Baseline serum protein levels associated with survival in axalimogene filolisbac (AXAL)-treated metastatic cervical cancer patients: The GOG/NRG-0265 trial

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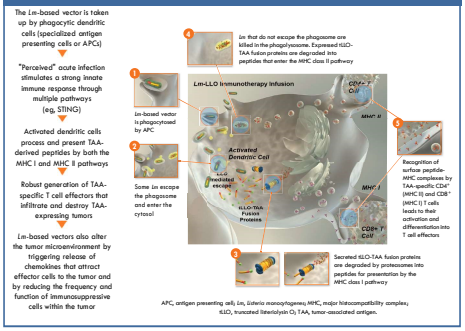
INTRODUCTION

- Cervical cancer is the most common human papillomavirus (HPV)-associated cancer and the fourth most common cancer in women worldwide.[1]
- Axalimogene filolisbac (AXAL) - a live attenuated *Listeria monocytogenes* (*Lm*)-based immunotherapy that expresses and secretes the full length E7 protein of HPV 16 - was developed as a vaccine-based immunotherapy for the treatment of cervical cancer as well as of other HPV-associated cancers.
- Advaxis' *Lm*-based immunotherapies act by stimulating innate immunity through multiple mechanisms including the STING pathway, by reducing the frequencies and functions of immunosuppressive cells in the tumor microenvironment, and by inducing the generation of tumor antigen-specific T cells that infiltrate and destroy the tumor (**Figure 1**).[2]
- Prognostic biomarkers that identify high-risk patients may guide treatment decisions and thus improve clinical outcomes for patients with persistent, recurrent or metastatic cervical cancer (PRmCC).
- To identify such biomarkers, we evaluated the association between baseline inflammation-related serum protein levels and overall survival (OS) in 45 of the 50 AXAL-treated PRmCC patients who participated in the phase 2 GOG/NRG-0265 trial.

Figure 1. *Lm* Technology™ Overview: Harnessing unique life cycle of Lm in APCs



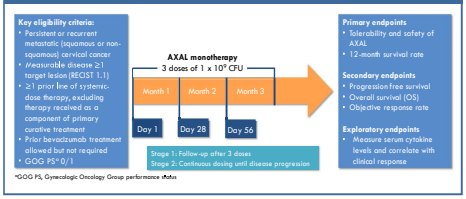
OBJECTIVE

- Evaluate the association between baseline inflammation-related serum protein levels and OS in AXAL-treated PRmCC patients in order to identify candidate prognostic biomarkers of clinical outcome.

MATERIALS AND METHODS

- The GOG/NRG-0265 trial is a phase 2 evaluation of AXAL in the treatment of persistent or recurrent squamous or non-squamous cell carcinoma of the cervix.[3] The study design for the GOG/NRG-0265 is summarized in **Figure 2**.
- Baseline levels of 54 serum analytes were measured using custom multiplex immunoassays (Myriad RBM, Austin, TX). Only those serum proteins who measurements were above the level of detection for all patients were analyzed further.
- Linear regression analysis, with a cut-off P value ≤.01, was used to assess the association between baseline serum proteins and OS.
- Unsupervised hierarchical clustering with complete linkage was used to subset or cluster patients based on their baseline expression patterns of the 4 identified serum proteins that associated with OS.
- Kaplan-Meier analysis with log-rank test was used to compare the survival curves of the two patient clusters identified by unsupervised hierarchical clustering after AXAL treatment.

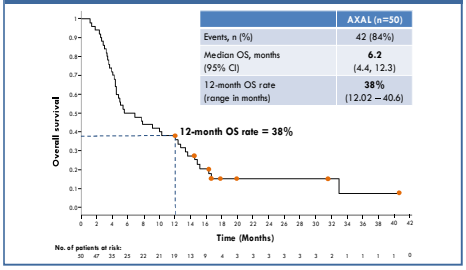
Figure 2. GOG/NRG-0265 study design



RESULTS

- In the GOG/NRG-0265 trial, which evaluated the safety and efficacy of AXAL in the treatment of PRmCC, AXAL demonstrated
  - a median overall survival of 6.2 months (**Figure 3**)
  - a 12-month OS rate of 38% (19/50) (**Figure 3**).[3]

Figure 3. Kaplan-Meier estimates of OS for all patients in GOG/NRG-0265 trial



	AXAL (n=50)
Events, n (%)	42 (84%)
Median OS, months (95% CI)	6.2 (4.4, 12.3)
12-month OS rate (range in months)	38% (12.02 – 40.6)

- Of the 54 serum proteins tested in 45 of the 50 AXAL-treated PRmCC patients who participated in the GOG/NRG-0265 trial, baseline levels of only 4 serum proteins - alpha-1 anti-trypsin (AAT), C-reactive protein (CRP), tissue inhibitor of metalloproteinases 1 (TIMP-1) and vascular endothelial growth factor (VEGF) (described in **Table 1**) - were found to associate significantly with OS (P≤.01).
  - ***Levels of all 4 serum proteins were negatively associated with OS*** (**Figure 4**).
- In addition, baseline levels of AAT, CRP, TIMP-1 and VEGF were significantly lower in patients who survived ≥12 months than in those who survived <12 months (**Figure 5**).

Table 1. Baseline serum proteins in PRmCC patients that associate with OS

Serum Protein	Cellular Source(s)	Functions
AAT[4]	• Liver • Monocytes • Tumor cells	• Acute phase reactant protein (ie, marker of inflammation) • Belongs to the serpin (serine protease inhibitors) superfamily of protease inhibitors • Limits tissue damage by targeting proteolytic enzymes secreted by neutrophils (eg, elastase and other serine proteases) • Significant positive correlations exist between serum AAT levels and tumor stage and between serum AAT levels and tumor growth and progression
CRP[5]	• Liver • Tumor tissue	• Acute phase reactant protein; serum CRP levels rise in response to elevated levels of IL-6 and other proinflammatory cytokines • Relationship exists between elevated serum/tumor CRP levels and prognosis in patients with solid tumors
TIMP-1[6,7]	• Macrophages • Variety of tissues including tumor tissue	• As a natural inhibitor of matrix metalloproteinases (MMPs), it regulates their ability to degrade extracellular matrix • Inhibits apoptosis and promotes cell growth as well as angiogenesis • High serum/tumor tissue levels in cancer patients correlate with poor prognosis
VEGF[8,9]	• Mast cells • Macrophages • Variety of tissues including tumor tissue	• Promotes angiogenesis and vasculogenesis • Overexpression is not only observed in multiple tumor types but is also associated with tumor stage and with a poor prognosis

Figure 4. High baseline levels of 4 serum proteins negatively associated with OS in PRmCC patients participating in GOG/NRG-0265

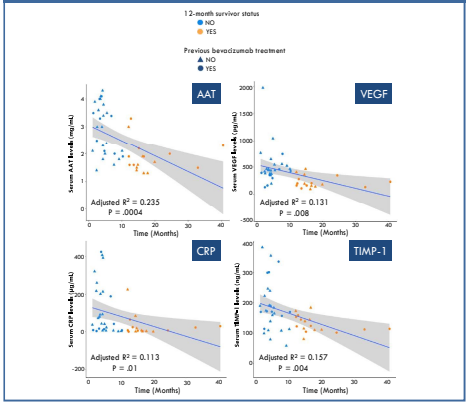
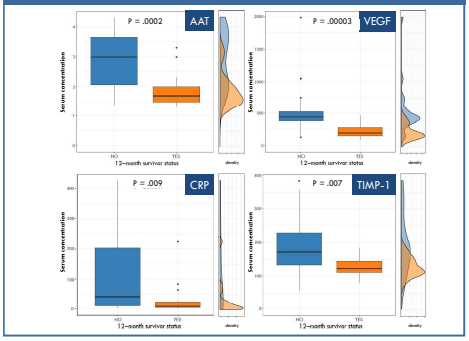


Figure 5. Patients who survived ≥12 months have significantly lower baseline levels of the 4 serum proteins than patients who survived <12 months



- In addition to evaluating the association of baseline levels of individual serum proteins with OS, we employed unsupervised two-dimensional hierarchical clustering with complete linkage to subset PRmCC patients based on the expression levels of the 4 serum proteins.
  - The resulting dendogram identified 2 patient clusters, which are distinguishable by a low expression pattern (cluster 1) or a high expression pattern (cluster 2) of the baseline levels of the 4 serum proteins (**Figure 6**).
  - 82% of the patients surviving ≥12 months and 39% of the patients surviving <12 months were found in patient cluster 1 (**Figure 7**)
  - 18% of the patients surviving ≥12 months and 61% of the patients surviving <12 months were found in patient cluster 2 (**Figure 7**).
- Survival analysis for both patient clusters after AXAL treatment revealed that
  - ***cluster 1 exhibited a 12-month OS rate of 56%***, whereas cluster 2 exhibited a rate of 15% (HR=0.23; 95% CI: 0.10-0.48; P<.001) (**Figure 8**)
  - ***cluster 1 exhibited a median OS of 12.32 months***, whereas cluster exhibited a median OS of 4.08 months (P<.0003) (**Figure 8**).
- Together, these findings suggest that the baseline levels of these 4 serum proteins have prognostic value for OS in AXAL-treated PRmCC patients.

SUMMARY AND CONCLUSIONS

- We have identified baseline levels of AAT, CRP, TIMP-1 and VEGF as candidate prognostic biomarkers of clinical outcome in PRmCC patients.
- Prospective validation of the utility of the baseline levels of the 4 serum proteins as prognostic biomarkers of clinical outcome in PRmCC patients is scheduled in the upcoming phase 3 ADVANCE trial, evaluating the safety and efficacy of the *Lm*-based immunotherapy ADXS-602 in combination with nivolumab compared with single-agent chemotherapy in PRmCC patients who have failed or were ineligible to receive first-line therapy with or without bevacizumab.
- Cluster 1 criteria may also identify PRmCC patients most likely to benefit from AXAL treatment.

Figure 6. Two dimensional hierarchical clustering identified 2 patient clusters

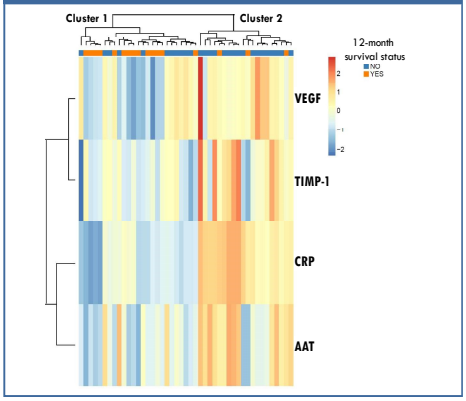


Figure 7. The majority of patients who survived ≥12 months are found in cluster 1

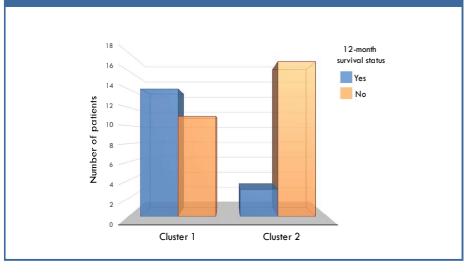
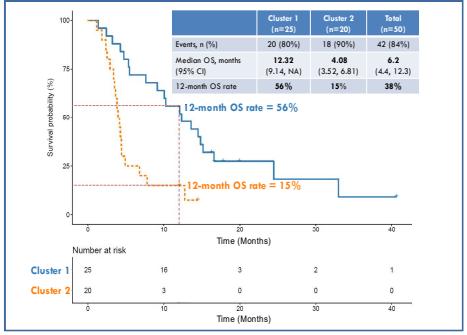


Figure 8. Kaplan-Meier estimates of OS for patients in clusters 1 and 2



	Cluster 1 (n=25)	Cluster 2 (n=20)	Total (n=50)
Events, n (%)	20 (80%)	18 (90%)	42 (84%)
Median OS, months (95% CI)	12.32 (9.14, NA)	4.08 (3.52, 6.81)	6.2 (4.4, 12.3)
12-month OS rate	56%	15%	38%

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DISCLOSURES

- S.M. Hayes, R.G. Petit, D. Balli and Q. Hong: Employees of Advaxis, Inc.
- W. Huh: Consulting or advisory role - THEVAX, Invio (DSMB)
- W.E. Brady: No conflicts of interest to disclose
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- C. Aghjanian: No conflicts of interest to disclose
- B.J. Monk: Consulting or advisory role - Advaxis, Inc.

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