Daily injections of the glutathione disulfide mimetic 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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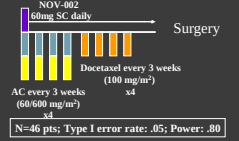
ABSTRACT

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). Owing to the increased risk of cancer mortality, tumor progression, and thrombovascular events associated with ESAs, other alternatives are needed. Febrile 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 and chemotherapy induced hematologic toxicities, weekly blood counts were obtained on all pts enrolled in the NEO-NOVO trial (Montero, et. al. SABCS 2008). Pts do not receive prophylactic G-CSF in the absence of prior febrile neutropenia with chemotherapy. To determine if NOV-002 is associated with induction of circulating dendritic cells (DCs), flow cytometry analysis is performed on whole peripheral blood prior to therapy and on day 1 of each cycle. DCs are defined as: lineage$^-$/HLA-DR$^+$; CD11b$^+$/CD11c$^+$. P-values were obtained from the signed rank test. **Results:** Thus far, 102 chemotherapy cycles have been administered to 16 pts. Baseline (BL) hemoglobin (Hgb), absolute neutrophil counts (ANC), and DCs are listed in Table 1. No grade 3-4 anemia has been observed. ESAs have been used in 2 pts (12.5%), and no blood transfusions were required. One pt (6.25%) had febrile neutropenia (0.9% of all cycles). Grade 3 neutropenia on day 1 of cycles 1-8 occurred in 2 pts (12.5%) or 1.9% 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 significantly higher on C8D1, while median Hgb remained stable between C5D1 and C8D1. **Conclusions:** Anemic events and febrile neutropenia were much lower than expected with the addition of NOV-002 to ACT, likely due to its hematopoietic properties. DC induction may be an important anti-tumor mechanism of action of NOV-002.

Trial Design, Patient Characteristics and Hematologic Data

Newly Diagnosed Invasive Breast Cancer
Clinical Stages II-III HER-2 –

Primary Endpoint: Pathologic + near pathologic complete response



N=46 pts; Type I error rate: .05; Power: .80

Table 2. Patient Characteristics and confirmed pathologic response rate	Number (%)
Total patients enrolled	**19**
	127
Total administered cycles	
Surgically evaluable patients	**15**
Pathologic complete response*	***6 (40)***
Clinical tumor size	
T2	**15****
T3	**5**
T4	**0**
Clinical node status	
N0	**12 (63)**
N1	**6 (32)**
N2	**1 (5)**
Age	
<40	**3 (16)**
40-50	**5 (26)**
50-60	**6 (32)**
>60	**5 (26)**
Hormone receptor status	
ER+PR+	**12 (60)**
ER+PR-	**3 (15)**
ER-PR+	**2 (10)**
ER-PR-	**3*(15)**

*Defined as N0 and residual invasive tumor <10mm
**1 patient with bilateral synchronous T2N0 breast cancer

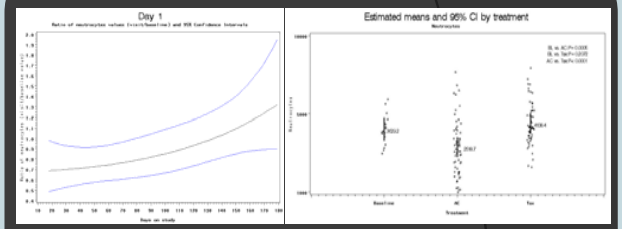
Table 1

Variable	BL MEAN (IQR)[1]	C5D1 MEDIAN (IQR)	C8D1 MEDIAN (IQR)
Hemoglobin (g/dL)	12.57 (11.9,13.5)	10.96* (10.6,11.6)	11* ^^ (10.3,11.6)
Hematocrit	38.1 (36.4, 41)	33.7* (31.8, 35.4)	34.7 ^^ (32.2, 36.5)
ANC/mm^3	3794 (3150,4140)	2633* (1380, 3880)	4558* § (3710, 5240)
Lymphs/mm^3	1925 (1590, 2620)	1270* (852, 1635)	1320* (1100, 1780)
DCs/mm^3	372 (97, 446)	544* (377, 592)	541^^ (216, 792)

[1]IQR: interquartile range (25th-75th percentiles); BL: baseline; C: cycle; D: day; *P ≤0.01 (vs. BL); §P=0.0002 (vs.C5D1); ^^P=not significant (vs. C5D1)

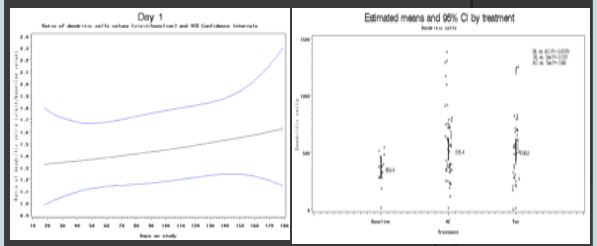
To determine the effect of daily NOV-002 on mitigation of hematologic toxicities associated with AC and Docetaxel, and its effect on circulating DCs, differences between pretreatment values of each variable for each patient was compared to levels measured on day 1 of cycles 5 (C5D1) and 8 (C8D1). P values 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.

Effect of daily NOV-002 on ANC



Longitudinal modelling 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 GCSF 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%

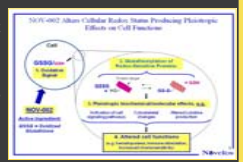
NOV-002 associated with increased frequency of circulating DCs



Blood for dendritic cell analysis by flow cytometry was collected on day 1 of each cycle. DCs were defined as: lineage$^-$/HLA-DR$^+$; CD11b$^+$/CD11c$^+$. Absolute counts were obtained by multiplying by WBC count (DCs/microliter). Left panel represents longitudinal modelling of ratio DCs/baseline value. Right panel represents values averaged during AC and Docetaxel (Tax).

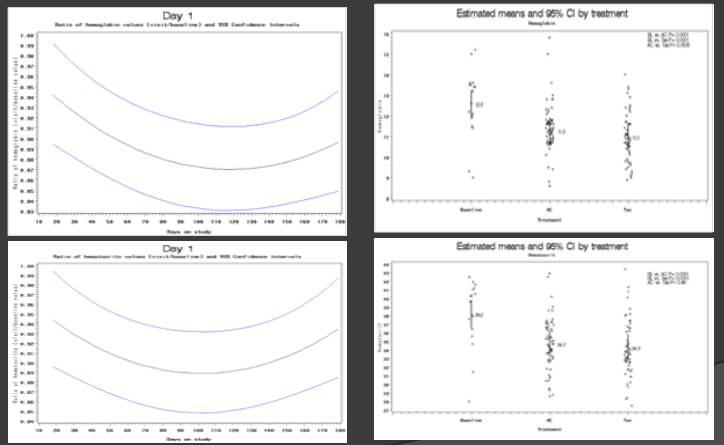
NOV-002 Background

- 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 functions including immune stimulation 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 doxorubicin-cyclophosphamide and docetaxel 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 HER-2 unamplified breast cancer patients results in a pathologic complete response (pCR) rate of <20%. Thus far in the ongoing phase II trial we have a confirmed pCR of 40% (Table 2).



References
Townsend, DM et al. *Cancer Res* 2008; 68 (8): 2870-2877.
von Minckwitz, *et al. JCO 2005; 23 (12): 2676-2685*
Townsend, DM et al. *Exp Opin Invest Drugs 2008; 17 (7): 1075-1083*

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 outcome variable was normalized by dividing by pretreatment (baseline) value 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 term of day on study and day. Upper left and lower left panels show HGB and hematocrit (HCT) levels, longitudinally with 95% confidence intervals (blue lines). Mixed regression models with patient specific intercepts were also created for HGB and HCT (upper right and lower right 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 HER-2 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.