Nectin-4-status assesment with High-Affinity Probes

Rationale: Current Nectin-4 status assessment using diagnostic antibodies (e.g. clone EPR15613-68) does not align with observed patient responses to the antibody-drug conjugate Enfortumab vedotin (EV) or other Nectin-4 targeted therapies, highlighting the need for more precise and functionally relevant diagnostic tools.

Method: We developed High-Affinity Probes (HAPs) to enable precise detection of the Enfortumab binding site on fixed tumor samples. HAPs are engineered molecular tools with exceptional specificity and sensitivity, designed to provide *in situ* quantification of therapeutic binding site accessibility and density across a wide range of therapeutic molecules.

Results: **HAPs Reveal Divergent Binding Patterns in Primary Tumors Compared to Traditional Assays**

Staining patterns and intensities obtained using Enfortumab HAPs were fundamentally different from conventional Nectin-4 IHC with established clones, with only weak correlation between the two methods (Fig. 1 and 2).

Enfortumab-HAP Signal Correlates with EV Sensitivity Across Tumor Cell Lines

Enfortumab HAPs were used to stain a panel of tumor cell lines. Quantitative HAP staining scores were then compared to cell viability following EV treatment. Across the models tested, there was a strong correlation between HAP-derived binding scores and sensitivity to Enfortumab vedotin, with higher HAP binding affinity associated with increased drug response (Fig. 3).

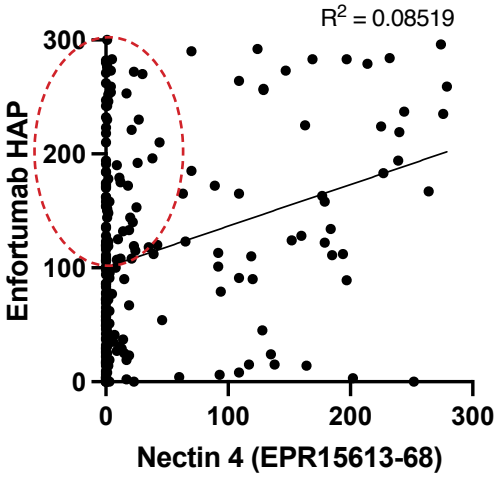

Fig. 1: Correlation analysis of Enfortumab HAP and Nectin-4 IHC (EPR15613-68) H-Scores in matched urothelial carcinoma cases (n =239). Red circle indicates the Nectin-4 low/negative group that still shows significant signal with Enfortumab HAP.

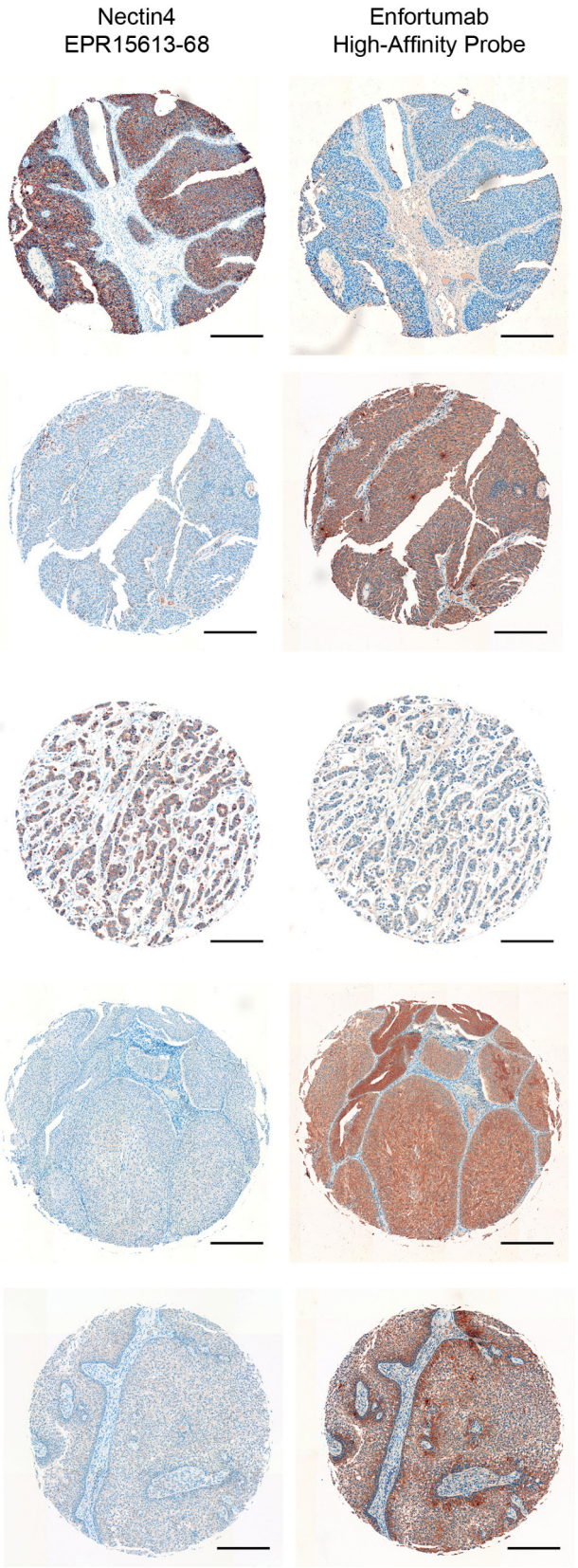

Fig. 2: Staining intensity and distribution of selected urothelial carcinoma cores from the screening cohort. Some cases show strong Nectin-4 staining while showing very little to no binding affinity to Enfortumab. Cores with low Nectin-4 scores still show high binding affinity for Enfortumab as assessed via HAPs.

Bavarian Biotech GmbH
Seeshaupterstr. 19
81476 Munich Germany

Dr. Alexander Beck
a.beck@bav.bio
www.bav.bio

Enfortumab-HAP Binding Assessed in Tumor Samples from EV-Treated Patients

HAP-based assessment of Enfortumab binding was applied to pretreatment tumor samples from two patients with metastasized urothelial carcinoma who later received Enfortumab vedotin. In Patient 1, no binding affinity was detected using the Enfortumab-HAP, and the patient subsequently showed disease progression under treatment. In contrast, Patient 2 exhibited high HAP binding in the tumor sample and experienced a complete and durable clinical response after two treatment cycles. While preliminary, these observations might suggest a potential association between HAP-derived binding-site accessibility and clinical outcome in EV-treated patients.

Conclusion: Existing stratification methods may not accurately reflect the therapeutic binding potential of Enfortumab in all patients, potentially leading to suboptimal treatment decisions. Our preliminary findings demonstrate that High-Affinity Probes (HAPs) specific to Enfortumab vedotin can reveal meaningful differences in drug-target binding across both preclinical and clinical settings. However, given the very limited clinical sample size, these findings should be viewed as exploratory. Further clinical validation in larger cohorts is needed to assess the consistency and predictive value of HAP-based stratification. Together, these results highlight the potential of Enfortumab-HAPs to provide functional insight into binding-site accessibility, which may support further investigation into their role in patient stratification and therapeutic decision-making.

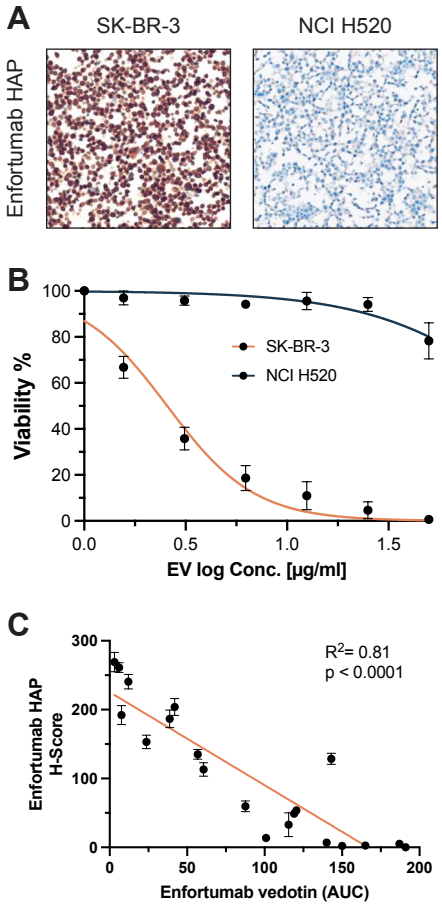

Fig. 3: Enfortumab-HAP staining of two cell lines (A) and their corresponding dose-response curves to EV (B). Correlation analysis between Enfortumab-HAP staining intensity and EV response in 20 carcinoma cell lines (C).

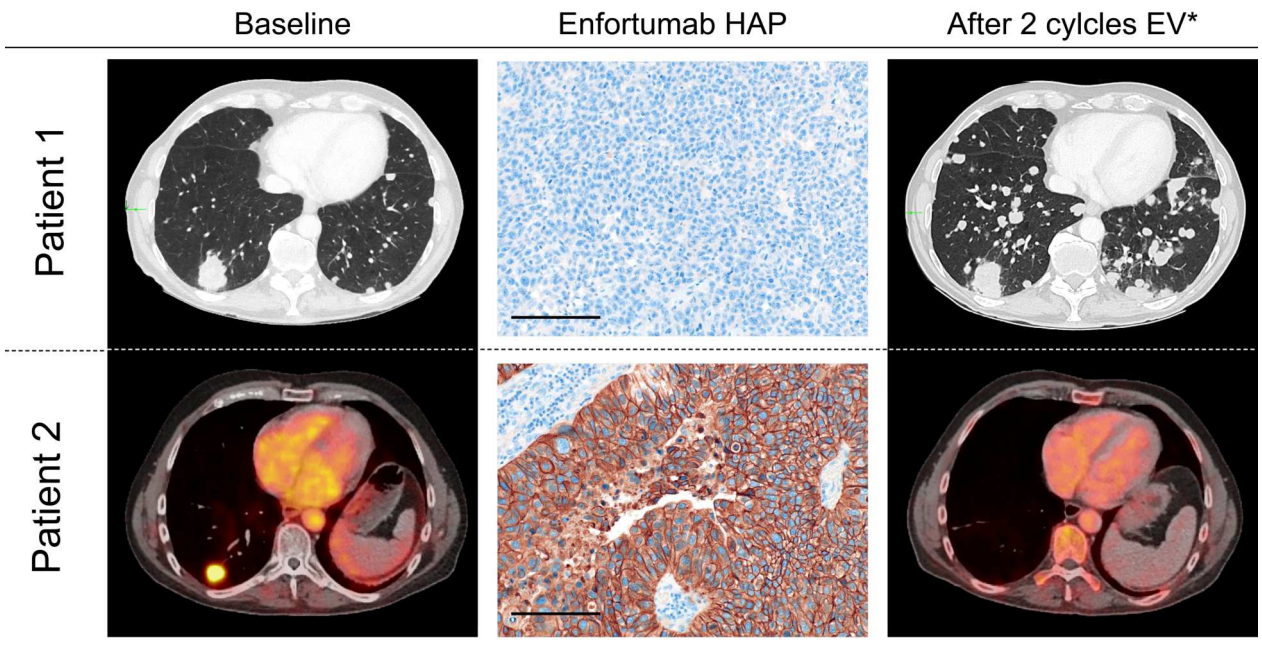

Fig. 4: (PET-)CT images of both patients at baseline show metastasis to the lung (left panel). Tumor tissue of Patient 1 shows no binding affinity to Enfortumab HAPs, while tumor tissue of Patient 2 shows strong and distinct binding affinity (middle panel). CT-staging after 2 cycles of EV show progressive disease in Patient 1 and complete response in Patient 2 (right panel).
*Enfortumab vedotin was given in combination with Pembrolizumab.