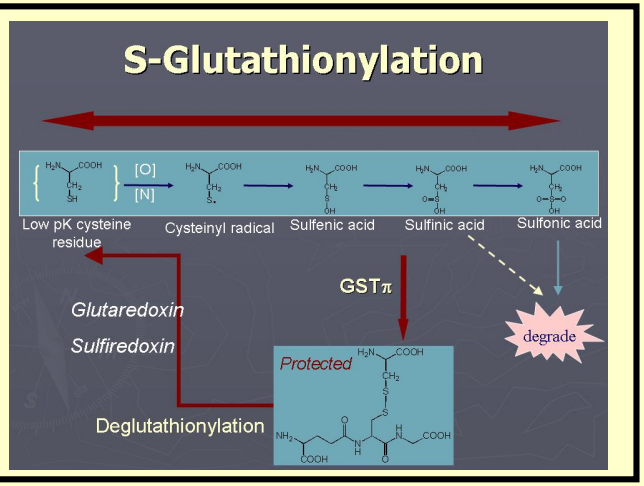
Serpin-A1 and A3 as potential pharmacodynamic biomarkers for NOV-002, a redox modulating anticancer agent

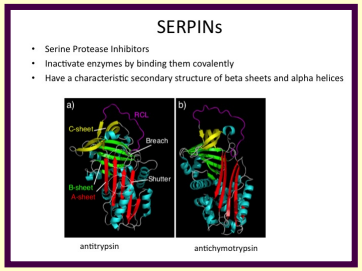
Christina L. Grek[2], Danyelle M. Townsend[1], Joachim D. Uys[2], Christopher J. Pazoles[3] and Kenneth D. Tew[2]

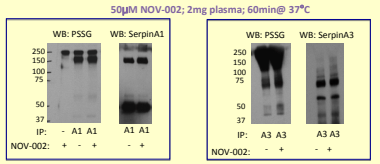
Depts. Biomedical and Pharmaceutical Sciences[1]; Cell and Molecular Pharmacology and Experimental Therapeutics,[2] Medical University of South Carolina 173 Ashley Ave., Charleston, SC 29425; Novelos Therapeutics, Inc.[3], One Gateway Ctr, Newton, MA 024584

Abstract

NOV-002 is a formulation of disodium glutathione disulfide (GSSG) that is in Phase II clinical trials for the treatment of breast in combination with standard chemotherapeutic regimens. NOV-002 has been shown to mitigate chemotherapy-induced hematological suppression. In animal studies it was found that, when administered after chemotherapy, NOV-002 stimulated proliferation of bone marrow progenitor cells and normalized peripheral blood levels of leukocytes and platelets. NOV-002's pharmacokinetic properties demonstrate first order absorption / elimination with a plasma half-life of ~13 mins. The redox regulation of protein thiols persist in plasma for ~4 hours. Using mass spectrometry, we identified that Serpin-A1 and -A3 were S-glutathionylated following NOV-002 administration to mice. Serpin-A1 and –A3 were also glutathionylated in a dose- and time- dependent S-glutathionylation following NOV-002 treatment of mouse or human plasma *in vitro*. There is evidence that members of the Serpin protein family can influence myeloproliferation and hematopoetic progenitor cell mobilization (Winkler et al., 2005; van Pel et al., 2006). Specifically, down regulation of serpins has been demonstrated in bone marrow during progenitor cell mobilization (van Pel et al., 2006), and this influences the marrow microenvironment and migratory behavior of hematopoetic precursor cells. Furthermore, glutathionylation of Serpins has been shown to inhibit their activity (Taygi et al., 1991 and 1992). Since our data shows that Serpins A1 and A3 are S-glutathionylated in plasma following NOV-002 treatment, there may be a mechanistic link between this effect and its *in vivo* myeloproliferative actions. Moreover, liberation of serpins into the peripheral circulation may also provide an indication that proteolytic pathways have been activated. Since the plasma half-life of S-glutathionylated proteins is ~4 hours (Townsend et al., 2006), it may be possible to identify and develop these post-translationally modified blood proteins as pharmacodynamic biomarkers.



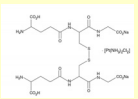




Immunoprecipitation of Serpin A1 and A3 from NOV-002 treated plasma confirms S-glutathionylation. Human plasma was treated with 50 uM NOV-002 for 1h. Biotinylated Serpin A1 and A3 rabbit polyclonal antibodies were used to pull down the proteins. The immunoprecipitated proteins were separated by non-reducing SDS-PAGE and S-glutathionylated proteins were evaluated by immunoblot with PSSG antibody. The blots were stripped and reprobed with anti-serpin A1 or A3 as a loading control. Due to high homology, there is significant cross reactivity of anti-A1 and A3.

Background:

NOV-002: a glutathione disulfide mimetic

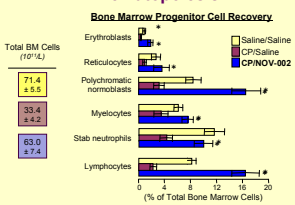


-Oxidized glutathione (GSSG) w/ cisplatin at a molar ratio of ~1000:1

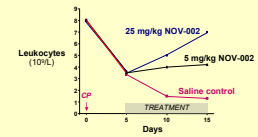
-Causes protein S-glutathionylation & myeloproliferation

-The goal of these studies is to understand and predict clinical response to treatment with NOV-002

NOV-002 Restores Chemosuppressed Hematopoiesis

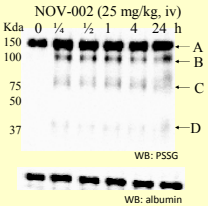


Rats (15/gp) were treated with cyclophosphamide (CP, 50 mg/kg, ip). Day 3 -- treated with NOV-002 (5 mg/kg, i.p.) or saline, daily for 10 days and femoral bone marrow analyzed. ✱ p<0.05 vs. chemosuppressed + saline group.



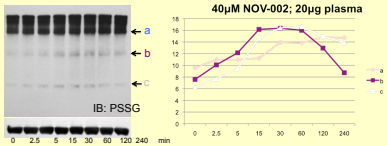
Mice (15/gp). Day 0 - treated with cyclophosphamide (CP, 50 mg/kg, ip). Days 5-15 - treated with NOV-002 (i.p.) or saline

NOV-002 induces protein S-glutathionylation in mouse and human plasma : a potential biomarker for myeloproliferation



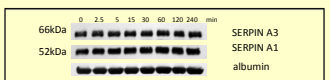
NOV-002 treatment induces S-glutathionylated mouse plasma proteins. Mice were treated with an i.v. bolus of the oxidized glutathione mimetic, NOV-002 at 25 mg/kg. Blood was collected at various time points via orbital bleed. The plasma proteins were separated by non-reducing SDS-PAGE and S-glutathionylated proteins were evaluated by immunoblot with PSSG antibody. The blots were stripped and reprobed with anti-albumin as a loading control.

Human Plasma



NOV-002 treatment induces S-glutathionylated human plasma proteins. Blood was collected from normal healthy volunteers and plasma was prepared. Plasma (20 ug) was treated with 40 uM NOV-002 for 0-240 min. The plasma proteins were separated by non-reducing SDS-PAGE and S-glutathionylated proteins were evaluated by immunoblot with PSSG antibody. The blots were stripped and reprobed with anti-albumin as a loading control.

S-glutathionylation of Serpins does not alter protein stability



Plasma (20 ug) was treated with 300 uM NOV-002 for 0-240 min. The plasma proteins were separated by SDS-PAGE and Serpin A3 and A1 were evaluated by immunoblot. The blots were stripped and reprobed with anti-albumin as a loading control.

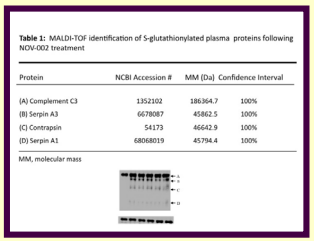
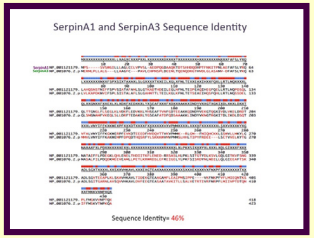


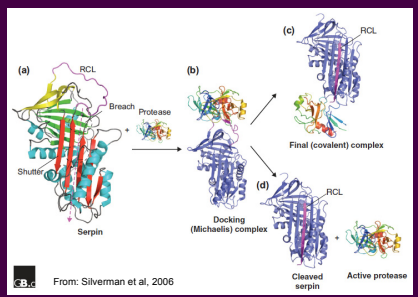
Table 1: MALDI-TOF identification of S-glutathionylated plasma proteins following NOV-002 treatment

Protein	NCBI Accession #	MM (Da)	Confidence Interval
(A) Complement C3	1352102	186364.7	100%
(B) Serpin A3	6678087	45862.5	100%
(C) Contrapsin	54173	46642.9	100%
(D) Serpin A1	68068019	45794.4	100%

MM, molecular mass



Possible pathway of activation of proteases by serpinA1 S-glutathionylation



Conclusions

- NOV-002 oxidative signaling results in S-glutathionylation of Serpins A1 and A3 in mouse (*in vivo*) and human (*in vitro*) plasma
- Serpin S-glutathionylation could serve as a pharmacodynamic biomarker for NOV-002 bioactivity
- Since glutathionylation is known to inhibit Serpin function, this effect of NOV-002 could contribute to its ability to reverse cyclophosphamide-induced hematological suppression