

Positive immunomodulatory effects of NOV-002, an oxidized glutathione mimetic, in a murine model of ovarian cancer

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ABSTRACT

Background:

Immune control in ovarian cancer is critically dependent on CTL activation against immunogenic determinants and antigen specific T cell migration into the tumor. NOV-002 is the subject of an ongoing, pivotal Phase III clinical trial in advanced non-small cell lung cancer. In this indication, NOV-002 administered in combination with standard chemotherapeutic regimens has demonstrated increased efficacy (survival, tumor response) and improved toleration (e.g. hematological recovery, immune stimulation). Here we report its observed immunomodulatory properties in a syngeneic model of ovarian cancer.

Methods:

FVB mice were injected intraperitoneally with BR5-FVB1 cells (day 1). This cell line has been previously shown to generate ovarian tumors that closely resemble papillary carcinoma in humans. From day 2 mice received daily intraperitoneal injections of NOV-002 (30mg/kg) or saline (n = 15) until clinical evidence of ascites. Post-mortem collection of plasma, ascitic fluid, and tumor tissue was then performed. After tissue processing and T cell extraction, the expression of CXCR4, IFN-gamma and T-cell immune profile (CD3+, CD4+, CD8+, CD25+, and FoxP3+) in tumor-infiltrating lymphocytes (TIL), ascites and spleen were analyzed by multi-parameter flow-cytometry. Production of IFN-gamma by splenocytes from experimental and control animals, stimulated with lysed tumor cells, was used to assess tumor specific T cell function. The Mann-Whitney test was used to compare the study groups.

Results:

No significant differences in survival, tumor weight and time to ascites development were detected between the experimental and control group. Ascitic fluid T cell subpopulations were not significantly different between the NOV-002 or saline treated group. However, a significantly higher number of CD4+CD25+ cells was detected in TIL (p = 0.03) and spleen (p=0.02) in the NOV-002 treated group compared to the control group. In contrast, no significant differences were seen with regards to the presence of CD4+CD25+FoxP3 cells in the tumor or spleen. Preliminary results showed higher IFN-gamma production in NOV-002 treated animals (2131 pg/ml vs 104 pg/ml, with FVB-non tumor bearing mice and unstimulated splenocytes as negative controls).

Conclusions:

Our studies to date reveal that NOV-002 increases the infiltration of tumors and spleen by cells bearing a memory T cell phenotype. In addition, this increased infiltration of memory T cells into the tumor apparently induced by NOV-002 was associated with increased IFN-gamma release in the context of stimulation by tumor cell lysates. These preliminary data support the view that NOV-002 may have immunomodulatory functions in addition to chemoprotective activity in the context of anti-tumor chemotherapy. Further studies of the breadth and depth of anti-tumor specific responses of the infiltrating T cells in the context of NOV-002 treatment are underway as well as an exploration of the potential mechanistic link between alteration of cellular redox status and changes in anti-tumor immune responses.

NOV-002

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 (= alutathionylation).

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 also the subject of an ongoing pivotal Phase 3 trial in advanced non-small cell lung cancer and an exploratory Phase 2 trial for neoadjuvant treatment of breast cancer.

Townsend DM et al, *Cancer Res* 2008;68(8):2870-7

Townsend DM et al, *Expert Opin Investig Drug* 2008;17(7):1075-83



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METHODS

DAY 1

Intraperitoneal injection of FVB-BR5 ovarian cancer cells into immunocompetent FVB mice

DAY 2-21*

Daily intraperitoneal injections of NOV-002 (NOV) (30mg/kg) versus N-saline (SAL)

* until clinical evidence of ascites

Sacrifice

Tumor extraction and processing

- Single cell suspension obtained by tumor disaggregation/digestion
- Mononuclear cells further purified by centrifugation on Ficoll-Hypaque (tumor cells, stromal cells and immune cells)

T-cell immune profile (TIL, spleen, ascites cells)

- Staining of fresh mononuclear cells with fluorophore conjugated CD3+, CD4+, CD8+, CD4+CD25+, CD4+CD8+FoxP3+
- T cell subpopulations quantified per gram of tumor tissue

Splenocytes extraction

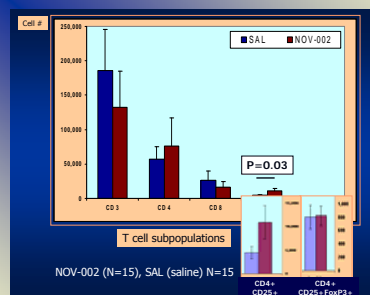
- Single cell suspension obtained by spleen disaggregation
- Mononuclear cells further purified by centrifugation on Ficoll-Hypaque

IFN-gamma detection

- In vitro culture of splenocytes with lysed tumor cells
- IFN-gamma detection (neg controls: non-tumor bearing mice, non-stimulated splenocytes)

RESULTS

T-cell subpopulations detection in tumor infiltrating lymphocytes (TIL) : NOV-002 versus control (SAL)

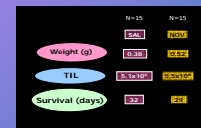


NOV-002 increases TIL CD4+CD25+FoxP3- subpopulation (P=0.03)

CD4+ T-cells are also higher (non significant) in the NOV-002 arm.

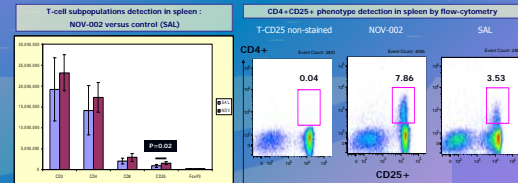
No differences were documented for T-effector/Tregs subpopulations

RESULTS



1. In absence of associated chemotherapy and in immunocompetent mice. No significant differences were detected for mouse survival or tumor weight between the two groups.

2. Significantly higher T-CD4+CD25+ cells were detected in the NOV-002 treated splenocytes compared to the saline control group (P=0.02)



3. Higher IFN-gamma production was detected in splenocytes from NOV-002 treated mice in response to tumor lysates in comparison to splenocytes from saline treated animals. (2131 pg/ml – NOV-002 vs 104 pg/ml – N-saline)

CONCLUSIONS

- NOV-002 increases the infiltration of cells bearing a memory T cell phenotype into syngeneic ovarian tumors
- Increased infiltration of T cells into the tumor is associated with increased IFN-gamma release in response to tumor lysates
- NOV-002 may have immunomodulatory functions in addition to chemoprotective activity in the context of anti-tumor chemotherapy

SUMMARY

