Nov-002, a cellular redox modulator, enhances the antitumor effect of adoptively transferred t cells in a murine melanoma model

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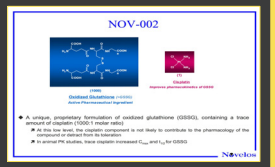
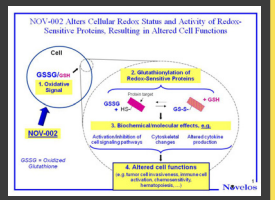
ABSTRACT

Oxidative signaling involving the glutathione system of redox control has been implicated in the regulation of T cell function. NOV-002, a glutathione disulfide mimetic, added to standard chemotherapy increased anti-tumor activity and survival in advanced non-small cell lung cancer patients compared to chemotherapy alone. Similarly, NOV-002 treatment significantly increased circulating T cell levels [$CD4^+$, $CD8^+$] in these patients. Here, we investigated whether the addition of NOV-002 to our previously established murine adoptive therapy (AT) regimen could enhance the anti-tumor activity of the transferred T cells. Adoptive transfer of early effector Pmel CD8+ cells activated in the presence of IL-12 ($Pmel^{IL12}$) into lymphopenic hosts is effective in regressing previously established B16 tumors. Daily administration of NOV-002 (25 mg/kg, i.p.) for 7 days following AT in C57BL/6 mice resulted in a significant delay in tumor progression when compared to mice subjected to AT alone, consistent with a marked enhancement of $Pmel^{IL12}$ anti-tumor immune activity. This enhanced anti-tumor effect was accompanied by a significantly longer median overall survival in mice treated with AT + NOV-002 compared to those treated with AT + saline [45 vs 28 days; 95% CI 34-56 days vs 23-32 days, respectively; p=0.02]. We then investigated, whether NOV-002 creates a more favorable micro-environment for expansion of transferred Pmel cells in a lymphopenic host. To this end, non-tumor bearing mice were treated with cyclophosphamide (CTX, 200mg/kg; to induce lymphopenia) or saline, and 7 days later $5x10^6$ Pmel cells were adoptively transferred into all mice. In addition, mice were treated with either NOV-002 (25 mg/kg, i.p.) or saline daily for 7 days following AT. All mice were vaccinated with gp100 (the melanoma tumor antigen towards which the Pmel T cells are specifically reactive) 24 hours after AT to elicit in vivo activation and proliferation of transferred Pmel cells. On day 3 after vaccination, peripheral blood was collected, and analyzed by flow cytometry for the presence of donor Pmel cells (= $Ly5.1^+$ cells) which represents T cell priming in response to vaccination. A significantly lower frequency of donor Pmel cells were seen in the animals treated with CTX relative to saline controls (9% vs. 20%; p=0.01). The addition of NOV-002 to CTX treatment, however, resulted in significantly higher frequencies of activated Pmel cells than CTX alone (18% vs 9%; p=0.03), and which were comparable to levels seen in saline controls. Taken together, these results indicate that NOV-002 enhances the effect of AT thereby generating a significantly greater anti-tumor T cell response, decreasing tumor growth and increasing survival. Moreover, NOV-002 enhances the expansion of CD8+ T cells in a lymphopenic host. These findings are consistent with the hypothesis that enhanced immune responsiveness may contribute to the clinical profile of NOV-002 in oncology trials.

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 alterations in cell functions including cell signaling pathways, cytoskeletal architecture and cytokine production and is associated with increased chemosensitivity of tumor cells, immune stimulation and hematopoiesis.
- NOV-002, in combination with standard chemotherapy, is the subject of an ongoing pivotal Phase 3 trial in advanced non-small cell lung cancer and 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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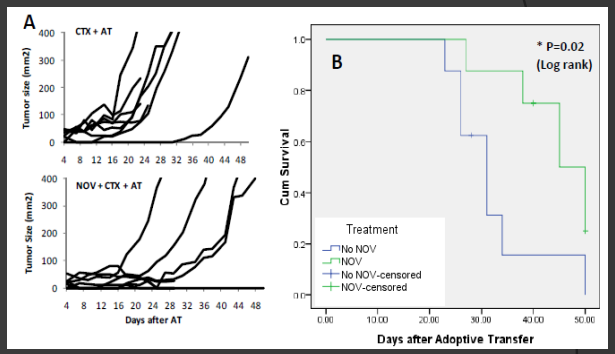
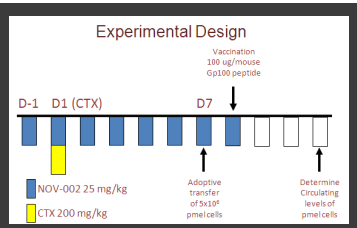
Townsend, DM et al. *Exp Opin Invest Drugs 2008; 17 (7): 1075-1083*

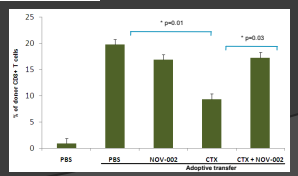
NOV-002 significantly enhances survival benefit and anti-tumor effect of adoptive transfer of $pmel^{IL12}$ cells



NOV-002 significantly enhances the therapeutic benefit of adoptively transferred CD8+ pmel cells. Daily NOV-002 injections for 7 days, beginning 2 days after adoptive transfer (AT) of $pmel^{IL12}$, and 1 day after CTX, resulted in significant delay in tumor growth (A) and also conferred a significant overall survival benefit (B). Data represents results from two independent experiments.

NOV-002 increases *in vivo* expansion of adoptively transferred Pmel cells in a lymphopenic host





On day 3 after vaccination, peripheral blood was collected, and analyzed by flow cytometry for the presence of donor Pmel cells, vis-à-vis donor Ly5.1 expression. Dot plots on the left show data from a representative animal. PBMCs were stained with anti- CD8 and anti-Ly5.1 mAbs. Data shows the percentage of donor (Ly5.1+) Pmel cells gated on the CD8+ fraction. Figure on the right shows the average of the percentage of donor CD8+ T cells in each experimental group ± SDEV. A significantly lower frequency of donor Pmel cells in the animals treated with CTX was observed. The addition of daily NOV-002 to CTX treatment, however restored frequencies of activated Pmel cells to levels similar to PBS controls.

Conclusions

- NOV-002 potentiated the ant-tumor effect of adoptively transferred pmelIL12 T-cells in a melanoma model, resulting in significant delays in tumor growth and increased survival relative to adoptive transfer alone.
- NOV-002 administration in a non-tumor bearing lymphopenic host resulted in significantly increased numbers of circulating adoptively transferred Pmel cells.
- Further studies are needed to determine precise mechanisms by which NOV-002 enhances expansion of adoptively transferred Pmel cells in a lymphopenic host in response to vaccination, and enhances their anti-tumor activity.
- These results extend earlier *in vivo* and *in vitro* findings (see references below), suggesting that NOV-002 possesses immunomodulatory activity that is of potential relevance to its clinical efficacy profile.

References

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