Mitochondria in a personalized cancer treatment approach

Valorization addendum

In this thesis, the scientific relevance of modulation of mitochondrial DNA and the role of mitochondria in the context of cancer treatment has been demonstrated. Scientific knowledge and findings should eventually benefit the society in one or another way. Worldwide, cancer is now-a-days one of the leading causes of death. In 2015, 104988 new patients were diagnosed with cancer in the Netherlands [1]. Next to the emotional burden of such a disease also the financial aspect plays an important role for patients and society. In the Netherlands, cancer treatment costs were already 4,8 billion euro in 2011 [2] and are rising every year. Currently, in our society a debate is ongoing on health and insurance policy in order to control the costs of cancer treatments combined with an improvement of survival rates and quality of life [3]. As not every patient is the same and there is not yet a perfect cancer therapy, stratification of patients is an important tool to select only the patients who will benefit the most from a certain (combination) treatment in order to reduce costs.

Clinical relevance and improvement for health care

As tumors are highly dynamic and heterogeneous in their appearance, treatment should be tailored to the specific needs of a patient; the so-called personalized treatment approach. Currently cancer researchers try to find better biomarkers to predict the patients' outcome to a specific treatment or to use as target for treatment. Mitochondrial function (chapter 2, 3 and 4), CAIX (chapter 4 and chapter 5), hypoxia (chapter 5 and 6) and GST expression (chapter 8) could serve as such biomarkers. Complementary to the use of specific drugs to eliminate cancer, possible normal tissue toxicity plays an important role in defining the optimal treatment approach. In lung cancer patients for example, the healthy tissue tolerance defines the radiation dose delivered to the tumor. Therefore, it is important to develop methods that can identify patients at risk for e.g. radiation-induced lung toxicity (RILT). Radiation therapy (RT) and mitochondria have common molecular processes. Where mitochondria are important for energy (ATP) and reactive oxygen species (ROS) production, regulation of apoptosis and cellular calcium homeostasis, RT induces the formation of ROS and can lead to cell death. Recently, it has been shown that ionizing radiation can elevate mitochondrial content and function. Furthermore, mitochondrial DNA (mtDNA) mutations have previously been reported to affect mitochondrial function (decreased ATP production, increased ROS production). As described in chapter 2 and 3, evaluation of the mitochondrial status and their functionality could result in a biomarker that helps us to identify new therapeutic targets, potentially limiting normal tissue toxicity or increasing tumor specific efficacy and provide us with better working therapies at the same time although further progress is necessary. Potentially this could not only work for radiotherapy as treatment modality, but could also be expanded to other treatments

like immunotherapy or chemotherapy and thereby contribute to a more optimal treatment of patients.

CAIX has been found to influence disease progression and is associated with a higher risk to develop metastasis. As CAIX is seen as an endogenous hypoxia marker since its expression is often HIF-1α regulated, identification of hypoxic areas could be beneficial on several distinctive levels. Different approaches could be pursued to increase the effectiveness of treatments by manipulating the tumoral hypoxic fraction. Drugs such as nitroglycerin, metformin or phenformin are suggested to reduce the hypoxic fraction within tumors. The CAIX inhibitors described in chapter 5 were shown not to be effective as single agents or didn't result in an enhanced efficiency compared to their parental compounds and to standard treatment modalities. Therefore, additional studies were not pursued. The combination of another biguanide metformin with radiotherapy is currently tested in clinical trials and recently mitochondrial targeted derivatives of metformin have been developed. Targeting a biguanide to mitochondria and making it more effective and consequently reduce side effects remains an interesting research area. These newly designed compounds are believed to be more effective than metformin itself and could therefore contribute to a beneficial therapy. Although these types of compounds require further extensive preclinical and clinical testing, the first results are promising. However, until they are proven more effective and equally safe as the parental drug, metformin would currently be the way to go forward.

In chapter 6, we indeed observed a decreased hypoxic fraction upon nitroglycerin administration in patients harboring hypoxic tumors. Since hypoxia causes resistance to radiotherapy, reducing the hypoxic fraction in these treated patients is of great importance. As metformin and nitroglycerin are FDA approved drugs, we know the possible side effects when patients are treated with these drugs. Therefore, these agents could be used relatively safe for repurposing. Of course, the effect on the tumor and its microenvironment still need to be carefully elucidated in patients when combined with conventional treatment modalities; so adverse tumor effects could be prevented. Since nitroglycerin reduced the hypoxic fraction only in patients with hypoxic tumors, assessing if a tumor is hypoxic is essential. Multiple hypoxia tracers and imaging modalities are currently available; however, it is important to validate these tracers in the same model systems to acquire advantages and limitations of each imaging modality. During our nitroglycerin study, we observed that performing several scans using different imaging modalities (HX4 (hypoxia PET/CT), DCE-CT (perfusion CT), FDG PET/CT) increased the burden on patients and physicians excessively resulting in premature termination of the study. Furthermore, we observed that it is utmost important to stratify patients to increase the chance of an effective non-small cell lung cancer (NSCLC) treatment after application of nitroglycerin.

Gain for society

The observations made in this thesis could potentially benefit cancer patients and the society in general. Although at first sight the introduction of a personalized medicine approach sounds like an expensive one. But unfortunately, from the economic point of view, a "one size fits all" strategy in cancer treatment does not lead to a favorable treatment outcome for the majority of patients. Therefore, an improvement in treatment response with low developmental costs can be considered as a good strategy. Repurposing of already FDA approved drugs or even the targeted re-use of drugs withdrawn from the market in the past due to toxicity problems in other diseases, could be interesting approaches. Also, implementing drugs in cancer treatment strategies with a known toxicity profile from other diseases would be an interesting approach. In this thesis, we have demonstrated that, with a correct patient stratification, previously developed drugs such as nitroglycerin (chapter 6) could reduce treatment limiting aspects, like for instance hypoxia. Derivatives of drugs such as phenformin or doxorubicin (chapter 5 and 7) in order to target specific areas of a tumor or to modulate their toxicity profile would help us to reduce the costs for society. Using this approach, investigating new potential targets and developing specific therapeutic agents for these targets with unknown toxicity profiles can be prevented. This would reduce the development costs of new treatments and eventually reduce the costs of a certain treatment. Repurposing of drugs is an approach that also gained the interest of the scientific community as it creates opportunities for new research. Gaining more knowledge on the biological processes involved in certain types of treatment as well as their functional consequences benefits not only researchers but also physicians and thereby patients in the long run. Patients however should be more often made aware of the fundamental and translational research essential to improve the treatment of their diseases.

Road to the market

Although it is still a long road, the studies performed in this thesis do have the potential to be implemented in daily practice and additional studies to prove the efficiency and safety are the next steps to be performed. The dual-targeting agents described in this thesis remain to be further developed with respect to efficacy and safety, as the ones described in this thesis were found not to be effective enough. On the other hand, CAIX expression levels themselves are also interesting for further research. Prior to developing a dual-targeting agent, used for homing a drug to the tumor, CAIX expression levels itself could be used as a biomarker for malignant and invasive tumors and thereby for predicting treatment outcome. Further preclinical and clinical research however is necessary before introducing such a biomarker, imaging agents and/or CAIX

targeting agents into clinical practice. Similarly, also the GST prodrugs investigated in the thesis need further evaluation and validation regarding efficacy, efficiency and safety for their use as a treatment for chemoresistant tumors. Previous developed GST activated prodrugs such as TLK-286 (Telcyta, canfosfamide) enrolled already into clinical trials, thereby demonstrating the potency of this approach.

For drugs already preclinically used in a safe manner, window-of-opportunity clinical trials can elucidate efficacy in patients in a cost-effective manner. Using this approach, non-effective adjuvant drugs will be filtered out in an earlier development phase instead of going through conventional clinical trials and therefore costs will be reduced. Adjuvant drugs will be evaluated for their specific therapeutic effects as monotherapy (e.g. reducing hypoxia after application), before combining them with standard therapy. When a drug is found to be effective and potentially interesting to continue, clinical trials should be performed. Nitroglycerin has been evaluated already in clinical trials as adjuvant NSCLC treatment, but didn't live up to the expectations, explained by the lack of patient stratification. In our study (chapter 6) we showed that there is an opportunity for the use of nitroglycerin in a subset of patients, namely those patients with hypoxic tumors. Since nitroglycerin targets tumor hypoxia, we believe that patient stratification based on hypoxia imaging is crucial for these types of drugs. Hypoxia imaging is already used in clinical settings and can be exploited for specific targeting of these hypoxic areas in tumors or for guiding proper patient stratification. Taking along better and less invasive patient stratification methods during drug development, will reduce toxicity and increase efficacy for the patient and will eventually lead to a significant cost reduction for the society.

In the future, treatments will become more tailor-made and more specific based on the patients' needs, although the financial pressure on our health system keeps on increasing. Improvement of patient stratification can eliminate unnecessary and unsuccessful treatments and will thereby reduce treatment costs and therapy burden for patients. Investing in modalities to improve stratification is therefore essential. Additionally, drug repurposing whether or not in combination with drug modifications for potential new treatment approaches could be a valid approach to reduce drug development costs and hopefully reduces the burden on our health system.

References

1. *Nederlandse Kankerregistratie*. webpage cited: 2016-09-30; Available from: http://www.cijfersoverkanker.nl.
2. *Kosten van Ziekten.* webpage cited: 2016-11-27; Available from: https://kostenvanziektentool.volksgezondheidenzorg.info/tool/nederlands/.
3. Rusman, F., *Hoeveel mag een mensenleven kosten?* NRC Handelsblad, 2015-11-14.