

# Abstract 4573: CXCL9:SPP1 Ratio: Macrophage Polarization and Outcomes with Pembrolizumab or Enfortumab Vedotin Plus Pembrolizumab in Metastatic Urothelial Cancer

Saad O. Atiq<sup>1</sup>, Tolulope Adeyelu<sup>2</sup>, Sudeh Izadmehr<sup>1</sup>, Jonathan Anker<sup>1,3</sup>, Eric J. Miller<sup>1</sup>, Teja Ganta<sup>1</sup>, Bobby Liaw<sup>1</sup>, Stephen Bohlman<sup>1</sup>, Alexander Karol<sup>1</sup>, Michelle Tran<sup>1,3</sup>, Byuri Angela Cho<sup>1,3</sup>, Andrew Elliott<sup>2</sup>, Pedro Barata<sup>4</sup>, Rana R. McKay<sup>5</sup>, Reza Mehrazin<sup>6</sup>, Amir Horowitz<sup>3</sup>, John P. Sfakianos<sup>6</sup>, Nina Bhardwaj<sup>1,3</sup>, Matthew D. Galsky<sup>1</sup>  
<sup>1</sup>Division of Hematology and Medical Oncology, Tisch Cancer Institute, Icahn School of Medicine at Mount Sinai, New York, NY, <sup>2</sup>Caris Life Sciences, Irving, TX, <sup>3</sup>Immunology Institute, Icahn School of Medicine at Mount Sinai, New York, NY, <sup>4</sup>Seidman Cancer Center, University Hospitals, Cleveland, OH, <sup>5</sup>Moore's Cancer Center, University of California San Diego, San Diego, CA, <sup>6</sup>Department of Urology, Icahn School of Medicine at Mount Sinai, New York, NY

## Background

- Enfortumab vedotin plus pembrolizumab (EV+P) is standard first-line treatment for metastatic urothelial cancer (mUC), yet responses vary considerably
- Single-cell RNA sequencing (RNA-Seq) across tumor types has revealed dichotomous tumor-associated macrophage (TAM) populations: SPP1+ TAMs exhibit tumor-promoting programs while CXCL9+ TAMs express programs associated with anti-tumor immunity
- The CXCL9:SPP1 gene expression ratio (CS ratio) from bulk RNA-Seq captures this balance and correlates with outcomes across solid tumors (Bill et al, Science, 2023)
- We explored CS ratio's association with outcomes in mUC patients receiving pembrolizumab monotherapy or EV+P

## Methods

- We retrospectively analyzed mUC patients who underwent molecular profiling at Caris Life Sciences using NGS (592-gene panel, DNA WES, or RNA WTS).
- CS ratios were calculated and patients stratified into quartiles (Q1–Q4; Q4=highest ratio).
- PD-L1 expression was assessed by IHC (22c3, positive (+) CPS  $\geq$ 10%).
- TMB-High was defined as  $\geq$ 10 Mut/Mb.
- Gene expression profiles were analyzed for transcriptomic signatures (IFN- $\gamma$ ) predictive of IO response.
- TME composition was inferred using quanTIseq.
- Real-world overall survival (rwOS) was derived from insurance claims and calculated from biopsy to last contact while time on treatment (TOT) was calculated from initiation to end of therapy. Hazard ratio (HR) was calculated by Cox proportional hazard method.
- Mann-Whitney U and  $\chi^2$ /Fisher exact tests were applied with adjustment for multiple comparisons.

- Lower CXCL9:SPP1 ratios associate with shorter time on treatment and real-world overall survival with both pembrolizumab and EV+P
- Further validation of the CXCL9:SPP1 ratio in patients treated with EV + P or P is warranted

Corresponding Author: Saad Atiq, [saad.atiq@mssm.edu](mailto:saad.atiq@mssm.edu), X: @SaadAtiq24

| CS Ratio        | Pembrolizumab Monotherapy            |                                      | EV + P                                 |  |
|-----------------|--------------------------------------|--------------------------------------|--|--|
|                 | TOT (months)                         | rwOS (months)                        | TOT (months)                           | rwOS (months)                          |
| CS-Q1           | 2.7 (2.1-3.0)                        | 5.7 (4.4-8.2)                        | 7.6 (6.2-8.8)                          | 17.8 (13.8-19.8)                       |
| CS-Q2           | 3.1 (2.2-4.1)                        | 7.2 (5.4-8.8)                        | 6.9 (6.0-8.0)                          | 16.4 (13.2-19.4)                       |
| CS-Q3           | 4.5 (3.9-5.6)                        | 13.5 (10.7-16.8)                     | 9.2 (8.1-10.8)                         | 17.2 (14.4-20.3)                       |
| CS-Q4           | 6.9 (4.9-8.7)                        | 18.7 (13.1-23.7)                     | 10.6 (9.3-12.2)                        | 21.8 (16.5-28.1)                       |
| CS-Q4 vs. CS-Q1 | HR 0.56, 95% CI 0.47-0.67, p < 0.001 | HR 0.53, 95% CI 0.44-0.64, p < 0.001 | HR 0.69, 95% CI 0.54-0.87, p = 0.00189 | HR 0.62, 95% CI 0.46-0.83, p = 0.00148 |

Table 1: Time on Treatment (ToT) and Real-World Overall Survival (rwOS)

## Results

- Among 6,708 mUC patients, 1,299 received pembrolizumab (Table 1) and 561 received EV+P
- Lower CS ratios (SPP1+ TAM predominance) were associated with shorter ToT and rwOS (Table 1)
- Low CS ratio was also associated with reduced PD-L1 expression (15.5% vs 66.7%, p < 0.001) and TMB-H prevalence (34.6% vs 52.3%, p = 0.006) (Figure 1a/b).

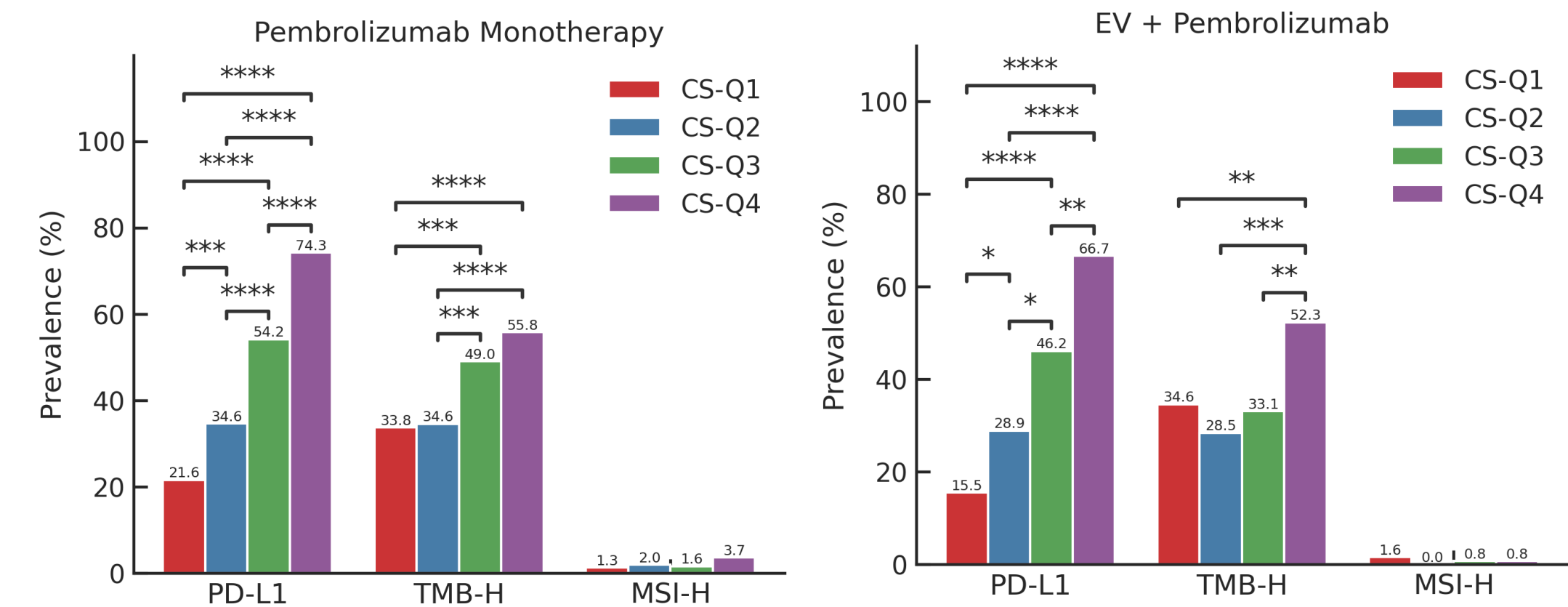


Figure 1: Biomarkers for Immunotherapy response (TMB-H/PD-L1/MSI-H) in (a) Pembrolizumab Only and (b) EV + P.

- CS ratio tumors demonstrated positive correlation with immune infiltration except for neutrophil (Figure 2). enrichment

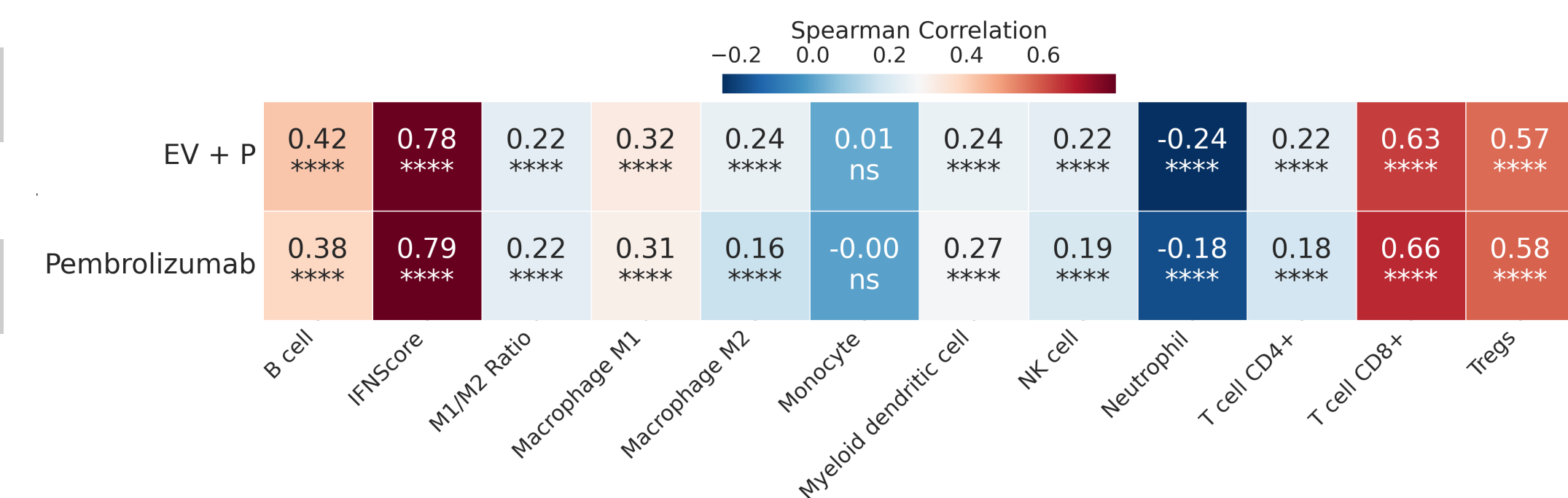


Figure 2: Spearman Correlation between CS ratio and TME cell fractions (quanTIseq) and Interferon Gamma Score.