other NET, including complete regression of established tumors and clearance of metastatic lesions. These findings warranted evaluation of AMG 757 (NCT03319940); the phase 1 study includes dose exploration (monotherapy and in combination with pembrolizumab) and dose expansion (monotherapy) in patients with SCLC (figure 2). A study of AMG 757 in patients with neuroendocrine prostate cancer is under development based on emerging data from the ongoing phase 1 study.

Conclusions AMG 757 engages and activates T cells to kill DLL3-expressing SCLC and other NET cells in vitro and induces significant antitumor activity against established xenograft tumors in mouse models. These preclinical data support evaluation of AMG 757 in clinical studies of patients with NET.

Ethics Approval All in vivo work was conducted under IACUC-approved protocol #2009-00046.

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628 IL-2 AUGMENTS IMMTAC-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 3] 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.[4]

Methods Tumour cell killing was determined by IncuCyte assay. Cell lines, healthy donor PBMC, tumour cell populations and cytokines including IL-2 (Proleukin, Novartis) were sourced commercially. ImmTAC molecules recognising peptides from either gp100 or the tumour antigen PRAME[5] 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

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629 TARGETING A SHARED TP53 NEOANTIGEN WITH BISPECIFIC T CELL RETARGETING ANTIBODY

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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 (HMTEVVRHC) can be presented by a common human leukocyte antigen (HLA-A*02:01) after proteasomal degradation.[1] 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-culturs 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 engrafted 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

HLA-A*02:01/p53 R175H pHLA complex with exquisite specificity. It effectively activated T cells and lysed tumor cells both in vitro and in vivo. This approach could in theory be used to target cancers containing mutations that are difficult to target in conventional ways.

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Trial Registration NA

Ethics Approval NA

Consent NA

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630 PD-L1/CD47 TUMOR DIRECTED B-BODYTM BISPECIFIC ANTIBODIES DEMONSTRATING SIGNIFICANT ANTI-TUMOR ACTIVITY WITH NO TOXICITY IN PRECLINICAL MODELS

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Background Tumor cells have been shown to utilize both innate and adaptive checkpoints to evade anti-tumor immune responses. CD47 and PD-L1 are two targets widely expressed on the cell surface of tumor cells and are predicted to coordinately suppress innate and adaptive sensing respectively to evade immune control. PD-L1 dampens T cell-mediated tumor killing (via PD-L1/PD-1 signaling) while CD47 protects tumor cells from phagocytosis (via CD47/SIRP-alpha signaling). Targeting each of the above pathways with monoclonal antibodies has shown promise with PD-L1/PD-1 inhibition showing durable responses and extended overall survival for several approved products, whereas the molecules targeting CD47 pathway are in early clinical trials. Given that a significant number of patients are either resistant or relapse on PD-L1/PD-1 therapy, combinations with anti-CD47 antibodies are being explored. However, the expression of CD47 on many normal cells such as hematopoietic cells, red blood cells (RBCs) and platelets provides a widespread antigen sink which impacts the PK and adverse event profile of these agents.

Methods Here, we describe the generation and testing of a large panel of bispecifics with combinations of different affinities to PD-L1 and CD47 using the B-Body™ bispecific screening platform. The bispecific antibodies were screened in various in vitro activity and developability assays. Selected leads from the screen were tested in multiple in vivo models with differential expression of CD47 and PD-L1.

Results The lead bispecific antibodies showed significant blockade of SIRPa/CD47 and PD-L1/PD-1 signaling in vitro and tumor growth inhibition in vivo. The studies also showed no significant binding to RBCs and induced minimal RBC phagocytosis in vitro. A summary of screened candidates and characterization of a lead candidate being developed further will be presented.

Conclusions We have identified multiple CD47/PD-L1 bispecific antibodies with favorable efficacy and safety profiles. Selection of a lead for further IND and clinical development is underway.

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631 DEVELOPMENT OF HIGHLY EFFICACIOUS AND SAFE TARGETED CANCER IMMUNOTHERAPY VIA IL12-BASED TMEKINE™ PLATFORM

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Background We developed tumor microenvironment-targeting immunocytokine or TMEkine™ utilizing strong anti-tumoral effect of interleukin 12 (IL-12). In this effort, we created a bi-specific 1+1 antibody fusion with conventional knob-in-hole technology where anti-CD20 was paired with IL-12 fc fusion arm. A couple of IL-12 muteins were used in our therapeutic molecules to reduce systemic toxicity. IL-12 has been known for a key orchestrator in immune response. The main actions of IL-12 include the induction of CD4+ Th0 cells toward Th1 type and enhancement of IFN-γ production, stimulation of cytotoxicity and growth of natural killer (NK) cells and CD8+ T cells. For these reasons, IL-12 has long been considered as a potential therapeutic molecule for treating cancers by enhancing immune activity toward tumor cells. However, systemic administration of IL-12 showed poor efficacy and severe adverse effects. With our therapeutic approach of tumor targeting and attenuated IL-12 mutein, we expect that our IL12-based TMEkine™ holds great promise for the future of cancer immunotherapy.In this study, we targeted CD-20 expressing cancers such as B-cell lymphoma with our anti-CD20/IL-12 mutein TMEkine. We evaluated the biological activity of our molecules with in vitro and in vivo efficacy and safety.

Methods The target specific binding to CD20 and IL-12 receptor was analyzed by FACS and ELISA. Biological activities as signaling transduction and T cell activation were confirmed in vitro using HEKblue IL12 cell line, primary human T cells and NK cells. The anti-tumor efficacy of TMEkine (CD20-IL-12) was assessed in A20 lymphoma syngeneic mouse model. To demonstrate long term protection to A20, the cured five mice after TMEkine administration were re-challenged with A20 and 4T1 cells.

Results First, we analyzed the specific binding of our TMEkine molecules to CD20 expressing B-cell lymphoma cell lines (such as Raji). We showed that TMEkine (CD20-IL-12) binds to Raji and Ramos, which express CD20, but not to Jurkat, which does not express CD20. We also showed that TMEkine molecules bind to IL-12 receptor in a dose-dependent manner. pSTAT4 alphaLISA assay revealed that TMEkine (CD20-IL-12) transduces STAT4 signaling. In our IL-12 mutein, key residues for heparin binding were mutated. The biological activity of our mutein molecule was attenuated due to this change in human PBMC. In addition, our TMEkine molecules significantly induced IFN-γ secretion from primary human T cells and NK cells. An A20 B-cell lymphoma syngeneic mouse model was utilized to investigate the anti-tumor activity of TMEkine (CD20-IL-12). TMEkine molecules were injected three times with Q3D intraperitoneally. Tumor growth was