OPTIMIZING A T CELL-ENGAGING BISPECIFIC ANTIBODY TARGETING THE HIGHLY RECURRENT P53 R175H NEOANTIGEN VIA AFFINITY MATURATION

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Background Targeting mutation-associated neoantigens (MANAs) is a highly-cancer specific strategy to selectively eliminate cells harboring common driver mutations in genes encoding intracellular proteins such as Ras and p53. T cell-engaging bispecific antibodies targeting MANAs redirect T cells to kill cancer cells presenting mutant peptides on human leukocyte antigens (pHLA). However, T cell-redirecting therapies’ efficacy can be limited by the low antigen density of these MANAs on the cell surface. Here, we investigate whether increasing the affinity of a T cell-engaging bispecific antibody (clone H2) targeting the p53 R175H MANA (HMTEVVRHC) presented on HLA-A*02:01 (R175H/A2) improves its efficacy in vitro and in vivo.

Methods To identify higher affinity variants, we screened a phage display library consisting of 1159 single-amino acid variants at 61 sites in the six complementarity determining regions of the H2 single chain variable fragment targeting R175H/A2. Variants retaining R175H/A2 specificity were selected over multiple rounds of panning followed by affinity enrichment via thiocyanate elution. Selected variants were compared to the original H2 bispecific antibody in co-cultures with primary human T cells and cancer cell lines expressing endogenous levels of HLA-A*02:01 and the mutant p53-R175H protein or isogenic control cell lines. For in vivo comparison, NSG mice inoculated with KMS26 or Nalm6 cells and human T cells were treated with a continuous infusion of bispecific antibody for 14 days.

Results Three variant bispecific antibodies were identified with higher affinity and retained specificity for R175H/A2 (KD of 12.9 nM, 6.8 nM, 3.3 nM vs. the original KD of 29.5 nM). The highest affinity variant was a double mutant incorporating two top single variants. Each higher affinity variant elicited greater T cell activation as measured by interferon gamma release and cytotoxicity in co-cultures with cell lines expressing endogenous levels of the R175H/A2 pHLA. In vivo testing demonstrated that the higher affinity bispecific antibodies had improved tumor control in xenograft models compared to the lower affinity bispecific, particularly at a lower treatment dose (0.075 mg/kg/d).

Conclusions Increasing affinity for the p53 R175H/A2 pHLA to the low nanomolar range yields increased T-cell activation and cancer cell killing without sacrificing specificity for the R175H mutation.

REFERENCES

Ethics Approval Animal studies were approved by the Johns Hopkins University Animal Care and Use Committee, #MO18M79.