Downregulation of T Cell Activity by Cyclophosphamide-Induced Myeloid Derived Suppressor Cells is Reversed by the Glutathione Disulfide Mimetic NOV-002

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ABSTRACT

NOV-002 is a unique glutathione disulfide mimetic that in early phase clinical trials has been shown to significantly enhance the anti-tumor activity of various types of cytotoxic chemotherapy in many different solid tumors, e.g. non-small cell lung cancer, ovarian cancer, and breast cancer. NOV-002 has been shown to have direct immunomodulatory effects in patients, e.g. increases in circulating activated CD3+ T lymphocytes and NK cells. This is consistent with other data showing that small changes in intracellular lymphocyte GSH/GSSG levels have profound effects on lymphocyte function and survival. We have previously shown that cyclophosphamide (CTX) at doses used as part of standard adjuvant therapy for breast cancer leads to transient increases in myeloid derived suppressor cells (MDSC), and that CTX-derived MDSC have suppressive effects similar to those derived MDSC. Although the exact mechanism of action of NOV-002 is unknown, one hypothesis is that NOV-002 improves T cell activation through a direct action on MDSC by decreasing production of reactive oxygen species (ROS). To test this hypothesis we utilized a model of CTX-induced MDSCs. A single intraperitoneal injection of CTX (4mg) results in transient lymphopenia. The recovery phase is characterized by a surge of MDSCs in both peripheral blood and spleen. Our results showed that NOV-002 restored MDSC downregulation of T cell activation in response to antigenic stimulation. In our model, CTX-induced MDSCs showed decreased downregulatory capabilities after in vivo treatment with NOV-002. Although the overall levels of circulating MDSCs remained unchanged, NOV-002 treated MDSCs had decreased ROS production, even after blockade of glutathione synthesis with buthionine sulfoximine. Moreover, NOV-002 treated MDSCs showed lowering expression levels of iNOS. In conclusion, one immunomodulatory effect of NOV-002 could be through alteration of GSH/GSSG levels in MDSC resulting in decreased generation of ROS and hence decreased suppressive capabilities.

Effect of NOV-002 on MDSC mediated suppression of in vitro T cell activation

Levels of myeloid derived suppressor cells (MDSC) which are CD11b+ Ly6G+ were determined at day 7 post-CTX injection in splenocytes by flow cytometry. Treatment with NOV-002 had no significant effect on CTX-induced mobilisation of MDSCs.

Splenocytes from treated mice (as indicated) were co-cultured with purified Pmel cells. Pmel cells are transgenic for a TCR specific for a peptide present in the gp100 protein. Pmel cells co-cultured with splenocytes from CTX treated mice demonstrated a 40% decrease in their proliferative capacity when compared with untreated controls. The decrease in proliferative capacity was absent when Pmel cells were co-cultured with splenocytes from mice treated with CTX and NOV-002.

Effect of NOV-002 on MDSC mediated suppression of in vivo T cell activation

On day 3 after vaccination, peripheral blood was collected, and analyzed by flow cytometry for the presence of donor Pmel cells, via a 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 Pmel cells co-cultured with MDSCs from NOV-002 treated mice vs. Pmel cells co-cultured with MDSCs from PBS treated mice. 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.

References

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

NOV-002 decreases iNOS expression and ROS production in CTX-induced MDSC

Splenocytes from mice treated as indicated were stained with mAbs against Ly5.1, CD11b, and iNOS. Intracellular expression levels of iNOS was measured in MDSCs (Ly5.1+CD11b+) and total splenocytes. NOS levels are expressed as the average (n=6 from two independent experiments) Mean Fluorescence Intensity (MFI) ± SDEV.

There was a significant decrease in the levels of expression of NOS in both MDSCs and total splenocytes after the addition of NOV-002 to CTX treatment. NOS expression in MDSCs from PBS treated animals was not shown since the frequency of circulating MDSCs are very low (5%).

Effect of NOV-002 on MDSC mediated suppression of in vitro Tcell activation

Splenocytes from treated mice (as indicated) were co-cultured with purified Pmel cells. Pmel cells are transgenic for a TCR specific for a peptide present in the gp100 protein. Pmel cells were co-cultured with CD11b+Ly6G+ splenocytes by flow cytometry using the fluorescent probe CM2-DCFDA. Fluorescence was detected on the Ly6G+/CD11b+ population and is expressed as Mean Fluorescence Intensity (MFI) ± SDEV.

Splenocytes from mice treated with NOV-002 showed decreased iNOS expression and reduced ROS levels in CTX-induced MDSC.

SUMMARY

• Recovery from CTX-induced lymphopenia results in MDSC accumulation, which in turn are able to directly suppress T cell activation.
• Daily NOV-002 administration resulted in a significant reversal of MDSC-induced suppression of antigen-specific CD8+ T cell proliferation.
• In vivo following antigen-dependent in vitro activation of Pmel cells co-cultured with MDSCs from NOV-002 treated mice.
• In vivo following vaccination after adoptive transfer of naïve Pmel cells in mice treated with NOV-002.
• MDSCs from mice treated with NOV-002 showed decreased iNOS expression levels, which also correlated with significantly decreased ROS production. Since a major mechanism of MDSC mediated T cell suppression is through ROS accumulation, NOV-002 appears to have a direct immunomodulatory effect on MDSC by decreasing ROS.

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 disulfides between protein cysteines and glutathione. Disulfides can regulate protein function by reversible formation of mixed disulfides between protein cysteines and glutathione.
• Protein glutathionylation by NOV-002 results in pleiotropic effects on cell functions including cell signaling pathways, cytoskeletal architecture and cytoskeleton dynamics and is associated with chemoresistance, in vivo chemoresistance and increased chemosensitivity of tumor cells.
• NOV-002, in combination with standard chemotherapy, is also the subject of an ongoing 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.

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