9

Applications of Gamma Knife Radiosurgery for Experimental Investigations in Small Animal Models

Gabriel Charest, Benoit Paquette and David Mathieu
Department of Nuclear Medicine and Radiobiology, Sherbrooke University
Canada

1. Introduction

The Gamma Knife (GK) was not originally designed for experimentations in small animals. In fact, there are no compatible custom accessories or stereotactic frames on the market for the spatial positioning of small animals in the GK. In addition, the GK is intensively used for patient treatments, and consequently the access for research with small animals is limited. On the other hand, devices specially designed for the irradiation of small animals are available on the market. For examples, small animal irradiators can be purchasable from Best Theratronics (Theratronics, 2011), Rad Source Technologies (Rad Source Technologies, 2011), Precision X-Ray Inc. (Precision X-Ray, 2011) and Xstrahl-Gulmay Medical Inc. (Xstrahl, 2011). Compared to the GK, these small animal irradiators have the following advantages: lower cost, smaller size, some are shielded and thus don't require a special shielded room, some can be combined with an imaging device that allows to image the animal and immediately irradiate the region of interest and, if needed, repeat imaging. Then, since irradiators designed for small animal already exist, why use a GK for animal experimentations? The answer should include technical as well as conceptual aspects. The most important benefit of using the GK for small animals is related to the difference between conventional external radiotherapy and GK radiosurgery (GKRS). Radiation deposition with a GK is produced by multiple concentric beams that allow high dose deposition in a very small volume. These converging beams of ionizing radiation in a precise volume allow a rapid fall-off of dose near the edges which limit adverse effects on the surrounding adjacent tissue.

This chapter is devoted to a review of some characteristics of "homemade" stereotactic frames allowing small animal fixation in the Gamma Knife, and explores small animal researches done with a Gamma Knife published in the literature.

2. In-house designed stereotactic frame for use with the Gamma Knife for small animals

Even if GK is reported to be used for irradiation of large animals like cat and baboon (Kondziolka et al., 2002; Kondziolka et al., 2000; Lunsford et al., 1990; Nilsson et al., 1978), this chapter is focused on small animals (mouse, rat) because of their frequent uses in

translational research, which is justified by their low cost, availability and reliable mimic of healthy or diseased human tissues/organs. When research groups plan to use the GK for experimental irradiations with small animals, the first step is to create a stereotactic device to hold the animals. These new devices need to show their efficiency and functionality and their approbation from the scientific community usually requires a publication that is peer-reviewed. For clinical application, GK radiosurgery is roughly separated in three steps: 1- imaging, 2- dose and localization planning with appropriate software and 3- positionning and irradiation. To mimic this, the animal stereotactic devices are generally designed to allow excellent transition from the imaging facility (MRI, CT-scan or X-ray radiography) to the planning software (i.e. Leksell Gamma Plan). After dose planning, the small animal stereotactic device must dock perfectly with the automatic positioning system (APS) of the GK, which is the part of the GK that allows movements in three dimensions to place the target at the exact irradiation coordinates. This is the basis for a stereotactic frame but, as we will see, some groups designed their device to bypass the conventional clinical stages or added features not related to radiosurgery to allow more functions. Fifteen articles concerning the use of new stereotactic frames for small animals were found in the literature (see Table 1).

Unfortunately, some of these articles do not mention clearly the details of their new devices, but they are suspected to be used as the first time in a series of experiments (Kouyama et al., 2003; Major et al., 2006; Pellerin et al., 2006; Rao et al., 1998; Takahashi et al., 1996; Xu et al., 2006; Zerris et al., 2002). The first mention of a stereotactic frame for small animals was reported by Kondziolka (Kondziolka et al., 1992b) in 1992. This device was a simple plate held by a modified Leksell model G stereotactic frame where the rat was maintained with adhesive restraints. This device allowed to be imaged by plain radiography and images are expected to be exported to a treatment planning software to assess the stereotactic coordinates. The Kondziolka device was subsequently used in other experiments (Kondziolka et al., 1992b; Kondziolka et al., 1996; Kondziolka et al., 1997; Mori et al., 2000; Niranjan et al., 2000; Niranjan et al., 2003) by the research group of the University of Pittsburgh. One year later, in 1993, Kamiryo *et al.* (Kamiryo et al., 1993) constructed a rat stereotactic device consisting of plate with earplugs and incisor bar to immobilize the animal, and a sliding Y and Z scales. This device has holes that adapt to the manual positioning system (trunnion) of the Gamma Knife. The coordinates of the target were "atlas-guided" using "the rat brain in stereotaxis coordinates" by Paxinos and Watson (Paxinos & Watson, 1986). A removable brain cutter and stereotactic arc to be used for surgical positioning according to the same coordinate system could be added to this device. In 1995, Kamiryo's group built another device to be used with magnetic resonance imaging (MRI). These two devices from Kamiryo's group were used in a few publications of the University of Virginia (Chen et al., 2001; Kamiryo et al., 1996; Kamiryo et al., 1996; Omary et al., 1995; Vincent et al., 2005). A similar atlas-guided stereotactic frame, known as the "Régis-Valliccioni stereotactic frame", was developed in 1996 (Rey et al., 1996) and used for research in Marseille (Bartolomei et al., 1998; Regis et al., 1996).

In 2002, a group from Czech Republic designed a rat stereotactic device compatible for GKRS and MR imaging (Liscak et al., 2002). This device has earplugs and incisor bar, and is used with the APS of the GK. Few publications are reported using this device (Herynek et al., 2004; Jirak et al., 2007b; Novotny et al., 2002a; Novotny et al., 2002b). In 2003, a new Régis-Valliccioni frame was used by a group from Tokyo (Kouyama et al., 2003; Tokumaru

First author	Location	Publication year	Objective	Target	Doses (Gy)	Positioning Methods
D. Kondziolka	Pittsburgh, USA	1992	Dose-response relationship and temporal effect of GKRS of the normal rat brain	Rat Brain: Right frontal lobe	30, 40, 50, 60, 70, 80, 100, 150, 200	Lateral and anterior-posterior radiography
T. Kamiryo	Charlottesville, USA	1993 1995	Technical note of new stereotactic device: Evaluation of geometric and dosimetric inaccuracies during GKRS. The device is built to accept a brain cutting system and an open stereotactic surgery system. In 1995 a new MRI device was developed to fit the same frame	Rat Brain: Right parietal cortex and phantom of thermoluminescence dosimetry (TLD) implanted in rat brain	4, 200, 300	Atlas-guided protocol and/or MRI
M. Rey	Marseille France	1996	Development of a stereotactic device for rat for use with GK. (Regis-Valliccioni model)	Rat Brain: Left striatum	100, 200	Atlasguided protocol
Z-R, Rao	Xi'an, China	1998	Expression and changes of GFAP after GKRS	Rat Brain: right caudate nucleus	100	MRI prior to GK
J. Novotny	Prague, Czech Republic	2002	Use of polymer gel dosimeter for evaluation of geometric and dosimetric inaccuracies during GKRS	Rat phantom	8	MRI prior to GK
N. Kouyama	Tokyo, Japan	2003	Survey of functional alteration in the rat striatum after GKRS. Preliminary results	Rat Brain: Unilateral striatum	150	?
C. DesRosiers	Indianapolis, USA	2003	Technical note about rat platform for GKRS and study of eye lens irradiation.	Rat eye: right eye lens of phantom and living rat	5, 10, 15	Observation through 4 mm collimator
Y-S, Im	Seoul, Korea	2006	Technical note: Stereotactic device for rats for GK model B and C. Testing for accuracy with phantom and normal and C6-glioma-inoculated rats	Film. Ionization chamber	150	Beam direction indicator

Table 1. Publications of new small animal stereotactic devices.

First author	Location	Publication year	Objective	Target	Doses (Gy)	Positioning Methods
D. Xu	Tianjin, China	2006	GKRS on C6 glioma in combination with adenoviral p53	Rat right frontal lobe: Tumor volume	15	MRI prior to GK
H-T. Chung	Goyang, Korea	2008	Development of a stereotactic device for rat for use with GK.	Phantom, ion chamber, radiochromic films, glioma bearing rat	30	X ray imaging
S. Takahashi	Sapporo, Japan	1996	Study of relaxation and contraction responses on common carotid artery following GKRS	Rat common carotid artery	100	Lateral and anterior-posterior radiogram
O. Major	Budapest, Hungary	2006	Modulation of blood vessel obliteration after GKRS and combination of paclitaxel.	Rat common carotid artery	15, 20, 25, 50, 80, 200	Atlasguided protocol
D. Wiant	Winston-Salem, USA	2009	High resolution treatment planning by MRi for GKRS	Radiographic film inserted in phantom and dead rat.	35	High resolution MRi prior to GK
G. Charest	Sherbrooke, Canada	2009	Technique evaluation: Stereotactic frame for GK model 4C docking with APS, Angiographic box and Bubble head measurement	Phantom: Polymer gel poured into skull of dead rats.	15	Radiography and recurrent position
G. Charest	Sherbrooke, Canada	Reported here	Stereotactic device (sarcophagus) for mice to use with GK 4C and Perfexion. For irradiation of mammary gland (breast cancer model) and hips (colorectal cancer model)	Gafchromic film. Ionization chamber	15	CT scan and recurrent positions
M. Pellerin	Sherbrooke, Canada	2006	Monitoring micro-vasculature damage of breast carcinoma induced by GKRS	Mice: breast cancer implanted in left hind limb	40	Direct measurement from the frame

Table 1. (Continued)

et al., 2005b). As the older version, this model uses earplugs and an incisor bar to fix the animal and enable target planning directly on the MR images. This new device appear in few publications (Hirano et al., 2009; Tokumaru et al., 2007a). In the series of devices equipped with earplugs and incisor bar, the most recent one from Chung *et al*. in 2008 (Chung et al., 2008) is a combination of a Leksell G-frame and KOPF rat adaptor. No publication that uses this tool has been found in the literature at the time of writing this chapter. One of the most simple device is reported in a publication of DesRosiers in 2003 (DesRosiers et al., 2003). This device is simply an 8" X 10" wood platform with holes to allow reproducible docking onto the GK manual positioning system. The rats were placed on another 6" X 8" wood resting plate that could be moved by hand in the X and Z direction and a leveling screw was set into the platform to allow motion in the Y direction. The targeting was performed by direct observation through 4 mm collimators. This visual targeting was deemed adequate because the target was the external eye of the animal, but could not be used for internal organ targeting. This device and method are reported elsewhere (Dynlacht et al., 2006; Dynlacht et al., 2008) and the coordinates were finally confirmed by CT scan (Tinnel et al., 2007). Im et al. (Im et al., 2006) also created a stereotactic device for rat consisting of a plate with ear plugs, incisor bar and a sliding Y and Z scales. The target was atlas-guided and a metallic bar that fitted exactly with one of the collimator holes of the collimator helmet was used as a beam direction indicator. This system is suspected to be used later by Lee et al. (Lee et al., 2006). It is to note that the positioning methods reported for the devices of DesRosiers (DesRosiers et al., 2003) and Im (Im et al., 2006) cannot be used with the newer Leksell Gamma Knife model Perfexion, because in this latest model, the internal collimators are inaccessible, which prevents their use as beam direction indicators. In 2009, the group of Wiant *et al*. (Wiant et al., 2009) developed a restrain jig with fiducial system small enough to be compatible with a small-bore 7T MR scanner aperture. This jig provides repeatability, accuracy, and interchangeability with MR scanner and the Gamma Knife headframe. This device was developed to gain a very high-resolution of MRI with a field of view of 50 mm^2 compare to 250 mm^2 with a clinical 3T MRI.

Our group at the University of Sherbrooke built three small animal stereotactic devices for GK. In 2006 Pellerin *et al.* (Pellerin et al., 2006) constructed a mouse frame where the animal was held in a 50 ml Falcon tube. The tube containing the mouse was positioned in relation to a mark onto the stereotactic frame. The use of this device is reported by Lemay *et al.* in 2011 (Lemay et al., 2011). In a long-term project that uses several animals, Charest *et al*. developed in 2009 a stereotactic frame for rat that allows constant and reproducible positioning using the same target coordinates (Charest et al., 2009). This device is a resin mold mimicking the rat body equipped with a silicon absorber on the sides to better fit the contour of the rat head. There is no metallic part in this frame, allowing MR imaging and radiographic imaging without interference. This frame was built to be used with clinical devices, such as the angiographic fiducial box, the bubble head measurement device and the APS. To test the accuracy of the coordinates of the focused radiation, the rat brain was removed and the intracranial cavity was washed and filled with a radiation sensitive polymer gel. After irradiation, the gel was removed, the polymerized area was measured and the coordinates were compiled to ensure the reproducible and accurate positioning of the rat head for GK radiosurgery. This project will be discussed in section 3 of this chapter, but briefly, our preclinical research effort focuses on the development or improvement of therapeutic modalities for malignant glioma. Our rat glioma model was extensively characterized and

showed that the implanted tumors always grow in the same position (Blanchard et al., 2006; Mathieu et al., 2007). Thus, after preliminary testing which showed reproducible and accurate positioning, we were able to use the same coordinates of irradiation for each animal with implanted tumor, bypassing the time-consuming step of imaging and planning. This frame was used for radioprotection (Belzile et al., 2009) and radiosensitizing (Charest et al., 2011; Charest et al., Article in preparation) experiments, and investigations on tumor invasion in irradiated brain are in progress. We recently upgraded our GK unit to the Perfexion model, and the reproducible and accurate positioning of our frame in this new model of GK was confirmed by intracranial polymer gel irradiation, ionic chamber measurement and Gafchromic film irradiation. Our rat frame fitting in the frame holder of the GK Perfexion is shown in figure 1. The department of Nuclear Medicine and Radiobiology of the University of Sherbrooke also conducts experiment using a mouse model. The mouse model is used for research on colorectal cancer, breast cancer and metastasis (details in section 3). As for rat glioblastoma experiments, the irradiation coordinates with mice are recurrent, focusing on the posterior thigh or the anterior mammary gland of the mouse. The stereotactic frame was built as a sarcophagus, to prevent movement of the animal (figure 2). Because some of these protocols require fractionated irradiation and because mice do not tolerate successive anaesthesias by i.v. injection, an isoflurane delivery system was also introduced in the design of this device. As with the rat frame, the mouse frame was tested for reproducible and accurate positioning, confirmed by ionic chamber measurements and Gafchromic film irradiations.

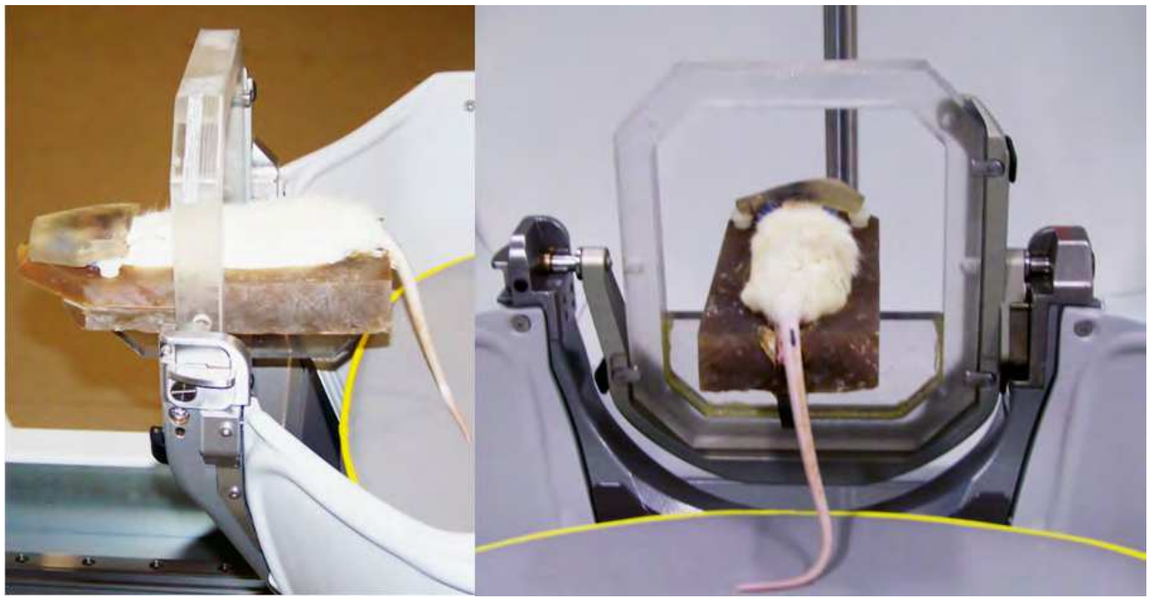


Fig. 1. Rat stereotactic frame held by the frame adaptator of the Gamma Knife Perfexion.

In this section, we described different devices that use various methods of positioning in the GK, from a simple visual confirmation of the target through the collimators, atlas-guided protocol, recurrent positioning and precise planning using MR or radiography imaging. In addition, some devices were not described clearly in the literature and we should assume that the efficiency is good enough to do GK radiosurgery. The design of these devices comes from the need of precise small animal stereotactic radiosurgery for different kind of researches that are reported in the next section.

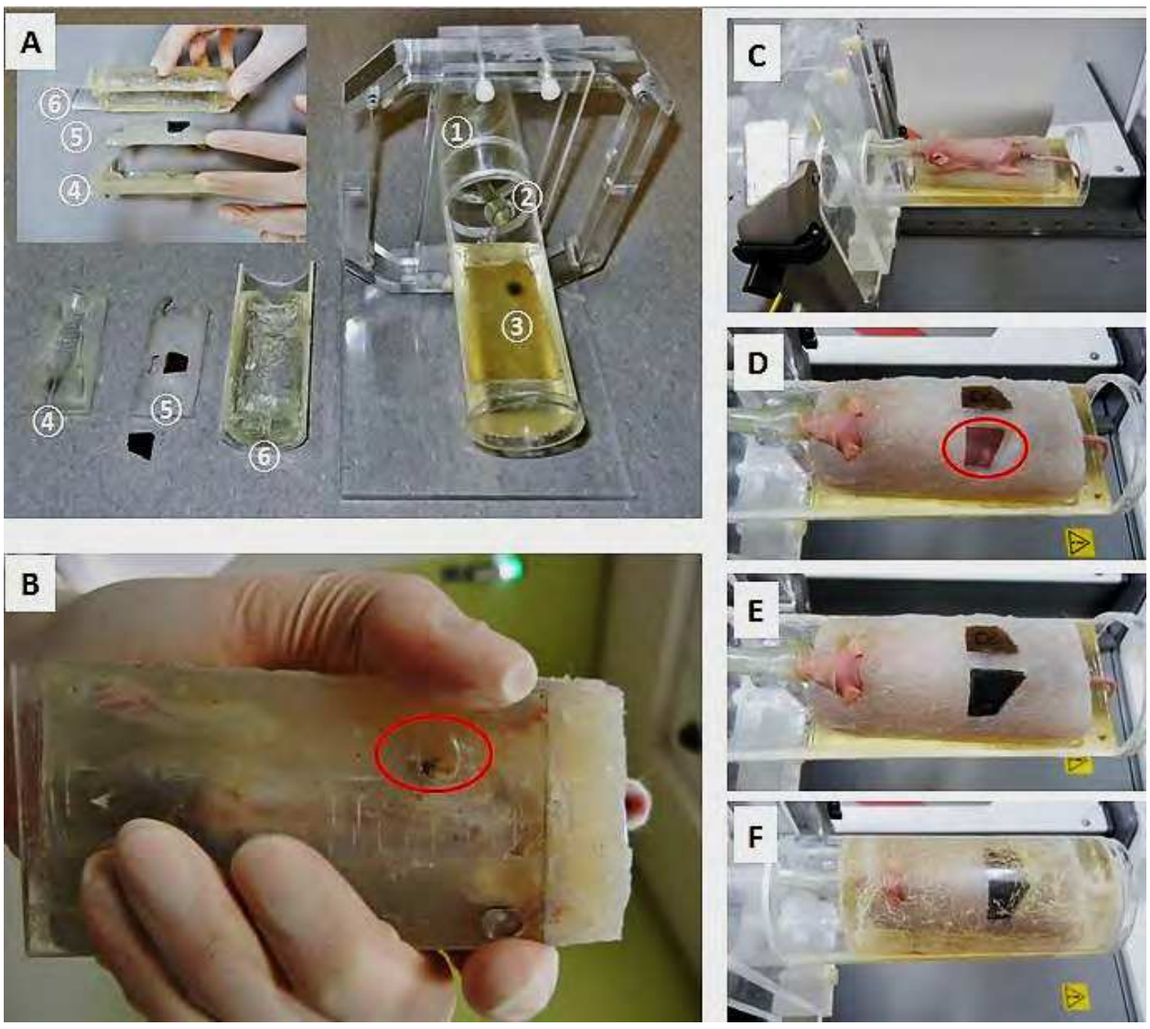


Fig. 2. Mouse sarcophagus stereotactic device.
A) Disassembled device. The numbers correspond to the following parts; 1: Compatible APS frame holding the tubular mouse platform, 2: Tubing for isoflurane delivery, 3: Bolus skin-equivalent layer, 4: lower part of mouse mold, 5: upper part of mouse mold with windows allowing fine positioning, 6:Top of the outer frame. B) Positioning window for mammary gland (red circle) under the lower part of the mouse mold (4). C) Nu/Nu nude mouse bearing colorectal cancer positioned on parts # 1, 2, 3, 4. D) Part #5 with open window for fine positioning of the subcutaneous cancer implanted into the thigh. E) Positioning windows closed with bolus skin-equivalent pieces. F) The sarcophagus is completed with part #6 and the mouse is ready for GKRS.

3. Experimental investigations using gamma knife technique with small animals

Five decades ago, Lars Leksell invented the Gamma Knife as a non-invasive method of delivering high-dose radiation to destroy discrete anatomical regions within the brain while minimizing the radiation effect on the surrounding tissues. Since 1991, over half a million

patients had radiosurgery using the Gamma Knife to treat malignant and benign tumors, vascular, functional and ocular disorders (ELEKTA, 2011). On the other hand, in the last 20 years, around fifty publications reporting small animal radiosurgery by GK were found in the literature (Table 2). These *in vivo* GK radiosurgery (GKRS) experiments were designed to evaluate the dose-response relationship and temporal effect of GKRS in normal and pathologic brain tissue. For more clarity, this section about experimental *in vivo* investigation is divided in the following subsections: healthy brain matter, functional disorders, target outside the brain and cancer.

3.1 Response of healthy brain to focused radiation

Radiation treatments affect all cells. When tumors or other brain pathologies are targeted by GKRS, inevitably some surrounding normal healthy cells are also irradiated in the dose fall-off volume around the target. The most basic irradiated brain tissue experiments were conducted to correlated the position planning of high dose of radiation with a necrotic area into the rat brain visualized by pathological observation (Chen et al., 2001; Kamiryo et al., 1993; Regis et al., 1996; Rey et al., 1996; Tokumaru et al., 2005a) or by MRi (Kamiryo et al., 1995; Kamiryo et al., 1996). The evolution of the histological changes after GKRS were also monitored in function of time (1-60 days) for the targeted volume and surrounding brain tissue (2 mm) (Kondziolka et al., 1992a). It was shown that at 90 days, no histological changes were observed for a dose lower than 70 Gy. At 70 Gy, shrunken neurons were observed, and vascular changes were reported to occur after a radiation dose between 80 and 100 Gy. A dose of 150 to 200 Gy generated necrosis and cavitation of the targeted area whereas the surrounding area demonstrated reactive astrocytosis, edema, microhemorrhage and thickened vessels. The same group has shown that the edema and vasculopathy caused by a high dose of radiation (100 Gy) can be prevented with high doses of a radio protective agent (Kondziolka et al., 1997). Subsequently, edema and metabolite changes were analyzed in the early stage (months) after irradiation. Researches on the levels of n-acethylaspartate (NAA), creatin and choline compounds (Cr/Cho) and lactate (Lac) did not detect any changes four weeks after GKRS by using ^{1}H magnetic resonance spectroscopy (MRS), but edema was observed using MRI (Omary et al., 1995). Another group using the same technique has shown that at 8 months after GKRS an increase of Lac and Cho was seen, whereas the level of Cr and NAA decreased (Herynek et al., 2004). These changes in metabolite levels were accompanied by edema.

A study conducted on expression of the proto-oncogene c-fos showed that a specific stress response following GKRS appears with two peaks (12-24h and at one month) in the target region but also in the surrounding forebrain regions (Duan et al., 1999a). The same group found that there are three types of Fos-immunoreactive cells induced after GKRS (Duan et al., 1999b). GKRS also upregulates the N-methyl-D-aspatate receptor (NMDARs) subunits NR1 and NR2A, which might represent a possible explanation for the therapeutic effect of GKRS on many neurological diseases because of the crucial roles of NMDARs in synaptic transmission, plasticity and neurodegeneration (Liang et al., 2008). The heat shock proteins (HSPs) are a group of stress proteins whose synthesis of some can be inducted and are suggested to play a role in neuron protection. The group of Rao has shown that from 3 hours to 30 days after GKRS, the expression of HSP70 changed in function of time and according to the cell types (Rao et al., 2000). This animal model was useful to perform assays

First author	Location	Publication year	Objective	Target	Doses (Gy)
Response of healthy brain matter to focused radiation					
D. Kondziolka	Pittsburgh, USA	1992a	Dose-response relationship and temporal effect of focused single dose irradiation of the normal rat brain.	Rat Brain: Right frontal lobe	30, 40, 50, 60, 70, 80, 100, 150, 200
D. Kondziolka	Pittsburgh, USA	1997	Evaluation of a radioprotector on brain tissue.	Rat Brain: Frontal lobe	50, 100, 150
T. Kamiryo	Charlottesville, USA	1993 1995 1996	Technical note of new stereotactic device: Evaluation of geometric and dosimetric inaccuracies during GKRS. The device is built to accept a brain cutting system and an open stereotactic surgery system. In 1995 a new MRI device were developed to fit the same frame.	Rat Brain: Right parietal cortex	4, 200, 300
R.A. Omary	Charlottesville, USA	1995	Study on blood-brain barrier breakdown caused by GKRS.	Rat Brain: Frontoparietal cortex	120
J. Régis	Marseille, France	1996	Effect on GKRS for epilepsy on biochemical differential functional effects for glutamate decarboxylase and choline acetyltransferase, excitatory amino acids (AAs) and non-excitatory AAs and gamma-aminobutyric acid.	Rat Brain: Left striatum.	50, 200
Z-F, Chen	Charlottesville, USA	2001	Anticonvulsant effect of GK for epilepsy.	Rat Brain: Bilateral irradiation of the ventral hippocampal formation.	10, 20, 40
D. A. Vincent	Plymouth, UK	2005	Evaluation of the effect on body weight after GKRS.	Obese Zucker rat brain: hypothalamus	40
M. Rey	Marseille France	1996	Development of a stereotactic device for rat for use with GK. (Régis-Vallicciоni model).	Rat Brain: Left striatum.	100, 200
X.Q. Duan	Xi'an and Guangzhou, China	1999a 1999b	Expression and change of Fos protein after GK irradiation.	Rat Brain: Caudate putamen, whole left forebrain.	100

Table 2. Radio-biological experiments with small animals.

Table 2. (Continued)

First author	Location	Publication year	Objective	Target	Doses (Gy)
Z-R, Rao	Xi'an, China	2000	Expression and changes of HSP70 after GK irradiation and survival.	Rat Brain: Right caudate putamen nucleus.	100
V. Herynek	Prague, Czech Republic	2004	Metabolite (Lac, Cho, Cr, NAA) and diffusion coefficient after GK irradiation.	Rat Brain: Bilateral irradiation of the hippocampus.	35
D. Jirak	Usti nad Labem, Czech Republic	2007	Lesion evolution after GKRS observed by MRI.	Rat Brain: Whole hippocampus	25, 50, 75
N. Kouyama	Tokyo, Japan	2003	Survey of functional alteration in the rat striatum after GK irradiation. Preliminary results.	Rat Brain: Unilateral striatum.	150
O. Tokumaru	Tokyo, Japan	2005a 2007	Survey of functional alteration in the rat striatum after GK irradiation.	Rat Brain: Left striatum.	150
M. Hirano	Tokyo, Japan	2009	Transcriptomic analysis of rat brain after GKRS.	Rat Brain: Striatum	60
C-d, Liang.	Shijiazhuang, China	2008	Effect of radiation on the expression of NMDAR subunits (NR1, NR2A, NR2B).	Rat Brain: Center at caudate putamen, whole left forebrain.	60
R. Liscak	Prague, Czech Republic	2002	Evaluation of changes in behavior (memory, orientation) and structural damage after GKRS.	Rat Brain: Bilateral irradiation of the hippocampus.	25, 50, 75, 100, 150
Epilepsy & Parkinson					
J. Régis	Marseille, France	1996	Effect on GKRS for epilepsy on biochemical differential functional effects for glutamate decarboxylase and choline acetyltransferase, excitatory amino acids (AAs) and non-excitatory AAs and gamma-aminobutyric acid.	Rat Brain: Left striatum.	50, 200
Y. Mori	Pittsburgh, USA	2000	Effect of GKRS on animal model of hippocampal epilepsy.	Rat Brain: hippocampus	20, 40, 60, 100
F. Bartolomei	Marseille, France	1998	Effect of GKRS on rat brain sodium channel subunit mRNA expression.	Rat Brain: Left dentate gyrus and upper thalamic region	100
Z-F, Chen	Charlottesville, USA	2001	Anticonvulsant effect of GK for epilepsy.	Rat Brain: Bilateral irradiation of the ventral hippocampal formation.	10, 20, 40

First author	Location	Publication year	Objective	Target	Doses (Gy)
Outside the brain matter					
C. DesRosiers	Indianapolis, USA	2003	Technical note about rat platform for GKRS and study of eye lens irradiation.	Rat eye: right eye lens of phantom and living rat	5, 10, 15
J. R. Dynlacht	Indianapolis, USA	2006 2008	Evaluation of estrogen as a radioprotector against cataractogenesis.	Rat right eye	10, 15
R.A. Omary	Charlottesville, USA	1995	Study on blood-brain barrier breakdown caused by GKRS.	Rat Brain: Frontoparietal cortex	120
S. Takahashi	Sapporo, Japan	1996	Study of relaxation and contraction responses on common carotid artery following GKRS.	Rat common carotid artery	100
O. Major	Budapest, Hungary	2006	Modulation of blood vessel obliteration after GKRS and combination of paclitaxel.	Rat common carotid artery	15, 20, 25, 50, 80, 200
B. Tinnel	Indianapolis, USA	2007	Evaluation of lung toxicity after GKRS in rat.	Rat right bronchus, GAF-chromic film	20, 40, 80
M. Pellerin	Sherbrooke, Canada	2006	Monitoring micro-vasculature damage of breast carcinoma induced by GKRS.	Mice: breast cancer implanted in left hind limb	40
M. Belzile	Sherbrooke, Canada	2009	Octreotide can be considered for prevention of radiation-induced salivary gland damage.	Rat parotid glands	30
CANCER **Brain cancer**					
D. Kondziolka	Pittsburgh, USA	1992b	Evaluation of tumoricidal effect of focused single dose irradiation of the rat C6 glioma model.	Rat Brain: Tumor in the right frontal lobe	30, 40, 50, 70, 100
D. Kondziolka	Pittsburgh, USA	1996	Evaluation of different techniques for irradiation of the rat C6 glioma model.	Rat Brain: Tumor in the right frontal lobe and WBRT	20, 35, 85
A. Niranjan	Pittsburgh, USA	2000	Treatment of mice glioblastoma by combination of TNF-alpha and HSV-tk gene transfer and GKRS.	Hsd nu/nu mice brain: Tumor in the right frontal lobe	21.4
A. Niranjan	Pittsburgh, USA	2003	Treatment of rat gliosarcoma by combination of HSV-based multigene therapy and GKRS.	Rat Brain: Tumor in the right frontal lobe	21.4

Table 2. (Continued)

Table 2. (Continued)

First author	Location	Publication year	Objective	Target	Doses (Gy)
Y-S, Im	Seoul, Korea	2006	Technical note: Stereotactic device for rats for GK model B and C. Testing for accuracy with phantom and normal and C6-glioma-inoculated rats.	Film. Ionization chamber	150
J-I, Lee	Kyoto, Japan	2006	Combination of GKRS and antiangiogenic agent in treatment of glioma.	Rat right frontal lobe: Tumor volume	5, 10, 20, 40, 80, 160
D. Xu	Tianjin, China	2006	GKRS on C6 glioma in combination with adenoviral p53.	Rat right frontal lobe: Tumor volume	15
Y. Li	Tianjin, China	2010	Combination therapy with GKRS and antisense EGFR for malignant glioma.	Rat right frontal lobe: Tumor volume	15
Q. Jia	Tianjin, China	2010	Radio sensitivity enhanced by RNA Ku70 for glioma treatment prior to GKRS.	Rat right frontal lobe: Tumor volume	15
H-T. Chung	Goyang, Korea	2008	Development of a stereotactic device for rat for use with GK.	Phantom, ion chamber, radiochromic films, glioma bearing rat	30
G. Desmarrais	Sherbrooke, Canada	submitted	Ongoing research on glioma (invasion, migration).	Rat brain	
G. Charest	Sherbrooke, Canada	2011 ongoing	Ongoing research on glioma (chemo-radio therapy).	Rat right frontal lobe: Tumor volume	15
Breast cancer					
R. Lemay	Sherbrooke, Canada	2011	Study of invasiveness of mammary cancer cells after irradiation.	Mice: thigh prior to cancer cells implantation.	30
G. Bouchard	Sherbrooke, Canada	ongoing	Ongoing research on breast cancer.	Mice: mammary gland	
Colorectal cancer					
T. Tippayamontri	Sherbrooke, Canada	ongoing	Ongoing research on colorectal cancer (radio-chemo therapy).	Mice: thigh implanted with colorectal tumor cells	15

at protein and metabolite level in brain tissue after GKRS. In fact, this field is probably just at its infancy. A recent study with rats (Hirano et al., 2009) showed that only 16 hours after a dose of 60 Gy, there are 230 induced and 144 repressed genes in the irradiated striatum and 432 induced and 239 repressed genes in the contralateral unirradiated striatum.

Interestingly, the biochemical changes of the brain following GKRS can be explored using behavioral experiments with the apomorphine test. Apomorphine is a dopamine agonist that stimulates dopamine receptors. After injection of apomorphine, a temporal increase of physical activity for about 30 minutes is observed. When the striatum is unilaterally irradiated, the dopaminergic function is then impaired on the irradiated site resulting in unbalanced dopaminergic activity between left and right striatum that results in a circling behavior (Kouyama et al., 2003; Tokumaru et al., 2005a; Tokumaru et al., 2007b). These studies emphasized the extreme precision of the GK and described how GKRS to the striatum can affect the behavior of small animals. The striatum is one site of dopaminergic function and the hippocampus is implicated in memory. In fact, irradiation of the rat hippocampus has been shown to affect memory (measured with the Morris water maze test) and the dose is correlated in function of time and size to the occurrence of edema following GKRS (Jirak et al., 2007a; Liscak et al., 2002). Finally, a pilot study of 40 Gy irradiation of the hypothalamus of obese Zucker rats produced an effect on weight control comparable to drug therapy with a metalloporphyrin. The authors hypothesized that this GKRS treatment leads to a resetting of the hypothalamic set point for body weight (Vincent et al., 2005).

In summary, small animal experiments have demonstrated that the effects of GK radiation onto the healthy brain tissue can appear a few hours to a few months after irradiation. The changes in the brain are observed not only at the dose deposition area but can be detected in the non-irradiated contralateral part of the brain. These changes were monitored by histological essays, external imaging and behavioral tests.

3.2 Focused radiation for epilepsy and Parkinson disease

We denoted only four studies using GKRS treatment for epilepsy in small animals. In 1996, Régis et al. showed that the biochemical effect on brain tissue obtained by GKRS could translate into a therapeutic effect for epilepsy. These biochemical changes were observed for glutamate decarboxylase, choline acetyl transferase, excitatory amino acids and non-excitatory amino acids as γ-aminobutyric acid (Regis et al., 1996) but no modification was observed in mRNA expression for the sodium channel subunit II and III up to 60 days following 100 Gy to the left dental gyrus and thalamus (Bartolomei et al., 1998). Thereafter GKRS was used in an epilepsy rat experiments. An animal model of hippocampal epileptic rat was produced by injection of kainic acid into the rat hippocampus and confirmed by electroencephalography (EEG). Different animal groups received doses of 20, 40, 60 or 100 Gy to the hippocampus and a significant dose-dependent reduction in the frequency of observed and EEG-defined seizures was reported (Mori et al., 2000). Another epileptic rat model was developed by repetitive electrical stimulations of the hippocampus in rats. The ventral hippocampus was irradiated and it is reported that a single dose of 20 or 40 Gy, but not 10 Gy, reduced substantially or eliminated the behavioral and EEG recognized seizures (Chen et al., 2001). These models of epileptic rats have improved our understanding of the fundamental mechanisms in epilepsy treatment by GKRS.

Parkinson's disease is another functional disorder for which GKRS experiments were performed. Only one rodent model of hemi-Parkinson was found in the literature. This animal model was developed by injection of 6-hydroxy-dopamine (6-OHDA) and the effect of unilateral dopaminergic loss was confirmed. The unilateral 6-OHDA lesion on rats showed ipsilateral rotation with the apomorphine test. A highly statistical reduction of apomorphine-induced rotation was observed at 2, 3 and 4 months after administration of 140 Gy to the striatum of these hemi-parkinsonian animals. The authors concluded that the focused radiation is potentially capable of inducing regeneration of dopaminergic pathway in the adult CNS.

3.3 Focused radiation targeted outside the brain

Few researches on irradiated organs and tissues located outside the brain using GKRS were reported. Eye cataractogenesis is a complication observed in patient receiving total-body irradiation prior to bone marrow transplantation, head and neck radiotherapy and for astronauts that receive low dose of densely ionizing space radiation. To study the effect of radiation on the eye, a research group at Indiana University (Indiana, IN) irradiated a single rat eye and lens using GKRS. They reported that the GK was precise enough to create a cataract in the irradiated eye while keeping the contralateral eye intact (DesRosiers et al., 2003). Later, they have shown that the cataractogenesis of the irradiated eye can be modulated with estrogen. When estrogen is administrated one week before GKRS and continuously thereafter, the incidence of cataractogenesis is increased (Dynlacht et al., 2006). On the other hand, when estrogen is administrated continuously, but starting only after irradiation, a decrease of incidence of cataractogenese is observed (Dynlacht et al., 2008). These researches have important implication for the management of astronauts and patient receiving radiotherapy.

The effect of ionizing radiation on vascular tissue was also studied. In 1996, a study on the relaxation and contraction response of the carotid artery after GKRS was conducted. The authors mentioned that the irradiated carotid artery had a reduction in the vasoconstriction response induced by norepinephrine, endothelin-1 and phorbol dibuthyrate. This impairment is biphasic and peaks one day and one month after irradiation. This phenomenon is apparently caused by alterations of both the vascular endothelial and smooth muscle cells (Takahashi et al., 1996). Other researchers experimented on the reaction of the middle cerebral artery to GKRS. Briefly, two groups of animal were studied: animals that received only irradiation and animals that received paclitaxel prior to GKRS. The authors reported that the constriction responses were decreased in the paclitaxel treated group and that complete recovery was faster for the paclitaxel group (12 months compared to 18 month). It appears that paclitaxel causes acceleration in the time course of the late vascular effect of GKRS (Major et al., 2006).

Another group has studied the influence of the volume of the dose deposition on bronchus integrity. In their experiment, the mainstream bronchus was irradiated with 4 mm or 8 mm collimators. It was found that the bronchus well tolerated small volume (4 mm) of very high dose of radiotherapy but a bigger volume (8 mm) encompassing the surrounding support stroma and normal tissue produced cellular atypia, interstitial pneumonitis, bronchial and vascular damages (Tinnel et al., 2007). Finally, Belzile et al. showed that administration of octreotide, a drug used in acromegaly and other types of digestive pathologies, prior to

GKRS acted as a radioprotective agent on rat parotid glands one month after irradiation (Belzile et al., 2009). Octreotide can then be considered for prevention of radiation-induced salivary gland damages. Other GKRS experiments outside the brain matter are included in the subchapter concerning cancer research.

3.4 Gamma knife for cancer research

Radiation for cancer therapy is widely used for many types of cancer. Brain cancer radiotherapy is certainly the main subject of research for GKRS. The emphasis on brain cancer research is easy to understand considering the clinical use of the Gamma Knife that is mainly limited to the head. However, with the new model PERFEXION, head and neck target are theoretically achievable and clinical research may eventually expand to these areas. However, in research with small animals, any regions of the animal can be targeted. This subchapter will discuss some animal models to study brain cancer but also models of breast and colorectal cancer.

3.4.1 Breast cancer

It has been reported that radiation treatment can enhance the invasiveness of many types of cancer by increasing the expression of matrix metalloproteinase type-2 (Ohuchida et al., 2004; Qian et al., 2002; Speake et al., 2005; Wang et al., 2000; Wild-Bode et al., 2001). It was also reported that the metastatic frequency was significantly higher in tumors implanted in pre-irradiated beds (Milas et al., 1988; Rofstad et al., 2005). The research group of Dr. Paquette of Sherbrooke University has studied whether irradiation of normal tissues could increase the invasiveness of breast cancer cells in a mouse model. They concluded that the implantation of non-irradiated mammary cancer cells in previously irradiated normal tissue enhanced the invasive capacity of the mammary cancer cells (Lemay et al., 2011). Another hypothesis from the same group is that there is an activation of transforming growth factor beta (TGF-β) from healthy tissues by radiation. Recent studies reported that the antiproliferative effect of TGF-β accompanied the stimulation of cancer cell migration. This phenomenon can lead to radioresistance since radiation mainly target dividing cells. These facts led them to believe that an inhibitor of TGF-β could reduce radio-induced invasion and promote a synergistic effect with radiotherapy treatments. Ongoing experiments are in progress to determine if an inhibitor of TGF-β maturation (chloroquin) can prevent radiation-enhancement of cancer cell invasion in a mouse model (Bouchard et al., Ongoing research).

3.4.2 Colorectal cancer

At the University of Sherbrooke, a study about the combination of platinum compounds and radiation to treat colorectal cancer in animal model is in progress. Nu/Nu nude mice implanted with colorectal cell cancer (HCT 116) are treated by intravenous injection of different platinum compounds prior to GKRS (see GKRS set up in figure 2). The investigators hope to show a potential synergistic effect of the combination of this chemo-radiotherapy (Tippayamontri et al., Ongoing research). One goal is to determine the optimal chemo-radiotherapy schedule to treat colorectal cancer in regards to pharmacokinetic properties of platinum-base drugs to obtain the best synergistic effects, by varying the time delays between administration of the drug and GKRS.

3.4.3 Brain cancer

A group of the University of Pittsburgh conducted a series of experiments involving small animals as models to study the treatment of brain cancer by GKRS. In 1992 Kondziolka et al. used the rat C6 glioma model to evaluate the potential role of GKRS for treatment of glial neoplasms. With a single fraction of focused irradiation to the tumor volume, they showed that the glioma bearing animals treated by GK had a longer mean survival compared to the control animals (39.2 days compared to 29.4 days respectively). At sacrifice, the mean tumor volume was smaller for the radiosurgery group (6.47 mm) compared to the control group (9.64 mm) and the tumors of the radiosurgery group showed significant hypocellular appearance and cellular edema (Kondziolka et al., 1992b). Later, using the same animal model, they tried different irradiation schemes resulting in a median survival time increasing as follows: control < single-fraction of 35 Gy hemibrain < whole brain radiation therapy (WBRT) 20 Gy in 5 fractions < radiosurgery to the margin of the tumor 35 Gy = radiosurgery to the margin of the tumor 35 Gy + WBRT 20 Gy in 5 fractions < fractioned hemibrain radiotherapy of 85 Gy in 10 fractions. They also reported edema after radiosurgery to the margin of the tumor 35 Gy and for combined treatment of radiosurgery to the margin of the tumor 35 Gy + WBRT 20 Gy in 5 fractions but not after fractionated radiotherapy or single-fraction of 35 Gy hemibrain (Kondziolka et al., 1996). After these studies about the effects of ionizing radiation on the C6 glioma model, the Pittsburgh research group published two articles about the combination of radiation and gene therapy. They used athymic nude mice in whom they implanted in the brain the U87MG human glioblastoma cell line. The mice were treated with different combinations of GKRS, herpes simplex virus thymidine kinase suicide gene therapy (SGT), tumor necrosis factor alpha (TNF-alpha) and ganciclovir (GCV). Compared to the median surviving time of the control animals (21 days), the combination therapies increased the median life span of the treated animal as follows: GKRS+TNF-alpha+GCV= 46 days < SGT+TNF-alpha+GCV= 60 days < GKRS+SGT+TNF-alpha+GCV = 75 days. The combination of conventional therapeutic methods and gene therapy was considered a promising treatment to improve the survival time of patients afflicted with glioblastoma (Niranjan et al., 2000). This group also reported the use of multigene therapy using the model of immunocompetent rats bearing 9L gliosarcoma. For these experiments, they used a new vector called NUREL-C2 that co-express TNF-alpha + gap-junction-forming protein connexin43 (Cx) + infected cell protein zero (ICP0) + viral thymidine kinase (HSV-tk) (Niranjan et al., 2003). The median survival time of GCV plus combined therapy and/or GKRS increases as follows: SGT+TNF-alpha = 39 days < SGT+TNF-alpha+Cx = 68 days < SGT+TNF-alpha+GKRS = 80 days < SGT+TNF-alpha+Cx+GKRS = 150 days. The results of this investigation on multigene therapy with NUREL-C2 have shown that this vector could be estimated as an efficient prototype vector in clinical trial in patient with recurrent malignant glioblastoma.

More recently, a group from Tianjin, of the Republic of China, also used gene therapy in two publications followed by lipofection of antisense therapy in a rat model bearing C6 glioma. Their first article mentioned the use of adenoviral therapy for expression of p53, this protein playing a role in improving radiosensitivity, cell cycle arrest and apoptosis. Their different therapies resulted in an increase of life survival from control < p53 < GKRS < p53 + GKRS (Xu et al., 2006). Their second study using lipofection tested the antisense EGFR (As-EGFR). EGFR the epidermal growth factor receptor is known to be associated with radioresistance of glioma and increased proliferation. Once again, the effects of their different therapies have shown an increase in survival from control < GKRS < As-EGFR < As-EGFR+GKRS (Li

et al., 2010). The same group from Tianjin published in 2010 a study using a recombinant adenovirus for the inhibition of Ku70 which play an important role in DNA double strands breaks repair. As in their other publications, they showed an increase in survival from control < inhibited Ku70 < GKRS < Inhibited Ku70+GKRS (Jia et al., 2010). Combination therapy with drugs and radiation was also reported by researchers from Kyoto, Japan. In their study, the authors combined GKRS and the antiangiogenic agent thalidomide (THD) or the chemotherapeutic agent temozolomide (TMZ). No surviving essay was reported in their article but they mentioned a significant decrease in tumor volume in rats bearing C6/LacZ glioma treated with the combination of GKRS and THD. Two other different groups (Chung et al., 2008; Im et al., 2006) published a new stereotactic frame for GKRS in small animals bearing a glioma tumor in their brain. Both groups mentioned that the biological data will be discussed in another articles in preparation.

To end this chapter on brain tumor experiments, we will talk about ongoing research in our department at the University of Sherbrooke. Radiotherapy remains one of the most effective treatment for glioblastoma (GBM), resulting in at least transient disease control in most patients. Since cancer cells always infiltrate adjacent normal brain, the target volume irradiated is 2-3 cm larger than the tumour volume detected by current imaging tools. Unfortunately, the radiation dose is not intended to restrain all cancer cells scattered in the brain, but is rather aimed at optimizing the number of cancer cells eliminated with minimal adverse effects. Therefore, the tumour frequently recurs in the brain volume previously irradiated. This observation raises the following question: Can the migration and proliferation capacity of cancer cells which are not eliminated by radiotherapy (or other modalities) be influenced by the surrounding microenvironment? To answer part of this question, we irradiated healthy brain tissue prior to tumour implantation (F98/Fischer rat glioma model) to observe the characteristics of the tumour development into an irradiated tissue, and how this might differ compared to normal non-irradiated tissue. Immunogenic reaction to radiation is an important aspect dictating the tumour growth and consequently altering the migration abilities of tumour cells. This investigation will allow us to have a better understanding of the tumour development and, hopefully, to translate this knowledge into an improved survival for GBM patients (Desmarrais et al., Submitted). Another study by our group is devoted to the potential synergistic effects of the combination of platinum compounds and GKRS. Platinum compounds were chosen because they are already approved for clinical use and are widely available. Zheng et al. demonstrated that the efficiency of low energy electrons produced by ionizing radiation to induce DNA strand breaks is significantly increased in presence of cisplatin (Zheng et al., 2008). Different routes of drug administration were evaluated in our study (Charest et al., Article in preparation). Five different platinum compounds were administrated by intravenous injection, intraarterial injection (carotid artery), or intraarterial injection after osmotic blood-brain barrier disruption. The drug treatments were also tested in combination with 15 Gy of radiation delivered by the Gamma Knife to the tumor volume. Surviving essays and drug uptake measurements were done. The biological and radiobiological data will be discussed in another article in preparation.

4. Conclusion

We reported here the development of our stereotactic devices for rat and mouse irradiation with the Leksell Gamma Knife models 4C and PERFEXION. Sixteen different stereotactic

devices for small animal irradiation by GK, and about fifty *in vivo* experiments were reviewed. This relatively small amount of *in vivo* experiments seems paradoxal compared to the half a million patients that received radiosurgery using the Gamma Knife. Nevertheless, the *in vivo* articles reported here are important because they are often the first publications in specific fields and have the potential to lead to significant clinical breakthroughs. In all these published articles, the GK was reported to be accurate and reliable for small animal irradiation experiments.

5. References

Bartolomei, F., Massacrier, A., Rey, M., Viale, M., Regis, J., Gastaldi, M & Cau, P. (1998). Effect of gamma knife radiosurgery on rat brain sodium channel subunit mRNA expression. *Stereotact Funct Neurosurg,* Vol. 70 Suppl 1, Oct, pp. 237-242, ISSN/ISBN: 1011-6125; 1011-6125.

Belzile, M., St-Amant, M., Mathieu, D., Doueik, AA., Fortier, PH & Dorion, D. (2009). Radiation-induced xerostomia: is octreotide the solution? *J Otolaryngol Head Neck Surg,* Vol. 38, No. 5, Oct, pp. 545-551, ISSN/ISBN: 1916-0216.

Blanchard, J., Mathieu, D., Patenaude, Y & Fortin, D. (2006). MR-pathological comparison in F98-Fischer glioma model using a human gantry. *Can J Neurol Sci,* Vol. 33, No. 1, Feb, pp. 86-91, ISSN/ISBN: 0317-1671.

Bouchard, G., Bujold, R., Saucier, C & Paquette, B. (Ongoing research). Ongoing research.

Charest, G., Mathieu, D., Lepage, M., Fortin, D., Paquette, B & Sanche, L. (2009). Polymer gel in rat skull to assess the accuracy of a new rat stereotactic device for use with the Gamma Knife. *Acta Neurochir (Wien),* Apr 18, ISSN/ISBN: 0942-0940.

Charest, G., Sanche, L., Fortin, D., Mathieu, D & Paquette, B. (2011). Glioblastoma treatment: Bypassing the toxicity of platinum compounds by using liposomal formulation and increasing treatment efficiency with concomitant radiotherapy. *Int J Radiat Oncol Biol Phys*, Accepted.

Chen, ZF., Kamiryo, T., Henson, SL., Yamamoto, H., Bertram, EH., Schottler, F., Patel, F., Steiner, L., Prasad, D., Kassell, NF., Shareghis, S & Lee, KS. (2001). Anticonvulsant effects of gamma surgery in a model of chronic spontaneous limbic epilepsy in rats. *J Neurosurg,* Vol. 94, No. 2, Feb, pp. 270-280, ISSN/ISBN: 0022-3085; 0022-3085.

Chung, HT., Chung, YS., Kim, DG., Paek, SH & Cho, KT. (2008). Development of a stereotactic device for gamma knife irradiation of small animals. *J Korean Neurosurg Soc,* Vol. 43, No. 1, Jan, pp. 26-30, ISSN/ISBN: 2005-3711; 1225-8245.

Desmarrais, G., Bujold, R., Fortin, D., Mathieu, D & Paquette, B. (Submitted).

DesRosiers, C., Mendonca, MS., Tyree, C., Moskvin, V., Bank, M., Massaro, L., Bigsby, RM., Caperall-Grant, A., Valluri, S., Dynlacht, JR & Timmerman, R. (2003). Use of the Leksell Gamma Knife for localized small field lens irradiation in rodents. *Technol Cancer Res Treat,* Vol. 2, No. 5, Oct, pp. 449-454, ISSN/ISBN: 1533-0346.

Duan, XQ., Wu, HX., Liu, HL., Rao, ZR & Ju, G. (1999a). Expression and changes of Fos protein in the rat forebrain after gamma knife irradiation targeted to the caudate putamen. *Neurosurgery,* Vol. 45, No. 1, Jul, pp. 139-45; discussion 145-6, ISSN/ISBN: 0148-396X; 0148-396X.

Duan, XQ., Wu, SL., Li, T., Liang, JC., Qiou, JY., Rao, ZR & Ju, G. (1999b). Expression and significance of three types of Fos-immunoreactive cells after gamma knife

irradiation of the forebrain in the rat. *Neurosci Res,* Vol. 33, No. 2, Feb, pp. 99-104, ISSN/ISBN: 0168-0102; 0168-0102.

Dynlacht, JR., Tyree, C., Valluri, S., DesRosiers, C., Caperell-Grant, A., Mendonca, MS., Timmerman, R & Bigsby, RM. (2006). Effect of estrogen on radiation-induced cataractogenesis. *Radiat Res,* Vol. 165, No. 1, Jan, pp. 9-15, ISSN/ISBN: 0033-7587; 0033-7587.

Dynlacht, JR., Valluri, S., Lopez, J., Greer, F., Desrosiers, C., Caperell-Grant, A., Mendonca, MS & Bigsby, RM. (2008). Estrogen protects against radiation-induced cataractogenesis. *Radiat Res,* Vol. 170, No. 6, Dec, pp. 758-764, ISSN/ISBN: 0033-7587; 0033-7587.

ELEKTA. *In: Gamma knife treatment stats,* Date of access: 2011, Available from: http://www.elekta.com/assets/Elekta-Neuroscience/Gamma-Knife-Surgery/pdfs/Gamma-Knife-Treatment-Stastics-2009.pdf.

Herynek, V., Burian, M., Jirak, D., Liscak, R., Namestkova, K., Hajek, M & Sykova, E. (2004). Metabolite and diffusion changes in the rat brain after Leksell Gamma Knife irradiation. *Magn Reson Med,* Vol. 52, No. 2, Aug, pp. 397-402, ISSN/ISBN: 0740-3194.

Hirano, M., Shibato, J., Rakwal, R., Kouyama, N., Katayama, Y., Hayashi, M & Masuo, Y. (2009). Transcriptomic analysis of rat brain tissue following gamma knife surgery: early and distinct bilateral effects in the un-irradiated striatum. *Mol Cells,* Vol. 27, No. 2, Feb 28, pp. 263-268, ISSN/ISBN: 1016-8478; 1016-8478.

Im, YS., Nam, DH., Kim, JS., Ju, SG., Lim, DH & Lee, JI. (2006). Stereotactic device for Gamma Knife radiosurgery in experimental animals: technical note. *Stereotact Funct Neurosurg,* Vol. 84, No. 2-3, pp. 97-102, ISSN/ISBN: 1011-6125.

Jia, Q., Li, Y., Xu, D., Li, Z., Zhang, Z., Zhang, Y., Liu, D., Liu, X., Pu, P & Kang, C. (2010). Radiosensitivity of glioma to Gamma Knife treatment enhanced in vitro and in vivo by RNA interfering Ku70 that is mediated by a recombinant adenovirus. *J Neurosurg,* Vol. 113 Suppl, Dec, pp. 228-235, ISSN/ISBN: 1933-0693; 0022-3085.

Jirak, D., Namestkova, K., Herynek, V., Liscak, R., Vymazal, J., Mares, V., Sykova, E & Hajek, M. (2007a). Lesion evolution after gamma knife irradiation observed by magnetic resonance imaging. *Int J Radiat Biol,* Vol. 83, No. 4, Apr, pp. 237-244, ISSN/ISBN: 0955-3002.

Jirak, D., Namestkova, K., Herynek, V., Liscak, R., Vymazal, J., Mares, V., Sykova, E & Hajek, M. (2007b). Lesion evolution after gamma knife irradiation observed by magnetic resonance imaging. *Int J Radiat Biol,* Vol. 83, No. 4, Apr, pp. 237-244, ISSN/ISBN: 0955-3002; 0955-3002.

Kamiryo, T., Berk, HW., Lee, KS., Kassell, NF & Steiner, L. (1993). A stereotactic device for experimental gamma knife radiosurgery in rats. A technical note. *Acta Neurochir (Wien),* Vol. 125, No. 1-4, pp. 156-160, ISSN/ISBN: 0001-6268.

Kamiryo, T., Berr, SS., Berk, HW., Lee, KS., Kassell, NF & Steiner, L. (1996). Accuracy of an experimental stereotactic system for MRI-based gamma knife irradiation in the rat. *Acta Neurochir (Wien),* Vol. 138, No. 9, pp. 1103-7; discussion 1107-8, ISSN/ISBN: 0001-6268.

Kamiryo, T., Berr, SS., Lee, KS., Kassell, NF & Steiner, L. (1995). Enhanced magnetic resonance imaging of the rat brain using a stereotactic device with a small head

coil: technical note. *Acta Neurochir (Wien),* Vol. 133, No. 1-2, pp. 87-92, ISSN/ISBN: 0001-6268; 0001-6268.

Kamiryo, T., Lopes, MB., Berr, SS., Lee, KS., Kassell, NF & Steiner, L. (1996). Occlusion of the anterior cerebral artery after Gamma Knife irradiation in a rat. *Acta Neurochir (Wien),* Vol. 138, No. 8, pp. 983-90; discussion 990-1, ISSN/ISBN: 0001-6268.

Kondziolka, D., Couce, M., Niranjan, A., Maesawa, S & Fellows, W. (2002). Histology of the 100-Gy Thalamotomy in the Baboon. *Radiosurgery.Basel, Karger,* Vol. 4, pp. 279-284, .

Kondziolka, D., Lacomis, D., Niranjan, A., Mori, Y., Maesawa, S., Fellows, W & Lunsford, LD. (2000). Histological effects of trigeminal nerve radiosurgery in a primate model: implications for trigeminal neuralgia radiosurgery. *Neurosurgery,* Vol. 46, No. 4, Apr, pp. 971-6; discussion 976-7, ISSN/ISBN: 0148-396X; 0148-396X.

Kondziolka, D., Lunsford, LD., Claassen, D., Maitz, AH & Flickinger, JC. (1992a). Radiobiology of radiosurgery: Part I. The normal rat brain model. *Neurosurgery,* Vol. 31, No. 2, Aug, pp. 271-279, ISSN/ISBN: 0148-396X; 0148-396X.

Kondziolka, D., Lunsford, LD., Claassen, D., Pandalai, S., Maitz, AH & Flickinger, JC. (1992b). Radiobiology of radiosurgery: Part II. The rat C6 glioma model. *Neurosurgery,* Vol. 31, No. 2, Aug, pp. 280-7; discussion 287-8, ISSN/ISBN: 0148-396X; 0148-396X.

Kondziolka, D., Somaza, S., Comey, C., Lunsford, LD., Claassen, D., Pandalai, S., Maitz, A & Flickinger, JC. (1996). Radiosurgery and fractionated radiation therapy: comparison of different techniques in an in vivo rat glioma model. *J Neurosurg,* Vol. 84, No. 6, Jun, pp. 1033-1038, ISSN/ISBN: 0022-3085; 0022-3085.

Kondziolka, D., Somaza, S., Martinez, AJ., Jacobsohn, J., Maitz, A., Lunsford, LD & Flickinger, JC. (1997). Radioprotective effects of the 21-aminosteroid U-74389G for stereotactic radiosurgery. *Neurosurgery,* Vol. 41, No. 1, Jul, pp. 203-208, ISSN/ISBN: 0148-396X; 0148-396X.

Kouyama, N., Katayama, Y., Hayashi, M., Tomida, M., Tokumaru, O & Kawakami, Y. (2003). On the survey of fonctional alterations in the rat striatum after gamma-knife irradiation. *Neuroscience Research,* Vol. 46 (Suppl 1), pp. S111, .

Lee, JI., Itasaka, S., Kim, JT & Nam, DH. (2006). Antiangiogenic agent, thalidomide increases the antitumor effect of single high dose irradiation (gamma knife radiosurgery) in the rat orthotopic glioma model. *Oncol Rep,* Vol. 15, No. 5, May, pp. 1163-1168, ISSN/ISBN: 1021-335X.

Lemay, R., Archambault, M., Tremblay, L., Bujold, R., Lepage, M & Paquette, B. (2011). Irradiation of normal mouse tissue increases the invasiveness of mammary cancer cells. *Int J Radiat Biol,* Jan 13, ISSN/ISBN: 1362-3095; 0955-3002.

Li, Y., Jia, Q., Zhang, J., Han, L., Xu, D., Zhang, A., Zhang, Y., Zhang, Z., Pu, P & Kang, C. (2010). Combination therapy with Gamma Knife radiosurgery and antisense EGFR for malignant glioma in vitro and orthotopic xenografts. *Oncol Rep,* Vol. 23, No. 6, Jun, pp. 1585-1591, ISSN/ISBN: 1791-2431; 1021-335X.

Liang, CD., Li, WL., Liu, N., Yin, Y., Hao, J & Zhao, WQ. (2008). Effects of gamma knife irradiation on the expression of NMDA receptor subunits in rat forebrain. *Neurosci Lett,* Vol. 439, No. 3, Jul 18, pp. 250-255, ISSN/ISBN: 0304-3940; 0304-3940.

Liscak, R., Vladyka, V., Novotny, J,Jr., Brozek, G., Namestkova, K., Mares, V., Herynek, V., Jirak, D., Hajek, M & Sykova, E. (2002). Leksell gamma knife lesioning of the rat

hippocampus: the relationship between radiation dose and functional and structural damage. *J Neurosurg,* Vol. 97, No. 5 Suppl, Dec, pp. 666-673, ISSN/ISBN: 0022-3085.

Lunsford, LD., Altschuler, EM., Flickinger, JC., Wu, A & Martinez, AJ. (1990). In vivo biological effects of stereotactic radiosurgery: a primate model. *Neurosurgery,* Vol. 27, No. 3, Sep, pp. 373-382, ISSN/ISBN: 0148-396X; 0148-396X.

Major, O., Walton, L., Goodden, J., Radatz, M., Szeifert, GT., Hanzely, Z., Kocsis, B., Nagy, Z & Kemeny, A. (2006). Radiosurgery of isolated cerebral vessels following administration of paclitaxel in the rat. *J Neurosurg,* Vol. 105 Suppl, Dec, pp. 214-221, ISSN/ISBN: 0022-3085; 0022-3085.

Mathieu, D., Lecomte, R., Tsanaclis, AM., Larouche, A & Fortin, D. (2007). Standardization and detailed characterization of the syngeneic Fischer/F98 glioma model. *Can J Neurol Sci,* Vol. 34, No. 3, Aug, pp. 296-306, ISSN/ISBN: 0317-1671.

Milas, L., Hirata, H., Hunter, N & Peters, LJ. (1988). Effect of radiation-induced injury of tumor bed stroma on metastatic spread of murine sarcomas and carcinomas. *Cancer Res,* Vol. 48, No. 8, Apr 15, pp. 2116-2120, ISSN/ISBN: 0008-5472; 0008-5472.

Mori, Y., Kondziolka, D., Balzer, J., Fellows, W., Flickinger, JC., Lunsford, LD & Thulborn, KR. (2000). Effects of stereotactic radiosurgery on an animal model of hippocampal epilepsy. *Neurosurgery,* Vol. 46, No. 1, Jan, pp. 157-65; discussion 165-8, ISSN/ISBN: 0148-396X; 0148-396X.

Nilsson, A., Wennerstrand, J., Leksell, D & Backlund, EO. (1978). Stereotactic gamma irradiation of basilar artery in cat. Preliminary experiences. *Acta Radiol Oncol Radiat Phys Biol,* Vol. 17, No. 2, pp. 150-160, ISSN/ISBN: 0348-5196; 0348-5196.

Niranjan, A., Moriuchi, S., Lunsford, LD., Kondziolka, D., Flickinger, JC., Fellows, W., Rajendiran, S., Tamura, M., Cohen, JB & Glorioso, JC. (2000). Effective treatment of experimental glioblastoma by HSV vector-mediated TNF alpha and HSV-tk gene transfer in combination with radiosurgery and ganciclovir administration. *Mol Ther,* Vol. 2, No. 2, Aug, pp. 114-120, ISSN/ISBN: 1525-0016; 1525-0016.

Niranjan, A., Wolfe, D., Tamura, M., Soares, MK., Krisky, DM., Lunsford, LD., Li, S., Fellows-Mayle, W., DeLuca, NA., Cohen, JB & Glorioso, JC. (2003). Treatment of rat gliosarcoma brain tumors by HSV-based multigene therapy combined with radiosurgery. *Mol Ther,* Vol. 8, No. 4, Oct, pp. 530-542, ISSN/ISBN: 1525-0016; 1525-0016.

Novotny, J,Jr., Novotny, J., Spevacek, V., Dvorak, P., Cechak, T., Liscak, R., Brozek, G., Tintera, J & Vymazal, J. (2002a). Application of polymer gel dosimetry in gamma knife radiosurgery. *J Neurosurg,* Vol. 97, No. 5 Suppl, Dec, pp. 556-562, ISSN/ISBN: 0022-3085.

Novotny, J,Jr., Novotny, J., Spevacek, V., Dvorak, P., Cechak, T., Liscak, R., Brozek, G., Tintera, J & Vymazal, J. (2002b). Evaluation of geometric and dosimetric inaccuracies of stereotactic irradiation in the rat brain. *Stereotact Funct Neurosurg,* Vol. 79, No. 2, pp. 57-74, ISSN/ISBN: 1011-6125.

Ohuchida, K., Mizumoto, K., Murakami, M., Qian, LW., Sato, N., Nagai, E., Matsumoto, K., Nakamura, T & Tanaka, M. (2004). Radiation to stromal fibroblasts increases invasiveness of pancreatic cancer cells through tumor-stromal interactions. *Cancer Res,* Vol. 64, No. 9, May 1, pp. 3215-3222, ISSN/ISBN: 0008-5472; 0008-5472.

Omary, RA., Berr, SS., Kamiryo, T., Lanzino, G., Kassell, NF., Lee, KS., Lopes, MB & Hillman, BJ. (1995). 1995 AUR Memorial Award. Gamma knife irradiation-induced changes in the normal rat brain studied with 1H magnetic resonance spectroscopy and imaging. *Acad Radiol,* Vol. 2, No. 12, Dec, pp. 1043-1051, ISSN/ISBN: 1076-6332; 1076-6332.

Paxinos G, Watson C. 1986. The rat brain in stereotaxic coordinates.

Pellerin, M., Tremblay, L., Paquette, B & Lepage, M. (2006). Monitoring micro-vasculature damage induced by radiotherapy on mouse breast carcinomas using DCE-MRI. *Proc Intl Soc Mag Reson Med,* Vol. 14, pp. 2910, .

Precision X-Ray. Date of access: 2011, Available from: http://www.pxinc.com/.

Qian, LW., Mizumoto, K., Urashima, T., Nagai, E., Maehara, N., Sato, N., Nakajima, M & Tanaka, M. (2002). Radiation-induced increase in invasive potential of human pancreatic cancer cells and its blockade by a matrix metalloproteinase inhibitor, CGS27023. *Clin Cancer Res,* Vol. 8, No. 4, Apr, pp. 1223-1227, ISSN/ISBN: 1078-0432; 1078-0432.

Rad Source Technologies. Date of access: 2011, Available from: http://www.radsource.com/.

Rao, ZR., Ge, X., Qiou, JY., Yang, T., Duan, L & Ju, G. (2000). Expression and changes of HSP70 in the rat forebrain subjected to gamma knife (100Gy) irradiation targeted on the caudate putamen and survived for different times. *Neurosci Res,* Vol. 38, No. 2, Oct, pp. 139-146, ISSN/ISBN: 0168-0102; 0168-0102.

Rao, ZR., Li, T., Wu, HX., Qiou, JY., Wu, SL., Tang, T., Huang, SH & Ju, G. (1998). Expression and changes of glial fibrillary acidic protein (GFAP) in normal rat brain irradiated with gamma knife. *Chines J Minim Invasive Neurosurg,* Vol. 3, No. 2, 1998, pp. 106-111.

Regis, J., Kerkerian-Legoff, L., Rey, M., Vial, M., Porcheron, D., Nieoullon, A & Peragut, JC. (1996). First biochemical evidence of differential functional effects following Gamma Knife surgery. *Stereotact Funct Neurosurg,* Vol. 66 Suppl 1, pp. 29-38, ISSN/ISBN: 1011-6125; 1011-6125.

Rey, M., Valliccioni, PA., Vial, M., Porcheron, D., Regis, J., Kerlerian-LE, GL., Nieoullon, A., Millet, Y & Peragut, JC. (1996). Radiochirurgie expérimentale chez le rat par Gamma knife : Description d'un cadre stéréotaxique pour le petit animal de laboratoire. Commentaire = Description of a stereotactic device for experimental gamma knife radiosurgery in rats. Commentary. *Neuro-chirurgie,* Vol. 42, No. 6, pp. 289-293, .

Rofstad, EK., Mathiesen, B., Henriksen, K., Kindem, K & Galappathi, K. (2005). The tumor bed effect: increased metastatic dissemination from hypoxia-induced up-regulation of metastasis-promoting gene products. *Cancer Res,* Vol. 65, No. 6, Mar 15, pp. 2387-2396, ISSN/ISBN: 0008-5472; 0008-5472.

Speake, WJ., Dean, RA., Kumar, A., Morris, TM., Scholefield, JH & Watson, SA. (2005). Radiation induced MMP expression from rectal cancer is short lived but contributes to in vitro invasion. *Eur J Surg Oncol,* Vol. 31, No. 8, Oct, pp. 869-874, ISSN/ISBN: 0748-7983; 0748-7983.

Takahashi, S., Toshima, M., Fukuoka, S., Seo, Y., Suematsu, K., Nakamura, J & Nagashima, K. (1996). Effect of gamma knife irradiation on relaxation and contraction responses

of the common carotid artery in the rat. *Acta Neurochir (Wien),* Vol. 138, No. 8, pp. 992-1001, ISSN/ISBN: 0001-6268; 0001-6268.

Theratronics. Date of access: 2011, Available from: http://www.theratronics.ca/product_gamma.html.

Tinnel, B., Mendonca, MS., Henderson, M., Cummings, O., Chin-Sinex, H., Timmerman, R & McGarry, RC. (2007). Pulmonary hilar stereotactic body radiation therapy in the rat. *Technol Cancer Res Treat,* Vol. 6, No. 5, Oct, pp. 425-431, ISSN/ISBN: 1533-0346; 1533-0338.

Tippayamontri, T., Kotb, R., Paquette, B & Sanche, L. (Ongoing research).

Tokumaru, O., Hayashi, M., Katayama, Y., Tomida, M., Kawakami, Y & Kouyama, N. (2007a). Gamma knife radiosurgery targeting protocols for the experiments with small animals. *Stereotact Funct Neurosurg,* Vol. 85, No. 4, pp. 135-143, ISSN/ISBN: 1011-6125.

Tokumaru, O., Hayashi, M., Katayama, Y., Tomida, M., Kawakami, Y & Kouyama, N. (2007b). Gamma knife radiosurgery targeting protocols for the experiments with small animals. *Stereotact Funct Neurosurg,* Vol. 85, No. 4, pp. 135-143, ISSN/ISBN: 1011-6125.

Tokumaru, O., Tomida, M., Katayama, Y., Hayashi, M., Kawakami, Y & Kouyama, N. (2005a). The effect of gamma knife irradiation on functions of striatum in rats. *J Neurosurg,* Vol. 102 Suppl, Jan, pp. 42-48, ISSN/ISBN: 0022-3085.

Tokumaru, O., Tomida, M., Katayama, Y., Hayashi, M., Kawakami, Y & Kouyama, N. (2005b). The effect of gamma knife irradiation on functions of striatum in rats. *J Neurosurg,* Vol. 102 Suppl, Jan, pp. 42-48, ISSN/ISBN: 0022-3085.

Vincent, DA., Alden, TD., Kamiryo, T., Lopez, B., Ellegala, D., Laurent, JJ., Butler, M., Vance, ML & Laws Jr, ER. (2005). The baromodulatory effect of gamma knife irradiation of the hypothalamus in the obese Zucker rat. *Stereotact Funct Neurosurg,* Vol. 83, No. 1, pp. 6-11, ISSN/ISBN: 1011-6125; 1011-6125.

Wang, JL., Sun, Y & Wu, S. (2000). Gamma-irradiation induces matrix metalloproteinase II expression in a p53-dependent manner. *Mol Carcinog,* Vol. 27, No. 4, Apr, pp. 252-258, ISSN/ISBN: 0899-1987; 0899-1987.

Wiant, D., Atwood, TF., Olson, J., Papagikos, M., Forbes, ME., Riddle, DR & Bourland, JD. (2009). Gamma knife radiosurgery treatment planning for small animals using high-resolution 7T micro-magnetic resonance imaging. *Radiat Res,* Vol. 172, No. 5, Nov, pp. 625-631, ISSN/ISBN: 1938-5404; 0033-7587.

Wild-Bode, C., Weller, M., Rimner, A., Dichgans, J & Wick, W. (2001). Sublethal irradiation promotes migration and invasiveness of glioma cells: implications for radiotherapy of human glioblastoma. *Cancer Res,* Vol. 61, No. 6, Mar 15, pp. 2744-2750, ISSN/ISBN: 0008-5472; 0008-5472.

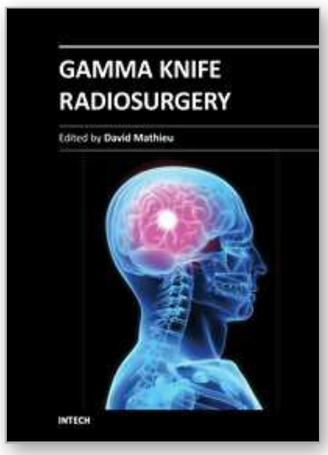
Xstrahl. Date of access: 2011, Available from: http://www.xstrahl.com/.

Xu, D., Jia, Q., Li, Y., Kang, C & Pu, P. (2006). Effects of Gamma Knife surgery on C6 glioma in combination with adenoviral p53 in vitro and in vivo. *J Neurosurg,* Vol. 105 Suppl, Dec, pp. 208-213, ISSN/ISBN: 0022-3085; 0022-3085.

Zerris, VA., Zheng, Z., Noren, G., Sungarian, A & Friehs, GM. (2002). Radiation and regeneration: behavioral improvement and GDNF expression after Gamma Knife

radiosurgery in the 6-OHDA rodent model of hemi-parkinsonism. *Acta Neurochir Suppl,* Vol. 84, pp. 99-105, ISSN/ISBN: 0065-1419; 0065-1419.

Zheng, Y., Hunting, DJ., Ayotte, P & Sanche, L. (2008). Role of secondary low-energy electrons in the concomitant chemoradiation therapy of cancer. *Phys Rev Lett,* Vol. 100, No. 19, May 16, pp. 198101, ISSN/ISBN: 0031-9007.



Gamma Knife Radiosurgery
Edited by Dr. David Mathieu

ISBN 978-953-307-888-5
Hard cover, 180 pages
Publisher InTech
Published online 16, December, 2011
Published in print edition December, 2011

Gamma knife radiosurgery is a minimally-invasive treatment alternative for intracranial disorders, including tumors, vascular malformations, facial pain and epilepsy. This book will allow the reader to learn when gamma knife radiosurgery is appropriate and what to expect as treatment results.

How to reference
In order to correctly reference this scholarly work, feel free to copy and paste the following:

Gabriel Charest, Benoit Paquette and David Mathieu (2011). Applications of Gamma Knife Radiosurgery for Experimental Investigations in Small Animal Models, Gamma Knife Radiosurgery, Dr. David Mathieu (Ed.), ISBN: 978-953-307-888-5, InTech, Available from: http://www.intechopen.com/books/gamma-knife-radiosurgery/applications-of-gamma-knife-radiosurgery-for-experimental-investigations-in-small-animal-models

INTECH
open science | open minds

InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

IntechOpen