Intracarotid Injection-Based Brain Metastasis Models Without Disrupting the Blood-Brain Barrier for Preclinical Drug Evaluation

Arianna Bandini[1,2], Gojko Bijelic[1], Melanie Heisler[1], Pia Norz[1], Philipp Metzger[1], Cynthia Obodozie[1], Holger Weber[1]

[1]Reaction Biology Europe GmbH, Freiburg, Germany; [2]Department of Translational Research and New Technologies in Medicine and Surgery, University of Pisa, Pisa, Italy

Introduction

Brain metastases are one of the most common and serious complications of solid tumors, affecting 20-40% of patients. They are common in lung, breast, and melanoma cancers and are associated with a poor prognosis and limited treatment options. Key challenges include the blood-brain barrier (BBB), which restricts penetration of many therapeutic agents, and the brain's historically immune-privileged status, limiting immune responses against tumor cells. Establishing in vivo models that accurately replicate the natural metastatic process to the brain is essential for understanding disease mechanisms and advancing therapeutic research.

Most current preclinical brain metastasis models rely on intracranial implantation of tumor cells directly into the brain or systemic delivery via intracardiac injection. Although intracranial implantation enables rapid local tumor formation, this approach disrupts the BBB integrity and limits physiological relevance for studying natural metastatic progression and evaluating compounds with specific BBB-crossing properties. In contrast, intracardiac injection allows systemic dissemination of tumor cells and can lead to brain metastases in ~50% of cases using MDA-MB-231 cells and 70%-80% using JIMT-1 cells while likely preserving BBB integrity. However, these models are limited by suboptimal brain metastasis rates or early euthanasia due to aggressive extracranial tumor growth, hindering study of brain-specific disease progression over time.

Method

In vitro proliferation
MDA-MB-231 human breast cancer cells were treated for 72 h with temozolomide (TMZ), paclitaxel (PTX), or ML00253764 (ML). Viable cells were counted and expressed relative to vehicle control. IC_{50} values were calculated by nonlinear regression. For combination studies, ML was combined with PTX or TMZ at a fixed 1:10 ratio. Drug interactions were assessed using the Chou–Talalay method (CI) and dose reduction indices (DRI) and confirmed using the Loewe additivity model.

Intracarotid injection model
Brain metastases were established by intracarotid injection of tumor cells in anesthetized mice. The carotid artery was exposed by gentle displacement of surrounding glands and muscles, enabling visualization of the bifurcation into internal and external branches. After ligation of the external carotid artery, tumor cells in PBS were injected using a fine-gauge needle over 1–2 minutes. Animals received inhalation anesthesia and peri-operative analgesia and were closely monitored throughout the study. Tumor development was assessed by bioluminescence imaging.

Efficacy evaluation in an intracarotid brain metastasis model
MDA-MB-231_Luc cells were injected via the intracarotid route, and tumor growth was monitored by bioluminescence imaging. Following randomization based on bioluminescence signal, animals were treated with Temozolomide, Abraxane, or ML00253764, either as monotherapies or in combination.

Method

- Despite the technically challenging surgical procedure, the majority of animals survive the intervention.
- The take rate for brain metastasis formation is high.
- Tumor growth was restricted to the brain, in contrast to intracardiac injection, which resulted in tumor dissemination to multiple organs with a higher overall tumor burden.
- In the efficacy study, Temozolomide significantly inhibited brain metastasis growth, whereas Abraxane showed no effect, consistent with their differing abilities to cross the blood–brain barrier.
- In contrast to the in vitro findings, synergistic effects could not be detected for the tested combination treatments under the in vivo conditions investigated.

Generation and characterization of CAR T cells

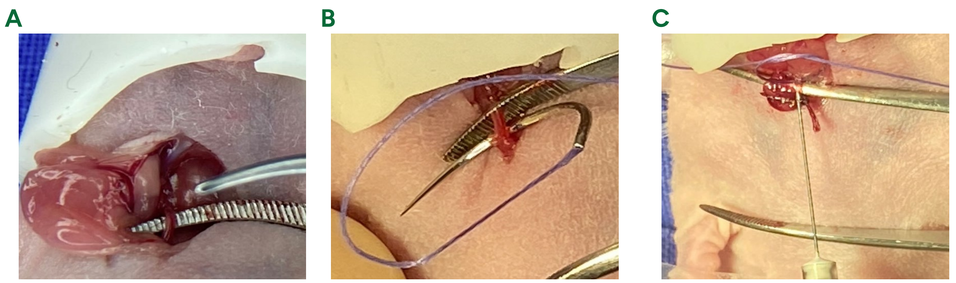


Figure 1: Images illustrating key steps of the intracarotid artery injection procedure. **(A)** Surgical exposure of the carotid artery by gentle displacement of surrounding glands and muscles, enabling visualization of the bifurcation into the internal and external carotid branches. **(B)** Ligation of the external carotid artery using absorbable sutures.
(C) Slow injection of tumor cells into the carotid artery using a fine-gauge needle over 1–2 minutes. Animals received inhalation anesthesia and peri-operative analgesia.

Comparison of brain metastasis models

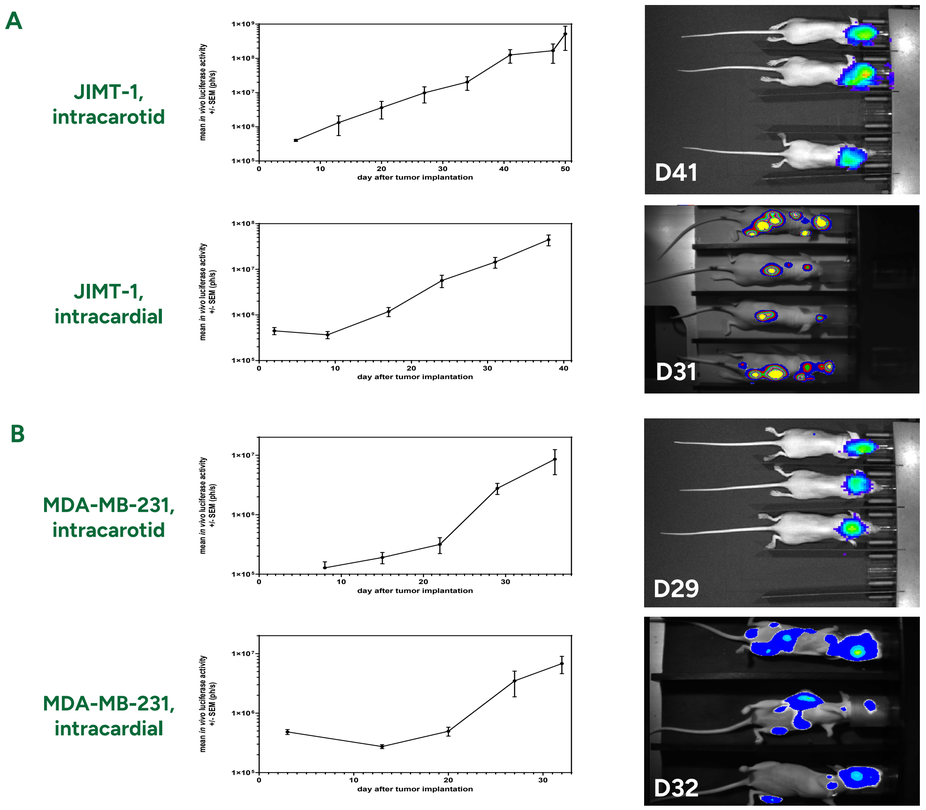
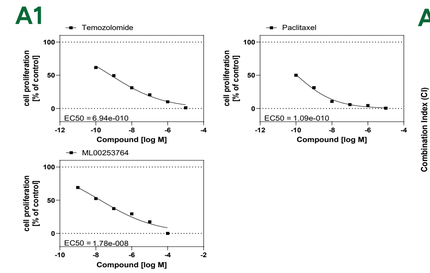


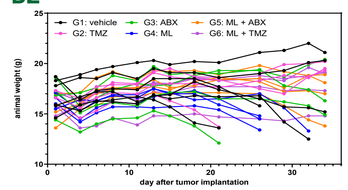
Figure 2: **(A)** JIMT-1_Luc cells (1 × 10^5 cells) were injected either into the left ventricle of the heart (intracardiac injection) or into the carotid artery. **(B)** Similarly, MDA-MB-231_Luc cells were administered via intracardiac injection (2.5 × 10^5 cells) or intracarotid injection (1 × 10^5 cells). Tumor growth was monitored longitudinally by bioluminescence imaging until humane endpoints were reached. Growth kinetics over time (left panels) and representative images (right panels) are shown. The two injection routes resulted in distinct metastasis patterns and growth dynamics. These data demonstrate the impact of injection route and cell line on brain metastasis establishment.

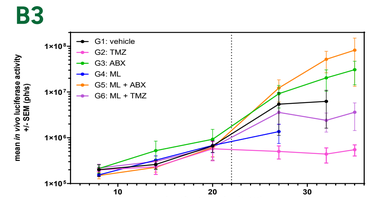
In vivo efficacy study

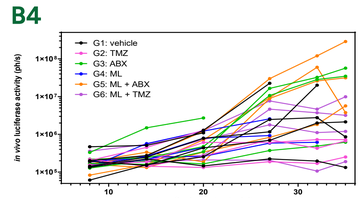


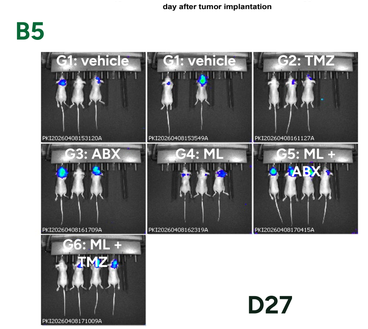
B1

Group	Test specimen	Dose (mg/kg)	Dose Volume (ml/kg)	Administration route	Dosing frequency	Number of animals
1	Vehicle	-	10	s.c.	daily	6
2	Vehicle + Temozolomide	- + 25	5 + 5	s.c. + i.p.	daily every 2 days	4
3	Vehicle + Abraxane (Nab-Paclitaxel)	- + 50	10 + 5	s.c. + i.v.	daily weekly	4
4	ML00253764	30	10	s.c.	daily	4
5	ML00253764 + Abraxane (Nab-Paclitaxel)	30 + 50	10 + 5	s.c. + i.v.	daily + weekly	4
6	ML00253764 + Temozolomide	30 + 25	10 + 5	s.c. + i.p.	daily every 2 days	4









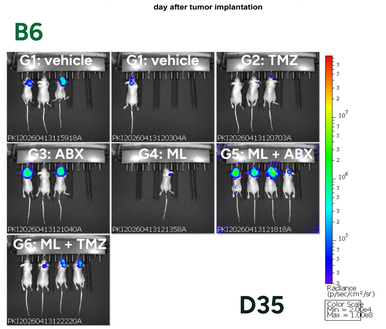


Figure 3: **(A)** (A1) MDA-MB-231 cells were treated with increasing concentrations of Temozolomide, Paclitaxel, or ML00253764 for 72 h, and cell proliferation was assessed. (A2) In addition, test compounds were applied in combination at a fixed 1:10 ratio (Temozolomide or Paclitaxel vs. ML00253764). Drug interactions were analyzed using the Chou–Talalay method, and the combination index (CI) versus fraction affected (Fa) is shown. Both combinations demonstrated synergistic effects, with the Paclitaxel/ML00253764 combination showing stronger synergy. For the in vivo study, MDA-MB-231 cells were injected via the carotid artery, and animals were randomized on day 20 post-injection. Treatment according to the scheme shown in (B1) started the following day. Animal body weight is shown in (B2). Tumor growth was assessed by bioluminescence imaging and is presented as group means (B3) or individual growth curves (B4). Representative bioluminescence image overlays from day 27 (B5) and day 35 (B6) are shown. TMZ = Temozolomide, ABX = Abraxane, ML = ML00253764

Contact Information

Holger Weber, PhD

Reaction Biology Europe
Engesserstr. 4
79108 Freiburg, Germany

+49-151-24000023
Holger.Weber@reactionbiology.com
www.reactionbiology.com