Daily injections of the glutathione disulfide mimic NOV-002 ameliorates hematologic toxicities from neoadjuvant chemotherapy in breast cancer patients enrolled in the NEO-NOVO trial, and significantly increases circulating dendritic cells.

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ABSTRACT

Background: Chemotherapy induced anemia is a common problem in breast cancer. Anemic events [Hgb<10 g/dL, use of erythropoietin stimulating agents (ESAs), or transfusion] are likely to occur in 30-40% of breast cancer patients (pts) receiving doxorubicin-cyclophosphamide (AC) and docetaxel (T). Due to the increased risk of cancer mortality, tumor progression, and thromboembolic events associated with ESAs, other alternatives are needed. Fibrin neutropenia with AC-T is a dose-limiting event and occurs in 10-20% of pts without prophylactic G-CSF. NOV-002 has been previously shown to stimulate hematopoiesis and reduce chemotherapy related hematologic toxicities, as well as have immunomodulatory properties. Methods: To determine the effect of daily NOV-002 on hematopoiesis, chemotherapy induced hematologic toxicities, weekly blood counts were obtained on all pts enrolled in the NEO-NOVO trial at SASCO 2007. Pts did not receive prophylactic G-CSF in the absence of prior fibrin neutropenia with chemotherapy. To determine if NOV-002 is associated with induction of circulating dendritic cells (DCs), flow cytometry analysis was performed on whole peripheral blood prior to therapy and on day 1 of each cycle. DCs are defined as Lin-CD14-CD11c+HLA-DR+. P-values were obtained from the signed rank test. Results: Thus far, 100 chemotherapy cycles have been administered in 19 pts. Baseline (BL) hemoglobin (Hgb), absolute neutrophil counts (ANC), and DCs are listed in Table 1. The grade 3-4 anemia has been observed. ESAs have been used in 9 pts (12.5%), and no blood transfusions were required. One (6.25%) had fibrin neutropenia (0.9% of all cycles). Grade 3 neutropenia on day 1 of cycle 1-8 occurred in 2 pts (12.5%) or 1.5% of all cycles. One pt had dose delay by 1 week due to neutropenia. Cycle 5 day 1 (C5D1) DCs were significantly higher relative to BL with a trend towards being higher at C8D1. Moreover, median ANCs were much lower than expected with the addition of NOV-002 to AC. Likely due to the hematopoietic properties, DC induction may be an important anti-tumor mechanism of action of NOV-002.

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.
• Protein glutathionylation by NOV-002 results in pleiotropic effects on cell function including increasing apoptosis and increased chemosensitivity of tumor cells.
• NOV-002, in combination with cytotoxic chemotherapy, is also 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 docetaxel- cyclophosphamide and doxalast as part of neoadjuvant treatment of breast cancer; and (ii) in combination with carboplatin in platinum refractory ovarian cancer.
• Four cycles of AC plus taxane in HDR-2- unamplified breast cancer patients results in a pathologic complete response (pCR) rate of >20%. Thus far in the ongoing phase 3 trial we have a confirmed pCR of 40% (Table 2).

REFERENCES

Von Minckwitz, G et al. JCO 2005; 23 (22): 4576-4586
Towatari, OM et al. Jpn J Clin Oncol 2008; 38 (7): 707-713

Trial Design, Patient Characteristics and Hematologic Data

Table 1

<table>
<thead>
<tr>
<th>Variable</th>
<th>BL MEAN (IQR)</th>
<th>C5D1 MEDIAN (IQR)</th>
<th>C8D1 MEDIAN (IQR)</th>
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<tbody>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>12.57 (11.9,13.5)</td>
<td>12.22 (11.4, 13.5)</td>
<td>10.96* (10.6,11.6)</td>
</tr>
<tr>
<td>Hematocrit</td>
<td>38.1 (36.4, 41)</td>
<td>33.7* (31.5, 35.9)</td>
<td>34.7* (32.2, 36.5)</td>
</tr>
<tr>
<td>ANC/mm^3</td>
<td>3794 (3380,4400)</td>
<td>2635* (3380, 3880)</td>
<td>4585* (3710,5240)</td>
</tr>
<tr>
<td>Lymphs/mm^3</td>
<td>1925 (1590,2620)</td>
<td>1270* (952, 1635)</td>
<td>1320* (1100, 1780)</td>
</tr>
<tr>
<td>DCs/mm^3</td>
<td>372 (97,446)</td>
<td>546* (377, 752)</td>
<td>541* (426,792)</td>
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</table>

Conclusions: To determine the effect of daily NOV-002 on mitigation of hematologic toxicities associated with AC and Docetaxel, and its effect on circulating DCs, differences between pretreatment values of each variable for each patient was compared to levels measured on day 1 of cycles 5 (C5D1) and 8 (C8D1). P values were calculated using the Wilcoxon signed rank test—a nonparametric analogue of the paired t-test which does not require the normality assumption. Clinical characteristics of patients enrolled in the trial are detailed on the table to the left as well as pathologic data after completion of neoadjuvant chemotherapy. Interestingly, both hemoglobin and hematocrit levels remained stable between completion of AC (C5D1) and on the last cycle of Docetaxel (C8D1). Thus far no grade 3 anemia has been observed, no blood transfusions, and EPO use in only 2/19 pts.

NOV-002 associated with increased frequency of circulating DCs

Blood for dendritic cell analysis by flow cytometry was collected on day 1 of each cycle. DC counts were defined as Lin-CD14-CD11c+HLA-DR+. Absolute counts were obtained by multiplying by WBC count (DCs/microliter). Left panel represents longitudinal modeling of ratio DCs/baseline value. Right panel represents values averaged during AC and Tax cycles (cycles 1-4) and AC and Tax cycles (cycles 5-8). P values were obtained from the signed rank test. P values for each comparison are listed in the upper right hand corner.

Effect of daily NOV-002 on ANC

Longitudinal modeling for ANCs (left panel) and averaged data during AC and T (right) suggest that ANCs become progressively higher during NOV-002 therapy particularly with docetaxel. Only 1 patient (5%) required G-CSF support with one cycle of chemotherapy or 0.8% of all cycles. Febrile neutropenia occurred in one patient (6.35%) or 0.8% of all chemotherapy cycles. By contrast, historic febrile neutropenia rates with docetaxel alone are approximately 20%.

NOV-002 ameliorates chemotherapy induced anemia

Weekly complete blood counts were obtained. Mixed regression models were estimated with subject specific intercepts which accounts for the longitudinal structure of the data. The baseline variance was normalized by dividing by pretreatment (baseline) variance and log-transformed. Covariates included the day on study and day on study squared values, the categorical variable of day (1, 8 and 15) and the (interaction) on study on day squared values. Lower lower limits were when day 1 was 1, lower lower limits were when day 8 was 8, and lower lower limits were when day 15 was 15. Lower lower limits had 95% confidence intervals (blue lines). Mixed regression models with patient specific intercepts were also created for HGB and HCT (upper right and lower lower panels, respectively). Baseline, AC (cycles 1-4) and Tax (cycles 5-8) were compared. P values for each comparison are listed in the upper right hand corner.

SUMMARY

• The addition of NOV-002 to AC-T is associated with PCR rates much higher than expected in HER2-negative breast cancer population.
• PCR rates with NOV-002 highest in patients with hormone receptor positive breast cancer, the subtype least likely to respond to chemotherapy.
• Anemic events and febrile neutropenia were much lower than expected with the addition of NOV-002 to AC and T.
• NOV-002 should be further explored as an alternative to ESAs and G-CSF in the context of ameliorating chemotherapy induced hematologic toxicities.
• NOV-002 was associated with significant induction of circulating DCs and may be an important anti-tumor mechanism of action.