) reported improvement in Karnofsky Performance Status (KPS) scores (10). Even patients presenting with multiple BM have viable treatment options. Surgery and radiosurgery both have comparable tumor control and survival outcomes for patients with 2–4 BM (11). Separately, a large-scale prospective study included patients with 1–10 newly diagnosed BM and found that stereotactic radiosurgery alone conferred similar survival benefit [HR 0.97, 95% CI 0.81–1.18 (less than non-inferiority margin), $p = 0.78$; p non-inferiority < 0.0001] and adverse event profiles for patients with a few BM or 5–10 BM (12). Repeat stereotactic radiosurgery can also lead to good metastatic brain tumor control without side effects of radiation necrosis (13). Furthermore, in addition to radiation therapy, developments in chemotherapy and oncologic immunotherapy have also been promising for BM patients with various primary cancers (14–16).

However, the treatments available—whether in the form of surgery, radiation, or chemotherapy—for patients once metastatic brain tumors have developed can also be taxing and present risks. Even though the BM patient population is heterogeneous, those presenting with multifocal BM or leptomeningeal disease may have additional challenges with their treatment as well.

Neurosurgical procedures inherently involve potential risks and complications following intracranial tumor resection, such as superficial or deep wound infection, perioperative stroke, or postoperative hematoma (17). In addition, patients who are preoperatively frail, according to an 11-factor modified Frailty Index, are significantly more likely to develop life-threatening complications or mortality in a population of benign meningioma patients who underwent cranial surgery (18). Similarly, in a cohort of 180 geriatric patients with surgically-resected BM, the frailest patients according to the modified Frailty Index had significantly shortened median overall survival compared to those considered "least frail" (3 vs. 18 months, $p < 0.0001$). Furthermore, not all cases of BM are amenable to surgery, particularly if the tumors are multifocal, located in eloquent areas or pose greater risk than benefit during surgical resection. In these cases, patients with BM could be eligible for other forms of treatment.

At times, patients must resort to radiation therapy or palliative radiation, even though some people's overall clinical response may be minimal (19). Common adverse effects from cranial radiation include headache, nausea, vomiting, and fatigue among others. While the overall toxicity from radiation courses, particularly with goal of palliation, is typically mild and rare, some patients may still experience various Grade 1 or 2 adverse effects, such as mucositis and skin reactions, as well as higher grade toxicities (20, 21). On the other hand, up to 90% of brain tumor patients who undergo radiation therapy experience cognitive changes, which may be exacerbated by the treatment length, radiation dose, fraction size, and volume treated (22–24). In other cases, whole brain radiation therapy (WBRT) may be indicated for palliation as well and is a standard therapy for patients with multiple BM (25). However, WBRT carries risk of new symptoms in the future for cancer patients. Memory loss and cognitive impairment have been reported for up to 50% of patients who had undergone WBRT with a higher rate of developing dementia in young cancer patients over time (26). Brown et al. designed a multi-institutional study on the cognitive effects of WBRT. They found that patients who received both WBRT and stereotactic radiation had significantly worse cognitive performance (decline in verbal fluency as well as both immediate and delayed memory tasks) at 3-month follow-up than patients who did not undergo WBRT (27). At present, hippocampus-avoiding WBRT is an option for patients with multiple BM as this treatment protocol minimizes hippocampal atrophy (28).

Various medications may be prescribed for symptomatic relief or prophylaxis for patients with BM. Some patients may present with or will be at risk of developing seizures, and

prophylactic anti-epileptic drugs like levetiracetam or phenytoin may be administered to decrease this symptomatic burden (29). However, a meta-analysis did not find significant decrease in seizure occurrence with prophylactic anti-epileptic medication compared to control [OR = 0.939, 95% confidence interval (CI) = 0.609–1.448, z = 0.29, $p = 0.775$] (30). Patients with BM commonly take corticosteroids to alle*via*te symptoms arising from vasogenic edema surrounding some intracranial metastatic tumors. Unfortunately, steroids have numerous adverse effects, such as mood shifts, hyperglycemia, and weight gain, and does not have permanent therapeutic effects.

Chemotherapy regimens are updated once a cancer patient develops metastatic brain tumors, in part due to the need for surmounting the blood-brain barrier and other factors that influence therapeutic levels of medications intracranially (31). However, even cancer patients without metastatic brain tumors can develop cognitive deficits from systemic chemotherapy in both short and long term cases (32). Targeted cancer immunotherapy has been a superb option for patients with metastatic cancer. However, the majority of early clinical trials assessing targeted therapies for advanced cancer patients excluded those with BM. Currently, many more clinical trials enroll patients with BM with primary cancer diagnoses of melanoma, non-small cell lung cancer, and breast cancer (33). Adverse effects from immunotherapy, some of which can be severe and debilitating, should not be overlooked even though they may herald good clinical response to therapy. In a cohort of 56 patients with Stage IV melanoma, 36% of the group experienced any immune-related adverse events associated with their anti-programmed death 1 (anti-PD1) treatment (34). Every patient had an adverse event while on ipilimumab treatment. In a separate retrospective chart-based study, it appeared that patients with melanoma and BM had longer median intracranial progression-free survival when they experienced severe adverse events following immunotherapy compared to those without severe adverse events, though the effect was not statistically significant (19.9 vs. 10.5 months, $p = 0.053$) (35).

The potential for patients' psychological distress must not be overlooked while providing healthcare for patients with BM. For patients with advanced cancer with or without BM, they typically experience high levels of distress and anxiety. For instance, a cross-sectional pilot study involved metastatic non-small cell lung cancers who did and did not have intracranial metastatic spread; 53% of the group of 78 patients had BM (36). Both groups of patients reported death anxiety that was significantly associated with demoralization ($p < 0.001$) and illness intrusiveness ($p = 0.001$). Cordes et al. studied groups of breast cancer patients with and without metastatic brain tumors, evaluating for measures of distress, depression, and anxiety. For patients with BM who underwent cranial radiotherapy in this study compared to people without intracranial metastases, a large proportion of the group (70 vs. 66%) experienced distress and reported higher measures of distress ($p = 0.029$) (37).

With increasing numbers of patients with BM, more families and caregivers also experience various challenges and burden. From a pilot study involving 21 family caregivers of patients with BM, Ketcher et al. (38) found that caregivers devoted extensive time and energy to providing care but lacked adequate support for numerous psychosocial aspects, such as coping mechanisms, anxiety, and depression. And, in general, caregivers of patients with BM with greater caregiving burden are at greater risk of suffering from anxiety and depression (39). Indeed, lower levels of resiliency appeared to correlate with high caregiver burden (OR = 0.76), according to the eQuiPe study, a prospective, longitudinal observational study involving advanced cancer patients and their family caregivers (40). Furthermore, caregivers reporting high burden were also less informed about the importance of self-care (OR = 0.39), pointing toward potential avenues for intervention in future prospective studies on building resiliency, reducing burden, and providing support for caregivers.

QUALITY OF LIFE IN PATIENTS WITH BRAIN METASTASES

Measuring Quality of Life

The World Health Organization (WHO) defines health related quality of life (QoL) as "an individual's perception of their position in life in the context of the culture and value systems in which they live and in relation to their goals, expectations, standards, and concerns" (41). While the complexities of capturing all elements of this multidimensional definition have been previously discussed across medical specialties, present research in metastatic brain tumors has focused on using validated scales to quantify functional status, neurocognitive abilities, and social wellbeing (42–45). The use of these scales has allowed providers and researchers to measure QoL throughout the course of treatment, and has served as a tool to document improvements, stability, or deterioration in a patient. Specifically, three scales have been commonly used: (1) the Karnofsky Performance Status (KPS), (2) Functional Assessment of Cancer Therapy-Brain (FACT-Br), and (3) EuroQoL-5D (EQ-5D) (**Table 1**).

The KPS was introduced to describe a patient's ability to carry their normal activity and work, and their ability to care for themselves (46). The scale places patients into one of three conditions (47):

- Able to carry on normal activity and work. No special care is needed.
- Unable to work. Able to live at home, care for most personal needs. A varying degree of assistance is needed.
- Unable to care for self. Requires equivalent of institutional or hospital care. Disease may be progressing rapidly.

One major limitation of the KPS is that it focuses on physical functioning and need for assistance but fails to isolate neurocognitive drivers of ability to work or care for self.

On the other hand, the FACT-Br is a tailored subscale used in conjunction with the FACT-G, the general scale. Together, they measure physical wellbeing (e.g., nausea, energy, pain), social and family wellbeing (e.g., emotional support, family communication), relationship with doctors, emotional wellbeing (e.g., worries about death), functional wellbeing (e.g., ability to work, sleep well), and additional neurologic-specific concerns (e.g., problems with vision or hearing, ability to read or write like

TABLE 1 | Commonly used Quality of Life (QoL) scales in BM literature.

Scale	Domains assessed	Scoring
Karnofsky Performance Status	Functional status, as defined by ability to carry on normal activity and work, as well as additional assistance necessary	0% (death) to 100% (no evidence of disease, no symptoms) • Scores between 80–100% are in Category A (able to carry on normal activity and to work; no special care needed) • Scores between 50–70% are in Category B (unable to work; able to live at home and care for most personal needs; varying amount of assistance needed) • Scores in 0–40% are in Category C (unable to care for self; requires equivalent of institutional or hospital care; disease may be progressing rapidly).
Functional Assessment of Cancer Therapy: General (FACT-G) and Brain (FACT-Br)	Physical wellbeing, social/family wellbeing, relationship with doctor, emotional wellbeing, functional wellbeing, additional concerns (FACT-Br specific)	0 (poorest QoL) to 200 (best QoL) • While some items are reverse scored, in general, an item is given a score of 4 if it is "not true at all" (indicating best QoL outcome for patient), score of 3 if it is true "a little bit," 2 if it is true "somewhat," 1 if it is true "quite a bit," and 0 if it is true "very much" (indicating worst QoL outcome for patient)
EuroQoL-5D	Mobility (walk), self-care (washing and dressing self), usual activities (work, study, housework, family or leisure activities), pain/ discomfort, and anxiety/ depression	11,111 (no problems in any of the domains) to 55,555 (severe problems/ inability to perform task in all five domains) • QoL is coded using a 1 if no problem, 2 if slight problems, 3 if moderate problems, 4 if severe problems, and 5 if unable to or have extreme problems for each of the five domains
Spitzer Quality of Life Index	Activity, daily living, health, support, outlook	0 (poorest QoL) to 10 (best QoL) • Each domain is given score of 0, 1, or 2 • 0 for each domain corresponds to not being able to perform activity or ADL, being very ill, receiving poor support, and being seriously confused, frightened or anxious • 1 for each domain corresponds to conducting normal activities and ADLs with assistance, feeling low on energy, perceiving limited family/friend support, and feeling some anxiety • 2 for each domain corresponds to performing normal activities and ADLs independently, feeling well, feeling strong relationships with others, and appearing calm
European Organization for Research and Treatment of Cancer (EORTC) Quality of Life Questionnaire-Brain Neoplasm (QLQ-BN20)	Symptoms (headaches, seizures, drowsiness, hair loos, itchy skin, leg weakness, bladder control), future uncertainty, visual disorder, motor dysfunction, communication deficit	0 (best QoL) to 100 (worst QoL) • 20 items rated on a four-point Likert Scale (not at all, a little, quite a bit, very much) • Linearly transformed to a 0–100 scale
European Organization for Research and Treatment of Cancer (EORTC) Quality of Life Questionnaire (QLQ-C30)	Five functioning scales (physical, role, cognitive, emotional, and social), symptom scales (fatigue, pain, nausea/vomiting), single symptoms (dyspnea, appetite loss, sleep disturbance, constipation, diarrhea, financial impact) and global health status	• For functioning and global health scales: 0 is worst QoL, 100 is best QoL • For symptom scales: 0 is best QoL, 100 is worst QoL

they used to) (48, 49). A limitation of this scale is that it uses a Likert scale to assess the presence of symptoms in the past 7 days, thus, recency or recall biases might affect the scoring. Moreover, it may not be appropriate to track deterioration, stability, or progress over longer time frames.

Lastly, the EQ-5D asks patients to rate their abilities across five domains using a descriptive scale ranging from "I have no problems [with activity]" to "I am unable to do [activity]." The five domains in the scale are mobility (ability to walk), self-care (ability to wash or dress self), usual activities (including work, study, housework, family or leisure activities), pain/ discomfort, and anxiety/depression (50). Importantly, this scale attempts to capture anxiety and depression, which have been shown to occur in advanced disease, including stage IV cancers (37, 51). However, the scale's brevity may prevent providers and researchers to understand specific drivers of poorer QoL. Given the wide range of symptoms that may result from brain metastases, assessment of QoL should seek to address both the concerns outlined by the WHO, as well as the functional, emotional, and psychiatric changes that may result from tumor burden.

It should be noted that numerous other QoL scales have been validated and are widely used. Other commonly used scales in the BM literature are included in **Table 1**.

Quality of Life in Brain Metastasis

BM without any intervention results in poor survival, and a rapid decline in QoL (52). However, the rise in availability of multiple treatment modalities for BM has led to a growing body of literature describing survival and QoL after intervention (**Table 2**). Most commonly, WBRT, Gamma Knife Surgery (GKS)/stereotactic surgery (SRS), and surgical resection, have been examined in observational and randomized controlled trials.

Whole Brain Radiation Therapy

Historically, WBRT was considered a mainstay of treatment for BMs. Early studies in QoL in BM provided prospective, descriptive analyses of QoL after WBRT. For instance, in one study conducted by Wong et al. (53), 217 patients who received WBRT between 2005 and 2012 were prospectively assessed for progression of symptoms and QoL using the KPS and FACT-Br scales, among others. In this study, overall QoL scores deteriorated over the three-month study period, and fatigue, drowsiness, and appetite were shown to deteriorate from baseline at statistically significant levels (53). In the first month following WBRT, weakness and appetite loss were the two elements which increased in severity at statistically significant levels, whereas in the second month, five symptoms (nausea, balance, headache, anxiety, and appetite loss) declined most severely. In the third month, anxiety was statistically significantly different than baseline. Notably, the authors did not find a difference in symptoms in patients taking dexamethasone (80% of patients), except for insomnia in the first month (53). In another prospective study of 46 patients by Steinmann et al., self-assessed global QoL remained stable in the 3-month follow-up period, but physical function deteriorated significantly (54). In this same study, QoL assessment by healthcare proxies, though, was found to be statistically significant lower at 3 months vs. baseline, and symptoms of fatigue, nausea, pain, dyspnea, appetite loss, and constipation were found to deteriorate, with statistically significant differences in fatigue and appetite loss, consistent with the findings by Wong et al. (54). Doyle et al. (55) also noted the poor concordance between proxies' assessment of health and patients' own self-assessment, and similarly found a trend toward poorer QoL, as defined by the FACT-G and FACT-Br scales. Moreover, similar to Steinmann et al.'s work, physical wellbeing at 2 months was found to deteriorate the most. However, in another study of 129 patients receiving WBRT, daily living and health, two elements of Spitzer Quality of Life Index, were found to significantly improve after treatment, and frequency of headache and fatigue declined (56). The authors suggest that while overall QoL may have not meaningfully improved, WBRT may have contributed to the stabilization of some symptoms. Improvement in QoL elements after WBRT was also noted in a study of 108 patients undergoing WBRT by Caissie et al., where improvements in sleep disturbance (insomnia), visual disorders, communication deficits, and future uncertainty were noted to improve (57).

Beyond the aforementioned observational studies, the 2016 Quality of Life after Treatment for Brain Metastases (QUARTZ) trial provided further evidence by assigning 538 patients with NSCLC to WBRT and supportive care or supportive care alone (58). This trial failed to show a difference in survival and QoL between the two treatment groups. Patients receiving WBRT and supportive care had 46.4 days quality-adjusted life-years (QALYs) vs. 41.7 days in the supportive care group alone. While these QALYs suggest a 4.7-day advantage for the WBRT group, the 90% confidence interval of −12.7 to 3.3 does not allow for a definitive conclusion proving survival and QoL advantage in the WBRT group. Moreover, the prevalence of severe or moderate QoL impairments, as measured by EQ-5D, was similar in patients with WBRT vs. those with supportive care, and deterioration, as measured by the KPS, was similar in both groups (58). Literature assessing QoL in patients receiving WBRT alone since the QUARTZ trial has been limited, largely due to pivot toward radiosurgery in select patients (66, 67).

Moreover, the utility of WBRT as adjuvant therapy in SRS or surgical resection was studied through in the European Organization for Research and Treatment of Cancer phase III trial, a study with 359 patients (68). While adjuvant WBRT was found to reduce intracranial relapses and neurologic deaths, the time period with functional independence was not increased, suggesting decreased QoL despite better tumor control (68). These findings are consistent with a later study by Brown et al. demonstrating that in patients with one to three metastases, SRS alone—without adjuvant WBRT—leads to less cognitive deterioration at 3 months, which may contribute to better QoL (67).

Stereotactic Radiosurgery

Quality of life in stereotactic radiosurgery (SRS) treatment of BM has been studied as early as 2002, when DiBiase et al. reported QoL outcomes in 20 patients undergoing Gamma Knife Radiosurgery (GKRS) (59). Using the Spitzer QoL survey and KPS, the authors demonstrated that among the 40% of patients whose tumor progressed after GKRS, QoL decreased. On the other hand, patients whose tumor did not progress, QoL remained stable or improved at one, three, and 6 months after treatment (59). As one of the earliest studies examining QoL in SRS, this study demonstrated a relationship between tumor burden in QoL, and showed that GKRS treatment can contribute toward stable or improved QoL. These findings have been replicated with larger samples, including a 97-patient study by Skeie et al. (60) which utilized the KPS and FACT-Br scales. Patients who had improved symptoms after GKRS had FACT-Br scores that were 4.6 points higher than those who experienced clinical deterioration. Importantly, a decline in QoL was noted among patients who required dexamethasone at the time of GKRS, and separately, this study found no association between prior WBRT status and post-GKRS QoL. Two important conclusions can be drawn from this study: first, reducing steroid use in the setting of peritumoral edema may confer a QoL benefit, and second, while WBRT alone may lead to cognitive decline and negatively impact QoL, WBRT does not seem to be a risk factor for better or worse QoL after GKRS (60). Skeie's findings regarding corticosteroids were also corroborated in a study by Habets et al. (61) assessing

TABLE 2 | Summary of literature describing quality of life in patients with BM receiving treatment and their caregivers.

References	Design and sample size	QoL scale(s)	Findings
Wong et al. (53)	Prospective, $n = 217$	KPS, QLQ-C30, QLQ-C15-PAL, QLQ-BN20, FACT-BR, Edmonton Symptom Assessment Scale, Spitzer Quality of Life	• In a 12-week study period, fatigue, drowsiness, and appetite deteriorated from baseline at statistically significant level • Appetite loss, weakness, and nausea significantly increased from baseline, while balance, headache, and anxiety decreased from baseline • At baseline all symptoms assessed (e.g., nausea, pain, insomnia, concentration) except for appetite loss were significantly correlated with overall QoL
Steinmann et al. (54)	Prospective, $n = 46$	QLQ-C30, QLQ-BN20, DEGRO-LQ	• Global QoL remained stable in 3-month study period • Overall physical functioning deteriorated in the 3-month study period at a statistically significant level. • There was a statistically significant deterioration in drowsiness, hair loss, and weakness but headaches and seizures improved
Doyle et al. (55)	Prospective, $n = 60$ patient/ caregiver pairs	FACT-BR	• In 2-month study period after WBRT, the physical wellbeing domain had the greatest absolute deterioration (statistically significant level)
Wong et al. (56)	Prospective, $n = 129$	Spitzer Quality of Life	• After WBRT, daily living, health, and headache improved in 12.2, 21.1, and 18.9% of patients, respectively • After WBRT, 56.7% of patients had worsened fatigue and 53.3% had poor neurofunctioning status
Caissie et al. (57)	Prospective, $n = 108$	QLQ-C15-PAL, QLQ-BN20	• Following WBRT, insomnia, future uncertainty, visual disorder, and concentration significantly improved • There was a decrease in physical function and increase in emotional functioning
Mulvenna et al. (58)	RCT, $n = 538$ (269 WBRT + OSC, 269 OSC alone)	EQ-5D	• There was no evidence of a difference in QoL between patients receiving WBRT + OSC and OSC alone • There is an increase in symptoms in patients after receiving WBRT (increased drowsiness, hair loss, nausea, and dry or itchy scalp)
DiBiase et al. (59)	Prospective, $n = 20$	Spitzer Quality of Life	• Extracranial tumor progression after GKRS is associated with worsened Spitzer QoL score, whereas in patients with stable or improved tumor control, Spitzer scores increased
Skeie et al. (60)	Prospective, $n = 97$	FACT-BR	• For 66% of patients, mean QoL score improved at 9 months after SRS, and remained unchanged for 6% of patients • Local control, improved symptoms, and reduced need for steroids after GKRS is associated with higher QoL • Low QoL is associated with local failure, increased need for steroids, and progression of the peripheral disease
Habets et al. (61)	Prospective, $n = 97$	QLQ-C30, QLQ-BN20	• Physical functioning and fatigue worsened at 6 months after SRT • KPS < 90 and tumor volume > 12.6 cm^3 were associated with lower QoL scores at 6 months after SRT
Verhaak et al. (62)	Cross-sectional, $n = 92$	FACT-BR, Hospital Anxiety and Depression Scale, Multidisciplinary Fatigue Inventory	• Compared to the general population and adult cancer patients, BM patients had lower QoL scores for emotional wellbeing and most (57.6%) of patients reported problems with emotional wellbeing • Compared to the general population, patients with BM had poorer functional wellbeing, and general QoL before treatment • Compared to the general population, BM patients had higher social wellbeing scores
Bragstad et al. (63)	Prospective, $n = 44$	FACT-BR	• 12 months after GKS, physical, social, emotional, and functional wellbeing average remained unchanged from baseline

(Continued)

TABLE 2 | Continued

References	Design and sample size	QoL scale(s)	Findings
			• Asymptomatic BMs at baseline, higher KPS score, and lower RPA classes were associated with higher QoL after GKS, whereas age, sex, number of BMs, prior treatment, SRS dose, extent of peritumoral edema, mutation status, and baseline metastases to other sites did not predict QoL
Salvati et al. (64)	Retrospective, $n = 62$ (32 multiple metastases, 30 with a single metastases)	KPS	• Preoperative KPS in patients with multiple metastases was 83.1 vs. 82.3 in patients with single metastases
Saria et al. (39)	Descriptive cross-sectional, $n = 56$ caregivers of patients with BM	NA	• Caregivers most commonly deployed the following coping strategies against cognitive dysfunction in their relatives: acceptance, planning, positive reinterpretation and growth
Papadakos et al. (65)	Cross-sectional, $n = 109$ patients with BM and 77 caregivers	NA	• The most important information patients and caregivers want belongs to the medical and physical health domains (e.g., symptoms, side effects, cognitive impairment) • Caregivers prefer one-on-one counseling for all informational domains, including medical, physical, emotional, social, and spiritual informational needs

97 patients with BM, though findings were not found to be statistically significant. Interestingly, though, corticosteroids were not found to negatively influence results of neurocognitive functioning over time, a measure that is often tested alongside QoL (61). Moreover, Habets et al. were among the first to establish a baseline difference in QoL among patients in BM vs. healthy controls, showing meaningful differences in global health status, physical functioning, emotional functioning, role functioning, and cognitive functioning as assessed by the QLQ-C30 scale (61). Similar to other studies, Habets et al. found that patients with progressive disease after SRS had poorer QoL scores over time, whereas those without disease progression had stable or even improved QoL scores. Declines in QoL were driven by poorer physical functioning, fatigue, and motor dysfunction, as assessed by the BN20 scale (61). Lastly, neurocognitive functioning was stable up to 6 months after SRS in patients with up to three BMs. In another prospective study by Veerhak et al., QoL was assessed in 92 patients set to undergo SRS. QoL prior to SRS were evaluated using the KPS, FACT-Br, Multidimensional Fatigue Inventory, and Hospital Anxiety and Depression scales to identify baseline deficits. Overall, 64.1% of patients had a clinically meaningful low QoL score in at least one of the subscales prior to SRS (62). Specifically, patients with BM were found to have significantly lower emotional wellbeing when compared to both general adult population and adult cancer patients. Patients with BM, though, were also found to have higher levels of social wellbeing, which the authors posit may be due to increased support patients experience just before undergoing treatments, such as SRS (62). When considering psychiatric wellbeing, 42.4 and 32.6% of patients met criteria for at least mild symptoms of anxiety and depression, respectively (62).

While many of the studies on SRS are from heterogenous patient samples with multiple primary tumor types, one study by Bragstad et al. focused on lung cancer, only, the most common origin of BMs. In their work, the authors identified baseline predictors for improved or stable QoL after GKRS. Total BM volume (≤ 5 cm^3 vs. >5 cm^3) at baseline was the only predictor associated with improved QoL after GKRS, as measured by the FACT-Br scale (63). On the other hand, asymptomatic BMs at baseline, higher KPS at baseline, lower recursive partitioning analysis (RPA) class at baseline were all predictors of high and stable QoL after GKRS. Importantly, in this subset of patients, baseline number of BMs, prior treatment, GKRS dose to the cranium, peritumoral edema, and baseline metastases to bone, liver, adrenals, or lymph nodes did not affect QoL scores (63). Overall, the authors found that 77% of patients improved and 82% had stable or improved cerebral symptoms at their last follow-up, supporting the use of GKRS as the preferred treatment modality in lung cancer patients with brain metastases (63).

Surgical Resection

While surgical resection is commonly used in the treatment of BMs, non-review research exploring QoL after surgical resection is limited. A 32-patient series of patients with one to three metastases reported patients' KPS preoperatively and used it as a surgical prognostic factor. Among the patients in this study, those with either single or multiple BM had similar proportions of metastatic tumor type, with lung metastases being most common. Notably, this sample's average KPS of 83.1 would place the average patient in Category A, meaning they can carry on normal activity and work with no special care necessary (64). Such a high preoperative KPS appears to reflect

TABLE 3 | Thematic analysis of the needs of patients with BM and their caregivers, as determined by drivers of poor QoL.

Needs of patients with BM and their caregivers	References
Support for declining physical and motor functioning, including services from physical therapists or physical medicine and rehabilitation physicians	(54–57, 61)
Early consultation of psychiatric and psychological support services for both the patient and caregivers	(39, 53, 61, 62, 71)
Ability to stay connected to social networks to preserve emotional wellbeing	(61, 69)
Frequent information sharing with caregivers, especially around expectations on physical and medical matters	(65, 69, 70)
Careful and frequent medication review to limit side effects, with special attention to dexamethasone	(53, 60, 61)

surgical candidacy and patient selection on the neurosurgeons' part, as the inclusion criteria for this study involved KPS > 60, isolated or up to three metastatic intracranial lesions, and, notably, controlled primary disease. There was no postoperative KPS reported.

Caregivers

Beyond patients' experiences, caregivers' QoL ought to be understood. As integral members of patients' care teams, caregivers take on significant emotional, physical, and load throughout their relatives' course of care (69). Patients with BM, specifically, represent a patient cohort that has advanced disease which may portend a greater load than a non-BM cancer patient, as advanced disease may indicate longer length of disease, rapid deterioration, or a terminal status. A study by Garzo Saria explored BM caregivers' experience specifically by analyzing patient's cognitive impairment against their caregiver's resiliency and coping strategies (70). The authors found that increased memory problems had a significant negative correlation with caregiver resilience, and acceptance, planning, positive reinterpretation, active coping, and suppression of competing activities serving as the most common coping mechanisms (70). Thus, it is important to preserve resilience and support caregivers in developing their coping strategies. Another need of caregivers is the ability to remain well informed in the caring of their relative. In one study by Papadakos et al., caregivers and patients were surveyed to understand their needs. Caregivers and patients prioritized information related to physical and medical matters (e.g., side effects, symptoms, headache management, seizure management). They preferred to receive this information *via* one-on-one counseling and pamphlets (65).

Drivers of Declining Quality of Life

Collectively, the studies described above demonstrate five key drivers for declining quality of life in patients with BM and their caregivers. These drivers can be reframed as opportunities to enhance end of life care (**Table 3**):

- Support declining physical and motor functioning
- Promptly consult psychiatric and psychological support services for both patients and caregivers
- Encourage and foster social connection to preserve emotional wellbeing
- Frequently share information with caregivers, especially around physical (e.g., symptoms) and medical (e.g., prognosis) matters
- Carefully review medications to limit side effects
- A multidisciplinary team is required to meet these diverse needs for both patients and caregivers as patients elect to receive treatment or opt for comfort measures.

ROLE OF MULTIDISCIPLINARY CARE AND PALLIATIVE/SUPPORTIVE CARE SERVICES

Metastatic brain tumor patients and their families benefit from effective patient-provider communication as well as comprehensive multidisciplinary care for surveillance, treatment, and preservation of high quality of life. Several randomized control trials have shown the benefits of early palliative and supportive care involvement for patients with advanced cancers (4, 72). However, implementing palliative care and other supportive care services requires organization at the provider and clinic levels. Danielson and Fairchild (73) describe the Rapid Access Palliative Radiotherapy Program (RAPRP) for the metastatic brain tumor clinic, with overarching goals of coordinating timely consultations and treatment and multidisciplinary care. The interdisciplinary team consisted of members from radiation oncology, nursing, social work, occupational therapy, and dietary services. Eighty six percentage of patients involved in the 6-month pilot study reported high satisfaction, with 97% of patients willing to recommend the program to other patients. In preparing a high-quality multidisciplinary care center for patients with BM, additional integral aspects also involve palliative care specialists interfacing with the treatment team consisting of oncologists, radiation oncologists and surgeons as well as involving various key stakeholders from social work, rehabilitation and nutrition services, nursing, psychological services, and more given the unique profile of challenges patients with BM face.

Specialty Palliative Care

The American Society of Clinical Oncology (ASCO) issued a provisional clinical opinion and recommendation for the timely introduction and integration of palliative care (PC), broadly defined as specialized care for patients with serious illnesses, into standard cancer care when the patient is diagnosed with metastatic cancer or high symptom burden (74). Temel et al.

randomized and fully evaluated 107 non-small cell lung cancer patients with metastatic burden to either standard oncologic care or early PC integrated into standard care (4). The early PC patient group demonstrated improvements across quality of life measures (mean FACT-L score 98.0 for early PC vs. 91.5, $p = 0.03$), proportions of patients suffering from depressive symptoms (16% for early PC vs. 38%, $p = 0.01$), and median survival (11.6 months for early PC vs. 8.9, $p = 0.02$) (4). More recently, Temel et al. also ran a multi-institutional randomized trial focused on early PC for patients with advanced, incurable cancer. Due to missing data and significant morbidity among the enrolled patient population, no measures were ultimately found to be statistically significant (75).

Other studies have found overall poor adherence to the ASCO recommendation (5, 76). For example, McDermott et al. investigated that only 48% of non-small cell lung cancer patients with BM received PC consultation during their disease course, although timing of PC consultation and rate of PC consultation have increased in 2016–2018 compared to trends in 2012–2015 (5). Only 19% thoracic oncologists from a single-institution study referred their patients with advanced lung cancer to PC specialty care. A separate nationwide database study found that metastatic non-small cell lung cancer patients did benefit from lower healthcare costs following specialty palliative care usage (77). Furthermore, oncology providers may have differing opinions about the breadth, meaning, and usage of PC, as evident from responses gleaned from semi-structured interviews conducted with oncology clinical trial investigators, researchers, nurses, and physicians (78).

ASCO recommends referral to interdisciplinary specialty PC for patients with advanced cancer (79). The ENABLE II study divided patients between advanced practice nursing PC and usual care, finding improved depression symptoms and QoL measures for the intervention group (80). Similarly, another cluster randomized trial demonstrated increased QoL at 4-month intervention follow-up for Stage III and IV patients enrolled in early PC at an independent PC clinic compared to standard care (81). However, additional studies, including randomized controlled trials, for specifically patients with BM are needed for insight on the role of specialty PC in comprehensive cancer care.

Social Work

Social workers may perform a variety of roles when caring for patients with intracranial metastases. With broad training in counseling, care coordination, community resource management, and other patient-centered skills, social workers are uniquely positioned to provide a number of critical services for cancer patients and families. Meier and Beresford argue that social workers in palliative care, for instance, have the specific knowledge and skillset to advocate and give psychosocial support for patients as well as facilitate care (82). In planning for end of life, social workers provide key communication skills while conducting advance care planning for patients, as they have more experience and expertise discussing advance directives than nurses or physicians (83). However, social workers, even those with additional training devoted to palliative care, face challenges in defining their roles within the multidisciplinary team: "social workers in palliative care need to make themselves heard [and] visible and conduct joint visits. . . . I get more buy-in after other team members watch me work," states Higgins who is a social work on a palliative care team at Brigham and Women's Hospital/Dana-Farber Cancer Center (82). Currently, relatively little is known about the role of social work in care for patients with BM, and the field would benefit from future research and attention to the important role social workers play.

Physical Therapy and Other Rehabilitation Services

A critical aspect of post-surgical and therapeutic recovery and maintenance of functional fortitude involves utilization of rehabilitation services for patients with BM. Not only do brain tumors themselves provoke various neurologic and systemic symptoms but the treatment courses patients undergo once BM develop is physically taxing and fraught with adapting to different potential deficits. For example, steroids are commonly prescribed for brain tumor patients to control manifestations of vasogenic swelling, but side effects include fatigue, muscle wasting, and weight changes (84). Rehabilitation services broadly involve the expertise of physical medicine and rehabilitation providers, physical and occupational therapists as well as speech and language therapists. Occupational therapists engage patients in exercises to overcome barriers that negatively impact an individual's physical, social, and emotional needs. Physical therapists work with patients to improve their strength, flexibility, balance and fine motor movements. Speech and language therapists perform various evaluations for speech, cognitive, language and swallowing abilities in addition to teaching patients exercises to improve their language and cognitive function.

Over 80% of patients with central nervous system tumors require rehabilitation services (85). In a separate study, Mukand et al. found that most brain tumor patients suffered from cognitive deficits (80%) and motor deficits (78%), with 39% of the cohort describing five or more separate neurologic deficits (86). A separate survey of 25 brain tumor patients revealed that 84% of the group reported recent fatigue, with worse symptoms experienced by those with recurrent lesions (87). However, following rehabilitation, the patients reported improvements across several functional scales, including KPS, Modified Barthel Index, and Motricity (motor function) Index. Similarly, another study of ten primary brain tumor patients indicated that total functional outcome significantly improved across three functional measures post-rehabilitation with a delayed enhancement in quality of life 1 month following discharge (88). Outcomes from inpatient rehabilitation are not significantly disparate between benign and malignant brain tumors or primary and secondary intracranial lesions, although more research is required for specifically metastatic brain tumor patients (89). Tang et al. included patients with BM as well as glioblastoma and other brain tumors who underwent inpatient rehabilitation, and patients demonstrated improved functional scores compared to measures on admission with a significant correlation

between high functional improvement and longer survival (90). As for evaluating outpatient rehabilitation, there are several potential indices, such as the Functional Assessment of Cancer Therapy, to identify brain tumor patients who could potentially benefit from rehabilitation services (48). A group based in Canada sought to understand the population of brain tumor patients who received occupational therapy by examining demographics of 3,199 patients, of which 78.2% had malignant lesions (91). A recent randomized controlled trial enrolled functionally independent glioma patients currently on treatment who either underwent standard rehabilitation care or a supervised rehabilitation course (92). The specialized rehabilitation course involved 6 weeks of physical therapy with a focus on cardiovascular and resistance training, evaluations of patients' progress and performance with activities of daily living, and individually tailored exercises when appropriate. The intervention group exhibited superior aerobic strength ($\beta = 2.6$), cognitive functioning ($\beta = 16.2$), and decreased fatigue ($\beta = -13.4$).

Cognitive support and rehabilitation services are an especially important aspect of holistic care for patients with BM as well. Cognitive dysfunction may manifest as impairments in memory, language, and executive function, which can impact decision-making capacity for treatment and personal decisions (93). The vast majority of brain tumor patients (80%) experience cognitive deficits depending on tumor location, size, and grade (86). There are some preventative methods to protect cognitive ability when patients are faced with treatment choices. Hippocampus-avoiding WBRT significantly curtails the risk of developing memory loss, and proton radiation therapy involves lower entrance and exit doses that can spare brain tissue and preserve cognition (94). Some providers may also consider prescribing neuroprotective agents, such as memantine and renin-angiotensin-aldosterone system blockers (95). Cognitive rehabilitation harnesses principles of neuroplasticity in retraining or promoting compensation training for brain tumor patients. Such rehabilitation exercises benefit patients most when implemented early, such as a study demonstrating that postoperative brain tumor patients regained some cognitive function just after a few weeks (96, 97).

Overall, research on multidisciplinary efforts to promote quality rehabilitation programs for brain tumor patients is still lacking. A particular challenge for rehabilitation specialists lies in the heterogeneity of needs within this patient population, since therapy programs are ideally personalized based on clinical status and needs (98). Such a premise necessitates open and timely communication among various members of the oncologic multidisciplinary team. A review of randomized and non-randomized clinical trials found one low-quality controlled clinical trial encompassing 106 glioma patients, some of whom were enrolled in an individualized, outpatient multidisciplinary rehabilitation program, involving occupational, social, psychological, and physical therapies (99). Despite high overall drop-out rate (20% at 6 month follow-up), patients in the specialized therapy group had improvements in self-care, mobility, locomotion, communication as well as cognition ($p < 0.05$ for all) at 3-month follow-up (99). As with social work's role in the interdisciplinary care team for BM patients, rehabilitation services also lack a firm place in most oncology practices, even though they can provide essential aid for patients at any stage in their cancer course (98).

Additional Gaps in Multidisciplinary Care

Patients with BM have unique perspectives on their prognoses and describe various needs. A qualitative study by Dorman and Pease involved collecting semi-structured interviews of nine patients with intracranial metastases from non-small cell lung cancer (100). Several patients expressed the importance of prioritizing quality of life along with preserving mobility and cognitive function. In particular, numerous studies have recapitulated the particular emotional and psychological distress cancer patients and patients with brain tumors experience. Personalized psychosocial support for patients with BM can lead to significant improvements in measures of distress, anxiety, and depression, as evident from a pilot study of 59 primary malignant brain tumor patients who worked with a certified psycho-oncologist. However, other patients with BM may indicate that they do not require additional help and, thus, reject services (101). Barriers to appropriate supportive care continue to exist and prevent some patients from accessing and utilizing services, pointing to a need for addressing patient knowledge and awareness.

Supporting Caregivers

Over time, awareness of the caregiver experience for patients with advanced cancers has increased. At present, caregiver burden—the multifaceted experience and reaction to patient needs and demands—is relatively well-studied in cancer research (102, 103). Among the unique burdens faced by caregivers of patients with BM is the extensive longitudinal cancer caregiving experience as patients with intracranial metastases are living longer with improved treatments. Furthermore, as the number of patients with metastatic brain tumors rises due to longer survival, the amount of caregivers will similarly increase, pointing to the importance of more research in this area of caregiving.

Caregiver wellbeing is a potentially fruitful aspect for investigation as well as for implementation of support services. Ketcher et al. collected self-reported information about caregiving responsibilities and wellbeing from 21 family caregivers of patients with BM. Overall, the study participants reported moderate levels of caregiver burden, which was itself significantly associated with time spent on caregiving ($R = 0.59$, $p < 0.01$), anxiety levels ($R = 0.54$, $p < 0.05$), depression levels ($R = 0.59$, $p < 0.01$), and efficacy of coping ($R = -0.54$, $p < 0.05$) (38). One small pilot study investigated outcomes after implementing a program that involved two 90-min in-person sessions at the patients and caregivers' homes and one 30-min telephone appointment (104). Trained oncology nurses facilitated the sessions with patient-caregiver dyads. Results demonstrated significantly improved measures of quality of life for caregivers ($t = 2.992$, $p < 0.006$), while the patients' emotional wellbeing trended toward a statistically significant improvement.

Overall, patient and caregiver communication and coordination with their healthcare providers remain critical throughout the cancer course. While one study of 600 stage IV cancer patients and 346 caregivers demonstrated that patients found communication with physicians to be well-executed compared to caregivers' opinions, both groups reported worse perceptions of physician communication and care coordination when anxious (105). Dionne-Odom et al. examined outcomes following implementation of a clinic-based telemedicine support system (FamilyStrong) for caregivers of patients with grade IV brain tumors (106). A palliative care nurse interfaced with caregivers on a weekly basis, evaluating for distress and advocating for various support services, including local counseling services and coordinating with the primary neuro-oncology team for patient care needs. However, overall there are few published studies specifically including and targeting caregivers of patients with BM.

CONCLUSION

As brain tumor and cancer treatments improve, patients with brain metastases (BM) have longer survival, though they still face numerous physical and psychosocial challenges from their disease and therapies. Patients with BM would benefit from coordinated multidisciplinary care consisting not only of their oncologists and surgeons but also among palliative care specialists, rehabilitation therapists, nursing, and other key healthcare providers. There is a dearth of published literature focused on quality of life studies, illness experiences, and the role of palliative and supportive care for this particular patient and caregiver population. This review highlights the important and gaps in understanding aspects of high-quality multidisciplinary care for patients with BM.

AUTHOR CONTRIBUTIONS

AW and GC contributed to draft manuscript preparation. AW, GC, and ML reviewed the manuscript. All authors designed the general outline for the manuscript. All authors contributed to the article and approved the submitted version.

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Management Strategies for Large Brain Metastases

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Brain metastases represent the most common intracranial neoplasm and pose a significant disease burden on the individual and the healthcare system. Although whole brain radiation therapy was historically a first line approach, subsequent research and technological advancements have resulted in a larger armamentarium of strategies for treatment of these patients. While chemotherapeutic options remain limited, surgical resection and stereotactic radiosurgery, as well as their combination therapies, have shifted the paradigms for managing intracranial metastatic disease. Ultimately, no single treatment is shown to be consistently effective across patient groups in terms of overall survival, local and distant control, neurocognitive function, and performance status. However, close consideration of patient and tumor characteristics may help delineate more favorable treatment strategies for individual patients. Here the authors present a review of the recent literature surrounding surgery, whole brain radiation therapy, stereotactic radiosurgery, and combination approaches.

Keywords: stereotactic radiosurgery (SRS) treatment, brain metastases (BM), whole brain radiotherapy (WBRT), large brain metastases, surgery for brain metastases

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INTRODUCTION

Brain metastases occur in up to 30% of systemic cancers and represent the most common type of intracranial tumor, with significant burden on patient survival and quality of life (1–3). Their management, however, remains complex and controversial. Multiple treatment modalities have been investigated, including surgical resection, radiotherapy (RT), stereotactic radiosurgery (SRS), and chemotherapy (3). Furthermore, large brain metastases, typically defined as ≥ 2 cm in maximum diameter or ≥ 4 cm3 in volume, present additional challenges in management due to their morphology, dosimetry, and anatomy that may be involved. While various chemotherapeutic mechanisms have yielded limited efficacy in the intracranial environment, both surgery and radiation are demonstrated to be promising approaches in this patient population.

The randomized, prospective trial described by Patchell et al. in 1990 remains pivotal in our understanding of the role of the neurosurgeon in the context of whole brain radiation therapy (WBRT) (4). In this study 48 patients were randomized to either surgical resection of their brain tumor followed by WBRT (surgical arm) or needle biopsy followed by WBRT (radiation arm). In the surgical arm, local recurrence was found to be reduced (20% vs. 52%), and overall survival was significantly improved (median 40 weeks vs. 15 weeks in the radiation arm). Additionally, surgically-treated patients retained functional independence over a longer period (38 weeks vs. 8 weeks in the radiation arm). The benefits of surgery were similarly shown by Vecht et al. in 1993 (5).

A prospectively randomized trial was conducted in 63 patients with solitary brain metastases, and the addition of surgery to WBRT resulted in significant longer survival and functional independence. These differences were especially notable when stratified according to stable extracranial disease (versus progressive). The utility of surgical resection, therefore, makes it an attractive modality for these patients.

Nonetheless, surgery remains one cornerstone in our paradigm for brain metastases management. The advancement of radiotherapies, including SRS, and our deepening understanding of patient and disease factors have revealed a multi-modal nature of brain metastases management. Here we provide an overview of the role of both surgical and radiation strategies for treatment of large brain metastases, as well as the implication for management of different patients.

OVERVIEW OF RADIATION AND SRS

Radiation therapy has been a key player in the treatment and palliation for brain metastases, and the technologies and techniques utilized have evolved over many decades (3). Chao et al. first described WBRT in brain metastases patients in 1954, and others have since reported on various outcomes following WBRT (6). As a non-invasive strategy, WBRT is shown to produce a median survival of 4 to 6 months and excellent improvement in ≥70% patients in terms of overall symptoms (7–9). WBRT regimens may also be tailored to the patient (e.g. 20-40 Gy over 1-4 weeks). Various fractionation schedules are utilized, and studies through the Radiation Therapy Oncology Group (RTOG) have revealed the importance of individual patient characteristics in guiding these treatment parameters (10, 11). Both short-term adverse effects, such as fatigue and reversible hair loss, as well as long-term effects, such as cognitive decline and urinary incontinence may influence the decision-making process between patient and physician (12). Some prior studies haves shown significant concern for neuro-cognitive decline within 5 to 36 months, including evidence of white matter changes and cortical atrophy; however, others have suggested that these long-term consequences may be irrelevant when looking at modern-day lower fractionation schemes (<3Gy per fraction) and that the risks of recurrent disease may in fact outweigh these side effects (12–14). In the setting of large brain metastases, WBRT appears to have limited efficacy as shown by Nieder et al. (15) Among 108 patients with 336 brain metastases, local failure was 48% in tumors <0.5cc while all lesions >10cc recurred. Complete response was only seen in tumors <6.4cc (16).

The advent of SRS systems has provided new options for patients in the context of radiation therapy, and its efficacy is supported across high-quality studies (2, 17–19). SRS utilizes multiple non-coplanar beams to deliver single or multi-fraction, highly concentrated radiation doses to a small, precise target volume. This results in a peak dose applied to the central portion of the tumor region of interest, with a steep fall-off gradient out to the periphery. SRS is an interdisciplinary treatment process involving typically a neurosurgeon, radiation oncologist, and radiation physicist to determine the optimal delivery plan.

SRS can be further divided into separate modalities based on the technological systems used, including multiple cobalt-60 sources (Gamma Knife or GK) or single-source linear accelerator (LINAC) (20). GK was initially developed and described by Lars Leksell in 1951, and this utilizes a stereotactic head frame. This tends to offer high conformality to irregularly shaped tumors and the ability to target multiple tumors in the same session. LINAC was developed later in the 1980s and utilizes a collimated, high-energy x-ray beam. Here the LINAC gantry is rotated around the region of interest to produce "multiple noncoplanar intersecting arcs of radiation" (21). Park et al. recently reviewed trends in SRS based on adult patients with non-small cell lung cancer using the National Cancer Database (21). Out of 1780 patients, 77% received GK and 23% underwent LINAC across the study time frame. The usage of LINAC increased steadily from 3.2% in 2003 to 30.8% in 2011 and appeared to be used more widely in community settings, possibly due to lower costs, easier use, less stringent federal regulations, and applicability of some LINAC systems to extra-cranial pathologies. Furthermore, volumetric modulated arc therapy (VMAT) is a more recent modification in LINAC systems, allowing for treatment of multiple targets *via* a single isocenter (single-isocenter multi-target,or SIMT) and reducing overall treatment time.

In a multi-institution series reported by Wen et al. the application of SRS for treatment of brain metastases showed excellent local control (65-90%) and survival (6-12 months). Doses of 15-30Gy were utilized in these patients with acceptable side effect profiles (17). In fact, the maximum tolerated radiation dose for single-fraction radiosurgery has been described as a function of tumor size in order to optimize treatment strength with toxicity profile. Shaw et al. reviewed 156 patients with recurrent primary brain tumors or brain metastases which were previously irradiated (22). They identified maximum tolerated doses (measure at the tumor margin) of 24 Gy, 18 Gy, and 15 Gy for tumors <2 cm, 2.1-3cm, and 3.1-4 cm in maximum diameter, respectively. Thus, larger tumors are typically subjected to lower radiation doses to mitigate toxicities.

Given such dose protocols established through RTOG 90-05, several studies have evaluated local recurrence rates. Vogelbaum et al. assessed 202 patients with 375 brain metastases in a single-center retrospective study after treatment with SRS (23). A dose of 24 Gy to the tumor margin had a significantly lower risk of local failure than 15 or 18 Gy ($p = 0.0005$), while the 15- and 18-Gy groups were not significantly different from each other ($p = 0.82$). At 1 year post-SRS, the local control rate was 85% (95% CI 78-92%) in the 24 Gy group, compared with 49% (CI 30-68%) in the 18 Gy group and 45% (CI 23-67%) in the 15 Gy group. Interestingly, overall survival was shown to be unrelated to tumor margin dose. Similarly Petrovich et al. showed that 1-year local control of lesions <3cc was improved compared to lesions >3cc (90% vs 78%), and Ebner et al. showed that large brain metastases with diameter at least 3cm had poorer 1-year

local control (68%) compared to smaller lesions (86%, p<0.001) (16, 24, 25).

Tumor size and consequently, radiation dose, also carries an impact on adverse effects. Specifically, radiation necrosis limits the deliverable dose and can have severe neurological impact requiring additional treatments such as steroids and anti-angiogenic drugs. Miller et al. evaluated 5747 brain metastatic lesions in 1939 patients to identify rates of radiation necrosis (26). After SRS treatment at a single tertiary-care center, it was shown that 427 lesions (7%) in 285 patients (15%) developed radiation necrosis at a median of 7.6 months. In multivariate analysis, the lesion diameter (HR 1.29; CI 1.20-1.39) as well as other biological characteristics were independent predictors of radiation necrosis in this population. This included graded prognostic assessment, renal pathology, and heterogeneity index. Certain subsets of pathologies such as HER2-amplified status, BRAF V600+ mutational status, lung adenocarcinoma histology, and ALK rearrangement were also associated with RN.

With respect to RN seen in specific SRS systems, Sebastian et al. recently described a multi-institutional experience including 391 patients treated for 2699 lesions (1014 LINAC-SIMT and 1685 GK) (27). GK was associated with similar overall survival compared to LINAC (9.5 vs 13.2 months), and after propensity score matching using a subset of 113 matched pairs, there remained no significant difference in survival (HR=0.86, p = 0.41). GK meanwhile was associated with higher rate of RN (HR=3.83, p = 0.002) compared to LINAC. Navarria et al. in 2018 presented a randomized clinical trial comparing GK (80 patients) with a LINAC-based Edge SRS system (88 patients) (28). For GK, a single dose of 20-24 Gy at the 50% isodose line was prescribed, whereas for LINAC a single dose of 24 Gy was prescribed; up to four brain metastases with maximum tumor diameter of 3cm were treated per patient. There was no significant difference in overall survival and local control rates between treatment arms. RN was similar except for grade III RN events, which were increased in the GK arm (3 cases at a median time of 3 months; 0 cases in LINAC arms). Thus, while GK remains more commonly used across treatment centers and provides higher dose conformality, data suggests that LINAC systems may have a favorable toxicity profile without negatively impacting survival outcomes.

Whereas SRS may be limited with certain tumor features and carries a risk for radiation necrosis, it offers key advantages compared to surgical resection (29–31). SRS is a less invasive intervention, has shorter procedural times and hospital length of stay, and has less risk of tumor seeding. Surgery alone allows for more immediate improvement of mass effect, formal tissue diagnosis, and no risk of radiation necrosis. The literature has shown the merits of both surgery and SRS in brain metastases patients. Bindal et al. in 1996 compared 13 patients who underwent SRS with 62 patients who underwent surgery, whom they retrospectively matched (32). SRS-treated tumors had a median size of 1.96 cm3 (range 0.41-8.25 cm3) and the median dose was 20 Gy (range 12-22 Gy). Median survival was 7.5 months for patients treated by SRS and 16.4 months for those treated by conventional surgery. Thus, the authors concluded that surgery was a superior option in these patients. Another study by Cho et al. assessed 225 single brain metastases in patients treated with WBRT alone, surgery plus WBRT, or SRS plus WBRT (33). Here the actuarial survival times were similar in the surgery and SRS groups, both of whom responded better than the WBRT alone group. The authors described that SRS may be a more desirable option compared to surgery when lesions are in surgical inaccessible locations and that it is potentially more cost-effective and less invasive to the patient.

Another consideration with SRS is the timing of recurrence compared with modalities such as surgery. Churrilla et al. reported a secondary analysis to compare patients treated with SRS or surgical resection from a phase 3 trial (34). 268 patients with one to three brain metastases were included, of whom 154 underwent SRS and 114 underwent surgery. The surgical arm tended to have larger metastases (median 2.8 cm vs. 2 cm, p<0.001) and more often 1 single brain metastasis (98.2% vs. 74%, p<0.001). Overall local recurrence was found to be similar between treatments (HR 1.15; 95% CI 0.72-1.83). Interestingly, when stratified by time intervals, surgery resulted in a higher risk of early (0-3 months) local recurrence compared with SRS (HR 5.94; 95% CI 1.72-20.45). By 9 months or longer, surgical patients showed a lower risk of local recurrence compared with SRS (HR 0.36; 95% CI 0.14-0.93). Thus, SRS-treated patients showed an advantage in reducing early local recurrences compared to surgery.

COMBINATION OF SRS AND WBRT

With adoption of SRS techniques, clinicians subsequently investigated the role of combination therapy with WBRT. Multiple studies have shown improved local control with this combination approach. Andrews et al. conducted a multi-institutional trial as part of RTOG to compare WBRT against WBRT followed by SRS boost (35). 333 patients with one to three brain metastases were randomly assigned to either treatment arm (167 received WBRT plus SRS; 164 received WBRT alone). Median survival time was significantly higher with combination therapy (6.5 vs 4.9 months, p=0.039). Also, functional status measured by Karnofsky Performance Status (KPS) was more likely to be stable or improved after combination therapy at 6 months (43% vs 27%, p=0.03).

Aoyama and associates described their phase 3 randomized, controlled trial comparing SRS alone with WBRT plus SRS boost in 132 patients (36). Each patient had one to four brain metastases, each less than 3 cm. 65 patients underwent WBRT plus SRS and 67 patients underwent SRS alone. At 1 year, the recurrence rate was significantly lower for combination therapy at 46.8%, compared to 76.4% after SRS alone (p<0.001). More patients required salvage therapy in the SRS group (29 patients versus 10 patients, p<0.001). Median survival was 7.5 months after combination therapy, which was similar to the 8 months survival after SRS alone (p=0.42). Toxicity and death related to neurologic dysfunction were also not shown to be significantly different between the treatment arms.

Kocher et al. reported findings from a phase 3 trial, which evaluated the effect of adding WBRT (30 Gy in 10 fractions) to surgery or SRS (37). Of 359 patients, 199 received SRS (100 patients subsequently observed; 81 subsequently underwent WBRT), and 160 received surgery (79 patients subsequently observed; 81 subsequently underwent WBRT). Here the primary endpoint was deterioration to a WHO performance status (WHO PS) of more than 2. The median time to WHO PS of more than 2 was similar across groups (10 months after observation and 9.5 months after WBRT, p=0.71). Overall survival was also similar at 10.9 months for WBRT and 10.7 months for observations (p=0.89). Of interest in the SRS group, the addition of WBRT resulted in lower 2-year progression rates at 2 years both at initial sites (31% vs. 19%, p = .040) and at new sites (48% vs. 33%, p = .023). Consequently, salvage therapies were also more often utilized.

The addition of WBRT to SRS treatment protocols has shown significant benefit for local control in patients with brain metastases, thus reducing the need for salvage therapies. In some studies, performance status and functional independence have also shown improvement. However, a survival benefit has not been consistently demonstrated.

STAGED SRS VERSUS FRACTIONATED SRS

Given the limitations of SRS at higher lesion sizes, strategies have emerged to help facilitate more effective application of SRS in brain metastases patients. As described earlier, SRS may be delivered as a stand-alone therapy through a single fraction in a single treatment session. In addition, staged and fractionated SRS schemes have been increasingly utilized depending on patient and tumor characteristics (38). Fractionated SRS (FSRS) involves several daily, consecutive treatments with a smaller dose (e.g. 9 Gy per fraction for 3 days). Staged SRS (SSRS) involves typically two fractions separated by approximately one month, utilizing a higher dose scheme (e.g. 15 Gy per fraction each month). Potentially, these alternative dosing schedules allow for better treatment of larger tumors and/or those too close to critical neural structures (39).

Oermann et al. reported a retrospective review across two centers, involving 214 patients with radiation-naïve brain metastases who received FSRS (39). Patients were given either a single dose or 2-5 fractions (74 patients), and local control was measured. Furthermore, 30 patients had radio-resistant tumors. No difference in local tumor control was found for single-fraction patients when comparing radiosensitive and radioresistant tumors (p=0.69). For the FSRS group, radioresistant tumors failed more frequently compared to radiosensitive (median local control of 14.4 months versus 41.5 months, p=0.001). Thus, radioresistant tumors appeared to respond better to higher dose, single-fraction therapy instead of FSRS dosing. Murai et al. evaluated 54 patients with 102 brain metastases, of which 61 were defined as large (≥2.5cm in maximum diameter) (40). These large brain metastases were treated with 18-30 Gy in three fractions (if ≥2.5 cm to <4 cm diameter) or 21-35 Gy in five fractions (≥4 cm). A dose escalation scheme was applied as long as patients showed no more than grade 2 toxicities. Here, overall survival was 52% and 31% at 6 and 12 months, respectively. For the large brain metastases, local tumor control rates were 77% and 69% at 6 and 12 months, respectively. These higher-dose FSRS schemes were overall well-tolerated and provided good local control and survival in these patients. Navarria et al. described their cohort of 102 patients treated with FSRS (41). They administered 27 Gy in 3 daily fractions to 51 brain metastases measuring 2.1-3cm in diameter; and 32 Gy in 4 fractions was administered for larger tumors measuring 3.1-5cm in diameter. The overall median local control was 30 months with a 1-year local control of 96%. The overall median survival was 14 months with a 1-year survival of 69%. No significant difference was found between the two size groups. Six patients in the cohort developed RN, and all these lesions were larger than 4.1cm in diameter. Overall, large brain metastases showed good response to FSRS.

In another large study of 289 patients with brain metastases >2 cm, Minniti et al. compared single-dose SRS with FSRS (9Gy x 3 days regimen) (42). At one year, local control rates were 77% in the single-dose group compared to 91% in the FSRS group (p=0.01). Radiation necrosis occurred in 31 patients (20%) in the single-dose group compared to 11 (8%) in the FSRS group (p=0.004). On the other hand, Fokas et al. reported their outcomes in a large-scale study of 260 patients treated with single-fraction SRS or FSRS (either 5 Gy × 7 or 4 Gy × 10) (43). Here, no difference was noted in local control at 1 year (73%, 75%, and 71%, respectively; p = 0.191). However, Grades 1–3 toxicity was significantly higher in the SRS group (14%) compared with the FSRS regimens (6% and 2%, respectively; p=0.01). Thus, the lower toxicity profile supported a FSRS scheme in this patient cohort.

Multiple studies have alternatively shown utility of SSRS in certain patient populations with brain metastases. Higuchi et al. evaluated 43 patients with large brain metastases, treated with 30 Gy in 3 staged fractions, delivered over 2 week intervals (44). The local control rates at 6 and 12 months were 89.8% and 75.9%, respectively, and only 1 patient developing a Grade 3 toxicity that required surgery. Of note, tumor volumes decreased by 18.8% (second SSRS) and 39.8% (third SSRS) (p<0.0001). This highlighted the importance of shrinking tumor volumetrics at each subsequent stage in order to achieve better efficacy.

Angelov et al. in 2018 evaluated a 2-stage SRS regimen in 54 patients with 63 large brain metastases (≥2cm) (2). Three primary outcomes were measured: response at first follow-up MRI, time to local progression, and overall patient survival. In this cohort, 46 patients (85%) had a single lesion, 7 patients (13%) had two lesions, and 1 patient had 3 lesions concurrently treated. 14 patients were classified as radioresistant tumors (renal or melanoma). In this staging schedule, the first median dose was 15 Gy (range 12-18) and second was 15 Gy (12-15Gy), in alignment with RTOG 90-05 guidelines. Median duration between stages was 34 days. Ultimately, 9 lesions (14.3%) showed local progression at a median of 5.2 months and 7

(11.1%) showed radiation necrosis (2 confirmed pathologically, 5 assessed based on imaging). Excellent local control at 3 months (95%) and 6 months (88%) was reported. Overall survival rates at 6 and 12 months were 65% ± 7% and 49% ± 8%, respectively. Furthermore, greater tumor volume at baseline was associated with shorter time to progression.

Overall, several retrospective and prospective studies have described individually FSRS and SSRS for management of large brain metastases (2). By increasing dose intensity and spacing out treatments, SSRS may offer improved local control with reduced adverse effects (45, 46). The change in tumor volumetrics at the second or subsequent stages may especially play a role in the overall treatment response. Enhanced tumor cell killing *via* a high dose, followed by an interval period to enable repair of normal cells, may be the mechanism through which SSRS facilitates good local tumor control. Meanwhile FSRS regimens may be a more important option when critical neural structures are involved, thus limiting absolute dosage.

SURGICAL RESECTION OF BRAIN METASTASES

In many patients, surgical resection remains the recommended initial step for treatment of mass effect and brain edema, as well as obtaining a definitive diagnosis. The main surgical techniques, are en-bloc resection which consist of a circumferential resection of the metastatic tumor with tumor capsule preservation, and piecemeal resection. As Patel et al. reported, en-bloc resection demonstrated superiority over piecemeal resection regarding leptomeningeal spread and local recurrence, except for significantly large tumors ≥9.7 cm^3, for which a 2-times local increased recurrence rate was shown, regardless of the resection technique used (47). Notably, surgical resection as a sole treatment option nowadays, is less acceptable treatment choice for brain metastases. Radiation treatment should accompany it, with appreciation of the radiation modality and timing suitable for each patient.

COMBINATION OF SURGERY AND WBRT

Literature reports indicated a significant value in irradiating the intracranial space to provide better local and distant control in proximity to the surgical resection. Nonetheless, the decision of which radiation modality to use relies on the patient's brain disease burden and expected neurocognitive effect following radiation (3). While some earlier studies suggested no clear benefit for adjuvant WBRT, others have shown encouraging data to support adding WBRT following surgical resection (37, 48–51).

Deangelis et al. evaluated 98 patients who underwent craniotomy for brain metastases resection followed by observation (19 patients) or WBRT (79 patients) (50). Adjuvant WBRT was found to significantly increase time to local or distant failure (p=0.034). At 1 year, the recurrence rate was 22% for WBRT-treated patients and 46% for observation. Median survival between groups was not statistically significantly different (20.6 vs. 14.4 months for WBRT and observation, respectively). Smalley et al. reviewed 85 patients who underwent brain metastases resection, and 34 patients went on to receive WBRT while 51 were observed only (51). The WBRT-treated patients demonstrated lower rates of recurrence (21% versus 85%) and also longer median survival (21 months vs. 11.5 months).

The study by Kocher et al., described earlier here, included one arm of surgery followed by observation or WBRT (37). Here, 160 patients underwent complete resection that was determined macroscopically, imagery or by a combination of both, of whom 79 were subsequently observed and 81 underwent adjuvant WBRT. Notably, the operated study arm included solitary large metastases, as these lesions more frequently required surgical resection. The authors noted that WBRT reduced the probability of relapse at initial sites from 59% to 27% (p<0.001) and at new sites from 42% to 23% (p=0.008). Overall survival and performance status were comparable between groups. Thus, WBRT appears to provide benefit especially in terms of local control without significantly enhancing overall survival.

COMBINATION OF SURGERY AND SRS

Surgery Followed by Adjuvant SRS

Given the potential neurocognitive toxicities associated with WBRT, post-operative adjuvant SRS offers another approach to improve local control when additional treatments are needed. Choi et al. retrospectively evaluated 112 patients with 120 surgical cavities, who subsequently underwent SRS (52). At 1 year, the local failure and distant failure rates were 9.5% and 54%, respectively. When a 2-mm margin was added to the surgical cavity for delivery of SRS, the local failure rates improved (3% versus 16%, p=0.042). There was no significant difference in toxicity at 1 year (3% versus 8%, p=0.27). Median overall survival was 17 months, and the 12-month overall survival rate was 62%. Of note, this methodology of applying a 2-mm margin to the treatment plan stems from prior work by Soltys et al. where 72 patients were treated with SRS alone, resulting in a 79% local control rate at 1 year (53). The authors described that increasing conformality indices (i.e. less conformal plans) were associated with improved local control. Hence, a 2-mm margin technique was advocated and has been adopted by many since then.

Mahajan et al. reported a randomized, controlled, single-center, phase 3 trial comparing post-operative SRS versus observation alone (54). 132 patients who underwent complete resection of one to three brain metastases were assigned to either observation (n=68) or SRS (n=64). In the SRS group, a 1-mm margin are added to the treatment plan. Dosage used was 16 Gy (<10cm3), 14 Gy (10.1-15 cm3), or 12 Gy (>15 cm3) based on cavity volume. Median follow-up was 11.1 months, and the 1-year local control was 43% in the observation group and 72% in the SRS group (HR 0.46, p=0·015). There were no adverse events in either group. Hence, the authors concluded that post-operative SRS offers a significant advantage in treatment.

Brown et al. directly evaluated postoperative SRS against WBRT in a randomized, controlled, phase 3 trial (55). In this multi-center study across 48 institutions, patients with one resected brain metastasis and resection cavity less than 5 cm diameter were eligible for enrollment. Overall 194 patients were assigned to either SRS (12-20 Gy single fraction, using 2-mm margin) or WBRT (30 Gy in 10 daily fractions or 37.5 Gy in 15 daily fractions). Median follow-up was 11.1 months. Importantly, the SRS arm showed a lower risk of cognitive deterioration (median 3.7 months, compared to 3 months for WBRT), and at 6 months the SRS patients had significantly lower rates of cognitive decline (52% compared to 85% of WBRT patients). Median survival was not significantly different (12.2 months for SRS; 11.6 months for WBRT). These findings suggest that SRS is associated with improved neurocognitive outcomes over time without reducing overall survival when compared with WBRT.

It is important to note also that radiation dosing is generally de-escalated for SRS and is variable between treatment centers. Interestingly, local tumor control in the study by Brown et al. was worsened following SRS (median time to progression of 6.4 months) compared with WBRT (median 27.5 months, $p<0.0001$) (55). The 1-year surgical bed control was 60.5% for SRS patients, relatively lower than that reported by Mahajan et al. (54) In an earlier observational study by Jensen et al. in 2011, 112 resection cavities were treated with SRS under different dosing protocols, reporting a median radiosurgical dose of 17 Gy to the tumor margin and a median cavity volume of 8 cc (56). Here median survival was 10.9 months while local tumor control was 80.3% at 1 year. The continued variability in SRS dosing protocols therefore limits direct comparisons across radiation-based studies.

In the course of post-SRS follow-up, multiple studies have suggested a high risk of leptomeningeal disease (LMD) in this patient population (1). Up to 30% of these patients may go on to develop LMD. Prabhu et al. reported a study of 125 patients who underwent surgical resection and adjunctive SRS to 1 brain metastatic lesion (1). Neurologic death (ND) was measured based on neurologic dysfunction attributable to brain metastases or the associated therapy, without systemic decline or progression. Ultimately, there were 107 patients (86%) who went on to receive LMD salvage treatment, and 82 (66%) also had cranial MRI follow up to characterize radiographic patterns of LMD including classical "sugar-coating" and nodular patterns. ND was seen in 99 patients (79%). These incidences of LMD and ND are in fact higher than the 14% to 48% rates reported in the literature for single-modality therapy (e.g. surgery or SRS) (36, 54, 57, 58).

Neo-Adjuvant SRS Followed by Surgery

The notable risk of LMD and ND after adjuvant SRS has led to the study of neo-adjuvant SRS (NSRS) as an alternative option to improve patient outcomes. NSRS may allow for more precise definition of the target volume and reduce intraoperative seeding of tumor cells. Asher et al. described a cohort of 47 patients (23 database, 24 prospectively accrued) with 51 lesions (59). NSRS was done a median of 1 day before surgical resection. Median lesion diameter was 3.04cm with a mdian volume of 8.49 cc). Median dose was 14 Gy to 80% isodose line. After a median follow-up of 12 months, overall survival was 77.8% and 60% at 6 and 12 months, respectively. Local control rates were 97.8% and 71.8% at 6 and 24 months, respectively. Interestingly, no LMD or other perioperative adverse events were reported. 8% of patients went on to develop radiation necrosis. Local failure was more likely with lesions >3.4 cm, and six of the 8 failures had a dural attachment or proximity to draining veins. Thus, NSRS yielded excellent response rates in this cohort with low rates of radiation necrosis and LMD.

Prabhu et al. in 2017 conducted a retrospective, multi-institutional study of 213 patients to determine outcomes of SRS alone or SRS plus surgery (60). 223 large brain metastases (≥4cm) were treated with either SRS alone (61), NSRS and surgery (62), or surgery with adjuvant SRS (94). Any complete resection with SRS was associated with improved local control (79.5%) compared with SRS alone (63.3%). Postoperative SRS resulted in the highest rate of radiation necrosis (22.6%) compared to SRS alone (12.3%) and NSRS (5%). In a more recent and updated analysis of their NSRS patients, Prabhu et al. in 2018 described 117 patients with 125 lesions treated with NSRS (63). Gross total resection was achieved in 95.2% of lesions, and median SRS dose was 15 Gy. Local recurrence at 2 years was 25.1% and distance failure was 60.2%. LMD was found in 4.3% of cases, and symptomatic radiation necrosis occurred in 4.8% of cases. Median overall survival was 17.2 months. Thus, NSRS resulted in good local control with an acceptable low toxicity profile.

INTRAOPERATIVE RADIOTHERAPY

While SRS and WBRT have been extensively evaluated in the last few decades for management of brain metastases, another mode of radiation therapy that is increasingly gaining attention is intraoperative radiotherapy (IORT) (64). IORT involves a single dose of radiation administered at the same time as the surgical biopsy or resection being performed. Three main categories are described: intraoperative electron radiotherapy (IOERT), low-energy X-ray intraoperative radiotherapy (LEX-IORT), and intraoperative high-dose brachytherapy (IOHDR). IOERT has historically been used in extracranial tumors such as breast, pancreas, head and neck, and colorectal cancers. Generally, it requires a cavity with clear line of sight given the structure of applicator tubes. LEX-IORT utilizes a 30- to 50-kV istotropic X-ray source and adapts more conformally to the resection cavity of interest while applying a more steep dose gradient. IOHDR involves a sealed radionuclide source being placed inside the resection cavity itself. This therapy has been used extensively in rectal cancers, soft tissue sarcomas, and head and neck cancers.

While there is limited data regarding intracranial effectiveness and risks with IORT, early studies do suggest potential benefits from this modality. Weil et al. evaluated 23 patients treated with 50 kV LEX-IORT, where 14 Gy was delivered to a 2mm depth from the applicator surface (62). Progression-free survival from

time of surgery was 22 months and overall survival was 30 months (1-year local control of 50%). In another study by Cifarelli et al. 54 patients were treated with LEX-IORT, using a median dose of 30 Gy to the applicator surface (65). The 1-year local control was 88%, and overall survival was 73%. LMD occurred in 3% of patients, and RN occurred in 7% of patients. Kahl et al. reported their cohort of 40 patients with 44 resected metastases, who were treated with LEX-IORT using a median dose of 20Gy (66). Median overall survival was 26.4 months (1-year survival of 61.6%), and the local control was 88.6% (1-year local control of 84.3%). They observed a low RN rate of 2.5%. The potential for favorable progression-free and survival outcomes coupled with a low toxicity profile that is demonstrated in these preliminary findings certainly warrants larger, prospective studies on IORT.

HISTOLOGIC CONSIDERATIONS IN TREATMENT

Our increased understanding of molecular genetics in tumor pathogenesis has allowed for more detailed diagnostics as well as tailored treatment options for cancer patients. In the context of patients suffering from brain metastases, it is therefore useful to evaluate histologic background in relation to treatment response. Few of the notable histological categories are discussed here.

In non-small-cell lung cancer (NSCLC), brain metastases may arise in 30% of patients in their disease course (61, 67). A unique subset of tumors carry the ALK rearrangement, which make these patients excellent candidates for targeted treatment with ALK-targeted tyrosine kinase inhibitors (TKI), including crizotinib. Nonetheless, brain metastases frequently occur, likely due to poor penetration of the drug across the blood-brain barrier. The role of radiotherapy in enhancing progression and survival in these patients is unclear. Johung et al. reviewed a cohort of 90 patients with ALK-rearranged NSCLC treated with a combination of SRS, WBRT, and TKI therapy (67). The median overall survival after diagnosis of brain metastases was 49.5 months and median intracranial progression-free survival was 11.9 months. Yang et al. reviewed outcomes from a smaller cohort of 34 patients, of which 19 were treated with combined TKI and radiotherapy, resulting in 70% overall survival at 3 years (68). Thomas et al. retrospectively reviewed 52 ALK-positive NSCLC patients and evaluated TKI combined with radiation versus newer CNS-penetrant TKI therapies alone (69). They reported similar time to intracranial progression (18.1 vs 21.8 mos, p=0.65) and time to overall progression (11.4 vs 13.4 months, p=0.98) for both groups. Thus, radiation with SRS or WBRT represents an important treatment option in these patients, but this should be further evaluated in the context of evolving TKI and other targeted therapies.

Melanoma represents another significant primary tumor histology, wherein 10-73% of patients go on to develop brain metastases (70). Median survival in these patients is 6.74 months, and the relatively radioresistant nature of these tumors makes SRS a more viable treatment option compared to WBRT. Goyal et al. reported a systematic review demonstrating favorable outcomes following SRS therapy in melanoma patients, while the addition of WBRT led to detrimental neurocognitive outcomes and no improvement in overall survival (71). Furthermore, half of melanoma patients carry the BRAF protein kinase mutation, and studies have shown favorable response to BRAF inhibitor therapy (BRAFi). Mastorakos et al. reviewed 198 patients in a multicenter retrospective cohort study to evaluate the role of SRS and BRAF mutation status in brain metastasis patients (70). They found that BRAF-mutated patients (45.5% or 90) receiving BRAFi had improved survival overall compared to wild-type BRAF. After receiving SRS in these two groups, median survival was improved in the BRAFi group compared to the wild-type group as well (13 vs 7 months). Furthermore, in terms of radiation timing, BRAFi given after SRS showed improved survival compared to giving it before or during SRS. While the authors concluded that SRS treatment followed by BRAFi may improves survival outcomes, this must also be weighed against the risks of therapy. Notably, BRAFi treatment was associated with a higher risk of intracerebral hemorrhage compared to no BRAFi treatment (10.4% vs 3%, p=0.03).

Small cell lung cancer (SCLC) has historically been excluded from randomized trials given its unique biology (72). SCLC carries a 40-50% risk of metastasis to the brain and is shown to have high radio- and chemo-sensitivity. The rapidly progressive nature of SCLC has led to the prevalent use of WBRT in its management, with prophylactic cranial irradiation (PCI) shown to increase survival when administered earlier in the course of this disease (73, 74). As imaging and clinical surveillance has improved and neurocognitive outcomes have become more relevant, there is renewed interest in SRS for these patients. Rusthoven et al. described a multi-center retrospective study evaluating 710 SCLC patients treated with SRS without prior PCI or WBRT (75). The median overall survival was 8.5 months, and median time to central nervous system progression was 8.1 months. After propensity matching a subset of 187 patients from the SRS cohort with 187 WBRT-treated patients, the overall survival was higher with SRS (median 6.5 months vs 5.2 months, p=0.003) while no difference was seen in progression-free survival (median 4 months for SRS vs 3.8 months for WBRT, p=0.79). Another study by Cifarelli et al. evaluated 293 patients treated with SRS for SCLC brain metastases across 10 centers (72). In this cohort, 79% had received SRS as salvage therapy following WBRT or PCI. At one year in the overall cohort, the local failure, distant brain failure, and overall survival were 31%, 49%, and 28%, respectively. On multivariate analysis, younger age for patients receiving salvage SRS was a significant predictor of overall survival. Robin et al. reviewed outcomes from the National Cancer Database comparing upfront SRS against upfront WBRT with or without SRS (76). After propensity score matching between 193 SRS patients and 1930 WBRT patients, overall survival was shown to be improved in the SRS-alone group (median 10.9 months vs 7.6 months, p<0.001). The encouraging outcomes with SRS warrant prospective trials to further elucidate its role in SCLC management (77).

DISCUSSION

Brain metastases represent a major healthcare burden, with significant impact on quality of life and survival (3). Survival length, however, is usually dependent on systemic disease control rather than CNS disease, and even though it may not reflect the efficacy of applied oncological treatment as local control do, it is a commonly measured outcome of studies in this field and therefore is extensively reported in this review as well.

While WBRT was initially a mainstay in treatment, its lack of specificity and risk of neurocognitive decline has required us to seek other modalities for therapy. Several alternative paradigms have been increasingly utilized in the last 30 years, including surgical resection; SRS *via* single, fractionated or staged approaches; and a combination of surgery with radiation (see **Table 1**). Early data evaluating IORT as an alternate mechanism for radiation delivery in intracranial disease remains limited yet encouraging. As the state of research evolves, it is imperative for the neurosurgical oncology community to continually update practice guidelines and metrics for evaluation of treatment modalities (78).

The availability of different options, therefore, allows a more tailored approach to each patient. While surgery offers a direct, immediate method for relieving mass effect and brain edema, SRS offers a less invasive approach with good local control and avoidance of peri-operative complications. In those patients for whom surgery would not be well-tolerated and mass effect is not of immediate concern, SRS alone may be a reasonable approach. Due to dose limitations of SRS in the context of large brain metastases and those lesions that are close to critical neural elements, a fractionated or staged approach may be pursued. Here, the goal is to maximize dose intensity in a safe manner to enhance tumor cell kill, while reducing risk of damage to surrounding structures. Staged SRS may also be valuable in radioresistant tumors where higher doses can be delivered. However, in patients who develop radiation necrosis, additional treatments may be needed, including anti-angiogenic agents.

Finally, a combination of surgery and SRS has been recommended in some cases to further improve local tumor control at the resection cavity. As described earlier, for large brain metastases where mass effect is of concern, surgical resection provides immediate symptomatic relief while SRS boost to a 1 to 2-mm margin is shown to enhance local control. Furthermore, adjuvant SRS may be superior to WBRT in terms of better neurocognitive outcomes. Interestingly, newer paradigms are emerging to address the neurocognitive risks associated with traditional WBRT. The use of memantine and hippocampal-sparing WBRT have been described more recently in brain metastases patients. Brown et al. in 2010 presented a phase III randomized trial evaluating 518 patients over a median follow-up of 7.9 months. Cognitive decline was significantly improved after hippocampal-sparing WBRT plus memantine versus WBRT plus memantine (HR 0.74, p=0.02). No significant difference was reported in progression-free survival or overall survival.

Regarding the notable risk of leptomeningeal disease after adjuvant SRS, several studies have argued that neoadjuvant SRS may be a better therapeutic strategy. Notably, radiation dosages varies in literature reports and further validation is needed as lower radiation doses given to large metastases may eventually lead to a higher recurrence rate. This removes the need for radiation to a post-operative cavity margin, thus reducing risk of radiation necrosis. Also, the sterilization of tumor cells pre-operatively appears to reduce the risk of seeding during surgery,

TABLE 1 | Overview of treatment strategies and their benefits and risks.

Treatment	Benefits	Risks
Surgical resection	• Relief of mass effect • Obtain pathological diagnosis • Improved survival compared to WBRT	• Most invasive • Peri-operative complications: hemorrhage, wound healing
a. Surgery plus WBRT	• Improved local and distant control compared to surgery alone.	• Long-term neurocognitive effects • Longer treatment duration for patient • Survival benefit inconsistent
b. Surgery plus SRS	• Improved local control compared to surgery alone • Reduced neurocognitive risks	• Leptomeningeal disease • Radiation necrosis
SRS	• High dose delivery in a single treatment session • Less invasive than surgery • Possibly lower rates of early local recurrence	• Radiation necrosis • Limited dose delivery with large brain metastases
a. Fractionated SRS	• Lower doses can be applied when close to sensitive neural elements	• Radiation necrosis
b. Staged SRS	• May help with large brain metastases requiring higher dosage overall	• Radiation necrosis
WBRT	• Less invasive • Good local and distant control	• Limited dose and targeting • Neurocognitive decline • Longer treatment duration for patient • More palliative in nature
a. SRS plus WBRT	• Improved progression-free survival • Preservation of functional status and cognitive function overall	• No consistent survival benefit • Not useful when significant brain edema is a concern

thus reducing the risk of leptomeningeal disease as well. En-bloc gross total resection is additionally important in improving patient outcomes in terms of local control and overall survival.

Ultimately, management of brain metastases remains a controversial issue as a single treatment plan may not apply to most patients. It is at the discretion of the treating neurosurgeon, along with radiation oncologist colleagues, to evaluate the benefits and risks of treatment with each patient.

AUTHOR CONTRIBUTIONS

NS: Compiled/interpreted resources and primary author for majority of the manuscript. TK: Assisted with manuscript writing/edits. AM: Conception and overall design of project/paper, manuscript editing/revisions, interpretation of resources to include in paper. All authors contributed to the article and approved the submitted version.

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REVIEW
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Improving Brain Metastases Outcomes Through Therapeutic Synergy Between Stereotactic Radiosurgery and Targeted Cancer Therapies

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Brain metastases are the most common form of brain cancer. Increasing knowledge of primary tumor biology, actionable molecular targets and continued improvements in systemic and radiotherapy regimens have helped improve survival but necessitate multidisciplinary collaboration between neurosurgical, medical and radiation oncologists. In this review, we will discuss the advances of targeted therapies to date and discuss findings of studies investigating the synergy between these therapies and stereotactic radiosurgery for non-small cell lung cancer, breast cancer, melanoma, and renal cell carcinoma brain metastases.

Keywords: targeted therapy, SRS, brain metastases, non-small cell lung cancer, breast cancer, melanoma, renal cell carcinoma

INTRODUCTION

Brain metastases are the most common malignant tumors found in the central nervous system (1). They are 10 times more common than primary central nervous system (CNS) brain tumors, affecting 20 to 40% of all patients with cancer, and greater than 100,000 new patients each year in the United States (2–4). With improved therapies, increased screening of neurologically asymptomatic patients, and patients living longer, the incidence of brain metastases continues to increase. The blood-brain barrier has long posed a challenge for traditional chemotherapeutics to enter the brain and effectively treat these lesions. Therefore, the mainstays of treatment, to date, have included surgery, stereotactic radiosurgery (SRS), and whole brain radiotherapy; with only a limited role for systemic therapies (5).

The current treatment algorithm for patients with brain metastases includes stratification by symptoms, as well as disease burden by number (single lesion, oligometastases, polymetastases) and size (6, 7). Symptomatic patients with poor performance status often benefit from best supportive care alone (8). Symptomatic patients with a favorable performance status may be candidates for surgery and/or radiotherapy (SRS, hypofractionated radiosurgery, or whole brain radiotherapy) depending on the number and size of the metastases, in addition to treatment with systemic therapy (either traditional chemotherapies, immunotherapies and/or targeted molecular therapies

depending on the molecular signature of the primary tumor) (9). Asymptomatic patients with small lesions may be treated with upfront systemic therapy, while saving radiotherapy and/or neurosurgery as salvage therapy (5).

Increasing knowledge of primary tumor biology, actionable molecular targets and continued improvements in systemic and radiotherapy regimens have helped improve survival but necessitates multidisciplinary collaboration between neurosurgical, medical and radiation oncologists. In this review, we will discuss the advances of targeted therapies to date and discuss findings of studies investigating the synergy between these therapies and SRS for the treatment of non-small cell lung cancer, breast cancer, melanoma, and renal cell carcinoma brain metastases.

Non-Small Cell Lung Cancer

Non-small cell lung cancer (NSCLC) accounts for approximately 85% of all lung cancers, with 16 to 34% of all NSCLC patients experiencing brain metastases and 40 to 50% of all patients with brain metastases having lung etiology (10–13). With evolution of targeted therapies, molecular testing for the following oncogenic driver mutations has become standard of care; *ALK* (Anaplastic lymphoma kinase) rearrangements, *BRAF* (B-Raf proto-oncogene, serine/threonine kinase) mutations, *EGFR* (epidermal growth factor receptor) mutations, *MET* (mesenchymal–epithelial transition) exon 14 skipping mutations, *NTRK* (Neurotrophic Tyrosine Receptor Kinase) 1/2/3 gene fusions, *RET* (ret proto-oncogene) rearrangements, and *ROS1* (c-ros oncogene 1) rearrangements (14–16). Mutations in *EGFR, ALK, BRAF, NTRK, MET, RET, ROS1, KRAS* (Kirsten rat sarcoma virus), *HER2* (human epidermal growth factor receptor 2) genes have all been found to be expressed in NSCLC and have targeted therapies inhibiting the abnormal proteins for which these mutated genes encode. First-generation EGFR inhibitors, erlotinib and gefitinib, and second-generation EGFR ErbB family inhibitor, afatinib, have been replaced by third generation EGFR tyrosine kinase inhibitor osimertinib as first-line therapy in patients with EGFR-mutated brain metastases secondary to improved CNS penetration, efficacy, longer response and survival duration (13, 17, 18). Alectinib, brigatinib, and loratinib are preferred first-line agents for patients with brain metastases containing *ALK* rearrangements (19–21). Selpercatinib (22) and pralsetinib (23) are selective RET inhibitors that are used in the treatment of patients with RET fusion-positive NSCLC, while Entrectinib (24) is a ROS1 fusion inhibitor used in the treatment of ROS1 fusion-positive NSCLC.

The individual efficacies of SRS and targeted therapies for NSCLC have led many to investigate the synergy between these two therapies and to investigate how it can best be maximized (**Table 1**). A retrospective study in 2018 by Yomo et al. assessed 133 patients with brain metastases arising from EGFR-mutant lung adenocarcinoma who received upfront gamma knife SRS and subsequently were administered EGFR tyrosine kinase inhibitors. 1-year and 2-year overall survival rates were 74 and 52%, respectively with a mean survival time of 24.8 months (27). These outcomes are significantly better than prior studies and showed median survival time from initial brain metastases treatment rose from 7 months between 1985 and 2005 to 12 months between 2006 and 2014 (29). The Oda study also reported 1-year and 2-year distant brain metastases recurrence rates were 34 and 53% respectively, and 1- and 2-year local tumor control per lesion were 97% and 95%, respectively. Multivariate analysis showed that being EGFR tyrosine kinase naïve was associated with longer overall survival (HR: 0.42, $P < 0.001$), a lower distant intracranial recurrence rate (HR: 0.61, P=0.037), and a higher local tumor control rate (HR: 0.28, P=0.001) (27). The underpinnings of synergy between SRS and targeted therapies are highlighted by these findings. The lower distant intracranial recurrence rate indicates that the targeted therapies help to address metastases beyond the SRS field, while the higher local tumor control rate may be theorized to occur secondary to improved breakdown of the blood-brain barrier within the SRS bed, therefore lending to increased efficacy of the targeted therapies in these regions.

Magnuson et al. conducted a multi-institutional retrospective pooled analysis of 351 patients with EGFR-mutant NSCLC-developed brain metastases who received SRS followed by EGFR-TKI, WBRT followed by EGFR-TKI, or EGFR-TKI followed by SRS or WBRT at intracranial progression and found best overall survival times in patients who received SRS followed by EGFR-TKI compared to those who received whole brain radiotherapy first followed by EGFR tyrosine kinase inhibitors and to those who received EGFR tyrosine kinase inhibitor treatment first followed by SRS or whole brain radiotherapy (25). SRS followed by EGFR-TKI resulted in the longest median survival time of 46 months while avoiding the neurocognitive impairment associated with whole brain radiotherapy.

A recent analysis of a prospective registry of 218 patients with NSCLC EGFR-mutated (EGFRm) and EGFR-wild-type brain metastases treated with SRS plus or minus systemic therapies, did not show a statistically significant difference in local failure or radionecrosis rate at 24 months in EGFRm patients with administration of tyrosine kinase inhibitor before SRS (3% and 3%) or after SRS (17% and 0%). Although not reaching statistical significance, receiving TKI before SRS led to a 3% local failure rate of 24 months compared to 17% when administered after SRS (26). The authors did not ascribe these results to lack of synergy but rather concluded that this highlights the importance of not delaying the initiation of systemic therapy with tyrosine kinase inhibitors. On multivariate analysis, brain metastases size and dose of radiation significantly correlated with a higher risk of local failure and brain metastases size correlated with a higher risk of radiation necrosis.

A retrospective study by Dohm et al. of 174 NSCLC brain metastases patients treated with SRS within 3 months of receiving systemic therapies found significantly improved distant intracranial control with EGFR-TKI therapy compared to conventional chemotherapy (HR 0.4; 95% CI 0.25-0.76; P = 0.04) and with receiving SRS before systemic therapy (HR 0.6; 95% CI 0.3-0.9; P = 0.03) (28). Local control was found to be significantly improved when patients received treatment with SRS before (HR 0.4; 95% CI 0.2-0.9; P = 0.03) or concurrent (HR 0.3; 95% CI 0.1-0.6; P = 0.003) with the receipt of systemic

TABLE 1 | Studies evaluating synergy between SRS and targeted therapies in patients with NSCLC brain metastases (BrM).

Study Identifier	Study Period	Study Size (n = patients)	Treatment/Intervention Groups	Results	References
Magnusen et al.	2008-2014	N = 351	Patients with EGFR-mutant NSCLC BrM treated with SRS followed by EGFR-tyrosine kinase inhibitor (TKI) *vs* WBRT followed by EGFR-TKI *vs* EGFR-TKI followed by SRS or WBRT at intracranial progression	• Median OS for SRS followed by EGFR-TKI, WBRT followed by TKI and EGFR-TKI followed by SRS or WBRT = 46, 30, and 25 months respectively ($p < .001$) • On MVA, SRS versus EGFR-TKI, WBRT versus EGFR-TKI, age, performance status, EGFR exon 19 mutation, and absence of extracranial metastases associated with improved OS	(25)
Moraes et al.	2008-2017	N = 218	Patients with EGFRm and EGFRwt NSCLC BrM treated with SRS ± systemic therapy (chemotherapy, TKI or immunotherapy)	• 24-month incidence of LF was 6% and 16% for EGFRm BrM and EGFRwt, respectively (0.43 (0.19-0.95); p = 0.037) • 24-month incidence of RN was 4% and 6% for EGFRm and EGFRwt BrM, respectively (0.8 (0.32-1.98) p = 0.63) • On MVA, BrM size and prescription dose (PD) significantly correlated with a higher risk of LF and BrM size correlated with a higher risk of RN	(26)
Yomo et al	2010-2016	N = 133	Patients with EGFR-mutant lung adenocarcinoma BrM who received upfront Gamma Knife SRS; post-SRS EGFR-TKI administered to 85% of cohort	• 1-year OS = 74%, 2-year OS 52% • 1-year and 2-year distant BrM recurrence rates (per patient) after SRS = 34% and 53% • 1-year and 2-year rates of local tumor control (per lesion) = 97% and 95% • MVA proportional hazards analyses found being EGFR-TKI naïve • associated with longer OS (HR: 0.42, $P < 0.001$), a lower distant intracranial recurrence rate (HR: 0.61, P = 0.037) and higher local tumor control rate (HR: 0.28, P = 0.001)	(27)
Dohm et al	2015-2019	N = 174	Patients with NSCLC BrM treated with single-fraction SRS sessions within 3 months of receiving immune checkpoint inhibitors (ICI), EGFR-tyrosine kinase inhibitors (TKI), chemotherapy and ICI, or standard chemotherapy	• 12-month DIC was 35%, 53%, 41%, and 20% (P = 0.02) for ICI, EGFR-TKI, ICI and chemotherapy, and chemotherapy alone groups, respectively • No differences were noted in LC (P = 0.1) and OS (P = 0.5) between treatment groups • On MVA, factors found to be significant for improved DIC included treatment with EGFR-TKI therapy compared to conventional chemotherapy (HR 0.4; 95% CI 0.25-0.76; P = 0.04) and treatment with SRS before systemic therapy (HR 0.6; 95% CI 0.3-0.9; P = 0.03) • On MVA, factors found to be significant for improved LC included treatment with SRS before (HR 0.4; 95% CI 0.2-0.9; P = 0.03) or concurrent (HR 0.3; 95% CI 0.1-0.6; P = 0.003) compared to following receipt of systemic therapy • Rates of radiation necrosis (RN) did not differ between treatment groups	(28)

therapy. These findings are consistent with immunotherapy literature reporting improved distant brain control for patients receiving stereotactic radiation during or prior to anti-PD1/PD L1 therapy (30, 31).

Breast Cancer

Breast cancer is the most common malignancy and second leading cause of cancer-related death in women in the United States (32) and the second most common pathology that metastasizes to the brain after lung cancer overall (33, 34). Approximately 10 to 30% of patients with breast cancer will develop brain metastases (35–37). The heterogeneity of breast cancer necessitates a multidisciplinary approach because there are a multitude of systemic therapies that vary depending on the type of breast cancer. There are 5 main types of breast cancer, which include luminal A (hormone receptor positive, HER-2 low expression, with low levels of Ki-67), luminal B (hormone receptor positive, either HER-2 positive or low expression, with high levels of Ki-67), HER-2 positive (hormone receptor low expression), triple negative (hormone receptor and HER-2 low expression) and finally normal breast-like (38).

Most of the literature investigating the synergy between SRS and breast cancer metastases involves HER-2 positive breast cancer brain metastases (**Table 2**). HER-2 is a member of the transmembrane tyrosine kinase EGFR family. It is overexpressed in approximately 14% of breast cancers but has a high incidence of brain metastases and accounts for approximately 44% of resected breast cancer brain metastases (45–47). Trastuzumab was the first anti-HER-2 antibody proved to enhance extracranial disease control and survival rates in patients with metastatic HER-2 positive breast cancer (48). This was followed by trastuzumab emtansine (T-DM1) which uses the trastuzumab antibody to deliver emtansine (DM1) to HER2 antigen expressing tumors (49–52). Unfortunately, several studies have

TABLE 2 | Studies evaluating synergy between SRS and targeted therapies in patients with breast cancer brain metastases (BrM).

Study Identifier	Study Period	Study Size (n = patients)	Treatment/Intervention Groups	Results	References
Mills et al.	2013-2019	N = 16	Patients with HER2+ breast cancer BrM treated with SRS/FSRT with T-DM1 delivered within 6 months	• Stereotactic radiation delivered concurrently with T-DM1 in (48%) • 1-year LC, DIC, systemic PFS, and OS were 75, 50, 30, and 67%, respectively • 1 case of leptomeningeal progression and 1 case (3%) of symptomatic radionecrosis	(39)
Gori et al.	2005-2014	N= 154	Patients with HER2+ breast cancer BrM treated with local and systemic therapies	• Median OS = 24.5 months • Patients receiving surgery/SRS experienced longer OS compared to those receiving whole-brain radiotherapy or no treatment (33.5 *vs*. 11.4 months; P < .001) • WBRT did not improve OS compared to no treatment (11.4 *vs*. 9.8 months; p = .99) • HER2-targeted therapy was associated with better OS compared to systemic therapy without HER2-targeted therapy or no systemic therapy (27.5 *vs*. 5.4 months; P < .001) • On MVA stratified by local treatments, systemic therapy, KPS, and neurologic symptoms significantly affected OS	(40)
Miller et al.	1998-2014	N = 547	Patients with different molecular subtypes of breast cancer and BrM treated with radiotherapy +/- targeted therapies	• Median OS = significantly shorter in the basal cohort (8.4 months) and progressively increased in luminal A (12.3 months), HER2-positive (15.4 months), and luminal B (18.8 months) cohorts (P < .001) • Among patients with HER2-amplified disease, the median OS increased with use of both HER2 antibodies (17.9 months *vs* 15.1 months; P5.04) and TKIs (21.1 months *vs* 15.4 months; P=.03) • 12-month cumulative incidences of local failure among molecular subtypes were 6.0% in the luminal A cohort, 10.3% in the luminal B cohort, 15.4% in the HER2-positive cohort, and 9.9% in the basal cohort (P = .01) • Concurrent HER2/EGFR TKIs with SRS significantly decreased the 12-month cumulative incidence of local failure from 15.1% to 5.7% (P < .001)	(41)
Kim et al.	2005-2014	N = 84	Patients with newly diagnosed HER2+ breast cancer BrM who treated with SRS and divided into 2 cohorts based on timing of treatment with lapatinib	• 132 lesions (27%) treated with SRS + concurrent lapatinib, 355 (73%) treated with SRS alone. • SRS + concurrent lapatinib group had higher rates of complete response (35% *vs* 11%, P = 0.008) • Per-lesion basis, best objective response superior in SRS + concurrent lapatinib group (median 100% *vs* 70% reduction, P < 0.001) • SRS + concurrent lapatinib group not associated with increased risk of grade 2+ RN (1.0% *vs* 3.5% without, P = 0.27)	(42)
Parsai et al.	1997-2015	N = 126	Patients with HER2+ breast cancer BrM who underwent treatment with lapatinib and SRS	• Concurrent lapatinib was associated with reduction in local failure at 12 months (5.7% *vs* 15.1%, p < 0.01) • For lesions ≤ 75th percentile by volume, concurrent lapatinib significantly decreased local failure • Any use of lapatinib after development of brain metastasis improved median survival compared to SRS without lapatinib (27.3 *vs* 19.5 months, p = 0.03) • 12-month risk of RN was consistently lower in the lapatinib cohort compared to the SRS-alone cohort (1.3% *vs* 6.3%, p < 0.01), despite extended survival	(43)
Figura et al.	2015-2018	N = 15	Patients who received stereotactic radiotherapy for HR+ BrM within 6 months of CDK4/6 inhibitor administration; RT was delivered concurrently, before, or after CDK4/6 inhibitors in 18 (43%), 9 (21%), and 15 (36%) lesions, respectively	• 6- and 12-month local control of treated lesions = 88% and 88%, respectively • 6- and 12-month distant brain control = 61% and 39%, respectively • Median OS was 36.7 months from the date of BrM diagnosis	(44)

shown the utilization of T-DM1 with SRS significantly increases symptomatic radiation necrosis rates when used concurrently or sequentially. For this reason, utilization of this drug decreased given that SRS is a mainstay of therapy for patients with brain metastases. However, a recent study by Mills at al reporting on a single institution series of 16 patients with HER-2 positive breast cancer who underwent SRS and T-DM1 therapy delivered within 6 months showed only 1 case (3%) of symptomatic radionecrosis (39). The authors hypothesized that those prior studies showing increased rates of radionecrosis included longer time intervals from radiation and potentially do not accurately reflect toxicity from the combined treatment. Furthermore, longer survival may also confound the incidence of radiation necrosis, which may not necessarily be caused by late toxicity of concurrent SRS and T-DM1 administration.

The Italian HERBA trial retrospectively evaluated 154 patients across 14 institutions and reported longer overall survival in patients receiving surgery/SRS (33.5 *vs*. 11.4 months for patients receiving WBRT or no treatment; HR = 0.34; 95% confidence interval, 0.22-0.52; P <.001) and in patients receiving HER-2 targeted therapies (27.5 *vs*. 5.4 months in patients receiving non-HER2-targeted therapy or no systemic therapy; HR = .26; 95% confidence interval, 0.17-0.41; P <.001) (40). However, this study did not investigate the timing of SRS with regards to systemic therapy. Miller at al reported on a large retrospective study of 547 patients presenting with 3224 brain metastases treated with radiotherapy and targeted therapies and found that concurrent HER-2/epidermal growth factor receptor tyrosine kinase inhibitors with gamma knife SRS significantly decreased 12-month cumulative incidence of local failure from 15.1% to 5.7% (P<0.001) (41). Similarly, they found that concurrent HER-2 antibody treatment with concurrent SRS decreased 12-month cumulative incidence of local failure from 18.4% to 10.2% (P = 0.003), demonstrating synergy with use of concurrent SRS and HER2/EGFR TKIs and HER-2 antibody therapies. Unfortunately, the same synergy was not found in hormone receptor positive breast cancer patients with brain metastases treated with concurrent hormone therapy and SRS.

Better blood-brain barrier penetrating small tyrosine kinase inhibitors were subsequently developed after first generation trastuzumab and include lapatinib, afatinib, epertanib, neratinib tucatinib, pyrotinib and are used as systemic targeted therapy for patients with HER-2 positive breast cancer (53). Lapatinib was one of the first small molecule dual tyrosine kinase inhibitors targeted against EGFR 1 and HER-2 pathways. Kim et al. reported on 84 patients with 487 HER-2 amplified breast cancer brain metastases and treatment with SRS alone versus concurrent SRS and lapatinib and found that patients with concurrent therapy had higher rates of complete response (35% versus 11%, p = 0.008) (42). Furthermore, best per-lesion objective response was superior in the concurrent lapatinib group with a median 100% objective response versus 70% reduction (p < 0.001). This group did not find an increased risk of grade 2 radiation necrosis with concurrent therapy. However, an interesting finding of the study was that lapatinib did not have protective effects on distant intracranial failure rates; one of the main avenues in which concurrent SRS and systemic therapies were thought to theoretically improve overall survival.

Parsai et al. recently reported on 126 patients with HER-2 positive breast cancer with 479 brain metastases; 24 patients received concurrent treatment with SRS and lapatinib. They found SRS with concurrent lapatinib was associated with reduction in local failure at 12 months reported as 5.7% compared to 15.1% in the nonconcurrent therapy group (P < 0.01) (43). Local failure decreased for lesions less than or equal to 75th percentile by volume but did not have a significantly improved local failure rate for lesions greater than the 75th percentile. Furthermore, any use of lapatinib after development of brain metastases improved median survival compared to SRS alone (27.3 months versus 19.5 months, p = 0.03). Unlike the Kim et al. study, this supports the theory that targeted therapies may improve overall survival by controlling distant intracranial failure and systemic extracranial disease.

The majority of breast cancers are hormone receptor (HR) positive with endocrine therapy being the mainstay of systemic therapy and including antiestrogen therapy with selective estrogen receptor modulators, aromatase inhibitors, and/or selective estrogen receptor (ER) degraders and combination with cyclin-dependent kinase 4/6 inhibitors (54). Unfortunately, 15 to 20% of ER positive breast cancers are intrinsically resistant to endocrine therapy and another 30 to 40% develop resistance after treatment (55, 56). One of the described escape mechanisms contributing to hormone resistance involves activation of the second depending kinase 4 and 6 pathways in the presence of hormone receptor antagonists (44, 57). A study by Mills et al. reported HR positive breast cancers patients with hormonal therapy prior to stereotactic radiotherapy (SRT) report 2-year overall survival as low as 24% (58), however there is little literature investigating concurrent SRS with systemic endocrine therapy.

Figura et al. report on a retrospective study involving 15 patients and 42 lesions in patients with HR positive brain metastases treated with SRS or fractionated stereotactic radiotherapy (FSRT) within 6 months of CDK 4/6 inhibitor administration. Radiotherapy was delivered concurrently, before, or after CDK4/6 inhibitors in 18 (43%), 9 (21%), and 15 (36%) lesions, respectively (44). Fourteen percent of the cohort received CDK inhibition alone, 48% of the cohort CDK inhibition plus fulvestrant and 38% CDK inhibition plus an aromatase inhibitor. 6- and 12-month local control of treated lesions were reported as 88% and 88%, respectively, while 6- and 12-month distant brain control was 61% and 39%, respectively, with median overall survival of 36.7 months from diagnosis of brain metastases (44). A significant portion of this cohort received concurrent therapies and the median overall survival was much higher at 36.7 months than the 13.3 month median overall survival recently reported in a 2021 ASCO meeting abstract by Wang et al. in patients who received SRS upfront for treatment of HR+/HER-2 negative breast cancer brain metastases (59). This led the Figura group to conclude that SRT to breast cancer brain metastases is well-tolerated without significant increase in neurotoxicity when

combined with CDK 4/6 inhibitors, and although brain metastases control rates were similar to prior historical data, there was a synergy between SRT and the systemic therapy which prolonged median overall survival.

Melanoma

Approximately 99,780 patients will be diagnosed with melanoma in the United States in 2022 (60), with nearly half developing brain metastases over the course of their disease (61). Approximately 40 to 60% of cutaneous melanoma patients have BRAF mutations which results in constitutive activation of BRAF, and downstream mitogen activated protein kinase (MAPK) pathway (62, 63). For this reason, many of the targeted therapies used in the treatment of metastatic melanoma include BRAF inhibitors such as dabrafenib, vemurafenib, encorafenib, and MEK1/2 inhibitors such as binimetinib (53).

A 2015 study by Ahmed at al evaluating LINAC-based SRS with concurrent vemurafenib found that patients had a median overall survival from the date of SRS of 7.2 months with a median survival from date of brain metastases diagnosis of 11.9 months (64). In this study, therapies were truly concurrent, with vemurafenib being held only 2 to 3 days pre- and post-SRS treatment. It did not show any evidence of increased toxicity with a combination of SRS and targeted therapy and concluded that concurrent therapy appeared to be safe and effective. Subsequent studies have further investigated and similarly reported synergy between SRS and targeted therapies in patients with melanoma brain metastases (**Table 3**).

Xu et al. subsequently evaluated use of BRAF kinase inhibitors in conjunction with SRS for patients with melanoma brain metastases and found that patients with BRAF mutations treated with BRAF inhibitors had improved median survival times from diagnosis, and after SRS, of 23 months and 13 months ($p < 0.01$), respectively (65). This was statistically significant compared to the BRAF wild-type group. In conjunction with SRS, they reported a local control rate of 92% at 1 year in patients with BRAF mutations treated with BRAF inhibitors, compared to 82.4% in patients with BRAF mutations not treated with BRAF inhibitors and 69.2% in patients who were BRAF wild-type.

A 2016 study by Ahmed at al investigating clinical outcomes in patients with melanoma brain metastases treated with SRS and anti-PD1, anti-CTLA4, BRAF/MEK inhibitors, BRAF inhibitors, and conventional therapy found distant 1-year disease control rate of 20% and 8% for BRAF/MEK inhibitors and BRAF inhibitors, respectively, and significantly improved overall survival for patients treated with anti-PD1, anti-CTLA4 and BRAF/MEK inhibitors when compared to those treated with conventional chemotherapy (66). This study is important because it demonstrated that targeted therapies and immunotherapies synergistically contribute to SRS by helping improve distant brain metastases control rates.

An important paper demonstrating synergy between multiple therapies is by Kotecha et al, in which 366 patients were treated for 1336 melanoma brain metastases. They found that younger age, lack of extracranial metastases, better Karnofsky performance status score, fewer melanoma brain metastases, as well as treatment with BRAF inhibitors, anti-PD1/CTLA4 therapies, or cytokine therapy were significantly associated with improved overall survival (67). Among patients who underwent SRS, patients with BRAF mutant lesions had a 12-month local failure rate of 6% compared to 22% and BRAF wild-type patients. Furthermore, 12-month local failure rates in patients treated with BRAF inhibitors and PD1/CTLA-4 agents were 1% and 7%, respectively. On multivariate analysis, BRAF inhibition within 30 days of SRS was protective against local failure ($p = 0.01$); 12-month radiation necrosis rates were 0% in patients treated with BRAF inhibitors, 2% in patients treated with PD1/CTLA-4 inhibitors, and 6% of patients treated with cytokine therapies.

Similarly, Murphy et al. found that following concurrent SRS and immunotherapy within 30 days, patients had significantly longer period of intracranial progression free survival than those treated without concurrent therapy, 19 months versus 3.4 months ($P < 0.0001$), with no grade 4-5 toxicities observed (68). A multicenter retrospective study by Mastorakos et al. evaluated patients with BRAF-mutated melanoma brain metastases and BRAF kinase inhibitor use in conjunction with SRS and found that BRAF-mutated patients who received BRAF inhibitors following SRS had improved survival compared to patients who received it before ($p<0.001$) or concurrently ($p = 0.007$) (69). This study supports synergy between use of targeted therapies and SRS but highlighted the importance of their timing in order to maximize clinical benefit.

Schaule et al. conducted a retrospective analysis of 110 patients treated with concurrent targeted or immunotherapy and stereotactic radiotherapy and found that cumulative brain metastases volume ($p = 0.04$), timing of metastases (syn-versus metachronous) ($p = 0.01$) and systemic therapy with concurrent immunotherapy ($p = 0.005$) significantly improved overall survival; with these findings they established a volume-timing-systemic therapy (VATS) score with point values ascribed to the aforementioned factors and median overall survival as of 34.5 months in patients with a VATS score of 2 ($p = 0.03$) (70).

With multiple studies demonstrating synergy between SRS and systemic therapies, Wang et al. sought to identify clinicopathologic characteristics and prognostic factors in patients with melanoma brain metastases. They found that in patients with BRAF-mutated melanoma brain metastases, first-line treatment with BRAF/MEK inhibitor therapy improved overall survival compared to patients treated with first-line therapy with anti-PD1 ($P = 0.043$) (71). This is the first study in the literature promoting BRAF/MEK inhibitors as a superior first-line therapy in patient with BRAF-mutated melanoma brain metastases. Although it did not specifically seek to elucidate synergy with SRS, 49% of this cohort also received SRS.

A 2021 study by Wegner et al. concluded that immunotherapy within 7 days of SRS had a statistically significant association with improved outcomes and 3-year survival rate of 55% (P equals 0.0153) (72). This study also illustrating that the timing of systemic therapy with relation to SRS delivery may affect clinical outcomes. Lastly, a 2021 meta-analysis including 8 studies and involving 976 patients with

TABLE 3 | Studies evaluating synergy between SRS and targeted therapies in patients with melanoma brain metastases (BrM).

Study Identifier	Study Period	Study Size (n = patients)	Treatment/Intervention Groups	Results	References
Ahmed et al.	2010-2013	N = 24	Patients with metastatic melanoma BrM treated with SRS while on vemurafenib	• Fourteen (58%) patients had distant brain failure at a median of 3.4 months • Median OS from the date of SRS = 7.2 months (range 1.5–26.8 months) • Median OS from date of BrM diagnosis = 11.9 months (range 1.5–28.5 months) • No evidence of increased toxicity with concurrent SRS + vemurafenib	(64)
Xu et al.	2010-2014	N = 65	Patients with metastatic melanoma BrM treated with SRS +/- BRAF inhibitors	• Median OS after diagnosis of BrM and after SRS were favorable in patients with BRAF mutation and treated with SRS + BRAFi (23 months and 13, respectively, $p < 0.01$) • SRS local tumor control rate of 89.4% in the entire cohort • Local control rate improved in the patients treated with SRS + BRAFi compared to BRAF mutated patients without BRAFi treatment and wild-type patients	(65)
Ahmed et al.	2007-2015	N = 96	Patients with metastatic melanoma BrM treated with single-session SRS and anti-PD-1 therapy, anti-CTLA-4 therapy, BRAF/MEK inhibitors(i), BRAFi, or conventional chemotherapy	• 12-month distant control rates = 38%, 21%, 20%, 8%, and 5% ($P = 0.008$) for SRS with anti-PD-1 therapies, anti-CTLA-4 therapy, BRAF/MEKi, BRAFi, and conventional chemotherapy, respectively. • No significant differences in local control rates • Treatment with anti-PD-1 therapy, anti-CTLA-4 therapy, or BRAF/MEKi significantly improved OS on both univariate and multivariate analyses when compared with conventional chemotherapy	(66)
Kotecha et al.	1987-2014	N = 366	Patients with metastatic melanoma BrM treated with SRS + targeted and immunotherapies	• On MVA, younger age, lack of extracranial mets, better KPS, and fewer BrM, and treatment with BRAF inhibitors, anti–PD-1/CTLA-4 therapy, or cytokine therapy were significantly associated with OS • For patients who underwent SRS, the 12-month LF rate was lower among those with BRAFm lesions *vs* BRAFwt lesions (6% *vs* 22%, $p < 0.01$) • 12-month LF rates among lesions treated with BRAFi and PD-1/CTLA-4 agents were 1% and 7%, respectively • On MVA, BRAF inhibition within 30 days of SRS was protective against LF (HR 0.08, 95% CI 0.01–0.55; $p = 0.01$) • 12-month rates of RN were low among lesions treated with BRAFi (0%), PD-1/CTLA-4 inhibitors (2%) and cytokine therapies (6%)	(67)
Murphy et al.	2011-2017	N = 26	Patients with metastatic melanoma BrM treated using pembrolizumab, nivolumab and/or ipilimumab, sequentially, or concurrently with SRS	• Median OS = 26.1 months • Following concurrent SRS and immunotherapy, patients had a significantly longer period of intracranial progression free survival than those treated with nonconcurrent therapy, 19 months versus 3.4 months ($P < 0.0001$) • No grade 4-5 toxicities were observed	(68)
Mastorakos et al.	2011-2015	N = 198	Patients with metastatic melanoma BrM treated with SRS +/- BRAF kinase inhibitors	• On MVA, BRAF mutation was an independent, positive prognostic factor with a hazard ratio of 0.59 • BRAF mutated patients who received BRAFi following SRS had improved survival compared to those who received it before ($P < .001$) or concurrently ($P = .007$) • PD-1 inhibitors improved survival, with more pronounced effect in patients not carrying the BRAF mutation • Among the patients treated with BRAFi, 10.4% developed intracerebral hematoma (ICH), in comparison to 3% of patients not treated with BRAFi ($P = .03$)	(69)
Schaule et al.	2011-2018	N = 110	Patients with metastatic melanoma BrM treated with targeted therapies or immunotherapy and concurrent (≤30 days) SRT	• Median OS = 8.4 months • Cumulative BrM volume, timing of metastases (syn- *vs*. metachronous) and systemic therapy with concurrent IT influenced OS significantly • Based on these parameters, the VTS (volume-timing-systemic therapy) score was established and stratified patients into three groups with a median OS of 5.1, 18.9 and 34.5 months, respectively ($p < 0.05$)	(70)
Wang et al.	2007-2019	N = 431	Patients with metastatic melanoma BrM treated with various local and systemic therapies	• Mucosal subtype ($p = 0.022$), LDH level ($p = 0.005$), no extracranial metastasis ($p = 0.01$), concurrent liver metastasis ($p = 0.004$), local treatment ($p = 0.001$) and use of PD-1 inhibitors ($p < 0.0001$) were independent prognostic factors for OS • Mucosal subtype BrM had poor response to PD-1 inhibitors ($p = 0.007$), with a shorter intracranial PFS than other subtypes	(71)

(Continued)

TABLE 3 | Continued

Study Identifier	Study Period	Study Size (n = patients)	Treatment/Intervention Groups	Results	References
Wegner et al.	2010-2015	N = 247	Patients with metastatic melanoma BrM treated with immunotherapy and SRS	• In patients with BRAF mutated melanoma BrM, first-line BRAF/MEK inhibitor therapy had an advantage in OS compared to the first-line anti-PD-1 therapy group (p = 0.043) • Immunotherapy prior to SRS, within 0–7 days of SRS, and greater than 7 days from SRS had 3-year survival rates of 21%, 55%, and 35%, respectively (p = 0.0153) • Multivariable Cox regression identified lack of extracranial disease, more recent year of treatment, and time from SRS to immunotherapy of 0–7 days as predictors of improved survival	(72)
Khan et al.	2010 meta-analysis	N = 976	Searched for studies comparing patients with metastatic melanoma BrM treated with SRS +/- BRAF inhibitors	• Survival significantly improved for patients receiving BRAF inhibitor plus SRS *vs* SRS alone as assessed from the time of SRS induction (p < 0.00001), from the time of brain metastasis diagnosis (p < 0.00001), or from the time of primary diagnosis (p = 0.02) • Dual therapy was also associated with improved local control (p = 0.03) • Intracranial hemorrhage was higher in patients receiving BRAF inhibitors plus SRS than in those receiving SRS alone (p = 0.004)	(73)

melanoma brain metastases found that dual therapy of BRAF inhibitors in combination with SRS improved survival (P < 0.00001) and local control (P = 0.03), further supporting the literature of synergy between these two therapies (73).

Renal Cell Carcinoma

Approximately 320,000 patients are diagnosed with renal cell carcinoma (RCC) worldwide (74), with 10% to 16% developing brain metastases (75, 76). Eighty percent of all renal cell carcinoma cases are clear-cell type and 90% of these develop a von-Hippel-Lindau tumor suppressor gene mutation that leads to activation of multiple genes including vascular endothelial growth factor (VEGF) with subsequent angiogenesis being a primary mechanism of progression in advanced RCC (77). For this reason, targeted therapies against VEGF-tyrosine kinases are included as part of first-line therapy for metastatic renal cell carcinoma. VEGF-tyrosine kinase inhibitors include sunitinib, pazopanib, and sorafenib; with newer multi-targeted tyrosine kinase inhibitors such as cabozantinib, which inhibits VEGFR/MET/AXL (78), and lenvatinib, which inhibits VEGFR 1, 2, 3/FGFR1, 2, 3, 4/PDGFR alpha/RET/KIT (79). Many studies assessing the synergy between SRS and targeted therapies utilize these agents (**Table 4**). Other targeted therapies including mammalian target of rapamycin complex 1 (mTORC1) inhibitors and immune checkpoint inhibitors, including anti-programmed death receptor 1 (PD-1) and anti-cytotoxic T-lymphocyte associated protein 4 (CTLA-4) monoclonal antibodies, have also been used to treat and improve overall survival in patients with extracranial metastatic renal cell carcinoma (87–91).

When reviewing outcomes for 61 patients with renal cell carcinoma brain metastases treated with targeted agents and gamma knife radiosurgery, Cochran et al. showed that the median survival for patients receiving targeted agents was 16.6 months compared to 7.2 months, with freedom from local failure at 1 year being 93% versus 60% (p = 0.01) (80). Their multivariate analysis also showed that utilization of targeted therapies was the only factor that predicted improved survival. Subsequently, Vickers et al. assessed prognostic factors for survival in patients with RCC brain metastases treated with targeted therapies and found KPS less than 80, diagnosis to treatment with targeted therapy less than a year, and greater than 4 brain metastases were associated with worse survival (81). In this study, 81.1% received whole brain radiotherapy (WBRT) and 24.8% received SRS and they found that patients diagnosed with brain metastases at the initiation of targeted therapy had a survival of 19.1 months, while patients who developed brain metastases while receiving targeted therapy had a survival of 6.3 months.

Bates et al. reviewed 25 consecutive patients who received radiotherapy consisting of WBRT and SRS in addition to targeted therapies and found no significant difference in overall survival or brain progression free survival with concurrent use of kinase inhibitors and radiotherapy (82). Although not statistically significant, there was a trend towards improved median overall survival in patients treated with concurrent kinase inhibitors compared to those not treated with concurrent kinase inhibitors, 7.3 months versus 4.1 months, respectively. Furthermore, this study only included first generation kinase inhibitors (sunitinib, sorafenib, or pazopanib) and not newer multi-targeted kinase inhibitors. Subsequent to these findings, Barata et al. reported on the effect of switching systemic treatment after SRS for oligoprogressive metastatic renal cell carcinoma and found no difference in median overall survival between patients who remain on the same systemic therapy and those who switched to another systemic therapy after SRS for their progressive disease (83). Those who remained on the same systemic therapy had a median overall survival of 24.2 months and those were switched 27.1 months (p = 0.381). Patients with progression outside of the SRS sites who switched systemic therapy had a significantly worse overall survival of 8.5

TABLE 4 | Studies evaluating synergy between SRS and targeted therapies in patients with renal cell carcinoma brain metastases (BrM).

Study Identifier	Study Period	Study Size (n = patients)	Treatment/Intervention Groups	Results	References
Cochran et al.	1999-2010	N = 61	Patients with metastatic renal cell carcinoma BrM treated with Gamma Knife surgery and targeted agents such as tyrosine kinase inhibitors, mammalian target of rapamycin inhibitors, and bevacizumab	• Median survival for patients receiving targeted agents was 16.6 months compared with 7.2 months for those not receiving targeted therapy (p = 0.04). • Freedom from local failure at 1 year was 93% versus 60% for patients receiving and those not receiving targeted agents, respectively (p = 0.01) • MVA showed use of targeted agents (hazard ratio 3.02, p = 0.003) was the only factor that predicted for improved survival	(80)
Vickers et al.	2005-2011	N = 106	Patients with metastatic renal cell carcinoma BrM treated with targeted therapies; 77 patients were treated with sunitinib, 23 patients with sorafenib, 5 with bevacizumab, and 1 with temsirolimus. 81.1% received WBRT and 24.8% received SRS	• On MVA, KPS < 80%, diagnosis to treatment with targeted therapy < 1 year, and a higher number of BrM (>4) was associated with worse survival from time of diagnosis with BrM • Patients diagnosed with BrM at the initiation of targeted therapy had a survival of 19.1 months while patients who developed BrM while receiving targeted therapy had a survival of 6.3 months	(81)
Bates et al.	2003-2014	N = 25	Patients with metastatic renal cell carcinoma BrM who received WBRT, SRS, or both; 28% of patients were receiving a concurrent kinase inhibitors (KI) at the time of radiotherapy	• No significant difference in overall survival or brain progression free survival (BPFS) for SRS compared with WBRT or WBRT and SRS combined • Concurrent use of KI was not associated with any change in OS or BPFS	(82)
Barata et al.	2005-2017	N = 95	Patients with metastatic clear cell renal carcinoma BrM treated with SRS and stratified by changing or continuing systemic treatment (VEGFR tyrosine kinase inhibitors, mTOR inhibitors, immune checkpoint, or other therapies)	• Local control with SRS was achieved in 85% of the patients • Most common systemic treatment at SRS included anti-vascular endothelial growth factor (67%), mammalian target of rapamycin (14%), and programmed cell death protein 1 inhibitors (9%) • No difference in median overall survival was found for the STAY and SWITCH groups (24.2 *vs*. 27.1 months; p = .381) but was significantly longer than patients with progression outside of the SRS sites who switched systemic therapy (8.5 months; p = .025)	(83)
Sperduto et al.	2006-2015	N = 711	Patients with metastatic renal cell carcinoma with new BrM treated with various regimens of radiotherapy/ targeted therapies	• Median survival 12 months • Four prognostic factors (Karnofsky performance status, extracranial metastases, number of BrM, and hemoglobin b) were significant for survival after the diagnosis of BrM • Of the 6 drug types studied, only cytokine use after BrM was associated with improved survival • Use of WBRT declined from 50% to 22%, and the use of SRS increased from 46% to 58%	(77)
Juloori et al.	1998-2015	N = 367	Patients with metastatic renal cell carcinoma BrM treated with various regimens of radiotherapy/targeted therapies	• Median OS was 9.7 months • KPS and number of BrM were the only factors prognostic for OS • 147 patients (39%) received VEGFR tyrosine kinase inhibitors (TKIs) • Median OS was significantly greater among patients receiving TKIs (16.8 *vs* 7.3 months, p < 0.001) • On MVA, KPS, number of metastases, and TKI use remained significantly associated with OS • TKIs did not significantly decrease the 12-month cumulative incidence of local failure (11.4% *vs* 14.5%, p = 0.11) • On MVA, age, number of BrM, and lesion size remained associated with local failure • 12-month cumulative incidence of radiation necrosis was 8.0%; use of TKIs within 30 days of SRS was associated with a significantly increased 12-month	(84)

(Continued)

TABLE 4 | Continued

Study Identifier	Study Period	Study Size (n = patients)	Treatment/Intervention Groups	Results	References
Khan et al.	2020 systematic review and meta-analysis	N = 897	Studies comparing TKIs in combination with SRS to SRS alone for treatment of patients with metastatic renal cell carcinoma BrM	• Cumulative incidence of radiation necrosis (10.9% *vs* 6.4%, p = 0.04) • TKI use associated with better survival (HR 0.60 [0.52, 0.69], p < 0.00001) and local brain control (HR 0.34 [0.11, 0.98], p = 0.05) • SRS subgroup revealed significantly better survival (HR 0.61 [0.44, 0.83], p = 0.002) and local brain control (HR 0.19 [0.08, 0.45], p = 0.0002)	(85)
Stenman et al.	2005-2014	N = 43	Patients with metastatic renal cell carcinoma BrM treated with single-fraction gamma knife radiosurgery (sf-GKRS) in era of targeted agents (TA) and immune checkpoint inhibitors	• LC rates at 12 and 18 months were 97% and 90%, respectively • Median OS from the first sf-GKRS was 15.7months • Low serum albumin (HR for death 5.3), corticosteroid use pre-sf-GKRS (HR for death 5.8) and KPS < 80 (HR for death 9.1) were independently associated with worse OS • Adverse radiation effects (ARE) were seldom symptomatic and were associated with tumor volume, 10-Gy volume and pre-treatment perifocal edema • ARE were less common among patients treated with TA within 1 month of sf- GKRS	(86)

months. The systemic therapies in this retrospective study included 67% anti-VEGF inhibitors, 14% mTOR1 inhibitors, and 9% program cell death protein 1 inhibitors. Furthermore, although not the main endpoint of the study, median overall survival of patients who switched systemic therapies after SRS for oligoprogressive renal cell brain metastases was 27.1 months, which was an improvement in previously reported overall survival and illustrated the positive effects of a multimodal, multidisciplinary approach to improving outcomes for patients with oligoprogressive disease.

In a large multi-institutional retrospective study assessing 711 renal cell carcinoma patients with new brain metastases, prognostic factors affecting survival included Karnofsky performance status, extracranial metastases, number of brain metastases, and hemoglobin; only cytokine use after brain metastases was associated with improved survival (77). Conversely, initiation of VEGF targeted TKI, mTOR targeted TKI, immunotherapy, antiangiogenic drugs, and cytotoxic chemotherapy prior to diagnosis of brain metastases was associated with greater risk of death. Although demonstrating benefit for cytokine use, it is important to note that newer multi-target tyrosine kinase inhibitors were likely excluded from this study, given that the study recruited participants up until 2015 and many of the newer multi-target tyrosine kinase inhibitors were subsequently developed.

In 2020, Juloori et al. reported on overall survival and response to radiation and targeted therapy in 367 patients with 912 renal cell brain metastases. They found that median overall survival was significantly greater among patients receiving TKI's (16.8 versus 7.3 months, p < 0.001) and that TKI use was significantly associated with improved overall survival after multivariate analysis (84). Similarly, a meta-analysis by Khan et al. evaluating the impact of TKI use combined with radiation therapy (n= 897) found that TKI use was associated with better survival (HR 0.60 [0.52, 0.69], p < 0.00001) and local control (HR 0.34 [0.11, 0.98], p = 0.05), although it did not affect distant brain control and brain progression free survival (85).

Finally, the most recent study by Stenman et al. in 2021 evaluated 43 patients with 194 targets that were irradiated with 88% of the cohort also receiving targeted therapies. This cohort was treated with single fraction gamma knife radiosurgery (sf-GKRS) after a median time of 8.5 months from metastatic renal cell carcinoma diagnosis and subsequent to sf-GKRS had a median overall survival of 15.7 months; reflecting a cumulative median overall survival of 24.2 months (86). Although the study did not show targeted agents to be associated with improved survival, when compared to historical data, a median overall survival at 24.2 months is an improvement in overall survival and supports the existence of synergy between targeted therapies and SRS. However, optimal administration timing of these therapies and physiologic explanation of their interaction remains to be elucidated.

DISCUSSION

Synergy between SRS and targeted therapies has been demonstrated and found to improve outcomes for patients with non-small cell lung, HER-2 positive and endocrine receptor positive breast, melanoma, and renal cell carcinoma brain metastases. The varied pathophysiological mechanisms behind radiation-induced synergy are beyond the scope of this review; they include but are not limited to: increase in expression of major histocompatibility complex class I, calreticulin, and Fas cell surface death receptor, release of high mobility group box 1 nuclear protein, activation of dendritic cells and enhanced tumor

antigen cross-presentation, increase in tumor-infiltrating lymphocyte density, and modulation of immune checkpoint molecule expression and regulatory T cells (92). Improvement in overall survival for patients with these diagnoses has only been made possible *via* a multidisciplinary approach between medical oncologists, radiation oncologists, and neurosurgeons. Several difficulties exist when trying to compare results from multiple studies assessing outcomes in patients with brain metastases. One challenge is that primary and secondary endpoints between studies vary, with some studies evaluating certain variables and other studies evaluating others. Some examples of primary and secondary endpoints include overall survival, rates of radiation necrosis, local and distant brain metastases control, and neurotoxicity. Standardization of primary and secondary endpoints would help to better compare outcomes of future studies. Another challenge with assessing the results of studies evaluating concurrent SRS with targeted therapy use is that the definition of "concurrent" also varies from study to study. Some studies define "concurrent" as actively on systemic therapy, others with systemic therapy only held for 1 to 2 days before and after SRS, while other studies define concurrent therapy as having occurred with the initiation of targeted therapy within 30 days, 3 months, 6 months or even within 12 months before or after SRS.

A final challenge is that the definition of synergy varies and that there are different types of synergy. One type of synergy involves additive, enhancing synergy from concurrent therapies in which the combined effects of two therapies contribute to a greater, durable clinical effect compared to if the individual therapies were applied alone and/or in sequential order at varying time points. This type of synergy has not been clearly supported in the literature. Another type of synergy involves cumulative synergy in which various therapies are applied at various time points to maximize therapeutic effects, increase progression free survival, overall survival, and quality of life for patients with brain metastases. Cumulative synergy is difficult to study given that comprehensive cancer care is tailored to each individual patient and that varied treatments may be implemented at varied timepoints depending on a patient's clinical status. Evaluation of cumulative synergy involves evaluation of treatment paradigms in their totality rather than a discrete response to a single treatment. The literature assessing cumulative synergy is lacking. An example of cumulative synergy was reported by Cristaudo et al. in which they described the clinical course of a patient with metastatic melanoma and 10 brain metastases which were treated with SRS with complete response (including untreated lesions), was then started on BRAF/MEK inhibitors and subsequently required other treatment modalities as new metastases occurred (93). Eight months after her initial SRS treatment she developed new metastases which responded to SRS once again and 7 months later had new lesions treated with whole brain radiotherapy and was started on immunotherapy. Twenty months after initial diagnosis the patient had a Karnofsky performance score of 100 with no radiologic signs of toxicity. This report demonstrates how therapies can exhibit a cumulative synergy and underscores the importance of a multidisciplinary approach in the treatment of patients with brain metastases. There is unlikely to be a one-time combination of therapies that will achieve permanent local and distant disease control. However, it is the cumulative synergy of various treatment modalities, used at various timepoints in clinical disease and in various combinations, that will likely lead to the most clinical benefit.

Future clinical trials with standardized inclusion criteria and shared endpoints are needed to better elucidate concurrent and cumulative synergy between SRS and targeted therapies in the treatment of patients with brain metastases. Newer targeted therapies proven to impact time to CNS progression and/or progression free survival must continue to be investigated for synergy with SRS. These future studies must include patients with active, symptomatic metastases and avoid over-recruitment of clinically silent, stable metastases. While important for future studies to focus on critical endpoints such as overall survival, response rate, and local control, it is equally important that they focus on understanding the toxicity associated with combination therapies. Additional basic science research is also needed to better understand brain metastases' molecular profiles, how they relate to their primary solid tumors, and how they may change after treatment with targeted therapy and radiotherapy. Lastly, multi-institutional collaboration is needed to achieve larger sample sizes, better external validity, faster accrual and, hopefully, more meaningful, positive results.

AUTHOR CONTRIBUTIONS

AE contributed to the conception of the manuscript. SR and AE contributed to the design of the manuscript. SR contributed to the data acquisition, assessment, and review; creation of initial manuscript/tables; and critical review and revision of the manuscript. DO, NT, MV, PF, HY, KA, and AE contributed to the critical review and revision of the manuscript. All authors contributed to the article and approved the submitted version.

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REVIEW
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Surgical Management of Brain Metastasis: Challenges and Nuances

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Brain metastasis is the most common type of intracranial tumor. The contemporary management of brain metastasis is a challenging issue and traditionally has carried a poor prognosis as these lesions typically occur in the setting of advanced cancer. However, improvement in systemic therapy, advances in radiation techniques and multimodal therapy tailored to the individual patient, has given hope to this patient population. Surgical resection has a well-established role in the management of brain metastasis. Here we discuss the evolving role of surgery in the treatment of this diverse patient population.

Keywords: brain metastasis, surgery, en bloc resection, LITT, LMD

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INTRODUCTION

Brain metastases represent the most common brain tumors in adults in the United States and outnumber primary brain tumors 5:1 (1, 2). Approximately 8-10% of patients with systemic cancer will develop brain metastasis (3–5). Lung cancer, breast cancer and melanoma represent the most common solid tumor pathologies to develop brain metastasis. Melanoma has the highest frequency with 40-60% of patients developing brain metastasis.6 While 37-50% of patients present with single brain metastasis, 50-63% have multiple brain lesions at presentation (6, 7). Historically brain metastasis prognosis is quite poor and more than half of the patients diagnosed with brain metastasis will die within 3-27 months of diagnosis (3–5). With advances in systemic therapy patients are living longer with advanced cancer with more opportunity to develop brain metastases (5–9). Brain metastasis represent a major source of morbidity in cancer patients and are a source of significant social and economic burden for patients and caregivers (10). Management of patients with brain metastases is complex and best performed by multispecialty teams consisting of medical oncologists, surgeons, and radiation oncologists and team members must appreciate the nuances of the available treatment paradigms in order to tailor individualized care. Surgery remains the cornerstone in brain metastasis management. Here we outline the surgical management of brain metastasis focusing on surgical challenges, nuances and decision-making.

SURGICAL MANAGEMENT OF SINGLE/SOLITARY BRAIN METASTASIS

A solitary brain metastasis is defined as one brain lesion without evidence of extracranial metastasis, whereas a single brain metastasis is one brain lesion with at least one other site of extracranial disease. The essential role of surgery in the treatment of single/solitary brain metastases is firmly established. Specifically, surgery can provide multiple pragmatic clinical benefits particularly in the

setting of a large (i.e. >2.5 cm maximal diameter) symptomatic lesion. Surgical resection is the most effective way to rapidly relieve mass effect, achieve cerebral decompression and subsequently reduce intracranial pressure (ICP). Further, brain metastases often cause cerebral edema, which can be severe and contribute to worsening neurological status. Steroid administration is typically the first option to address edema, but in the circumstance of refractory symptomatic edema, tumor resection is beneficial. Resection also reduces the length of time patients require steroid treatment thereby potentially limiting the development of steroid-induced medical complications. Lesions located in the posterior fossa (e.g. cerebellum) or intraventricular metastases can obstruct cerebrospinal fluid (CSF) flow resulting in hydrocephalus, which can also be addressed with resection of the obstructing mass. Additionally, brain metastases can cause seizures due to irritation of the surrounding cortex and surgery may help in optimizing seizure control. Finally, surgery can aid diagnosis when the etiology/pathology of the brain lesion is unclear; specifically in the circumstance of a new brain lesion with a negative systemic workup or in a patient with a history of an unknown primary. Notably approximately 11% of patients with a diagnosis of a primary cancer may have a non-metastatic brain lesion such as glioma (11). As such, if imaging characteristics favor a primary glial neoplasm, surgical biopsy may be warranted to guide the subsequent treatment plan.

In addition to the clinical benefits, surgical resection also provides a known survival advantage in the setting of single metastasis. The positive impact of surgery was solidified after the completion of two pivotal randomized clinical trials. The first was conducted by Patchell and colleagues, who randomized patients with a single brain metastasis to receive tumor resection followed by whole-brain radiation therapy (WBRT) (n = 25) versus WBRT alone (n = 23) (11). The authors found that patients in the surgical resection group survived significantly longer than patients treated with WBRT alone (median survival of 40 weeks versus 15 weeks, respectively). Surgery was also associated with significantly lower risk of local recurrence (20%) relative to WBRT alone (52%). Finally, surgical patients maintained functional independence [defined by a Karnofsky Performance Scale (KPS) score of >70] significantly longer (median, 38 weeks) relative to patients treated with only WBRT (median, 8 weeks). A second prospective randomized study by Vecht et al. also compared combination surgical resection plus radiation versus radiation alone in patients with a single brain lesion (12). Primary outcomes measures were overall survival and functionally independent survival (FIS). Combined treatment led to longer patient survival (p = 0.04) and a longer FIS (p = 0.06) compared with radiotherapy alone. This was most pronounced in patients with stable extracranial disease (median survival, 12 versus 7 months; median FIS, 9 versus 4 months). Overall, these two historic trials verified the substantial advantage surgery imparts.

In the modern treatment of single/solitary brain metastases, WBRT has given way to stereotactic radiosurgery (SRS) as an upfront treatment option, primarily due to the detrimental cognitive effects of WBRT (13–15). SRS is a specialized radiation technique in which a targeted dose of radiation is delivered to one or more intracranial lesions with high precision. SRS can be delivered in a single or multiple fractions and has become a standard of care in the management of brain metastasis. Even with the availability of this effective, minimally invasive treatment option, surgery continues to play a powerful role, particularly in the setting of large brain metastases. Prabhu et al. conducted a retrospective analysis of 213 patients with large brain metastases treated with single fraction SRS alone or surgery + SRS between 2005 and 2013 from two institutions (16). In this study, large brain metastases were defined as ≥4 cm^3 (2 cm in diameter) and surgical gross total resection (GTR) was required for inclusion. Overall, 213 patients with 223 treated brain metastases were included; 66 (30%) were treated with SRS alone and 157 (70%) with combination surgery + SRS (pre-operative or post-operative). Patients in the combination therapy group had higher tumor volumes (median 9.6 cm^3) compared to patients receiving SRS alone (5.9 cm^3; p<0.001). Patients receiving surgery + SRS demonstrated significantly longer survival compared with those receiving SRS alone with a median survival of 15.2 months versus 10 months (p < 0.01) respectively. Overall survival was significantly higher in the surgery + SRS group (2-year OS rate, 38.9% vs 19.8%; p= 0.01). Finally, the local recurrence (LR) rate was significantly lower with surgery + SRS (1-year LR rate, 36.7% versus 20.5%; p= 0.07) (16). This study highlights the critical role of surgery even with the availability of SRS.

Patient Selection

Thoughtful patient selection is the foundation of surgical decision-making. The survival benefit of surgical resection can be significantly diluted if surgical candidates are not carefully selected. Brain metastasis patients are a challenging population with unique factors that should be balanced when considering surgery. As brain metastases are often a consequence of advanced systemic cancer, many patients are elderly and may have age-related medical co-morbidities that increase surgical risk (17). Cancer patients are higher risk for thromboembolic complications (e.g. deep vein thrombosis, pulmonary emboli) throughout the course of their illness requiring anti-coagulation and this must be taken into consideration for surgical planning to reduce the risk of intra- and post-operative bleeding complications. Furthermore, venous thromboembolic complications are reported to be the most common post-operative medical complication of brain metastases surgery (17). Another critical consideration is that surgery will typically delay the initiation of systemic therapy and/or radiation for weeks to allow for post-operative healing. Notably, even minor post-operative wound healing issues or surgical site infections can delay therapy even longer and be detrimental to patient care, particularly if re-operation/open surgical debridement is required to address infection. Finally, metastases in eloquent cortex (motor and language centers) pose a particular concern as the development of a new neurological deficit can significantly impact quality of life. Further, a major post-operative neurological deficit may significantly reduce a patient's

functional status and harm candidacy for aggressive adjuvant therapy and/or clinical trial enrollment. Additionally, surgeries in functional cortical locations may require longer recovery and rehabilitation times, which must be carefully balanced with a patient's life expectancy.

Overall, younger patients (<65 years) with high functional status (KPS score ≥ 70), controlled systemic disease and no extracranial metastases are considered to be the most suitable candidates based on the classic recursive partitioning analysis (RPA) classification system developed by the RTOG (Radiation Therapy Oncology Group). In a pivotal study by Tenduklar et al. (18) the authors analyzed the outcome of 271 patients undergoing resection for a solitary brain metastasis. They reported that patient survival was significantly correlated with RPA class and specifically patients with the above-mentioned attributes had the best prognosis suggesting this patient population most suitable for surgical resection (18). A diagnosis specific graded prognostic assessment (GPA) is another prognostic algorithm that accounts for tumor histology and was developed based on the analysis of over 4000 patients with brain metastasis including breast, lung, GI, melanoma and renal cell carcinoma (19–21).

Even though the characteristics of the "ideal" surgical candidate are well described, there are circumstances where surgery may be considered in patients that do not meet these specific criteria. First in the setting of an emergency, there is likely not adequate time for establishment of systemic disease status prior to proceeding with resection as the priority would be to immediately relieve life-threatening elevated ICP. Second, patient functional status at presentation may be modifiable and improved with surgical intervention. For example, a patient may present with a metastasis in the motor area causing hemiplegia and an associated low functional status. However, resection of the symptomatic lesion can restore functional status, improve KPS and allow the patient to be a candidate for systemic therapy post-operatively. Hence, surgery may be considered in a patient with lower functional status, if that condition is a direct consequence of the metastatic lesion and is potentially reversible. Third, in regards to patients with uncontrolled systemic disease, it may be prudent to consider whether the patient's uncontrolled disease is at initial presentation (where systemic treatment options remain available) or in the setting of refractory disease progression and multiple failed treatment regimens as these represent vastly different clinical scenarios. Moreover, with advances in molecular testing, targeted therapy and immunotherapy, subpopulations with advanced disease are surviving longer so surgical consideration may be at times reasonable, particularly if the patient is symptomatic. Additionally, it is important to note that the classic prognostic algorithms do not factor in patient medical co-morbidities that can impact post-operative morbidity, re-admission and mortality (17). The current algorithms focus on age, however a healthy, 75-year old patient with no medical comorbidities maybe be a more desirable surgical candidate than a 60 year-old with multiple crippling medial ailments. Overall, the careful consideration of surgical candidacy is critical in the management of brain metastasis. Brain metastasis patients represent a complex population patient, selection for surgical resection can be highly nuanced and a multi-disciplinary evaluation is invaluable.

Impact of Surgical Technique

The maximal benefit of surgical resection is dependent on both extent of resection and surgical technique. In metastasis surgery, radiographic gross total resection (GTR) is the goal whenever feasible as it improves patient outcome (18, 22). A retrospective analysis of 271 patients from single institution (1984-2004) found that GTR of metastasis was associated with a median overall survival of 10.6 months versus subtotal resection (STR; 8.7 months) (18). Although these results did not reach statistical significance (p=0.07). A more recent study (1995-2011) retrospectively evaluated the outcomes of 157 patients with single brain metastasis and reported a median post-operative survival of 19.3 months (22). Among the 157 patients; the majority received post-operative radiation; whole-brain radiotherapy (11%) and radiosurgery (69%). Multivariate analysis showed that extent of surgical resection was significantly correlated with survival. Median survival was 20.4 months following GTR and 15.1 months after STR (p= 0.016).

In addition to extent of resection, there is substantial data underscoring the importance of surgical technique on the outcome of brain metastasis surgery. Historically, brain metastasis surgery was often accomplished *via* a piecemeal resection. This method of resection involves internal debulking of the mass followed by removal of the tumor capsule in multiple pieces. En bloc resection, on the other hand, entails circumferential dissection of the tumor along the brain-tumor interface without violating the tumor capsule. This method avoids spillage of tumor contents into the resection cavity. From a purely technical standpoint, en bloc resection is helpful as brain metastasis can be vascular and dissection along the tumor margin (as opposed to entering a vascular lesion) may reduce intraoperative bleeding and reduce operative time. Furthermore, dissection along the brain-tumor interface allows for better definition of tumor borders, aiding in the accomplishment of a complete resection.

Beyond the technical aspects, en bloc resection also positively impacts patient outcome. In a landmark study by Patel et al., the authors evaluated the predictors of local recurrence after resection of untreated single brain metastasis. This was a single institution study that included 570 surgical cases; 35% of cases done with a piecemeal resection technique and 65% en bloc. The overall rate of local recurrence was 15%. This study identified two factors that impacted local recurrence: tumor volume (greater than 9.7cm^3) and resection technique. Specifically, the authors reported that patients who had piecemeal GTR were 1.7 times more likely to develop local recurrence compared to patients who had an en bloc resection (p = 0.03) (23). This was one of the earliest studies advocating for en bloc resection. A follow-up study at the same institution, which included an analysis of 1033 patients with single brain metastases also determined that en bloc resection was not associated with increased complication rates even for tumors in functional

areas of the brain (i.e. eloquent cortex) emphasizing that en bloc resection is both effective and safe (24).

In addition to local recurrence, distance recurrence and/or the development of leptomeningeal spread is major concern in the management of brain metastases. Leptomeningeal disease (LMD), which entails tumor spread to the leptomeninges and/or CSF, is a devastating form of metastatic dissemination associated with a very poor prognosis (25, 26). Notably, another reported advantage of en bloc resection includes a lower risk of LMD. A single institution study examined the risk of LMD following resection of posterior fossa metastasis (27). Posterior fossa/infratentorial (e.g. cerebellum/vermis) metastases are of particular concern for LMD due to their proximity to ventricular/CSF spaces and the opportunity for CSF spread. In this study, Suki et al., analyzed the outcome of 379 patients with posterior lesions undergoing either SRS (n = 119) or open surgical resection (n = 260). The primary outcome measure was development of LMD. Interestingly piecemeal resection was associated with significantly higher LMD risk compared to en bloc resection (p = 0.006) or SRS (p = 0.006). Specifically, of the patients undergoing en bloc resection only 5.7% developed LMD compared with 13.9% of piecemeal resection patients. It is hypothesized that an en bloc resection provides this advantage because it avoids violation of the tumor capsule, which could lead to spillage of tumor contents into CSF space. A similar investigation was conducted in patients with supratentorial brain metastasis (28). This study included 827 patients with a supratentorial brain metastasis that underwent surgical resection (191 piecemeal and 351 en bloc) or SRS (n = 295). Once again the authors reported that en bloc resection was associated with a lower incidence of LMD compared to piecemeal resection. This difference was most pronounced in patients with melanoma brain metastases (28).

SURGICAL MANAGEMENT OF MULTIPLE BRAIN METATASES

Approximately 30–50% of brain metastasis patients present with multiple lesions (6, 7). In contrast to single/solitary brain metastasis, in which the beneficial role of surgical resection has been established by prospective, randomized trials (11, 12), no class I evidence exists for the role of surgery patients with multiple brain metastases. There are specifically are no prospective randomized studies formally evaluating the impact of surgery on patient survival in the setting of multiple brain metastases. However, in patients with multiple brain metastases, surgery may be beneficial to provide symptomatic relief and/or improve KPS, particularly after resection of large dominant lesion(s). In a recent multi-center, retrospective study, the authors analyzed the outcome of 750 surgical patients following resection (29). This study included patients with multiple brain metastases (39% of cases). The authors reported that functional status was significantly improved by surgical resection, with a median preoperative KPS of 80 increasing to 90 post-resection (p<0.0001). Furthermore, systemic treatment was more frequently provided to patients with KPS >70 (p<0.0001) and this was associated with improved patient survival (16 versus 7 months; p<0.0001).

Even though prospective data is limited, retrospective studies on multiple brain metastases indicate that the best survival outcome is obtained when all lesions are resected if feasible (30–32). Bindal et al. evaluated 56 patients who underwent resection for multiple brain metastases (30). Thirty patients had one or more lesions left unresected (Group A) and 26 patients had all lesions resected (Group B). This study also included a matched cohort of patients with a single metastasis resected for comparison (n = 26; Group C). These authors reported that symptoms improved in 65% of Group A patients compared to 83% in Group B. Furthermore, the survival of patients who had all lesions resected was also significantly longer than in patients who had residual lesions (14 versus 6 months respectively). Notably there was no significant survival difference between patients who had multiple metastases with complete resection of all (Group B) and those who had a single metastasis removed (Group C) (30). These results have been duplicated in other surgical series. Salvalti et al. retrospectively analyzed the outcome of 32 patients undergoing resection for multiple brain metastases (2-3 lesions). They compared the outcome of this cohort to 30 patients undergoing resection for a single brain metastasis. Neurological status improved in approximately 60% of multiple metastases patients post resection and there was no significant difference in survival between patients who had multiple metastases resected compared to those with a single lesion resected (31). Another study by Schakert et al. evaluated 127 patients with multiple brain metastasis (32). Similar to the prior study, patients who had all lesions resected had prolonged survival compared to patients with residual lesions (10.6 versus 5.8 months respectively) (32).

In summary, there is increasing data to support surgical resection in patients with multiple brain metastases. Resection can improve functional outcome and potentially improve candidacy for adjuvant therapy, which is critical to overall cancer prognosis. However, most studies are small series and larger prospective studies are needed. Additionally, the majority of surgical series are not pathology specific. With advances in molecular profiling and targeted systemic treatments, tumor specific studies are warranted to fully capture the benefit of resection in this population.

SURGICAL MANAGEMENT OF RECURRENT BRAIN METATASES AND RADIATION NECROSIS

Even with maximal therapy, including resection, brain metastases can recur locally or distantly, requiring further intervention. The challenge is that most patients with recurrent lesions have already undergone extensive cranial treatment (resection, SRS, and/or WBRT), limiting additional therapeutic options. In the setting of large, symptomatic, and/or previously treated brain metastases, repeat surgical resection is a reasonable treatment option in

appropriately selected patients. A retrospective analysis reported the outcome of 67 patients with recurrent brain metastasis undergoing repeat resection. All patients had surgery as a component of their initial treatment. The majority of patients had a distant recurrence (n = 35) and GTR was achieved in most patients with solitary metastases. The overall median post-operative survival time was 7.5 months. Multivariate analysis demonstrated that RPA class and time to recurrence were both significant predictors of patient survival. Specifically, in patients who recurred occurred within 200 days of the initial resection, the median survival time was only 6 months compared with patients who recurred after 200 days (9.2 months) (33).

Management of brain metastasis after failed SRS is a particular challenge. Local progression and/or radiation necrosis is reported to occur in approximately 20% of brain metastasis treated with SRS (34–36). This patient population will pose a growing concern as it becomes more common to treat a higher number of brain metastases with upfront SRS in order to avoid the cognitive side effects of WBRT (13, 37). Each lesion treated with SRS theoretically has the potential to fail or develop into radiation necrosis. Radiation necrosis is a known complication of SRS and is characterized by a progressive radiation-induced inflammatory reaction, which can result in neurological symptoms (38, 39). It can be a difficult condition to manage for several reasons. First, radiographically, it can be difficult to distinguish radiation necrosis from true tumor progression as both enhance on post contrast imaging and can cause cerebral edema and mass effect. Even with advanced imaging modalities such as MR mass spectroscopy, perfusion and diffusion studies and positron emission tomography (PET) (40–46), diagnosis cannot be confirmed without pathological diagnosis. The correct diagnosis can be critical for deciding the next treatment step since radiation necrosis can be observed (especially if small and/or asymptomatic) while tumor progression necessitates treatment escalation. Furthermore, the results of imaging studies can be inconclusive and these recurrent/failed treatment lesions can sometimes be mixed, with components of both radiation necrosis and active progressive tumor. Second, patients with radiation necrosis can have severe symptoms; particularly because the intense inflammatory reaction can cause extensive cerebral edema, which sometimes can be disproportionate to the size of the enhancing mass itself. Third, often the first line of therapy is steroid treatment to reduce symptomatic edema. And for some patients, a slow steroid taper (over 2-3 weeks) will be sufficient to address clinical symptoms and stabilize or improve radiographic changes without the need for additional intervention. However, in a subset of patients, radiation necrosis can become progressive and refractory to steroid therapy. Since long-term steroid use is suboptimal due to risk of medical complications, in such cases additional interventions may be required. There are effective medical therapies such as Avastin (bevacizumab), which has shown notable benefit in the treatment of radiation necrosis (47, 48) however; we will focus the surgical treatment options.

There are several studies evaluating the effectiveness of salvage surgery for recurrent metastasis/failed SRS/radiation necrosis (49–53). Kano et al., retrospectively analyzed the outcome of 58 patients who required resection of brain metastases following previous treatment with SRS. Median time between SRS and surgical resection was 7.1 months. Median follow-up was 7.6 months and median overall survival following resection was 7.7 months. Post-operatively, the local tumor control rate was 62% at 12 months and peri-operative morbidity was reported in 6.9% of cases. Interestingly these authors reported that a short interval between initial SRS and surgical resection (< 3 months) was significantly associated with poor survival (p = 0.001). In fact, no patient having a salvage surgery within three months of SRS lived more than one year post-operatively. This finding is an important consideration in determining surgical candidacy in the setting of recurrent metastasis (49). A more recent study by Mitsuya et al. (50), also evaluated the efficacy of salvage surgery in a cohort of 48 surgical patients (54 surgeries). In this study the median post-operative survival was 20 months with a reported local control rate of 76% at one year. Further, this study highlighted the palliative benefit of surgery as among the patients with pre-operative neurological deficits, 75% of cases had neurological improvement following salvage surgical resection (50). Overall, in select patients, salvage surgery for failed SRS is a reasonable treatment option that can aid in symptom management with a reasonable rate of local control and low surgical mortality.

In cases were surgical resection is not feasible due to an inaccessible location or suboptimal patient candidacy for an open craniotomy, laser interstitial thermal therapy (LITT) may serve as a salvage treatment option (54–57). The principle of LITT is selective ablation of target tissue by heat. Laser electromagnetic radiation is focused energy that is transformed into thermal energy, which spreads to tissues to induce coagulation. LITT is a minimally invasive procedure that consists of a probe inserted under stereotactic guidance into the target lesion. When the laser interacts with the target tissue, the tissue absorbs the laser photons, which are then transformed into thermal energy inside the target tissue. The heat generated heat leads to thermal damage of the target tissue with the goal of inducing necrosis through protein denaturation, while avoiding damage to surrounding normal tissues (57, 58). The ideal lesion for LITT is a well-circumscribed lesion with a diameter 3-3.5 cm or less (57). For larger lesions; multiple fibers could be used to cover the entire target. Lesions located inside the ventricles or near heat sink areas (porencephalic cysts, dura large venous lakes, large caliber vessels) might represent a challenge for thermal spread and should be evaluated on a case-by-case basis (57).

The main advantage of LITT is the ability to treat lesions not amenable to surgical resection due to difficult locations. The minimally invasive nature of the procedures allows for potentially shorter hospital stays, and faster transition to adjuvant treatments. LITT can also be repeated if progression is found after the procedure with no concern of accumulated ionizing radiation damage. LITT also it does not preclude a future open surgery in the case of treatment failure. Bastos et al. performed a retrospective study with consecutive brain

metastasis patients treated with LITT (59). Based on radiological aspects, lesions were divided into progressive disease after SRS (recurrence or radiation necrosis) and new, untreated lesions. The primary endpoint was time to local recurrence. A total of 61 consecutive patients with 82 lesions (5 newly diagnosed, 46 recurrence, and 31 radiation necrosis) were included for analysis. Freedom from local recurrence at 6 months was 69.6% and 59.4% at 12, months. Shorter time to recurrence was significantly associated with incompletely ablated lesions (p <.001), larger lesion volume (>6 cc) (p = 0.03) and dural-based lesions (p = 0.01). Tumor recurrence/newly diagnosed metastases also had shorter time to local recurrence when compared to radiation necrosis (p = 0.01). Patients receiving systemic therapy after LITT had longer time to local recurrence (p = 0.01). In multivariate analysis the hazard ratio for incompletely ablated lesions was 4.88 (p <.001), 3.12 (p= 0.03) for recurrent tumors, and 2.56 (p = 0.02) for patients not receiving systemic therapy after LITT. The procedural complication rate in this series was 26%. Notably, this complication rate is higher than reported in surgical resection series. However, it is important to consider that patients may be dispositioned for LITT due to lesions in high-risk locations and/or higher risk medical conditions, potentially contributing the elevated complication rate. One additional consideration is the initial inflammatory response caused by LITT, which typically requires steroids post-operatively. This period can be prolonged depending on the duration of previous steroid use and degree of pre-operative peri-lesional edema. However, a recent study comparing the post-operative outcome of LITT versus craniotomy for failed SRS lesions reported no significant differences in the rates of steroid cessation at 1-month follow-up between the LITT and surgical resection (60). The authors also reported no significant delay in the resumption or initiation of immunotherapy between the two treatment modalities, which is an important consideration in the setting of extended steroid use. In summary, LITT is a valid salvage strategy for recurrent brain metastasis or radiation necrosis. The current data would be strengthened by a prospective, randomized study.

CONCLUSION

As patients live longer with advanced cancer, brain metastasis will continue to be a growing issue. Brain metastases are a major contributor to cancer mortality and can have a significant impact on patient quality of life. Even with the significant advances in systemic therapy and radiation techniques, surgery remains a critical aspect of patient management. The maximal benefit of surgery can be achieved with careful patient selection and attention to surgical technique.

AUTHOR CONTRIBUTIONS

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Systemic Therapy Type and Timing Effects on Radiation Necrosis Risk in HER2+ Breast Cancer Brain Metastases Patients Treated With Stereotactic Radiosurgery

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Background: There is a concern that HER2-directed systemic therapies, when administered concurrently with stereotactic radiosurgery (SRS), may increase the risk of radiation necrosis (RN). This study explores the impact of timing and type of systemic therapies on the development of RN in patients treated with SRS for HER2+ breast cancer brain metastasis (BCBrM).

Methods: This was a single-institution, retrospective study including patients >18 years of age with HER2+ BCBrM who received SRS between 2013 and 2018 and with at least 12-month post-SRS follow-up. Presence of RN was determined *via* imaging at one-year post-SRS, with confirmation by biopsy in some patients. Demographics, radiotherapy parameters, and timing ("during" defined as four weeks pre- to four weeks post-SRS) and type of systemic therapy (e.g., chemotherapy, HER2-directed) were evaluated.

Results: Among 46 patients with HER2+ BCBrM who received SRS, 28 (60.9%) developed RN and 18 (39.1%) did not based on imaging criteria. Of the 11 patients who underwent biopsy, 10/10 (100%) who were diagnosed with RN on imaging were confirmed to be RN positive on biopsy and 1/1 (100%) who was not diagnosed with RN was confirmed to be RN negative on biopsy. Age (mean 53.3 vs 50.4 years, respectively), radiotherapy parameters (including total dose, fractionation, CTV and size target volume, all $p>0.05$), and receipt of any type of systemic therapy during SRS (60.7% vs 55.6%, $p=0.97$) did not differ between patients who did or did not develop RN. However, there was a trend for patients who developed RN to have received more than one agent of HER2-directed therapy independent of SRS timing compared to those who did not

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develop RN (75.0% vs 44.4%, p=0.08). Moreover, a significantly higher proportion of those who developed RN received more than one agent of HER2-directed therapy *during* SRS treatment compared to those who did not develop RN (35.7% vs 5.6%, p=0.047).

Conclusions: Patients with HER2 BCBrM who receive multiple HER2-directed therapies during SRS for BCBrM may be at higher risk of RN. Collectively, these data suggest that, in the eight-week window around SRS administration, if HER2-directed therapy is medically necessary, it is preferable that patients receive a single agent.

Keywords: breast cancer, brain metastasis, stereotactic radiotherapy, systemic therapy, radiation necrosis

INTRODUCTION

Breast cancer is the most common malignancy diagnosed in women and the second leading cause of cancer-related mortality among women worldwide (1). Increased survival has been observed in breast cancer patients due to advances in early diagnosis/screening methods and improved treatments. However, long-term survival is complicated by increased prevalence of breast cancer brain metastasis (BCBrM), which is associated with poor prognosis and decreased quality of life (2). Specifically, breast cancer is the second most common primary origin of BrM, with 15-30% of patients estimated to develop BrM during the course of advanced disease (3, 4).

Human epidermal growth factor receptor 2-positive (HER2+) breast cancer is a subtype of breast cancer with a predilection for BrM (5). As many as 30% of patients with advanced, metastatic HER2+ breast cancer will develop BrM (6). Current standard of care options for HER2+ BCBrM include radiation therapy (stereotactic radiosurgery [SRS] or whole brain radiation therapy [WBRT]), brain permeable systemic therapies, and/or neurosurgical resection when appropriate (7). A multimodal approach combining these different treatment modalities has improved the overall survival and functional outcomes of patients with BCBrM. Specifically for radiation therapy, SRS is a highly effective form of radiation therapy that offers meaningful control of BrM (8). Because the vast majority of patients who present with BrM have both intracranial and extracranial disease, most of them will also receive systemic treatment.

Radiation-induced injury is one of the most significant complications of brain tumor irradiation (9). One of the important adverse effects associated with SRS is radiation necrosis (RN), which is a late complication of radiation injury and occurs in about 5-25% of treated patients (10, 11). RN often significantly impacts quality of life; for example, it often presents with neurological deficits such as headaches, nausea and seizures (12, 13). The mechanism of RN remains unclear, but the pathology involves inflammation and angiogenesis in a region of coagulative necrosis associated with breakdown of the blood-brain barrier. resulting in perilesional edema and heterogeneous contrast enhancement (14, 15). RN commonly occurs 3-12 months after radiotherapy (16, 17), though it can be observed as late as several years post radiosurgery, in our experience. Because the likelihood of RN depends on factors such as timing of radiation therapy, total dose, dose per fraction and volume irradiated (18–20), efforts to decrease the rate of RN have focused on controlling these radiotherapy parameters. However, recent studies have shown that rates of RN are higher in patients who received both SRS and immunotherapies or targeted therapies compared to those who received SRS alone (21, 22). This association is significant because most BCBrM patients receive concurrent systemic therapy as part of their treatment regimen. This study explores the impact of timing and type of systemic therapies on the development of RN in patients with HER2+ BCBrM treated with SRS.

METHODS

This was a single-institution, retrospective study (approved by the Institutional Review Board) of patients >18 years of age with HER2+ BCBrM who received SRS between 2013 and 2018 with at least 12-month post-SRS follow-up. Demographics and baseline characteristics including age at the time of SRS, race, location of irradiated BrM, and number of BrM were collected. Relevant systemic and radiation treatment details were also recorded. Presence of RN was determined *via* magnetic resonance (MR) imaging one-year post-SRS (see details below). The rate of RN was also determined using biopsy reports of brain lesions if they were available at any time after SRS (i.e., not restricted to within one-year post-SRS). Patients with incomplete follow-up data and/or who were deceased within one-year post-SRS were excluded. Demographics and lesion characteristics considered included age at the time of SRS, race/ethnicity, time to SRS from date of brain metastasis, and location of brain metastasis.

Brain Metastases

Location of irradiated BrM was categorized as follows: frontal, parietal, temporal, occipital, cerebellar, midbrain/brainstem, and multiple. The number of BrM was considered as a binary variable (single or multiple).

Imaging and Pathological Criteria for Radiation Necrosis

MR imaging was used to diagnose RN within 12 months of receipt of SRS. For a given area, the diagnosis of RN was determined by: 1) the degree of hyperintensity on T2-weighted image and enhancement on contrast-enhanced T1-weighted image and

2) assessment and confirmation by neuroradiologists. For biopsy-confirmed cases with associated pathology reports, pure (tumor absent) and mixed (tumor present) RN were all considered as RN.

Radiotherapy Parameters

The total administered dose (in grays) and the number of fractions were collected. Other relevant radiotherapy parameters considered were clinical target volume (CTV), gross tumor volume (GTV), conformity index (CI), and volume receiving 12 gray (V12Gy). For V12Gy, only single-fraction SRS was considered.

Systemic Therapy

Types of systemic therapy included HER2-directed therapy (T-DM1, trastuzumab, pertuzumab, lapatinib), mitosis inhibitors (taxanes, vinca alkaloids, eribulin), DNA synthesis inhibitors (capecitabine, platinum, anthracycline, pemetrexed, cyclophosphamide, doxorubicin, gemcitabine), and other (all other systemic treatments). Timing of systemic therapy with regard to radiotherapy was defined as a binary variable as follows: 1) systemic therapy "during" radiotherapy meant the systemic therapy was administered within 4 weeks prior to Day 1 of SRS treatment through 4 weeks post-SRS Day 1; 2) "not during" radiotherapy meant systemic therapy was administered outside the 8-week window surrounding SRS. Number of systemic therapy agents overall, number of HER2-directed therapies, and use of T-DM1 were also considered.

Statistical Analysis

Categorical variables were summarized with frequencies and percentages and analyzed using Pearson's Chi-squared test with Yates' continuity correction. Continuous variables were summarized with means/standard deviations and medians/minimum and maximum and analyzed using the Wilcoxon rank sum test with continuity correction. Statistical significance was assessed at level alpha = 0.05. All statistical analyses were conducted using both SAS 9.4 (SAS Institute Inc., Cary, NC, USA) and R (RStudio, Inc; Boston, MA).

RESULTS

An initial sample of 386 adult patients who were diagnosed with BCBrM were identified between 2013 and 2018. From this sample, 264 patients were excluded as they did not have HER2+ BCBrM. 68 patients were further excluded as they did not receive systemic therapy and/or SRS at our institution. Finally, seven patients who were deceased within one year following SRS were excluded. A final cohort of 46 patients remained after applying these exclusion criteria (**Figure 1**).

Patient Demographics and Lesion Characteristics

Among 46 patients with HER2+ BCBrM who received both SRS and systemic therapies, the mean age at time of SRS was 52.1 years and the cohort was predominantly white (76.1% vs 23.9% non-white). A majority of patients had a single treated BrM (63%) vs multiple (37%).

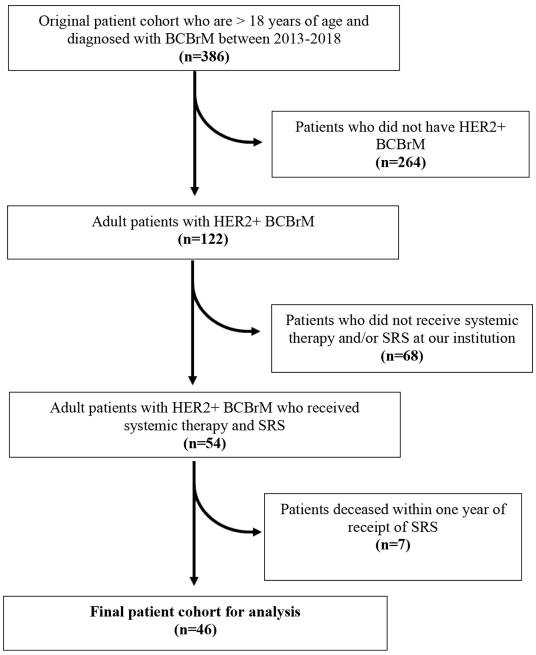

FIGURE 1 | Consort diagram of selection of patient cohort. Inclusion and exclusion criteria applied for derivation of final sample cohort.

In this cohort of 46 patients, 28 (60.9%) developed RN and 18 (39.1%) did not based on imaging parameters. Of the 11 patients from whom tissue biopsy samples were obtained (average date of biopsy was 1.5 years after SRS), 10/10 (100%) patients who were diagnosed with RN on imaging were confirmed to have RN on biopsy (with 4 of those reported as pure RN and 6 as mixed RN/recurrence) and 1/1 (100%) patient who was determined to not have RN on imaging was confirmed to not have RN on biopsy. Age at time of SRS did not differ between those who developed RN and those who did not (mean 53.3 vs 50.4 years, respectively; p=0.54). There was a higher, though not statistically significant, percentage of African Americans in the RN group (28.6% vs 11.1%, p=0.24). Although there was no statistically significant difference between the anatomic location of BrM irradiation between the two cohorts, more patients who developed RN had a single BrM lesion (78.6%) as opposed to multiple BrM lesions (21.4%) (p=0.016). Conversely, more patients who did not develop RN had multiple BrM (61.1%) compared to those with a single lesion (38.9%) (p=0.016). The results are summarized in **Table 1**.

Radiation Treatment

Overall, the mean total dose of SRS administered was 21.9 ± 4.10 Gy. 60.9% of the patients underwent single-fraction SRS. The mean values for the measured radiotherapy parameters were as follows: CTV of 9.15 ± 13.0 cc, GTV of 5.39 ± 7.51 cc, CI of 1.32 ± 0.27, and V12Gy (for single-fraction only) of 7.14 ± 6.28 cc. When we compared the two groups, there were no significant differences in the total dose, fraction (1 vs 5), and all measured radiotherapy parameters (all p>0.05) as shown in **Table 2**.

TABLE 1 | Demographics and lesion characteristics.

Variable	No RN (n=18)	RN (n=28)	Total (n=46)	p-value
Age at time of SRS, years				0.54
Mean (SD)	50.4 (13.0)	53.3 (11.7)	52.1 (12.1)	
Median [Min, Max]	51.5 [28.0, 74.0]	55.0 [33.0, 78.0]	53.0 [28.0, 78.0]	
Race, n (%)				0.24
White	16 (88.9)	19 (67.9)	35 (76.1)	
Black	2 (11.1)	8 (28.6)	10 (21.7)	
Other	0 (0)	1 (3.6)	1 (2.2)	
Location of irradiated brain metastasis, n (%)				0.14
Frontal	3 (16.7)	4 (14.3)	12 (26.1)	
Parietal	1 (5.6)	4 (14.3)	5 (10.9)	
Temporal	0 (0)	2 (7.1)	2 (4.3)	
Occipital	0 (0)	2 (7.1)	2 (4.3)	
Cerebellar	3 (16.7)	9 (32.1)	12 (26.1)	
Midbrain/Brainstem	0 (0)	1 (3.6)	1 (2.2)	
Multiple	11 (61.1)	6 (21.4)	17 (37.0)	
Number of brain metastasis (binary), n (%)				**0.016**
Single	7 (38.9)	22 (78.6)	29 (63.0)	
Multiple	11 (61.1)	6 (21.4)	17 (37.0)	

RN, radiation necrosis; SD, standard deviation; SRS, stereotactic radiosurgery. Significant p values (p<0.05) in bold.

Systemic Treatment

In the entire cohort, 58.7% of patients received any type of systemic therapy (i.e., HER2-directed therapy, mitosis inhibitors, DNA synthesis inhibitors, others) during SRS. Specifically, 43.5% received a HER2-directed therapy, 2.2% received a mitosis inhibitor therapy, 8.7% received both HER2-directed and mitosis inhibitor therapy, and 4.3% received other systemic therapy.

Receipt of any systemic therapy during SRS did not differ between patients who did or did not develop RN (60.7% vs 55.6%, p=0.97) (**Table 3**). However, patients who developed RN more commonly received more than one agent of HER2-directed therapy, independent of SRS timing, compared to those who did not develop RN (75.0% vs 44.4%, p=0.08). A significantly higher proportion of those who developed RN received more than one agent of HER2-directed therapy *during* SRS compared to those did not develop RN (35.7% vs 5.6%, p=0.047).

DISCUSSION

In our cohort of 46 patients with HER2+ breast cancer and BrM who received SRS, 60.9% of them were determined to have RN on imaging. Of the 11 patients with RN who had a BrM biopsy

TABLE 2 | Radiation parameters.

Variable	No RN (n=18)	RN (n=28)	Total (n=46)	p-value
Total dose (Gy)				0.19
Mean (SD)	22.9 (3.52)	21.3 (4.37)	21.9 (4.10)	
Median [Min, Max]	22.5 [18.0, 30.0]	20.0 [10.0, 27.5]	20.0 [10.0, 30.0]	
Fractions				>0.95
1	11 (61.1%)	17 (60.7%)	28 (60.9%)	
5	7 (38.9%)	11 (39.3%)	18 (39.1%)	
CTV (cc)				0.86
Mean (SD)	8.21 (11.3)	9.75 (14.2)	9.15 (13.0)	
Median [Min, Max]	5.74 [0.312, 44.9]	4.19 [0.104, 54.1]	5.15 [0.104, 54.1]	
Missing	2 (11.1%)	3 (10.7%)	5 (10.9%)	
GTV (cc) (single-fraction only)				0.84
Mean (SD)	4.72 (5.81)	5.82 (8.51)	5.39 (7.51)	
Median [Min, Max]	3.27 [0.138, 21.5]	2.60 [0.0264, 34.8]	2.63 [0.0264, 34.8]	
Missing	2 (18.2%)	2 (11.8%)	4 (14.3%)	
CI				0.55
Mean (SD)	1.30 (0.240)	1.34 (0.29)	1.32 (0.27)	
Median [Min, Max]	1.23 [1.03, 1.84]	1.29 [1.03, 2.35]	1.25 [1.03, 2.35]	
Missing	2 (11.1%)	3 (10.7%)	5 (10.9%)	
V12Gy (cc)				0.59
Mean (SD)	6.55 (6.74)	7.49 (6.20)	7.14 (6.28)	
Median [Min, Max]	3.96 [0.947, 20.2]	5.23 [1.66, 23.1]	5.18 [0.947, 23.1]	
Missing	2 (11.1%)	3 (10.7%)	5 (10.9%)	

CI, conformity index; CTV, clinical target volume; GTV, gross tumor volume; Gy, gray; SD, standard deviation; V12Gy, volume receiving 12Gy.

TABLE 3 | Systemic therapy and SRS.

Variable	No RN (n=18)	RN (n=28)	Total (n=46)	p-value
Time to first SRS from date of BrM (mo)				0.84
Mean (SD)	4.65 (6.63)	4.33 (7.64)	4.46 (7.19)	
Median [Min, Max]	1.03 [0.131, 22.0]	0.986 [0, 28.5]	1.00 [0, 28.5]	
Agents of systemic therapies, n (%)				>0.95
≤4	11 (61.1%)	18 (64.3%)	29 (63.0%)	
>4	7 (38.9%)	10 (35.7%)	17 (37.0%)	
Systemic therapy during SRS, n (%)				0.97
No	8 (44.4%)	11 (39.3%)	19 (41.3%)	
Yes	10 (55.6%)	17 (60.7%)	27 (58.7%)	
Type of systemic therapy received during SRS, n (%)				0.62
No systemic therapy	8 (44.4%)	11 (39.3%)	19 (41.3%)	
HER2-directed inhibitors	6 (33.3%)	14 (50.0%)	20 (43.5%)	
Mitosis inhibitors	1 (5.6%)	0 (0%)	1 (2.2%)	
HER2-directed inhibitors + mitosis inhibitors	2 (11.1%)	2 (7.1%)	4 (8.7%)	
Other	1 (5.6%)	1 (3.6%)	2 (4.3%)	
HER2-directed inhibitor during SRS, n (%)				0.59
No	10 (55.6%)	12 (42.9%)	22 (47.8%)	
Yes	8 (44.4%)	16 (57.1%)	24 (52.2%)	
Number of HER2-directed inhibiting agents during SRS, n (%)				**0.047**
0-1	17 (94.4%)	18 (64.3%)	35 (76.1%)	
2	1 (5.6%)	10 (35.7%)	11 (23.9%)	

SRS, stereotactic radiosurgery; SD, standard deviation; T-DM1, trastuzumab emtansine. "During" SRS defined as 4 weeks prior to through 4 weeks after Day 1 of SRS (an 8 week window). Significant p values (p<0.05) in bold.

specimen to evaluate, 10/10 who were diagnosed with RN on imaging were positive for RN on biopsy and 1/1 (100%) who was not diagnosed with RN on imaging was negative for RN. While the radiation treatment parameters did not differ significantly between those who did and did not develop RN, patients with RN were more often treated with more than one agent of HER2-directed therapy during SRS, as defined by 4 weeks prior to or after radiosurgery.

Challenges in Diagnosing Radiation Necrosis

There is no single best imaging modality used to diagnose RN. However, in current practice, MR imaging is the most common modality used to explore RN. Because it is often difficult to distinguish between RN and recurrence, surgical resection remains the best way to establish the histopathological diagnosis of RN along with providing relief from any symptomatic effects. Depending on the method of assessing RN, the rate of RN can vary widely from 7% (biopsy-proven) to 24% (imaging based) (20, 23). Hence, it is possible that our high rate of RN (60.9%) observed in our study could have been overestimated based on our primarily imaging-based diagnosis of RN. Biopsy was available for 10 of the 28 of those diagnosed with RN, of which all 10 were confirmed. If the rate of RN was calculated from biopsy-proven RN only, the rate would have been 21.7% which is still higher than those reported for biopsy-proven series (24, 25).

Although our ability to make a definitive diagnosis of RN versus recurrent tumor is currently based on histopathology, the pathology results can be limited by the variability of surgical site sampling which can give confounding results in the presence of both RN and tumor. To augment the current diagnostic capacity, attention has been focused on identifying the features and techniques that are important for making the distinction between RN and recurrence. For example, various imaging modalities using different sequences have been studied. Magnetic resonance spectroscopy, diffusion-weighted imaging, diffusion tensor imaging, and positron emission tomography (PET), among others, have surfaced as promising candidates for improving the current diagnostic rates (26, 27). Specifically for PET, fluciclovine is an amino acid radiotracer that has recently demonstrated good initial results for distinguishing RN from recurrent tumor among patients with brain metastases who were treated with SRS (28, 29). Furthermore, there also have been efforts to use radiomics and data mining to develop models that can effectively differentiate tumor from RN (30).

Significance of the Use of SRS and HER2-Directed Therapy on RN

BCBrM remains a significant challenge in the current era of improved extracranial disease control, owing to the lower efficacy of many systemic therapies in the brain (31). Given that HER2-driven cancers seem to preferentially metastasize to the brain, and the apparent brain-penetrance of some therapies (32, 33), we focused on the HER2-directed therapies. The effect of combination of HER2- directed therapies and SRS on overall survival/local control and RN is still unclear. For example, numerous studies have illustrated the benefit of concurrent use of lapatinib and SRS on overall survival and RN. Parsai et al. demonstrated that the use of lapatinib at any time of SRS administration was associated with improved overall survival (27.3 vs 19.5 months, p=0.03) with a lower risk of RN (1.3% vs 6.3% at 12 months, p<0.01) compared to those who received SRS without lapatinib in patients with HER2+ BCBrM (34). Miller et al. also showed that concurrent use of lapatinib/HER2-

directed antibody treatment with SRS was associated with a lower 12-month cumulative incidence of RN (1.3% vs. 6.3%, p=0.001) compared to those who only received SRS. Furthermore, Kim et al. reported that the use of concurrent lapatinib with SRS did not increase the risk of RN compared to those who underwent SRS only (1.0% vs 3.5%, p=0.13) (35). On the other hand, the outcomes for T-DM1 are suboptimal. Carlson et al. showed in their case series that the overall rate of clinically significant RN among the patients in the treatment group who received SRS and T-DM1 was 57% (36). Geraud et al. also reported that RN was observed in 50% of patients who received T-DM1 concurrently with SRS compared to those who received T-DM1 sequentially to SRS (37). Thus, prior studies suggest that different agents (i.e. lapatinib as a tyrosine kinase inhibitor vs T-DM1 as an antibody drug conjugate) within the same class (HER2-directed) may have different risks on development of RN. While the current study was unable to assess differences between specific agents due to low numbers, this is a question warranting further exploration in future larger studies.

Although previous studies have looked into the effect of a specific therapy on the rate of RN for patients undergoing SRS, they have not considered the effect of different types or the number of systemic therapies (particularly HER2-directed therapies) on the development of RN during/following SRS. We found that a higher number (2 or more) of HER2-directed agents administered during SRS may increase the risk of development of RN. This result can be interpreted in two ways: 1) the toxicity of the HER2-directed therapies increases when used in combination and concurrently with SRS or 2) use of more HER2-directed therapies leads to longer survival and the observed higher rate of RN is a result of time bias. That is, there is an increased risk for RN in patients who live longer after their SRS treatment, allowing more time to observe the natural progression of RN (38). This is further supported by the observation that the diagnosis of RN was not made in the patients who deceased before their one-year follow-up. Nevertheless, our results suggest that timing and number of HER2-directed therapies matter when considering SRS for this patient population. These findings may be used to identify the group of patients with HER2+ BCBrMs with the highest risk for RN who would be candidates for preventative strategies in the future.

Limitations

Our study is limited by its retrospective design which is subject to selection and misclassification bias. Some patients with HER2+ BCBrM who received SRS and systemic therapy might not have been included in our cohort if they received their treatment outside of our institution and were screened out in the initial phase. Also, the seven patients who were diagnosed with RN *via* imaging only could have been misclassified because the difference between RN and local recurrence is difficult to discern with conventional imaging techniques. There could also be confounding factors that may be present that were not accounted for which could have affected the statistical significance of the results. Furthermore, our cohort is largely homogenous with regards to race which is predominantly Caucasian. Hence, the results may not be applicable to the greater population. Finally, the small sample size and diversity of treatment histories (e.g., the wide variety of systemic agents) contributed to the lack of power to detect statistical difference in the measured outcomes. The lack of adequate sample size also made it difficult to draw meaningful conclusions through logistic regression modeling.

CONCLUSION

Patients with HER2+ BCBrM who receive multiple agents of HER2-directed therapy during SRS for BCBrM may be at higher risk of RN. These data suggest during the eight-week window around SRS administration, if HER2-directed therapy is medically necessary, use of a single HER2-directed agent may lead to lower RN rates. Further investigation of next generation HER2-directed therapies, particularly comparing specific agents, in a larger cohort of patients will help refine best practices to minimize RN.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Duke University Institutional Review Board (IRB). Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

CP, AV, JK, SF, and CA were involved with the conception of the study. CP, EB, WG, and JH participated in analysis and interpretation of data. CP formulated the initial draft of the manuscript. All co-authors reviewed the final draft of the manuscript.

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Review of Current Principles of the Diagnosis and Management of Brain Metastases

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Brain metastases are the most common intracranial tumors and are increasing in incidence as overall cancer survival improves. Diagnosis of brain metastases involves both clinical examination and magnetic resonance imaging. Treatment may involve a combination of surgery, radiotherapy, and systemic medical therapy depending on the patient's neurologic status, performance status, and overall oncologic burden. Advances in these domains have substantially impacted the management of brain metastases and improved performance status and survival for some patients. Indications for surgery have expanded with improved patient selection, imaging, and intraoperative monitoring. Robust evidence supports the use of whole brain radiotherapy and stereotactic radiosurgery, for both standalone and adjuvant indications, in almost all patients. Lastly, while systemic medical therapy has historically provided little benefit, modern immunotherapeutic agents have demonstrated promise. Current investigation seeks to determine the utility of neoadjuvant radiotherapy and laser interstitial thermal therapy, which have shown benefit in limited studies to date. This article provides a review of the epidemiology, pathology, diagnosis, and treatment of brain metastases and the corresponding supporting evidence.

Keywords: metastasis, radiosurgery, laser interstitial thermal therapy (LITT), craniotomy, immunotherapy, whole brain radiotherapy (WBRT), SRS

INTRODUCTION

The incidence of brain metastases is difficult to quantify but is estimated to reach up to 100,000 new cases per year in the United States (1) and continues to increase due to improved diagnostic testing, increasing cancer survival, and an overall increase in life expectancy throughout the population irrespective of a diagnosis of cancer (2). Metastases are by far the most common brain tumors, accounting for over half of all intracranial neoplasms, and outnumber both malignant and benign primary brain tumors combined (2).

EPIDEMIOLOGY

The most common primary source of brain metastases is lung cancer, accounting for over half of all instances; other prominent etiologies include breast cancer, melanoma, renal cell carcinoma, and colorectal cancer (2). These statistics largely reflect the relative incidence of primary cancers in general with the notable exception of prostate cancer, which, despite its distinction as the most common malignancy affecting men in the United states, rarely metastasizes to the brain (3).

Melanoma is the primary malignancy with the highest rate of metastasis to the brain, in up to 60% of cases (4), and while age of incidence varies by primary pathology, most brain metastases occur between the sixth to eight decades.

At the time of diagnosis, many patients—up to 85% based on radiographic and pathologic studies—are found to have multiple intracranial metastases; melanoma is also the most likely primary pathology to produce multiple lesions (5).

The incidence of brain metastases is also increasing due to advances in the efficacy of systemic therapy for cancer. For example, the advent of trastuzumab has dramatically improved survival in human epidermal growth factor receptor 2 (HER2)-positive breast cancer, leading to increasing incidence of brain metastases in patients as they live longer with systemic disease (6). A similar phenomenon has been observed in colorectal cancer; as brain involvement is a late-stage finding, and advances in systemic therapy have improved median survival nearly fourfold, the incidence of brain metastases has increased (7).

Brain metastases are both the most commonly occurring brain tumors and an increasingly common secondary complication of systemic cancers, and therefore warrant ever increasing clinical attention.

PATHOLOGY

Brain metastases, like metastases in other organs, take on the histologic appearance of the primary pathology from which they arise. They most commonly spread *via* hematogenous dissemination to the junction of the gray and white matter of the brain, where changes in microvascular anatomy cause microemboli to be trapped. Metastatic tumors are more discrete and focal than primary glial neoplasms and tend to cause local displacement and compression of native brain tissue rather than diffuse infiltration. However, in some instances they may have infiltrating features, usually confined to within 5mm of the tumor capsule (2).

Between 80 and 85% of brain metastases are located in the cerebrum, with between 10 and 15% located in the cerebellum, and fewer than 5% in the brainstem (8).

DIAGNOSIS

The most common presenting signs and symptoms of brain metastases are those associated with any intracranial mass lesion, including headache, nausea, vomiting, focal neurologic deficits, seizure, and, in severe cases, disorders of consciousness. Prompt acquisition of cranial imaging is indicated. In an acute setting, computed tomography (CT) imaging can often be acquired more rapidly and may demonstrate the presence of a mass lesion, the extent of edema and mass effect on brain parenchyma, and the presence of tumor-associated hemorrhage. However, magnetic resonance imaging (MRI) is the gold standard diagnostic imaging modality in the evaluation of brain metastases. Metastases usually demonstrate contrast enhancement on T1 imaging and can have irregular internal appearance due to intralesional necrosis. As with other intracranial neoplasms, metastases often cause local vasogenic edema, which appears hyperintense on T2-weighted sequences and respects the borders of white matter tracts.

In patients with a symptomatic intracranial lesion and a known diagnosis of a primary cancer originating from outside the central nervous system, these imaging findings are highly suggestive of a diagnosis of brain metastasis (9); however, in patients without a history of cancer, a solitary brain mass is unlikely to be a metastatic lesion and is more probably a primary brain tumor. The presence of multiple lesions, especially when involving multiple intracranial compartments, is effectively diagnostic of metastasis.

With improved survival associated with advances in systemic oncologic therapies and increasing accessibility of advanced imaging, some brain metastases are diagnosed incidentally on imaging obtained for unrelated indications. Routine screening imaging of the brain is not necessary in patients with systemic cancer without neurologic symptoms.

TREATMENT

Treatment modalities for brain metastases include surgery, radiation, and systemic therapy. Recent advances in all of these areas have resulted in improved survival of patients with brain metastases. Selection of treatment modalities depends on the size and location of the metastasis, the extent of intracranial and extracranial disease, and the patient's performance status. Treatment paradigms are informed by consensus guidelines from several organizations, including the Congress of Neurological Surgeons (10–13) and the consortium of the American Society of Clinical Oncology, the Society for Neuro-Oncology, and the American Society for Radiation Oncology (14).

Initial treatment for symptomatic metastases includes dexamethasone to reduce edema (10), which can cause both focal neurologic deficits and increased intracranial pressure. In patients without a history of seizure, prophylactic antiepileptic medications are not indicated (11). In patients with altered mental status or abnormal vital signs, assessment of airway security and hemodynamic stability is of the highest priority.

Systemic Medical Therapy

Historically, systemic cytotoxic chemotherapy has been ineffective in treating metastatic disease of the central nervous system both because these agents may be difficult to deliver across the blood-brain barrier and because patients with brain

metastases usually present in advanced, sometimes treatment-resistant phases of disease.

However, the advent of modern small-molecule antineoplastic medications, such as systemic tyrosine kinase inhibitors (TKI), and immunotherapeutic monoclonal antibody agents, such as PD-1 and CTLA-4 inhibitors, has significantly changed the standard of care for many types of systemic cancer, including lung cancer, breast cancer, melanoma, and renal cell carcinoma, which are all among the most common malignancies to metastasize to the brain. Additionally, molecular and genetic subtyping of systemic malignancies has also allowed for increased precision in designing and delivering targeted therapy, which in some cases has been shown to be effective in treating brain metastases.

Immunotherapy has revolutionized the systemic treatment of some cancers to the extent that immunotherapy alone—without surgery or radiation therapy—is the preferred management for certain patients who present with brain metastases. In patients with melanoma and asymptomatic brain metastases, combination immunotherapy alone resulted in a three-year progression-free survival rate of 54% and an overall survival rate of 72%, without the need for radiation therapy or surgery (15, 16). Similarly, patients with HER-2 positive breast cancer and newly diagnosed, asymptomatic intracranial metastases may be offered targeted combination immunotherapy or TKI upfront as an alternative to radiation (17), although direct comparison data are limited. In patients with non-small cell lung cancer with ALK mutation, a variety of ALK-targeting TKI are considered first-line therapy and are FDA approved for both asymptomatic and symptomatic brain metastases without severe mass effect (18, 19), and furthermore, may even be offered as second-line agents for patients whose intracranial disease progresses on initial TKI therapy (20).

The viability of systemic treatment for patients with metastatic intracranial disease, including as both first- and second-line therapy without radiation or surgery, is a significant achievement in medical oncology in the modern era. However, its role in addressing acutely symptomatic, large lesions with risk for acute neurologic compromise is limited.

Surgery

The role of surgery in the management of brain metastases has grown over time and is now considered the standard care for select patients. Resection of a metastatic lesion offers immediate relief of mass effect caused by the lesion, which can improve symptoms far more rapidly than radiation therapy to the same lesion. Additionally, resection of an edematous tumor can reduce the need for high-dose steroids in the short term, which may lead to reduction in steroid-related side effects.

Surgery also offers an opportunity for definitive histologic diagnosis, which radiation or systemic therapy alone does not. Although the majority of solitary brain tumors in patients with cancer are metastases from the known primary, in a minority of cases the tumor is a primary brain tumor, metastasis of a second systemic malignancy, or even a non-neoplastic lesion (9). Histopathologic diagnosis in these cases may inform further treatment, though surgery for biopsy and diagnostic confirmation alone is rarely indicated.

Patients with a single brain metastasis and a good overall preoperative performance status, generally measured as a score of > 70 by the Karnofsky Performance Scale (KPS), may be candidates for surgery to attain local control of metastatic disease. Evidence from randomized controlled trials suggests that surgery does not offer a benefit over radiotherapy when patients are not selected based on performance status and systemic disease burden (21). Patient selection for resection requires consideration of age, KPS, and the extent of systemic disease. These factors were evaluated by recursive partitioning analysis (RPA) in a landmark analysis which established RPA classes for patient selection, demonstrating that RPA class 1 patients—with age under 65, KPS of at least 70, and controlled primary disease without additional sites of metastasis—are most likely to benefit from surgery (22). In appropriately selected patients with tumor size > 2.5cm, resection carries a benefit in overall survival and local control over whole-brain radiation alone as demonstrated in studies of both disease-specific cohorts and all patients with oligometastatic disease (9, 23–26). Resection should be considered first-line means of local control for metastatic lesions > 3cm in diameter, as lesions of this size are less responsive to radiotherapy regardless of modality.

The decision to offer surgery must take into account the likelihood of the patient incurring a neurologic deficit. With modern microsurgical techniques and technology including frameless stereotactic navigation, intraoperative ultrasound, and intraoperative neuromonitoring (including awake and asleep mapping and stimulation), the risk of surgical morbidity for anatomically resectable lesions is low. However, not all metastatic lesions are amenable to surgery. Tumors located in deep nuclei and white matter tracts including the brainstem, thalamus, and basal ganglia are usually not considered resectable due to the risk of morbidity. In patients with cancer, avoiding a postoperative neurologic deficit is of utmost importance as patients with impaired performance status may not be offered further disease-directed therapy.

Surgical technique has also been shown to affect outcomes. Multiple studies have demonstrated that en bloc resection is superior to piecemeal resection both for oncologic benefit and perioperative morbidity. En bloc resection has been shown to decrease risk of leptomeningeal dissemination in both supratentorial and infratentorial metastases compared to piecemeal resection and even stereotactic radiosurgery (SRS) (27, 28) and is also associated with lower risk of local recurrence prior to adjuvant radiotherapy (29). A retrospective study evaluating postoperative complications based on surgical technique found that en bloc resection was associated with a lower rate of complications than piecemeal resection (30).

Advances in radiotherapy and systemic therapy have led to an expanded role for surgery in patients with multiple brain metastases and created a new indication for surgery in patients with symptomatic brain metastases: resection to reduce steroid dependence. Since dexamethasone interferes with the mechanism of action of certain types of modern immunotherapy, removing the edematous focus can facilitate rapid weaning of steroids and allow patients to resume systemic treatment with these highly effective

agents. In these instances, resection of highly edematous lesions, even if smaller than 2cm and/or in patients with many intracranial metastases for which radiation would otherwise be the preferred treatment modality, may be offered for systemic oncologic benefit.

In modern neurosurgical practice, craniotomy for resection is not the only option for surgical management of brain metastases. Laser interstitial thermal therapy (LITT) involves magnetic resonance-guided thermal ablation of tissue *via* a relatively less invasive surgical approach than open craniotomy. Although ablation does not immediately address the mass effect of lesions requiring surgical resection, studies have demonstrated it is effective in achieving local control of recurrent previously irradiated brain metastases and reducing steroid requirement (31). Furthermore, LITT can be considered as a surgical alternative to craniotomy for local control for lesions that are anatomically unresectable due to the risk of neurologic morbidity (32). In patients with advanced disease and metastases which progress to recurrence or radiation necrosis following radiotherapy, LITT has been shown to prevent worsening of KPS, reduce steroid requirement, and preserve quality of life and cognitive function over 12 weeks postoperatively (33). Therefore, LITT may be an increasingly appropriate palliative option for patients with advanced disease who may not be able to tolerate craniotomy, or who may not benefit from open surgery in light of their systemic disease burden and limited life expectancy. However, its availability is limited by operator expertise and availability of advanced laser and intraoperative thermosensitive MR technology.

Radiation Therapy

Radiation therapy is the mainstay of treatment of intracranial metastases to attain local control and prevent growth and recurrence. Several modalities of radiation therapy are used in modern practice, both alone and in conjunction with surgery. The main modalities are whole brain radiation therapy (WBRT), stereotactic radiosurgery (SRS), and, more recently, brachytherapy.

Whole brain radiation therapy has been used to treat intracranial metastases for nearly 70 years. Advantages of this modality include treating the entire brain, providing therapy to all sites of metastasis, including microscopic deposits potentially not identifiable on imaging. For this reason, prophylactic WBRT is offered to many patients with small cell lung cancer, which characteristically metastasizes to the brain early in its clinical course; multiple studies have supported this indication even in patients with no clinical or radiographic evidence of intracranial disease (34, 35). Additionally, it is the easiest modality to deliver to patients who may not tolerate the placement of a stereotactic headframe for radiosurgery.

However, WBRT is inherently imprecise and targets both malignant and normal brain tissue. This results in a higher risk of deleterious neurologic side effects, including memory and cognitive deficits in the long term, and headache, nausea, and vomiting in the short term. Most patients who receive WBRT should be prescribed memantine to mitigate neurologic side effects (36). Additionally, for patients with metastases not involving the hippocampus, hippocampal-sparing WBRT offers improved cognitive outcomes compared to conventional WBRT (37, 38).

Due to the potential for delayed neurologic injury, WBRT is best suited to patients with a relatively shorter life expectancy, which coincides with its suitability for patients with multiple intracranial lesions. Neurologic injury is associated with higher fractionated doses; therefore, a paradigm of smaller doses in more fractions has been shown to be superior (12). A standard regimen may include 30 Gy in 10 or 15 fractions; alterations in dose and fractionation do not result in improvement in survival or local control, which has been established *via* multiple randomized controlled trials (39, 40).

In many patients with extensive intracranial disease burden, prognosis is poor despite treatment with WBRT. Therefore, symptomatic treatment with steroids and supportive care is also an option for some of these patients, especially those with low performance status; a randomized controlled trial demonstrated that optimal supportive care was non-inferior to optimal supportive care with WBRT in patients with non-small cell lung cancer in terms of overall survival and quality of life (41).

WBRT can also be used in an adjuvant role to prevent local recurrence after surgery and further distant metastasis. Adjuvant WBRT has been shown to reduce the risk of both local recurrence and distant metastasis by more than half but does not affect overall survival or functional independence in this population (42, 43).

Stereotactic radiosurgery (SRS) has emerged as a favorable alternative to WBRT in many patients with brain metastases. SRS delivers radiation to discrete sites at the intersection of highly collimated sources, resulting in high doses at the site of intersection with rapid falloff in the delivered dose away from the target. It therefore carries the advantage of effective treatment to discrete lesions without the harmful off-target effects associated with WBRT but is less effective in treating many lesions. However, linear accelerator-based stereotactic radiotherapy has recently emerged as an option for the targeted treatment of multiple brain metastases in fractionated doses (44, 45).

SRS is the preferred first-line treatment for patients with oligometastatic disease and lesions < 3cm in maximal diameter without acutely life-threatening presentation, especially when located in an eloquent area or when surgical resection may otherwise result in neurologic deficit. Current guidelines suggest that patients with up to 4 metastases, and with >4 metastases if the total tumor volume is < 7cc, should be treated with SRS upfront instead of WBRT (13). Although some evidence exists for improved intracranial disease control with SRS plus WBRT for oligometastatic disease (46), more recent randomized trials have shown that SRS plus WBRT carries no advantage in overall survival compared to SRS alone and is associated with greater neurocognitive morbidity (43, 47, 48).

SRS has also been established as a mainstay of adjuvant radiotherapy in patients with surgical metastatic disease. Current neurosurgical guidelines recommend consideration of SRS to the postoperative cavity following resection of a solitary metastasis based on studies demonstrating effective local control and decreased morbidity compared to adjuvant WBRT (49–51).

Another recent randomized trial demonstrated that adjuvant SRS in patients with up to three resected metastases significantly lowered the rate of local recurrence, suggesting that, even for patients with multiple resected lesions, SRS may be an effective alternative to WBRT (52).

More recently, investigators have explored the role for neoadjuvant SRS in the treatment of oligometastatic disease. While no clinical trial to date has evaluated the efficacy of SRS in the neoadjuvant role (53), one combination prospective/retrospective study has suggested that it is safe and does not increase the risk of radiation necrosis or leptomeningeal spread of disease (54); further studies are currently underway (55, 56).

Brachytherapy is the third modality of radiation therapy employed in the treatment of brain metastases. Conceptually, the implantation of radioactive source material into the tumor cavity at the time of surgical resection dates back to the 1930s and the early age of neurologic surgery, but has been limited by the danger of systemic toxicity of indwelling radioisotopes to both the patient and bystanders (57). However, more recent advances in bioabsorbable materials science and the use of ^{125}I and especially ^{131}Cs offer the possibility of delivering high-dose radiotherapy to disease sites with limited off-target effects in a safe biodegradable delivery system. Early trials have demonstrated safety and efficacy in limited applications (58–60).

DISCUSSION

Metastasis to the brain is a frequent complication of many of the most common types of systemic malignancies. Over the last generation, dramatic advancements in systemic medical oncology, surgical technique and technology, and radiation therapy have resulted in both a dramatic increase in the incidence of brain metastases—due to increased overall cancer survival—and a dramatic improvement in the options available to neurosurgeons, radiation oncologists, and medical oncologists in their management.

Future advancements in the treatment of brain metastases may emerge from cutting-edge fields including genomics, radiomics, and artificial intelligence. Basic science and translational studies have identified mutations and epigenetic factors that drive expression of brain metastases in animal models (61, 62), which may present an option for targeted therapy in the future. As imaging technology continues to evolve, radiologic characteristics of brain metastases are being evaluated for their utility as non-invasive biomarkers that may guide prognosis and treatment (63). Machine learning may further be able to incorporate radiologic (64, 65), genomic, and clinical characteristics of individual patients to offer more precise and individualized diagnosis and treatment options than consensus-based guidelines can offer.

The nature of metastatic cancer renders each patient with brain metastases unique, and therefore no single treatment paradigm is appropriate for every such patient. Patients with oligometastatic disease and good functional status benefit most from surgical resection with adjuvant stereotactic radiosurgery and medical therapy where indicated. Patients with widespread metastatic disease still benefit most from palliative whole brain radiation therapy to achieve maximal local control and prevent neurologic worsening. For patients whose disease burden falls in between these extremes, a variety of treatment modalities are available with various levels of supporting evidence. Ultimately, each patient's optimal treatment paradigm must be developed in collaboration between the neurosurgeon, the radiation oncologist, the medical oncologist, and the patient. As further advances in systemic therapy, neurosurgical technique, and radiation therapy are achieved, more options will be available to treat patients with metastatic cancer involving the brain.

AUTHOR CONTRIBUTIONS

AB – First author. AP – Senior author. All authors contributed to the article and approved the submitted version.

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Comprehensive Analysis of the Immunogenomics of Triple-Negative Breast Cancer Brain Metastases From LCCC1419

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Background: Triple negative breast cancer (TNBC) is an aggressive variant of breast cancer that lacks the expression of estrogen and progesterone receptors (ER and PR) and HER2. Nearly 50% of patients with advanced TNBC will develop brain metastases (BrM), commonly with progressive extracranial disease. Immunotherapy has shown promise in the treatment of advanced TNBC; however, the immune contexture of BrM remains largely unknown. We conducted a comprehensive analysis of TNBC BrM and matched primary tumors to characterize the genomic and immune landscape of TNBC BrM to inform the development of immunotherapy strategies in this aggressive disease.

Methods: Whole-exome sequencing (WES) and RNA sequencing were conducted on formalin-fixed, paraffin-embedded samples of BrM and primary tumors of patients with clinical TNBC ($n = 25$, $n = 9$ matched pairs) from the LCCC1419 biobank at UNC—Chapel Hill. Matched blood was analyzed by DNA sequencing as a comparison for tumor WES for the identification of somatic variants. A comprehensive genomics assessment, including mutational and copy number alteration analyses, neoantigen prediction, and transcriptomic analysis of the tumor immune microenvironment were performed.

Results: Primary and BrM tissues were confirmed as TNBC (23/25 primaries, 16/17 BrM) by immunohistochemistry and of the basal intrinsic subtype (13/15 primaries and 16/19 BrM) by PAM50. Compared to primary tumors, BrM demonstrated a higher tumor mutational burden. *TP53* was the most frequently mutated gene and was altered in

50% of the samples. Neoantigen prediction showed elevated cancer testis antigen- and endogenous retrovirus-derived MHC class I-binding peptides in both primary tumors and BrM and predicted that single-nucleotide variant (SNV)-derived peptides were significantly higher in BrM. BrM demonstrated a reduced immune gene signature expression, although a signature associated with fibroblast-associated wound healing was elevated in BrM. Metrics of T and B cell receptor diversity were also reduced in BrM.

Conclusions: BrM harbored higher mutational burden and SNV-derived neoantigen expression along with reduced immune gene signature expression relative to primary TNBC. Immune signatures correlated with improved survival, including T cell signatures. Further research will expand these findings to other breast cancer subtypes in the same biobank. Exploration of immunomodulatory approaches including vaccine applications and immune checkpoint inhibition to enhance anti-tumor immunity in TNBC BrM is warranted.

Keywords: triple-negative breast cancer, brain metastases, immunogenomics, whole-exome sequencing, mRNA sequencing, biobank

INTRODUCTION

Triple-negative breast cancer (TNBC) lacks the expression of hormone receptors estrogen (ER) and progesterone (PR) as well as human epidermal growth factor receptor 2 (HER2). TNBC is also the most aggressive subtype of breast cancer, with a predilection for brain metastases; up to 50% of patients with metastatic TNBC will develop brain metastases (BrM) during their disease course (1). Patients with TNBC BrM face a poor prognosis with a median survival following diagnosis of less than 6 months (2). Despite progress in the treatment of ER+ and HER2+ breast cancer BrM with the advent of brain-penetrant targeted therapies, the prognosis for TNBC BrM remains largely unchanged over the past decade (3). Thus, studies to better understand the biology of TNBC BrM to identify new therapeutic targets are needed.

Previous studies have conducted sequencing of primary and metastatic tissues, including BrM, from melanoma (4), lung cancer (5), breast cancer (6–8), and multiple solid tumor types (9). A seminal work by Brastianos et al. demonstrated that some solid tumors (including BrM) undergo branched evolution during the metastatic process. These studies have led to a growing appreciation that BrM can be biologically different from not just their primary tumors but also extracranial metastases, including differential acquisition or loss of targetable alterations. Breast cancer brain metastasis (BCBrM) have demonstrated mutations and/or copy number alterations in clinically targetable genes and pathways such as HER2 (6, 7, 9), BRAF (8), PI3K/Akt (9), CDK (6), ATM (8), and CRYAB (10) not seen in primary tumors. BrM can also be metabolically different from primaries, with increased oxidative phosphorylation (4). Preclinical studies have demonstrated that these targets can alter the brain metastatic potential and/or growth of BrM in breast cancer models (4, 11–13). These findings have led to the first genomically guided clinical trial in BrM to match alterations present in BrM to an appropriate brain-penetrant inhibitor (NCT03994796).

While these and other prior studies have made significant progress in the genomic characterization of BrM in recent years, no studies have yet focused exclusively on TNBC BrM specifically, and few studies have looked at comprehensive RNA and DNA sequencing-derived features of the tumor immune microenvironment. Using the LCCC1419 Biobank of metastatic breast cancer samples, we have collected and analyzed BrM, matched primaries, and normal tissue from 25 patients with TNBC through both whole-exome sequencing (WES) and mRNA sequencing. We report the somatic mutational landscape of TNBC BrM compared to primary tumors and implement a comprehensive neoantigen prediction pipeline to elucidate potentially immunogenic peptides arising from traditional and alternative neoantigen sources. Utilizing mRNA gene expression, we evaluated the tumor immune microenvironment of TNBC BrM relative to primary tumors and correlate these features with overall survival. To our knowledge, this study represents the largest evaluation of TNBC BrM through WES and RNA-seq and is the first to analyze gene expression and immunogenomics in addition to the mutational landscape.

MATERIALS AND METHODS

Patient Consent and Tissue Collection

Archival formalin-fixed, paraffin embedded (FFPE) tumor tissues were obtained from patients with clinically determined triple-negative breast cancer (TNBC) based on either a primary or metastatic site, with known metastasis to the brain. The patients consented to participation in either the UNC Health Registry (UNC IRB 09-0605), opened on 04/16/2009 and consented between 11/2014 and 06/2016, or to a clinically annotated biobank study at the University of North Carolina at Chapel Hill under an Institutional Review Board (IRB)-approved protocol (LCCC1419) which opened on 10/31/2014 and consented from 11/2014 to 11/2018. Brain metastases tissues

were available from n = 19 patients (n = 19 with RNA, n = 17 with DNA), while primary tumor tissue was available from n = 16 patients (n = 15 with RNA, n = 13 with DNA). Matched whole blood samples were available for n = 22 patients (DNA). Of these cases, n = 9 included matched RNA primary TNBC and BrM tissue pairs from the same patient, and n = 6 had matched DNA triplet samples (primary, BrM, and blood), all n = 6 of which also had RNA for both primary and BrM samples.

DNA Whole-Exome Sequencing and Variant Calling

FFPE tumor/tissue blocks and normal fresh frozen blood samples were collected and inventoried through honest brokers in accordance with IRB standards through the UNC Health Registry. UNC patient samples were inventoried through UNC Surgical Pathology Core (SP), while patient samples from outside UNC were inventoried through UNC Tissue Procurement Facility. Tissue blocks were sectioned by UNC Translational Pathology Laboratory (TPL). Twenty-two sections were made at a time: two 5-µm sections at the beginning and end of sectioning for pathologist review and 20 10-µm sections for DNA/RNA isolation on glass slides. TPL pathologists reviewed the samples, circling areas with more than 50% tumor mass for DNA/RNA isolation. When required, the process was repeated to collect more DNA/RNA. UNC BioSpecimen Processing Facility performed all DNA/RNA isolations. DNA from tumor-enriched cores were extracted using the Maxwell 16 FFPE Tissue LEV DNA Purification Kit. DNA was exome-captured and amplified with Agilent SureSelect XT (G9611B) and capture library (5190-8881). WES was performed using the Illumina HiSeq 2500 or NextSeq 500 platform with multiple samples per lane using 2 × 100 paired-end chemistry. RNA was isolated from the same sections with QIAGEN AllPrep FFPE, and libraries were prepared with Illumina TruSeq Stranded with RiboZero Gold (RS-122-2301). mRNA-Seq libraries were run at 2 samples per lane on an Illumina HiSeq2500 sequencer in high-output mode using 2 × 50 paired-end chemistry.

WES was performed on FFPE tumor tissue, with peripheral blood mononuclear cells serving as a matched normal. Library preparation was performed with the SureSelect XT Human All Exon V6 + UTR kit (Agilent, Santa Clara, CA, USA), and pooled samples were sequenced on the HiSeq2500 platform (Illumina). The resulting somatic and germline WES sequencing files were aligned to Hg38 using bwa (v0.7.17), sorted, and indexed, and duplicates were marked using biobambam2 (v2.0.87). BAMs were re-aligned with Abra2 (v2.22), followed by somatic and germline variant detection with Strelka2 (v2.9.10), Cadabra (from Abra2 v2.22), and Mutect2 (GATK v4.1.4.0). The capture of exonic sequences was verified using the Picard (v2.21.1) CollectHsMetrics tool, and the quality of sequencing data was verified using FastQC (v0.11.8) and the Picard suite's CollectAlignmentSummaryMetrics, CollectInsertSizeMetrics, QualityScoreDistribution, and MeanQualityByCycle tools. Variants with matched normals were filtered by the following criteria: protein-coding mutations only, Cadabra indel quality >10.5, Mutect2 indel quality >6.8, or (single-nucleotide variant) SNV quality >9.2, Strelka2 indel quality >15.2 or SNV quality >19.7. Additionally, Cadabra indels with quality <35 required a supporting high-quality call from either Strelka2 or Mutect2, and Strelka2 calls with SomaticEVS <20 similarly required a matching call from either Mutect2 or Cadabra. Variants for tumor-only samples were detected by Mutect2 and filtered to retain the protein-coding mutations. The remaining variants required at least 5 supporting reads and a minimum read depth of 40 or 10 supporting reads and a minimum read depth of 80 if MAF <5%. The variants with a MAF >5% in normal tissue were dropped, as were the variants appearing at rates above 1% in any subpopulation in either GnomAD or 1000 Genomes databases. To counter FFPE artifacts, C>T and G>A substitutions required a minimum MAF of 10%. Tumor mutational burden (TMB) was calculated from small indels and substitutions identified by WES and divided by the megabases adequately covered by sequencing reads. Whole-exome sequencing data for both tumor and germline was available for 28 samples (representing 22 unique patients) at baseline. Tumor-only whole-exome sequencing data—without matched normal—was available for a further 2 samples (representing 2 patients), for a total of 30 samples across 24 patients with WES data. Oncoplots were created in R using maftools v2.10.0, with variant genes limited to those implicated in breast cancer from the COSMIC Cancer Gene Census Tier 1 list (https://cancer.sanger.ac.uk/cosmic).

Copy Number Variation and Subclonal Heterogeneity Analysis

LCCC1419 patients with matched DNA normal and DNA tumor samples (n = 22) were processed through trim galore v. 0.6.2. The resulting trimmed FASTQs were aligned to the human reference genome FASTA file Homo_sapiens_assembly38.fasta (from the GATK hg38 file bundle) with the bwa mem command from BWA v. 0.7.17 with default parameters. The resulting SAM files were sorted, converted to BAM files, and indexed using SAMtools v. 1.9. The matched samples from each patient were then processed through the Sequenza v. 3.0.0 workflow (gc_wiggle with window size of 50 basepairs, bam2seqz, seqz_binning, sequenza.extract, sequenza.fit, and sequenza.results all with default parameters). The resulting patient-level segment files were then modified to conform to the format required by GISTIC. This conversion was performed with standard BASH scripting and included a log2(x) - 1 transformation of Sequenza's raw depth ratio value for GISTIC. The resulting modified segment files were then concatenated across samples and ran through GISTIC v. 2.0.23 using the hg38.UCSC.add_miR.160920.refgene.mat file (from docker://shixiangwang/gistic:1.2) for the -refgene parameter. CNVKit was run using the "batch" mode for primary tumors and BrM separately (with their matched normal samples, respectively). All normal samples within each group were pooled together to generate a pan-sample normal control. Agilent's SureSelect Human All Exon V6+UTRs probed bed file was provided for the –targets parameter, a standard hg38 refFlat.txt file was provided for annotations, and a k50 umap

mappability BED file was provided for the –access parameter. Outputs from Sequenza were paired with each patient's corresponding MuTect2 somatic variant calls to create PyClone-VI input files using Sequenza copy number information and MuTect2's variant read support information. Files were created for each primary tumor and BrM sample separately, and paired input files were created for patients that had both primary tumor and BrM data available. The resulting files were run through PyClone-VI using default parameters.

Neoantigen Prediction Using Genomics Data

Tumor antigens were predicted from a comprehensive set of genomic sources (single-nucleotide variations, insertions/deletions, gene fusions, alternative splice variants, cancer testis antigens, overexpressed self-antigens, and viral and endogenous retroviral antigens) using methods developed by our group and implementations of methods developed by others (14–22). Briefly, whole-exome sequencing was used to identify tumor-specific genetic variants (single-nucleotide variations, insertions/deletions, gene fusions), and RNA sequencing was used to confirm the expression of these variants. RNA sequencing data alone were used to evaluate for expressed alternative splice variants, viral and endogenous retroviral antigens. Cancer testis antigens/overexpressed self-antigens were evaluated using RNAseq data, but WES data was used to incorporate germline variants. RNA sequencing data was also used to infer tumor MHC haplotypes *via* HLAProfiler, the most accurate tool for MHC haplotype inference (23). Peptide fragments generated *in silico* are evaluated for predicted binding affinity to tumor MHC alleles using NetMHCpan-4.1 (24). Peptides with predicted binding affinity <500 nM were considered positive binders (*e.g.*, potential tumor antigens), while peptides with predicted binding affinity <50 nM considered strong binders and more likely to be tumor antigens (25).

RNA-Seq Data Processing

RNA-Seq Paired FASTQs were run through trim galore v. 0.6.2 using –paired parameter. STAR v. 2.7.0f was used to index the reference genome Homo_sapiens.GRCh38.dna_sm.primary assembly.fa from GATK and to map trimmed reads to reference (using parameters –quantMode TranscriptomeSAM –outSAMtype BAM SortedByCoordinate –sjdbGTFfile Homo_sapiens.GRCh38.100.gtf). Gffread v. 0.11.7 was used to create a transcriptome reference using the reference genome and the gtf file Homo_sapiens.GRCh38.100.gtf. The "toTranscriptome" alignments from STAR were used with Salmon v. 1.1.0 using "salmon quant -l a". Sample quality was assessed using MultiQC v1.9 with Picard CollectRnaSeqMetrics, and samples with less than 20 M coding reads were excluded as this threshold has been found to approximate the minimal sequencing depth to achieve equivalent detection to microarrays (26). Counts were log2-transformed and the upper quartile normalized for further downstream analysis. Some patients' tumors were sequenced multiple times (technical replicates), and in such cases, gene-level expression values were averaged across technical replicates.

Intrinsic Subtype Analysis

Intrinsic subtype analysis was performed according to the methods described in Picornell et al (27). The R package heatmaply (28) was used for heat map visualization with hierarchical clustering based on average linkage.

Immune Gene Signature Expression Analysis

Thirty-two immune gene signatures were chosen to reflect the diversity of tumor-infiltrating immune cell populations and to minimize redundancy (refer to associated.gmt file in the **Supplementary Material**). The *binfotron* R package (29) was used to compute the differential gene expression [along with the DESeq2 dependency (30)], produce a volcano plot, and calculate immune signature metagene scores (median log2 expression values) for downstream analysis. ssGSEA was performed using the R packages *GSVA* and *GSEABase* (31, 32). CIBERSORTx immune cell fraction imputation using the LM22 matrix was also performed (33).

TCR/BCR Repertoire Analysis

Immune chain inference was performed on RNA-Seq samples *via* MiXCR 2.1.2 for TCR chains (34) and V'DJer 0.12 (35) for BCR chains. The consensus BCR contigs from V'DJer were quantified using Salmon 0.13.11 (36). Repertoire diversity was calculated using a model-based approach, which improves estimations of diversity in part by minimizing known sources of estimate bias (37).

Survival Analyses

Multivariable Cox proportional hazards modeling was performed using the *survival (*38*)* R package. The model included cancer stage, age at primary tumor diagnosis, and race. Time from initial diagnosis, from diagnosis of any metastasis regardless of anatomical location, or from diagnosis of BrM to an event was interrogated. Hazard ratios and 95% confidence intervals were returned for gene signature covariates and visualized using the *forestplot (*39) R package.

Accession Numbers and Data Sharing

Sample information for RNA-seq and DNA-seq fastQ runs, including the clinical information, were uploaded to the NCBI's dbGaP repository (accession no. phs002457.v1.p1) and SRA.

RESULTS

Patient Cohort Characteristics

Tissues and blood from patients with clinical TNBC (n = 25) were included in this analysis, including BrM tissues (n = 19), primary breast tumors (n = 17), and whole blood samples (n = 22). The specimen numbers by tissue type and analysis (IHC, RNA, or DNA) are outlined in **Supplementary Figure S1**. Patient demographics are included in **Table 1**, with individual clinical–pathological characteristics and specimen availability

presented in **Supplementary Table S1**. The majority of patients were Caucasian (n = 17, 68%), with African American, Asian, and other ethnicities represented [n = 6 (24%), n = 1 (4%), and n = 1 (4%), respectively]. One male was included in the cohort. Median age at breast cancer diagnosis was 46.7 years (range, 29–70.9), while median age at BrM diagnosis was 51.5 years (range, 31.7–72). The majority (n = 12, 48%) of patients were initially diagnosed with stage II disease prior to recurrence; a minority (n = 2, 8%) were diagnosed with *de novo* stage IV TNBC. In addition to BrM, other sites of disease included the liver (n = 8, 32%), bone (n = 15, 60%), lung (n = 16, 64%), and non-local lymph nodes (n = 18, 72%). Ten patients (40%) were initially diagnosed with a solitary BrM, while 7 patients were diagnosed with 5 or greater BrM (28%). BrM was supratentorial in n = 23 (92%) patients and infratentorial in n = 13 (52%) patients. The median progression-free survival (*e.g.*, time from primary TNBC diagnosis to the diagnosis of any metastasis) was 1.8 years (range, 0–19.5). The median OS from primary TNBC diagnosis was 3.7 years (range, 0.9–19.8), while the median OS from BrM diagnosis was 1.2 years (range, 0 – 8.9).

TABLE 1 | Relevant demographic, subtype, and clinical diagnostic information for the LCCC1419 TNBC cohort.

Characteristics	Number (%)
Demographic information	
Means of enrollment (n = 25)	
LCCC 1419 consent	6 (24%)
Health Registry consent	16 (64%)
Waiver of consent	3 (12%)
Race (n = 25)	
African American	6 (24%)
Asian	1 (4%)
Caucasian	17 (68%)
Other	1 (4%)
Ethnicity (n = 25)	
Hispanic	2 (8%)
Not Hispanic	22 (88%)
Unknown	1 (4%)
Sex (n = 25)	
Female	24 (96%)
Male	1 (4%)
Smoking status (n = 25)	
Never smoker	14 (56%)
Current smoker	4 (1%)
Former smoker	7 (28%)
Subtype information	
Subtypes by primary resection (n = 25)	
Luminal A (ER/PR+, HER2-)	1 (4%)
Luminal B (ER/PR+, HER2+)	0 (0%)
HER2 (ER-, PR-, HER2+)	0 (0%)
TNBC (ER-, PR-, HER2-)	23 (92%)
Mixed (two primaries tested with different results)	0 (0%)
Unknown	1 (4%)
Subtypes by CNS resection (n = 21)	
Luminal A (ER/PR+, HER2-)	1 (5%)
Luminal B (ER/PR+, HER2+)	0 (0%)
HER2 (ER-, PR-, HER2+)	0 (0%)
TNBC (ER-, PR-, HER2-)	15 (71%)
Radiation necrosis	1 (5%)
Unknown	4 (19%)

Intrinsic Subtype Classification of Primary TNBC and Brain Metastases

Intrinsic molecular subtype analysis using RNAseq data illustrated that the majority of clinically determined TNBC samples were of the basal subtype (**Supplementary Figure S2**). Of the BrM (n = 19), 16 were classified as basal (84%), with normal-like (n = 2) and HER2-enriched (n = 1) comprising a small fraction of the cohort. Similarly, primary tumors (n = 15) were predominantly classified as basal (n = 13, 87%), with the remaining tumors being normal-like (n = 2). Notably, the 4 samples that were called normal-like by PAM50 analysis had a basal subtype as the second highest identity probability. There were 2 cases with discordant receptor classification between primary tumor and BrM by immunohistochemistry (n = 1 ER +/PR+/HER2- Luminal A primary converted to a TNBC BrM, and n = 1 TNBC primary converted to an ER+/PR+/HER2- Luminal A BrM) (**Table 1**; **Supplementary Figure S2**). Despite the potential subtype switching between primary and BrM, these samples were included in the downstream analyses.

Mutational, Somatic Copy Number Alteration, and Subclonal Analyses of Primary TNBC Tumors and Brain Metastases

First, we analyzed the tumor mutational burden (TMB) of primary tumors (n = 13) relative to BrM (n = 17) using WES. On average, BrM harbored a greater mutational load than primary tumors (median 3.33 *vs*. 1.78 mutations/Mb, respectively, $p < 0.05$; **Figure 1A**). Upon analysis of matched primary-BrM WES pairs (n = 6), however, there was no significant difference between tumor location and TMB (median 2.80 *vs*. 1.88 mutations/Mb, respectively, p = 0.69; **Figure 1B**). An analysis of shared mutations within matched pairs revealed varying degrees of mutational conservation between anatomical locations (**Figure 1C**). The degree of variant sharing between matched pairs (**Figure 1C**) was generally greater than the degree of mutations shared between primaries and BrM globally (**Supplementary Figure S3**), highlighting interpatient tumor heterogeneity and mutational divergence. We assessed whether a survival benefit was conferred by increasing TMB, as TMB has been considered a proxy for neoantigen burden (40, 41). There was no significant association between TMB and survival (p = 0.07) from the time of primary TNBC diagnosis in the context of a model that included standard clinicopathological features (age at diagnosis of primary tumor, stage, and race) (**Supplementary Figure S4**). Next, we examined the mutational spectrum of genes with known associations to breast cancer development (42). We found that these genes were altered in 70% of combined primary and BrM samples, with *TP53* being the most commonly mutated gene (mutated in 50% of samples, n = 15), in accordance with its known relevance to TNBC (43) (**Figure 1D**). The next most frequently altered genes were *MAP3K13* and *PIK3CA*, which were mutated in 13% (n = 4) and 10% (n = 3) of samples, respectively; all other genes were mutated less frequently, occurring in 7% or less ($n \leq 2$) of samples (**Figure 1D**).

We next analyzed the recurrent copy number alteration patterns in primary and BrM samples using Sequenza/GISTIC 2.0 (44, 45). The primary TNBC samples harbored 3 significant amplicons and 1 deleted region, whereas BrM was more profoundly altered with 15 significant amplicons and 12 regions of deletion ($q < 0.25$; **Supplementary Figure S5**). At this level of genomic resolution, only 2 regions were commonly altered between primary and BrM (11p13 amplicon and 13q11 deletion). In breast cancer, these two sites are previously known to be amplified or deleted, respectively (46, 47). Interestingly, a number of amplicons/deleted regions identified in this cohort are known to be associated with breast cancer/aggressive basal breast cancer, such as gains of 1q, 8p11-12, 8q, 12p13, 13q34, 17q, and 19q and deletions of 3p, 4p16.3, 8p, 11p15, 17p, and 19p13 (48–58). Documented breast cancer oncogenes (*NOTCH2*, *ENSA*, *PIK3CA*, *CD44*, *WT1*, *BCL2L2*, *AKT2*, and *TFF3*) and tumor suppressors (*BRCA2* and *PRKCDBP*) reside or are in close proximity to some of these significantly amplified/deleted genomic regions, and these alterations potentially contribute to TNBC progression and BrM development.

Since somatic copy number alteration (SCNA) detection tools are prone to high false positive rates as well as issues with precision and accuracy (59, 60), we also performed SCNA calling using CNVkit (61), which combines all normal samples into a pooled reference to increase performance. In contrast to Sequenza, this method identified a greater number of SCNA in primary TNBC relative to BrM, showing that, as a collective group, primary TNBC samples harbored 27 significant amplicons and 17 deleted regions, whereas BrM had 8 significant amplicons and 4 regions of deletion ($q < 0.25$; **Figures 1E, F**). Despite notable differences between the two methods, there was a concordance in the results as well, with corroboration of 8p11.22 and 11p13 amplification in primary TNBC and validation of 14q11.2 and 19q13.2 amplification and 4p16.3 deletion in BrM (**Figures 1E, F; Supplementary Figure S5**). The CNVkit SCNA analysis also highlights the potential importance of oncogenes (*e.g.*, RCP, CD44, WT1, BCL2L2, and AKT2) and tumor suppressors (*e.g.*, PRKCDBP) to TNBC etiology and metastatic progression, as significant amplicons/deleted regions harbor these genes.

Finally, we examined the ploidy, tumor purity, and subclonal makeup of tumors in this cohort. No differences in cellular ploidy were noted between primary TNBC and BrM (median ploidy of 3.2 and 3.25, respectively; **Supplementary Figure S6A**). Similarly, no significant differences in tumor purity were observed between groups (median purity of 0.57 and 0.76,

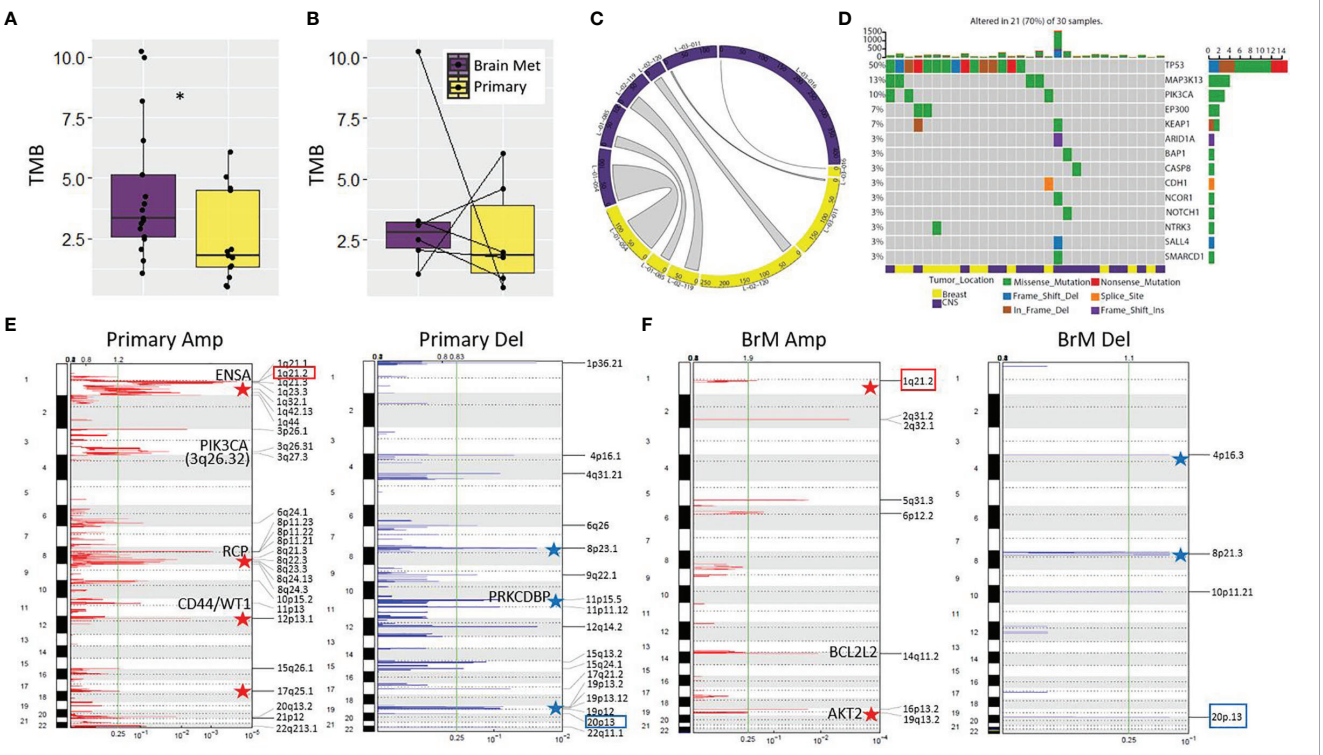

FIGURE 1 | Mutational analysis of the LCCC1419 cohort. **(A)** Increased tumor mutational burden (TMB) was observed for BrM ($n = 17$) relative to primary ($n = 13$); *$p < 0.05$, Wilcoxon rank-sum test. **(B)** No difference was observed between TMB for matched pairs ($n = 6$). **(C)** Circos plot showing the total number of variants and the proportion of variants shared between matched primary/BrM ($n = 6$). **(D)** Oncoprint displaying the mutational spectrum of cancer-associated genes with known etiology to breast cancer (COSMIC Tier 1, Sanger Institute). Representation of the somatic copy number alterations in **(E)** primary TNBC and **(F)** BrM as determined by CNVkit/GISTIC 2.0 analysis. Significant amplicons (Amp) or deleted (Del) regions are annotated ($q < 0.25$, green line). The 1q21.2 amplification and the 20p13 deletion, which are shared genomic features of primary and BrM TNBC, are highlighted by colored boxes. Relevant oncogenes and tumor suppressors are annotated on the plots, and stars indicate genomic regions where copy number alterations are known to contribute to breast cancer/aggressive basal breast cancer.

respectively; **Supplementary Figure S6B**). An analysis of subclonal tumoral architecture using Pyclone-VI (62) showed no difference between the number of clones per tumor in each location, where a median of 3.5 clones per tumor in primary TNBC (range, 1–5 clones/tumor) and a median of 4 clones per tumor in BrM (range, 3–6 clones/tumor) were observed (**Supplementary Figure S6C**). A subclonal assessment in patients with matched primary/BrM pairs showed that some pairs had similar clonal constituency between anatomical sites (*e.g.*, patients L-01-054, L-01-085, L-02-119, and L-03-016), whereas other pairs showed signs of divergent clonal evolution (*e.g.*, L-02-120 and L-03-011) (**Supplementary Figure S6D**). Interestingly, 5 of 6 matched pairs the dominant subclone harbored the highest mutational burden, which was reflected in the analysis of unmatched tumors as well (not shown), suggesting that increased mutational load may endow these subclones with a selective growth advantage.

Tumor Antigen Landscape

We next performed a comprehensive analysis of the neoantigen landscape in this cohort. We queried a range of neoantigen sources, including single-nucleotide variants (SNVs), insertion/deletion events (InDels), splice variants, structural fusion events, cancer testis antigens (CTAs)/self-antigens, endogenous retroviruses (ERVs), and viral sources excluding ERVs (63) (**Figure 2**). The predominant antigen sources in both primary TNBC and BrM were CTAs/self-antigens and ERVs (**Figures 2A, B**). Upon comparison of the number of predicted neoantigen-derived peptides, there were significantly more SNV-derived MHC class I-binding peptides in BrM as compared to primary TNBC ($p = 0.005$), with no differences seen between groups with respect to other neoantigen sources (**Figure 2B**). This analysis together shows that TNBC harbors a diverse set of potentially therapeutically actionable neoantigen-derived peptides.

Comparison of Immune Gene Signatures Between Primary TNBC and BrM

Immune gene signatures (IGS) representing multiple components of the immune system, including B cells, T cells, natural killer cells, and innate immune cells along with immune cell phenotype frequencies, were evaluated between primary TNBC tumors ($n = 15$) and BrM ($n = 19$) using RNA-Seq (**Figure 3**; **Supplementary Figure S7**; **Supplementary Data S1**). The majority of IGS across each of these categories were lower in TNBC BrM compared to primary TNBC. A gene signature associated with fibroblast-associated wound healing [Chang_Serum_Response_Up (64)] was significantly higher in BrM relative to primary tumors ($q < 0.05$). RNAseq expression data from primary tumors and BrM were also assessed using CIBERSORTx (65) to determine relative frequencies of 22 immune cell subtypes (LM22) to tumor composition. In this analysis, naïve B cells and M1 macrophages were lower in BrM compared to primary tumors, while eosinophils and neutrophils were higher in BrM tissues ($q < 0.05$) (**Supplementary Figure S8**). The expression of the 20-gene immunologic constant of rejection signature [ICR (66)], which is representative of Th1-mediated immunity, cytotoxic function, and tissue-specific destruction (*e.g.*, GVHD, autoimmunity, and allograft rejection), was also significantly reduced in BrM relative to primary TNBC (**Supplementary Figure S9A**). Additionally, the blood transcriptional modules reported by Rinchai et al. (67) were queried against our dataset and showed a significant reduction of B and T cell modules in BrM relative to primary TNBC (**Supplementary Figure S9B**), concordant with the abovementioned data. These results are together consistent with an overall immune-excluded brain tumor microenvironment (TME) in the context of TNBC BrM.

T and B Cell Repertoire Analysis

We used RNA-Seq data from primary TNBC tumors ($n = 15$) and BrM ($n = 19$) to perform T cell and B cell repertoire (TCR/BCR) profiling. Relative to primary TNBC, TNBC BrM had lower read counts of T cell receptor alpha and beta (TRA, $p < 0.001$ and TRB, $p < 0.01$), with BCR heavy chain and light chain abundance showing trending but non-significant differences (**Figure 4A**). This result is in accordance with RNA-seq data that showed less T cell and B cell abundance in the primary samples relative to BrM (**Figure 3**). Repertoire diversity was indexed as modeled Shannon entropy (37), which is a diversity index that accounts for both the *richness* of the sample (*e.g.*, the number of unique TCR/BCR sequences) and relative species *abundance* (evenness) (68, 69). Thus, a large Shannon entropy score reflects a more diverse distribution of TCR/BCR sequences. The modeled Shannon entropy (TCR/BCR diversity) was lower for BrM compared to primary tumors (TRA, $p < 0.01$ and TRB, $p < 0.05$) (**Figure 4B**). A comparison of matched BrM and primary TNBC pairs *only*, however, did not show a reduction of TCR/BCR read counts and modeled Shannon entropy (**Figures 4C, D**).

Differential Gene Expression and Pathway Analysis Support an Immune Cell Deficit in TNBC BrM Relative to Primary TNBC Tumors

Gene expression was evaluated by utilizing RNA-Seq data between the primary tumor ($n = 15$) and BrM ($n = 19$) tissues. In total, there were 1,669 differentially expressed genes (DEGs) between these 2 groups, with 935 genes upregulated and 734 genes downregulated in the BrM tissues compared to primary tumors ($q \leq 0.1$; **Figure 5A**; **Supplementary Data 2**). Gene ontology (GO) analysis of DEGs revealed a significant enrichment of immune-related terms in the primary TNBC tumors compared to the BrM (particularly terms reflecting adaptive immune system involvement), whereas GO terms associated with the nervous system were significantly higher in the BrM relative to the primary TNBC tumors (**Figure 5B**). Canonical pathway analysis (Ingenuity Pathway Analysis, IPA) of DEGs in primary tumors *versus* BrM illustrated a similar preponderance of immune signaling-related pathways as well as nervous system-related pathways associated with DEGs in BrM relative to primary tumors (**Figure 5C**). Upstream regulator analysis (IPA) further demonstrated an association of immune-related signaling activity with DEGs in primary tumors relative to BrM (*e.g.*, IFNG, NFKB, CD3, CSF2, and IL-1β) and an association

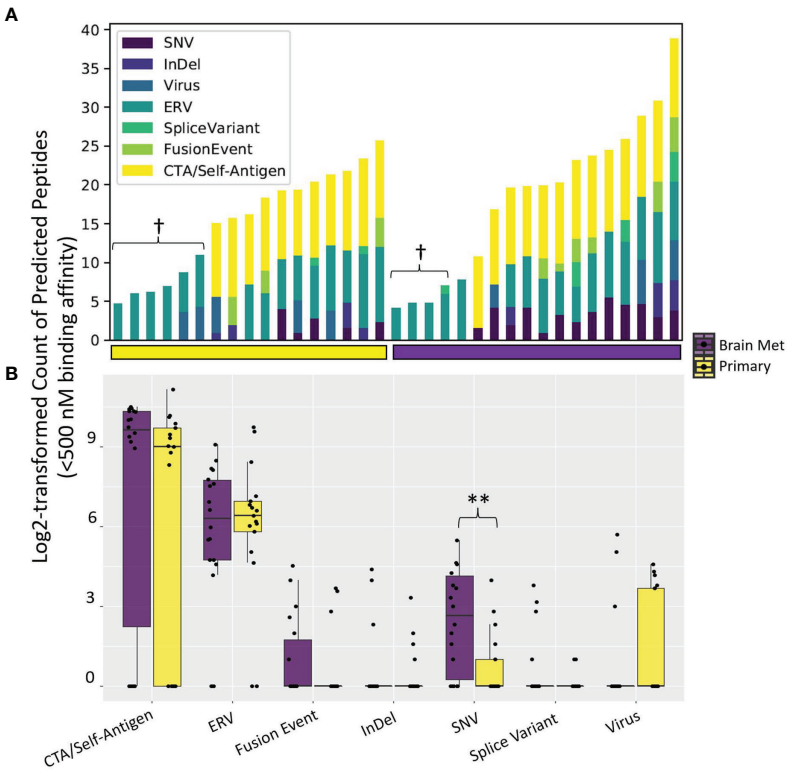

FIGURE 2 | Tumor antigen sources among the LCCC1419 patients. A comprehensive bioinformatics prediction pipeline that exports **(A)** the number of neoantigen-derived MHC class I-binding peptides (Kd < 500 nM) broken down by antigen source was employed. Some patients' tumors did not have associated tumor or normal whole-exome sequencing data, and as such, antigen sources that require DNA sequencing data (single-nucleotide variants, InDels, cancer testis antigens, or fusion events) are not able to be queried in these cases (denoted by †). **(B)** Distribution of the number of neoantigen-derived MHC class I-binding peptides (Kd < 500 nM) broken down by antigen source, corresponding to **(A)**; $^{**}p$ <0.01 (Wilcoxon rank-sum test).

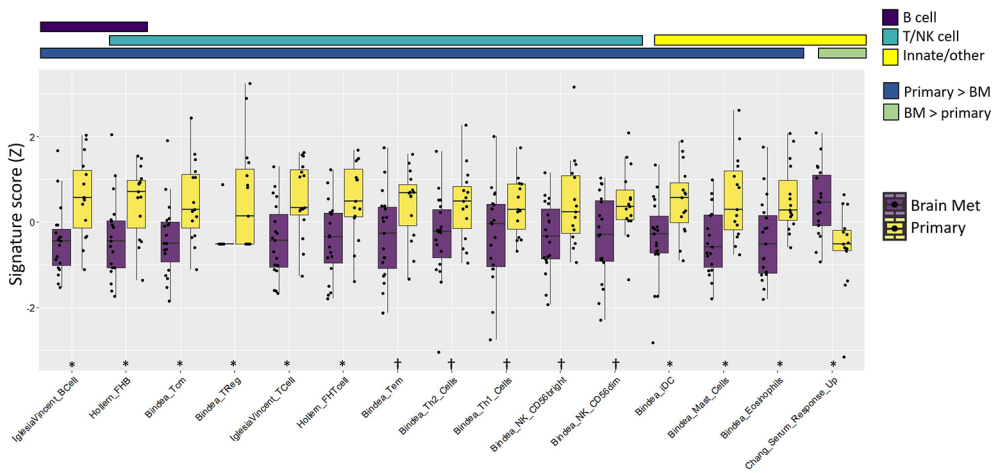

FIGURE 3 | Immune gene signature metagene analysis showed an overall immune cell deficit in BrM relative to primary triple-negative breast cancer. The colored bars above the plot indicate both the immune cell category assigned to the respective signatures and whether the signatures were increased/decreased in the primary tumor relative to BrM. Wilcoxon rank-sum test was performed on Z-transformed signature scores to determine the statistical significance after false discovery rate correction. Significance codes: $^{\dagger}q < 0.1$, $^{*}q < 0.05$.

of potential oncogenic drivers (*e.g.*, TCF7L2, mTOR, and SH3TC2) with regulation of BrM DEGs (**Figure 5D**; **Supplementary Data S3**).

Adaptive Immune Cell Signatures Are Associated With Improved Survival for Patients With TNBC BrM

We examined the survival association of standard clinicopathological variables (age at diagnosis of primary tumor, stage, and race) with different time metrics to event: (1) time from diagnosis of primary TNBC to death, (2) time from diagnosis of any metastatic disease to death, and (3) time from diagnosis of BrM to death. Of these variables, only older age was significantly associated with poor survival using each of these time metrics (**Supplementary Figure S10**), which was similar to other recent reports (70, 71). Next, survival associations relative to IGS expression were evaluated using multivariable CoxPH models in both primary TNBC and BrM. The IGS features in primary TNBC tumors which were associated with improved survival following metastatic diagnosis included T cell, B cell, and dendritic cell (DC) signatures (**Supplementary Figure S11A**). Interestingly, a fibroblast serum response/wound healing signature (64) ("Chang_Serum_Response_Up") was associated with a significantly poorer survival (p = 0.025) in BrM (**Supplementary Figure S11B**) after a diagnosis of metastasis.

DISCUSSION

In this study, we examined the genomic and transcriptomic landscape of TNBC BrM and primary tumors to further the understanding of TNBC BrM etiology and the tumor immune microenvironment. Despite recent progress in the treatment of ER+ and HER2+ BCBrM with newer brain-penetrant, targeted therapies, the treatment options for TNBC BrM remain largely restricted to chemotherapy and local therapy due to lack of known targets. A growing appreciation for the role of immunotherapy in the treatment of TNBC highlights the need to better understand the immune context of BrM as we consider incorporation of immunotherapy into the care of our patients (72).

Through whole-exome sequencing, we report that BrM, as a group, exhibited a greater TMB than primary tumors, though this observation was not recapitulated in matched tissue pairs.

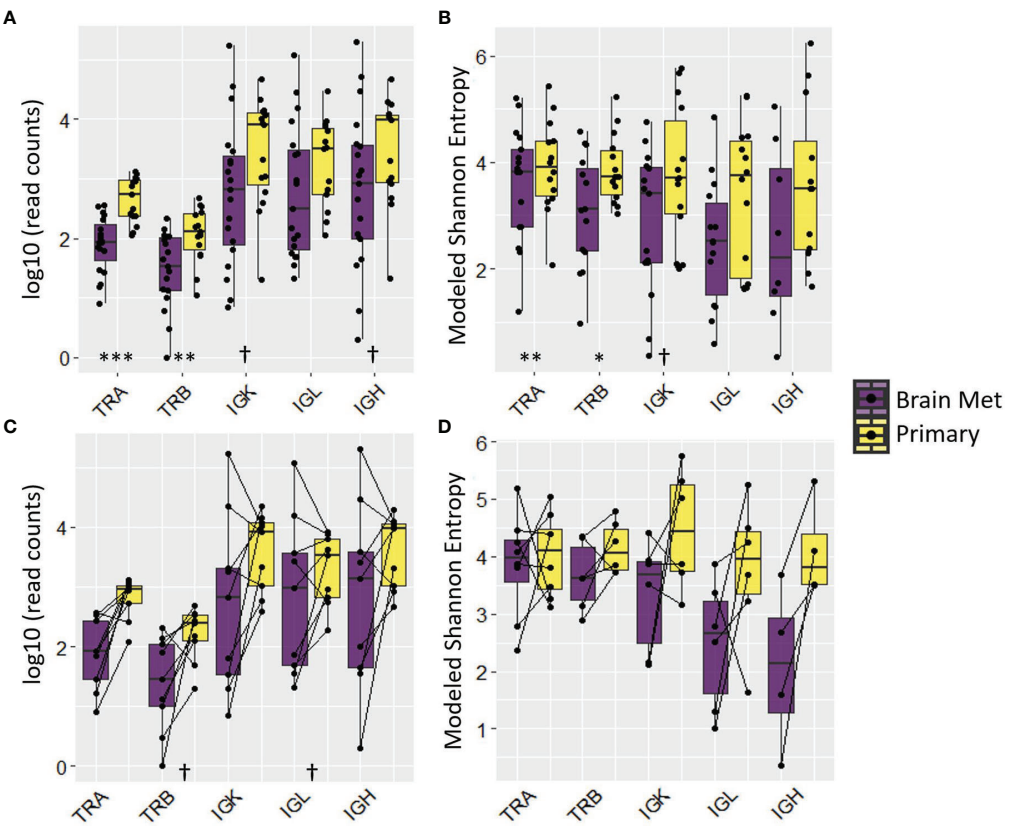

FIGURE 4 | T cell and B cell repertoire analysis revealed adaptive immune cell deficit in BrM relative to primary triple-negative breast cancer. The distribution of read counts and modeled Shannon entropy for all samples is displayed in **(A, B)**, respectively. The same information is displayed, respectively, in **(C, D)** for matched pairs (note that the number of matched pairs varies due to the presence/absence of relevant reads). Wilcoxon rank-sum test was performed on $\log_{10}$-transformed (read counts) or raw (modeled Shannon entropy) values to determine the statistical significance. Significance codes: $^{\dagger}p < 0.1$; $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.

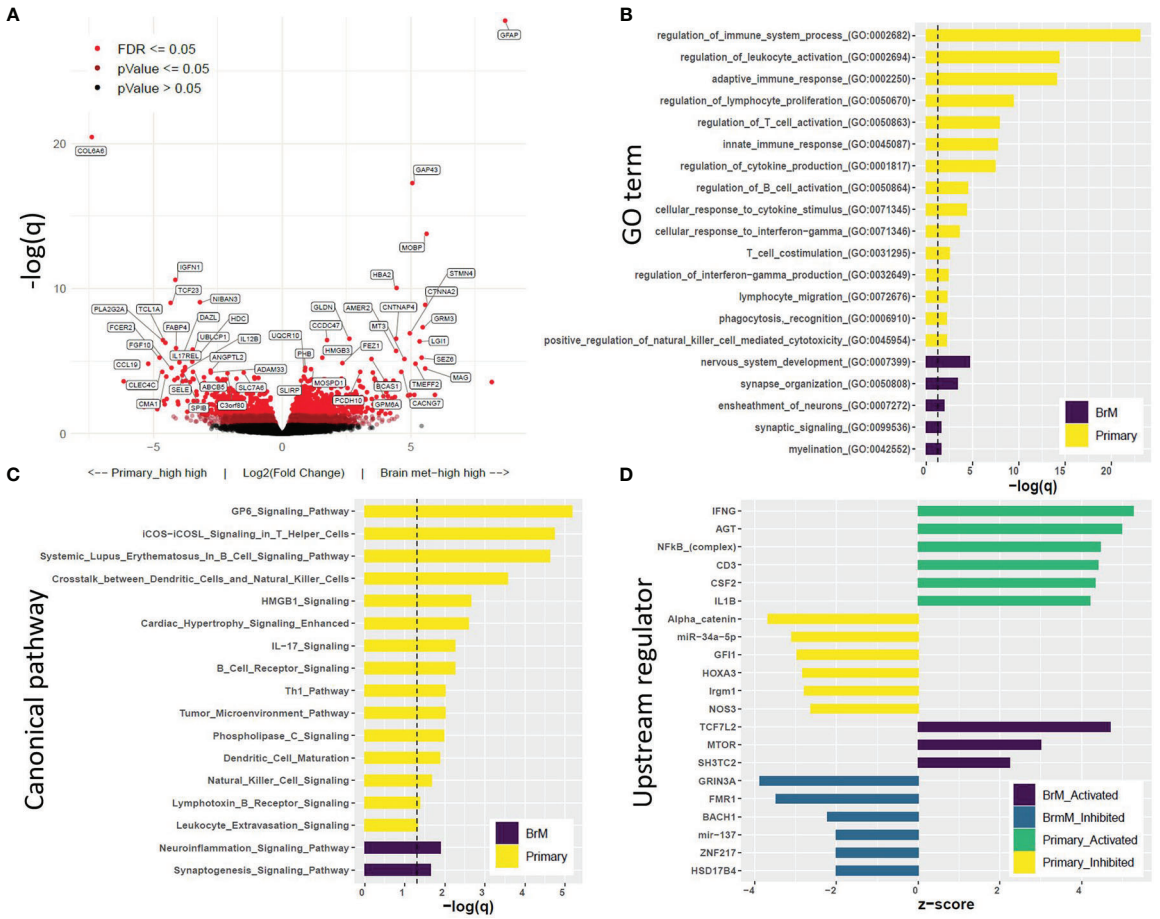

FIGURE 5 | Differential gene expression analysis and Gene Set Enrichment Analysis further support the immune cell deficit in BrM relative to primary triple-negative breast cancer (TNBC). **(A)** Volcano plot displaying differentially expressed genes (DEGs) in primary TNBC relative to BrM, with the legend showing color-coded levels of significance. **(B)** Top Gene Ontology terms associated with DEGs. DEGs with LFC > |1| and $q < 0.1$ (equating to 468 genes for primary_vs_BrM and 463 genes for BrM_vs_primary) were subjected to PANTHER overrepresentation test (dotted line represents $q = 0.1$). **(C)** Canonical pathway analysis (Ingenuity Pathway Analysis, IPA) of DEGs. The pathways displayed were significant at $q < 0.05$ (dotted line) and were associated with a significant z-score ($z > |2|$) which indicates associative activity. **(D)** Upstream regulator analysis (IPA) displaying top regulators ($z > |2|$, $q < 0.1$) identified to be associated with an active or inhibited state in primary *versus* BrM TNBC [see **Supplementary Data S2, S3** for the full list of DEGs ($q < 0.1$) and upstream regulators ($z > |2|$, $q < 0.1$).

We suspect that this was due to underpowering of our study to assess matched pairs ($n = 6$) in the context of TNBC tumor biological heterogeneity (73). An analysis of shared variants showed that matched primary TNBC and BrM samples were more alike than inter-patient primaries and inter-patient BrM, showing that TNBC is a heterogeneous disease with potentially non-redundant mechanisms of tumorigenesis. A subclonal analysis of matched pairs also showed that some patients displayed patterns of divergent evolution between primary tumors and BrM. The mutational spectrum of genes with known causality to breast cancer tumorigenesis was also queried. *TP53* was commonly mutated in this cohort (50%), supporting its causal role in the development of TNBC, while other genes such as *MAP3K13* and *PIK3CA* were mutated at a lower frequency.

Copy number variation analysis revealed common and unique genomic alteration events between primary TNBC and BrM. 11p13 was commonly amplified in both primary tumors and BrM. This genomic location harbors the *CD44* gene, which is used to discern breast CSCs, although it has been shown that it is not likely a driver of amplification of this region in basal breast cancer (74). *WT1*, which also resides at 11p13, has been shown to promote a mesenchymal phenotype in breast cancer cells as well as to elicit resistance to taxane therapy (47). Regions of 1q were also commonly amplified, which supports a known role for this genomic location in breast cancer development (48). The amplification and increased expression of ENSA (1q21.3) have recently been shown to drive TNBC progression *via* positive regulation of cholesterol biosynthesis (58). 13q11 was deleted in both primary and BrM TNBC, and this site is proximal to *BRCA2* (located on 13q13.1). Whether or not the loss of 13q11 has any *BRCA2*-regulatory functionality is unknown, although deletions in 13q and 14q are common in *BRCA2*-mutated breast cancers (75). Common deletion of 20p13 was also observed. While this

location is known to be deleted in colon cancer (76), its association with breast cancer has yet to be explored. Other common deletions identified included 1p36 and 11p15.5. In ductal breast carcinoma, the 1p36 deletion is associated with grade, *ERBB2* loss, and loss of BCL2 expression (57) and is known to be a common feature underlying breast cancer development and the carcinogenesis of various cancer types (77). BrM-specific deletion at 11p15.5 (region harboring *PRKCDBP*) was also observed, and the chromosomal loss of this region is associated with BCBrM, with *PRKCDBP* identified as a putative tumor suppressor (53).

In primary TNBC, notable amplicons were associated with both arms of chromosomes 1 and 8. These locations are associated with breast cancer cytogenetics and pathology (48, 78, 79) and harbor genes (*NOTCH2* and *RCP*, respectively) associated with breast cancer etiology (49, 80). There were also several notable alterations specific to BrM. Regions harboring the oncogenes *BCL2L2* (14q11.2), *AKT2* (19q13.2), and *TFF3* (21q22.3) were amplified in BrM. BCL2L2 is an anti-apoptotic protein that has an oncogenic role in many solid tumor types, and it has been found to contribute to breast cancer progression through its upregulation *via* hypermethylation of the negative-regulatory miR-129-2 (81). While *PIK3CA* was only mutated in 10% of evaluated samples in this study, *AKT2* upregulation *via* genomic amplification may have a significant impact on TNBC BrM progression. Dysregulation of the PI3K/AKT/mTOR axis is a common feature of TNBC (82), and this pathway represents a promising target in this disease context. *TFF3* is also associated with breast cancer metastasis, where its expression predicts poor survival (83), and it is also associated with residual invasive disease following neoadjuvant chemotherapy in breast carcinoma (84). Interestingly, *TFF3* was also found to be significantly upregulated in T cell-cold tumors of diverse tissue types, and it was in the top percentile of genes differentially expressed in T cell-cold *versus* T cell-hot breast cancers (85), which suggests its potential as an immunotherapy target. These mutational and copy number analyses together highlight potential causative genomic alterations contributing to TNBC progression and BrM.

A systematic evaluation of the neoantigen landscape in LCCC1419 was undertaken here. Using a suite of bioinformatics prediction software, we analyzed tumor-associated antigens (*e.g.*, CTAs/self-antigens), traditional tumor-specific antigens (TSAs; *e.g.*, SNVs), and alternative TSAs [*e.g.*, derived from splice variants, chromosomal structural variants, InDels, ERVs, and other viral antigens (63)]. We found that both primary TNBC and BrM harbored substantial numbers of high-affinity MHC class I-binding peptides derived from CTAs and ERVs relative to other antigen sources. CTAs are known to be associated with aggressive hormone-negative breast cancers and poor survival; however, they have also been associated with robust immunogenicity in some contexts (86). ERVs, which are evolutionary remnants of viral insertional mutagenesis, are also potentially powerful immunogens (18). Although ERV transcriptional regulation is often epigenetically silenced in normal cells, tumor cell-specific derepression is known to occur and is associated with a response to immune checkpoint blockade (ICB) in multiple cancer types (18, 87, 88). As such, antigens derived from CTAs and ERVs may be invaluable immunotherapeutic targets for vaccine strategies targeting TNBC and BrM lesions. Relative to primary TNBC, we also found an elevated SNV mutational load associated with BrM. This augmented TSA burden in BrM also represents a potential vulnerability to be targeted by combination immunotherapeutic approaches, including neoantigen vaccine strategies.

A comprehensive analysis of transcriptomic data derived from this cohort was performed to further understand the difference between the tumor immune microenvironment of primary and BrM TNBC. We found that BrM lesions harbored significantly less immune infiltrate than primary tumors. This is not surprising, as the brain has historically been considered an immunologically protected organ (89). A recent study with RNA array data in BCBrM, agnostic to subtype of BC, has similarly reported reduced immune scores in BCBrM relative to primary tumors (90). The general dearth of immune involvement in the BrM spanned both adaptive (T and B cell) and innate (DC, eosinophils, and mast cells) immune populations, indicative of a broad immune deficit relative to primary tumors and again similar to recent reports (90). Interestingly, BrM displayed an elevated expression level of genes involved in a serum-induced fibroblast wound healing response (64). This finding may suggest that, relative to primary TNBC, BrM lesions are more reliant on aberrant wound healing properties, requiring increased levels of stromal involvement for growth and maintenance, as seminally put forth by Dvorak (91). We also observed that BrM had significantly decreased TCR (TRA/TRB) abundance and diversity as compared to primary tumors, and this association was verging on significance for certain immunoglobulin classes. These metrics are important, as increased TCR abundance and diversity have been associated with a response to ICB in multiple solid tumor types (92). DEGs between primary and BrM TNBC also reflected a BrM-specific immune deficit. Gene Ontology and canonical pathway analysis showed that genes that exhibited relatively lower expression levels in BrM *versus* primary tumors were enriched for terms related primarily to an adaptive immune response. An upstream regulator analysis further supported these findings, with IFNG being the putative regulator with the highest significance. In BrM, this upstream regulator analysis further demonstrated the importance of mTOR signaling but also showed that *TCF7L2* and *SH3TC2* may be important players in BrM development. TCF7L2 variants have been found to be associated with breast cancer incidence (93, 94). Additionally, this gene is a positive regulator of Wnt signaling, regulates the MYC oncogene, represses the cell cycle inhibitors CDKN2C/CDKN2D, and is a transcriptional driver of various oncogenes, contributing to the progression of colon cancer and other cancer types (95).

We performed survival analyses examining the prognostic potential of IGS in the context of standard clinicopathological

features. Signatures representing levels of T cells, B cells, NK cells, and DC cells in the primary tumors were associated with improved survival. The collective association of these IGS with favorable survival is likely indicative of the high degree of expression correlation structure (and thus co-infiltration levels of the associated immune cell types) observed in this cohort (**Supplementary Figure S5**). Moreover, the favorable association between T cell, B cell, and DC IGS and survival may indicate that patients with higher anti-tumor immune infiltrate in their primary TNBC may have a higher propensity to develop long-lasting immunological memory that functions to stave off metastatic spread. Similarly, levels of signatures reflective of gamma-delta T cells and ICB responsiveness [*e.g.*, Vincent_IPRES_Responder signature (96)] in BrM were associated with improved survival from the time of BrM diagnosis, indicating that elevated immune involvement in the brain TME may be beneficial to patient survival. Conversely, the aforementioned fibroblast wound healing signature [Chang_Serum_Response_Up (64)] was associated with poor survival in BrM, indicative of a deleterious quality of this signature and the underlying biology that it represents.

While this study represents the largest series focused on TNBC BrM to date, to our knowledge, it is mainly limited by low power, particularly regarding matched pairs (with only $n = 6$ matched WES and $n = 9$ matched RNA-seq pairs). An additional limitation is the inability to corroborate adaptive immune receptor repertoire inference with amplicon sequencing, which was precluded due to inadequate specimen nucleic acid abundance. Future work will expand these, and additional analyses to additional TNBC samples, as well as to other BC subtypes in the LCCC1419 biobank, including HER2+ and ER/PR+ BCBrM, to enable a comparison of BrM across the spectrum of BC. Utilization of *in vivo* murine models for testing the relevance of these findings, including the assessment of vaccine strategies and ICB as potential therapeutic approaches for TNBC BrM, is warranted.

In summary, we report the genomic characterization of BrM compared to primary tumors from TNBC patients, including some matched pairs, with a focus on the immune landscape. Utilizing both WES and RNA-seq analytical pipelines, we demonstrated that BrM exhibited increased TMB and SNV mutational load, reduced immune gene signature expression and TCR receptor abundance/diversity metrics, and increased expression of a wound healing signature. A prediction of elevated levels of CTA- and ERV-specific neoantigen peptides was confirmed in both anatomical locations, supporting the continued development of vaccine and immune checkpoint inhibition approaches in TNBC. IGS, including T cell-related immune signatures in primary and BrM TNBC, correlated with improved survival in this patient cohort. We expect that these results and the data reported herein will be valuable in understanding TNBC BrM biology going forward and provide further rationale for the application of immunotherapeutic approaches in this disease.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The name of the repository and accession number can be found below: https://www.ncbi.nlm.nih.gov/projects/gap/cgi-bin/study.cgi?study_id=phs002457.v1.p1.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the University of North Carolina at Chapel Hill Institutional Review Board. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

Conception: CA, BV, MM, and LCa. Design: ER, AVS, MS, MM, BV, SC, CA, and LiC. Acquisition/analysis: MS, LuC, LiC, AG, AW, ER, SV, SC, MW, JP, MM, AVS, BV, CA, and AH. Interpretation: ER, AVS, MS, SC, JP, MM, MW, CA, and BV. Manuscript preparation/editing: all authors. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2022.818693/full#supplementary-material

Supplemental Figure 1 | Samples included in immunohistochemical and sequencing (RNA and DNA) analyses. Primary breast tumor, BrM, and normal blood specimens from a total of 25 patients with BrM from TNBC were included in the analyses. Matched blood was analyzed by DNA sequencing as a comparison for tumor WES for identification of somatic variants. Final sample numbers by tissue and analysis type are provided.

Supplemental Table 1 | Clinicopathological characteristics and specimen availability by patient in the LCCC1419 study cohort. Grayed boxes indicate tumors that switched subtypes between the primary tumor and the BrM based on IHC (n=2 patients). "Y" indicates a specimen was analyzed by the indicated method.

Supplemental Figure 2 | PAM50 intrinsic molecular subtype analysis of RNA-Seq expression data. **(A)** Heatmap displaying that the majority of samples in 1419 were of the basal subtype. Subtype analysis was performed according to methods described in Picornell et al27. Red circles highlight samples from the two patients where IHC indicated subtype switching (see **Table 1**). The R package heatmaply97 was used for heatmap visualization with hierarchical clustering based on average linkage. **(B)** Stacked barplot displaying percentage of primary and BrM tumors belonging to respective molecular subtypes.

Supplemental Figure 3 | Intrasample variant sharing is minimal in primary and BrM TNBC. Circos plot showing total number of variants and proportion of variants shared between primary TNBC (yellow; n=13) and BrM (purple; n=17).

Supplemental Figure 4 | TMB is associated with survival in the LCCC1419 TNBC cohort. Multivariable survival analysis including age at diagnosis, race, stage, and TMB of primary tumor as covariates relative to time to event, which for this analysis was the time from diagnosis of the primary tumor to death. Patients with unknown race or stage were excluded from analysis (n=11 patients included). Additionally for this analysis stage I and II were binned, and stage III and IV were binned.

Supplemental Figure 5 | Somatic copy number alteration assessment. SCNA in **(A)** primary TNBC (n=12) and **(B)** BrM (n=16) as determined by Sequenza/GISTIC 2.0 analysis. Significant amplicons (Amp) or deleted (Del) regions are annotated (q<0.25, green line). 11p13 amplification and 13q11 deletion, which are shared genomic features of primary and BrM TNBC, are highlighted by colored boxes. Potential oncogenes and tumor suppressors are annotated on the plots, and stars indicate genomic regions where copy number alterations are known to contribute to breast cancer/aggressive basal breast cancer.

Supplemental Figure 6 | Ploidy, tumor purity, and subclonal heterogeneity assessment of LCCC1419. **(A)** Ploidy and **(B)** tumor purity were assessed using Sequenza, where no significant differences were observed between primary TNBC (n=12) and BrM (n=16); matched pairs shown on right of each panel (n=6). **(C)** Distribution of subclone number per tumor. **(D)** Cellular prevalence and mutational load of subclones in matched pairs (n=6).

Supplemental Figure 7 | Correlation matrix of expression of immune gene signatures (IGS) used in this study. **(A)** IGS (n=32; refer to Supplemental Data 1 for genes comprising respective signatures) were quantified from RNA-Seq expression data from primary TNBC (n=15) and BrM (n=19), and analyzed by Spearman correlation analysis. **(B)** IGS correlation matrix applied to the same dataset from **(A)** using a compendium of IGS (64 signatures; refer to Supplemental Data 1 for genes comprising respective signatures) curated by our group. Due to the high degree of correlation of some of the subsets of IGS, we culled this list to the 32 signatures shown in **(A)** so as to reduce signature redundancy while maintaining breadth of represented immune cell types/features. Color denotes Spearman rho, and X indicates a non-significant relationship (p>0.05). The R package Ggcorrplot98 was used to generate correlation plot.

Supplemental Figure 8 | Immune cell type deconvolution of RNA-Seq expression data. RNA-Seq expression data from primary TNBC (n=15) and BrM (n=19) was input into CIBERSORTx (33) (https://cibersortx.stanford.edu/) to determine relative contributions of 22 immune cell subtypes (LM22) to tumor composition. Wilcoxon rank-sum test was performed on Z-transformed cell fraction values to determine statistical significance. Significance codes: *q<0.05; **q<0.01.

Supplemental Figure 9 | Analysis of additional immune modules further demonstrate immune cell deficit in BrM relative to primary TNBC. **(A)** Expression of the 20-gene Immunologic Constant of Rejection (ICR) signature was significantly reduced in BrM (n=19) relative to primary TNBC (n=15). **(B)** Blood transcriptional module repertoires reported by Rinchai et al (DOI: 10.1093/bioinformatics/btab121) were applied to the LCCC1419 RNAseq dataset using the Bioconductor R package BloodGen3Module Groupcomparison function. Significant immune modules are annotated on the plot. This analysis corroborates data showing a reduction of B and T cells in the BrM relative to primary TNBC (q<0.1).

Supplemental Figure 10 | Multivariable survival analysis of clinicopathological variables. Clinicopathological variables (age at primary tumor diagnosis, race, and stage) were analyzed by multivariable CoxPH. Category labels indicate time metric to event that was applied. Patients with unknown race (including one Asian patient) or stage were excluded from analysis (n=21 patients included). Additionally for this analysis stage I and II were binned, and stage III and IV were binned. Significance codes: *q<0.05; **q<0.01; ns, not significant.

Supplemental Figure 11 | Multivariable CoxPH analysis of IGS in primary and BrM TNBC. Each of 32 IGS (refer to **Supplemental Figure 7**) was included as a covariate along with clinicopathological variables (age at diagnosis, race, stage), which yielded odds ratios (OR; hazard ratios) and p values. These p values were then FDR-adjusted. This analysis was performed using three different time metrics to death, as indicated in **(A)** primary TNBC and **(B)** BrM analyses. Only IGS that had unadjusted p values of < 0.1 are shown. Note that none of the IGS achieved significant association with survival after false discovery correction, which is likely due to low sample number.

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