In vivo pharmacokinetic and pharmacodynamic behavior of NOV-002, a redox modulating glutathione disulfide mimetic

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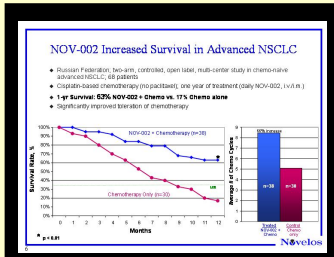
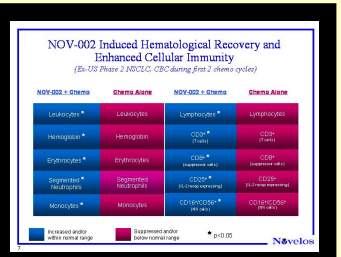
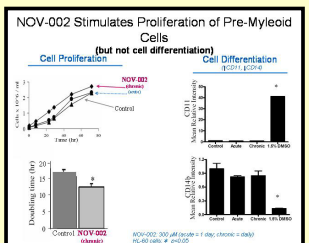
Abstract

Nov-002 is a novel formulation of oxidized glutathione (GSSG) currently in a pivotal Phase 3 clinical trial in advanced non-small cell lung cancer. In clinical trials conducted to date, NOV-002 administered in combination with standard chemotherapeutic regimens has resulted in increased efficacy (survival, tumor response) and improved tolerance of standard chemotherapy (e.g. enhanced hematological recovery, immmune stimulation. Recently, we showed that the proliferative effects of NOV-002 in the pre-myeloid HL-60 cell line is commensurate with stress-induced S-glutathionylation and activation of kinase pathways (AKT, JAK2 and STAT5) that are known to regulate hematopoiesis. The present investigation was undertaken to characterize the plasma pharmacokinetics of NOV-002 in C57 black6 mice and to determine if the drug caused S-glutathionylation of plasma proteins that might be used as surrogate biomarkers for drug efficacy. Using HPLC-MS, NOV-002 was measured in plasma at various times following a single intraperitoneal dose. Peak plasma concentrations were observed 10 min following administration of NOV-002 and were diminished by 60 min. Fluorescence based methods were used for quantification of protein thiol content and three S-glutathionylated plasma proteins were identified by mass spectrometry, serine proteinase inhibitor, contrapsin and alpha-1-antitrypsin 1-6 precursor. These preclinical pharmacokinetic and pharmacodynamic results provide evidence that, as in cell systems, NOV-002 administration in vivo results in generation of a transient oxidative signal that may trigger a variety of redox-regulated biochemical cascades. Such correlates could help to understand and predict clinical responses to treatment with NOV-002.

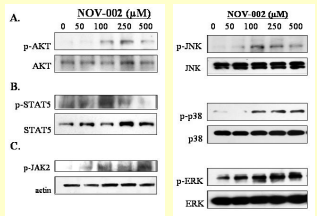






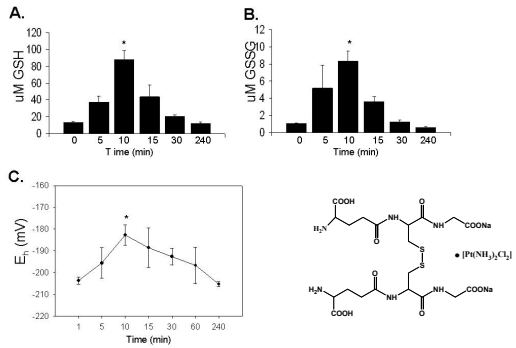
Effects of NOV-002 on growth and differentiation in HL-60 cells. The growth rate of untreated HL60 cells (▲), HL60 cells + 300 uM NOV-002, "acute" (●) or HL60 cells + 300 uM NOV-002 every 24h, "chronic" (◆) was measured using a cell coulter counter every 6-12 hours . Flow cytometry was used to measure cell surface markers for differentiation, cd11 and cd14b. The results are expressed as the mean ± S.E. , N=3.

Cellular Kinase Signaling



Concentration dependent effects of NOV-002 on stress kinases and their phosphorylated products are accompanied by an increase in actin S-glutathionylation and cytoskeletal changes. HL60 cells were treated for 1h with 0-500 μM NOV-002 in complete media.

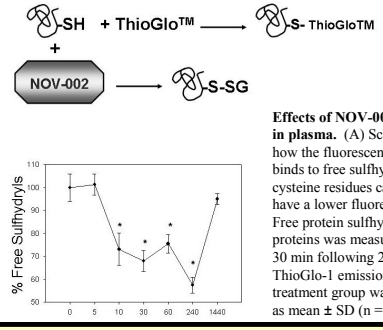
Pharmacokinetic Analysis of NOV-002



Effect of NOV-002 on plasma GSH, GSSG and redox potential. Mice were treated with 250 mg/kg NOV-002 (ip). Orbital blood was collected at 0, 5, 10, 15, 30 and 240 min. Mean plasma concentration-time profiles for (A) GSH and (B) GSSG were obtained by HPLC-MS analysis. The Eh GSSG/GSH was calculated from the GSSG and GSH concentrations using the Nernst equation (C). Data are represented as mean ± SD, n=7 animals / time-point.

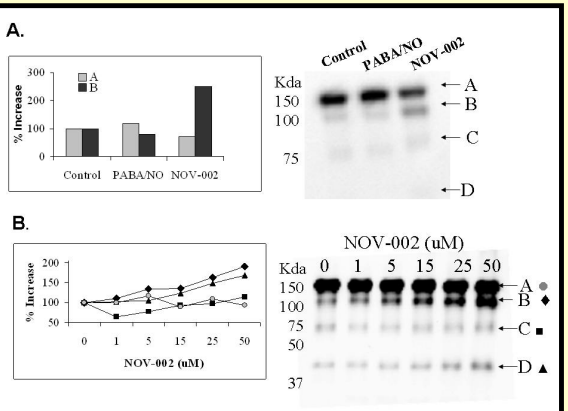
PK Parameter	GSH	GSSG
C_o (ug/mL)	4.1 ± 0.2	0.65 ± 0.02
C_{max} (ug/mL)	27.1 ± 3.3	5.4 ± 1.2
$T_{1/2}$ (min)	16.6 ± 1.9	13.1 ± 1.6
AUC $_{0\text{-}30min}$ (ug.h/mL)	1249 ± 161	114 ± 14

NOV-002 treatment leads to a decrease in plasma protein free thiol level



Effects of NOV-002 on free sulfhydryls in plasma. (A) Schematic illustrating how the fluorescent probe, ThioGloTM, binds to free sulfhydryls. Modified cysteine residues cannot bind and thereby have a lower fluorescent intensity. (B) Free protein sulfhydryl content of plasma proteins was measured at 0, 5, 10, 15 and 30 min following 250 mg/kg (ip). The ThioGlo-1 emission (at 513nm) for each treatment group was averaged and plotted as mean ± SD (n = 6); (*=p≤0.001).

Identification of S-glutathionylated plasma proteins



NOV-002 treatment induces S-glutathionylated plasma proteins. Mice were treated with an i.v. bolus of the oxidized glutathione mimetic, NOV-002 at 25 mg/kg or PABA/NO at 5 mg/kg. Blood was collected at various time points via orbital bleed. The plasma proteins (A) were separated by non-reducing SDS-PAGE and S-glutathionylated proteins were evaluated by immunoblot with PSSG antibody. Plasma from untreated controls (B) were treated with 0-50 μM NOV-002 for 15 min. Equivalent protein was separated and evaluated by immunoblot. The corresponding relative abundance of S-glutathionylated proteins (A, B, C and D) was plotted as the relative ratio to albumin.

Table 3: MALDI-TOF identification of S-glutathionylated plasma proteins following NOV-002 treatment

Protein	NCBI Accession #	MM (Da)	Confidence Interval
(A) Complement C3	1352102	186364.7	100%
(B) Serine proteinase inhibitor	6678087	45862.5	100%
(C) Contrapsin	54173	46642.9	100%
(D) α-1-antitrypsin 1-6 precursor	68068019	45794.4	100%

MM, molecular mass

Summary

- NOV-002 causes a transient change in physiological redox balance *in vitro and in vivo*
- This redox change may underlie the altered kinase response and, plausibly, the clinical effects of NOV-002
- Specific plasma protein S-glutathionylation represents a potential NOV-002 pharmacodynamic biomarker