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Manuscript title: An *in vivo* mouse model of intraosseous spinal cancer causing evolving paraplegia

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Abstract

Background: The spine is the commonest site of skeletal metastatic disease and uncontrolled growth of cancer in the spine will inevitably cause pain and neurologic compromise. Improved understanding of the pathobiology behind this devastating condition is urgently needed. For this reason, the aim of this study was to establish a clinically relevant, animal model of spinal cancer. **Methods:** A percutaneous orthotopic injection of human breast (MDA-MB-231) or human prostate (PC-3) cancer cells was administered into the upper lumbar spine of nude mice (n=6). Animals were monitored twice daily for general welfare, gait asymmetry or disturbance, and hindlimb weakness. After sacrifice, plain radiographs, micro-CT imaging and histological analysis of the spines were performed on each mouse. **Results:** All mice recovered fully from the inoculation procedure and displayed normal gait and behaviour patterns for at least 3 weeks post-inoculation. Subsequently, between 3 to 5 weeks post-inoculation, each mouse developed evolving paralysis in their hindlimbs over 48 to 72 hours. All followed the same pattern of decline following onset of neurological dysfunction; from gait asymmetry and unilateral hindlimb weakness, to complete unilateral hindlimb paralysis and finally to complete bilateral hindlimb paralysis. Plain radiographs, micro-CT scanning and histological analysis confirmed local tumour growth and destruction of the spine in all six mice. **Conclusion:** An *in vivo* mouse model of human intraosseous spinal cancer has been established forming cancers that grow within the spine and cause epidural spinal cord compression, resulting in a reproducible, evolving neurological deficit and paralysis that closely resembles the human condition.

Key words: spinal metastasis, animal model, cancer, prostate, breast

Introduction:

Spinal metastases are the commonest site of skeletal metastatic disease and metastatic spread of cancer to the spine will more often than not occur in patients with advanced cancer. The commonest primary tumours are breast cancer in women and prostate cancer in men [1-4]. The vertebral column is essential for structural support of the bony skeleton and each vertebra encapsulates and protects the spinal cord and exiting nerve roots, so destruction by cancer inevitably causes pathological fracture, spinal cord and nerve root compression, intractable pain, weakness, paralysis and inability to weight bear and ambulate [5-7]. Conventional therapies including chemotherapy, hormonotherapy and radiation therapy and are currently the mainstay of treatment for patients with asymptomatic spinal metastases however, each of these treatment modalities have their own toxic side effects and can often not stop tumour progression nor reverse symptoms of spinal instability or neural compression when they occur [7-9]. Spinal surgery in the form of tumour debulking, spinal decompression and stabilization has been shown to improve quality of life in selected patients [10]. However, surgery is purely palliative and usually reserved for patients with an estimated survival prognosis of greater than six months who have intractable symptoms or spinal cord compression. Despite recent advances, surgery is not usually able to reverse established neurological deficit or paralysis, and surgery on patients with advanced cancer carries a risk of morbidity and mortality, especially in patients who are immunocompromised or who have received local radiotherapy. Furthermore, local tumour regrowth causing symptom recurrence is often seen if patients continue to live or who have cancers unresponsive to chemo- or radiotherapy. Salvage options are currently very limited for patients with primary or metastatic tumours who respond poorly to conventional medical treatments.

Novel therapies are urgently needed in addition to conventional therapies in order to improve the local control of spinal cancer, to prevent or reduce the destruction of bone that causes spinal instability and fracture, and to minimize the growth of cancer to compress the neural structures causing radiculopathy, neurological deficit, incontinence and paralysis. Any advances in this field would lead to improvement in pain, functional status and quality of life in these patients. Currently, our understanding of the pathophysiological processes behind growth of cancer in the spine and its invasion of local tissues is limited. The threshold for when sufficient bony destruction has occurred to

cause pathological fracture or instability, or sufficient epidural spinal cord compression has occurred to cause weakness or paralysis remains unknown. Reproducible *in vivo* animal models that are clinically relevant are essential in order to improve understanding of the temporospatial pattern of cancer growth and invasion of the spine, to enable analysis of the interaction between host and cancer cells that govern the growth and spread of spinal cancer and the neurological deficit it causes, and to develop and assess novel therapies. Important considerations include the origin and metastatic characteristics of the experimental cancer cell lines, the host species and its genetic profile, the method of tumour establishment in the spine, and the clinical, radiological and histologic assessment of the cancer once established and growing in the spine [5]. The aim of the present study was to establish a reproducible and clinically relevant *in vivo* small animal model of intraosseous spinal cancer that causes progressive paralysis and mimics the clinical sequelae of metastatic epidural spinal cord compression in humans.

Materials and Methods:

Animal protocols

6 BALB-C/Nu-Nu athymic nude mice between 5-6 weeks of age and weighing between 15-20g were purchased from the Animal Resources Centre (ARC; Western Australia) and were housed in the Bio Resources Facility (BRF) at the Austin hospital (Melbourne, Australia). All animals were housed in standard facilities under aseptic sterile conditions with a maximum of 4 mice per cage, and had free access to water and food. Before procedures commenced, animals were anesthetized with a mixture comprising ketamine hydrochloride (60mg/kg) and xylazine (5mg/kg), administered via an intra-peritoneal injection at a volume 100µl/20g of body weight. Following the cancer cell inoculation procedure, animals were given the analgesic buprenorphine (0.1mg/kg) as a preventative measure, in a total volume of 100µl/20g of body weight via an intraperitoneal injection using a 25G needle. Thereafter, animals were administered analgesic for the following consecutive three days again (am), as a preventative measure followed by administration at the first sign of gait asymmetry or disturbance (time dependent upon when gait asymmetry or disturbance was observed). All procedures were approved by the Austin Health Ethics Committee (A2012-04395) and in accordance with University of Melbourne, Australia, guidelines.

Cell culture

The human breast cancer line MDA-MB-231 (passage #8) was a generous gift from Dr. John Mariadasson (Ludwig Institute for Medical Research, Heidelberg, Victoria) and the human prostate cancer cell line PC-3 (passage #129) was a generous gift from Prof. Ian Davis (Ludwig Institute for Medical Research, Heidelberg, Victoria). Mycoplasma testing was routinely performed on these cell lines, which confirmed both were free of Mycoplasma infection. Both cell lines were maintained in plastic T-75 cm^2 culture flasks (VWR International Ltd, Melbourne, Australia) in RPMI culture medium (VWR International Ltd, Melbourne, Australia), which contained 10% fetal bovine serum (FBS) (VWR International Ltd, Melbourne, Australia), 1% GlutaMAX (Life Technologies Inc. Australia) and 1% penicillin and streptomycin (50 mg/mL each) (Life Technologies Inc. Australia). Cells were grown at 37°C in humidified 5% CO_2 and maintained at sub-confluent levels and passaged sequentially.

Orthotopic inoculation of cancer cells

Monolayers of both MDA-MB-231 and PC-3 cells were detached by trypsinization using EDTA (Ethylenediamine tetra-acetic acid) (Life Technologies Inc. Australia), washed in phosphate-buffered saline (PBS), and counted so that 2 x 10^5 cells of each cell line re-suspended in a total volume of 5µL PBS was ready for administration. After animals had been adequately anaesthetized, the skin overlying the thoracolumbar spine was cleansed using a sterile iodine swab. Animals received a percutaneous intra-osseous injection of the total volume of the cancer cell preparation into the upper lumbar vertebral body (VB) via a direct posterolateral approach to the spine (Fig.1). The thoracic spine of the mouse was held firmly with the thumb and index finger of the senior author and the VB of interest was held stably between with this and blunt end forceps, whilst a percutaneous injection containing either the MDA-MB-231 tumour cell-suspension (in female mice) or PC-3 tumour cell-suspension (in male mice) was administered directly into the VB using a Hamilton Leur-tip syringe with a 26G needle (Fig. 1). The needle tip was advanced through the skin until it reached hard cortical bone of the VB. It was then passed slowly through the VB bone cortex, using a gentle drilling motion, until decreased resistance was encountered, signifying breach of hard cortical bone and entry into the softer cancellous bone of the VB. The needle was allowed to remain in place in the VB for approximately ten seconds before gradual removal in order to minimize any leakage of cells. No vascular complications were encountered during the inoculation process. Buprenorphine (0.1mg/kg)

in a total volume of 100µl/20g of body weight was administered for post-procedure analgesia via an intraperitoneal injection. Animals were monitored hourly for 6 hours post-procedure to ensure complete recovery. Thereafter, mice were monitored twice daily for general welfare which included general behaviour, grooming, food and water consumption, weight gain and gait. Gait observations included cadence and placement of both forelimbs and hindlimbs and the tail. If gait disturbance or asymmetry was detected, the reason for this was noted, such as dragging of one limb, and which hindlimb joints displayed decreased or no movement.

Animal sacrifice, radiological imaging, tissue harvesting and processing

Animals were sacrificed once complete bilateral hindlimb paraplegia was observed with an injection of a lethal dose (200mg/kg) of Pentobarbitone Sodium (Nembutal®; Boehhringer Ingelheim Pty. Ltd. NSW, Australia) administered intra-peritoneally. The thoracolumbar spine junction containing the tumour was harvested *en bloc* for each animal and fixed in 10% formalin (Livingstone International NSW, Australia). Plain radiographs and micro-Computed Tomography (CT) imaging were performed on the spines using the vivaCT 40 *in vivo* animal x-ray and micro-CT (Scanco Medical, Basserdorf, Switzerland). 3-dimensional reconstructions were performed using the micro-CT Evaluation Program V5.0 (Scanco Medical, Basserdorf, Switzerland). After the spines had been fixed for one week, they were placed in decalcifying solution comprising of paraformaldehyde in EDTA and PBS on a shaker at 4°C for 2 weeks, refreshing the decalcifying solution every 2-3 days. The spines were then processed through standard methods and embedded in paraffin wax blocks.

Histological analysis

Histological analysis was performed through Haematoxylin and Eosin staining on 10µm paraffin sections following standard staining protocols. Briefly, tissue sections were dewaxed through (2 x 5 mins) immersions in xylene (Chem Supply, Gillman, South Australia) and then rehydrated through a series of graded ethanol: 100% (2 x 5 mins), 100%, 70%, 30% (v/v) (2 x 5 mins each) concentration. Slides were then rinsed in running tap water for 3-4 mins. Slides were immersed in Harris Haematoxylin Solution (Sigma Chemical Co. MO, USA) for 4 mins, rinsed under running tap water for 5 mins, dipped in 0.5% acid-ethanol for 10 secs and immersed in 1% Eosin Y (Accustain™; Sigma Chemical Co. MO, USA) solution for 2 mins. Excess eosin was rinsed off by running tap water

followed by 3 sequential (2 x 2 mins) washes through a graded ethanol series of 30%, 70% and 100% (v/v). Finally, slides were cleared by immersing in xylene for 2 x 2 mins and cover-slips were placed immediately on the sections using DePeX mounting medium (BDH Chemicals Pty. Ltd, Victoria, Australia). Slides were then allowed to dry overnight in a fume-hood before imaging on a Leica DM4000 B Upright Microscope System.

Results:

Clinical sequelae following orthotopic inoculation of cancer cells

All mice fully recovered from both anaesthesia and the orthotopic cancer cell inoculation procedure as demonstrated by their ability to be fully mobile and resuming food and water consumption within 10 – 15 minutes post-inoculation. Up to three weeks, all mice displayed normal healthy behaviour and activity including regular eating and drinking and steady weight gain, normal grooming, symmetrical movement and gait, plantar placement of paws whilst walking and running, tail held consistently up rather than dragging downwards (Fig. 2, Table 1). From 3 weeks post-inoculation of cancer cells, the first mouse began to exhibit a disturbance in their gait whilst maintaining ability to walk, with the other mice soon following (range, 21 to 40 days). One of the hindlimbs began to show a subtle, occasional dorsal placement of the paw, or foot-drop, during gait. It was also noticed that the affected paw was rotated intermittently either externally or internally and not in parallel with its body whilst either stationary or during gait. At the same time, a limp was observed, as demonstrated by decreased time spent during stance phase on the affected limb. These observations were not observed consistently during gait; intermittently the gait would return to normal. Following the initial observation of subtle gait disturbance and asymmetry, the dorsal placement of the foot became more consistent, with less time spent on the affected side during weight-bearing and accompanied by intermittent tail dragging along the ground.

Between 1 to 7 days following evidence of gait asymmetry, total loss of movement distal to the hip joint in one hindlimb occurred, resulting in a complete unilateral hindlimb paraplegia and consistent dragging of the paralysed limb along the ground. The contralateral hindlimb remained functional and mice could still intermittently stand upright and walk as a result of this. Tail dragging was also consistently observed in affected mice. At this stage of neurological decline, 3 out of 6 mice

developed tail sores. From the point of unilateral hindlimb paralysis, all mice developed complete bilateral hindlimb paraplegia within 24 to 72 hours. Animals displayed total loss of movement from the hip joint distally resulting in both hindlimbs being dragged along the ground, were incapable of standing upright and could only mobilize and propel themselves forward using their forelimbs (Fig. 2).

Due to the consistent pattern of neurological decline and hindlimb paraplegia, a grading system from 0 to 3 was devised (Fig. 2 and Table 1). Grade 0 = normal movement and function of both forelimbs and hindlimbs and a smooth, symmetric gait. Grade 1 = gait disturbance or asymmetry due to dorsal placement of one foot and decreased time spent during stance phase (weight-bearing) of the affected hindlimb. Grade 2 = complete unilateral hindlimb paralysis with good function of the contralateral hindlimb. Grade 3 = complete bilateral hindlimb paralysis with mobilization only possible using the forelimbs. Mice developed the increasing neurological deficits at differing time-points (Table 1), however they all followed the same pattern of decline upon onset of the first symptoms.

Radiological and histological analysis

Plain radiographs and micro-CT scanning showed the presence of lytic lesions within the upper lumbar vertebra of all mice (Fig. 3). Histological analysis confirmed that the tumour had established within the vertebral body of the upper lumbar spine (Fig. 4). Normal bone marrow cells were present adjacent to the cancer cells, as well as blood vessels and osteoclasts. In some sections, tumour was seen invading into the spinal canal and causing epidural spinal cord compression (Fig. 4).

Discussion

An *in vivo* animal model of human spinal cancer that is relevant to the human condition assumes that the host vertebral anatomy and location of neurological structures are similar to that of humans, a cancer that can establish, grow within and invade bone and if untreated causes epidural spinal cord compression that results in neurological decline exemplified by gait disturbance, weakness and eventually paralysis. We describe one such small animal model, using a percutaneous technique of orthotopic inoculation of human breast or prostate cancer cells directly into the upper lumbar vertebra of nude mice. The resulting cancers that grew within the spine caused epidural spinal cord compression and progressive neurological deficit, eventually leading to bilateral complete hindlimb

paralysis. Importantly, the observed pattern of functional decline was reproducible and consistent in all mice and closely resembles what happens in humans. If left untreated, the natural history of metastatic epidural spinal cord compression and continual growth of cancer in the spine of human patients and is for progressive neurological deficit and/or weakness that inevitably results in the loss of ability to ambulate and paraplegia.

Although the timing of development of neurological deficit in our model was unpredictable, the pattern of subsequent neurologic decline was consistent in all animals. As a result, a four-part grading system was devised; from 0 (normal gait), to 1 (asymmetrical gait due to weakness or pain), to 2 (unilateral hindlimb paralysis and 3 (bilateral hindlimb paralysis). This grading system is simple and concise, descriptive, easily interpretable, mimics what happens in humans, and may be applied to other *in vivo* models of spinal cancer. The cause of the original gait asymmetry may either be due to pain or weakness, as in human patients. The development of tail sores that occurred in half of the mice may similarly be due to impaired movement and sensation. More complex grading systems for neurological deficit exist, such as the Basso, Beatie and Breshnahan (BBB) Locomotor Rating Scale, which was originally described in a rat spinal cord contusion model [11]. This was later modified to be applicable in mice as the Basso Mouse Scale (BMS) for Locomotion [12]. These scales are based on ankle, knee and hip movements, plantar or dorsal stepping, coordination, trunk stability, tail position and paw position and are graded between 0 – 9. Although relevant to spinal cord injury, these scales are yet to be adopted in any model of spinal cancer. Attempt to minimize leakage of cells following the orthotopic inoculation into the VB was made by allowing the needle to stay in the VB for approximately ten seconds before slowly withdrawing the needle, however some leakage of cells out of the VB may have occurred, which might potentially account for the differing timing of neurological deficit seen in the mice.

Our model is not a true model of spinal metastasis as it does not involve the spontaneous spread of cancer through the systemic circulation to the spine and as such, fails to demonstrate the molecular mechanisms by which cancer cells are attracted to the spine. Indeed, *in vivo* models of spontaneous bone metastasis are extremely rare [5, 13]. Systemic administration of cancer cells directly into the systemic circulation via the lateral tail vein or left ventricle of the heart has been able to produce

multiple-site metastases but the locations of these are unpredictable and not reproducible, and the spine may or may not be involved [14-20]. Mantha *et al*. [21] described an *in vivo* model of orthotopic inoculation of mammary carcinoma cells into the lumbar spine of rats via an anterior surgical midline incision of the abdominal wall, however this is technically demanding, requires animal dissection and recovery, and may lead to secondary complications [22, 23]. More recently, two separate research groups have described direct implantation of cancer cells via a posterior approach into the laminae of Fischer rats or immunodeficient mice [24, 25]. Both techniques involved animal anaesthesia and surgery using an operating microscope, a dorsal skin incision, dissection of the paraspinal miscles and high-speed burring to decorticate the posterior elements of the spine. In the rat model, tumour-induced paresis occured relatively rapidly, at median time of 8.7 days post-operatively [24]. In the mouse posterior spinal tumour model, the initial 4 out of a total of 12 mice suffered neurologic injury during surgery, were immediately paraplegic post-operatively and subsequently expired, suggesting that a learning curve is required for this technique [25]. We did not observe any immediate intra- or post-operative neurologic or vascular complication following our technique of percutaneous orthotopic inoculation. The relevance of any animal spinal metastasis model to the human condition assumes a cancer that can establish, grow and invade bone, have a similar host spinal anatomy and physiology to humans, and has a correlation between the onset of neurological decline with tumour progression and spinal cord compression. Our percutaneous orthotopic inoculation technique utilizes a known quantity and volume of cancer cells that are directly introduced into the vertebral body, producing tumours that develop consistently within the spine and are capable of growing within the bone microenvironment, eventually causing spinal cord compression. The procedure has low morbidity, does not require a skin incision or tissue dissection, and the post-procedure recovery time is shorter than more invasive inoculation techniques [21-31]. Using a percutaneous approach rather than open surgery means that the VB is not directly visualized, however it is likely that we are injecting into the vertebral body because once the tumour was established, plain radiographs, micro-CT scanning and histological analysis confirmed lesions within the VB in all mice. Furthermore, we presume that if the spinal canal and/or spinal cord was perforated at the time of cancer cell inoculation, this would cause immediate post-operative paralysis or neurological deficit, which was not observed in a single mouse. Our model allows investigation of the interactions of the cancer cells that establish in the spine with the surrounding bone microenvironment and neural structures, the molecular mechanisms behind

tumour growth and destruction of bone, the difference in behaviour and growth of different cancer cell types within the spine, and the threshold of spinal cord compression and onset of neurological deficit. This is the focus of current work in our laboratory.

Histological analysis confirmed the establishment and growth of tumour within the vertebra of the mouse and radiological evaluation confirmed lytic bony destruction. Cross sections of the tumour showed the tumour-bone and tumour-spinal cord interfaces, which enables potential future analysis of the temporospatial pattern and molecular mechanisms by which spinal cancer grows within the vertebra, destroys bone and causes epidural spinal cord compression. Our model utilizes athymic nude mice which have no immune system in order to obviate the immunological rejection response that would normally occur following introduction of cancer cells from a different species (human). This precludes analysis of the important host immune responses that combat the establishment, growth and progression of cancer. Nevertheless, it allows us to examine other molecular mechanisms by which cancer cells invade and destroy bone, such as the angiogenesis and osteolysis pathways. Another advantage of our model is that it does not limit us to the study of breast and prostate cancer in the spine; it allows us to investigate all other cancer cell types, including primary cancers such as osteosarcoma and chondrosarcoma, and other cancers that commonly metastasize to the spine such as lung and renal cancer. The use of immunodeficient mice enables investigation of any human cancer cell line; these cancer cell lines may be established from bone or spinal metastases in human patients, such as the PC-3 human prostate cancer cell line used in our model, and thus have direct clinical relevance in terms of studying the biology and molecular mechanisms of spinal metastasis. For these reasons, immunodeficient mice are currently the commonest host animals used in *in vivo* cancer models. Indeed, in a recent review of animal models of skeletal metastasis, nude or severe combined immunodeficiency (SCID) mice were utilized in 75% of established models [32].

No obvious differences were observed in terms of the clinical, radiological and pathological features between the two different cancer cell types used in the present study. Although metastatic prostate cancer lesions are often osteoblastic in nature, they may also be mixed or lytic, and require osteolysis in order to progress. Similar to our findings, human PC-3 prostate cancer cells have been shown to produce purely osteolytic lesions in orthotopic xenograft models [33, 34]. Other less aggressive

prostate cancer cell lines such as Du-145, LnCaP and C42B have been shown to produce mixed sclerotic/lytic bone lesions in mouse tibial injection models [34-36].

In light of the reproducible and consistent pattern of neurologic decline, our model may provide a suitable platform to test novel therapies. For example, experimental treatment may be initiated at the first sign of gait disturbance (Grade 1), to see if it can delay or prevent progression to unilateral (Grade 2) or bilateral (Grade 3) hindlimb paraplegia that would have otherwise been inevitable if left untreated. This is the equivalent of implementing treatment in human patients with spinal metastases upon symptoms of pain, neurological deficit or impairment of walking ability.

The need for a better understanding of the molecular mechanisms behind cancer spread to the spine, establishment and growth within the bony vertebral column, and subsequent destruction of bone and invasion into surrounding soft tissues to cause pathological fracture and epidural spinal cord compression is critical in order to minimize the devastating clinical impact spinal metastases have on patients with advanced cancer. In the present study, we have established an *in vivo* mouse model of human intraosseous spinal cancer that is induced by a percutaneous orthotopic inoculation of human breast or prostate cancer cells into the upper lumbar vertebra of nude mice. This causes a reproducible and evolving neurological decline resulting in eventual paraplegia that resembles the human condition of epidural spinal cord compression by cancer. This model has the potential to enable temporospatial analysis of the pattern and molecular mechanisms behind cancer growth in the spine and provides us with a suitable platform to identify and test novel therapies, in order to improve the local control of primary or secondary spinal cancer and the pain and functional impairment it inevitably causes for patients.

Acknowledgments

This work was supported by the National Health and Medical Research Council of Australia (Fellowship No. 558418), the Victorian Orthopaedic Research Trust and the Austin Health Medical Research Foundation. We thank Dr. Ali Ghasem Zadeh, Department of Medicine, University of Melbourne, Austin Health for the reconstruction of the micro-CT images.

Disclosure Statement:

The authors declare no conflict of interest and all procedures were approved by the Austin Health Ethics Committee (A2012-04395) and in accordance with University of Melbourne, Australia, guidelines.

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Figure Legend

Fig 1 Percutaneous orthotopic inoculation of human cancer cells

The thoracolumbar spine junction was immobilized between fingers and forceps. An orthotopic inoculation of cancer cells was delivered into the vertebral body via a percutaneous, direct posterolateral approach.

Fig 2 Graded clinical stages of evolving neurological deficit post-inoculation of cancer cells

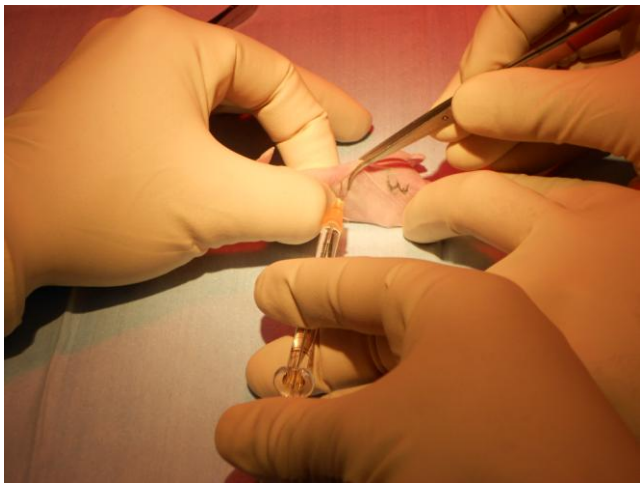
a. 0: Normal movement of hindlimbs and symmetric gait. **b. 1: Gait asymmetry** due to intermittent dorsal placement of one foot (white arrow) and decreased time spent on this foot during the weight-bearing phase of gait. **c. 2: Complete unilateral hindlimb paralysis** from the hip joint and good function of the contralateral hindlimb. **d. 3: Complete bilateral hindlimb paralysis** with mobilization only possible using the forelimbs.

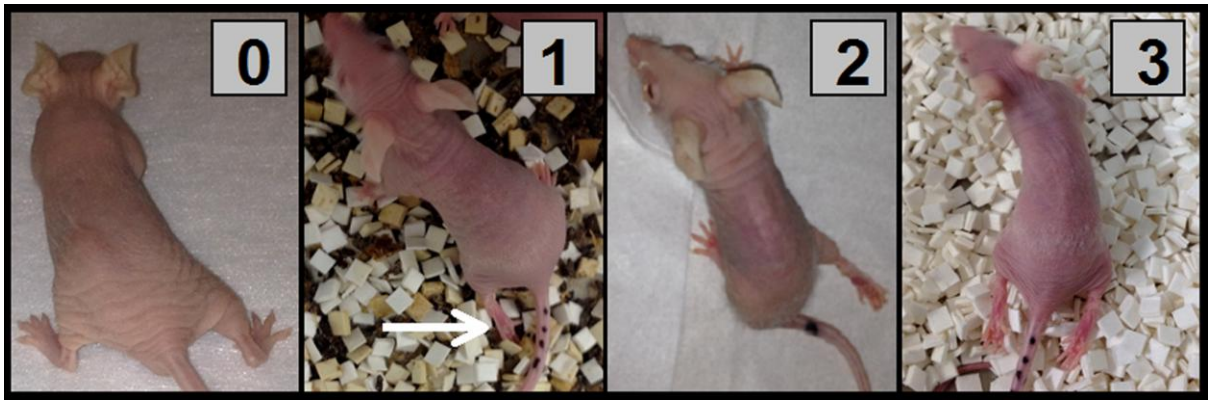
Fig 3 Plain Radiographs and Micro-CT Imaging at 3 to 6 Weeks Post-Inoculation of Human Cancer Cells

a. Whole body plain radiograph showing lytic lesion in the upper lumbar vertebra of a mouse post-inoculation of human PC-3 prostate cancer cells (arrow). **b.** Control axial CT image showing normal vertebral body (VB) and spinal canal (SC). **c.** Axial CT image showing tumour growth within the vertebral body (arrow) and posterior vertebral body cortical destruction. **d.** 3D micro-CT reconstruction showing lytic destruction of the spine (outlined), at 4 weeks post-inoculation of human MDA-MB-231 breast cancer cells. **e.** 3D micro-CT reconstruction showing lytic bony destruction of the posterior elements of the spine at 5 weeks post-inoculation of human PC-3 prostate cancer cells.

Fig 4 Histological analysis of the spine in cancer-inoculated mice

a. *En bloc* resected specimen of the spine, showing the internal section and tumour mass in the upper lumbar vertebra (arrow). **b.** Axial cross section of normal mouse spine below the tumour, showing the vertebral body (VB) and normal bone marrow (BM) surrounding the spinal cord (SC) (x4 magnification). **c.** Axial cross section of the spine with prostate cancer (T) present in the vertebral body (x4 magnification). **d.** Boxed area in panel C showing the tumour-spinal cord interface (arrow) and tumour-bone marrow interface laterally (x10 magnification).

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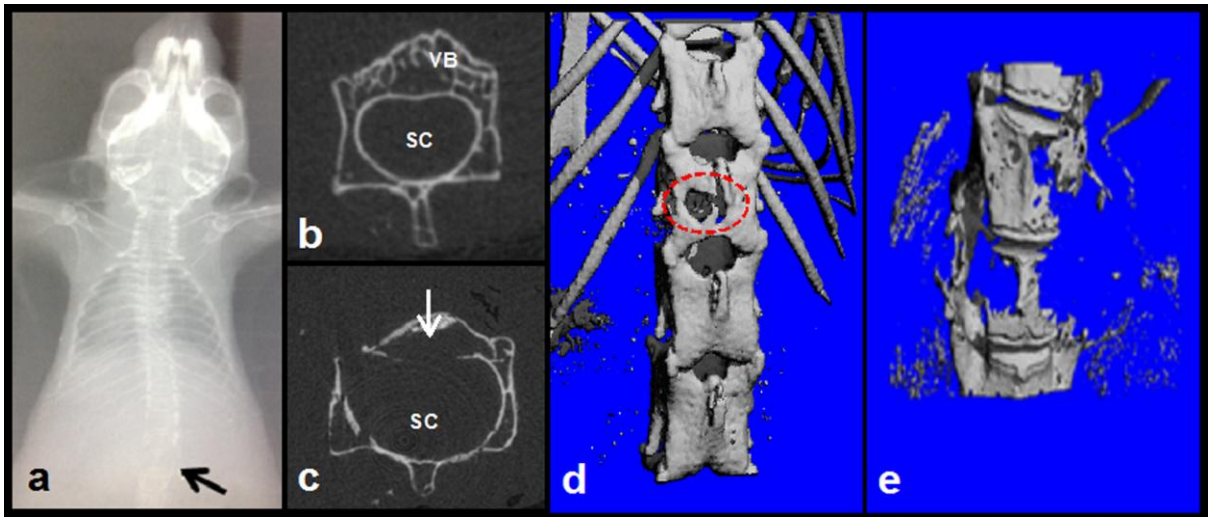
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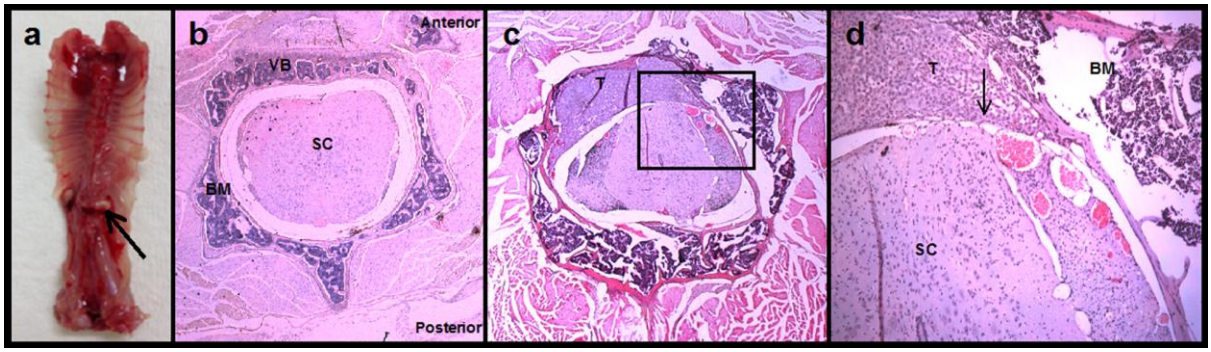

TABLES

Table 1. Timepoint of neurological decline in each mouse.

Neurological Decline		
Mouse	**Days Post-Inoculation**	**Score**
Female #1 MDA-MB-231	1 - 20	0
	21	1
	29	2
	31	3
Female #2 MDA-MB-231	1 - 26	0
	27	1
	28	2
	29	3
Female #3 MDA-MB-231	1 - 29	0
	30	1
	31	2
	32	3
Female #4 MDA-MB-231	1 -34	0
	35	1
	37	2
	40	3

Male #1 [PC-3]	1- 21	0
	22	1
	24	2
	26	3
Male #2 [PC-3]	1 - 39	0
	40	1
	44	2
	45	3

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Title:
An in vivo mouse model of intraosseous spinal cancer causing evolving paraplegia

Date:
2013-11-01

Citation:
Cossigny, D. A. F., Mouhtouris, E., Dushyanthen, S., Gonzalvo, A. & Quan, G. M. Y. (2013). An in vivo mouse model of intraosseous spinal cancer causing evolving paraplegia. JOURNAL OF NEURO-ONCOLOGY, 115 (2), pp.189-196. https://doi.org/10.1007/s11060-013-1226-z.