



Universiteit
Leiden
The Netherlands

Probing proteasome activity and function : cancer diagnostics and mechanism of antigen processing

Berkers, C.R.

Citation

Berkers, C. R. (2010, October 5). *Probing proteasome activity and function : cancer diagnostics and mechanism of antigen processing*. Retrieved from <https://hdl.handle.net/1887/16011>

Version: Corrected Publisher's Version

License: [Licence agreement concerning inclusion of doctoral thesis in the Institutional Repository of the University of Leiden](#)

Downloaded from: <https://hdl.handle.net/1887/16011>

Note: To cite this publication please use the final published version (if applicable).

Chapter 2.4

**Summary and prospects:
development and use of chemical
proteasome probes**

Summary and prospects: development and use of chemical proteasome probes

The ubiquitin-proteasome system is responsible for the degradation of proteins involved in a wide variety of cellular processes, including cell proliferation and survival, cell-cycle control, transcriptional regulation and cellular stress responses, as well as for the destruction of redundant and misfolded proteins. The ubiquitin proteasome system is an attractive pharmacological target for the treatment of cancer, because of the higher sensitivity of malignant cells to proteasome inhibition compared to normal cells, and the proteasome inhibitor bortezomib has been approved for the treatment of multiple myeloma (MM) and mantle cell lymphoma. Bortezomib treatment is however associated with substantial adverse effects, and the occurrence of both primary and secondary resistance has been reported. Consequently, there is an ongoing search for novel proteasome inhibitors that are able to overcome bortezomib resistance and that are better tolerated to increase their therapeutic potential. Several second-generation proteasome inhibitors, which differ in their mode of inhibition and subunit specificity, have entered clinical trials, including marizomib and CEP-18770.

Techniques that are commonly used to monitor proteasome activity include the application of fluorogenic substrates, small molecule-based activity assays and models based on recombinant reporter proteins. Traditionally, fluorogenic substrates are used to mea-

sure the activity of the different proteasome active sites, but most fluorogenic substrates cannot be used in cells, and prior cell lysis is required before activity measurements can be performed. Reporter proteins can be used in living cells, but their use is limited to genetically altered cells or organisms and therefore does not allow profiling of patient material. In addition, their activity readout depends on the balance between synthesis and degradation of fusion proteins, which involves many cellular factors other than the proteasome, including the rate of fusion-protein synthesis. With proteasome inhibitors in use in the clinic and with clinical trials in progress investigating the treatment of several proteasome inhibitors for a variety of hematologic and solid malignancies, accurate methods that allow profiling of proteasome inhibitor specificity and efficacy in patients are in demand.

In **chapter 1**, the development of chemical proteasome probes that provide such methods is described. In **chapter 1.2**, we describe the first small molecule-based activity assay in its class that could be used to profile the specificity of proteasome inhibitors in living cells. This dansylated vinylsulfone based proteasome probe contains an α,β -unsaturated sulfone part that reacts through a Michael addition reaction with the γ -hydroxy moiety of the N-terminal threonine residue of catalytic β -subunits of the proteasome, resulting in the formation of a β -sulfonyl ether link-

age. Antibodies against the dansyl moiety are used for detection of labeled active subunits by SDS-PAGE and Western blot analysis. Using this probe, we investigated the *in vivo* subunit specificities of bortezomib and another inhibitor, MG132. When we compared the specificities in cultured cells and in crude cell extracts, we observed substantial differences in activity patterns, stressing the importance for bioassays compatible with live cells to ensure accuracy of such measurements. In **chapter 1.3**, we describe the further optimization of this probe by replacement of the environment sensitive and low quantum yield dansyl fluorophore by high quantum yield fluorophores that allowed for direct scanning of the SDS-PAGE gel for fluorescence emission of fluorescently labeled subunits. In order to be able to select for effective fluorescent proteasome inhibitors with good biochemical and biophysical properties, we embarked on the synthesis of a range of fluorescent inhibitors. Two differentially labeled probes tested proved suitable to study proteasome activity in cell extracts, living cells and murine tissues using a range of techniques including SDS-PAGE, confocal laser scanning microscopy and flow cytometry.

In **chapter 2**, these fluorescent proteasome probes were used to study the proteasome inhibitory profiles of two second-generation proteasome inhibitors, CEP-18770 and marizomib, in a preclinical model of MM and in patients in clinical trials, respectively. In **chapter 2.1**, we compared bortezomib and CEP-18770 in a variety of *in vitro* and preclinical *ex vivo* models. In a panel of cell lysates and cell lines, both proteasome inhibitors were equipotent in inhibiting distinct subunits of the proteasome, with proteasome activity recovering similarly after withdrawal of either inhibitor. In contrast, in a preclinical MM model, CEP-

18770 inhibited tumor proteasome activity to a significantly higher extent compared to bortezomib, while the two inhibitors had an equal capacity to inhibit the proteasome in normal tissues. In addition, we showed that CEP-18770 was able to inhibit proteasomes and overcome bortezomib resistance in cells with acquired bortezomib resistance *in vitro*. Marizomib is currently being evaluated in phase I clinical trials for the treatment of both solid and hematologic tumors, enabling a detailed study of patient response to marizomib treatment. In **chapter 2.2**, we validated the use of a fluorescent proteasome activity probe for profiling proteasome inhibition in both packed whole blood (PWB) and peripheral blood mononuclear cells (PBMCs) using marizomib- or bortezomib-treated rats. In addition, we developed a protocol that enables the use of this fluorescent probe to profile blood samples from patients that are treated with proteasome inhibitors. We showed that PWB, which contains >98% red blood cells, responded differently to proteasome inhibitors and recovered slower from proteasome inhibition compared to PBMCs. These data caution against the use of PWB as a model system to monitor the effects of proteasome inhibitors in animals or patients, as is routinely done. In addition, we used the fluorescent activity-based probe to demonstrate that both initial proteasome activity level in cells and the ability of cells to change their proteasomal subunit composition in response to marizomib treatment may be important determinants of sensitivity to proteasome inhibitors.

Collectively, the findings presented in **chapter 2** demonstrate that chemical proteasome activity probes can reliably assay proteasome activity *in vitro* and *ex vivo* in a broad range of tissue types, both in preclinical models and during clinical trials. Furthermore, these

probes may enable prediction of patient responses to proteasome inhibitors, which can contribute to a more personalized proteasome inhibition strategy to improve the therapeutic potential of this class of drugs.

We expect that the development of second-generation proteasome inhibitors will likely result in the generation of inhibitors that can overcome resistance and may ultimately contribute to better patient outcome. Intravenously administered proteasome inhibitors likely saturate proteasomes in red blood cells, which predominantly display constitutive activity, before peripheral sites are reached. Specific immunoproteasome inhibitors may saturate blood proteasome at relatively low concentrations, resulting in higher inhibitor concentrations in tumor cells. This suggests that immunoproteasome inhibitors may in future provide a good alternative for tumors that have high intrinsic immunoproteasome activity. An interesting strategy to overcome resistance to the current generation of proteasome inhibitors may be the development of inhibitors that do not interfere with the 20S core, but with other elements of the UPS. Experiments setting up a high-throughput flow cytometry-based assay that can be used to measure both proteasome activation and proteasome inhibition in both cultured and primary cells are currently ongoing. This assay allows screening of large compound or siRNA libraries to identify either direct regulators of proteasome activity, such as inhibitors of the 20S core, or modulators that interfere elsewhere in the ubiquitin-proteasome pathway. This assay is currently optimized for the flow cytometry assisted measurement of overall proteasome inhibition in tumor cells originating from complex patient samples. We expect that our flow-cytometry based proteasome activity assay will not only identify proteasome inhibitors, but also activators

of the ubiquitin proteasome system. These activators may find use in the treatment of neurodegenerative diseases, which are often characterized by a decrease in intracellular proteasome activity. In addition, the development of proteasome inhibitors that are specific for proteasome subtypes or bacterial proteasome may broaden the clinical application of UPS-modulating compounds in cancer, infectious- and autoimmune diseases.

