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

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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 agents results in enhanced anti-tumor activity due to an anti-tumor immune mediated effect significantly enhancing tumor failure rates. 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+ , NK1.1+ ) in non-lieu 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 day 16. In these 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 (96 vs 47 days, P=0.005). Interestingly, all mice receiving CTX alone developed tumors, while approximately 40% of CTX/NOV mice showed no tumor growth after rechallenge with CTX. To test whether tumor failure associated with NOV-002/CTX, BALB/c mice with tumor failure were sacrificed and marrows were collected from mice receiving NOV-002 plus CTX. BA12/mice were subcutaneously injected with 2.5x105 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 regression. 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 represent a combination of results from five independent experiments.

Conclusions

- The active ingredient in NOV-002 is oxidized glutathione.
- Changes in the ratio of oxidized/reduced glutathione control 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 function including cell signaling pathways, cytoskeletal architecture and cytokine production and is associated with hematopoiesis, immune stimulation and increased tumor progression.
- NOV-002, in combination with standard chemotherapy, is the subject of two phase 2 trials. (i) in combination with docetaxel-cyclophosphamide and doxorubicin as part of neoadjuvant treatment of breast cancer and (ii) in combination with carboplatin in platinum refractory ovarian cancer.

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

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

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 adopting NOV-002 therapy which typically occurred after the last mouse in the CTX group was sacrificed due to tumor >400mm2. In three independent experiments mice were subcutaneously injected with 2.5x105 CT-26 cells. Approximately 6% of mice developed tumors upon rechallenge (n=115). To further determine whether this effect is immune-mediated, marrows from BALB/c mice who never developed tumors were collected. Novel BALB/c mice were adoptive transfered AT. To test if CT-26 tumor injection. CTX 3 mg/mouse IP was administered days 5 post tumor injection to reduce transient lymphopenia prior to AT. To expand CT-26 mice were vaccinated on day after AT (i.m. 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-Rag1null IL2rnull 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 Rag1(-/-) and gamma(-/-) mice were treated with NOV-002/CTX. Cisplatin was administered for 14 consecutive days, starting on day +5, then Mon-Fri until tumor regression. 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 45%.
- The effect of NOV-002 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.

NOV-002 Background

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- Changes in the ratio of oxidized/reduced glutathione control 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 function including cell signaling pathways, cytoskeletal architecture and cytokine production and is associated with hematopoiesis, immune stimulation and increased tumor progression.
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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 2.5x105 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 regression. 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 represent a combination of results from five independent experiments.