PEDIATRIC ACUTE LEUKEMIA

Acute leukemia is characterized by the uncontrolled proliferation of hematopoietic precursor cells in the bone marrow, leading to the replacement of normal blood cells. The two most common types of acute leukemia are acute lymphatic leukemia (ALL) and acute myeloid leukemia (AML), which are derived from different white blood cell lineages. While ALL is more common in children, AML is more common in adults. ALL is a leukemia of the lymphoid lineage, and is the most common pediatric cancer, accounting for 75% of all cases of childhood cancer. In the Netherlands, about 120 children are diagnosed with ALL each year. Survival of patients suffering from leukemia has improved enormously over the last decades due to optimal use of existing chemotherapeutics and better supportive care. Nowadays, for pediatric ALL the 5-year overall survival is over 85% in high-income countries. This has been achieved by combination chemotherapy regimens including glucocorticoids, vincristine, and asparaginase for remission induction therapy, with an initial remission rate of >95%. Unfortunately, pediatric relapsed ALL is the fourth most common pediatric malignancy. ALL relapse occurs in about 20% of patients and outcome after relapse remains poor with remission rates of 40% to as low as 10% for bad-prognostic subtypes such as T-cell ALL and patients with an early relapse. However patients can achieve second and even third remissions, survival after a third remission is lower than 10%. AML is a leukemia of the myeloid lineage. Children with AML treated with cytarabine and anthracyclin-based regimen, currently experience a probability of long-term survival of about 70%. However, 30-40% of pediatric AML patients will experience a relapse, and outcome from relapsed AML is poor. The age of diagnosis is a prognostic factor in acute leukemia. Adult patients with ALL have a substantial worse prognosis than younger patients with a 5-year survival ranging from 50% for younger adults, until 20% for patients older than 45. Similarly, adult AML has a substantial worse prognosis with a 5-year survival from around 40% which declines gradually with age until approximately 4% for patients older than 75 years. Accordingly, for the treatment-refractory and relapsed patients with low chances of survival, there is still room for improvement of therapeutic options.

MULTIPLE MYELOMA

Multiple myeloma (MM) is a malignant disease characterized by the abnormal proliferation of plasma cells and is the second most common hematologic cancer. MM is most prevalent in older people, and to date no definitive curative treatment exists. Nonetheless, survival has improved over the last decades due to the introduction of new treatment options, including the immunomodulatory drugs (IMiDs) thalidomide and lenalidomide, and bortezomib, a proteasome inhibitor. Initially used as single agent in the relapsed setting, proteasome inhibitors have become the backbone of frontline therapy. These front-line treatment options normally precede autologous stem cell transplantation in patients younger than 65 years. However, most patients relapse after induction therapy. A novel IMiD, pomalidomide is currently being evaluated for these patients, and in addition, the next generation proteasome inhibitor carfilzomib was recently approved for MM patients that relapsed after treatment with bortezomib and an IMiD. The promising results of proteasome inhibitors in MM patients have initiated the experimental exploration of proteasome
inhibitors in acute leukemia, which set the stage for studies described in this thesis.

THE PROTEASOME

The ubiquitin-proteasome system (UPS) controls normal protein homeostasis in cells and thereby influences cellular processes such as intracellular protein processing and degradation, apoptosis, inflammation, antigen presentation, cell growth and survival, and cell cycle control. Proteins marked for degradation after ubiquitin conjugation will be proteolysed by the proteasome\textsuperscript{10}. Upon protein degradation, shorter peptides are generated that can either be trimmed to 8-10 mer peptides for presentation on major histocompatibility complex (MHC) class I molecules on the cell surface to initiate an immune response, or further fully hydrolyzed by aminopeptidases to amino acids reutilized for protein synthesis\textsuperscript{11} (Figure 1). The constitutive 26S proteasome consists of two outer 19S regulatory particles and an inner 20S core particle with 2 identical rings of 7 α-subunits and 2 identical rings of seven β-subunits. The α-subunits are responsible for recognizing and unfolding the ubiquitin-bound proteins and the three catalytically active β-subunits; β1 (\textit{PSMB6}, caspase-like activity), β2 (\textit{PSMB7}, trypsin-like activity), and β5 (\textit{PSMB5}, chymotrypsin-like activity) facilitate proteolysis (Figure 2). Beyond constitutive proteasomes, the immunoproteasome represents an additional variant that is dominantly expressed in hematopoietic cells\textsuperscript{12–15}. The immunoproteasome differs from the constitutive proteasome in the 11S regulatory particles and the replacement of the catalytically active β-subunits by: β1i (\textit{PSMB9}), β2i (\textit{PSMB10}), and β5i (\textit{PSMB8}).
Immunoproteasome expression is markedly induced upon stimulation by inflammatory cytokines such as IFN-γ and to a lesser extent TNF-α. The dominant function of immunoproteasomes was assigned to increase the generation of antigenic peptides for presentation on MHC class I molecules. Recently, an alternative function of the immunoproteasome was proposed in facilitating efficient clearance of protein aggregates that arise upon interferon-induced oxidative stress, thereby preventing cell death\(^\text{16}\). However, this hypothesis was recently disputed by Nathan et al\(^\text{17}\) who reported that immunoproteasomes were not more proficient in degrading ubiquitinated proteins compared to constitutive proteasomes. Next to immunoproteasomes, two additional proteasome hybrid types (β1+β2+β5i) and (β1i+β2+β5i) were identified, each of which harboring the capacity to process different tumor antigens\(^\text{18}\).

**PROTEASOME INHIBITION**

Because diverse human diseases are linked to deregulation of UPS-controlled processes, the UPS has become an important therapeutic target. In MM cells, sensitivity to proteasome inhibition comes with the fact that these proliferative cells are highly dependent on their protein synthesis and turnover, relying on the UPS for processing of defective proteins\(^\text{19,20}\). Disruption of proteasome activity results in rapid accumulation of regulatory proteins in the cell that will cause endoplasmic reticulum stress and consequently lead to apoptosis. Moreover, the induction of the unfolded protein response, which would normally
block protein translation and induction of alternative degradation pathways in stress situations, is probably disturbed in MM cells, leading to imbalanced protein homeostasis rendering these cells highly susceptible to proteasome inhibitors\textsuperscript{13}. Bortezomib is the first proteasome inhibitor entering clinical practice 10 years ago for the treatment of relapsed/refractory MM\textsuperscript{21}, and subsequently in 2008 as frontline therapy for MM. In addition, next generation proteasome inhibitors were developed to improve on bortezomib with respect of oral availability, reduced toxicity and overcoming bortezomib-resistance\textsuperscript{22}. Given original observations that leukemia cells express higher levels of proteasomes\textsuperscript{23} and thus potentially more prone to undergo apoptosis after proteasome inhibition than normal cells\textsuperscript{24}, the proteasome has also gained increasing interest as therapeutic target for leukemia.

**AIM OF THE THESIS**

With respect to novel therapeutic opportunities for relapsed acute leukemia, proteasome inhibitors are attractive candidates given their proven track record in the treatment of MM and mantle cell lymphoma. The aim of this thesis was therefore to evaluate the efficacy of proteasome inhibitors against leukemic cell line models and ex-vivo clinical samples. Additionally, we verified potential benefits of combination treatment of proteasome inhibitors with conventional anti-leukemic chemotherapeutics. Because emergence of acquired resistance is often observed during therapy of MM patients, and thus a potential limiting factor of the efficacy of proteasome inhibitor treatment, we also aimed to further decipher the molecular basis of acquired resistance to proteasome inhibitors. Based on these studies, we set out to define parameters that could help to identify patients who may benefit from bortezomib-containing therapy and/or examine strategies to overcome resistance to bortezomib. Lastly, we evaluated the applicability of next generation proteasome inhibitors for acute leukemia was evaluated.
INTRODUCTION TO THE CHAPTERS

Chapter 2 covers a review on proteasome inhibitors in acute leukemia. Building on the results obtained with bortezomib treatment in multiple myeloma therapy, the current status of proteasome inhibitors (for pre-clinical and clinical studies) in the treatment of acute leukemia is outlined.

In Chapters 3-5, in vitro studies are described, examining the antileukemic effects of bortezomib and two new generation proteasome inhibitors against leukemia cell lines and also explored potential mechanisms of resistance to these drugs following long term exposure to leukemia cells. Specifically, in Chapter 3 we report on sensitivity profiling and discuss molecular mechanisms of acquired resistance to the founding member of proteasome inhibitors, i.e. bortezomib, in leukemia and MM cells. These studies were extended in Chapter 4 to the immunoproteasome inhibitor PR-924, and in Chapter 5 to the novel marine Salinispora tropica proteasome inhibitor salinosporamide A (Marizomib).

In Chapters 3-5 we also verified the ability of novel generation proteasome inhibitors for their capacity to overcome acquired resistance to bortezomib, PR924 and salinosporamide A.

In Chapter 6 we describe another strategy to overcome bortezomib resistance in leukemia and MM cells in vitro by upregulation of immunoproteasome levels by exposure to interferon-γ. This approach introduced sensitization for, in particular, immunoproteasome inhibitors and the molecular basis for this are discussed.

In Chapters 7 and 8, parameters of bortezomib and next generation proteasome inhibitors were examined in clinical samples of pediatric leukemia patients (ALL and AML), and relapsed pediatric acute leukemia patients participating in a phase II clinical trial with bortezomib-containing reinduction chemotherapy. From both studies, a novel indicator for sensitivity to bortezomib emerged, i.e. ratio of immunoproteasome over constitutive proteasome levels, which merits further clinical validation.
REFERENCES