Abstract 53

Assessing the mechanism of action of tisotumab vedotin in head and neck squamous cell carcinoma with digital pathology analysis of H&E images from innovaTV 207 part C

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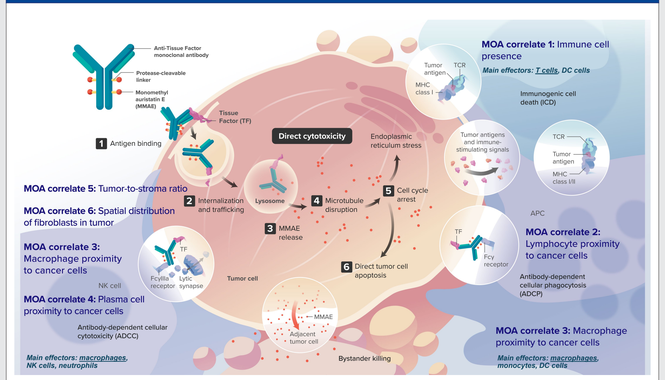
Background

- Recent progress in artificial intelligence (AI) and digital pathology has facilitated in-depth characterization of the tumor microenvironment (TME)
- Complex ADC MOAs may be better understood by elucidating the spatial relationship between cellular effectors or immune cells and cancer cells in the TME
- TV, a tissue factor–directed ADC approved to treat recurrent/metastatic cervical cancer, is being investigated as mono- or combination therapy for advanced solid tumors in the global, open-label, phase 2 innovaTV 207 trial (NCT03485209)[1]
- Based on data from preclinical models and RNA sequencing of clinical specimens, TV is hypothesized to drive cell death via several MOAs, including direct cytotoxicity as well as ADCC, ADCP, and ICD, with the latter 3 depending on immune cell presence in the TME (**Figure 1**)[2,3]
- In this exploratory analysis from innovaTV 207 part C (HNSCC cohort), an AI-trained digital pathology algorithm was used to provide correlative evidence for described and hypothesized TV MOAs

Figure 1. Proposed MOA for TV and hypothesized MOA correlates with TV response

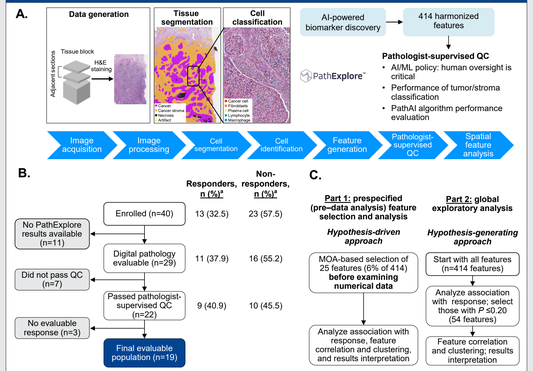


APC, antigen-presenting cell; DC, dendritic cell; MHC, major histocompatibility complex; MMAE, monomethyl auristatin E; NK, natural killer; TCR, T-cell receptor; TF, tissue factor; TV, tisotumab vedotin.

Methods

- Patients enrolled in the HNSCC cohort of innovaTV 207 (part C) had previously treated metastatic disease and received TV monotherapy (1.7 mg/kg IV Q2W)
- In a 2-part analysis, pre-treatment biopsy samples collected at screening were used to systematically identify features potentially associated with TV efficacy
- H&E–stained whole-slide images were used to identify baseline histological features associated with treatment response, using the AI-trained digital pathology algorithm PathExplore (PathAI) (**Figure 2**)
- Pathologist-supervised quality control (QC) of the H&E–stained tissue was performed after PathExplore cell identification to exclude images with <90% accuracy of cancer, stroma or immune cell identification (24% of slides reviewed)
- **Part 1:** prespecified features corresponding to known TV MOAs
  - The six hypothesized MOA correlates included: (1) immune cell presence, (2) lymphocyte proximity to cancer cells, (3) macrophage proximity to cancer cells, (4) plasma cell proximity to cancer cells, (5) tumor-to-stroma ratio, and (6) spatial distribution of fibroblasts in tumor (**Figure 1**)
  - The features were selected prior to examination of numerical data and analyzed for association with response and feature correlation
- **Part 2:** all 414 features examined by the PathExplore algorithm were analyzed for association with TV response
  - Features with $P \leq 0.20$ were analyzed for feature correlation and clustering and predictive modeling
- Unadjusted ("raw") P values were calculated using the Wilcoxon rank sum test and were adjusted to control the false discovery rate using the Benjamini-Hochberg procedure. A hypergeometric test (without adjustment for any multiple testing) was used in **Figures 3** and **6** to examine enrichment

Figure 2. Study design showing the (A) study workflow, (B) study population, and (C) 2-part approach



Images included with the permission of pathAI. AI, artificial intelligence; ML, machine learning.
[a]For response rate (%) calculations, denominators included patients not evaluable for response.

Results

19 patients were included in the final evaluable population

- Of 40 enrolled patients, 19 were included in the final digital pathology analysis dataset (see **Figure 2B** for patient disposition)
  - Of these 19 patients, 9 patients were responders and 10 were non-responders
- Baseline characteristics of the final evaluable population were consistent with those of the intention-to-treat population[1]

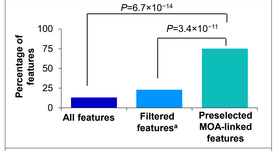
Part 1. The preselected MOA-linked feature set showed strong enrichment of features associated with response

- In the prespecified approach, 25 of 414 features (6%) were preselected for potential association with TV MOAs
  - Features were prespecified to test the hypothesized MOA correlates **before** any examination or analysis of numerical data
  - Of these 25 features, 10 features (40%) had a raw $P<0.05$, and 19 (76%) had an adjusted $P \leq 0.20$ for association with response (**Table 1**)

Table 1. Correlation of preselected MOA-related features with response

MOA correlates	Cellular components	No. of features	Features with raw $P<0.05$	Features with adjusted $P \leq 0.20$
1. Immune cell presence	Immune cells	6	1 (17%)	6 (100%)
2. Lymphocyte proximity to cancer cells	Lymphocytes	4	1 (25%)	4 (100%)
3. Macrophage proximity to cancer cells	Macrophages	3	3 (100%)	3 (100%)
4. Plasma cell proximity to cancer cells	Plasma cells	4	1 (25%)	1 (25%)
5. Tumor-to-stroma ratio	Tumor tissue	3	1 (33%)	2 (67%)
6. Spatial distribution of fibroblasts in tumor	Fibroblasts	5	3 (60%)	3 (60%)

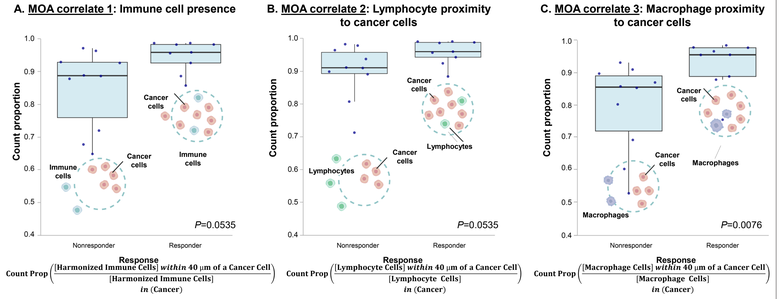
Figure 3. Percentage of features with unadjusted $P \leq 0.20$



[a]Restricted to measurement categories in the preselected MOA-linked set.

- Low raw P values were highly enriched in the preselected feature set vs the overall set (**Table 1**; **Figure 3**)
- Each putative TV MOA correlate had ≥1 feature associated with response (**Table 1**; **Figure 4**)
  - Spatial proximity of immune cells (lymphocytes and macrophages) to cancer cells in the TME was associated with response to TV
  - Responders and non-responders had overlapping distributions for all MOA correlates, indicating that selection based on these features may not be feasible

Figure 4. Association of MOA correlates with TV response



P values are unadjusted for multiple testing.

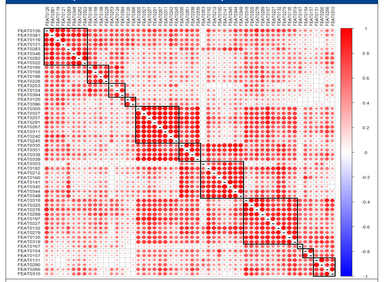
Part 2.1 Many features are highly correlated, complicating overall interpretation

- In the global exploratory analysis, 54 of 414 features were potentially associated with response (raw $P \leq 0.20$)
- Hierarchical clustering based on Pearson's r (either raw Pearson's r or absolute value of Pearson's r) showed clear, strong clusters of features (absolute Pearson's r; **Figure 5**)
- Examination of feature clusters showed that in a given cluster, there were often some features that were clearly closely related in description but also some features that were not obviously linked to other features
  - High correlation between features, especially unrelated features, may indicate underlying cancer states

Part 2.2. Preliminary predictive modeling supported the association of MOA-linked features with response

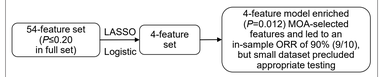
- We fit a logistic regression with an L1 (LASSO) penalty, which resulted in a model with 4 non-zero coefficients
- All 4 features selected by the model were among the preselected MOA-based set (**Figure 6**), supporting the association of MOA-linked features with response
  - However, the small dataset precluded conclusions regarding response enrichment
- MOA-linked features were also identified using other predictive modeling approaches (Gradient Boosting Machine (GBM) and elastic net–penalized logistic regression), supporting the importance of MOA-linked features

Figure 5. Clustering analysis of global exploratory features (n=54 with raw $P \leq 0.20$)



Clustering based on absolute value of Pearson's r. Larger circles and darker red indicate higher correlation values.

Figure 6. L1-penalized (LASSO) logistic regression-selected four-feature set



All 4 selected features were from the preselected MOA set

Feature	Coefficient	Feature description	Prespecified MOA feature
1	0.79	Proportionate area of cancer epithelium relative to total area of harmonized tissue	**MOA correlate 5**: Tumor-to-stroma ratio (**Figure 4E**)
2	0.46	Fraction of fibroblast cells that are within 40 μm of any cancer cell, in cancer epithelium	**MOA correlate 6**: Spatial distribution of fibroblasts in tumor
3	−0.42	Fraction of cancer cells that are within 40 μm of any harmonized immune cell, in cancer stroma	**MOA correlate 1**: Immune cell presence
4	0.13	Fraction of macrophage cells that are within 40 μm of any cancer cell, in cancer epithelium	**MOA correlate 3**: Macrophage proximity to cancer cells (**Figure 4C**)

Conclusions

- This proof-of-concept analysis of head and neck squamous cell carcinoma (HNSCC) tumor samples from innovaTV 207 highlights a novel approach, using the routine and cost-effective hematoxylin and eosin (H&E) staining method to identify features potentially associated with response to tisotumab vedotin (TV) and to support hypothesized concepts regarding TV mechanisms of action (MOAs)
- A preselected ("hypothesis-driven") subset of features linked to TV MOAs showed enrichment of features associated with response compared with the overall set and provided clinical support for all 6 hypothesized MOA correlates, including those supporting TV MOAs of immunogenic cell death (ICD), antibody-dependent cellular cytotoxicity (ADCC), and antibody-dependent cellular phagocytosis (ADCP)
  - For all features, we observed overlapping distributions of values for responders and non-responders, suggesting no clear basis for patient selection from these analyses
- Preliminary predictive modeling supported the association of response with MOA-linked features, which were important to differentiate responders from non-responders
- This work provides correlative clinical support for hypothesized MOAs supported by preclinical evidence and also for other hypothesized MOAs. However, results are limited by the small sample size, the analysis of only pre-treatment samples, and strong correlations among some features, meaning that inferences regarding MOA are indirect
- Further validation of digital pathology capabilities may aid in the elucidation of multifaceted antibody-drug conjugate (ADC) MOAs

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References: 1. Sun L, et al. J Clin Oncol. 2024; 42:S6012. 2. Gray E, et al. J Immunother Cancer. 2020; 8:A371. 3. Sridhar S, et al. Cancer Res. 2025;85:3093.

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Disclosures: Mark Bieda (presenter) declares employment by Pfizer

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