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

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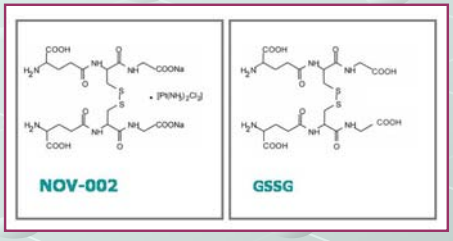
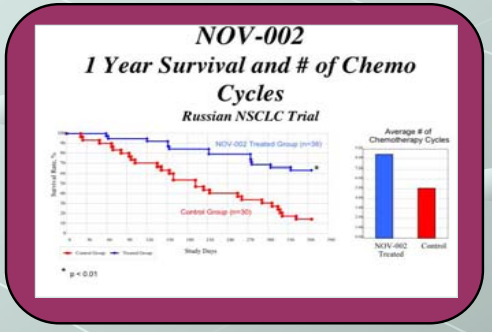
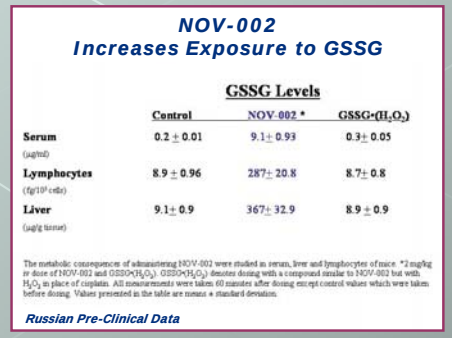
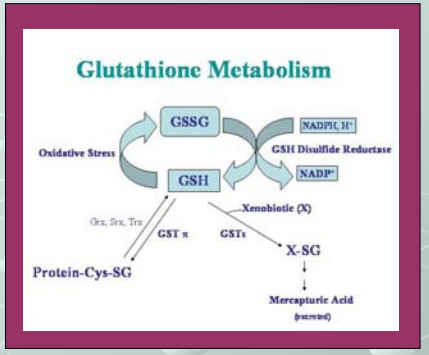
ABSTRACT

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 500μM 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.





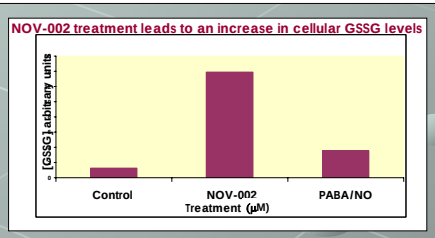




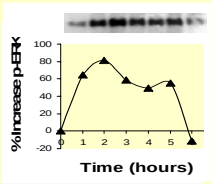
NOV-002 increases the half-life and bioavailability of GSSG without inducing cytotoxicity

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

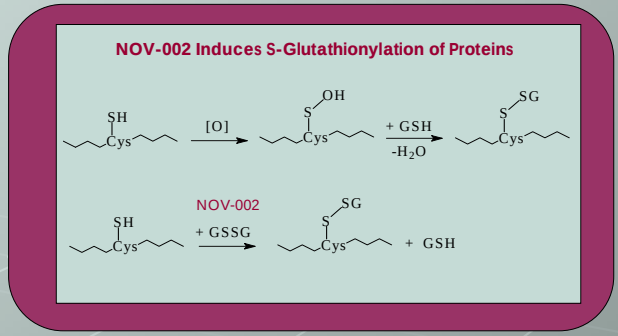
		MEF WT	MEF GSTπ KO
NOV-002:			
	200μM	114.99 ± 9.43%	113.68 ± 14.39%
	500μM	107.67 ± 14.86%	94.59 ± 4.01%
GSSG:			
	200μM	87.74 ± 9.27%	83.20 ± 9.76%
	500μM	101.48 ± 8.02%	83.19 ± 13.40%

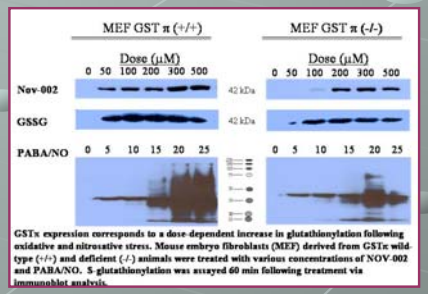


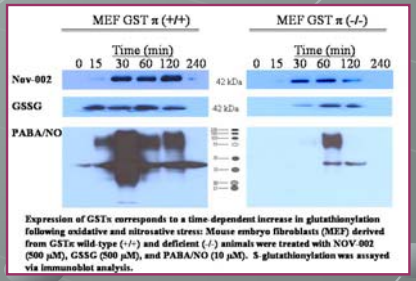
Nov-002 treatment induces ERK activation



HL60 cells were treated with 50 μM NOV-002 for 1h. Phosphorylation of ERK was evaluated by immuno-blot 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