Results Using radiolabeled synTacs/Immuno-STATs loaded with the appropriate peptides, we employed positron emission tomography to localize human papillomavirus (HPV16)-specific CD8 T cells to implanted HPV16-positive tumors in mice, as well as influenza A virus (IAV)-specific CD8 T cells in the lungs of IAV-infected mice. In vivo administration of HIV- and CMV-specific synTacs/Immuno-STATs to immunodeficient mice intrasplenically engrafted with human donor PBMCs resulted in the marked and selective expansion of HIV-specific and CMV-specific human CD8 T cells populating their spleens, respectively.

Conclusions We demonstrate the remarkable flexibility of the synTacs/Immuno-STAT platform for addressing a broad range of applications, including the first report of the in vivo imaging of antigen-specific CD8 T cell populations and in vivo antigen-selective expansion of human CD8 T cells. These results suggest that, in addition to broad therapeutic applications, synTac/Immuno-STATs may provide prognostic/diagnostic information. Most notably, these results demonstrate the presence of synTacs/Immuno-STAT biologics in the tumor or infected tissues where they can elicit T cell restimulation and expansion necessary for target killing and enhanced therapeutic efficacy.

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IFN γ SECRETED BY TEBENTAFUSP (IMCGP100)-REDIRECTED T CELLS INHIBITS EXPRESSION OF MELANIN SYNTHESIS PATHWAY GENES IN HEALTHY MELANOCYTES

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Background Tebentafusp (IMCgp100) is a bispecific T cell redirector comprised of an affinity-enhanced TCR recognising melanocyte lineage antigen gp100 and a T cell engaging anti-CD3 scFv domain. Tebentafusp has shown activity as monotherapy in advanced cutaneous and uveal melanoma (Middleton *et al.*, ASCO 2019), and we have previously reported that over half of uveal melanoma patients treated with tebentafusp display melanocyte-related adverse events (MRAE). These include vitiligo/skin hypopigmentation, leukotrichia, and hyperpigmentation and, collectively, are associated with better overall survival in uveal patients receiving tebentafusp (Orloff *et al.*, AACR 2020). In this study, we dissected the mechanisms by which tebentafusp may induce MRAE and highlight the potential clinical significance.

Methods In vitro studies were conducted to assess the direct and indirect effects of tebentafusp on epidermal melanocytes from healthy donors. Expression of gp100 and the gp100:HLA*02:01 target complex by melanocytes were quantified at the mRNA level and on the cell surface by confocal microscopy, respectively. Melanocytes co-cultured with PBMC and increasing concentrations of tebentafusp were assessed for their susceptibility to lysis and/or ability to stimulate cytokine production. These readouts were compared to gp100-positive and negative melanoma cancer cell lines. Melanin production by melanocytes was quantified and the melanin synthesis pathway interrogated at the mRNA and protein level following exposure to secretomes from tebentafusp-redirected PBMC against melanoma cancer cells.

Results Healthy melanocytes expressed 2 to 3-fold lower levels of gp100 peptide-HLA complexes on their surface compared to gp100-positive melanoma cell lines. In the presence of tebentafusp, this lower target expression translated into 3–6 fold lower levels of IFN γ and more than 100 fold lower granzyme B production by redirected T cells and these melanocytes were resistant to direct tebentafusp-induced killing (EC50 for melanocytes greater than 1nM vs EC50 melanoma cell lines of 23–50 pM). Supernatants from T cells activated in response to melanoma cancer cells by tebentafusp downregulated the melanin content of healthy melanocytes (20–30% reduction). Western blotting revealed 30–40% inhibition of two key components of the melanin synthesis pathway; the tyrosinase-related protein (TRP)-1 and TRP-2. This inhibition was reversed by blocking IFN γ in supernatants from activated T cells.

Conclusions MRAEs, especially vitiligo, associated with response to tebentafusp, may be explained, at least in part, by the downregulation of melanin biosynthesis pathway genes by IFN γ secreted by tebentafusp-activated T cells.

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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BISPECIFIC PERSONALIZED APTAMER FOR THE TREATMENT OF SOLID TUMORS

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Background Despite substantial progress observed in the field of targeted therapies for cancer, there is still a major unmet clinical need for truly personalized medicine approaches. Aimmune's innovative proprietary technology enables a personalized anti-cancer treatment based on a process of selecting and identifying specific functional aptamers – structured single-stranded DNA (ssDNA) oligonucleotides, that are able to bind a large variety of targets with high affinity and specificity.

Methods The Bispecific Personalized Aptamer is comprised of two ssDNA oligonucleotides arms joined together by a dimerization site. One arm of the Bispecific Personalized Aptamer is the outcome of Aimmune's innovative platform,¹ identifying functional aptamer sequences with the ability to kill tumor target cells, while leaving healthy tissue intact. The second arm is a constant aptamer sequence that binds to cytotoxic T lymphocytes. The two aptamer arms of the bispecific structure are bridged together by complementary sequences that form a CpG-rich domain designed to induce TLR9-mediated Antigen Presenting Cells (APCs) stimulation.

Results We have demonstrated a successful identification of a personalized aptamer arm using HCT116 colon carcinoma cells as a target. When hybridized to the constant, T cell engager arm, the bispecific entity has demonstrated potent yet

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selective tumor cell death induction. The Bispecific Aptamer has been further shown to significantly attenuate HCT116 tumor growth in vivo, an effect that was translated into a benefit to survival of treated mice.

Conclusions We have provided a proof-of-concept for Aumune's platform ability to identify an effective functional personalized aptamer, which did not harm healthy cells. The Bispecific Aptamer's exerted function in vitro has translated into a significant effect in vivo. Based on the personal approach and multiplicity of modes of action, the Bispecific Personalized Aptamer could have an effect in a broad spectrum of cancer indications.

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IMPROVING THE YEAST TRANSFORMATION EFFICIENCY FOR YEAST DISPLAY IN ANTIBODY DEVELOPMENT

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Background In the therapeutic antibody development process, the yeast display technology which expresses a large library of antibodies is very useful for increasing the affinity of a lead antibody. Ideally, a yeast library should exceed the size of 10E10 to 10E11 to get close to the real affinity maturation process. However, due to low transformation efficiency with yeast, it requires tremendous scaling-up efforts to simply reach the 10E9 library size.

Methods To address the transformation problem, we developed a new electroporation device that applies a high voltage on a sealed electroporation tube containing the yeast and plasmids in a low conductance buffer.

Results The new device is arcing free due to the sealed design and each single reaction could generate 10E8 library size, far exceeding the 10E6 size that was previously reported in a single reaction.

Conclusions With the improved transformation efficiency, it becomes very straightforward to reach the currently difficult size of 10E9. Further more, it is possible to reach the 10E10 to 10E11 library size with reaction scaling-up. Our new method could be very useful for the field of antibody development.

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THE DLL3-TARGETED HALF-LIFE EXTENDED BISPECIFIC T CELL ENGAGER (HLE BiTE®) IMMUNE-ONCOLOGY THERAPY AMG 757 HAS POTENT ANTITUMOR ACTIVITY IN NEUROENDOCRINE CANCER

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Background Neuroendocrine tumors (NET), including small cell lung cancer (SCLC), have poor prognosis and limited therapeutic options. AMG 757 is an HLE BiTE® immune therapy designed to redirect T cell cytotoxicity to NET cells by binding to Delta-like ligand 3 (DLL3) expressed on the tumor cell surface and CD3 on T cells.

Abstract 627 Figure 1 AMG 757 Significantly reduced tumor growth in orthotopic SCLC mouse models

Methods We evaluated activity of AMG 757 in NET cells in vitro and in mouse models of neuroendocrine cancer in vivo. In vitro, co-cultures of NET cells and human T cells were treated with AMG 757 in a concentration range and T cell activation, cytokine production, and tumor cell killing were assessed. In vivo, AMG 757 antitumor efficacy was evaluated in xenograft NET and in orthotopic models designed to mimic primary and metastatic SCLC lesions. NSG mice bearing established NET were administered human T cells and then treated once weekly with AMG 757 or control HLE BiTE molecule; tumor growth inhibition was assessed. Pharmacodynamic effects of AMG 757 in tumors were also evaluated in SCLC models following a single administration of human T cells and AMG 757 or control HLE BiTE molecule.

Results AMG 757 induced T cell activation, cytokine production, and potent T cell redirected killing of DLL3-expressing SCLC, neuroendocrine prostate cancer, and other DLL3-expressing NET cell lines in vitro. AMG 757-mediated redirected lysis was specific for DLL3-expressing cells. In patient-derived xenograft and orthotopic models of SCLC, single-dose AMG 757 effectively engaged human T cells administered systemically, leading to a significant increase in the number of human CD4+ and CD8+ T cells in primary and metastatic tumor lesions. Weekly administration of AMG 757 induced significant tumor growth inhibition of SCLC (figure 1) and

ED, extensive disease; MTD, maximum tolerated dose; RP2D, recommended phase 2 dose; RR, relapsed or refractory; SCLC, small-cell lung cancer.
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Abstract 627 Figure 2 AMG 757 Phase 1 study design