and renal failure in one patient treated at 0.1 mg/m^2 and Grade 4 neutropenia of 3 days duration in one patient treated at 0.112 mg/m^2, otherwise drug related adverse events have been unremarkable at the highest dose level tested. Preliminary pharmacokinetic data indicate an elimination half-life of approximately 2−5 minutes with clearance of 5.5−15 mL/min and volume of distribution of 16.5–103.5 L. In all clinical trials with NPI-0052, proteasome inhibition has been assayed in whole blood from patients treated at 0.0125 mg/m^2 through 0.45 mg/m^2 demonstrating inhibition of CT-L, increasing with time and dose, up to 92%. Seven patients (24%) have had stable disease for at least two months (8 weeks; 2 months), including patients with hepatocellular carcinoma (6 cycles) adenoid cystic carcinoma (4 & 5 cycles), melanoma (4 cycles), CRC (6 cycles), ovarian (4 cycles) and cervical carcinoma (12 cycles).
Conclusions: NPI-0052 produces dose and time-dependent pharmacologic effects into the predicted efficacious range at well tolerated doses. Chronic dosing can be maintained with prolonged stable disease without toxicity emerging. Safety and clinical benefit continues to be evaluated to further characterize the significance of the initial findings. Based on these results, clinical trials have been initiated in combination with other cancer therapies.

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Preclinical and clinical monitoring of cell-and circulating plasma specific proteasome biomarkers after treatment with the proteasome inhibitor, NPI-0052

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Background: Pharmacodynamic profiling of proteasome inhibitors has focused on monitoring activities in packed whole blood (PWB), rather than peripheral blood mononuclear cells (PBMC) or the tumor. Proteasome activities in plasma of cancer patients may correlate with prognosis suggesting they are tumor derived. Therefore studies were performed to evaluate changes in proteasome activities during preclinical studies and Phase I trials with NPI-0052, a novel proteasome inhibitor that produces prolonged inhibition of all three proteasome 20S proteolytic activities.
Material and Methods: Chymotrypsin-(CT-L), trypsin-(T-L) and caspase-like (C-L) activities were measured before and after NPI-0052 treatment by monitoring the production of AMC from specific fluorogenic peptides and plasma proteasome levels by ELISA.
Results: Treatment of mice with NPI-0052 inhibited CT-L, T-L and C-L activities in PWB in a dose-dependent manner which recovered significantly by day seven. Since PWB contains mainly RBCs that do not regenerate proteasomes, the effects of NPI-0052 were evaluated in PBMCs. CT-L activity in PBMCs markedly recovered within 2−3 days after NPI-0052 treatment. In Phase I trials, inhibition of CT-L activity in PWB after treatment at 0.45 mg/m^2 reached ~60% after the first (day 1) and ~90% after the third treatment (day 15). A similar profile was also obtained in PBMC. In a patient with cervical carcinoma with stable disease for ~12 months, treated at 0.025 mg/m^2 and 0.05 mg/m^2, significant inhibition of all three activities was observed in PWB. When proteasome levels and enzymatic activity were compared in plasma of patients treated with low (0.025 or 0.05 mg/m^2) and higher-doses (0.075 or 0.112 mg/m^2), there were significant changes in median proteasome levels at 1 h and 4 h. Therefore the plasma proteasome activity was normalized to proteasome levels. We demonstrate significant changes in the median normalized levels of CT-L, C-L and T-L activity at 1 h in low-dose and higher-dose groups that remained detectable at 4 h in the higher-dose for CT-L and C-L but not the low-dose groups. Normalized T-L activity returned to pre-therapy level at 4 h in both groups.
Summary: The data indicate a complex and dynamic interaction between proteasome inhibitors, proteasome levels and activities in tissues. We are evaluating these parameters at higher clinical doses to determine whether they provide a prognostic value to assess disease progression and treatment response.

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Phase I clinical trial of the 2nd generation proteasome inhibitor NPI-0052 in patients with advanced malignancies with a CLL RP2D cohort

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Background: NPI-0052 is a novel proteasome inhibitor that produces prolonged inhibition of all three catalytic activities of the 20S proteasome. NPI-0052 has a novel structure leading to a unique proteasome inhibition and signal transduction profile. Preclinical models suggest NPI-0052 may demonstrate an improved therapeutic ratio and activity in hematologic (myeloma, lymphoma, leukemia) and solid tumor models. Secondary to these findings, clinical trials are currently being conducted in patients with myeloma, lymphomas, leukemias and solid tumors.
Materials and Methods: Patients with solid tumor, lymphoma or leukemia were treated with NPI-0052 administered weekly, for 3 weeks in 4-week cycles in this 3+3 design dose escalation study. The dose of NPI-0052 was escalated in 50–100% increments dependent on observed adverse events (AE). In addition to regular safety monitoring, proteasome inhibition (PI) (baseline, D1 & D15) and PK (D1 & D15) were assayed in blood. Once a Recommended Phase 2 Dose (RP2D) is identified, a RP2D cohort of patients with CLL will be enrolled. **Preliminary Results:** 22 patients have been treated at doses ranging from 0.1 mg/m^2 to 0.55 mg/m^2 without reaching an MTD. The AE profile has been tolerable with fatigue, transient peri-infusion site arm discomfort and lymphopenia being commonly ascribed to NPI-0052. Preliminary PK data indicate T1/2 of 2−5 min, a mean clearance of 12.6–22.8 L/min and Vss of 72.7–176.3 L. There is a linear relationship between AUC and Cmax. PI has been assayed in blood, indicating a dose:response relationship with inhibition of up to 92% observed. A total of 5 patients (23%) have had stable disease for at least 2 cycles (8 weeks; 2 months), including two with melanoma (4 cycles), one each with mantle cell lymphoma (4 cycles), Hodgkin's lymphoma (4 cycles), follicular lymphoma (4 cycles) and sarcoma (5 cycles).
Conclusions: NPI-0052 produces dose-dependent pharmacologic effects into the predicted efficacious range at doses below the MTD. Enrollment continues to identify a RP2D based on safety, efficacy and pharmacodynamics. These data have supported additional studies being initiated in combination with other targeted agents.

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Leaving groups prolong the duration of 20S proteasome inhibition and enhance the inhibition profile of salinosporamides

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Background: The 26S proteasome, a multicatalytic enzyme complex that degrades intracellular proteins, has emerged as an important target in cancer chemotherapy. Its 20S proteasome core particle contains three pairs of proteolytic subunits with chymotrypsin-like (CT-L), trypsin-like (T-L) and caspase-like (C-L) activities. NPI-0052 (salinosporamide A) is a mono-chlorinated natural product in clinical trials for cancer that exhibits potent and prolonged duration inhibition of all three proteolytic activities (IC50 CT-L < T-L $\ll$ C-L). The crystal structure of NPI-0052/yeast 20S proteasome complex indicated that the chlorine acts as a leaving group (LG) within the proteolytic sites. In this study, we investigated the mechanism whereby the LG enhances the duration and potency of 20S proteasome inhibition and developed analogs that more effectively inhibit all three sites.
Materials and Methods: Analogs with a range of LG potentials were prepared by semi- or total synthesis and evaluated for: (i) stability and hydrolysis product identity by LC-MS; (ii) inhibition of isolated rabbit 20S proteasomes before and after attempted removal of inhibitors by dialysis; (iii) cytotoxicity and inhibition of CT-L activity in human multiple myeloma

cell line RPMI 8226; (iv) crystal structures in complex with yeast 20S proteasomes.
Results: Synthetic methodology was established for analogs with a variety of LG potentials and identities. Analogs bearing good LGs generally exhibited enhanced potency in bioassays. LG analogs gave rise to prolonged duration proteasome inhibition compared to non-LG analogs. Intermediate results were observed for fluorosalinosporamide, with poor LG potential. A model for the mechanism of irreversible inhibition by LG analogs versus slow substrate, non-LG analogs was developed. Crystal structures of inhibitor-20S proteasome complexes offered insights into inhibitor-active site interactions. A subset of LG analogs showed enhanced inhibition of C-L activity while maintaining good potency against CT-L and T-L sites.
Conclusions: Proteasome inhibition is enhanced by the presence of a good LG. Analogs that bear a substituent with good LG potential give rise to a common, highly stable cyclic ether product that cannot be deacylated and thus induces prolonged duration of proteasome inhibition. This "irreversible binding" holds true for all three proteolytic subunits. Specific LG identities resulted in enhanced potency against the C-L site.

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The selective proteasome inhibitor carfilzomib in combination with chemotherapeutic agents improves anti-tumor response in solid tumor xenograft models

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Background: Since treatment options for previously treated solid tumors are limited, novel combination therapies are warranted. Carfilzomib is a first in class selective epoxyketone proteasome inhibitor that is currently being evaluated in Phase 2 trials in solid tumors and multiple myeloma. Because the proteasome plays a central role in the regulation of a broad spectrum of cell signaling and protein homeostatic pathways, combining proteasome inhibition with standard chemotherapies represents a promising avenue for increasing anti-tumor responses and overcoming drug resistance in solid tumor cells. **Aim:** To determine the tolerance and efficacy of treatment regimens combining carfilzomib and approved chemotherapeutic agents for solid tumors in mouse tumor models.
Methods: Maximum tolerated doses (MTDs) of cisplatin (CDDP), carboplatin, irinotecan, docetaxel or Doxil on clinically relevant dose schedules in combination with carfilzomib were determined in immunocompromised (BNX) mice. Toxicity was assessed by body weight changes and clinical observations. BNX mice bearing established subcutaneous tumors of lung (A549) and colorectal (HT-29) human tumor cells were treated with carfilzomib, docetaxel, Doxil or combinations of carfilzomib and docetaxel or Doxil.
Results: Carfilzomib treatment was well tolerated in combination with a DNA-cross linking agent (carboplatin), a topoisomerase inhibitor (irinotecan), a microtubule disrupting agent (docetaxel) and an anthracycline (Doxil) at the MTD for each individual agent. A carfilzomib and CDDP combination resulted in increased toxicity if dosing commenced on the same day, but was well tolerated with a staggered dose schedule. The combination of carfilzomib and docetaxel resulted in a significant reduction in A549 tumor growth compared to vehicle controls or treatment with either single agent ($p < 0.001$ vs. control; $p < 0.01$ vs. carfilzomib or docetaxel alone). Similar observations were noted in the HT-29 xenograft model where a carfilzomib and Doxil combination significantly reduced tumor burden ($p < 0.001$ vs. control or carfilzomib alone; $p < 0.01$ vs. Doxil alone).
Conclusions: These results demonstrate that carfilzomib can be combined with chemotherapeutic agents of multiple classes. More importantly, the combination of carfilzomib with either docetaxel or Doxil improved anti-tumor responses in multiple solid tumor models. Clinical investigation of combining carfilzomib to standard of care in solid tumors is merited.