# SUMMARY

The convergence of molecular imaging with cell and molecular biology started around two decades ago and has since led to new insights in understanding biological processes and developing new diagnostic tools as well as novel therapeutics. Bioluminescence imaging, one of the numerous imaging modalities now available, has been extensively used in pre-clinical studies notably in cancer research. This naturally occurring phenomenon gleans its information from photons emitted upon oxidization of a substrate by its specific luciferase.

The luciferase from the marine copepod *Gaussia princeps* (Gluc) which we characterize in this thesis has several advantages over other commonly used luciferases: This small, ATP-independent luciferase is naturally secreted thus allowing live cell assays with high sensitivity. We have optimized this bioluminescent reporter and used it to study several biological processes as well as to develop novel therapeutics against glioblastoma, the most malignant form of primary brain tumors. Despite all efforts, treatment options for glioblastoma are still limited and the survival rate remains poor. We used a multi-disciplinary-bioluminescence imaging-approach to study cellular and physiological processes as well as genetic hallmarks related to cancer and applied it to develop efficient cancer gene therapy and to screen for new glioblastoma therapeutics.

In **Chapter 2**, we developed a new assay to study protein trafficking through the secretory pathway using Gluc natural properties. This highly sensitive assay allows monitoring of the endoplasmic reticulum (ER) stress, a common signature in many tumor types that helps support survival and promotes resistance of tumor cells to therapy. In **Chapters 3** and **4**, Gluc secretion was used for *in vivo* monitoring of biological applications. By measuring the level of secreted Gluc in blood or urine samples, it is possible to monitor several biological events such as cell growth and survival, tumor cells response to therapy as well as tracking the fate of small numbers of stem cells implanted in mice. This assay allows *ex vivo* monitoring of such events and complements *in vivo* bioluminescence imaging using the CCD-camera. In **Chapter 5**, Gluc was used as a reporter to directly monitor gene activity, in particular NFkB, a transcription factor constitutively active in several tumor types. An optimized expression cassette with tandem repeats of NFkB DNA-binding sites combined to a minimal promoter was engineered to drive the expression of Gluc. This assay proved useful in studying the kinetics of NFkB activation and inhibition in cultured tumor cells as well as in animal models. In addition to being sensitive and versatile for NFkB monitoring, this reporter is amenable for high-throughput screening of NFkB inhibitors, highly desirable for cancer treatment.

The tools described above were ultimately used in order to develop new glioblastoma therapeutics. In **Chapter 6**, we used the NFkB construct to drive the expression of a toxic gene along with Gluc. Since NFkB is constitutively active in tumors but not in normal tissue, we sought to design a tumor specific-NFkB-driven suicidal gene therapy approach. A combination of cytosine deaminase and uracil phosphoribosyltransferase (CD-UPRT) as well as Gluc under the control of the NFkB "promoter system" was shown to be effective in killing glioblastoma as well as other tumor cell lines. In addition to the therapeutic effect, monitoring NFkB activation and therefore transgene expression was achieved using the secreted Gluc in the conditioned media in cultured cells and in blood of mice *in vivo*. In **Chapter 7**, we developed a cell-based bioluminescent screening assay to identify small molecules with anti-glioma effect as well as TRAIL sensitizers. Our lead hit was a cardiac glycoside, lanatoside C, which sensitized glioblastoma cell lines as well as primary glioblastomas to TRAIL induced-apoptosis. Interestingly, lanatoside C on its own showed toxicity towards glioblastomas at higher concentrations through a non-apoptotic, necrotic cell death mechanism. This combined cell death therapy -which would induce an apoptotic and necrotic cell death- could be of benefit towards apoptosis-resistant tumor types such as glioblastomas.

The work presented herein provides new opportunities to help investigate glioblastoma's behavior and to identify novel therapeutics. It illustrates the use of bioluminescent tools commonly available combined with common cell and molecular biology methods to cast light on multifaceted and unwieldy pathologies such as cancer and help with drug discovery and development at the pre-clinical level laying the grounds for potential clinical trials.