NOV-002, a glutathione disulfide mimetic, decreases Cisplatin-induced nephrotoxicity and S-glutathionylates serum proteins in mice.

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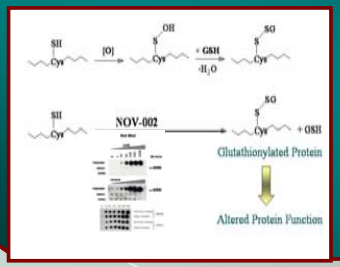
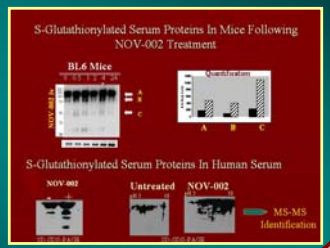
Abstract

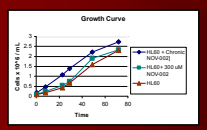
NOV-002 is a glutathione disulfide (GSSG) mimetic with chemoprotective activity. Clinical studies in the Russian Federation have demonstrated significantly improved survival and decreased tumor progression rates in non-small cell lung (NSCLC) and ovarian cancer patients treated with NOV-002 + cytotoxic chemotherapy compared to those receiving chemotherapy alone. The efficacy of NOV-002 in NSCLC has been confirmed in a recent US randomized Phase 1/2 clinical study. The increased efficacy seen with NOV-002 + chemotherapy occurred in the context of significantly better tolerance for the chemotherapeutic regimen as evidenced by improvement/normalization of hematologic indices and indices of liver and kidney toxicity compared to chemotherapy alone. Cisplatin-induced nephrotoxicity requires activation of a glutathione-platinum conjugate by γ-glutamyl-transpeptidase (GGT). Because oxidized glutathione is an endogenous substrate of GGT, the protective effect of NOV-002 may be partly attributable to its ability to act as a competitive substrate for the enzyme. As such, 8-week old Bl6 mice were treated with a single nephrotoxic dose of Cisplatin (15 mg/kg, ip) and sacrificed on day 5. One group of animals was treated with NOV-002 (15 mg/kg, im) daily. The Cisplatin-treated mice had significantly elevated levels of plasma creatinine compared to mice treated with NOV-002 (4.7 vs 2.9 mg/dL, respectively). Cisplatin-induced weight loss was diminished in the NOV-002 treated animals (22% vs 14.5%). In prior studies, we have shown that NOV-002 treatment of cancer cells leads to S-glutathionylation (glutathione conjugation) of cysteine residues with a low pKa values in redox sensitive proteins. Analysis of blood in drug treated mice showed that NOV-002 causes S-glutathionylation of three distinct proteins in plasma in <30 minutes. Using two-dimensional SDS-PAGE analysis and mass spectroscopy we are identifying these target proteins. S-glutathionylation of one or more of these proteins will be used as marker(s) for following drug pharmacokinetics/dynamics and as plausible surrogates for clinical efficacy.

NOV-002 induced S-glutathionylation

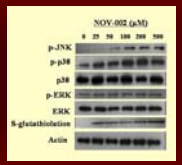




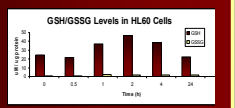
Cellular Effects of NOV-002 Treatment on HL60 Cells



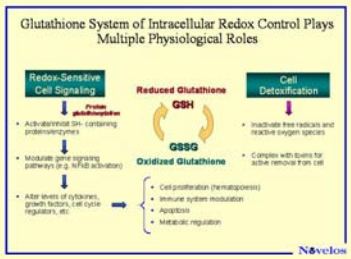
NOV-002 increases cell growth rate. HL60 cells were treated with saline, a single treatment of NOV-002 or chronic NOV-002 treatment. The cells were counted at various times. The doubling time of HL60 cells was 15.5h whereas NOV-002 chronic treatment decreased the doubling time to 12.5h, p<0.01. N=3 independent experiments.

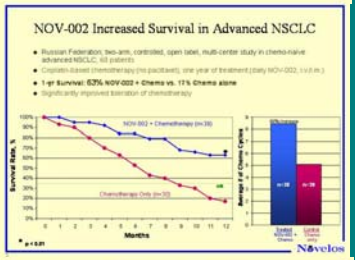


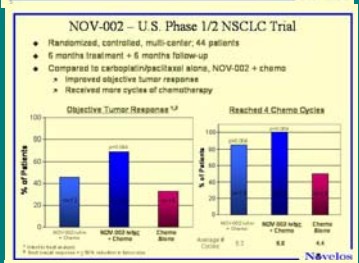
NOV-002 treatment leads to activation of JNK and p38. HL60 cells were treated with increasing concentrations of NOV-002 for 1h. Cell lysates were separated by SDS-PAGE. Activation of signaling pathways was evaluated with specific antibodies.

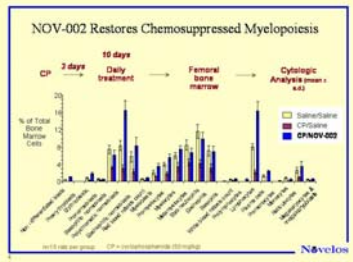


NOV-002 treatment leads to a transient increase in intracellular GSH/GSSG levels. HL60 cells were treated with 300 μM NOV-002. The cells were harvested at various times. GSH/GSSG levels were measured using the Tietz method.

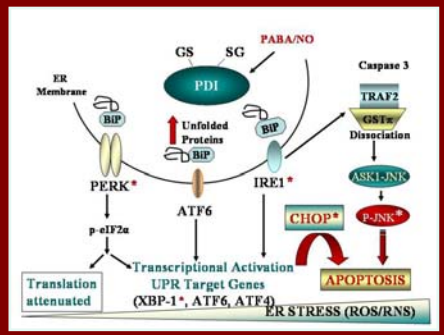


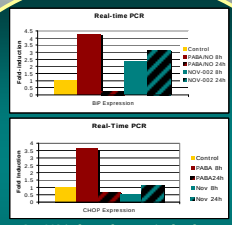






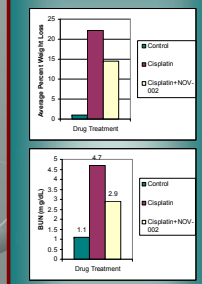
The Unfolded Protein Response





NOV-002 induces the UPR in the absence of cell death, as indicated by the lack of CHOP induction HL60 cells were treated with 30 μM PABA/NO or 300 μM NOV-002. Transcriptional activation of UPR responsive genes was assayed by Realtime PCR.

Nephroprotective Effects of NOV-002



Female Bl6 mice were treated with saline, 15 mg/kg Cisplatin or 15 mg/kg Cisplatin plus 15 mg/kg NOV-002. The animals were weighed and sacrificed on day 5. BUN was evaluated with a BUN endpoint assay (Sigma).

Conclusions:

- NOV-002 is an effective therapy when combined with standard cytotoxic drugs.
- NOV-002 provides protection of normal tissues and permits administration of more cycles of therapy.
- NOV-002 cause S-glutathionylation of a variety of proteins
- Some of the glutathionylated proteins in the serum may prove to be effective biomarkers for drug effects
- Induction of the UPR by NOV-002 occurs through redox balance changes and is independent of cell death