IL-2 AUGMENTS IMMTC-DEPENDENT T CELL ACTIVATION AND TUMOUR CELL KILLING

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Background Immune mobilising TCRs against cancer (ImmTAC) are a novel class of soluble bispecifics which bind both CD3 on T cells and specific peptide/MHC antigen on tumour cells.1 Four ImmTAC molecules are currently in clinical trials, one of these being tebentafusp which targets a gp100-derived HLA-A*02:01 peptide. Tebentafusp has demonstrated monotherapy activity in cutaneous and uveal melanoma.2 Here, we explored approaches to enhance ImmTAC-mediated T cell activation and anti-tumour activity. We hypothesised that the cytokine IL-2, which is approved for treatment of cutaneous melanoma and renal cell cancer, might augment ImmTAC-mediated T cell responses.3

Methods Tumour cell killing was determined by IncuCyte assay. Cell lines, healthy donor PBMC, tumour cell populations and cytokines including IL-2 (Proleneuk, Novartis) were sourced commercially. ImmTAC molecules recognising peptides from either gp100 or the tumour antigen PRAME were used for in vitro assays. Surface marker expression was determined by flow cytometry.

Results Clinically relevant IL-2 levels (60 to 600U/ml) improved both the speed (maximum killing after ~15 hours following 60 U/ml IL-2 pre-treatment versus ~30 hours without IL-2) and potency (mean EC50 13.6pm with IL-2 versus 81.2pm in absence of supplementation) of antigen-specific ImmTAC-dependent tumour killing in vitro by either PBMC or purified T cell populations. Addition of IL-2 was sufficient to improve killing responses when either T cell numbers or target peptide:HLA levels were limiting, and IL-2-cultured TILs exhibited ImmTAC-dependent killing. Dose-dependent cytokine enhancement of ImmTAC-mediated killing response was also consistent with increased surface CD3 and CD69 expression on T cell populations. Notably, weak PBMC donor populations with minimal intrinsic killing activity could be induced to provide robust ImmTAC-dependent tumour cell killing responses by physiological IL-2 treatment.

Conclusions These results suggest that the anti-tumour activity of ImmTAC molecules could be enhanced by combination treatment with the cytokine IL-2.

Ethics Approval The study was approved by the South Central Oxford A Research Ethics Committee (UK), REC reference 13/SC/0226

REFERENCES
4. Tang and Harding Cytokine: X 2019
5. Moureau, et al. (2020) AACR poster

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TARGETING A SHARED TP53 NEOANTIGEN WITH BISPECIFIC T CELL RETARGETING ANTIBODY


Background TP53 is the most commonly mutated cancer driver gene but drugs that target TP53 are not yet available. A peptide derived from the most common p53 mutation R175H (HMTEVVRHCA) can be presented by a common human leukocyte antigen (HLA-A*02:01) after proteasomal degradation.3 We aimed to develop T cell receptor (TCR)-mimic antibody targeting this shared neoantigen.

Methods We constructed a single-chain variable fragment (scFv) phage display library that presents scFvs at an estimated diversity of 3.6e10. Mutant peptide-HLA (pHLA)-specific scFvs were enriched through five rounds of positive and negative selections. Mutant pHLA-specific scFv clones were converted into bispecific T cell retargeting antibodies in the single-chain diabody (scDb) format by linking with the anti-CD3 scFv UCHT1.2 These scDbs were tested in T cell co-cultures in the presence of target pulsed cells or target cells that either overexpress the p53 neoantigen or present the p53 neoantigen at endogenous levels. In vivo efficacy was assessed by administering scDb in NSG xenograft mouse model. The structural basis of the binding specificity was evaluated by X-ray crystallography.

Results We identified an scFv, termed clone H2, that specifically binds p53 R175H/HLA-A*02:01 pHLA and not its wild-type counterpart at a Kd of 86 nM (figure 1A). H2-scDb induced T cell cytokine release and cytotoxicity in the presence of 1) HLA-A*02:01-expressing cells pulsed with the p53R175H peptide, 2) cells transfected with HLA-A*02:01 and p53 R175H, and 3) cancer cell lines KMS26, KLE, and TYK-nu that express endogenous HLA-A*02:01/p53 R175H (figure 1B-E). T cell activation was diminished when TP53 was knocked out from these cancer cell lines using CRISPR (figure 1E). When administered to NOD scid gamma (NSG) mice systemically engraved with the KMS-26 cell line, H2-scDb significantly suppressed tumor growth (figure 1 F, G). The structure of p53 R175H/HLA-A2 bound to the H2-Fab fragment shows that four complementarity-determining region loops of the H2 antibody formed a cage-like configuration around the C-terminus of the p53 R175H peptide, trapping the mutant histidine (His175) and the adjacent arginine (Arg174) residues in a stable interaction, which provides the structural basis for the specificity (figure 2).

Conclusions We have developed a TCR-mimic bispecific T cell engager H2-scDb that recognized the shared neoantigen...