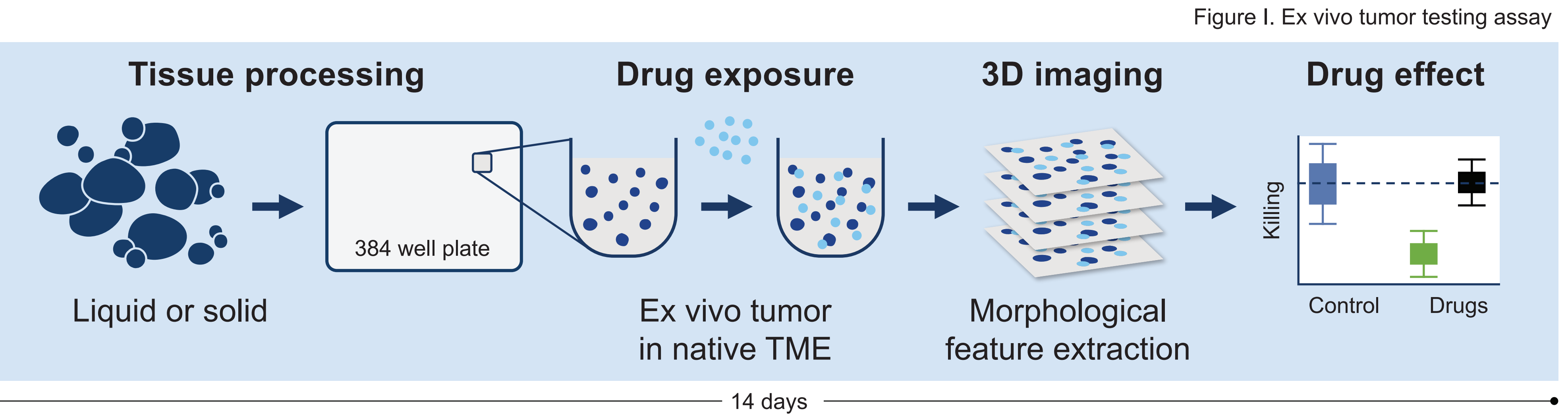


**BACKGROUND**

- Immunotherapy brings great progress for cancers that are difficult to treat but it **remains challenging to select sensitive patients upfront**. PD-L1 expression, TMB and MSI/MSS status fail to optimize patient stratification for ovarian cancer.
- Patient-derived ex vivo tumor tissue with preserved tumor microenvironment (TME)** represents the ideal model for patient-specific testing of immunotherapy sensitivities.

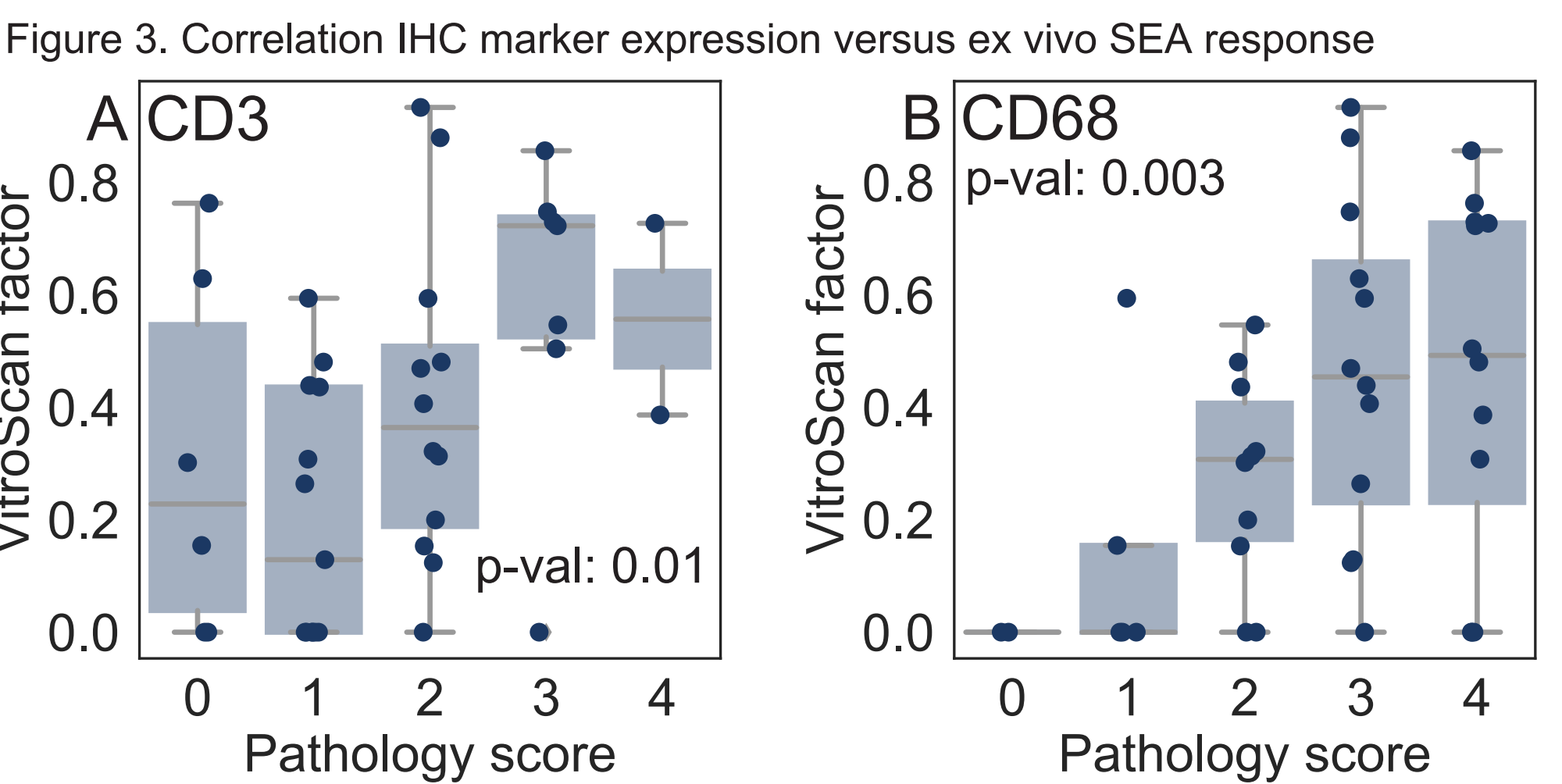
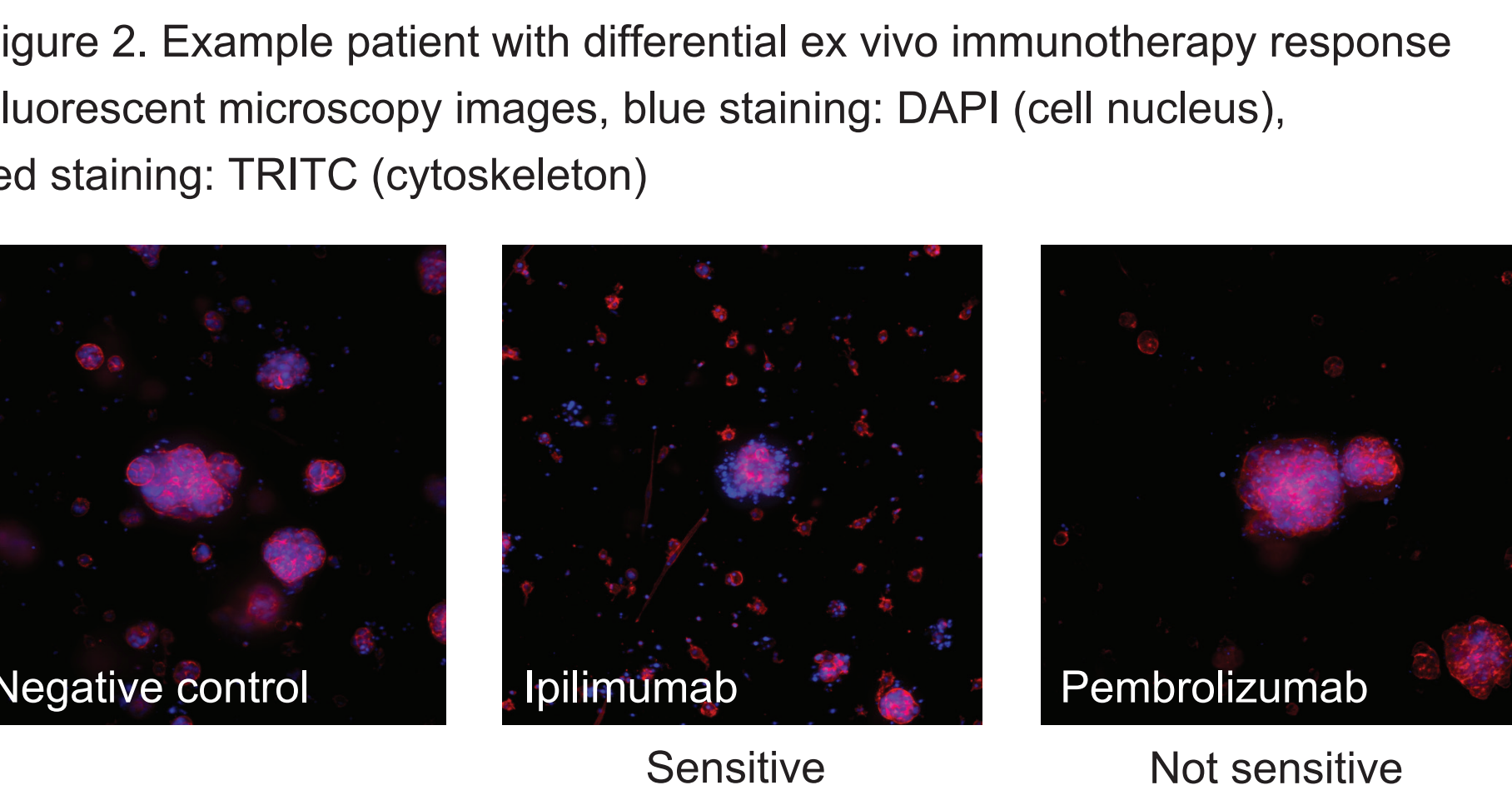
**METHODS**

- Solid tumor tissue or ascites was collected for 86 patients with predominantly high-grade serous ovarian cancer.
- Fresh tumor clusters with preserved native TME were exposed to six immunotherapies and the positive control enterotoxin A (SEA). Tumor killing and immune cell proliferation were quantified using 3D imaging (Figure 1).
- Ex vivo tumor sensitivity was classified by percentage of tumor killing as no response (<10%), weak (10-20%), strong (20-50%) and very strong (>50%). Its statistical significance is expressed in two categories: significant (p-value < 0.05) and highly significant (p-value < 3.33e-4, Bonferroni corrected, shown as \* in the large heatmap)
- Complementary assays were performed on the tissue prior to drug exposure:
  - Immunohistochemical staining (IHC) for immune cell markers (CD3, CD68) (n=40). Pathology scoring was based on the percentage positive cells: 0 (none), 1 (0-5%), 2 (10-25%), 3 (25-50%), 4 (>50%).
  - Proteomics on cell pellets and supernatant (n=24) comparing expression profiles in response subgroups.



**RESULTS**

- Differential patient response profiles were observed, with 40% of the patients tissues displaying highly significant sensitivity (\* annotation in large heatmap) for at least one of the therapies tested. Example response in Fig 2.
- A significant positive correlation was found between measured ex vivo SEA sensitivity and CD3 and CD68 marker expression. See Figure 3A-B with p-values of Pearson correlation coefficients.
- In addition, a significant correlation was found between the measured immune cell proliferation of SEA and CD3/CD28-beads (Pearson coefficient: 0.80, p-value < 0.002)
- Protein profiling showed significantly higher levels of chemokines and interleukins in SEA responders. Immune checkpoint inhibitors with different modes of action demonstrated distinct protein abundance profiles (Figure 4)
- Our results showcase the presence of relevant immune components in the ex vivo assay, and confirm the credibility of using an image-based approach for quantifying immunotherapy responses.

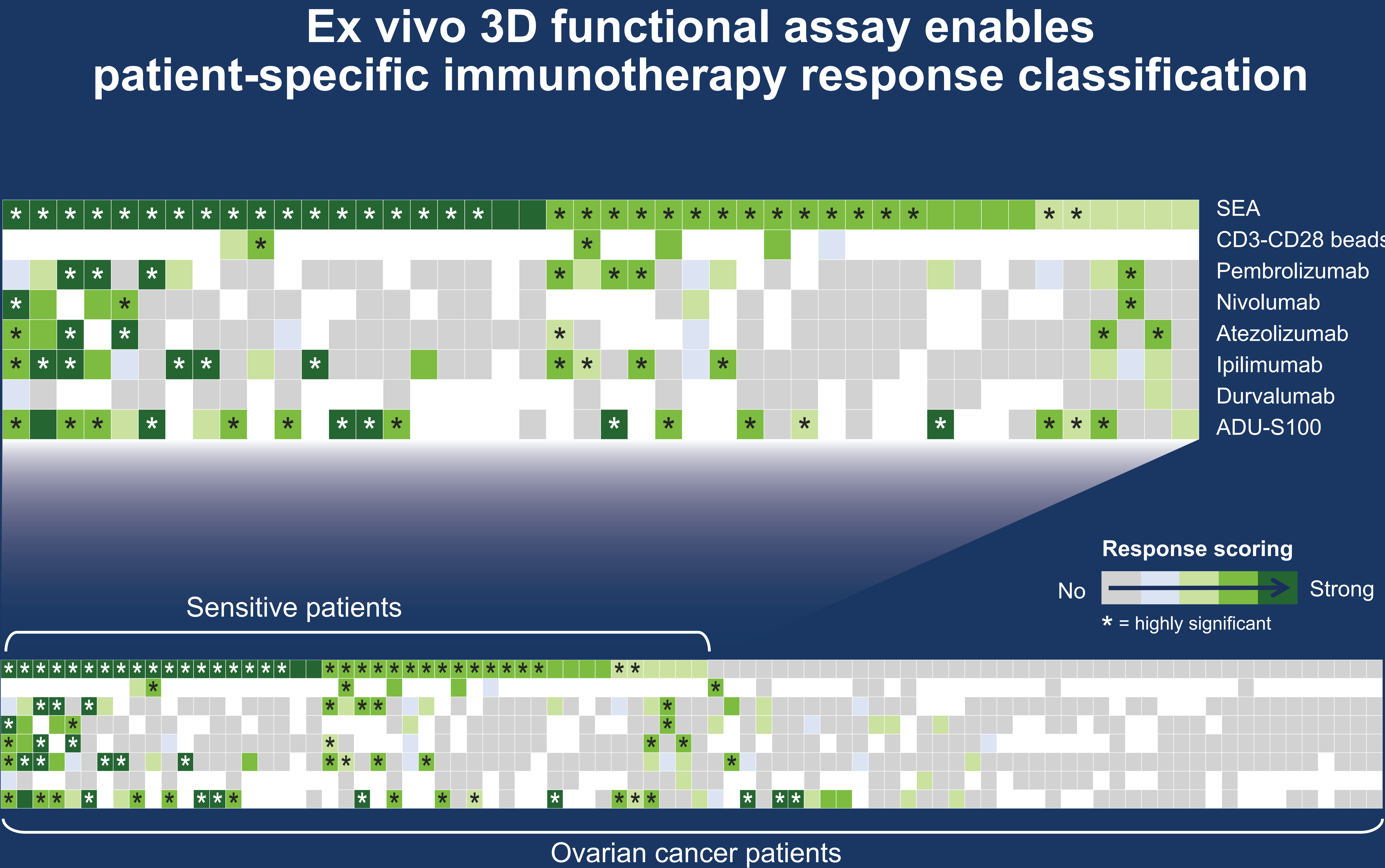


**CONCLUSIONS**

- Ex vivo functional tumor testing combined with IHC staining and protein profiling enables classification and characterization of patient-specific sensitivity to 6 immunotherapies.
- A positive correlation was shown between the ex vivo sensitivity to immunotherapies and the presence of functional immune components in the patient's TME.

**DISCUSSION and OUTLOOK**

- The predictive value of the platform was previously demonstrated by correlation of ex vivo sensitivity to clinical chemotherapy responses of ovarian cancer patients.
- A clinical trial is currently ongoing to establish the correlation of the assay with NSCLC patients' response to immunotherapy.
- An integrated approach using ex vivo functional tumor testing and advanced biomarker discovery will facilitate development of effective immunotherapies for difficult to treat cancers.



Ex vivo immune response correlates significantly with the presence of CD3+ and CD68+ cells