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Differential *in vitro* activity of amivantamab, cetuximab, and panitumumab against EGFR ECD resistance mutations

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Key Takeaway

Amivantamab is active against a broader range of EGFR ectodomain (ECD) mutations, which are known resistance mechanisms to EGFR monoclonal antibodies, compared with cetuximab and panitumumab

Conclusions

Amivantamab demonstrated increased binding and functional activity against a panel of EGFR ECD mutations, including those that confer resistance to cetuximab and panitumumab

Structural modeling projected that the amivantamab epitope is shifted away from the EGFR domain III residues that interact with cetuximab and panitumumab, which may contribute to the differential activity of amivantamab

A participant enrolled in OrigAMI-1 (ClinicalTrials.gov Identifier: NCT05379595) harboring an *EGFR* S492R mutation achieved a partial response, which suggests that amivantamab is active against an ECD mutation that confers resistance to cetuximab

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Disclosures

All authors are employees of and may hold stock in Johnson & Johnson.

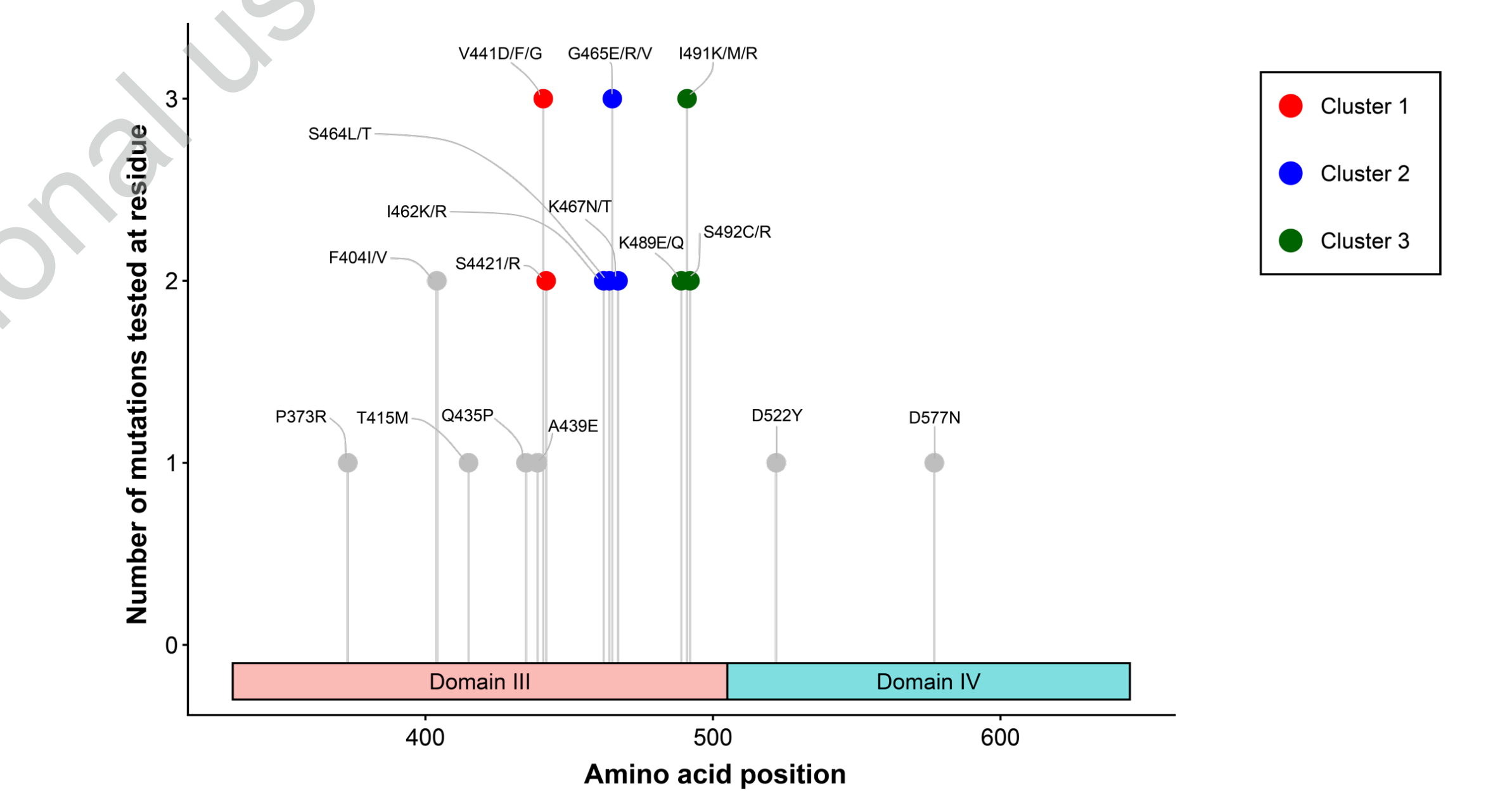
Background

- Epidermal growth factor receptor (EGFR) ectodomain (ECD) mutations are a known mechanism of acquired resistance to the EGFR monoclonal antibodies cetuximab and panitumumab[1]
- Amivantamab, an EGFR-MET bispecific antibody with immune cell–directing activity,[2,3] was evaluated among participants with *RAS/BRAF* wild-type (WT) metastatic colorectal cancer (mCRC) in the OrigAMI-1 study (NCT05379595)
- Amivantamab, cetuximab, and panitumumab bind to unique but overlapping epitopes in EGFR domain III[4,5]
- Previous studies have demonstrated that disparities in EGFR epitope binding between cetuximab and panitumumab can confer differential activity against EGFR ECD mutations,[6,7] which suggests that amivantamab may exhibit activity against ECD mutations that lead to cetuximab and/or panitumumab resistance

Methods

- A panel of 29 commonly occurring EGFR ECD mutations reported to confer resistance to EGFR monoclonal antibodies was identified, with frequently occurring mutations[8] highlighted (**Figure 1**)
- EGFR ECD mutants and WT EGFR were transiently expressed in HEK293Ta cells, and amivantamab, cetuximab, and panitumumab binding was assessed through 2 orthogonal approaches: flow cytometry–based analyses to measure antibody binding and AlphaLISA and western blotting to measure inhibition of EGF-induced EGFR phosphorylation
- Structure of the binary complex composed of amivantamab variable domain Fv and human EGFR domain III was modeled using AlphaFold Multimer v2.3.2 software
- The epitope region was defined as EGFR residues within 4 Å of a complementarity-determining region (CDR) residue using MOE 2024.06, and *EGFR* single-point mutations were modeled using Residue-Scan with Amber forcefield option in MOE 2024.06

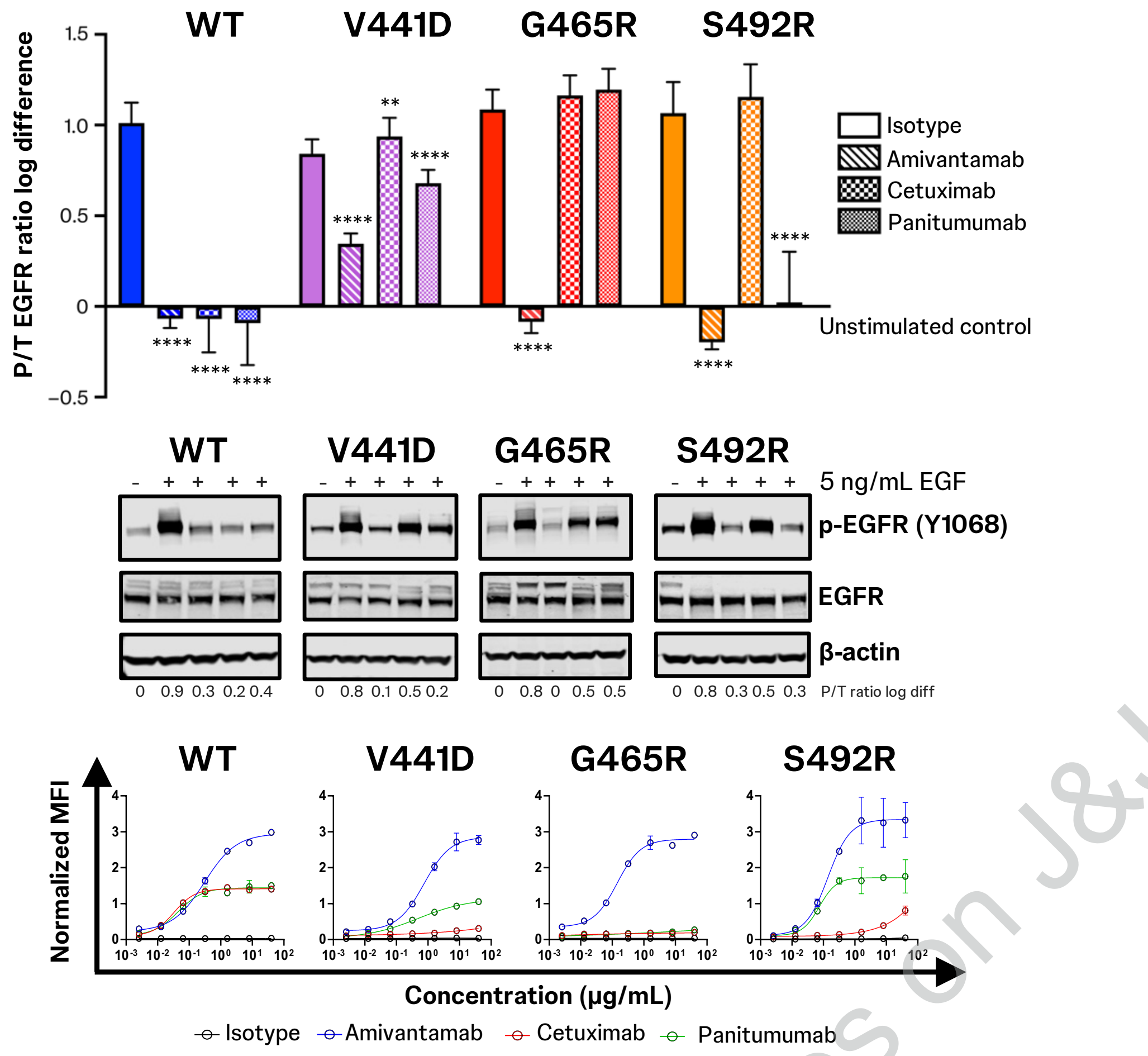
Figure 1: EGFR ECD mutations evaluated for antibody activity



Amivantamab, cetuximab, and panitumumab functional and binding activity were tested against the 29 EGFR ECD mutations depicted in the lollipop plot above. Clusters of frequently occurring mutations identified by Strickler et al[8] are highlighted.

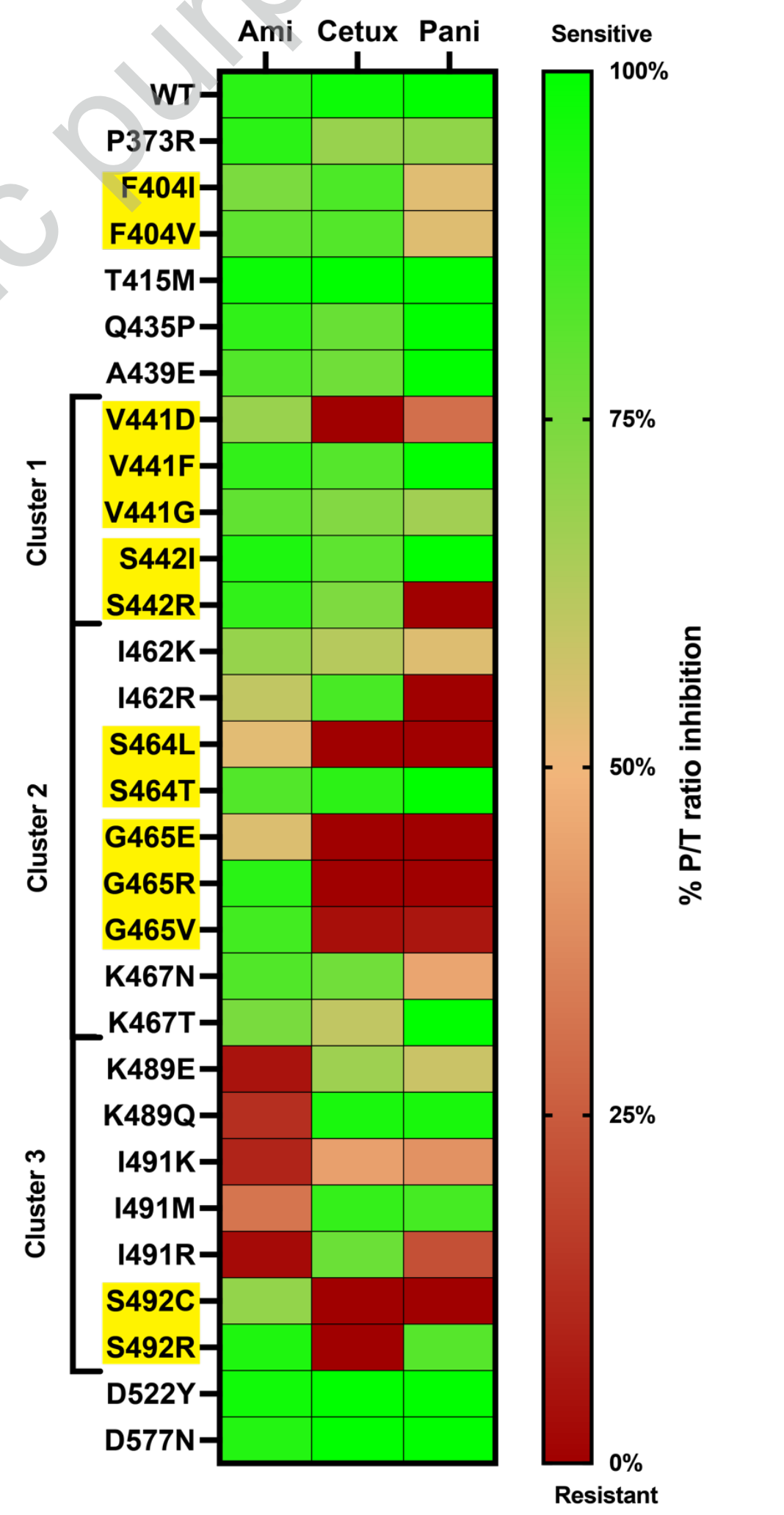
Results

Figure 2: Anti-EGFR activity among selected EGFR ECD resistance mutations



- AlphaLISA, western blotting, and flow-based binding assays validated literature reports that V441D, G465R, and S492R confer resistance to cetuximab and/or panitumumab (**Figure 2**)
- Conversely, amivantamab retained activity against these EGFR ECD mutants, which demonstrates that they do not prohibit sensitivity to amivantamab
- A heat map summary of AlphaLISA data demonstrated that amivantamab retains full or partial activity against most ECD mutants that confer resistance to EGFR monoclonal antibodies (**Figure 3**)
- All AlphaLISA results were orthogonally validated by flow cytometry–based binding assays (not shown)

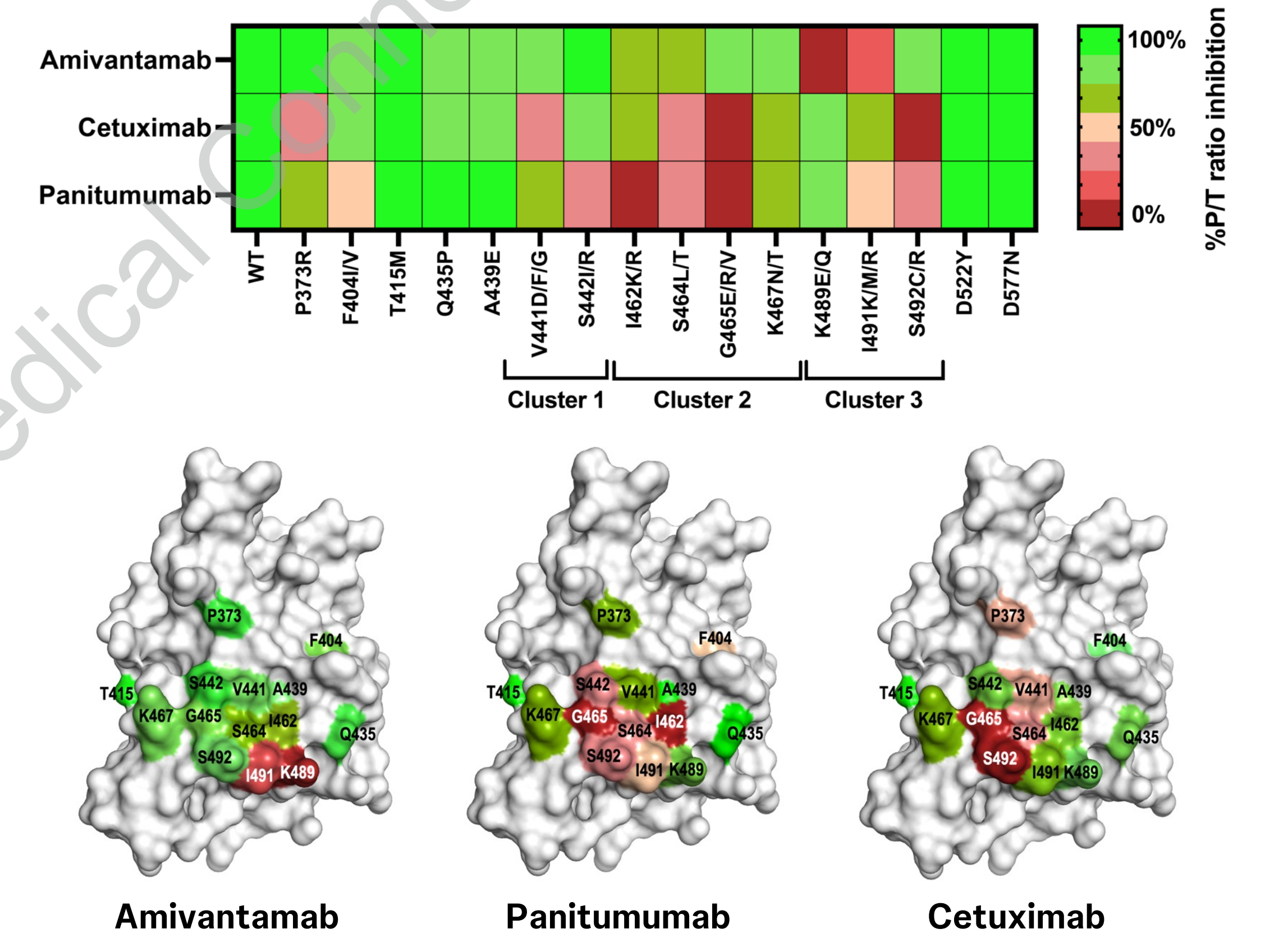
Figure 3: Anti-EGFR activity across EGFR ECD mutations



Note: Highlighted residues indicate a mutation frequency >5%. Ami, amivantamab; cetux, cetuximab; pani, panitumumab.

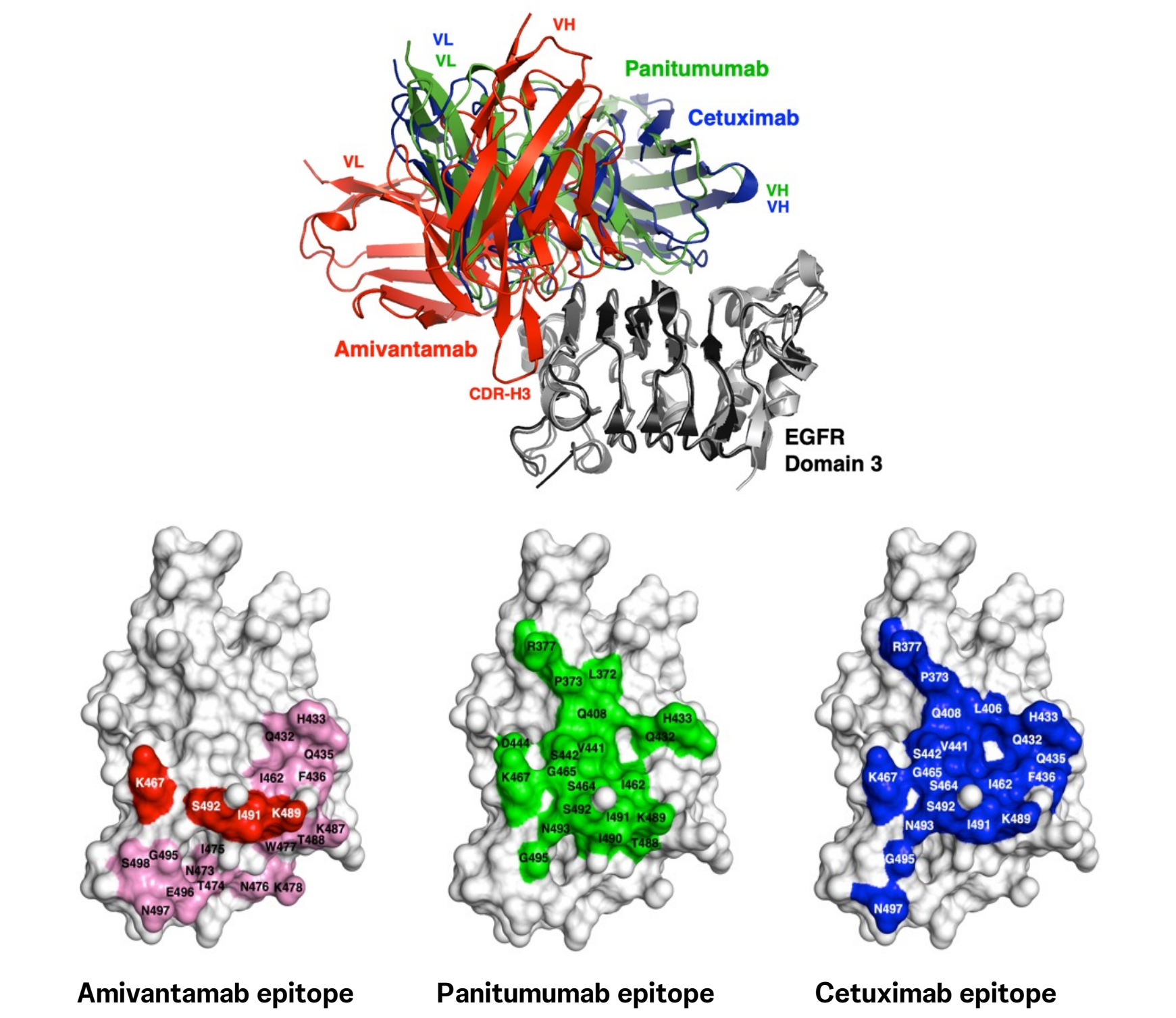
- AlphaLISA data were averaged across all ECD mutations at each residue and mapped onto the EGFR domain III face (**Figure 4**)
- The 3 clusters of frequently mutated ECD residues were found to be located within the same region of domain III, suggesting that the amivantamab epitope diverges from that of cetuximab and panitumumab

Figure 4: Visualization of sites of differential antibody activity



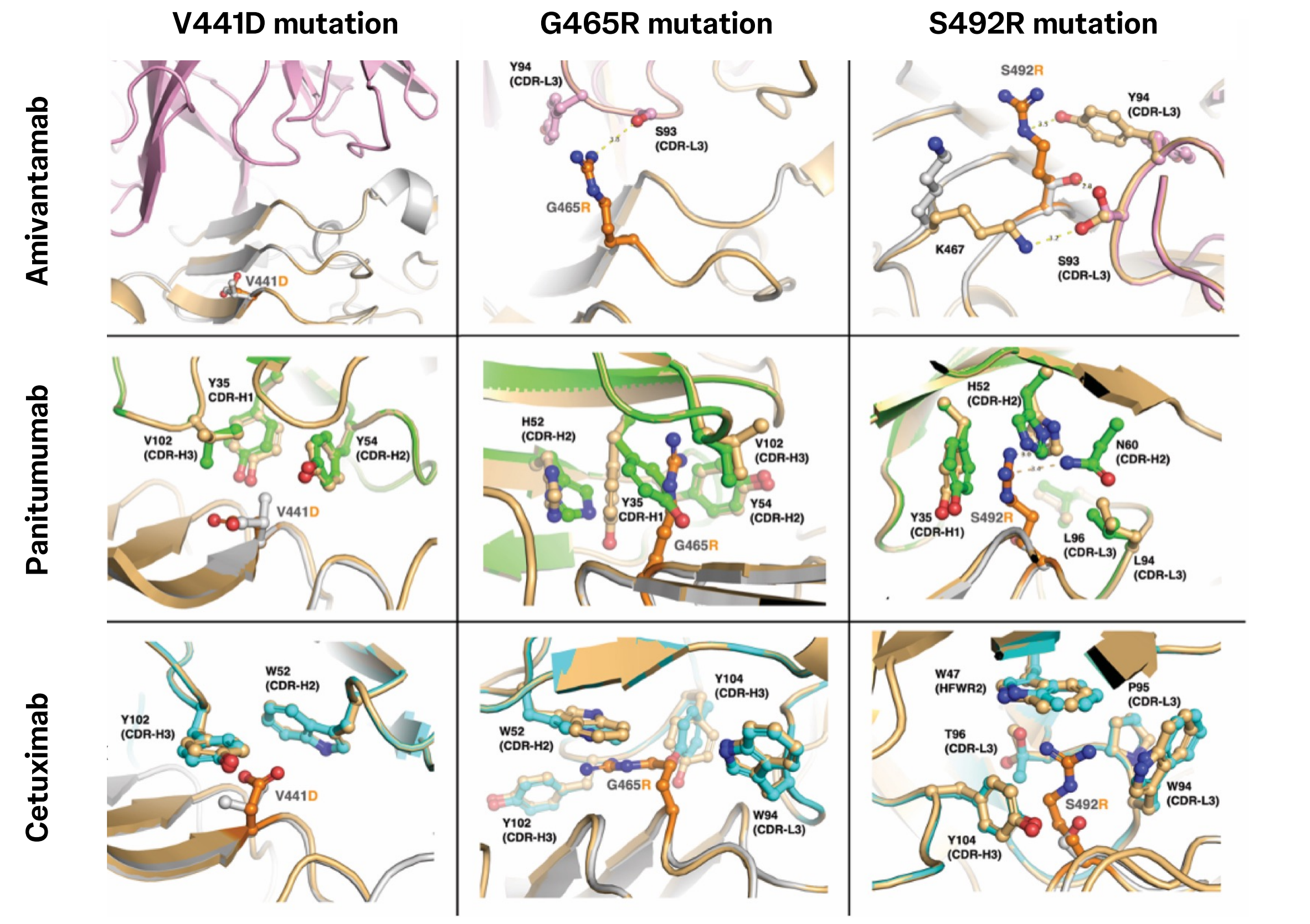
- Structural modeling demonstrated that the variable domain of amivantamab adopts a distinct position on EGFR domain III as compared with cetuximab and panitumumab (**Figure 5**)
  - Most notably, amivantamab's CDR-H3 forms unique interactions with domain III, which results in an epitope shifted away from residues 441 to 444 and 464 to 465 and toward residues 473 to 478 and 496 to 498

Figure 5: Comparison of binding interactions between amivantamab, cetuximab, or panitumumab and EGFR



- Structural modeling, which was consistent with the epitope footprints in **Figure 5**, projected that V441D, G465R, and S492R mutations introduce steric hindrance and/or disrupt hydrogen bonding with cetuximab and panitumumab while having minimal impact on amivantamab and domain III interactions (**Figure 6**)

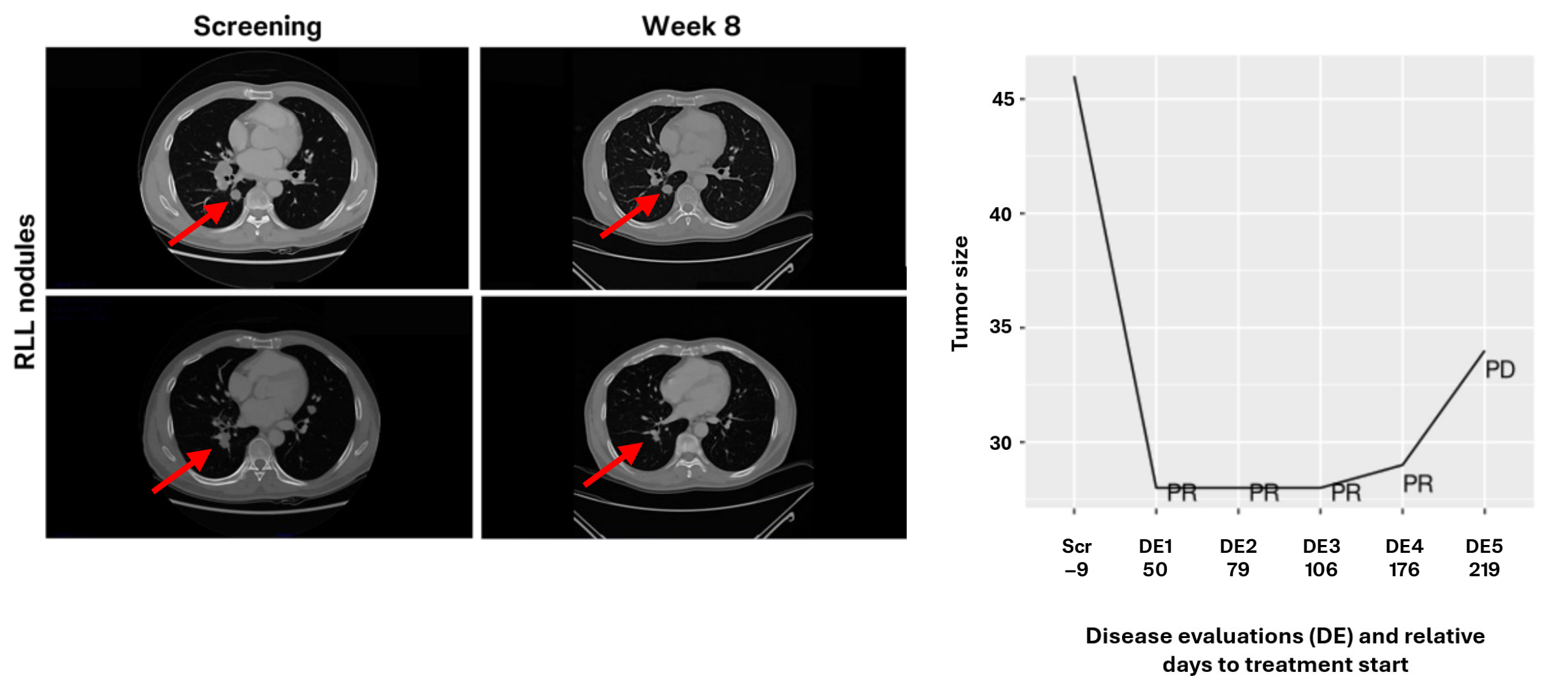
Figure 6: Structural modeling of interactions between antibodies and selected EGFR ECD mutations



Case study from OrigAMI-1

- While patients with EGFR ECD mutations detected by central circulating tumor DNA were excluded from OrigAMI-1, 1 participant with *RAS/BRAF* WT mCRC was retrospectively determined to harbor an S492R mutation by tissue next-generation sequencing (**Figure 7**)
  - This participant who received amivantamab monotherapy (had also received prior cetuximab) demonstrated a partial response with a duration of response of ~6 months, which provides preliminary clinical evidence that amivantamab is active against an EGFR ECD mutation associated with cetuximab resistance

Figure 7: Clinical case study of amivantamab monotherapy antitumor activity against EGFR S492R mutation



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Solid Tumors

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