Secreted reporter proteins, a valuable complementary tool for non-invasive preclinical monitoring of brain tumour growth

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Malignant glioma and glioblastoma multiforme (GBM) are the most common and aggressive primary brain cancers with a very low dismal mean survival after initial diagnosis (1), emphasizing the need for novel treatment options. Several novel therapeutics have been identified for the treatment of malignant brain tumours, but despite strong preclinical data, very few successes have been achieved in clinical trials (2). The dichotomy between potential successful preclinical therapeutic agents and the ineffectiveness of the drug in patients underscores the need for better predictive preclinical models. The ideal model is a highly reproducible tumour model that mimics the key histopathological, genetic and imaging characteristics encountered in human GBMs. The most relevant models are isogenic orthotopic models, in which the complex micro-environmental interaction between glioma cells, the central nervous system and the immune system are present during tumour growth and treatment (3). The major challenge is to monitor the development of the orthotopic models in a non-invasive manner. A PubMed search (search terms: non-invasive, brain, orthotopic, mice) revealed that bioluminescence imaging (BLI, 39.5%) and magnetic resonance imaging (MRI, 21%) are the most commonly used imaging modalities for non-invasive monitoring of brain tumours. Fluorescence imaging and computed tomography (CT) account both for 16%, while positron emission tomography (PET, 5%) and ultrasound (US, 2.5%) are rarely used.

With interest we read the article of Alessandrini *et al*. "Noninvasive monitoring of glioma growth in the mouse" recently published in the *Journal of Cancer* (4), in which they evaluated secreted Gaussia luciferase (Gluc), as a reporter to monitor glioma tumour burden directly from the blood. Gluc is naturally secreted by cells, does not require ATP for its activity and despite its fast 'flash' kinetics the initial activity is 100–1,000 fold more sensitive compared to other luciferases. Although Gluc has been used as a tumour biomarker, correlations between Gluc expression levels in blood and tumour burden have been lacking so far. For immune incompetent Nod/Scid mice bearing the human L0306 glioma, exponential Gluc curves were derived which correlated at several time-points with the estimated tumour size determined after excision, irrespective of the tumour location. In contrast, in an immune competent Balb/c mice model bearing HCG (vIII) intracranial gliomas, there was no consistent relationship between Gluc in the blood and intracranial tumour growth attributed to the presence Gluc neutralizing antibodies in these animals. Whether there was an enhanced immune response against these isogenic tumours was not reported. Direct correlations of blood and tumour Gluc *in vivo* or *ex vivo* were not conducted which would be informative to address this point further and understand the benefits and limitations of Gluc as a reporter. Taken together the Gluc blood assay does appear suitable in immune competent animals bearing isogenic orthotopic tumours.

Non-invasive tumour growth monitoring using the most relevant model remains to be done with imaging modalities as described above. BLI is a cheap, fast and sensitive non-invasive imaging modality well suited for treatment response monitoring because it provides

functional information on tumour growth in a quantitative spatial and temporal manner. Anatomical information can be obtained using contrast-enhanced (CE)-CT, MRI or US from which MRI is probably the most commonly used modality. Small animal radiotherapy platforms, relying on CT information rather than MRI, are increasingly used in preclinical research (5). Therefore, several teams, including ours, have investigated the complementary use of CE-CT and BLI in context of radiotherapy combination treatments (6-10) and observed high correlations between both imaging modalities. CE-CT is suitable for longitudinal monitoring and accurate delineation of intracranial tumours, especially important when using patient-derived non-luciferase expressing xenograft models. However, repeated—daily or multiple times per week for several weeks—use of CE-CT should be avoided because of the cumulative radiation dose to healthy tissues (5,11). Both imaging modalities can complement each other using BLI to frequently monitor tumour progression and treatment response and CE-CT for tumour delineation essential for radiotherapy treatment planning and dose calculations (10,12-14).

The results from Alessandrini *et al*., indicate that secreted Gluc expands the toolbox for preclinical non-invasive imaging of tumours, however caution is warranted when using immune competent models. Gluc pre-immunization may potentially overcome some of these limitations. Furthermore, expanding these results to additional tumour models and comparing Gluc directly to BLI will shed light on the future applicability of secreted Gluc proteins as biomarkers for tumour growth and response.

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Footnote

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Dubois et al. Secreted luciferases as cancer biomarker

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