IDENTIFICATION OF FULLY HUMAN TCR-MIMIC ANTIBODIES TARGETING THE KRAS G12V/HLA COMPLEX GENERATED IN HLA-TRANSGENIC RENMAB™ MICE


Background Mutations in the small GTPase protein KRAS drive several cancers, owing to accelerated growth and differentiation phenotypes resulting from altered GDP-GTP exchange and GTP hydrolysis rates. As targeting RAS proteins with traditional drug modalities has proved to be challenging, new therapeutic strategies are needed. Here, we generated and screened TCR-mimic antibody hits targeting the mutant KRAS G12V peptide/HLA complex, as the majority of KRAS mutations occur at this residue.1

Methods HLA-transgenic RenMice™ were immunized with KRAS G12V/HLA-A0301 or KRAS G12V/HLA-A1101 peptide-MHC (pMHC) complexes. The specificity of antibody hits recovered from the mice, as well as a positive control antibody previously described,2 was tested on HLA-A overexpressing cells in the context of various KRAS peptides by flow cytometry. Positive hits were assessed for heavy and light chain germline gene usage and CDR3 length, and K_D values were measured. To assess specificity of the antibodies, binding of two candidate antibody hits to other peptide and/or HLA complexes were tested. Alanine scanning substitutions of the KRAS G12V peptide were also performed to identify the residues critical for TCR-mimic antibody recognition. Killing of HLA-A0301+ cells with KRAS G12V or wildtype KRAS by TCR-mimic antibody candidate 2 conjugated to a CD3-targeting antibody was subsequently measured (by LDH activity) in the context of human T cells.

Results We identified TCR-mimic antibodies with specificity for KRAS G12V residues 7–16 complexed with HLA-A0301 or HLA-A1101 by flow cytometry. KRAS G12V/HLA-A1101 and KRAS G12V/HLA-A0301 antibody hits exhibited varying degrees of germline gene diversity and CDR3 length, with the majority of KRAS G12V/HLA-A0301 antibodies exhibiting higher affinity than the positive control. KRAS G12V/HLA-A0301 antibodies specifically bound HLA-A0301 overexpressing cells pulsed with KRAS G12V decamer peptide in a dose-dependent manner, but did not bind KRAS G12C, G12D, WT peptide or unpulsed controls. Furthermore, KRAS G12V/HLA-A0301 antibodies did not bind HLA-A1101 overexpressing cells loaded with KRAS mutant or WT peptides, or HLA-A0301 overexpressing cells loaded with off-target peptide. Alanine substitutions revealed that residue V6 of KRAS G12V was required for antibody binding. Cytotoxicity assays demonstrate specificity of KRAS G12V/HLA-A0301 antibody for KRAS G12V+/HLA-A0301+ cells.

Conclusions Using our novel fully human HLA-transgenic RenMice, we have successfully generated and screened TCR-mimic antibodies specific for KRAS G12V/HLA-A0301 pMHC or KRAS G12V/HLA-A1101 pMHC complexes for further preclinical investigation.

REFERENCES

Ethics Approval All animal studies were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of Biocytogen Beijing Co., Ltd.

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