

1213

HYDROPHOBIC INTERACTIONS UNDERLIE RECOGNITION OF A KRAS G12V NEOANTIGEN BY A TCR-MIMIC ANTIBODY

¹Katharine Wright*, ¹Sarah DiNapoli, ²Michele Miller, ¹Sandra Gabelli, ¹P Aitana Azumendi, ¹Jacqueline Douglass, ¹Kenneth Kinzler, ¹Bert Vogelstein, ¹Shibin Zhou, ³XiaoWei Zhao, ³Zhiheng Yu. ¹Johns Hopkins School of Medicine, Baltimore, MD, USA; ²Johns Hopkins University, Baltimore, MD, USA; ³Janeila, Ashburn, VA, USA

Background Antibodies' exquisite specificity enables the development of new targeted immunotherapies for treatment in the fight against cancer. Antibodies can be engineered to target peptides derived from oncogenic proteins that are presented on the cell surface by human leukocyte antigens (pHLA).^{1, 2} Here, we describe the structural basis of recognition of a T cell receptor (TCR)-mimic antibody, termed V2, against the HLA-A*03:01-restricted KRAS^{G12V} epitope (VVVGAVGVGK), highlighting its specificity.

Methods For structural studies, the KRAS^{G12V}-specific full-length antibody (V2-IgG) was expressed in mammalian cells to generate an optimal protein fold. Structure determination of the V2-IgG in complex with KRAS^{G12V}/HLA-A*03:01 was performed by single particle cryo-electron microscopy (cryoEM). Two-dimensional and three-dimensional classifications were performed to produce a final refined map at 3.1 Å resolution. To fully understand determinants of the specificity of the V2 antibody, we determined the X-ray crystal structure of the KRAS^{WT}/HLA-A*03:01 monomer (resolution 2.6 Å). Biophysical characterization included carrying out differential scanning fluorimetry (DSF) and binding kinetics experiments using surface plasmon resonance (SPR). Furthermore, we screened a single variant library of the V2 TCRm antibody to identify key residues for peptide specificity and affinity enhancement.

Results The cryoEM complex structure revealed the V2 TCRm antibody sits on top of the KRAS^{G12V}-HLA-A*03:01 binding groove, making multiple contacts and leaning heavily towards the C-terminus of the KRAS^{G12V} peptide. All contacts made between the V2 TCRm antibody and KRAS^{G12V} peptide were aliphatic and hydrophobic in nature, with no hydrogen bonds made directly with the peptide. Three complementarity determining regions (CDRs) of the V2 TCRm antibody formed a loose cage-like configuration around the G12V neoantigen. Structural alignment of the V2 bound KRAS^{G12V}-HLA-A*03:01 and unbound KRAS^{WT}-HLA-A*03:01 structures revealed the KRAS^{G12V} peptide underwent a conformational change upon antibody binding. This observation is in congruence with the SPR data that showed a two-state binding model. Moreover, the affinity matured process yielded V2 variant antibodies with comparable or enhanced affinity, but none retained both specificity and increased T-cell activation.

Conclusions This complex structure is the first cryoEM structure of an antibody fragment binding a neoantigen-HLA target and the first structures of the KRAS^{WT/G12V} peptides presented by HLA-A*03:01 with or without an antibody in complex. Characterizing how the V2 TCRm antibody recognizes KRAS^{G12V}-HLA-A*03:01 and differences in KRAS^{WT} and KRAS^{G12V} peptide binding to HLA-A*03:01 offers insight into how highly hydrophobic peptide neoantigens can be targeted with antibody-based therapies.

REFERENCES

1. Hsiue EH, Wright KM, Douglass J, Hwang MS, Mog BJ, Pearlman AH, Paul S, DiNapoli SR, Konig MF, Wang Q, Schaefer A, Miller MS, Skora AD, Azumendi PA, Murphy MB, Liu Q, Watson E, Li Y, Pardoll DM, Bettegowda C, Papadopoulos N, Kinzler KW, Vogelstein B, Gabelli SB, Zhou S. Targeting a neoantigen derived from a common TP53 mutation. *Science*. 2021;**371**:eabc8697.

2. Douglass J, Hsiue EH, Mog BJ, Hwang MS, DiNapoli SR, Pearlman AH, Miller MS, Wright KM, Azumendi PA, Wang Q, Paul S, Schaefer A, Skora AD, Molin MD, Konig MF, Liu Q, Watson E, Li Y, Murphy MB, Pardoll DM, Bettegowda C, Papadopoulos N, Gabelli SB, Kinzler KW, Vogelstein B & Zhou S. Bispecific antibodies targeting mutant RAS neoantigens. *Sci Immunol*. 2021;**6**:eabd5515.

<http://dx.doi.org/10.1136/jitc-2022-SITC2022.1213>