Results Results show > 4 log enrichment of tumor cells and average recovery of spiked CTC > 85% in the clinical relative range <100 cells per ml of whole blood (R2=0.929) with a throughput of 60 ml/hr. Isolated cells were confirmed to be cancer cells with imaging analysis and single cell genomic sequencing. The protocol was also validated with other cell line cells such as A549. The purity of the cells prepared by MARS are ideal for single cell genomics platforms.
Conclusions The fluidics of MARS is also replaceable and can be sterilized to minimize sample to sample contamination. The high molecular debris removal achieved by MARS is ideal for single cell genomics platforms, as is the first-to-market automated and integrated sample preparation and cell separation system designed to be a versatile tool for downstream cell analysis.

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12 DEVELOPMENT OF AN IN VITRO ASSAY TO ASSESS BISPECIFIC T CELL ENGAGER USING T CELLS FROM CD3E HUMANIZED MICE

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Background Bispecific T cell engagers (BiTE) is a fast-growing class of immunotherapies. They are bispecific antibody that bind to T cell-surface protein (for example, CD3e) and a specific tumor associate antigen (TAA) on tumor cells, by which to redirect T cells against tumor cells in a MHC-independent manner. A successful example in the clinical is Blinatumomab, a BiTE antibody against CD3/CD19 approved in 2014 to treat acute lymphoblastic leukemia. Currently, many CD3-based BiTE are in clinical trials, including BCMAxCD3, Her2xCD3, CEAxCD3, and PSMAxCD3. To evaluate the efficacy of BiTE in vitro, human peripheral blood monocyte cells (hPBMC) are commonly being used as a source of T cells to co-culture with tumor cells. The disadvantage of using hPBMC is donor-to-donor variability and the availability of the original donor if a study needs to be repeated.
Methods To overcome this, we proposed to replace hPBMC with T cells from human CD3e (hCD3) genetically engineered mouse models mice (GEMM) for in in vitro coculture assay. T cells were isolated from hCD3 GEMM mice using negative selection mouse T cell isolation kit. Conventional tumor cell lines or luciferase-engineered patient-derived-xenograft (PDX)-derived organoids (PDXO) expressing specific antigens are co-cultured with hCD3 T cells in 96-well plates in the presence of BiTE antibody.
Results We measured the killing of tumor cells using either flow cytometry or luciferase activity as readouts. To analyze tumor-reactivity of T cells to cancer cell line or organoids, IFN-gamma in the culture medium was measured and activation markers on T cells was assessed.
Conclusions Our data showed the feasibility of using humanized mice T cells as a replacement for hPBMCs to assess BiTE antibody in vitro. We are further validating the application of murine hCD3 T cells for in vivo models to test bispecific T cell engagers.

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13 USE OF ANTI-VIRAL T CELLS TO MODEL HLA-RESTRICTED ANTI-TUMOR CYTOTOXIC LYMPHOCYTE RESPONSES

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Background With the success of T cell checkpoint antagonists in treating cancer, we must better understand treatment response heterogeneity and develop more physiological preclinical models for evaluating the next wave of candidate therapeutics. Several hurdles limit the successful recapitulation of the cellular and molecular interactions between human T cells and tumor cells, not the least of which involves the challenge of access to – and ex vivo manipulation of – bona fide tumor antigen-specific T cells.
Methods In order to improve on our understanding of checkpoint therapy using human model antigens, we developed an antigen-specific T cell-mediated cytotoxicity model using anti-viral human T cells co-cultured with a human tumor cell line expressing viral peptide epitopes.
Results We found that anti-viral T cells could be used to model cytotoxic HLA-restricted anti-tumor responses and these responses varied by donor according to peptide antigen density, antigen quality, T cell numbers, and time. By identifying sub-optimal conditions in a donor-specific fashion, we demonstrated enhanced cytolytic function of T cells in vitro when combined with multiple disparate anti-tumor modalities, including immune checkpoint blockade, growth factor blockade, and chemotherapy. This in vitro model was then successfully adapted to an in vivo tumor model system that demonstrated control of tumor growth in an antigen-dependent manner that was responsive to checkpoint blockade.
Conclusions These in vitro and in vivo systems represent a simple, yet elegant and robust platform for testing human T cell-directed immuno-oncology (IO) therapeutics and IO combinations.
Ethics Approval All animal experiments were conducted in a facility accredited by the Association for Assessment and Accreditation of Laboratory Animal Care in accordance with institutional animal care and use committee guidelines and after appropriate approvals.

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14 NOVEL CD3 EPSILON HUMANIZED N-TERMINAL EPITOPE MODEL FOR ASSESSMENT OF EFFICACY OF T-CELL ENGAGERS

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Background T-cell engagers have proved to be a promising therapeutic strategy in immunotherapy, for redirecting T cells activity against tumor cells. To facilitate the preclinical assessment of novel T-cell engagers and their translatability, we have developed an immunocompetent CD3 epsilon N-terminal epitope humanized mouse model.
Methods This model was developed to express the human epitope of the CD3 epsilon chain, which is recognized by approximately 70% of the T-cell engagers (clone SP34). The rest of the extracellular domain was kept from mouse origin to preserve the amino acids involved in the interaction with CD3 gamma and delta. Similarly, the transmembrane domains

and the intracellular domains where kept murine to enable salt bridges interaction, interaction with the CD3 zeta and the signaling into mouse cells.

Results T cells from CD3 epsilon epitope humanized mice are found in comparable frequency in spleen, blood and bone marrow from WT mice. B cells, monocytes, dendritic cells and NK frequencies are also similar to the frequencies of these cell types in WT mice, suggesting that the humanization of the epitope of CD3 epsilon did not alter the immune cells distribution in these mice. Activation of T cells with antibodies targeting human CD3 (clone SP34) induced CD4 and CD8 T cell proliferation, as well as production of IL-2 and IFN-gamma. The CD3 functionality was demonstrated in vitro by the ability of B cells to produce IgM upon activation of T cells, suggesting a proper cooperation between T and B cells. Additionally, a first class of T-cell engagers targeting both human CD3 and a tumoral antigen, induced tumor cell lysis of MC38-Ag in a concentration-dependent manner. A second class of T cell engagers, also targeting CD3 and a tumoral antigen, showed an anti-tumor effect in vivo, and this effect was also shown to be dose-dependent.

Conclusions These data suggest that the CD3 epsilon N-terminal epitope humanized mouse model enables the assessment of efficacy and mechanism of action of T-cell engagers.This model is currently being intercrossed with immunostimulatory humanized mouse models to provide new opportunities for assessment of bi-specific antibodies targeting the CD3 and immunostimulatory molecules. This model is the first generation of a broader program aiming at developping a Pan CD3 humanized model, where the gamma, delta and epsilon chains of the CD3 complex will be humanized. The Pan CD3 humanized mice are currently being investigated for immune responses and would provide a broader tool for assessment of T-cell engagers.

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15 A NOVEL CD28 HUMANIZED MOUSE MODEL FOR EFFICACY ASSESSMENT OF CD28-TARGETING THERAPIES

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Background Immuno-intervention through targeting of activating and inhibitory immune checkpoints (ICP), has shown promising results in the clinic over the last years. To facilitate these researches, mouse models expressing humanized ICP instead of their mouse counterparts were developed. Herein, we describe a novel CD28 humanized mouse model (hCD28 model), designed to test compounds targeting human CD28 (hCD28).

Methods Human and mouse CD28 (mCD28) have different signalling responses, with hCD28 being known for inducing higher levels of pro-inflammatory cytokines upon stimulation with ligands/superagonists. This can be explained by the expression of CD28i, a hCD28 amplifier isoform which is not found in mouse. Additionally, evidences suggested that the different signalling between human and mCD28 relies on one amino acid change in the intracellular domain (ICD).[1] Because the hCD28 model was developed to assess hCD28-targeting therapeutics, we decided to keep the expression of both canonical and CD28i isoforms to avoid undermining the biological effects of the testing antibodies. Although keeping the human ICD could favour the evaluation of cytokine production and therefore the safety of the test therapeutics, we decided to keep the mouse ICD to enable a proper interaction of CD28 with its signalling partners, allowing a physiological stimulation of CD28 in efficacy studies.

Results hCD28 mice express hCD28 on T cells and the frequency of CD3 T cells is comparable in both WT and hCD28 mice. Stimulation of hCD28 mice-isolated T cells with hCD28 ligands and agonist antibodies resulted in T cell proliferation and cytokine production, suggesting that hCD28 is functional in mouse cells. MC38 uptake rate and kinetic of growth were comparable in WT and hCD28 mice, suggesting no major defect in the immune response in the hCD28 mice. Importantly, splenocytes and tumor draining lymph nodes cells isolated from tumor-bearing hCD28 mice showed higher production of IL-2 and IFN-gamma upon in vitro re-challenged with MC38 when compared to WT cells. Since the frequency of CD3 cells (Treg, $CD4^+$ and $CD8^+$) is comparable to WT mice, this could be explained by the expression of the amplifier CD28i isoform, which is absent in WT mice.

Conclusions The hCD28 model described here supports the efficacy assessment of hCD28-targeting biologics, enabling PK/PD studies as hCD28 expression levels and pattern are physiological. However, after careful consideration of the CD28 biology, we decided to keep the mouse ICD, although it triggers lower pro-inflammatory cytokine production than CD28 human ICD. As such, this model is not suitable for toxicology/safety studies.

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16 ANTIBODY PROFILING OF PROSTATE CANCER PATIENTS REVEALS DIFFERENCES IN ANTIBODY SIGNATURES AMONG DISEASE STAGES AND FOLLOWING TREATMENT

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Background Previous studies of prostate cancer autoantibodies have largely focused on diagnostic applications. So far, there have been no reports attempting to more comprehensively profile the landscape of prostate cancer-associated antibodies. Specifically, it is unknown whether the quantity of antibodies or the types of proteins recognized change with disease progression or treatment.

Methods A peptide microarray spanning the amino acid sequences of the gene products of 1611 prostate cancer-associated genes was synthesized. Serum samples from healthy male volunteers (n=15) and prostate cancer patients (n=85) were used to probe the array. These samples included patients with various clinical stages of disease: newly diagnosed localized prostate cancer, castration-sensitive non-metastatic prostate cancer (nmCSPC), castration-resistant non-metastatic prostate cancer (nmCRPC), and castration-resistant metastatic disease (mCRPC). Serial sera samples from individuals who received treatment with either standard androgen deprivation therapy (ADT) or an anti-tumor vaccine were also used to probe the