Immunomodulatory activity of NOV-002 potentiates the anti-tumor efficacy of cyclophosphamide (CTX) in the CT26 murine colon cancer model

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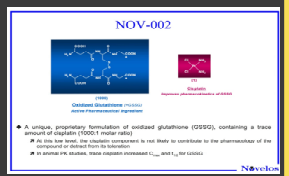
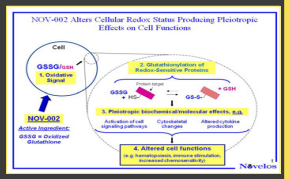
ABSTRACT

Clinical trials in a number of solid tumor indications have shown that the addition of NOV-002 (a formulation of disodium glutathione disulfide) to a range of cytotoxic chemotherapeutic regimens potentiates their anti-tumor efficacy. However, the specific mechanisms involved remain unclear. Previous *in vitro* studies demonstrated that NOV-002 generates oxidative signals in human tumor cell lines resulting in apoptosis and decreased proliferation. In addition, NOV-002 has been reported to display *in vivo* immunomodulatory properties in oncology settings. Hence, it (i) significantly increases circulating T-lymphocyte subsets [CD4+, CD8+, NK-T lymphocytes] in non-small lung cancer patients receiving chemotherapy plus NOV-002 vs. chemotherapy alone; (ii) reverses T-cell suppressive effect of myeloid suppressor cells in mice; (iii) potentiates anti-tumor effect of adoptive immunotherapy in a mouse melanoma model; (iv) increases intra-tumor memory T-cells and anti-tumor immune responsiveness in a mouse model of ovarian cancer. Here, we sought to further the hypothesis that NOV-002 potentiates the anti-tumor effect of chemotherapy through immune-mediated mechanisms. To this end in the CT26 murine colon cancer model, tumor-bearing mice were treated with NOV-002 (25 mg/kg, i.p.) or saline (daily on days 1-14, then Monday-Friday, beginning 5 days post-tumor injection until tumor progression), followed by 200mg/kg cyclophosphamide (CTX) on days 6 and 9. In three independent experiments we found that while NOV-002 alone had only a modest effect on tumor growth rate, the addition of NOV-002 to CTX resulted in significantly slower tumor growth compared to CTX alone. Mean overall survival was significantly longer in CTX/NOV mice [90 vs 47 days; P=0.005]. Interestingly, all mice receiving CTX alone developed tumors, while approximately 40% of CTX/NOV-002 mice showed no tumor development. To further investigate whether failure of tumor development in these mice was due to an immune mechanism, they underwent tumor rechallenge. In two independent experiments only 1/7 mice (14%) developed tumors after rechallenge. To test the contribution of memory T-cells, which are known to home to the bone marrow, CTX/NOV-002 mice with tumor failure were sacrificed and marrows adoptively transferred to mice bearing 7 day old CT26 tumors. Significant delay in tumor progression was observed in mice receiving adoptively transferred marrow suggesting that the immunologic memory generated after CTX/NOV-002 treatment was sufficient to effectively prevent or regress tumor formation. Taken together, these data provide further support to the hypothesis that, when combined with chemotherapy, NOV-002's anti-tumor efficacy may be due at least in part to enhancement of anti-tumor cellular immune responsiveness.

NOV-002 Background

- The active ingredient in NOV-002 is **oxidized glutathione**
- Changes in the ratio of oxidized: reduced glutathione controls cellular redox state and can regulate protein function by the reversible formation of mixed disulfides between protein cysteines and glutathione (= **glutathionylation**).
- Protein glutathionylation by NOV-002 results in pleiotropic effects on cell functions including cell signaling pathways, cytoskeletal architecture and cytokine production and is associated with hematopoiesis, immune stimulation and increased chemosensitivity of tumor cells.
- NOV-002, in combination with standard chemotherapy, is the subject of two phase 2 trials: (i) in combination with doxorubicin-cyclophosphamide and docetaxel as part of neoadjuvant treatment of breast cancer; and (ii) in combination with carboplatin in platinum refractory ovarian cancer.





References
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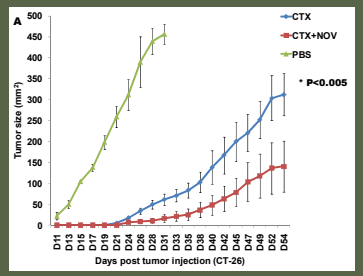
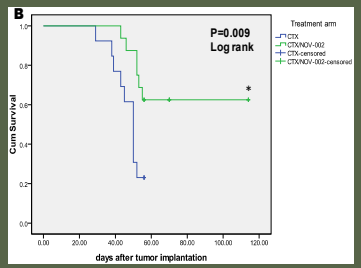
Townsend, DM et al. *Exp Opin Invest Drugs 2008; 17 (7): 1075-1083*

Addition of NOV-002 to Cyclophosphamide (CTX) significantly enhances tumor failure rates

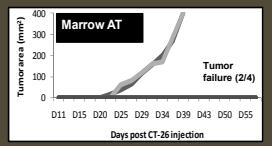




NOV-002 plus CTX delays tumor growth and enhances survival in the CT-26 tumor model. BALB/c mice were subcutaneously injected with 2.5x10^5 CT-26 cells. CTX 3 mg/mouse IP was administered days 6 and 9 post tumor injection when tumors were palpable. Daily NOV-002 injections (25mg/kg) were administered for 14 consecutive days, starting on day +5, then Mon-Fri until tumor >400mm^2. The addition of NOV-002 to CTX, resulted in significant delay in tumor progression (A) where tumor failure rate was ≅ 40% in 5 independent experiments; however tumor failure was not observed in any mice treated with CTX alone. This also translated into a significant overall survival benefit (B). Data representative of results from five independent experiments.

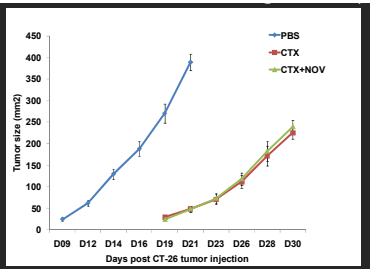
Synergistic Effect of NOV-002 with CTX may be due to an anti-tumor immune mediated effect

Mice with Tumor Failure after NOV-002 +CTX	Tumor growth after rechallenge with 2.5x10^5 CT-26 cells
N=15	1/15 (6%)



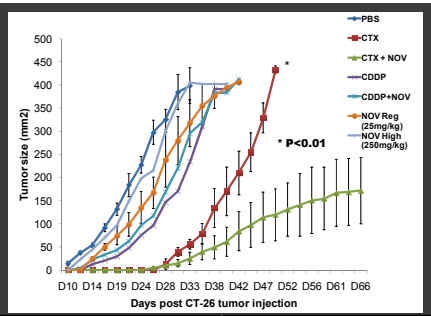
To test whether tumor failure associated with NOV-002/CTX, BALB/c mice with tumor failure were rechallenged with CT-26 cells approximately 30 days after stopping NOV-002 therapy which typically occurred after the last mouse in the CTX group was sacrificed due to tumor >400mm^2. In three independent experiments, mice were subcutaneously injected with 2.5x10^5 CT-26 cells. Approximately 6% of mice developed tumors upon rechallenge (n=1/15). To further determine whether this effect is immune-mediated, marrows from BALB/c mice who never developed tumors were collected. Naïve BALB/c ice received adoptive transfer (AT) of marrow on day +7 post tumor injection. CTX 3 mg/mouse IP was administered days 5 post tumor injection to induce transient lymphopenia prior to AT. To expand T-cells, mice were vaccinated on day after AT (irradiated CT26 cells). Mice did not receive any NOV-002. Interestingly, approximately 50% of mice who received AT had tumor failure.

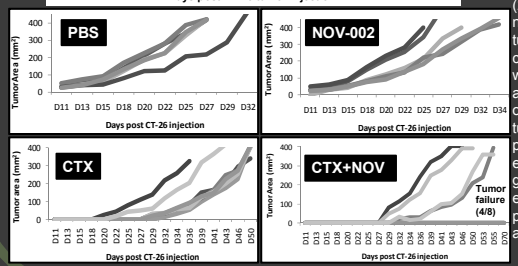
Tumor-failure effect with NOV-002 and CTX not seen in immunodeficient NOD-*Rag1^{null} IL2rγ^{null}* mice



To further test whether the effect of NOV-002 when combined with CTX is due to an immunomodulatory effect, similar experiments were performed where RAG (-/-) and gamma (-/-) double knockout mice were subcutaneously injected with 2.5x10^5 CT-26 cells. CTX 3 mg/mouse IP was administered days 6 and 9 post tumor injection when tumors were palpable. Daily NOV-002 injections (25mg/kg) were administered for 14 consecutive days, starting on day +5, then Mon-Fri until tumor >400mm^2. In two independent experiments, tumor re-growth rates were nearly identical for both groups.

NOV-002 Induced Tumor Failure Is Chemotherapy Specific





BALB/c mice were subcutaneously injected with 2.5x10^5 CT-26 cells. CTX 3 mg/mouse IP was administered days 6 and 9 post tumor injection when tumors were palpable. Daily NOV-002 injections (25mg/kg) were administered for 14 consecutive days, then Mon-Fri until tumor >400mm^2. While the addition of NOV-002 to CTX, resulted in significant delay in tumor growth (top and bottom panels), NOV-002 in the absence of CTX did not delay tumor growth and tumor failure was not seen even at a higher dose of NOV-002 (250mg/kg). Interestingly, NOV-002 did not result in any significant delay in tumor growth (upper panel) when combined with cisplatin as was seen with CTX. Cisplatin was administered as a single dose (200 micrograms) IP on day 6 post CT-26 injection when tumors were palpable. Top and bottom panel represent data from separate experiments. In the former, 10 mice per group, error bars represent standard error of the mean. Curves in bottom panel represent individual animals from a separate experiment.

CONCLUSIONS

- The addition of NOV-002 to CTX, but not cisplatin, resulted in tumor failure rates of approximately 40%.
- The vast majority of mice with CTX+NOV-002 associated tumor failure did not develop tumors upon rechallenge with CT-26 cells.
- Adoptive transfer of marrow cells collected from mice with tumor failure after NOV/CTX therapy resulted in regression of tumor in 50% of mice in a therapeutic setting.
- NOV-002 does not delay tumor growth of CTX-treated immunodeficient mice suggesting an immunomodulatory mechanism.
- Further studies are needed to determine the anti-tumor immunomodulatory mechanism of NOV-002.