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REVIEW

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Beyond the brain: exploring the impact of animal models of leptomeningeal disease from solid tumors

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Abstract

Leptomeningeal disease (LMD) is a devastating manifestation of late-stage cancer which currently suffers from a lack of effective therapeutics. Unfortunately, a significant obstacle preventing the widespread development and testing of therapeutics for LMD is the lack of biologically accurate animal models. We provide overviews of six types of animal models of leptomeningeal metastasis from solid tumors: injection of tumor cells into the cerebrospinal fluid (CSF), blood, or brain parenchyma; subcutaneous or mammary fat pad injection of tumor cells; the LeptoM/LM-phenotype model; and genetic manipulation. We identify the pros and cons of each model and suggest broad areas of future research that could improve each model in terms of its similarity to human LMD.

Keywords Leptomeningeal disease, Leptomeningeal metastasis, LMD, Animal model, Cerebrospinal fluid, CSF

Introduction

LMD diagnostics, treatment, and prognosis

Leptomeningeal disease (LMD), also known as leptomeningeal carcinomatosis (LMC) or leptomeningeal metastasis (LM), is a rare and generally lethal complication of systemic cancer. It occurs when cancer cells seed the leptomeningeal space, which is found under the dura mater and is comprised of the pia mater, subarachnoid space, and arachnoid mater of the brain and spinal cord (Fig. 1A). LMD can occur in patients with solid tumors or hematologic malignancies; in this review, we will focus on LMD arising from solid tumors. LMD prevalence varies widely among cancer types, with studies reporting a prevalence of up to 30% in some cancer types (Table 1) [64]. Based on clinical data, the incidence of LMD may be as high as 132,000 new cases per year in the United States (US) alone, and this number may be higher based on autopsy studies. Unfortunately, LMD incidence is only expected to increase as cancer patients live longer due to improvements in diagnostics and therapeutics.

LMD is generally diagnosed in patients with advanced-stage solid tumors. Patients can present with a wide range of symptoms that can lead to a diagnosis of LMD due to the disease's ability to affect any part of the brain or spinal cord. Symptoms may occur due to the dysfunction of cranial or spinal nerves in the subarachnoid space or a mass effect on the underlying brain or spinal cord. LMD may be diagnosed through clinical features, imaging findings, or cerebrospinal fluid (CSF) analysis. The most commonly used imaging modality for LMD

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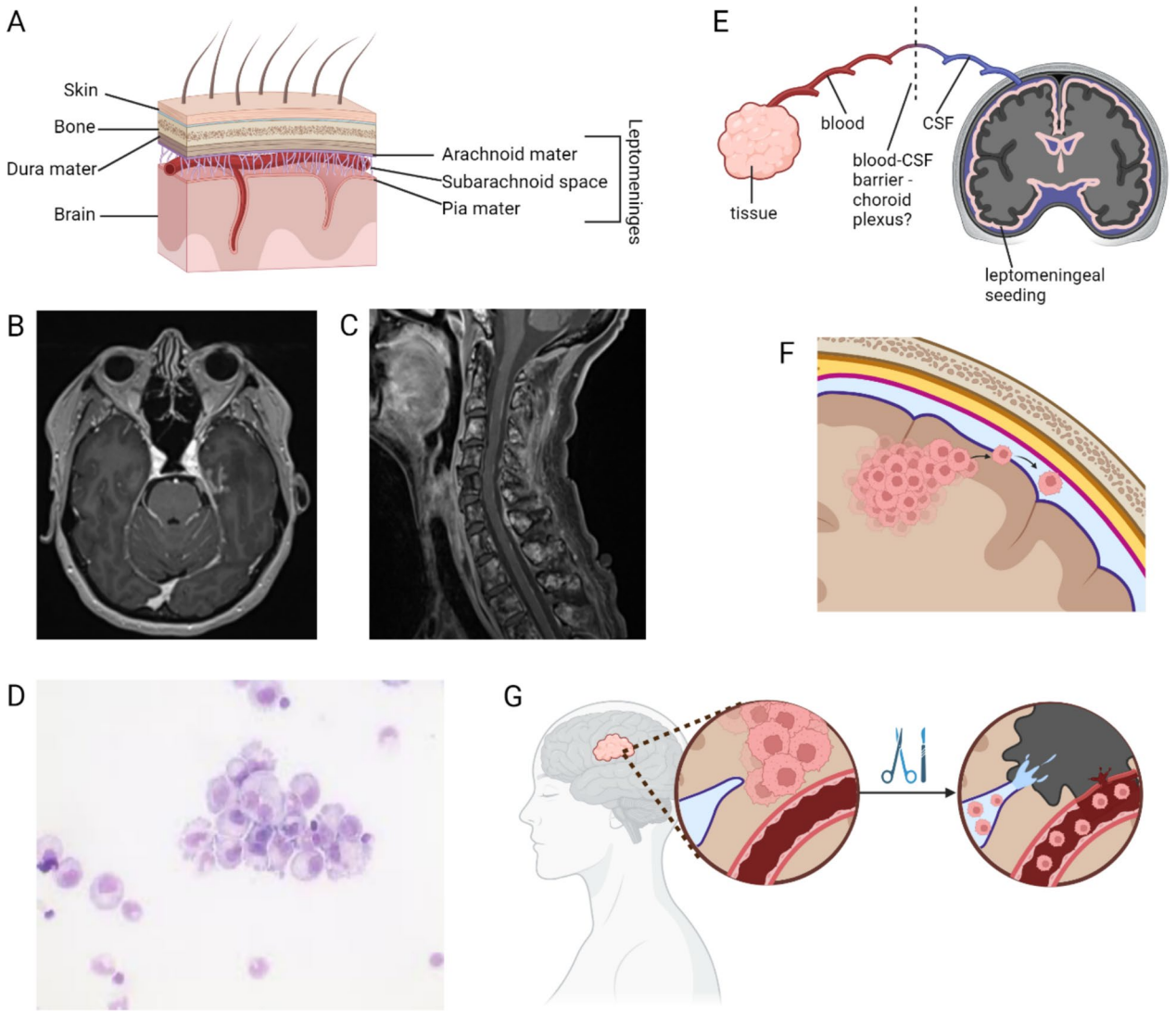

Fig. 1 LMD imaging and pathology and hypotheses of spread. The leptomeningeal space consists of the pia mater, subarachnoid space, and arachnoid mater (**A**). Brain MRI with contrast showing leptomeningeal enhancement in supratentorial and infratentorial sulci, consistent with LMD (**B**). Spine MRI with contrast demonstrating linear enhancement along the cervical and thoracic regions of the spinal cord, consistent with LMD (**C**). CSF showing large multinucleated tumor cells, consistent with LMD (**D**). A main hypothesis of leptomeningeal spread is escape of tumor cells into the blood; cells cross the blood-CSF barrier, which is likely the choroid plexus, and enter the CSF, where they have access to seed the leptomeninges (**E**). Another hypothesis of leptomeningeal spread is contiguous spread from parenchymal metastasis (**F**). Another hypothesis of leptomeningeal spread is that tumor cells enter the CSF and blood during or after surgical resection of a parenchymal metastasis (**G**). Images A, E, F, G created in https://BioRender.com. Images B, C, D courtesy of PK

diagnosis is magnetic resonance imaging (MRI) of the brain and spine with and without contrast, although computed tomography (CT) and positron emission tomography (PET) can supplement MRI findings. Cranial MRI findings indicative of LMD include leptomeningeal enhancement along the sulci, cerebellar folia, cranial nerves, and spinal nerve roots (Fig. 1B). Spinal MRI findings indicative of LMD include linear or nodular spinal cord enhancement and nerve root thickening (Fig. 1C). CSF analysis is considered the gold standard for LMD diagnosis; a positive CSF cytology reveals the presence of malignant cells (Fig. 1D). Both imaging and CSF analysis are repeated throughout a patient's disease to assess treatment response and disease progression.

The prognosis of LMD is 4–6 weeks overall survival without treatment [90]. However, a variety of treatment options are available. Radiation therapy, which can be focal, whole-brain, or cranio-spinal, is usually

Table 1 Estimated incidence of leptomeningeal metastasis by primary tumor location

References	Primary tumor location	2024 estimated new cancer cases in US [85]	% Cancer patients developing LMD	2024 estimated LMD incidence in US
[10]	Digestive system	353,820	0.61	2158
[64]	Lungs	234,580	2–12	4692–28,150
[64]	Melanoma	100,640	5–30	5032–30,192
[4]	Breast	313,510	5–15	15,676–47,027
[101]	Genitourinary system	597,160	0.03	179
[66]	Glioma	8467	16	1355
[24]	Thyroid	44,020	1	440
[11]	Non-Hodgkin Lymphoma	89,190	5–15	4460–13,379
[14]	Myeloma	35,780	1	358
[11]	Leukemia	62,770	5–15	3139–9416
	Other	161,203	NA	NA
	Total	2,001,140	1.9–6.6	37,489–132,654

Estimated cases for 2024 exclude basal and squamous cell carcinomas and carcinomas in situ, except urinary bladder

the first line of treatment for symptomatic patients [90]. Chemotherapy is commonly prescribed; select agents that cannot cross the blood–brain barrier (BBB) may be administered intrathecally via lumbar puncture or an Ommaya reservoir. Molecularly targeted therapies and immunotherapies may also be available depending on the patient's primary cancer and underlying biology. Unfortunately, even with treatment, the prognosis of LMD is generally 2–7 months, depending on the primary cancer [63, 90].

LMD pathophysiology

The BBB and the blood-CSF barrier theoretically protect the CSF from the circulatory factors leading to metastases, yet LMD still occurs and spreads throughout the CSF. While the mechanism by which metastatic cells enter the CSF has not been definitively described, there are a few leading hypotheses. In 1983, Kokkoris and colleagues hypothesized that cancer cells in arterial blood penetrate the CSF via the choroid plexus, an epithelium in the ventricles that filters blood into the CSF (Fig. 1E) [46]. Boire and colleagues expanded on this hypothesis, showing that complement component 3 is upregulated in leptomeningeal metastasis. It disrupts choroid plexus tight junctions and the blood-CSF barrier and alters CSF composition to promote cancer cell growth [13]. It is also thought that LMD may arise from the contiguous spread of parenchymal brain metastases and spinal metastases to the leptomeninges (Fig. 1F) [90]. Parenchymal brain metastasis surgery may also contribute to tumor cell seeding in the leptomeninges (Fig. 1G). Suki and colleagues found that piecemeal resection of solid tumor metastasis to the posterior fossa was associated with a significantly higher risk of subsequent LMD compared to en-bloc resection or stereotactic radiosurgery [89]. Ahn and colleagues confirmed these results in a patient population with metastases in brain parenchymal locations other than the posterior fossa; additionally, they found that the use of aspiration during surgery and proximity of the resected metastasis to CSF spaces were also associated with a higher risk of subsequent LMD [3].

Once cancer cells enter the CSF, they face a protein- and metabolite-deficient environment; therefore, they have several features that allow them to survive. Chi and colleagues showed that cancer cells in the CSF outcompete macrophages for iron. Iron enables cancer cells to grow and proliferate, and a lack of iron inhibits macrophage function; therefore, cancer cells that outcompete macrophages for iron increase their metabolism while decreasing the immune response against them [18]. Additional evidence that LMD thrives in an immunosuppressed environment comes from Smalley and colleagues, who demonstrated that the CSF of melanoma LMD patients whose disease followed the expected clinical course was higher in immunosuppressive markers, including myeloid-derived suppressor cells and transforming growth factor β (TGF-β), than the CSF of non-LMD, non-melanoma controls. Additionally, the CSF of melanoma LMD patients showed high levels of T cell exhaustion and dysfunction [86, 87].

LMD affects at least tens to hundreds of thousands of patients per year in the United States alone, yet due to a lack of treatment options, each of these patients has only months to live after their diagnosis. This lack of treatment is partly due to our lack of knowledge about the mechanisms of this disease; thus, research is urgently needed. This requires reliable and representative disease

models to elucidate the intricate mechanisms of LMD seeding and progression, ultimately paving the way for the development of effective therapies. However, the complexity of accurately recapitulating the clinical disease's pathology in animal models has been a significant hurdle. In this review, we will comprehensively explore the existing models for LMD and the advantages and limitations of each. By critically assessing the current state of these models, we aim to provide insights and future perspectives that could guide the development of more refined and representative models. We argue that the optimal model would represent human disease not only visually but also molecularly, with tumor cells and a leptomeningeal space that reflect the molecular changes required for cells to invade into, and proliferate within, the CSF. The presence of brain parenchymal and systemic metastases may also indicate a representative model without interfering with analysis of the disease, as human patients often have metastases to multiple locations but ultimately succumb to their leptomeningeal metastases. While previous models have approached aspects of this optimal model, none has addressed all of these factors. The creation of more representative models is vital for advancing our understanding of LMD and opening new avenues for therapeutic interventions.

LMD models

Injection of tumor cells into the CSF

LMD spreads throughout the CSF. It makes sense, then, that the most commonly reported method of generating animal models of leptomeningeal metastasis from solid tumors is injecting tumor cells into an animal's CSF. This method has been used to model LMD from breast carcinoma, lung carcinoma, medulloblastoma (MB), glioma, rhabdomyosarcoma, squamous cell carcinoma, and melanoma. This method is also the oldest, with papers reporting this method beginning in the 1970s. A summary of papers reporting this method is found in Supplementary Table 1. Mice, rats, rabbits, and zebrafish have been used for this method. The most common injection strategy is to use a syringe to inject cells into the cisterna magna of rodents (Fig. 2A). The second most common injection strategy is to place a catheter in the subarachnoid space of the spinal cord of rodents so that cells are injected into the external end of the cervical spine and are delivered to the subarachnoid space of the lumbar spine (Fig. 2B). Injections into the right lateral ventricle and cerebral subarachnoid spaces other than the cisterna magna have also been reported in rodents (Fig. 2C). Recently, a new model of breast cancer LMD was developed in transparent zebrafish larvae in which a microinjector was used

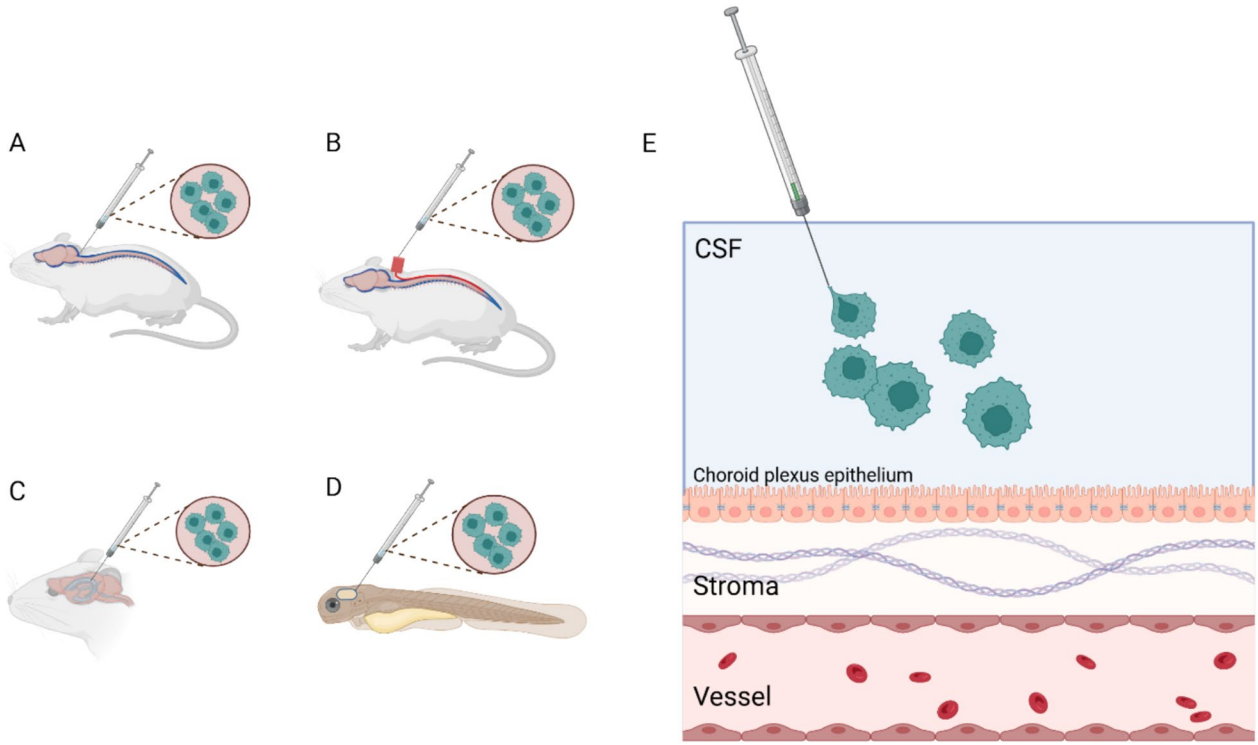

Fig. 2 The intra-CSF injection model. Cancer cells can be injected into the CSF of rodents via injection into the subarachnoid space or cisterna magna (**A**), injection into a catheter extending from the cervical spine to the lumbar spine (**B**), or injection into the lateral ventricle (**C**). Cancer cells can also be injected into the CSF of zebrafish embryos via injection into the fourth ventricle (**D**). Cancer cells injected into the CSF have no obvious barriers to seeding the leptomeninges (**E**). Created in BioRender. Ahmed, A. (2025) https://BioRender.com/i85y916

to inject 10^2 breast cancer cells into the fourth ventricle (Fig. 2D) [34]. The number of cells injected in rodents varies widely but is generally between 10^4 and 10^7. The development of LMD in an animal can be confirmed by histological examination or in vivo imaging, including MRI. Interestingly, one study reported that MRI showed meningeal enhancement in both LMD and non-LMD controls; therefore, MRI should always be supplemented by a second confirmation method [19].

With adequately trained experimenters and an appropriate number of cells, the efficacy of this procedure can be up to 100%. Survival varies widely according to the quantity and identity of cells injected, but similarly to human disease, untreated animals usually die within a few weeks. Upon histological examination, LMD within these animals often mirrors human disease, with findings of thin layers of cells coating the brain and spinal cord, especially the sulci, cranial nerves, and spinal nerve roots. Some animals may also display nodular disease with a mass effect. Thus, a significant strength of this model is that it visually mirrors human disease. However, models which inject primary tumor cells into the CSF reveal little about the biology of LMD and its microenvironment. As previously discussed, LM cells upregulate complement component 3 to disrupt the choroid plexus and alter CSF composition, likely allowing their spread from the blood to the CSF [13]. This implies that cancer cell molecular characteristics change during the metastatic process and that cells from the primary tumor may have different molecular characteristics compared to cells from a distant site of metastasis. Therefore, injecting a primary tumor cell line into the leptomeningeal space may not reflect an accurate molecular profile of LMD despite visual similarities (Fig. 2E).

Several studies have aimed to overcome this issue by injecting cells derived from leptomeningeal metastases into the CSF. Two of these studies used cell lines derived from human bulky leptomeningeal metastases which were resected and processed [6, 12]. Three other studies used cell lines derived from human circulating tumor cells, or CTCs, which are thought to contribute to LMD [27, 52, 79]. CTCs have been observed in the blood and CSF of LMD patients, and CSF CTC count is correlated with prognosis and is being studied as a potential new diagnostic method for LMD [58, 68]. Within these animal models, cells that reach the leptomeninges may harbor molecular characteristics that allow their passage from the blood to the leptomeningeal space [32]. Additionally, patient-derived cell lines have likely been passaged fewer times than established primary tumor cell lines; given that repeated passaging of cells can cause their molecular characteristics to change over time, this may also indicate that patient-derived cells are more similar to human disease [50]. Despite the similarity of cells, a potential weakness of some of these models is their reliance on a few patient samples to model a diverse disease. Many cancers are affected differently by each patient's genetic and epigenetic factors; if this is true of LMD, then a model of one patient's disease should not be considered a model of disease for all patients with the same primary cancer. A deeper exploration of the molecular characteristics of LMD may guide the development of models from patient-derived cells. An additional critique of some of these CSF injection studies is the animal used for the model. Several studies have demonstrated immunosuppression in the leptomeningeal space in the setting of LMD [18, 86, 87]. Injecting tumor cells into the leptomeningeal space of previously healthy animals, as some CSF injection studies did, means that the environment in which LMD grows has not undergone the immunosuppression that contributes to LMD seeding and spread in human patients. However, many CSF injection studies overcame this issue by using immunosuppressed animals such as athymic nude or NOD-SCID/NSG rodents.

A critique of all CSF injection models is the lack of metastases to areas other than the leptomeninges. Clarke and colleagues' study of human LMD revealed that 58% of LMD occurred in the setting of prior or concurrent parenchymal brain metastasis [21]. Few studies of tumor cell injection into the CSF reported parenchymal metastasis with leptomeningeal metastasis [30, 39, 91, 100]. Even in those studies, no metastasis outside of the central nervous system (CNS) was reported, even though extra-CNS metastasis is common in human patients with LMD. This may be an indication that the environment in which LMD develops in these models does not reflect the environment in which LMD develops in human patients. Thus, while injecting primary tumor cells into the CSF may create an animal model of LMD that visually resembles human disease, the molecular characteristics of the cancer cells and their microenvironment in some models may not.

Injection of tumor cells into the bloodstream

A hypothesis for the seeding of leptomeningeal metastases is that tumor cells access the CSF via the arterial circulation of the choroid plexus [46]. Thus, injecting tumor cells into an animal's bloodstream is another standard method of generating animal models of leptomeningeal metastasis from solid tumors. This method has been used to model LMD from breast carcinoma, lung carcinoma, glioma, and melanoma. A summary of papers reporting this method is found in Supplementary Table 2. Typically, mice and rats have been used for this method. To create this model, a glass cannula or needle is utilized to inject

tumor cells into rodents' internal or external carotid artery (ICA, ECA) (Fig. 3A). Two groups attempted to inject tumor cells into the left cardiac ventricle of rodents, and 0%–40% of the rodents developed LMD [25, 27]. The number of cells injected varies but is generally between 10^4 and 10^7. The development of LMD in an animal can be confirmed by histological examination or in vivo imaging. The efficacy of the intracarotid injection method is highly dependent on the cell line injected; within the same study, with the same experimental technique, efficacy could range between 0 and 100% depending on the cell line used [26, 49, 102]. Survival is also variable but death generally occurs within a few weeks in untreated animals. In successful models, LMD can mirror human disease, with histology showing tumor cells in the leptomeningeal space of the brain sulci and cranial nerves; few studies examined the spinal cord.

Consistent with the previously held idea that intracarotid injection of primary tumor cells is more likely than CSF injection to produce systemic metastases, the studies discussed here were generally more likely to report metastases in a variety of extra-leptomeningeal locations compared to the previously discussed studies of CSF injection. The most common extracranial metastasis site reported in these papers was the lungs, although metastases to the liver, bone, adrenal gland, kidney, eye, and heart were also reported [23, 25, 26, 95]. Within the brain, most blood injection studies reported metastases to the brain parenchyma in at least some animals, regardless of whether metastasis also occurred in the leptomeninges. One study also reported metastasis to the dura mater, although the dura and leptomeninges were not well differentiated in the text [43]. The greater diversity of metastasis location in this model may indicate that it is more representative of human disease than the CSF injection model. Furthermore, one study that did not report LMD did report metastasis to the choroid plexus, which is thought to be implicated in leptomeningeal metastasis [13, 46, 78]. These studies also contained interesting molecular analyses of brain and leptomeningeal metastases: they reported high LAT1, Ki67, MMP2, and MMP9 expression in metastatic cells, which are markers of cancer cell growth and proliferation [23, 95]. Upon examination of human patient samples, LAT1 was overexpressed similarly in both primary tumors and the brain metastases that arose from them [23]. However, the expression of growth and proliferation genes should be further examined in human samples and animal models to better understand how they contribute to LMD seeding and spread.

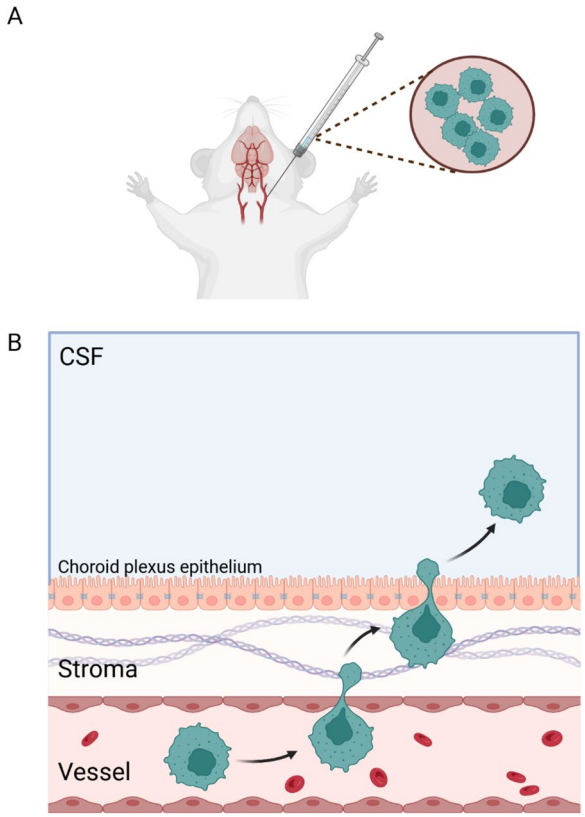

Fig. 3 The intracarotid injection model. Cancer cells can be injected into the bloodstream of rodents via injection into a carotid artery (**A**). Cancer cells injected into the bloodstream must cross the blood-CSF barrier, which may be the choroid plexus, to gain access to the CSF and leptomeninges (**B**). Created in BioRender. Ahmed. (2025) https://BioRender.com/h09i048

Tumor cells in the bloodstream may resemble CTCs, and the LMD which results from this injection method may express molecular changes required for the passage of tumor cells from the blood to the CSF (Fig. 3B). In fact, one study injected CSF-derived CTCs into the bloodstream, and LMD seeding was 40% successful; however, the molecular characteristics of the animals' disease were not examined [27]. In some models, another of our previous critiques still stands: some studies used immunocompetent mice, where the disease microenvironment likely does not resemble that of human disease. Shi and colleagues attempted to address the issue of molecular changes between primary tumor and metastasis by injecting into the bloodstream a cell line derived from leptomeningeal metastases which arose after subcutaneous injection of small cell lung cancer cells. As these cells need to exit the primary tumor and travel through both blood and CSF to reach the leptomeninges, this cell line may possess mutations allowing metastasis into both the blood and CSF; further molecular characterization should be performed on this cell line [82].

Injection of tumor cells into the brain parenchyma

LMD has been associated with the proximity of parenchymal brain metastases to CSF spaces of the brain [3]. Because of the connection between LMD and parenchymal brain metastases, another method of generating animal models of leptomeningeal metastasis from solid tumors is injecting tumor cells into an animal's brain parenchyma. This method has been used to model LMD from breast cancer, glioma, and medulloblastoma (MB). A summary of papers reporting this method is found in Supplementary Table 3. Mice are normally used for this method. To create this model, a needle injects tumor cells into the brain parenchyma, such as the frontal lobe, putamen, pons, or cerebellum (Fig. 4A). The number of cells injected varies but is generally between 10^4 and 10^7. The development of LMD in an animal can be confirmed by histological examination or in vivo imaging. One study also attempted to use PET imaging, but it did not have a high enough resolution to image the mouse brain field effectively [17]. The efficacy of the intraparenchymal injection method is highly dependent on the cell line and quantity of cells injected; efficacy can vary between 10 and 100%. An interesting technique used by Garzia and colleagues is parabiosis surgery, in which one of two sister mice is intracranially injected with tumor cells. Then, both mice are given skin incisions at the olecranon joint and tied to each other at the olecranon joint. The skin is sutured shut around the joints, and the mice will begin to share a blood supply during the healing process (Fig. 4B). In this experiment, 3 of 6 recipient mice developed LMD despite the lack of a primary tumor [32]. Most brain parenchymal injection studies reported the expected survival of a few weeks. One paper reported a mean survival of 69–98 days, depending on the cell line; however, the control mice in this paper received a dead virus, which could have affected their survival [98].

Models created via the injection of tumor cells into the brain parenchyma generally mirror human disease, with metastasis occurring throughout the leptomeninges of the brain and spinal cord. Seven of the nine models discussed here used brain tumor cell lines; therefore, this model addresses some of our critiques of the intra-CSF and intracarotid injection models. Because the tumor cells are injected into the brain parenchyma, their native tumor site, their metastatic process may reflect the interactions with the tumor microenvironment and

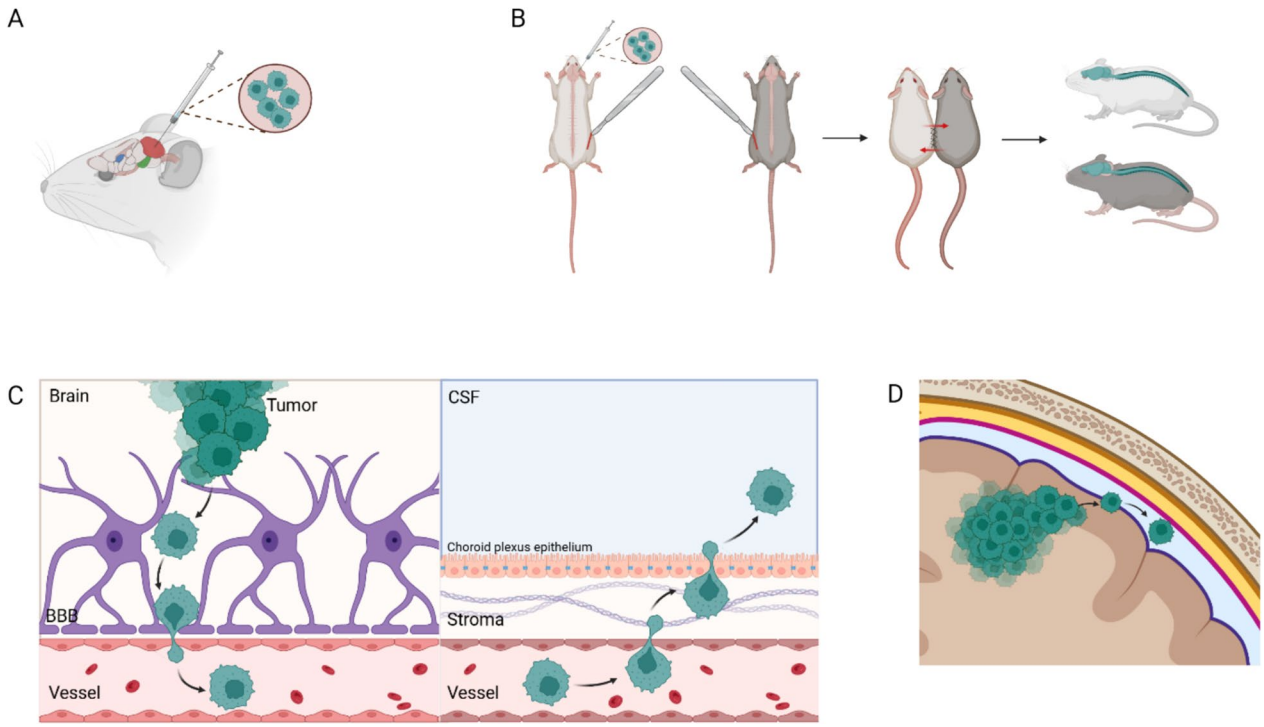

Fig. 4 The intracranial injection model. Cancer cells can be injected into the brain parenchyma of rodents via injection into the frontal lobe, cerebellum (red), pons (green), or putamen (blue) (**A**). In the parabiosis method, one mouse is given an intraparenchymal injection of cancer cells; then the injected mouse and a non-injected mouse are incised and connected at the olecranon joint, allowing them to share a blood supply. Eventually, both mice may develop LMD (**B**). Cancer cells injected into the brain parenchyma may spread from the brain parenchyma to the blood, cross the blood-CSF barrier, and enter the CSF before seeding the leptomeninges (**C**). Alternatively, they may access the leptomeninges via contiguous spread (**D**). Created in BioRender. Ahmed. (2025) https://BioRender.com/k92d786

the priming of the leptomeningeal space which lead to metastasis in humans. Additionally, the contiguous spread of tumors from the brain parenchyma to the leptomeninges, which has been posited in human disease, is especially possible in the small mouse brain (Fig. 4D). In Garzia and colleagues' parabiotic model, some mice that lacked a primary tumor and shared a blood supply with tumor-bearing mice developed LMD. This suggests that medulloblastoma primary tumor cells can spread through the blood to reach and seed the leptomeninges. Indeed, CTCs were present in other mouse models of MB LMD in this study (the parabiotic mice were not tested for CTCs) [32]. Thus, the development of LMD in mice with intraparenchymal tumor cell injections may also be due to the dissemination of tumor cells to the leptomeninges via the blood (Fig. 4C). The tumor cell content of the blood and CSF should be more deeply examined in these models to explore these possibilities. No extra-CNS metastases were noted in any of these studies. However, this is to be expected, as seven of the nine studies used brain tumor cell lines, which rarely metastasize beyond the CNS.

Two of the studies reported here used breast cancer cell lines: one used primary breast cancer cells, with an LMD seeding success rate of 94%, and the other used patient-derived breast cancer brain metastasis cells, with an LMD seeding success rate of 42% [71, 98]. While it is unclear how similar disease derived from the injection of primary breast tumor cells into the brain parenchyma is to human disease, there may be strong similarities between animal and human disease when cells derived from human brain metastasis are injected into the brain parenchyma and allowed to proliferate. The LMD that develops from this cell line should be further characterized to examine these similarities. However, it is important to note that LMD does not always develop in the context of prior brain parenchymal metastasis, so this model's disease may represent only a subset of LMD in human patients [21].

Injection of tumor cells subcutaneously or into the mammary fat pad

A few groups have created models of LMD via subcutaneous or mammary fat pad injection of tumor cells. This method has been used to model LMD from melanoma, medulloblastoma (MB), and breast cancer. A summary of papers reporting this method is found in Supplementary Table 4. Mice are commonly used for this method. To create this model, between 10^5 and 10^6 primary tumor cells are injected subcutaneously into the flank, or into the mammary fat pad. Tumors must be removed when they reach the maximum acceptable size defined by an institution's animal care office. Alterman and Stackpole saw LMD development only after resection of the subcutaneous flank tumor; they saw a higher incidence of leptomeningeal metastasis when brains with visible metastases arising from the initial subcutaneous flank injection were broken down and injected subcutaneously into the flanks of different mice [5]. Cruz-Munoz and colleagues resected the subcutaneous tumor, resected and cultured metastases that developed in mice who survived long-term chemotherapeutic regimens, and then subcutaneously injected the cultured metastasis cells into different mice. While all metastasis-derived cell lines metastasized similarly to the lung, liver, and kidneys, brain-metastatic cell lines were the most likely to metastasize to the brain. The group concluded that brain-metastatic cell lines were not better able to metastasize overall but instead had acquired changes that facilitated metastasis to the brain specifically [22]. However, the nature of these changes was not explored. In Garzia and colleagues' MB model, there was no reinjection of tumor cells after removing the original flank tumor. They attributed the subsequent development of LMD to flank tumor-derived CTCs in the bloodstream; indeed, CTCs were proven to be present in animal models in their study [32]. Fitzpatrick and colleagues collected CTCs from the CSF of patients with metastatic breast cancer, cultured the CTCs as organoids, and then injected the cultured CTCs into mouse mammary fat pads. The maximum tumor size was not reached, so tumors were not resected; the presence of CTCs was not assessed in the mice after injection [27]. Subcutaneous and mammary fat pad injection methods are summarized in Fig. 5A. The efficacy of these models can be up to 45% if the flank tumor is resected. The presence of LMD can be confirmed via microscopic examination or imaging.

Models created via the subcutaneous or mammary fat pad injection of tumor cells generally mirror human disease, with metastasis occurring throughout the leptomeninges of the brain and spinal cord. They address some of our critiques of previous models; namely, primary tumor cells are not injected directly into the CSF or blood, which would allow them more immediate access to the leptomeningeal space. Instead, primary tumor cells must develop the potential to metastasize into the blood and then cross the blood-CSF barrier into the leptomeningeal space, which may increase their similarity to cells of human LMD (Fig. 5B). Additionally, the time between tumor cell injection and LMD development may allow for changes to the leptomeningeal space which facilitate tumor cell seeding. Importantly, Cruz-Munoz and colleagues' model derived cell lines from metastases of mice subjected to long-term chemotherapy regimens. Human patients often develop LMD after years of treatment for their primary tumor and non-leptomeningeal metastases, and it is thought that LMD may develop in part due

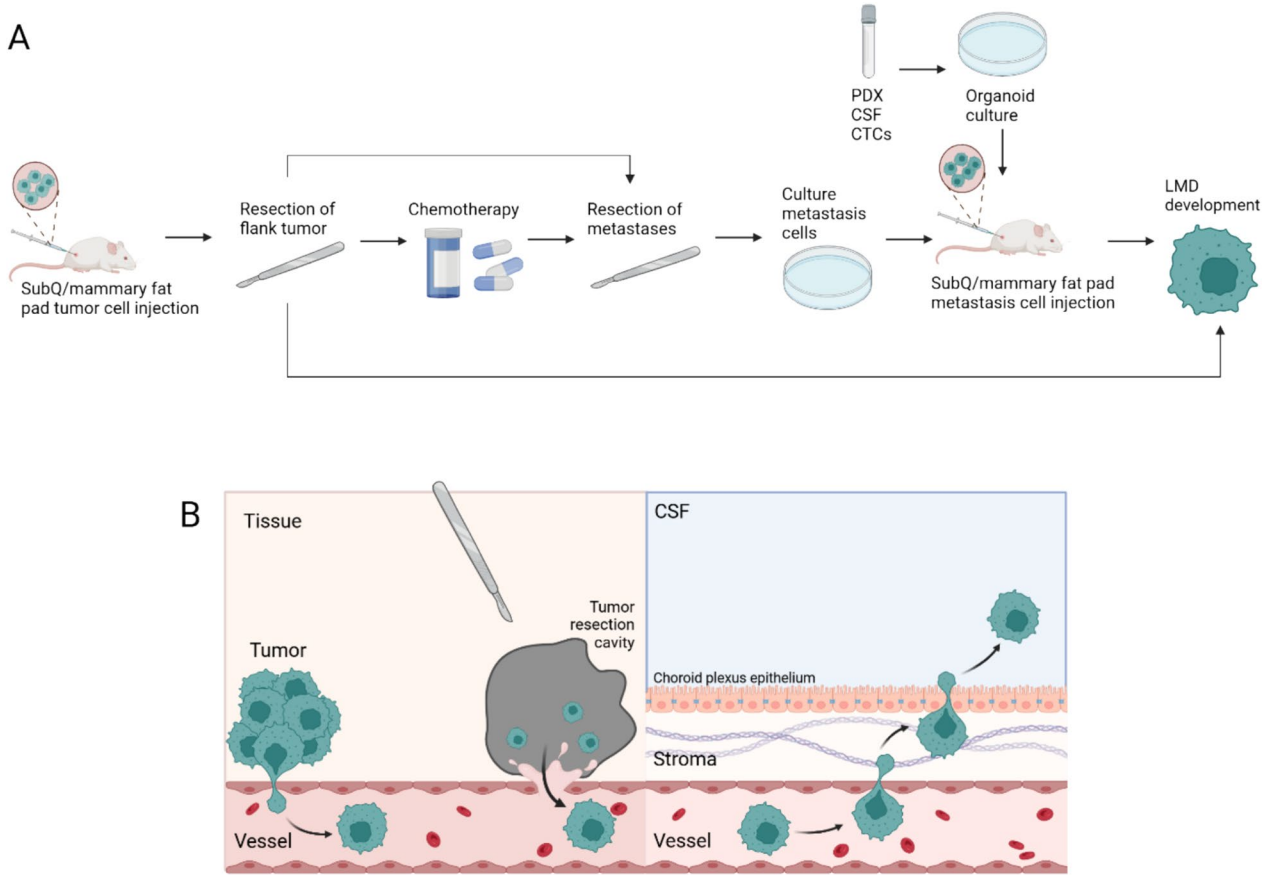

Fig. 5 The subcutaneous/mammary fat pad injection model. Cancer cells can be injected subcutaneously or into the mammary fat pad of rodents; LMD development can occur after resection of the flank tumor or after resection of the flank tumor and metastases, culturing of the metastasis cells, and subcutaneous/mammary fat pad injection of the cultured metastasis cells into different mice (**A**). Cancer cells injected subcutaneously may travel from tissue to blood, cross the blood-CSF barrier, and enter the CSF before seeding the leptomeninges; alternatively, cancer cells may be released into the bloodstream during resection of the subcutaneous tumor (**B**). Created in BioRender. Ahmed. (2025) https://BioRender.com/t09i860

to the immunosuppression in the leptomeningeal space caused by years of anti-cancer therapy. Incorporating chemotherapy into an animal model may allow it to better model human disease compared to models without treatment [22]. Another way these models may mirror human disease is the resection of the primary tumor; surgical resection is often a first-line treatment for solid tumors. Surgical resection may promote LMD by augmenting the effects of previously circulating CTCs or by promoting CTC circulation in animals or human patients without pre-existing CTCs [1]. CTC content should be examined at several points in time to elucidate this timeline. Finally, three of the four studies discussed here all reported extra-CNS metastases, with liver and lung metastases being the most common, further increasing the similarity of these models to human disease. However, an important caveat is that none of these models can be considered an orthotopic model, where tumor cells are injected into their original site of growth. The subcutaneous injection models injected cells into the flank rather than the mammary fat pad, and the mammary fat pad injection model used CSF-derived CTCs rather than primary breast tumor cells. To best recapitulate the metastatic process from breast to leptomeninges, a true orthotopic model would inject primary breast tumor cells into the mammary fat pad and observe any potential metastasis to the leptomeninges. This will be an important consideration when improving this model type in future experiments.

The LeptoM/LM-phenotype model

Two similar animal models of LMD have been created which involve multiple rounds of selection for cells that metastasize to the leptomeninges. These models, called the LeptoM model and the LM-phenotype model, have been developed to model LMD from breast cancer, lung cancer, and melanoma. A summary of papers reporting this method is found in Supplementary Table 5. Mice are

normally used for this method. Primary tumor cells from established cell lines are injected into the cisterna magna and allowed to metastasize. Those cells metastasizing to the leptomeninges are collected, cultured ex vivo, and reinjected either intracardially or intracisternally. This process is repeated until a cell line results which is highly selective for metastasis to the leptomeninges (Fig. 6). The number of cells injected varies between 10^2 and 10^5, depending on the procedure stage. The development of LMD in an animal can be confirmed by histological examination or in vivo imaging. Efficacy was only reported by two LeptoM papers; both reported success rates of 95% or greater, depending on the cell line used [74, 75]. Survival tends to mirror human disease, with untreated animals living only a few weeks. The LeptoM/LM-phenotype model generally mirrors human disease, with tumor cells coating the supratentorial and infratentorial brain; the spine was not examined.

A major strength of this model is that it isolates cells that selectively seed the leptomeninges, requiring them to track to the leptomeninges from the CSF and the bloodstream. These cells can be compared to the parent cell line to investigate properties that promote leptomeningeal seeding. Some differences that have been found are increased C3a levels, increased IFNy levels, and CD8 + T cell depletion in LMD cells compared to parental cells [13, 53, 75]. Additionally, the spatial and temporal properties of leptomeningeal seeding can be examined. For example, Boire and colleagues found that LeptoM cells invade the choroid plexus, then the CSF a few days later, and then the brain parenchyma a few days later [13]. Remsik and colleagues found that LeptoM cells are 75% adherent, but the 25% of cells that remain floating in the CSF are more aggressively metastatic than the adherent cells[74]. However, our primary critique of this model is that its similarity to the biological process of leptomeningeal seeding is unclear. The LeptoM/LM-phenotype cells are derived from commercially available primary tumor cell lines. While it has been shown that the leptomeningeal-selective derivatives are transcriptionally and phenotypically different from the parent cell line, it is not clear whether these differences are due to the isolation and expansion of one small group of cells from a genetically diverse parent cell line or whether differences arose during the process of metastasis to the leptomeninges [53]. Additionally, direct injection of cells into the CSF, regardless of the prior selection process, may not accurately model the molecular processes that occur in the human leptomeningeal space due to non-leptomeningeal cancer growth and treatment. Comparison of LeptoM/LM-phenotype cells to human LMD cells should be performed beyond simply confirming animal model findings in human specimens [53]. Adding to the question of similarity to biological seeding processes, none of these models reported extra-CNS metastases, which are common in human patients with LMD.

Animal models created via genetic manipulation

To work around some of the critiques of injection-based animal models of LMD, some models have been created in which genetic manipulation gives rise to LMD and/or the primary tumor. Genetic manipulation

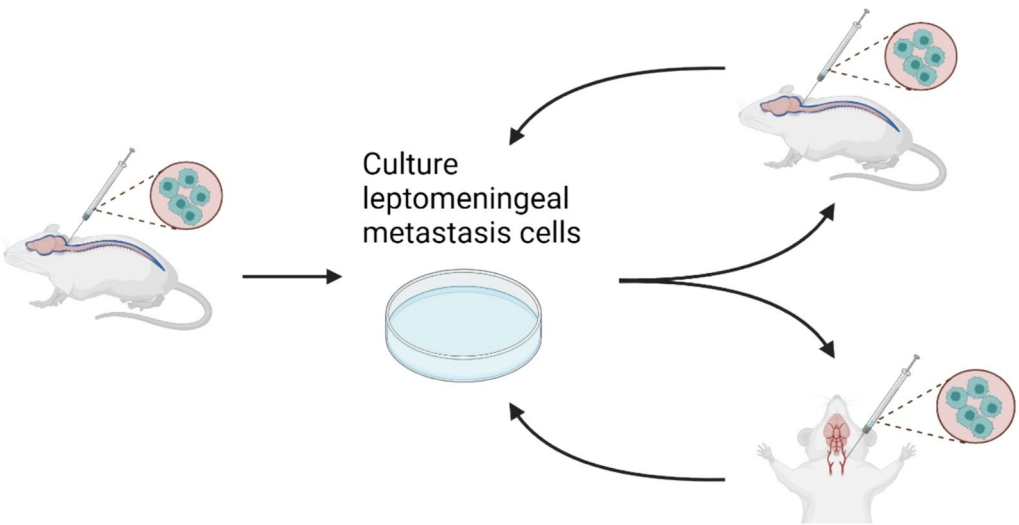

Fig. 6 The LeptoM/LM Phenotype model. The LeptoM/LM phenotype model begins with injection of cancer cells into the cisterna magna of mice; leptomeningeal metastasis cells are cultured and injected into the cisterna magna or blood (via intracardiac or intracarotid injection) of mice without tumors; and the process is repeated until a cell line is established which is highly selective for metastasis to the leptomeninges. Created in BioRender. Ahmed (2025) https://BioRender.com/p94p748

has been used to model LMD from medulloblastoma (MB) and pineoblastoma. A summary of papers reporting this method is found in Supplementary Table 6. Mice are normally used for this method. Five of the six genetic manipulation studies used a mouse model that spontaneously develops the primary brain tumor, and its metastasis to the leptomeninges is also spontaneous [20, 35, 38, 88, 96]. Jenkins and colleagues used a mouse model that produced MB and LMD upon injection of 10^5 DF-1 cells producing a recombinant retrovirus into the cerebellum bilaterally [41]. The development of LMD in an animal can be confirmed by histological examination or in vivo imaging, including MRI. Efficacy was only reported by three genetic manipulation studies; it varied between 14 and 100% [35, 41, 88]. Survival was only reported in one study with a mean survival time of 71 days [88].

Models created via genetic manipulation generally mirror human disease, with metastasis occurring throughout the leptomeninges of the brain and spinal cord. These models are useful when information about the genetics leading to a primary cancer is known, as in MB and pineoblastoma. Modeling a disease via genetic manipulation allows for observation of the timeline of the metastatic process; for example, Wu and colleagues showed that a primary tumor and its leptomeningeal metastases arose from a common origin cell and subsequently underwent genetic divergence [96]. Grausam and colleagues showed that tumor cells infiltrate the leptomeningeal space at an early stage of primary tumor development, and these develop into LMD in late-stage disease; for example, one mouse strain showed tumor cells in the CSF at 6 weeks of age, while another mouse strain developed MB and LMD within 8 months [35]. While some cancers have well-known genetic drivers, it is still important to exercise caution in applying findings from these models to patients with those cancers. Any cancer is driven by a complex array of genetics that varies between patients and can never be fully replicated by an animal model. Furthermore, when little is known about the genetic background of a primary tumor and its metastasis, creating this model becomes much more difficult. Examination of the genetics of non-brain human solid tumors is necessary to expand this model to cancers originating outside of the brain.

Summary and future directions

Summary

LMD is a devastating complication of late-stage cancer which currently suffers from a paucity of effective therapeutics. Unfortunately, a significant challenge preventing widespread development and testing of therapeutics for LMD is the lack of biologically accurate animal models. In this review, we have provided overviews of six types of animal models of leptomeningeal metastasis from solid tumors. We have identified pros and cons of each model and have suggested broad areas of future research which could improve each model in terms of its similarity to human LMD. Table 2 displays a summary of the most

Table 2 Important features of model types

	Visually resembles human LMD	Cancer cells genetically similar to human disease	Reveals changes to leptomeningeal space	Involves brain parenchymal and systemic metastasis	Involves treatment (surgery, chemotherapy, etc.)	Can model many cancer types	Spontaneous generation of primary tumor and leptomeningeal metastasis
Intra-CSF injection of tumor cells	Yes	Variable				Yes	
Intracarotid injection of tumor cells	Yes	?	?	Yes		Yes	
Brain parenchymal injection of tumor cells	Yes	Variable	?				
Subcutaneous/mammary fat pad injection of tumor cells	Yes	?	?	Yes	Yes	Yes	
LeptoM/LM-phenotype	Yes	?	?			Yes	
Genetic manipulation	Yes	?	?				Yes

Question marks denote areas of future research which could be explored by the model

important features of each model type. Question marks denote areas of future research that could be explored using the model.

The injection of tumor cells into the CSF creates models that visually resemble human LMD and can be used to model many cancer types. However, the genetic similarity between animal and human disease likely varies. When primary tumor cells are injected directly into the leptomeningeal space, they do not possess the genetic changes required to cross the blood-CSF barrier. However, CSF-derived CTCs may represent cells containing these genetic changes. Regardless, there may be changes to the leptomeningeal space during the metastatic process that prime it to accept tumor cells. In that case, these changes are not present in an animal model in which cells are directly injected into the CSF.

The injection of tumor cells into the blood creates models that visually resemble human LMD, involve brain parenchymal and systemic metastasis, and can be used to model many cancer types. There may be genetic similarity between animal and human disease since cells injected into the blood either develop molecular characteristics required to cross the blood-CSF barrier, as in primary tumor cells, or already contain those characteristics, as in CSF-derived CTCs. However, the extent of these genetic similarities should be an area of future study. It should also be studied whether there are changes to the leptomeningeal space during the metastatic processes seen in this model.

The injection of tumor cells into the brain parenchyma creates models which visually resemble human LMD, but the genetic similarity between animal and human disease likely depends on the type of tumor cell injected. Primary brain tumor cells and brain metastasis cells from non-brain primary tumors may undergo metastasis into the blood then travel into the CSF, or they may simply create LMD through contiguous spread, but either way the resultant LMD is likely similar to human disease because the brain is the tissue of origin for these tumor cells. Whether there are changes to the leptomeningeal space prior to LMD seeding should be studied in these animals. When non-brain tumor cells are used, however, it is unclear how the resultant LMD compares to human LMD since the brain is not the tissue of origin.

Subcutaneous or mammary fat pad injection of tumor cells creates models that visually resemble human LMD, involve brain parenchymal and systemic metastasis, involve treatment such as surgery and chemotherapy, and can model many cancer types. As cells must travel from tissue to blood to CSF, they may be genetically similar to human disease, and the full metastatic process may change the leptomeningeal space of these animals, but these possibilities should be further examined.

The LeptoM/LM-phenotype model creates models which visually resemble human LMD and can be used to model many cancer types. While genetic differences between primary tumor and metastasis cells have been discovered, it is not clear whether these differences are due to the isolation and expansion of one small group of cells from a genetically diverse parent cell line or whether differences arose during the process of metastasis to the leptomeninges, and this should be further studied. Also, further characterization of the leptomeningeal space should be performed to determine whether there are changes during the metastatic processes seen in this model.

Models which create LMD through genetic manipulation visually resemble human LMD, utilize genetic mutations seen in human patients, and spontaneously generate both the primary tumor and the subsequent leptomeningeal metastasis. Changes to the leptomeningeal space throughout the metastatic process should be further studied in these models. It is important to consider genetic variations in patients with even the most well-studied cancers. Thus far, this technique has only been used in brain tumors with known genetic mutations.

Future directions

The six models summarized above inspire exciting ideas for further study. For example, future research should address molecular changes that allow tumor cells to cross the blood-CSF barrier. This subject may be addressed by the intracarotid injection model, if one compares the cells injected into blood to the cells that end up in the CSF in animals that develop LMD. This subject could also be addressed by the mammary fat pad injection model, especially if an orthotopic injection model is created where primary tumor cells are injected into their native organ. In this case, primary tumor cells could be compared to cells in the blood and to cells in the CSF to examine the molecular characteristics of cells in multiple stages of metastasis. Additionally, Boire and colleagues have addressed this subject with their LeptoM model, showing that the upregulation of complement component 3 in leptomeningeal metastasis cells contributes to disruption of the blood-CSF barrier [13].

Another important area of future study is the incorporation of human cells, such as CTCs, into in vivo models. It has been shown that CTCs in the blood and CSF of human LMD patients are implicated in their disease. The intracarotid injection model injects primary tumor cells into the bloodstream and may resemble CTC dissemination. Additionally, generating metastases via brain parenchymal or mammary fat pad injection of primary tumor cells may involve CTCs as tumor cells must travel from solid tissue to the leptomeninges. Human CTCs have

been used in several animal models of LMD. Still, their utility should be further explored – which injection location is most productive when injecting CTCs, and how similar are the resultant models to the original human disease? It should be noted, however, that while human cells may be more representative of human disease than established primary tumor cell lines, there is the danger of a model being overly specific, representing only one or a few patients.

It should also be noted that tumor cells are only one piece of the puzzle. In human patients, the leptomeningeal space is often affected by years of harsh, immunosuppressive cancer treatment. Further molecular characterization of the leptomeningeal space and comparisons between patients who have received different treatment regimens may inspire ideas of how this immunosuppression should be represented in an animal model. We hope that future experimentation will improve animal models of LMD and lead to a better understanding of the disease's mechanisms and how novel treatments can target them.

Abbreviations

BBB	Blood–brain barrier
CNS	Central nervous system
CSF	Cerebrospinal fluid
CT	Computed tomography
CTC	Circulating tumor cell
ECA	External carotid artery
F	Female
ICA	Internal carotid artery
IVIS	In vivo imaging system
L	Left
LM	Leptomeningeal metastasis
LMC	Leptomeningeal carcinomatosis
LMD	Leptomeningeal disease
M	Male
MB	Medulloblastoma
MRI	Magnetic resonance imaging
MST	Mean survival time
NA	Not available
PDX	Patient-derived
PET	Positron electron tomography
R	Right
TGF-β	Transforming growth factor β
US	United States
WBFIS	Whole body fluorescent imaging system

Supplementary Information

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Supplementary file 1

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Declarations

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References

1. Adachi H, Ito H, Sawabata N (2022) Circulating tumor cells and the non-touch isolation technique in surgery for non-small-cell lung cancer. Cancers (Basel) 14:1448. https://doi.org/10.3390/cancers14061448
2. Adile AA, Bakhshinyan D, Suk Y, Uehling D, Saini M, Aman A, Magolan J, Subapanditha MK, McKenna D, Chokshi C et al (2023) An effective kinase inhibition strategy for metastatic recurrent childhood medulloblastoma. J Neurooncol 163:635–645. https://doi.org/10.1007/s11060-023-04372-w
3. Ahn JH, Lee SH, Kim S, Joo J, Yoo H, Lee SH, Shin SH, Gwak HS (2012) Risk for leptomeningeal seeding after resection for brain metastases: implication of tumor location with mode of resection. J Neurosurg 116:984–993. https://doi.org/10.3171/2012.1.JNS111560
4. Alder L, Trapani D, Bradbury C, Van Swearingen AED, Tolaney SM, Khasraw M, Anders CK, Lascola CD, Hsu L, Lin NU et al (2023) Durable responses in patients with HER2+ breast cancer and leptomeningeal metastases treated with trastuzumab deruxtecan. NPJ Breast Cancer 9:19. https://doi.org/10.1038/s41523-023-00519-0
5. Alterman AL, Stackpole CW (1989) B16 melanoma spontaneous brain metastasis: occurrence and development within leptomeninges blood vessels. Clin Exp Metastasis 7:15–23. https://doi.org/10.1007/BF02057178
6. Ansari KI, Bhan A, Saotome M, Tyagi A, De Kumar B, Chen C, Takaku M, Jandial R (2021) Autocrine GMCSF signaling contributes to growth of HER2(+) breast leptomeningeal carcinomatosis. Cancer Res 81:4723–4735. https://doi.org/10.1158/0008-5472.CAN-21-0259
7. Arai S, Takeuchi S, Fukuda K, Taniguchi H, Nishiyama A, Tanimoto A, Satouchi M, Yamashita K, Ohtsubo K, Nanjo S et al (2020) Osimertinib overcomes alectinib resistance caused by amphiregulin in a leptomeningeal carcinomatosis model of ALK-rearranged lung cancer. J Thorac Oncol 15:752–765. https://doi.org/10.1016/j.jtho.2020.01.001
8. Archer GE, Sampson JH, Lorimer IA, McLendon RE, Kuan CT, Friedman AH, Friedman HS, Pastan IH, Bigner DD (1999) Regional treatment of epidermal growth factor receptor vIII-expressing neoplastic meningitis with a single-chain immunotoxin, MR-1. Clin Cancer Res 5:2646–2652
9. Archer GE, Sampson JH, McLendon RE, Friedman AH, Colvin OM, Rose M, Sands H, McCullough W, Fuchs HE, Bigner DD et al (1999) Intrathecal

busulfan treatment of human neoplastic meningitis in athymic nude rats. J Neurooncol 44:233–241. https://doi.org/10.1023/a:1006304424346
10. Baccili Cury Megid T, Baskurt Z, Ma LX, Barron CC, Farooq A, Saltiel MP, Wang X, Bach Y, Ayoama H, Jang RW et al (2024) Leptomeningeal carcinomatosis and brain metastases in gastroesophageal carcinoma: a real-world analysis of clinical and pathologic characteristics and outcomes. J Neurooncol 167:111–122. https://doi.org/10.1007/s11060-024-04576-8
11. Beauchesne P (2010) Intrathecal chemotherapy for treatment of leptomeningeal dissemination of metastatic tumours. Lancet Oncol 11:871–879. https://doi.org/10.1016/S1470-2045(10)70034-6
12. Bhan A, Ansari KI, Chen MY, Jandial R (2021) Inhibition of jumonji histone demethylases selectively suppresses HER2(+) breast leptomeningeal carcinomatosis growth via inhibition of GMCSF expression. Cancer Res 81:3200–3214. https://doi.org/10.1158/0008-5472.CAN-20-3317
13. Boire A, Zou Y, Shieh J, Macalinao DG, Pentsova E, Massague J (2017) Complement component 3 adapts the cerebrospinal fluid for leptomeningeal metastasis. Cell 168(1101–1113):e1113. https://doi.org/10.1016/j.cell.2017.02.025
14. Bommer M, Kull M, Teleanu V, Schwarzwalder P, Feuring-Buske M, Kroenke J, Bunjes D, Langer C (2018) Leptomeningeal myelomatosis: a rare but devastating manifestation of multiple myeloma diagnosed using cytology, flow cytometry, and fluorescent in situ hybridization. Acta Haematol 139:247–254. https://doi.org/10.1159/000489484
15. Brandsma D, Taphoorn MJ, Reijneveld JC, Nas TM, Voest EE, Nicolay K, Blezer E (2004) MR imaging of mouse leptomeningeal metastases. J Neurooncol 68:123–130. https://doi.org/10.1023/b:neon.0000027742.78828.99
16. Cancer M, Hutter S, Holmberg KO, Rosen G, Sundstrom A, Tailor J, Bergstrom T, Garancher A, Essand M, Wechsler-Reya RJ et al (2019) Humanized stem cell models of pediatric medulloblastoma reveal an Oct4/mTOR axis that promotes malignancy. Cell Stem Cell 25(855–870):e811. https://doi.org/10.1016/j.stem.2019.10.005
17. Caretti V, Zondervan I, Meijer DH, Idema S, Vos W, Hamans B, Bugiani M, Hulleman E, Wesseling P, Vandertop WP et al (2011) Monitoring of tumor growth and post-irradiation recurrence in a diffuse intrinsic pontine glioma mouse model. Brain Pathol 21:441–451. https://doi.org/10.1111/j.1750-3639.2010.00468.x
18. Chi Y, Remsik J, Kiseliovas V, Derderian C, Sener U, Alghader M, Saadeh F, Nikishina K, Bale T, Iacobuzio-Donahue C et al (2020) Cancer cells deploy lipocalin-2 to collect limiting iron in leptomeningeal metastasis. Science 369:276–282. https://doi.org/10.1126/science.aaz2193
19. Cho HR, Wen H, Ryu YJ, An YJ, Kim HC, Moon WK, Han MH, Park S, Choi SH (2012) An NMR metabolomics approach for the diagnosis of leptomeningeal carcinomatosis. Cancer Res 72:5179–5187. https://doi.org/10.1158/0008-5472.CAN-12-0755
20. Chung PED, Gendoo DMA, Ghanbari-Azarnier R, Liu JC, Jiang Z, Tsui J, Wang DY, Xiao X, Li B, Dubuc A et al (2020) Modeling germline mutations in pineoblastoma uncovers lysosome disruption-based therapy. Nat Commun 11:1825. https://doi.org/10.1038/s41467-020-15585-2
21. Clarke JL, Perez HR, Jacks LM, Panageas KS, Deangelis LM (2010) Leptomeningeal metastases in the MRI era. Neurology 74:1449–1454. https://doi.org/10.1212/WNL.0b013e3181dc1a69
22. Cruz-Munoz W, Man S, Xu P, Kerbel RS (2008) Development of a preclinical model of spontaneous human melanoma central nervous system metastasis. Cancer Res 68:4500–4505. https://doi.org/10.1158/0008-5472.CAN-08-0041
23. Deng J, Chernikova SB, Wang Y, Rodriguez ML, Andersen SJ, Umeh-Garcia MC, Godfrey BO, Ahmadian SS, Fischer WN, Koller KJ et al (2021) A novel brain-permeant chemotherapeutic agent for the treatment of brain metastasis in triple-negative breast cancer. Mol Cancer Ther 20:2110–2116. https://doi.org/10.1158/1535-7163.MCT-21-0140
24. Elansari RA, Abada R, Rouadi S, Roubal M (2016) Leptomeningeal carcinomatosis unusual clinical presentation from insidious anaplasic thyroid carcinoma. Biomark Gene. https://doi.org/10.15761/BG.1000101
25. Engebraaten O, Fodstad O (1999) Site-specific experimental metastasis patterns of two human breast cancer cell lines in nude rats. Int J Cancer 82:219–225. https://doi.org/10.1002/(sici)1097-0215(19990719)82:2<219::aid-ijc12>3.0.co;2-#
26. Fidler IJ, Schackert G, Zhang RD, Radinsky R, Fujimaki T (1999) The biology of melanoma brain metastasis. Cancer Metastasis Rev 18:387–400. https://doi.org/10.1023/a:1006329410433
27. Fitzpatrick A, Iravani M, Mills A, Vicente D, Alaguthurai T, Roxanis I, Turner NC, Haider S, Tutt ANJ, Isacke CM (2023) Genomic profiling and preclinical modelling of breast cancer leptomeningeal metastasis reveals acquisition of a lobular-like phenotype. Nat Commun 14:7408. https://doi.org/10.1038/s41467-023-43242-x
28. Friedman HS, Archer GE, McLendon RE, Schuster JM, Colvin OM, Guaspari A, Blum R, Savina PA, Fuchs HE, Bigner DD (1994) Intrathecal melphalan therapy of human neoplastic meningitis in athymic nude rats. Cancer Res 54:4710–4714
29. Fuchs HE, Archer GE, Colvin OM, Bigner SH, Schuster JM, Fuller GN, Muhlbaier LH, Schold SC Jr, Friedman HS, Bigner DD (1990) Activity of intrathecal 4-hydroperoxycyclophosphamide in a nude rat model of human neoplastic meningitis. Cancer Res 50:1954–1959
30. Fujimaki T, Price JE, Fan D, Bucana CD, Itoh K, Kirino T, Fidler IJ (1996) Selective growth of human melanoma cells in the brain parenchyma of nude mice. Melanoma Res 6:363–371. https://doi.org/10.1097/00008390-199610000-00003
31. Garner EF, Stafman LL, Williams AP, Aye JM, Goolsby C, Atigadda VR, Moore BP, Nan L, Stewart JE, Hjelmeland AB et al (2018) UAB30, a novel RXR agonist, decreases tumorigenesis and leptomeningeal disease in group 3 medulloblastoma patient-derived xenografts. J Neurooncol 140:209–224. https://doi.org/10.1007/s11060-018-2950-1
32. Garzia L, Kijima N, Morrissy AS, De Antonellis P, Guerreiro-Stucklin A, Holgado BL, Wu X, Wang X, Parsons M, Zayne K et al (2018) A hematogenous route for medulloblastoma leptomeningeal metastases. Cell 172(1050–1062):e1014. https://doi.org/10.1016/j.cell.2018.01.038
33. Gholamin S, Mitra SS, Feroze AH, Liu J, Kahn SA, Zhang M, Esparza R, Richard C, Ramaswamy V, Remke M et al (2017) Disrupting the CD47-SIRPalpha anti-phagocytic axis by a humanized anti-CD47 antibody is an efficacious treatment for malignant pediatric brain tumors. Sci Transl Med 9:eaaf2968. https://doi.org/10.1126/scitranslmed.aaf2968
34. Gopal U, Monroe JD, Marudamuthu AS, Begum S, Walters BJ, Stewart RA, Washington CW, Gibert Y, Zachariah MA (2023) Development of a triple-negative breast cancer leptomeningeal disease model in zebrafish. Cells 12:995. https://doi.org/10.3390/cells12070995
35. Grausam KB, Dooyema SDR, Bihannic L, Premathilake H, Morrissy AS, Forget A, Schaefer AM, Gundelach JH, Macura S, Maher DM et al (2017) ATOH1 promotes leptomeningeal dissemination and metastasis of sonic hedgehog subgroup medulloblastomas. Cancer Res 77:3766–3777. https://doi.org/10.1158/0008-5472.CAN-16-1836
36. Gu C, Li S, Tokuyama T, Yokota N, Namba H (2010) Therapeutic effect of genetically engineered mesenchymal stem cells in rat experimental leptomeningeal glioma model. Cancer Lett 291:256–262. https://doi.org/10.1016/j.canlet.2009.10.020
37. Hall WA, Myklebust A, Godal A, Nesland JM, Fodstad O (1994) In vivo efficacy of intrathecal transferrin-pseudomonas exotoxin A immunotoxin against LOX melanoma. Neurosurgery 34:649–655; Discussion 655–646. https://doi.org/10.1227/00006123-199404000-00012
38. Hatton BA, Villavicencio EH, Tsuchiya KD, Pritchard JI, Ditzler S, Pullar B, Hansen S, Knoblaugh SE, Lee D, Eberhart CG et al (2008) The Smo/Smo model: hedgehog-induced medulloblastoma with 90% incidence and leptomeningeal spread. Cancer Res 68:1768–1776. https://doi.org/10.1158/0008-5472.CAN-07-5092
39. Herrlinger U, Buchholz R, Jachimczak P, Schabet M (1996) Intrathecal treatment of C6 glioma leptomeningeal metastasis in Wistar rats with interleukin-2. J Neurooncol 27:193–203. https://doi.org/10.1007/BF00165475
40. Janczewski KH, Chalk CL, Pyles RB, Parysek LM, Unger LW, Warnick RE (1998) A simple, reproducible technique for establishing leptomeningeal tumors in nude rats. J Neurosci Methods 85:45–49. https://doi.org/10.1016/s0165-0270(98)00115-0
41. Jenkins NC, Kalra RR, Dubuc A, Sivakumar W, Pedone CA, Wu X, Taylor MD, Fults DW (2014) Genetic drivers of metastatic dissemination in sonic hedgehog medulloblastoma. Acta Neuropathol Commun 2:85. https://doi.org/10.1186/s40478-014-0085-y
42. Kanaya N, Kitamura Y, Lopez Vazquez M, Franco A, Chen KS, van Schaik TA, Farzani TA, Borges P, Ichinose T, Seddiq W et al (2023) Gene-edited and -engineered stem cell platform drives immunotherapy for brain

metastatic melanomas. Sci Transl Med 15:eade732. https://doi.org/10.1126/scitranslmed.ade8732
43. Kawaguchi T, Kawaguchi M, Lembo TM, Nicolson GL (1989) Differential tumor growth of blood-borne B16 melanoma variants in cerebral dura mater is related to tumor-host cell reactions. Clin Exp Metastasis 7:1–14. https://doi.org/10.1007/BF02057177
44. Kitamura Y, Kanaya N, Moleirinho S, Du W, Reinshagen C, Attia N, Bronisz A, Revai Lechtich E, Sasaki H, Mora JL et al (2021) Anti-EGFR VHH-armed death receptor ligand-engineered allogeneic stem cells have therapeutic efficacy in diverse brain metastatic breast cancers. Sci Adv 7:abe8671. https://doi.org/10.1126/sciadv.abe8671
45. Knox AJ, Van Court B, Oweida A, Barsh E, DeSisto J, Flannery P, Lemma R, Chatwin H, Vibhakar R, Dorris K et al (2023) A novel preclinical model of craniospinal irradiation in pediatric diffuse midline glioma demonstrates decreased metastatic disease. Front Oncol 13:1105395. https://doi.org/10.3389/fonc.2023.1105395
46. Kokkoris CP (1983) Leptomeningeal carcinomatosis. How does cancer reach the pia-arachnoid? Cancer 51:154–160. https://doi.org/10.1002/1097-0142(19830101)51:1%3c154::aid-cncr2820510130%3e3.0.co;2-k
47. Kooistra KL, Rodriguez M, Powis G, Yaksh TL, Harty GJ, Hilton JF, Laws ER Jr (1986) Development of experimental models for meningeal neoplasia using intrathecal injection of 9L gliosarcoma and Walker 256 carcinosarcoma in the rat. Cancer Res 46:317–323
48. Kramm CM, Rainov NG, Sena-Esteves M, Chase M, Pechan PA, Chiocca EA, Breakefield XO (1996) Herpes vector-mediated delivery of marker genes to disseminated central nervous system tumors. Hum Gene Ther 7:291–300. https://doi.org/10.1089/hum.1996.7.3-291
49. Kusters B, Westphal JR, Smits D, Ruiter DJ, Wesseling P, Keilholz U, de Waal RM (2001) The pattern of metastasis of human melanoma to the central nervous system is not influenced by integrin alpha(v)beta(3) expression. Int J Cancer 92:176–180. https://doi.org/10.1002/1097-0215(200102)9999:9999%3c::aid-ijc1173%3e3.0.co;2-l
50. Kwist K, Bridges WC, Burg KJ (2016) The effect of cell passage number on osteogenic and adipogenic characteristics of D1 cells. Cytotechnology 68:1661–1667. https://doi.org/10.1007/s10616-015-9883-8
51. Law V, Baldwin M, Ramamoorthi G, Kodumudi K, Tran N, Smalley I, Duckett D, Kalinski P, Czerniecki B, Smalley KSM et al (2021) A murine ommaya xenograft model to study direct-targeted therapy of leptomeningeal disease. J Vis Exp. https://doi.org/10.3791/62033
52. Law V, Chen Z, Vena F, Smalley I, Macaulay R, Evernden BR, Tran N, Pina Y, Puskas J, Caceres G et al (2022) A preclinical model of patient-derived cerebrospinal fluid circulating tumor cells for experimental therapeutics in leptomeningeal disease from melanoma. Neuro Oncol 24:1673–1686. https://doi.org/10.1093/neuonc/noac054
53. Li J, Huang D, Lei B, Huang J, Yang L, Nie M, Su S, Zhao Q, Wang Y (2022) VLA-4 suppression by senescence signals regulates meningeal immunity and leptomeningeal metastasis. Elife 11:e83272. https://doi.org/10.7554/eLife.83272
54. Li K, Zhang G, Zhao J, Wang X, Li Y, Hu Y (2011) Vascular endothelial growth factor antisense oligonucleotides inhibit leptomeningeal metastasis in vivo. Med Oncol 28:1116–1122. https://doi.org/10.1007/s12032-010-9580-6
55. Liu Y, Li Y, Li Z, Li C, He J, Bu H (2020) Knockdown of IKKbeta inhibits tumor development in a leptomeningeal metastasis mouse model and proliferation of lung cancer cells. Cancer Manag Res 12:6007–6017. https://doi.org/10.2147/CMAR.S252184
56. Miree J Jr, Gold S (1973) Relationship of survival with number of V-2 carcinoma cells implanted in the subarachnoid space of rabbits. J Natl Med Assoc 65:407–409
57. Miree J Jr, Harwood TR, Gold S (1972) The implantation of V-2 carcinoma in the subarachnoid space of rabbits. J Natl Med Assoc 64:305–307
58. Morganti S, Parsons HA, Lin NU, Grinshpun A (2023) Liquid biopsy for brain metastases and leptomeningeal disease in patients with breast cancer. NPJ Breast Cancer 9:43. https://doi.org/10.1038/s41523-023-00550-1
59. Myklebust AT, Godal A, Fodstad O (1994) Targeted therapy with immunotoxins in a nude rat model for leptomeningeal growth of human small cell lung cancer. Cancer Res 54:2146–2150
60. Nakagawa H, Yui Y, Sasagawa S, Itoh K (2018) Evidence for intrathecal sodium butyrate as a novel option for leptomeningeal metastasis. J Neurooncol 139:43–50. https://doi.org/10.1007/s11060-018-2852-2
61. Nanjo S, Arai S, Wang W, Takeuchi S, Yamada T, Hata A, Katakami N, Okada Y, Yano S (2017) MET copy number gain is associated with gefitinib resistance in leptomeningeal carcinomatosis of EGFR-mutant lung cancer. Mol Cancer Ther 16:506–515. https://doi.org/10.1158/1535-7163.MCT-16-0522
62. Nanjo S, Ebi H, Arai S, Takeuchi S, Yamada T, Mochizuki S, Okada Y, Nakada M, Murakami T, Yano S (2016) High efficacy of third generation EGFR inhibitor AZD9291 in a leptomeningeal carcinomatosis model with EGFR-mutant lung cancer cells. Oncotarget 7:3847–3856. https://doi.org/10.18632/oncotarget.6758
63. Nayar G, Ejikeme T, Chongsathidkiet P, Elsamadicy AA, Blackwell KL, Clarke JM, Lad SP, Fecci PE (2017) Leptomeningeal disease: current diagnostic and therapeutic strategies. Oncotarget 8:73312–73328. https://doi.org/10.18632/oncotarget.20272
64. Nguyen A, Nguyen A, Dada OT, Desai PD, Ricci JC, Godbole NB, Pierre K, Lucke-Wold B (2023) Leptomeningeal metastasis: a review of the pathophysiology, diagnostic methodology, and therapeutic landscape. Curr Oncol 30:5906–5931. https://doi.org/10.3390/curroncol30060442
65. Pan S, Ye D, Yue Y, Yang L, Pacia CP, DeFreitas D, Esakky P, Dahiya S, Limbrick DD, Rubin JB et al (2022) Leptomeningeal disease and tumor dissemination in a murine diffuse intrinsic pontine glioma model: implications for the study of the tumor-cerebrospinal fluid-ependymal microenvironment. Neurooncol Adv 4:vac059. https://doi.org/10.1093/noajnl/vdac059
66. Park YW, Han K, Park JE, Ahn SS, Kim EH, Kim J, Kang SG, Chang JH, Kim SH, Lee SK (2023) Leptomeningeal metastases in glioma revisited: incidence and molecular predictors based on postcontrast fluid-attenuated inversion recovery imaging. J Neurosurg 139:38–48. https://doi.org/10.3171/2022.9.JNS221659
67. Pastan IH, Archer GE, McLendon RE, Friedman HS, Fuchs HE, Wang QC, Pai LH, Herndon J, Bigner DD (1995) Intrathecal administration of single-chain immunotoxin, LMB-7 [B3(Fv)-PE38], produces cures of carcinomatous meningitis in a rat model. Proc Natl Acad Sci U S A 92:2765–2769. https://doi.org/10.1073/pnas.92.7.2765
68. Patel AS, Allen JE, Dicker DT, Peters KL, Sheehan JM, Glantz MJ, El-Deiry WS (2011) Identification and enumeration of circulating tumor cells in the cerebrospinal fluid of breast cancer patients with central nervous system metastases. Oncotarget 2:752–760. https://doi.org/10.18632/oncotarget.336
69. Phi JH, Choi SA, Lim SH, Lee J, Wang KC, Park SH, Kim SK (2013) ID3 contributes to cerebrospinal fluid seeding and poor prognosis in medulloblastoma. BMC Cancer 13:291. https://doi.org/10.1186/1471-2407-13-291
70. Phillips PC, Than TT, Cork LC, Hilton J, Carson BS, Colvin OM, Grochow LB (1992) Intrathecal 4-hydroperoxycyclophosphamide: neurotoxicity, cerebrospinal fluid pharmacokinetics, and antitumor activity in a rabbit model of VX2 leptomeningeal carcinomatosis. Cancer Res 52:6168–6174
71. Priceman SJ, Tilakawardane D, Jeang B, Aguilar B, Murad JP, Park AK, Chang WC, Ostberg JR, Neman J, Jandial R et al (2018) Regional delivery of chimeric antigen receptor-engineered T cells effectively targets HER2(+) breast cancer metastasis to the brain. Clin Cancer Res 24:95–105. https://doi.org/10.1158/1078-0432.CCR-17-2041
72. Reijneveld JC, Taphoorn MJ, Kerckhaert OA, Drixler TA, Boogerd W, Voest EE (2003) Angiostatin prolongs the survival of mice with leptomeningeal metastases. Eur J Clin Invest 33:76–81. https://doi.org/10.1046/j.1365-2362.2003.01056.x
73. Reijneveld JC, Taphoorn MJ, Voest EE (1999) A simple mouse model for leptomeningeal metastases and repeated intrathecal therapy. J Neurooncol 42:137–142. https://doi.org/10.1023/a:1006237917632
74. Remsik J, Chi Y, Tong X, Sener U, Derderian C, Park A, Saadeh F, Bale T, Boire A (2022) Leptomeningeal metastatic cells adopt two phenotypic states. Cancer Rep (Hoboken) 5:e1236. https://doi.org/10.1002/cnr2.1236
75. Remsik J, Tong X, Kunes RZ, Li MJ, Osman A, Chabot K, Sener UT, Wilcox JA, Isakov D, Snyder J et al (2023) Leptomeningeal anti-tumor immunity follows unique signaling principles. BioRxiv. https://doi.org/10.1101/2023.03.17.533041
76. Rodrigues AJ, Chernikova SB, Wang Y, Trinh TTH, Solow-Cordero DE, Alexandrova L, Casey KM, Alli E, Aggarwal A, Quill T et al (2024) Repurposing mebendazole against triple-negative breast cancer

CNS metastasis. J Neurooncol 168:125–138. https://doi.org/10.1007/s11060-024-04654-x
77. Sagar SM, Price KJ (1995) An experimental model of leptomeningeal metastases employing rat mammary carcinoma cells. J Neurooncol 23:15–21. https://doi.org/10.1007/BF01058455
78. Saito N, Hatori T, Murata N, Zhang ZA, Nonaka H, Aoki K, Iwabuchi S, Ueda M (2008) Comparison of metastatic brain tumour models using three different methods: the morphological role of the pia mater. Int J Exp Pathol 89:38–44. https://doi.org/10.1111/j.1365-2613.2007.00563.x
79. Schabet M, Martos J, Buchholz R, Pietsch T (1997) Animal model of human medulloblastoma: clinical, magnetic resonance imaging, and histopathological findings after intra-cisternal injection of MHH-MED-1 cells into nude rats. Med Pediatr Oncol 29:92–97. https://doi.org/10.1002/(sici)1096-911x(199708)29:2%3c92::aid-mpo5%3e3.0.co;2-m
80. Schabet M, Ohneseit P, Buchholz R, Santo-Holtje L, Schmidberger H (1992) Intrathecal ACNU treatment of B16 melanoma leptomeningeal metastasis in a new athymic rat model. J Neurooncol 14:169–175. https://doi.org/10.1007/BF00177621
81. Shackleford GM, Mahdi MY, Moats RA, Hawes D, Tran HC, Finlay JL, Hoang TQ, Meng EF, Erdreich-Epstein A (2019) Continuous and bolus intraventricular topotecan prolong survival in a mouse model of leptomeningeal medulloblastoma. PLoS ONE 14:e0206394. https://doi.org/10.1371/journal.pone.0206394
82. Shi MX, Ding X, Tang L, Cao WJ, Su B, Zhang J (2024) PROTAC EZH2 degrader-1 overcomes the resistance of podophyllotoxin derivatives in refractory small cell lung cancer with leptomeningeal metastasis. BMC Cancer 24:504. https://doi.org/10.1186/s12885-024-12244-3
83. Shimizu K, Ushio Y, Hayakawa T, Mogami H (1980) Combination chemotherapy with 1-(4-amino-2-methyl-5-pyrimidinyl)methyl-3-(2-chloroethyl)-3-nitrosourea hydrochloride and bleomycin in meningeal carcinomatosis in rats. Cancer Res 40:1341–1343
84. Siegal T, Sandbank U, Gabizon A, Siegal T, Mizrachi R, Ben-David E, Catane R (1987) Alteration of blood-brain-CSF barrier in experimental meningeal carcinomatosis. A morphologic and adriamycin-penetration study. J Neurooncol 4:233–242. https://doi.org/10.1007/BF00150615
85. Siegel RL, Giaquinto AN, Jemal A (2024) Cancer statistics, 2024. CA Cancer J Clin 74:12–49. https://doi.org/10.3322/caac.21820
86. Smalley I, Chen Z, Phadke M, Li J, Yu X, Wyatt C, Evernden B, Messina JL, Sarnaik A, Sondak VK et al (2021) Single-cell characterization of the immune microenvironment of melanoma brain and leptomeningeal metastases. Clin Cancer Res 27:4109–4125. https://doi.org/10.1158/1078-0432.CCR-21-1694
87. Smalley I, Law V, Wyatt C, Evernden B, Fang B, Koomen JM, Welsh EA, Macaulay RJB, Forsyth PA, Smalley KSM (2020) Proteomic analysis of CSF from patients with leptomeningeal melanoma metastases identifies signatures associated with disease progression and therapeutic resistance. Clin Cancer Res 26:2163–2175. https://doi.org/10.1158/1078-0432.CCR-19-2840
88. Soltys BJ, Grausam KB, Messerli SM, Hsia CJC, Zhao H (2023) Inhibition of metastatic brain cancer in Sonic Hedgehog medulloblastoma using caged nitric oxide albumin nanoparticles. Front Oncol 13:1129533. https://doi.org/10.3389/fonc.2023.1129533
89. Suki D, Abouassi H, Patel AJ, Sawaya R, Weinberg JS, Groves MD (2008) Comparative risk of leptomeningeal disease after resection or stereotactic radiosurgery for solid tumor metastasis to the posterior fossa. J Neurosurg 108:248–257. https://doi.org/10.3171/JNS/2008/108/2/0248
90. Thakkar JP, Kumthekar P, Dixit KS, Stupp R, Lukas RV (2020) Leptomeningeal metastasis from solid tumors. J Neurol Sci 411:116706. https://doi.org/10.1016/j.jns.2020.116706
91. Ushio Y, Chernik NL, Posner JB, Shapiro WR (1977) Meningeal carcinomatosis: development of an experimental model. J Neuropathol Exp Neurol 36:228–244. https://doi.org/10.1097/00005072-197703000-00003
92. Ushio Y, Shimizu K, Aragaki Y, Arita N, Hayakawa T, Mogami H (1981) Alteration of blood-CSF barrier by tumor invasion into the meninges. J Neurosurg 55:445–449. https://doi.org/10.3171/jns.1981.55.3.0445
93. Vincent AJ, Esandi MD, van Someren G, Noteboom JL, Avezaat CJ, Vecht C, Smitt PA, van Bekkum DW, Valerio D, Hoogerbrugge PM et al (1996) Treatment of leptomeningeal metastases in a rat model using a recombinant adenovirus containing the HSV-tk gene. J Neurosurg 85:648–654. https://doi.org/10.3171/jns.1996.85.4.0648
94. Vrionis FD, Wu JK, Qi P, Cano WG, Cherington V (1996) Tumor cells expressing the herpes simplex virus-thymidine kinase gene in the treatment of Walker 256 meningeal neoplasia in rats. J Neurosurg 84:250–257. https://doi.org/10.3171/jns.1996.84.2.0250
95. Wasita B, Kamitani H, Kinoshita Y, Mamun MH, Watanabe T (2009) A rat glioblastoma model with diffuse leptomeningeal gliomatosis induced by intracarotid injection of C6 glioma cells. Neurol Res 31:453–462. https://doi.org/10.1179/174313209X403904
96. Wu X, Northcott PA, Dubuc A, Dupuy AJ, Shih DJ, Witt H, Croul S, Bouffet E, Fults DW, Eberhart CG et al (2012) Clonal selection drives genetic divergence of metastatic medulloblastoma. Nature 482:529–533. https://doi.org/10.1038/nature10825
97. Yamada M, Shimizu K, Tamura K, Moriuchi S, Mabuchi E, Park KC, Miyao Y, Hayakawa T (1991) Murine model of leptomeningeal dissemination using human medulloblastoma cells. Neurol Med Chir (Tokyo) 31:763–767. https://doi.org/10.2176/nmc.31.763
98. Yang WQ, Senger DL, Lun XQ, Muzik H, Shi ZQ, Dyck RH, Norman K, Brasher PM, Rewcastle NB, George D et al (2004) Reovirus as an experimental therapeutic for brain and leptomeningeal metastases from breast cancer. Gene Ther 11:1579–1589. https://doi.org/10.1038/sj.gt.3302319
99. Yang Z, Guo Q, Wang Y, Chen K, Zhang L, Cheng Z, Xu Y, Yin X, Bai Y, Rabbie S et al (2016) AZD3759, a BBB-penetrating EGFR inhibitor for the treatment of EGFR mutant NSCLC with CNS metastases. Sci Transl Med 8:368ra172. https://doi.org/10.1126/scitranslmed.aag0976
100. Yoshida T, Shimizu K, Ushio Y, Hayakawa T, Arita N, Mogami H (1986) Development of experimental meningeal gliomatosis models in rats. J Neurosurg 65:503–507. https://doi.org/10.3171/jns.1986.65.4.0503
101. Yust-Katz S, Mathis S, Groves MD (2013) Leptomeningeal metastases from genitourinary cancer: the University of Texas MD Anderson Cancer Center experience. Med Oncol 30:429. https://doi.org/10.1007/s12032-012-0429-z
102. Zhang C, Zhang F, Tsan R, Fidler IJ (2009) Transforming growth factor-beta2 is a molecular determinant for site-specific melanoma metastasis in the brain. Cancer Res 69:828–835. https://doi.org/10.1158/0008-5472.CAN-08-2588

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