1168 IDENTIFICATION OF FULLY HUMAN TCR-MIMIC ANTIBODIES TARGETING THE KRAS G12V/HLA COMPLEX GENERATED IN HLA-TRANSGENIC RENMAB™ MICE

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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

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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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