NOV-002, a Glutathione Disulfide Mimetic, Is a Pleiotropic Modulator of Cellular Redox Balance

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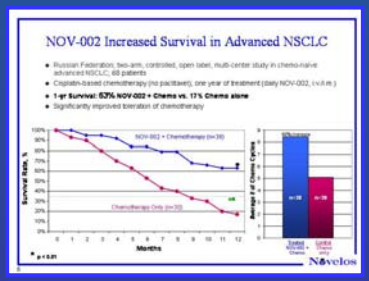
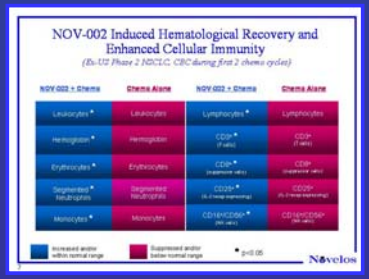
ABSTRACT

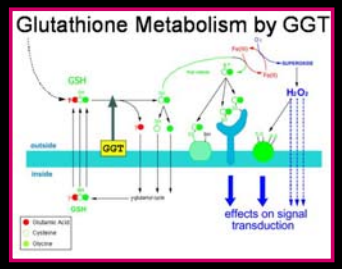
NOV-002 is a novel formulation of oxidized glutathione (GSSG) currently in a pivotal Phase 3 clinical trial in advanced non-small cell lung cancer. In clinical trials conducted to date, NOV-002 administered in combination with standard chemotherapeutic regimens has resulted in increased efficacy (survival, tumor response) and improved toleration (e.g. hematological recovery, immune stimulation). The studies reported here were aimed at further elucidating its cellular and *in vivo* pharmacologic profile. The effects of NOV-002 were assessed on a range of cellular and *in vivo* endpoints reflecting its proposed key pharmacological action – modulation of redox balance. NOV-002 treatment of the pre-myeloid cell line HL60 resulted in mild and transient time- and concentration-dependent oxidative signals at the cell surface (reduction in protein thiols) and intracellularly (altered GSSG and GSH levels and ratio). These oxidative signals were associated with an increase in S-glutathionylation of cell proteins, particularly actin, with a concomitant decrease in focal adhesions as detected by fluorescence microscopy. Intravenous administration of NOV-002 to mice also resulted in a fingerprint of glutathionylated serum proteins which could represent a useful pharmacodynamic biomarker. Commensurate with the above *in vitro* effects, NOV-002 treatment of HL60 cells resulted in increases in activated (phosphorylated) forms of the signaling kinases p38, JNK and ERK and caused a dose-dependent increase in phosphorylation of three proteins that have previously been linked with hematopoiesis, AKT, JAK2 and STAT5. The effect of NOV-002 on enzymes involved in glutathione metabolism was evaluated. These multiple redox-associated cell-signaling actions occurred in the context of increased HL-60 cell proliferation after treatment with NOV-002. We conclude that the pleiotropic pharmacological effects of NOV-002 can be attributed to the GSSG component of the drug, and that modulation of cellular redox balance is a feature central to NOV-002's mechanism of action. Such modulation may underlie its clinical actions, including hematological recovery and immunostimulation in the face of chemosuppression.

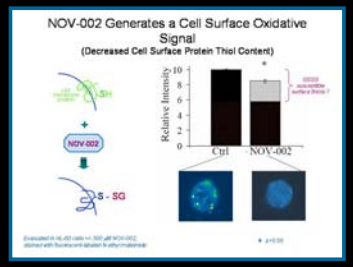
BACKGROUND





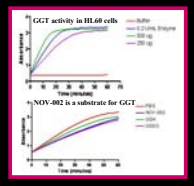
Extracellular Redox Signaling

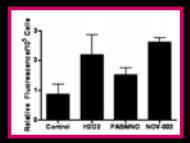




Effects of NOV-002 on cell surface and intracellular protein S-glutathionylation in HL60 cells. HL60 cells were treated with 300 µM NOV-002 or vehicle for 1h, placed on ice in the presence fluorescently labeled NEM. Cell surface cysteine modifications were semi-quantitated by flow cytometry

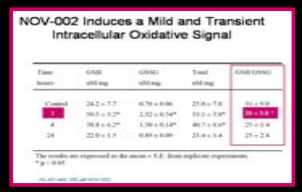
GGT is a cell surface enzyme that metabolizes extracellular glutathione. The activity of GGT was measured in HL60 cells. Elevated levels of GGT in HL60 cells provide the rational for altered intracellular levels of GSH/GSSG following NOV-002 treatment. Purified GGT was used to confirm that NOV-002 is a substrate of the enzyme.

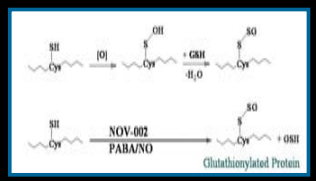


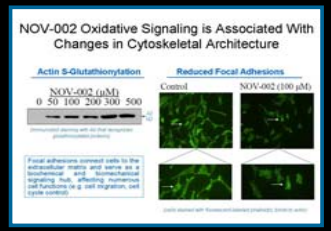


Extracellular ROS levels were measured in HL60 cells 1h following 100 uM H202; 25 uM PABA/NO or 300 uM NOV-002 treatment. N=3 +/- STD

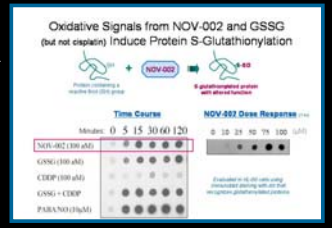
Intracellular Redox Signaling







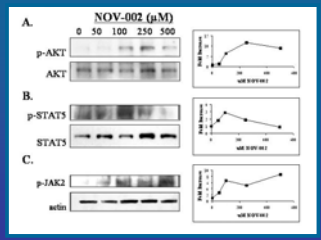
Concentration and time dependent effects of NOV-002 on actin S-glutathionylation in HL60 cells. 1.5 x 106 cells were treated with varying concentrations of NOV-002 for 1h. Following treatment, 20 µg of lysate was separated by SDS-PAGE under non-reducing conditions and analyzed for S-glutathionylation by immunoblot. Actin modification was observed by placing control and treated cells on poly-lysine coated cover slips and staining for phalloidin.



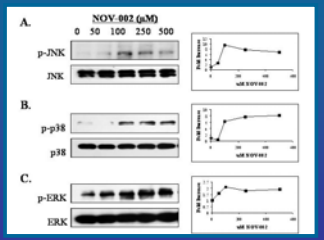
Concentration and time dependent effects of NOV-002 on intracellular protein S-glutathionylation in HL60 cells were evaluated by dot-blot. 1.5 x 106 cells were treated with varying concentrations of NOV-002, GSSG, PABA/NO or cisplatin (CDDP). Following treatment for 1 hr, 1 µL was dotted onto a nitrocellulose membrane and analyzed for S-glutathionylation by immunoblot.

Intracellular Kinase Signaling

JAK-STAT Pathway

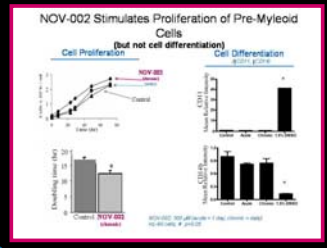


MAP Kinase Pathway



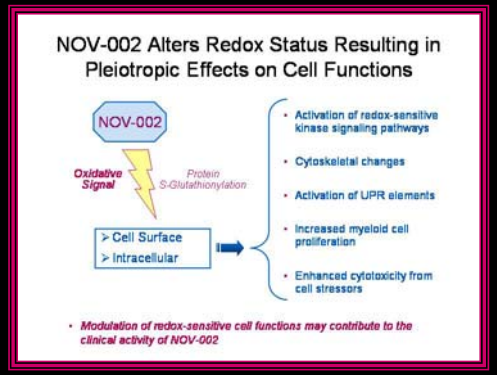
Concentration dependent effects of NOV-002 on stress kinases and their phosphorylated products are consistent with actin glutathionylation patterns. HL60 cells were treated for 1h with 0-500 µM NOV-002 in complete media. 50 µg protein lysate was separated by SDS-PAGE

Pre-Myeloid Cell Proliferation



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.

SUMMARY



Novelos Therapeutics Inc.