Mid-Term Scientific Report – September 29, 2020

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Radiation Nanomedicine for Intraoperative Treatment of Glioblastoma Multiforme (GBM)

Lay Summary of Progress

This research project aims to develop a new radiation treatment for glioblastoma multiforme (GBM) called a radiation nanomedicine that could be infused at surgery into the brain to eradicate any tumour that the surgeon is unable to remove. The long-term goal is to reduce the risk for recurrence of GBM and thereby improve the long-term outcome of patients with this very poor prognosis form of brain tumour. In the first year of the project, we synthesised the radiation nanomedicine and studied its retention in human GBM tumours implanted into the brain in mice. The radiation nanomedicine was strongly retained in the brain tumour and did not re-distribute into the normal brain or to normal organs outside the brain. This is a very promising finding since it means that the radiation nanomedicine will deposit radiation only in the tumour and not in the normal brain or other normal organs, which should maximize its effectiveness for treatment of GBM, while minimizing any toxicity to the normal brain or normal organs. Indeed, we conducted a study in the past year to evaluate the normal organ toxicity of the radiation nanomedicine, which showed no evidence of toxicity against any normal organs. We still need to study if the radiation nanomedicine has any toxicity against the normal brain, but this is not expected due to the local retention in the tumour. In the second year of the project, we will study the effectiveness of the radiation nanomedicine for treating human GBM tumours in mice and examine the toxicity on normal brain. The project is on track. In addition, the support of the BTFC has allowed us to be successful in obtaining additional major funding from the Canadian Cancer Society as an Innovation Grant (2020-2022) to extend the idea to another radioisotope, mercury-197 that emits a different type of radiation (Auger electrons) and combine the radiation nanomedicine with immunotherapy for treatment of GBM

Brief Synopsis of the Project: This project will study if a locally infused radiation nanomedicine composed of 5 nm sized gold nanoparticles (AuNPs) labeled with ^{177}Lu with/without modification to target epidermal growth factor receptors (EGFR) overexpressed on glioblastoma multiforme (GBM) could be effective for intra-operative treatment of GBM. The approach will be studied in NRG mice stereotaxically implanted with human GBM tumours and infused intra-tumourally with the radiation nanomedicine.

Hypothesis and Aims: The hypothesis is that stereotactic intratumoural infusion of EGFR-targeted ^{177}Lu-AuNPs will be effective for treating human GBM tumours in mice and will not cause toxicity to normal organs or the normal brain. The aims are: 1. To measure the retention of the ^{177}Lu-AuNPs in the tumour and any re-distribution to normal organs or normal brain and estimate the tumour and normal organ radiation doses. 2. To study the effectiveness of the ^{177}Lu-AuNPs for treatment of GBM tumours and assess the normal organ toxicity including to the normal brain.

Progress on Aim 1. We have synthesized ^{177}Lu-AuNPs with/without modification with panitumumab to target EGFR overexpression on GBM cells (**Fig. 1**).

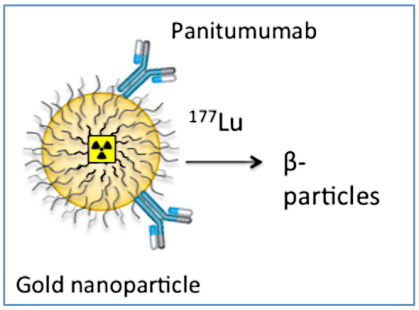


Fig. 1. 5 nm AuNPs were modified with a metal-chelating polymer that binds the β-particle emitter, ^{177}Lu with/without modification with panitumumab to target EGFR overexpressed on GBM cells. ^{177}Lu emits moderate energy β-particles (Eβ=0.5 MeV) for radiation treatment of GBM and γ-photons (Eγ = 208 keV) that can be used for SPECT imaging of the biodistribution of the radiation nanomedicine. The γ-photons also allow measurement of uptake of the radiation nanomedicine in GBM tumours, normal brain and other organs by γ-counting.

We compared the binding of non-targeted and EGFR-targeted ^{177}Lu-AuNPs to U-251 human GBM cells. There was increased cell binding for the EGFR-targeted ^{177}Lu-AuNPs (**Fig. 2**) which suggests that EGFR-targeting may be offer an advantage for uptake by GBM cells.

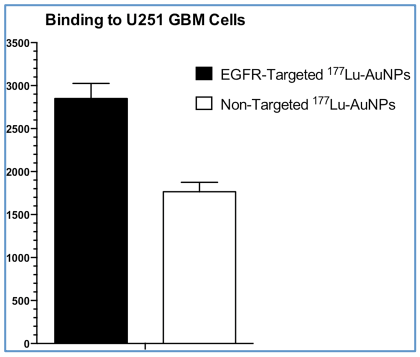


Fig. 2. Binding of EGFR-targeted vs. non-targeted ^{177}Lu-AuNPs to U251 GBM cells.

We have established a tumour model of GBM by stereotoxic inoculation of U251 cells into the brain of NRG mice. These cells were transfected with the luciferase gene which allows confirmation of establishment of the tumour by bioluminescence imaging (BLI) (**Fig. 3**). After the tumour was established (in 5-7 days) we stereotaxically infused the radiation nanomedicine into the tumour. MicroSPECT/CT imaging revealed that the radiation nanomedicine was retained in the tumour and did not re-distribute to other regions of the brain or to other normal organs outside the brain for up to 21 days (**Fig. 4**). In contrast, the ^{177}Lu-labeled metal-chelating polymer was rapidly eliminated from the brain within 3 days. Biodistribution studies were performed at 14 days post-infusion of the radiation nanomedicine into a mouse with a GBM tumour in the right side of the brain. These studies showed that there was >50-fold higher concentrations of ^{177}Lu in the right side of the brain where the tumour was located [150 percent injected dose/g (% ID/g)] than on the left side of the brain which was normal (<3% ID/g)] (**Fig. 5**). In addition, other normal organ uptake was very low (<1% ID/g). These results are very promising because they indicate that the AuNPs act as an "anchor" to retain ^{177}Lu in the tumour, and avoid re-destribution of ^{177}Lu to normal regions of the brain or to normal organs outside the brain. Combined with the short range of the β-particles emitted by ^{177}Lu (~2 mm), this should result in high radiation doses deposited in the tumour but very low doses deposited in normal brain or other normal organs. This may provide an effective radiation treatment for GBM that minimizes harm to normal brain or other normal organs. Based on the imaging and biodistribution data, we plan to estimate the radiation doses deposited in the tumour, normal brain and normal organs. We also plan to compare the tumour retention of EGFR-targeted vs. non-targeted ^{177}Lu-AuNPs.

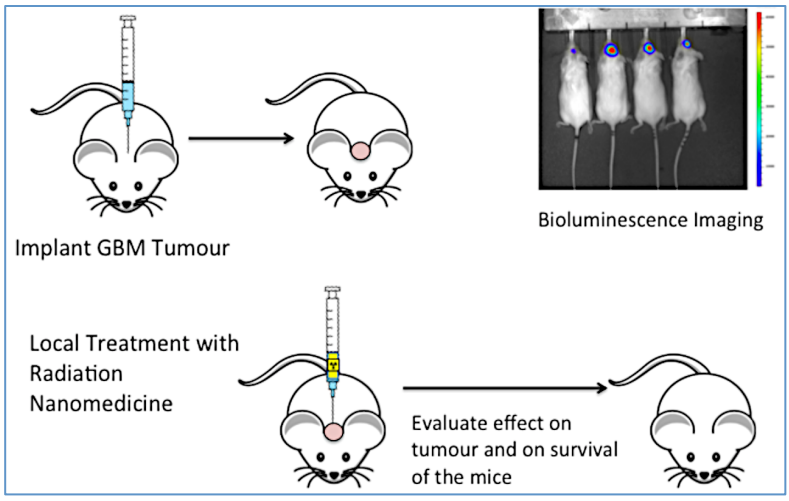
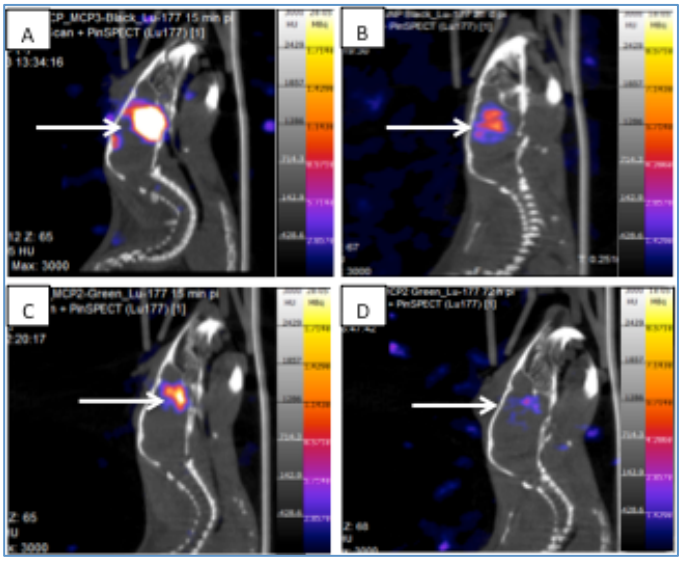


Fig. 3. Approach to establishing human GBM tumours in NRG mice by stereotaxic inoculation of U251 human GBM cells and local infusion of the radiation nanomedicine for treatment of these tumours. The tumour cells are transfected with the luciferase gene which enables confirmation of establishment of the GBM tumours by bioluminescence imaging (BLI). The radiation nanomedicine will also be infused stereotaxically into the tumour. The effect of treatment with the radiation nanomedicine will be evaluated by monitoring the tumour growth by BLI and the effect on the survival of the mice.

Fig. 4. MicroSPECT/CT images of an NRG mouse with a U251 human GBM tumour in the brain infused stereotaxically with the radiation nanomedicine (^{177}Lu-AuNPs; arrows) at 1 day (A) and 21 days (B) showing that the radiation nanomedicine is reained in the tumour. In contrast, the metal-chelating polymer labeled with ^{177}Lu (arrows) is eliminated from the brain after 3 days (D) compared to 1 day (C) after injection.



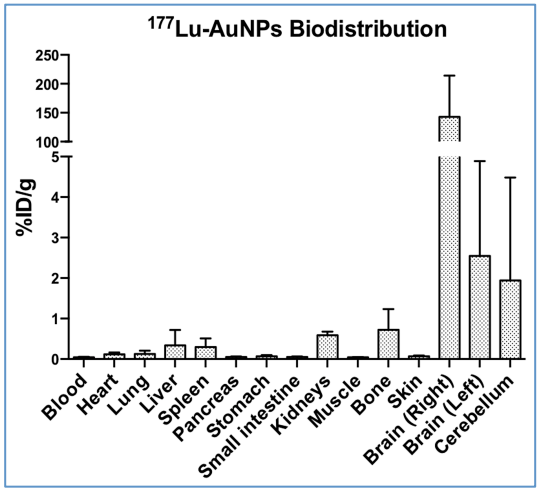
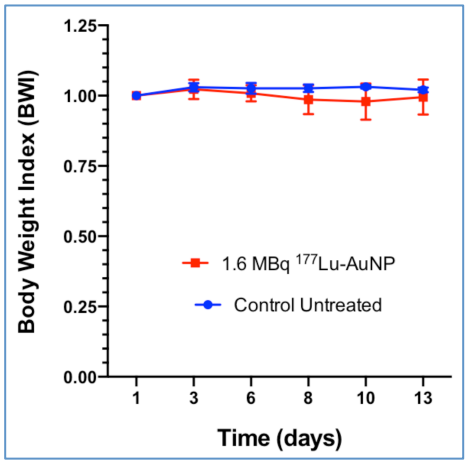


Fig. 5. Biodistribution of ^{177}Lu-AuNPs a 14 days after intratumoural infusion in NRG mice with U251 human GBM tumours in the right side of the brain. There is very high retention (>150% ID/g) in the right brain where the tumour is located but very low uptake (<3% ID/g) of the ^{177}Lu-AuNPs in the left brain or cerebellum which is normal. There was also very low uptake in normal organs outside the brain (<1% ID/g). These results indicate that the AuNPs provide an "anchor" to retain ^{177}Lu in the tumour which combined with the short range (2 mm) of the β-particles emitted by ^{177}Lu should deposit high radiation doses in the tumour while avoiding irradiating normal brain or other normal organs.

Progress on Aim 2. W have studied the toxicity of the radiation nanomedicine on normal organs after infusion of 1.6 MBq into the brain of NRG mice, which is expected to be the dose required for treatment of the GBM tumours. There was no significant decrease in the body weight of the mice compared to control untreated mice (**Fig. 6**) and there was no evidence of decreased blood cell counts or increased serum alanine aminotransferase (ALT) or creatinine (Cr) that could indicate toxicity to the liver or kidneys, respectively (**Table 1**). There were also no changes in other serum biochemistry. These results indicate that a dose of 1.6 MBq of ^{177}Lu-AuNPs is not toxic to normal organs in the mouse. We plan to extend these toxicity studies to evaluation of the toxicity of the radiation nanomedicine on normal brain by histological examination of the brain in tumour-bearing NRG mice after intratumoural infusion of the ^{177}Lu-AuNPs.



Our future plans are to study the effectiveness of treatment of U251 human GBM tumours in the brain of NRG mice following intratumoural infusion of 1.6 MBq of ^{177}Lu-AuNPs.

Fig. 6. Body weight of NRG mice over 14 days after infusion of 1.6 MBq of ^{177}Lu-AuNPs into the brain demonstrating no decreased body weight compared to control, untreated NRG mice.

Table 1. Toxicity Evaluation of the Radiation Nanomedicine in NRG Mice after Infusion into the Brain.

	Untreated Control Mice	Mice Infused with 1.6 MBq ^{177}Lu-AuNPs
ALT (U/L)	19.8±1.7	27.0±2.9
Cr (μmol/L)	18.3±0.5	18.0±0.0
GLU (mmol/L)	12.6±1.3	8.5±4.2
Total Protein (g/L)	47.3±2.6	45.0±2.1
WBC (10^9/L)	0.9±0.3	2.4±2.9
RBC (10^9/L)	8.7±0.3	8.6±0.3
Hemoglobin (g/dL)	13.1±0.6	12.1±1.4
PLT (10^9/L)	441.3±227.8	428.3±166.1
ALP (U/L)	61.0±6.4	55.8±6.3
BUN (mmol/L)	8.2±0.4	7.2±1.5
Hematocrit (%)	38.2±1.2	38.0±1.3