

Mechanism of Action Studies of NOV-002; a Novel Glutathione Disulfide Anti-cancer Drug

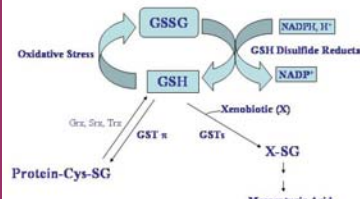
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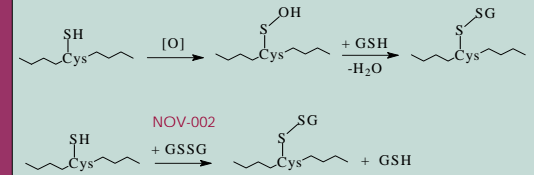
ABSTRACT

Building on an glutathione-based drug discovery platform, Novelos Inc. are developing NOV-002, a drug that contains 99.95% oxidized glutathione (GSSG) as the disodium salt coordinated with 0.05% cisplatin. The drug has shown clinical benefit and a good safety profile in Russia, and in a smaller US Phase I/II trial in non-small cell lung cancer. At effective *in vivo* drug concentrations, the cisplatin component is at sub-therapeutic levels. As such, we have focused preclinical studies on the oxidized glutathione (GSSG) constituent. NOV-002 is an alternative substrate for glutathione reductase with kinetics equivalent to GSSG. Using an immunoblot approach with monoclonal antibodies against cysteine-glutathione, concentrations of 50 to 500 μ M NOV-002 were shown to cause a rapid (15 minutes) S-glutathionylation of proteins, most predominantly actin. Less S-glutathionylation was observed in Mouse Embryo Fibroblasts (MEF) GST p^{-/-} cells as compared to MEF wild type cells. This result implies that GST p plays a role in GSSG, or NOV-002 induced S-glutathionylation of cellular proteins. S-glutathionylation is an important post-translational modification of low pKa cysteine residues, triggered by cell exposure to oxidative or nitrosative stress. NOV-002 also causes phosphorylation of both ERK and p38, two kinases with critical regulatory roles in governing cell proliferation and apoptosis. The data parallel in both a time and dose dependent manner those results obtained with GSSG, emphasizing that this is the active component of the drug. Overall, these data support the general importance of GSSG in regulating proliferation in normal cells and perhaps, apoptosis in cancer cells. NOV-002 provides a source of GSSG with bioavailability and kinetic properties that permit therapeutic activity.

Glutathione Metabolism



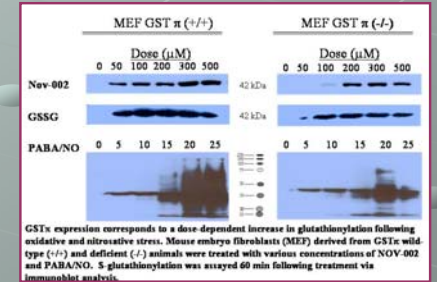
NOV-002 Induces S-Glutathionylation of Proteins



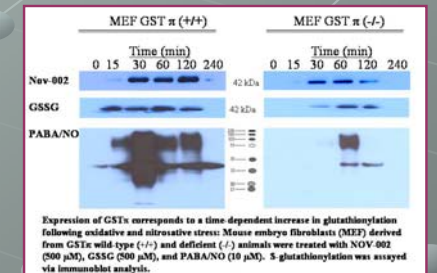
NOV-002 increases the half-life and bioavailability of GSSG without inducing cytotoxicity

MT cytotoxicity assays for NOV-002 and GSSG (% cell viability)

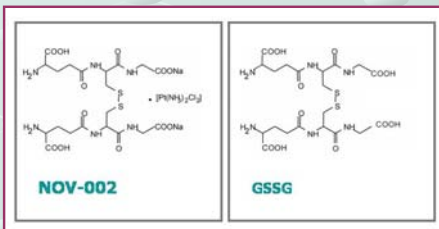
		MEF WT	MEF GST π KO
NOV-002:	200 μ M	114.99 \pm 9.43%	113.68 \pm 14.39%
	500 μ M	107.67 \pm 14.86%	94.59 \pm 4.01%
GSSG:	200 μ M	87.74 \pm 9.27%	83.20 \pm 9.76%
	500 μ M	101.48 \pm 8.02%	83.19 \pm 13.40%



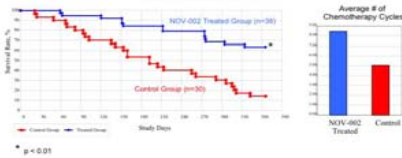
GST π expression corresponds to a dose dependent increase in glutathionylation following oxidative and nitrosative stress. Mouse embryo fibroblasts (MEF) derived from GST π wild type (+/+) and deficient (-/-) animals were treated with various concentrations of NOV-002 and PABA/NO. S-glutathionylation was assayed 60 min following treatment via immunoblot analysis.



Expression of GST π corresponds to a time dependent increase in glutathionylation following oxidative and nitrosative stress. Mouse embryo fibroblasts (MEF) derived from GST π wild type (+/+) and deficient (-/-) animals were treated with NOV-002 (500 μ M), GSSG (500 μ M), and PABA/NO (10 μ M). S-glutathionylation was assayed via immunoblot analysis.



NOV-002 1 Year Survival and # of Chemo Cycles Russian NSCLC Trial



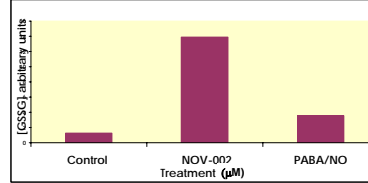
NOV-002 Increases Exposure to GSSG

	Control	NOV-002 *	GSSG-(H ₂ O ₂)
Serum (μg/ml)	0.2 \pm 0.01	9.1 \pm 0.93	0.3 \pm 0.05
Lymphocytes (fg/10 ⁶ cells)	8.9 \pm 0.96	287 \pm 20.8	8.7 \pm 0.8
Liver (μg/g tissue)	9.1 \pm 0.9	367 \pm 32.9	8.9 \pm 0.9

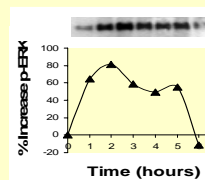
The metabolic consequences of administering NOV-002 were studied in serum, liver and lymphocytes of mice. * 2 mg/kg of NOV-002 and GSSG (500 μ M). GSSG (500 μ M) dosing with a compound similar to NOV-002 but with H₂O₂ in place of cisplatin. All measurements were taken 60 minutes after dosing except control values which were taken before dosing. Values presented in the table are mean \pm standard deviation.

Russian Pre-Clinical Data

NOV-002 treatment leads to an increase in cellular GSSG levels



Nov-002 treatment induces ERK activation



HL60 cells were treated with 50 μ M NOV-002 for 1h. Phosphorylation of ERK was evaluated by immunoblot and quantitated.

Conclusions:

NOV-002 potentiates the therapeutic response in NSCLC
NOV-002 is not toxic
NOV-002 activates the ERK signaling pathway
NOV-002 leads to time & dose dependent S-glutathionylation