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More people die every year in the European Union from lung cancer than from any other form of cancer. Early stages of the disease are often asymptomatic and diagnosis is typically made at a late stage when there is no more cure possible.

Chemotherapy can sometimes help to delay the progression of inoperable disease but often coincides with considerable side effects. So-called “resistance” to chemotherapy is common in lung cancer, a disease known for its heterogeneity. Our knowledge on the biology of cancer has greatly increased over the past decades. Compared to normal cells, cancer cells have an abnormal cellular architecture which drives them to grow in an uncontrolled fashion, to invade surrounding tissues and to spread throughout the body. Rather than simply trying to kill all rapidly dividing cells, recently developed treatment strategies, so called “targeted therapies”, aim to exploit the different characteristics of cancer cells to selectively kill them whilst sparing normal cells. Even though a cure for lung cancer still seems distant, the first targeted drugs are now used to treat patients, with sometimes remarkable improvements in outcome compared to chemotherapy.

For a cancer cell to grow and divide it needs to undergo changes which require an effective cellular metabolism. Crucial hereby is the timely activation and break-down of intracellular proteins. A certain cell structure, the “proteasome”, plays a central role in this process. It contains several enzymes that can cut and degrade proteins, helping to clear the cell from damaged and redundant proteins. Although normal cells also rely on the function of the proteasome for their homeostasis, prior research has shown that cancer cells are more likely to die when the activity of the proteasome is (temporarily) inhibited. In part this probably has to do with an aberrant protein metabolism in most malignant cells. The proteasome became therefore an attractive candidate “target” for treating malignant disease. In the late 90s, a new drug called bortezomib was developed which is capable of inhibiting the function of the proteasome. Early clinical studies showed a remarkable activity of this drug in treating certain cancer patients, including lung cancer patients.

We have investigated in this thesis, entitled: "The proteasome, a viable target in non-small cell lung cancer?", whether "targeting" (inhibiting) the proteasome function, holds promise for the treatment of non-small cell lung
cancer (NSCLC), a subtype of lung cancer which represents around 85% of cases.

Chapter 1 provides a general introduction and background to the research described in this thesis. In Chapter 2, we investigated the molecular mechanisms of cell death induced by bortezomib in NSCLC cells. We compared this mechanism to treatment with cisplatin, a chemotherapeutic drug commonly used to treat NSCLC patients. We focused our research on characterization of apoptosis activation by both drugs. Apoptosis is a regulated form of cell death which is triggered upon certain forms of cellular stress. Apoptosis plays an important role in normal physiology as it prevents accumulation of aberrant and damaged cells. There are two main apoptotic pathways, the so-called “intrinsic or mitochondria-dependent” pathway and the “extrinsic or death receptor mediated” pathway. Aberrant apoptosis activation or apoptosis resistance is a common feature in cancer cells. Previously our group showed that mitochondria-dependent apoptosis activation in NSCLC cells is dysregulated upon treatment with cisplatin. We observed that treatment with bortezomib effectively induced mitochondria-dependent apoptosis in NSCLC cells and was able to overcome apoptosis resistance. This is thought to be partly due to bortezomib-induced up-regulation of a protein called “Noxa”.

Not all NSCLC cells are equally sensitive to treatment with bortezomib and in Chapter 3 we were able to relate a difference in sensitivity to bortezomib to a difference in proteasome enzyme activity as well as differences in apoptosis activation in different NSCLC cell lines.

In Chapter 4, we investigated a combination of bortezomib and another drug called TRAIL, which induces apoptosis via the death receptor mediated pathway. This combination of drugs proved very toxic for NSCLC cells and different molecular mechanisms for the synergistic apoptosis activation were found.

About 30% of NSCLC patients respond to cisplatin and gemcitabine chemotherapy. Chapter 5 describes a clinical study which was performed to determine whether bortezomib can be safely combined with cisplatin and gemcitabine and can potentially result in a more effective treatment of NSCLC patients. Although this new combination of drugs was relatively well tolerated, preliminary results did not show a great enhancement in treatment efficacy.
A major disadvantage of chemotherapy are the, sometimes unexpected, and potentially life threatening side effects. It is therefore important to report and study toxicity of all new treatments or combinations carefully. In our clinical study some patients experienced serious treatment-related side effects and in Chapter 6 a case is described of a patient who suffered from cardiac failure whilst on treatment. In Chapter 7 a review of the literature regarding bortezomib-related neurotoxicity is provided, one of its main side effects. Quite unexpectedly, in the patients we treated we did not observe severe treatment-related neurotoxicity, even though cisplatin and bortezomib are both neurotoxic drugs.

As part of the clinical study we evaluated if the addition of bortezomib affected the drug levels of gemcitabine and cisplatin (“pharmacokinetic studies”). As initial studies in human blood cells showed that bortezomib might negatively affect the level of gemcitabine we conducted additional studies, described in Chapter 8, which surprisingly showed a different interaction pattern in NSCLC cells compared to human blood cells. In the NSCLC cells the addition of bortezomib was shown to positively enhance the activity of gemcitabine, suggesting human blood cells are not adequate to evaluate the anticancer activity of bortezomib/gemcitabine combinations.

In Chapter 9 we describe preliminary results of a study in which we investigate blood serum samples from patients participating in the clinical study and healthy volunteers for protein (fragment) patterns (“proteomics analysis”). Our aim is to find protein patterns that distinguish healthy people from cancer patients. Furthermore, we found certain serum protein patterns which could distinguish patients benefitting from the treatment from those who did not or less. Potentially these type of studies contribute to future development of tests for early detection of NSCLC and prediction of treatment outcome in individual patients, enabling a more personalized form of treatment.

Chapter 10 provides a summary and discussion of our research. In summary, our results from the clinical study, as well as the results from studies reported by others, do not suggest bortezomib is a very active drug in NSCLC. Nevertheless, our as well as other preclinical studies in NSCLC cell lines do suggest bortezomib can dramatically increase the activity of other (targeted) drugs when combined rationally. Further (pre)clinical studies are therefore warranted.