nature portfolio

Corresponding author(s): Rashmi K. Murthy

Last updated by author(s): Jul 30, 2025

Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our Editorial Policies and the Editorial Policy Checklist.

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed
☐	☒ The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒ A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒ The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☐	☒ A description of all covariates tested
☐	☒ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒ A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒ For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☐	☒ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☒	☐ Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	RedCap
Data analysis	Pharmacokinetic analyses were conducted using WinNonlin Version 8.1 (Certara L.P., Princeton, NJ) and plots were constructed using GraphPad Prism version 8.1.0 for Windows (GraphPad Software, San Diego, CA).

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

Data underlying the findings described in this article may be obtained in accordance with the TBCRC data sharing policy. Requests for TBCRC data or tissue for the purpose of carrying out ancillary analyses that are not part of the objectives of the parent TBCRC study must be reviewed by the TBCRC Correlative Science Review Committee.

Research involving human participants, their data, or biological material

Policy information about studies with human participants or human data. See also policy information about sex, gender (identity/presentation), and sexual orientation and race, ethnicity and racism.

Reporting on sex and gender	From February 20, 2019 through June 22, 2021, 17 female patients were enrolled and received at least one dose of tucatinib, capecitabine, and trastuzumab at four sites in the United States
Reporting on race, ethnicity, or other socially relevant groupings	Race was self reported. Five patients identified as Black (29%) and 12 patients as White (71%).
Population characteristics	The median patient age at study treatment initiation was 53 years (range: 33-67). Five patients identified as Black (29%) and 12 patients as White (71%).
Recruitment	The study enrolled at four sites in the United States , The University of Texas MD Anderson Cancer Center (Houston, TX); University of Alabama at Birmingham O'Neal Comprehensive Cancer Center (Birmingham, AL); University of California San Francisco (San Francisco, CA); University of Michigan (Ann Arbor, MI) through the Translational Breast Cancer Research Consortium (TBCRC). Eligible patients were adults (men and women, based on self-report), aged ≥18 years at the time of consent with histologically proven metastatic HER2+ breast carcinoma by immunohistochemistry (IHC) 3+ and/or fluorescence in situ hybridization (FISH) ratio > 2.0, or average HER2 copy number > 6.0 signals per cell or per current American Society of Clinical Oncology – College of American Pathologists (ASCO-CAP) or National Comprehensive Cancer Network (NCCN) guidelines, and newly diagnosed LM, defined as positive CSF cytology and/or radiographic evidence of LM plus neurologic signs/symptoms attributed to LM, and KPS ≥ 50.
Ethics oversight	The study protocol was reviewed and approved by the institutional review board at each participating institution.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	17 ; The TBCRC049 study employed a Gehan-like two-stage trial design, aiming to enroll a total of 30 patients. This design incorporated an interim futility analysis after the enrollment of the first 15 patients to allow early termination if the treatment lacked sufficient clinical activity. The early efficacy endpoint for this analysis was progression-free survival (PFS) in the CNS at 12 weeks, with "success" defined as the absence of CNS progression or death from any cause during this period. If fewer than two successes were observed among the first 15 patients, enrollment would have been halted for futility. As this threshold was met, the trial proceeded toward full enrollment with the goal of estimating the median overall survival (OS).The sample size was determined not to power a formal hypothesis test but rather to allow for an initial screen for activity and a descriptive estimate of OS. The threshold for promising activity was based on comparison to a historical control median OS of 4.4 months, derived from a retrospective cohort of 56 patients with HER2+ LM treated at a single tertiary care center. The study team estimated that if the true median OS were 6.0 months, the trial would yield an observed median OS >4.4 months with approximately 88% probability, supporting further evaluation of the regimen. However, the study closed early after enrolling 17 patients due to poor accrual following the FDA approval of tucatinib in April 2020, which made the drug commercially available and limited further trial participation. Rationale for Sufficiency: Although the trial did not reach the planned 30 patients, the adaptive design and early efficacy signal provided initial support for treatment activity in a rare and understudied population. The use of a historical control and the probabilistic estimate of detecting a clinically meaningful improvement in OS provide a reasonable justification for the sample size given the rarity of HER2+ LM and the feasibility challenges of recruiting such patients in a post-approval setting.
Data exclusions	Pre-established exclusions : Not HER2+ breast cancer; no newly diagnosed leptomeningeal (LM); previous LM - directed therapy; received whole brain radiotherapy
Replication	To replicate the findings of this phase 2 study, a clinical trial should enroll HER2+ breast cancer patients with newly diagnosed leptomeningeal metastases (LM) who meet eligibility criteria, including MRI-confirmed LM and either abnormal CSF cytology or neurologic symptoms. Patients should receive tucatinib (300 mg BID), capecitabine (1000 mg/m² BID, Days 1–14 of a 21-day cycle), and trastuzumab (6 mg/kg IV every 21 days, with an 8 mg/kg loading dose if needed).The primary endpoint is overall survival (OS), with secondary endpoints assessing CNS progression-free survival, composite LM response (via CSF cytology, MRI, and neurologic function), and patient-reported quality of life. Safety should be monitored using CTCAE criteria, tracking common adverse events such as diarrhea, nausea, and liver function changes. Pharmacokinetic analysis should measure tucatinib and ONT-993 levels in CSF and plasma, alongside ctDNA analysis to explore biomarkers.

	The study should follow ethical guidelines, use historical controls for OS comparison, and aim for ~30 patients in a Gehan-like design. All attempts at replication were successful
Randomization	No, the study was not randomized; The study was a single-arm, non-randomized phase 2 trial due to the rarity and aggressive nature of leptomeningeal metastases (LM) from HER2+ breast cancer, which makes large-scale randomization challenging. Ethical concerns also played a role, as withholding effective systemic therapy from a control group would be inappropriate given the historically poor prognosis. Instead, the study used historical controls to evaluate efficacy. A single-arm design allowed for faster enrollment and treatment initiation, crucial for this rapidly progressing disease.
Blinding	Since there was no randomized control arm, blinding was unnecessary to prevent bias in treatment allocation.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☒	☐ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☒	☐ Animals and other organisms
☐	☒ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Clinical data

Policy information about clinical studies

All manuscripts should comply with the ICMJE guidelines for publication of clinical research and a completed CONSORT checklist must be included with all submissions.

Clinical trial registration	The clinical trial registration number for this study is NCT03501979.
Study protocol	The study protocol can be accessed in accordance with the Translational Breast Cancer Research Consortium (TBCRC) data sharing policy. Requests for data or study materials, including the protocol, must be reviewed by the TBCRC Correlative Science Review Committee. You may contact the corresponding author, Dr. Rashmi K. Murthy at rmurthy1@mdanderson.org, for further details on obtaining access.
Data collection	The TBCRC049 (NCT03501979) study was conducted at four U.S. sites (University of Texas MD Anderson Cancer Center (Houston, TX) ; University of Alabama at Birmingham O'Neal Comprehensive Cancer Center (Birmingham, AL); University of California San Francisco (San Francisco, CA);University of Michigan (Ann Arbor, MI)).These institutions are part of the Translational Breast Cancer Research Consortium (TBCRC), which coordinated the study. All data were collected according to protocol at these sites, and assessments were performed by site investigators, radiologists, and neuro-oncologists, with data reviewed centrally when applicable, enrolling 17 patients from February 20, 2019, to June 22, 2021. Data collection included baseline assessments such as MRI imaging, CSF cytology, neurologic examinations, and prior treatment history. Patients received tucatinib, trastuzumab, and capecitabine in 21-day cycles, with treatment monitoring through MRI scans every other cycle, CSF analysis via lumbar puncture or Ommaya reservoir, and neurologic function assessment using the NANO scale. Patient-reported outcomes, including quality of life and symptom burden, were measured using MDASI-BT and LASA questionnaires. Safety was closely monitored, with adverse events graded per CTCAE v4, while pharmacokinetic data were collected from plasma and CSF samples to analyze tucatinib and ONT-993 concentrations.
Outcomes	The study's primary outcome was overall survival. Primary Outcome: Overall Survival (OS) - Defined as the time from initiation of study treatment to death from any cause. All patients who initiated treatment were included in the modified intent-to-treat analysis for this endpoint. Secondary outcomes included safety (Adverse events were assessed and graded using CTCAE version 4. All patients receiving at least one dose of therapy were included in safety analyses), time to CNS progression (Defined as the time from treatment initiation to the first occurrence of CNS progression, based on clinical and radiographic evaluation) , the LM composite objective response (Assessed using a composite measure adapted from the Response Assessment in Neuro-Oncology – Leptomeningeal Metastases (RANO-LM) criteria. Included three domains: CSF cytopathology – Conversion from positive to negative CSF cytology (confirmed on a subsequent sample). Neuroaxis imaging – Radiologic partial or complete response (PR or CR) per MRI.Neurologic clinical evaluation – Stability or improvement in pre-identified LM-related neurologic signs/symptoms using: The Neurologic Assessment in Neuro-Oncology (NANO) scale (exam findings), and The MD Anderson Symptom Inventory – Brain Tumor module (MDASI-BT) (patient-reported neurologic symptoms like headache, seizure) and clinical benefit (Clinical benefit was defined as stable disease (SD), partial response (PR), or complete response (CR)), pharmacokinetics (Plasma and CSF samples were collected at specific timepoints during Cycles 1 and 2. Concentrations of tucatinib and its metabolite ONT-993 were quantified using validated HPLC-MS/MS methods), and patient-reported outcomes(Evaluated using: MDASI-BT: Collected every cycle to assess symptom burden. Linear Analog Self-Assessment

(LASA): Administered every other cycle to assess overall QoL. Changes from baseline in these scores were analyzed descriptively over time) including quality-of-life assessments

Plants

Seed stocks: N/A

Novel plant genotypes: N/A

Authentication: N/A