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The role of immune checkpoint blockade in the cure of HIV infection

Sharon Lewin

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2

The anti-cancer immunotherapy landscape

Irv Weissman

Not available at time of printing

3

Tumour antigen-directed anti-cancer therapy in the era of checkpoint blockade

Jonathan Cebon

Not available at time of printing

4

CAR's and armoured CARs

Renier Brentjens

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5

CAR-T cell therapy for the GD2 tumour target

Michael Brown[1]

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We have enrolled 6 metastatic melanoma patients into the Phase 1 CARPETS clinical trial of autologous third-generation CAR-T cell therapy targeted toward GD2. No significant adverse events were attributable to the CAR-T cell therapy. In vivo persistence of the adoptively transferred CAR-T cells was not observed beyond two months post-infusion except in the one patient who received prior lymphodepleting chemotherapy using cyclophosphamide and fludarabine. We have in vitro evidence to suggest that the lack of in vivo persistence may have resulted from activation-induced cell death (AICD). We are now modifying our ex vivo culture conditions both to favour transduction of T cells with a memory phenotype rather than an effector phenotype and reduce the likelihood of AICD. Our clinical protocol modification will also include broadening the eligibility to enrol patients with other types of GD2-expressing malignancy

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Pre-clinical assessment of EphA2-directed CAR-T cell adoptive therapy for paediatric solid tumours

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The establishment of Chimeric Antigen Receptor (CAR) T cell immunotherapy as a treatment for refractory and relapsed B cell leukaemia demonstrates that genetic modification of immune cells to circumvent tumour cell immune escape is a powerful strategy to combat paediatric cancers. However, efforts to develop effective CAR T cell therapies for solid tumours face a number of challenges: establishing an effective dose, route of administration, tumour cell mediated dampening of immune cell function, and overcoming heterogeneous antigen expression across the tumour cell population.

We have developed reagents and methodology to generate CAR T cells specific for Erythropoietin-producing Hepatocellular receptor tyrosine kinase class A2 (EphA2), expressed on a range of paediatric solid tumours. We showed that EphA2 redirected CAR T cells specifically targeted and killed various EphA2+ pediatric cancer cell lines in vitro, alongside secretion of cytokines. In an in vivo osteosarcoma mouse model, injection of EphA2 CAR T cells directly into established tumours resulted in complete regression of tumours, and extended survival of mice compare to mice receiving control CAR T cells or vehicle alone. We also tested the efficacy on systematic delivery of CAR T cells. Further work focussing on effective clinical CAR T production, safety and possible off-target effects, improve design of gene therapy vectors and delivery, and combinatorial testing of EphA2 CAR T cells with CAR T cells targeting alternative antigens, or immune checkpoint blockade will elucidate a way forwards effective clinical usage of CAR T cell therapy for refractory and/or recurrent pediatric solid tumours.

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Development of dual specific CAR-T cells – a one-two anti-cancer punch

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The recent revolution in bioengineering of the cellular immune system, offers a promising new frontier of medicine with the potential to ultimately defeat cancer. Supercharging the anti-cancer power of the immune system by genetically engineering killer T lymphocytes to co-express chimeric cancer-specific antibody fragment molecules with cytoplasmic activation domains (Chimeric Antigen Receptor T cells; CAR-T cells) is leading this remarkable new clinical approach. We are developing multi-specificity CAR-T cells, initially targeted at ovarian cancer but the specificities chosen are equally applicable to a variety of adenocarcinomas including those of the gastrointestinal tract, breast, pancreas, lung, and prostate. Targeting multiple determinants should mitigate against tumour escape via mutation. We have identified two primary target molecules expressed on the cell surface of the target cells: TAG 72, a glycosylation mutant, and a second determinant (CTH CAR) commonly upregulated on many cancer cells. We initially developed a range of second generation CAR constructs with either CD28 or 4-1-BB as the signal activation domain. The relative cytotoxic efficacies of these variant CAR-T cells were evaluated using the real time impedence-based xCelligence assay. This system was also to reveal the importance of cytokine and CAR-T receptor activation on the specificity and longevity of the cytotoxic capacity. The resultant CAR-T induction, activation, specificity and safety strategies are being developed as a platform for an autologous Phase I clinical trial in ovarian cancer patients..

An additional approach that may dramatically enhance T cell clinical utility is to transfect such anti-cancer CAR constructs into iPSC cells, which themselves maybe derived from cancer specific TCR T cells (the rearranged TCR specificity is thus embedded in the iPSC). These are then re-differentiated into cytotoxic T cells to create a "limitless" expansion of such cancer killing T cells. These cells retain both the TCR specificity to cytoplasmic derived determinants plus the scFv to membrane (in our case glycosylation) determinants. By creating multiple lines of iPSC-derived "multi-antigen, cancer specific" killer T cells, we aim to service a wide number of people with this first-line cancer immunotherapy. The Australian Government has recently awarded Cartherics, (in partnership with Cell Therapies, Mesoblast, the Hudson Institute and Monash University), a grant of AUD$3million under the Cooperative Research Centre's Project (CRC-P) program. This CRC-P for Allogeneic Stem Cell Cancer Immunotherapies* aims to produce unique allogeneic CAR-T cells derived from homozygous haplotype iPSCs that can be administered to very large numbers of histocompatible cancer patients. These cells are produced from healthy donors rather than seriously ill cancer patients, manufactured and expanded at a fraction of the cost of autologous CAR-T cells and banked until needed.

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Multi-pronged approaches to augmenting CAR T cell therapy for solid tumours

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Adoptive cellular immunotherapy (ACT) involving genetic modification of T cells with antigen-specific chimeric single-chain receptors (CAR) has been highly successful in treating B cell malignancies particularly ALL. We have successfully completed a Phase I clinical trial using CAR T cells directed against the Lewis Y antigen in patients with Acute Myeloid Leukaemia; the therapy was well tolerated and the transferred cells persisted long term and trafficked to the site of disease1. However, for effective treatment of solid cancers by ACT, a major problem is the immunosuppressive mechanisms utilized by tumors to suppress immune clearance. One such pathway that has largely been ignored is the generation of adenosine by CD73 expressed on tumor cells. In this study, we investigated whether blockade of this pathway could enhance ACT using CAR T cells. Blockade of the A2A adenosine receptor with the small molecule antagonist SCH58621 enhanced the ability of CAR T cells targeting Her-2 to produce cytokines when cocultured with Her-2+ tumors. Moreover, CAR T cells generated from A2A-/- mice demonstrated enhanced activity against Her-2+ tumors compared with wildtype CAR T cells, highlighting a role for A2A mediated suppression in vivo. Interestingly, we found that although combined CAR T therapy and A2A blockade only moderately reduced the growth of established Her-2+ tumors, the addition of anti-PD-1 mAb in the treatment regimen resulted in a striking increase in efficacy. In conclusion, this study demonstrates that simultaneous blockade of adenosine and PD-1 can potently enhance CAR T-cell action, with potentially significant implications for future trials of CAR T cell therapy in solid malignancies.

1. Ritchie DS et al., Molecular Therapy, 2013

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Genome editing provides solution for cell therapy safety and immune tolerance: a guide for a One4All pluripotent cell line

Andras Nagy

Pluripotent stem cells have accelerated the development of new avenues for targeting degenerative diseases and cancer with cell-based therapies. Numerous human therapies are currently on their way to treat devastating conditions. However, concerns about the cell-safety hold back the full utilisation of these promising new treatments. Here we introduce a concept and show the associated genome engineering strategy that addresses this issue and provides a solution for "fail-safe" cell therapies.

To ensure the reliable expression of a suicide transgene system in proliferating cells, we transcriptionally linked it to a cell division essential endogenous locus (CDEL) in a homozygous manner. Our prototype suicide gene was the herpes simplex virus-thymidine kinase (HSV-TK), and the prototype CDEL was CDK1. The coding regions of these two kinases were connected with a viral 2A sequence. Using mouse and human embryonic stem cell lines with the above homozygous modification, we showed an extremely efficient and reliable ablation of proliferating cells both *in vitro* and *in vivo* by ganciclovir treatment, the pro-drug for HSV-TK.

Using published and our experimental measures of forward mutation rates, we defined mathematically the level of safety of therapeutic batches of these cells. Our general approach to assess and quantify the safety will be critical to make informed decisions by the regulators, doctors, and patients to advance the modern medicine-transforming cell therapies.

Building on the fail-safe technology, we addressed the next hurdle faced by cell therapies; a solution for induced allograft tolerance. We showed that the expression of eight local-acting, immunomodulatory transgenes introduced into embryonic stem cells is sufficient to protect cell derivatives against rejection in allogenic, immune-competent recipients. Allografts survive long-term, in different MHC-mismatched recipients, and without immunosuppressive drugs. Most importantly, the recipients of these engineered cells do not have suppressed systemic immune function.

The combination of the fail-safe and immune tolerance genome editing makes the One4All cell line and therapeutic cell development a reality.

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Transposon CAR T-cells for Relapsed Leukaemia and Lymphoma

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Chimeric antigen receptor T-cells targeting CD19 (CAR19 T-cells) are revolutionising the treatment of relapsed and refractory B-cell leukaemia and lymphoma. High rates of remission in otherwise incurable tumours has led to the recent approval of two CAR19 T-cell products for routine use in young patients with relapsed acute lymphoblastic leukaemia and adults with relapsed diffuse large B-cell lymphoma. However, many challenges to widespread use remain, including distribution and cost of a highly personalised product. We have developed a very simple, inexpensive procedure for the production of CAR19 T-cells using the PiggyBac Transposon system of gene modification. We are testing the clinical safety and efficacy of these PiggyBac CAR19 T-cells in a series of clinical trials. The use of our simplified protocol may enable localised CAR19 T-cell production at suitable facilities, hastening the widespread adoption of CAR19 T-cells as standard therapy for patients with relapsed B-cell malignancies in Australia.

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Adoptive T cell Therapy: targeting solid tumors through antigen discovery

Cassian Yee

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The potential therapeutic role of personalized neoantigen cancer vaccines

Bruce Robinson[1], **Shaokang Ma**[1], **Jonathan Chee**[1], **Craig Rive**[1], **Paula Van Miert**[1], **Rob Holt**[1], **Jenette Creaney**[1]

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Tumours typically express many mutations, As these mutations are 'neo' to the body, i.e. would not have undergone self tolerance, it is logical to postulate that they might be able to induce specific cytotoxic T lymphocytes (CTLs), especially in the context of immune checkpoint blockade immunotherapy (ICPB). Positive outcomes to ICPB are associated with high neo-antigen loads and with neo-antigen specific CTL responses. However only around 20% of patients respond to ICPB.

In order to examine ways in which the non-responders might become responders we examined several strategies to improve response rates, using anti-CTLA4 initially as the ICPB therapy in BALB/c mice in which Uqcrc2 has been defined as a DNA/RNAseq-identified neo-antigen in AB1 tumor lines induced by a relevant human carcinogen(*1*).

1. a) We first examined *therapy-induced changes in T-cell responses* against Uqcrc2. Anti-CTLA4 alone increased the magnitude of responses against Uqcrc2. Anti-CTLA4 combined with anti-GITR induced determinant spreading, unmasked responses against a new neo-antigen UNC45A that was undetectable during normal tumour growth. Immunogenic chemotherapy also unmasked responses against UNC45A, suggesting that subdominant neo-antigens can be unmasked by the appropriate immunotherapy.

2. b) We then evaluated *neo-antigen vaccination strategies*. Uqcrc2 vaccination best protected against tumor growth when administered in combination with partial Treg depletion (Foxp3.DTR mice), suggesting that neo-antigen vaccination will only be maximally effective when administered in combination with therapies that modulate existing immune restraints, including Tregs.
3. c) We determined *optimal anatomical location for tracking neo-antigen CTL responses* and identified the draining lymph node as an optimal, though not exclusive, location for response testing compared to blood or tumor.

We are currently studying the immunogenicity of non-SNVs and the relationship between NGS and proteomic analysis of neo-antigens. We will commence a neo-antigen vaccine trial in early 2018. Overall, these observations have important translational implications for identification of key neo-antigens, choice of therapy and monitoring of anti-tumor responses.

1. J. Creaney et al., Strong spontaneous tumor neoantigen responses induced by a natural human carcinogen. Oncoimmunology 4, e1011492 (2015).

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Unconventional T cell targets for cancer Immunotherapy

Dale Godfrey

Not available at time of printing

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Molecular sculpturing of scFv's and their payloads to optimise anti-cancer targeting

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Immunotherapies are now providing unprecedented therapeutic benefits across many cancer indications, with the most spectacular advances only in the last 5 years. This presentation will summarise the latest immunotherapy strategies based on antibody-directed delivery of therapeutic payloads. All these strategies rely on careful sculpturing of both the antibody module and the payload to provide a stable, high-affinity and exquisitely-specific cancer therapy. Applications range from the latest 3rd generation drug-loaded antibodies (ADCs) to check-point inhibitors (I/O therapy) to the latest Car-T (active antibody-retargeted T-cells). The antibody delivery modules are usually based on an scFv fragment which has been Bio-Nano-engineered to provide precise structural features and deliver unique pharmacokinetic properties. For the next generation of ADC therapeutics, Avipep designed and produced Diabodies (Avibodies™; scFv dimers) with unique surface disulphides for precise loading of either cytotoxic drug payloads (ADC-therapy) or radionuclides (for PET-imaging/ RIT-therapy). With PEGylation, Tag72-targeting diabodies demonstrated remarkable xenograft-tumour uptake >70% ID/g over 24-48hrs with fast blood clearance and low kidney uptake (<10% ID/gm). GMP-manufacture has exceeded 1gm/litre in bacterial fermentation and the diabody is stable for >36 months. A first-in-man Phase-1 Biodistribution (124-I PET imaging) trial in prostate/ovarian cancer was completed in 2016, demonstrating PEG-diabodies delivered high tumour loads in metastatic disease, with no specific uptake in normal tissues, no adverse events nor immunogenicity. Metastases were identified within 1-2 days and tumor-load persisted for 7 days with ideal tumor:blood ratios, AUC and T½ beta of ~48 hrs. The clinical trial validated PEG-diabodies for imaging cancer or for delivery of radioisotopes (RIT) or cytotoxic drug payloads (ADC). Preclinical xenograft evaluation with the latest ADC/RIT therapeutic formulations will be reported.

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Putative neoantigens predicted from frame-shift mutations of malignant pleural mesothelioma

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Background:

Malignant pleural mesothelioma (MPM) is a rare cancer of the pleura, currently without curative treatment, and tumour neoantigens are of interest in the development of targeted immunotherapy for its treatment. Neoantigens derived from frameshift mutations are likely to generate longer aberrant sequences than those of point mutations, and thereby are potentially more immunogenic against tumour cells. We hypothesized that, although the point mutation frequency is relatively low in mesothelioma compared with other tumours, immunogenic neoantigens may arise from frame-shift mutations. Our aim was to identify putative neoantigens arising from somatic frameshift mutations, and predict their binding affinity to HLA phenotypes.

Method:

Frameshift aberrations were identified from whole-genome sequencing (WGS) of tumour biopsies (80x) and peripheral blood (30x) in three MPM cases (MM1, MM2, MM3). Putative neoantigen sequences were extracted by obtaining wildtype coding sequence from RefSeq, inserting the variant of interestand generating new amino acid sequence using EMBOSS TranSeq. HLA phenotypes of each case were predicted using OptiType. Binding affinities of each neoantigen sequence against corresponding HLA phenotypes were evaluated using netMHCpan version 3.0 and 4.0, setting the threshold for strong binders to 0.5%, and weak binders to 2.0%.

Results:

HLA–A, –B and –C serotypes were predicted for both HLA alleles of each tumour. Twenty putative neoantigen sequences were identified from SNVs and fusion events in the following genes – **MM1:** NF2, TCF7L2, HSP90AB1, WBSCR17; **MM2:** NF2, SESN3, CRMP1; **MM3:** NF2, LATS2, SUMF2, ME1, KLHL1, ASIC2, COLQ, TP53BP1, ITGA1, RFX6. netMHCpan server predicted strong- and weak-HLA binding sequences from the candidates. Minor variations in the predictions were found between

versions 3.0 and 4.0 of netMHCpan, resulting in changes to predicted rank scores and classifications of strong- or weak-binding candidates.

Conclusion:

WGS and *in silico* analysis identified several putative neoantigens in MPM, and revealed candidate sequences that may be immunogenic against tumour cells. These candidates will be assessed further using other *in silico* approaches to determine their binding properties to HLA phenotypes, and by experimental methods.

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Predicting the future of novel immunotherapy agents for Haematological Malignancies: one size does not fit all

Miles Prince

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Developing novel therapies to treat cancer based on targeting the nucleolus

Ross Hannan

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Interferon Epsilon – a new weapon in innate immunity against ovarian cancer

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Ovarian cancer is one of the most common and deadly types of cancer in women and effective treatments are lacking. Patients with the most prevalent form of the disease — high-grade serous cancers (HGSC) — mostly present with advanced disease. The standard of care is debulking surgery and chemotherapy to which a majority develop resistance. Taken together these data highlight the need for new insights into the development and pathogenesis of these cancers in order to develop new treatments. Previously, we discovered interferon-ε (IFNε) as a novel cytokine that is constitutively expressed and hormonally regulated by epithelial cells in the female reproductive tract (FRT) and drives protection against common sexually transmitted infections [Fung et al Science 2013]. Here, we report that IFNε also shapes a unique anti-tumour response in the FRT that is specifically tailored to the tissue. We used syngeneic mouse models of ovarian cancer together with genomic analysis of large cohorts of women with HGSC to demonstrate that loss of IFNε results in increased tumourigenesis, poor disease prognosis and reduced survival. In mouse models of developing, established and advanced ovarian cancer, treatment with recombinant human IFNε inhibited peritoneal tumourigenesis by two main mechanisms. First, IFNε directly inhibited the proliferation of tumour cells and induced tumour cell apoptosis. Second, IFNε activated peritoneum anti-tumour immune cells including CD8 T cells and NK cells and modulated the expression of immune checkpoint molecules. Comparative studies showed that IFNε was a more effective inhibitor of ovarian cancer than IFNβ. Thus, we demonstrate that IFNε is a novel, intrinsic, tissue-specific tumour suppressor in the FRT and potential treatment for HGSC, whose repertoire of activities may be suitable for mono-, adjuvant or combination therapy with immune- or chemotherapy.

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Using genomic innate immune function (IIF) profiling to predict patient response to immunotherapy

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We present Innate Immune Function (IIF) profiling metrics derived from whole exome sequencing (WES) of cancer genomes that can be used to improve the ability of oncologists to predict the efficacy of cancer treatment regimes. These include drugs (or drug combinations) designed for hormone therapy or targeted checkpoint immunotherapies. They include classes of drugs and proteins (monoclonal antibodies) that are specific for markers associated with different types of cancer. These all work by exploiting the immune system to treat cancer through several mechanisms. The genomic IIF profile is predicated upon our growing understanding of the relationship between the highly targeted and regulated nature of processes involving endogenous cytosine (C-site) and adenosine (A-site) deaminases (APOBEC/ADAR) that are crucial for Innate Immune system processes viz. the mutagenic targeting early in infections of the DNA and RNA genomes of pathogens. When such deamination activities are dysregulated, they often target host cell genomes leading to the accumulation of many unwanted mutations, and thus eventually to the potential for full blown cancer. We have found that abnormal variations in various C-site and A-site IIF profiling metrics associated with deaminase activity (i.e. outside of the calculated range intervals for 'normals', or 'responders'), provide an excellent indicator of whether or not a particular patient will respond to immunotherapy. Using mutation data from several clinical trials, we provide IIF profile examples to predict patient response to: Pembrolizumab (Keytruda), PD-1 blockade, in non-small cell carcinoma of lung [1]; Atezolizumab (Tecentriq), PD-L1 blockade, for metastatic urothelial cancer [2]; Trastuzumab (Herceptin), HER2/neu, for HER2+ve breast cancer [3]; Asatinib, ErB family inhibitor for HER2+ve inflammatory breast cancer [4]; and, Ipilimumab (Yervoy), CTLA-4 blockade in metastatic melanoma [5]. We show that IIF profiling using WES data is effective at

differentiating between Responders and Non-Responders for a particular treatment, and that the personal profile of a patient provides clinicians with additional information that can help to guide further care.

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Multipotential mesenchymal stem/progenitor cells – a novel player in immuno-oncology

Geraldine Storton

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Exploring the interface between ethics and practice: unproven cell therapies in the twilight zone?

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Immunotherapies including checkpoint inhibitors and CAR-T cells have captured the attention of many scientists, physicians and cancer sufferers. The convergence of substantial incremental technical advances towards combined cell and gene therapy has led to improved clinical outcomes in immune deficiencies, haemoglobinopathies, immunotherapies and other inherited diseases. However in parallel with objectively proven therapies 'stem cell tourism' has become a billion dollar industry with increasing examples of false claims. Embryonic and induced pluripotent stem cells have been mired in controversy and clinical development has been forestalled. We reported an analysis of the global distribution of more than 400 unique businesses marketing stem cell-based interventions. Many of these online entities promote clinical applications of 'stem cells' beyond present-day standards of care. These data should be of immediate concern to governments and ethicist being lobbied to amend laws governing the manufacture, distribution and clinical use of human cell-based medical products. Unregulated, untested or unsafe stem cell 'therapies' place the field at a difficult crossroad. Blurring the lines that distinguish evidence-based cell therapies from those that are not remains a fundamental public health concern.

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Revealing the immune changes that associate with breast cancer risk to develop prophylactic immune therapies

Kara Britt

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Targeting RAS/MAPK signalling to enhance tumour immunogenicity

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Previously we have shown that tumor infiltrating lymphocytes (TILs) are an important prognostic factor in triple negative breast cancer (TNBC); where their presence is associated with improved survival[1]. Ras/MAPK pathway activation is associated with significantly lower levels of TILs in TNBC and whilst MEK inhibition can promote recruitment of TILs to the tumor, here we show that MEKi adversely affects early onset T cell effector function. Our studies[2,3] have demonstrated that the MEK targeted inhibitor (trametinib) increased immunogenicity (MHCI/II,) of tumors and reduced tumor growth. However, whilst MEK inhibition effectively inhibited tumor growth, blockade of this pathway triggered adverse inhibitory effects on crucial ERK signaling in immune cells, thus reducing their functional activity and overall efficacy of this treatment. We have previously shown that combining anti PD-1 with trametinib can enhance anti-tumor effects[2]. In the current study immune agonists anti-4-1BB or anti-OX-40 were combined with trametinib in order to recover immune cell function through rescue of T cell function independently of the MEK1/2 pathway[3]. These agonists restored T cell proliferation (Ki67), cytokine production (IFNy) and redirected signaling through p38/JNK activation following trametinib treatment, leading to enhanced TIL function and more effective anti-tumor immune responses *in vivo.* Accordingly, this data suggests that immune agonists with anti-PD-1 checkpoint blockade may be an effective strategy as the combination can directly overcome T cell suppression induced by MEK inhibition. As such, we propose that combining trametinib with agonistic immunotherapy could be a promising strategy in the clinic for TNBC patients.

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IL-18 critically drives multiple myeloma progression by generating an immunosuppressive microenvironment.

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Although novel anti-myeloma agents have been developed, multiple myeloma (MM) is still an incurable disease. The growth of MM cells highly depends on the bone marrow (BM), where cellular components and soluble factors cooperatively orchestrate the pro-survival and immunosuppressive microenvironment. Tumor-promoting inflammation and avoiding immune destruction are hallmarks of cancer, however the molecular and cellular mechanisms of MM-associated inflammation and immunosuppression remain poorly understood. Here, we demonstrate that the pro-inflammatory cytokine IL-18 is critically involved in these hallmarks in MM. Using Vk*MYC preclinical mouse models, a comprehensive analysis of the transcriptional landscape of the immune microenvironment in MM patients, and BM cytokine analyses, we demonstrate that dysregulated production of IL-18 is a key driving force for immunosuppression in the MM microenvironment and a potential therapeutic target. Mice deficient for IL-18 were remarkably protected from Vk*MYC MM progression in a $CD8^+$ T cell-dependent manner. The MM-niche derived IL-18 drove generation of myeloid-derived suppressor cells (MDSCs), leading to accelerated disease progression. A global transcriptome analysis of the immune microenvironment in 73 MM patients strongly supported the negative impact of IL-18-driven MDSCs on T cell responses. Strikingly, high levels of bone marrow plasma IL-18 were associated with poor overall survival in MM patients. Our results reveal the critical role of IL-18 in the MM immunopathology, which provides insight into therapeutic strategies against MM.

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Mass cytometric analysis of life and death decisions in blood cancer (cells)

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Multiple myeloma is an incurable and fatal plasma cell cancer. Most patients harbor plasma cells that resist current treatments, causing the disease to relapse following therapy. Hence, there is a pressing need to determine the best way to kill therapy-resistant multiple myeloma. In this study, we used mass cytometry (or CyTOF) to profile the molecular mechanisms that multiple myeloma cells employ to survive treatment with the standard-of-care treatments, bortezomib and dexamethasone. We developed a unique suite of 26 probes for high-throughput, simultaneous detection cell survival/death, cell cycle, signalling and tumour suppressor pathways by mass cytometry at the single cell level. These data were were analysed by purpose-built FLOWMap computational algorithms to organise the high dimensional single-cell data into an interpretable 2D graph, allowing visualization of changes in multiple markers over time. Classification analysis was then used to define the predictive power of identified markers in drug resistance. Our data reveal a simple metric involving just six death or survival proteins was a strong predictor of cell resistance or sensitivity for both drugs. We tested and confirmed these predictions using new compounds targeting distinct survival proteins, offering new combination therapies the may be effective in eradicating resistant cells. These findings provide the first time-resolved, deep profiling of multiple myeloma cells undergoing cell death following treatment. They reveal new metrics of resistance versus sensitivity and identify potential targets for salvage therapy during relapse. In addition, we demonstrate how new computational approaches like FLOWMap and statistical modeling can provide a general framework for understanding the diverse responses of tumor populations to anti-cancer drugs.

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Integrin-linked kinase expression in myeloid cells promotes colon tumorigenesis

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Integrin-linked kinase (ILK) is a ubiquitously expressed serine/threonine protein kinase involved in focal adhesion formation and regulates many cellular processes, such as cell adhesion, proliferation, migration, invasion, embryonic development and tissue homeostasis. Interestingly, aberrantly elevated ILK activity is also associated with a variety of human cancers. ILK is also known to be implicated in signalling pathways, such as WNT/b-catenin, MAPK and PI3K, which are often dysregulated in many cancers, including colorectal cancer (CRC). We identified and reported a novel role for ILK as a key mediator of innate immune Toll-like receptor (TLR) signalling via non-classical pathway activation of NF-kB (Ahmed *et al*., *JBC* 289:27776), a major pro-inflammatory transcription factor that plays an essential role in promoting CRC. Since ILK knockout mice are not viable and immune cells play critical roles in CRC, we generated mice with a selective deletion of the *Ilk* gene in myeloid cells ($Ilk^{loxP/loxP}$;LysMCre). Using a mouse model of colitis, we have recently shown that the myeloid-ILK deficiency ameliorated the pathology of disease along with reduced neutrophil infiltration, impaired inflammation response and elevated epithelial regeneration (Ahmed *et al*., *J Immunol* 199:2128). Moreover, myeloid-ILK-dependent inflammatory signalling in the mucosal epithelium was also therapeutically targeted using a global inhibitor of ILK during experimental colitis (Ahmed *et al*., *J Immunol* 199:2128). Since patients with inflammatory bowel diseases are at increased risk for colon cancer, we have investigated the effect of myeloid-ILK in mouse models of colitis-associated and $APC^{min/+}$-driven colon carcinogenesis. We aim to provide an insight into tumour biology by investigating the role of ILK in tumour-associated macrophage (TAM)-mediated tumour progression. Our data suggest a role for ILK in macrophage M2 polarization. Our observations also show that myeloid cell specific ILK deficient mice have a significant reduction in tumour burden compared with the control mice. Overall, our findings implicate ILK as a potential target for therapeutic intervention in both inflammatory diseases and cancers.

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Adoptive transfer of genetically engineered monocytes for the tumour targeted delivery of IFN-alpha

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The establishment of an immunosuppressive microenvironment is one of the hallmarks of cancer and the major impediment to the successful application of cancer immunotherapy. To re-activate an anti-tumoural response, we have previously developed a strategy which is able to reverse the immunosuppressive tumour microenvironment. Specifically, we turned a pro-tumoural population of macrophages, the TIE2-expressing monocyte/macrophages (TEMs), into cellular vehicles for the tumour targeted delivery of a potent immune-stimulatory molecule: interferon-α (IFNα). This was achieved by exploiting their tumour homing capability and selective expression of the angiopoietin TIE2 receptor. Using lentiviral vectors (LVs), we introduced an IFNα transgene regulated by the enhancer/promoter of the mouse Tie2/Tek gene into transplanted murine hematopoietic stem cells (HSCs), and showed selective activation of IFN-α expression in their TEM progeny recruited to tumours. This cell- and gene-based delivery therapy strongly inhibited primary breast cancer tumours and lung metastasis in mouse and human hematochimeric models with no evidence of toxicity(1,2). However, despite clinical evidences for safe and effective HSC gene transfer by LVs in clinical trials, autologous HSC transplantation is not currently used in breast cancer patients. On these grounds, as a clinically applicable alternative to HSC transplantation in the setting of breast cancer, we explored adoptive transfer of autologous genetically engineered monocytes and have preliminary data supporting tumour homing of adoptively transferred monocytes and delivery of IFNα to both primary and metastatic lesions in mouse and humanized models of breast cancer, thus providing a valid strategy for the delivery of anti-tumoural biomolecules to the tumour microenvironment.

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2. Escobar et al. Sci Transl Med 2014, 4, 217ra3

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CD19 CAR-T cell immunotherapy for B cell malignancies

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Lymphodepletion chemotherapy followed by infusion of T cells that are genetically modified to express a chimeric antigen receptor (CAR) targeted to CD19 is a promising and novel therapy for patients with relapsed and/or refractory B cell acute lymphoblastic leukemia, non-Hodgkin lymphoma, and chronic lymphocytic leukemia. Identification of factors that govern outcomes after CAR-T cell immunotherapy has been hindered in part due to the functional heterogeneity of infused CAR-T cell products. We are conducting the first clinical trial in which CD19 CAR-T cells are manufactured from distinct subsets of T cells and formulated in a defined composition for infusion to patients with B cell malignancies. Clinical responses, toxicities and factors governing outcomes will be presented.

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Using Chimeric Antigen Receptor (CAR) T cell to Treat Solid Tumours

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Chimeric antigen receptor (CAR) T cell therapy is a novel form of adoptive cellular therapy and has generated remarkable effects in patients with haematological cancers. However, the success against solid cancers has been modest. The major challenges are the hostile tumour microenvironment and the low efficiency of CAR T cells infiltrating the tumour. Here, we present a major

advancement in CAR T therapy that eradicated large established solid cancers, some in excess of 150 mm^2, in immunocompetent mice.

We hypothesized that a vaccine composed of a recombinant poxvirus could be used as an antigen delivery vehicle to specifically activate CAR T cells through their T cell receptor (TCR) and simultaneously change the tumour microenvironment, allowing the recruitment and activation of CAR T cells. The approach involves adoptive cell transfer incorporating vaccination (ACTIV) therapy. We generated dual-specific T cells expressing a CAR specific for the tumour antigen Her2 and a TCR specific for the melanocyte protein (gp100). Injection of T cells, together with recombinant vaccinia virus expressing gp100, induced durable complete remission of a variety of $Her2^+$ tumours and established metastases, some in excess of 150 mm^2, in immunocompetent mice expressing Her2 in normal tissues, including the breast and brain. Tumour destruction mediated by dual-specific T cells occurred rapidly over a period of seven days and was associated with an extensive proliferation and infiltration of the dual-specific CAR T cells. Mice that had rejected tumours were resistant to rechallenge with the same $Her2^+$ tumour cells and partially resistant to rechallenge with $Her2^-$ tumour cells, indicating the formation of immune memory and epitope spreading. This mouse model study supports the view that it is possible to design a highly effective CAR T cell therapy for solid cancers and metastases, even when the target antigen is also expressed in vital tissues.

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Generating dual-specific T cells in treating cancers

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While immunotherapy can eliminate substantial burdens of some leukaemias, the ultimate challenge remains the eradication of large solid tumours and metastases for most cancers. In our laboratory, we generated dual-specific T cells expressing a chimeric antigen receptor (CAR) specific for Her2 and a TCR specific for the melanocyte protein (gp100). Injection of these dual specific T cells, together with recombinant vaccinia virus expressing gp100, induced durable complete remission of a variety of $Her2^+$ tumours and established metastases, in immunocompetent mice expressing Her2 in normal tissues, including the breast and brain. To explore the translational potential for using the dual specific CAR T cell strategy, I established methods to transduce the T cells from human peripheral blood with both a TCR specific for gp100 and a CAR for Her2. From as little as 1 ml of human buffy coat, I could generate more than 10^9 dual-specific CAR T cells, which is sufficient for a course of treatment. The human dual-specific CAR T cells were functional in secreting IFN-γ and killing human cancer cells when co-cultured with the gp100 or Her2 expressing human cancer cells. The stimulation of gp100 through TCR enhanced the dual-specific CAR T cell proliferation, secretion of IFN-γ and killing of $Her2^+$ human cancer cells *in vitro.* These characteristics were identified to be important for eradicating tumours in the mouse models. Taken together, my data provide valuable information for the development of CAR T cell therapies for patients with solid cancers. My next step is to further characterise these dual-specific T cells functions *in vitro* and *in vivo* using immunodeficient NSG mice injected with human cancer cells.

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The development, functional evaluation and optimisation of CAR-T cells attacking novel epitopes on ovarian cancer

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Chimeric antigen receptor (CAR-) T cells are designed to exploit the intrinsic cytotoxic function of T cells whilst manipulating specificity by expressing a nominal antigen-specific receptor containing a cytoplasmic activation domain. Such cells have recently revealed remarkable success in the clinic, with multiple studies reporting the ability to ameliorate CD19+ malignancies in particular. However, transitioning this technology to the treatment of solid tumours has posed many challenges, in particular the identity of appropriate target antigens and the need to penetrate the protective cancer stromal microenvironment. Accordingly, we developed second generation CAR-T constructs against an ovarian cancer antigen TAG-72 using lentiviral transduction of T cells from donated healthy blood. Resultant CAR^{+ve} T cells were isolated by flow cytometry and maintained in basal media supplemented with IL-2, IL-7, IL-15 or IL-21 ± serum supplements for 24-48h. Cell activation status was determined by flow cytometry examining expression levels of CD137 and HLA-DR relative to baseline. Function was monitored *in vitro* using the real-time xCELLigence platform where both TAG-72^{hi} and TAG-$72^{low/neg}$ target ovarian cancer cell lines were exposed to CAR-T for at least 20h. Changes in cell impedance were monitored throughout, where a reduction is indicative of target cell death. Parallel studies were performed with non-transduced T cells from the same donors. High levels of IL-2 resulted in T cell hyper-stimulation and potent, indiscriminate elimination of target cells induced by both CAR-T and non-transduced T cells. This was ameliorated in part by the reduction of IL-2 in the culture system. In contrast, addition of IL-7 alone maintained low levels of activation of CAR-T cells, as determined by comparable levels of HLA-DR/CD137 expression to baseline levels. This translated to complete elimination of TAG-72^{hi} but not TAG-$72^{low/neg}$ cells *in vitro,* suggesting antigen-specific killing; non-transduced "resting" T cells showed no killing. Through sensitive, label-free, real-time cell monitoring we have been able to identify a collection of culture conditions which augment CAR-T function *in vitro*. These studies highlight the importance of the production process in the ability to achieve the fine balance between highly antigen-specific but potent cytotoxicity CAR-T cells for the eradication of cancer.

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Chimeric antigen receptor T cells and the immune synapse

Misty Jenkins

Chimeric antigen receptor T cells and the immune synapse

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Validation of a novel CAR-T cell targeting non functional P_2X7

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One of the most significant hurdles to the wide scale delivery of cancer immunotherapies is the need for novel broad cancer specific targets. We have developed a novel CAR-T cell against the extracellular domain of a non functional form of the ATP transporter P_2X7, (nfP_2x7) which is expressed on multiple cancer cells, but not normal cells. P_2X7 is widely expressed on many cell types and is an active ATP transporter linked to cell survival, however, the non functional form of P_2X7 is conformationally altered, which reveals an epitope not present on the WT P_2X7 receptor. We have generated a set of binders to this epitope and assembled them into a chimeric antigen receptor. Using lentiviral gene delivery we have expressed this novel CAR-T on human CD8 cells and performed specificity screening and functional assay of cytotoxicity. We have pilot data suggesting this CAR-T cell can directly target breast cancer cells in vitro, and only the nfP2X7 epitope is recognised by the CAR-T. We have validated these findings with killing assays on a number of other cancer cell lines, suggesting that nfP2x7 is a valid target on a number of cancer types. We now aim to demonstrate broad anti cancer specificity using tissue arrays and *in vivo* tumour killing assays as part of pre-clinical validation.

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Optimisation of third-generation CAR T cells for the treatment of solid tumours

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Chimeric antigen receptor (CAR) T cells are genetically engineered to recognize tumour-associated antigens and have potent cytolytic activity against tumours. Adoptive therapy with CAR T cells has been highly successful in haematological cancers. However in solid cancers, CAR T cells face a particularly hostile tumour microenvironment and clinical trials of solid tumour antigen-targeted CAR T cells have shown limited efficacy. Hence, it is clear that CAR T-cell technology requires further optimization before it becomes a viable treatment for non-haematological malignancies. Our research team is currently conducting the CARPETS study, a phase I clinical trial of third generation GD2-specific CAR T cells in metastatic melanoma patients at the Royal Adelaide Hospital. This trial was a world-first in using CAR T-cell therapy for melanoma, and in combining CAR T cells with standard kinase inhibitor-targeted therapy. So far, six patients have been treated safely with a further six to be recruited. Dose escalation has been completed and the treatment has been shown to be safe with no serious adverse events. However CAR T cells failed to persist beyond four weeks in five of six patients; the only patient to demonstrate long-term persistence was pre-treated with lympho-depleting chemotherapy. Our research suggests that GD2-specific CAR T cells have a predominately effector memory phenotype and a subset of CAR T cells upregulate and maintain PD-1 expression following antigen encounter. CAR T cells also demonstrate loss of function and increased susceptibility to activation-induced cell death with repeated stimulation. To counter this we have adjusted the CAR T cell manufacturing conditions, and investigated a combination therapy approach with anti-PD1 monoclonal antibody, with the aim of promoting a central memory phenotype and improved persistence and function of CAR T cells. Our hope is that this will improve CAR T cell efficacy for solid cancer patients.

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Immunotherapy with antigen specific T cells

Helen Heslop

Cellular immunotherapies have immense potential as they can specifically target tumor antigens through native or artificial receptors. We have evaluated expanded T cells recognizing tumor antigens though their native receptor to target viruses such as EBV or HPV expressed on tumor cells with encouraging response rates when using either autologous cells or donor derived cells post transplant. More recent studies have infused closely matched third-party virus-specific T cells and reported encouraging response rates between 50 and 90%. As most tumors do not express viral antigens we have also evaluated tumor-associated antigens (TAAs) such as cancer testis antigens (CTAs) MAGE, BAGE, GAGE, NY-ESO-1, SSX and PRAME which are expressed by many malignancies but otherwise are found only in germline tissues that are immune privileged and therefore not susceptible to T-cell attack. We have used peptide libraries that can present both HLA-class I- and class II-restricted epitopes to reactivate TAA-specific T cells and to overcome the possibility of tumor escape by targeting multiple epitopes in 5 antigens in clinical trials in lymphoma, myeloma and acute leukemia. Clinical responses have been seen in all studies correlating with the detection of tumor-reactive T cells in patient peripheral blood post-infusion directed against both targeted antigens as well as non-targeted TAAs indicating antigen/epitope spreading. Antigen specific T cells may also be used as target cells for transfer of artificial receptors such as chimeric antigen receptors (CARs).

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Mass cytometry and a systems biology – new tools to explore the biological effects of adoptive T cell therapy

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Adoptive T cell therapy with pathogen specific T cells for the treatment of immune deficiency following allogeneic haemopoietic stem cell transplant (HSCT) has shown clinical efficacy with little toxicity. Graft manipulation strategies and adoptive cell transfer after HSCT are ever more complex but the biological effects are not well understood. Small patient numbers and complex clinical features pose significant challenges.

We have employed high dimensional mass cytometry and a bioinformatics pipeline to study immune reconstitution in recipients of allogeneic HSCT with and without adoptive T cell therapy using a 37 marker panel producing 75 gated canonical cell subsets per patient sample. We have used bioinformatics tools including t-stochastic neighbour embedding (t-SNE), principle component analysis (PCA) and unsupervised consensus clustering algorithm SC3 to analyse this large dataset. Immunological profiles have been identified that are influenced by clinical parameters such as transplant conditioning, donor source, T cell depletion and post-transplant events, in particular, CMV reactivation, adoptive T cell transfer of pathogen specific T cells and time post-transplant. Immune recovery within each individual can be visualised in detail using spanning-tree progressive algorithm of density normalised events (SPADE) or visualisation of t-SNE (ViSNE).

The system level immunological changes form cell therapy interventions can be understood using mass cytometry immune profiling. This technology has numerous potential applications in cancer immunotherapy.

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Using cell banks to treat opportunistic infection and haematological malignancy

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Infusion of HLA matched antigen specific T-cells targeting viruses such as Epstein-Barr virus and cytomegalovirus is an effective therapy to prevent and treat opportunistic infections in immunocompromised individuals. In the setting of stem cell transplantation, cell products are most frequently derived from the HLA matched stem cell donor and are prepared in advance of the infection in order to avoid a prolonged delay between diagnosis and treatment. Inevitably, this approach results in some products never being administered, since many patients fail to develop the specific infection for which the T-cell therapy has been generated. To avoid the human, reagent and storage costs associated with product wastage, the safety and efficacy of infusing cryopreserved antigen specific T-cells from partially HLA matched unrelated donors has been investigated. The need for only partially HLA matching reduces the number of products required for a bank to provide wide patient coverage if donors with common HLA alleles are chosen for cell generation. We have investigated the use of 3rd party partially HLA matched virus-specific T-cells for refractory CMV, EBV and adenovirus infections after allogeneic stem cell transplant and demonstrated both short and long term efficacy. We are currently evaluating the same strategy in allogeneic transplant recipients at the first evidence of viral reactivation. Products consisting of T-cells responding to fungal antigens can also be created. Fungus-specific T-cells are mostly CD4+ and release cytokines including tumour necrosis factor alpha and interferon gamma in response to antigen stimulation. Specific cytokine secretion and proliferation responses are mediated via HLA-DR. We propose to establish a trial of 3rd party HLA-DR matched fungus-specific T-cells for allogeneic stem cell transplant recipients with invasive fungal disease. Finally, infusion of T-cells recognising tissue or cancer antigens from cryopreserved banks is a practical option for cancer immunotherapy. We created a "virtual" bank of cell products from donated haemopoietic progenitor cells or donor lymphocytes that were due for discard from participating laboratories. We treated 4 elderly AML patients with chemotherapy followed by 2-3 infusions of partially HLA matched products from unrelated donors. Infusions were associated with fever, infection and rash but no patient developed graft versus host disease. 2 patients relapsed but 2 remain alive up to 432 days after treatment. Donor cell persistence was observed up to 60 days post infusion. Cryopreserved banks of antigen specific T-cells targeting infectious, tissue and cancer antigens may facilitate the application of cell therapy in infections and cancer.

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Orthogonal approaches to regulate CAR T cell activity and safety

Daniel Powell

Adoptive transfer of T cells expressing a chimeric antigen receptor (CAR) has yielded impressive results in patients with select hematologic malignancies. However, CAR T cell therapy has been less effective in solid tumors with heterogeneous antigen expression and the inability to regulate the activity of CAR T cells after infusion is a significant safety concern. To address these issues, we developed highly adaptable CAR T cell systems that consist of tagged bi-functional targeting ligands that selectively and exclusively bind to universal immune receptors expressed by engineered T lymphocytes, and the desired antigen, thus selectively redirecting CAR T cell activity via a bi-functional protein bridge. Unlike conventional CAR T cells, this platform enables precision control over engineered CAR T cell function and specificity through antibody dosing to deliver the desired antitumor activity and improve safety. Further, the adaptable specificity enables either simultaneous or sequential multi-antigen attack to address tumor antigen heterogeneity and antigen loss/escape. Since our original discovery, a variety of universal immune receptors have been developed and tested, and clinical trials initiated. While the promise of these approaches remains significant, the impacts of architecture, geometry, valency, orientation and avidity are being investigated. Our most recent data suggests, that incorporation of covalent binding partners into universal immune receptor systems can stabilize CAR formation, redirect antigen specificity on demand, and permit studies of receptor turnover. We anticipate that the dose dependent control of CAR T cell activity provided by universal immune receptor systems will aid in preventing cytokine release syndrome in patients receiving CAR T cell infusion, address issues of tumor antigen heterogeneity/loss, and permit better control over CAR T cell activation and persistence after administration.

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Cbl-b – towards a new paradigm in T cell based anti-cancer therapy

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Checkpoints in immune cells fine tune the immune response to activation or tolerance. We have identified the E3 ligase Cbl-b as a key negative modulator of immune cells activation extending from T cells, NK cells and recently dendritic cells. Loss of Cbl-b licenses immune cells to spontaneously kill multiple cancers or even revert lethal fungal infections without severe side-effects to the tested animals. Thus, targeting Cbl-b provides a novel modality to activate the immune system to kill tumors which is currently explored in phase I clinical trials using a rapid and direct bed-side cell therapy approach.

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Allogeneic Graft vs Tumor effect: pros, cons and pointers for the future

Eric Wong

Not available at time of printing

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SOCS protein regulation of NK cell-mediated tumour immunity

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Natural killer (NK) cells have evolved to detect and kill aberrant cells. NK cell function is governed by the cytokine interleukin (IL)-15 and the detection of foreign and self-ligands, which together generate an integrated intracellular signal cascade. There is a great deal of interest in understanding the inhibitory signals that curb NK cell responses, with the aim of developing new immunotherapies which enhance NK cell detection and killing of cancer cells. The Suppressor of cytokine signalling (SOCS) proteins are induced in response to JAK/STAT activation and act to limit the extent of cytokine receptor signalling. We have identified CIS (Cytokine-inducible SH2-containing protein; *Cish gene*) as the critical SOCS protein regulating IL-15 signalling in NK cells. *Cish* was rapidly induced in response to IL-15 and deletion of *Cish* rendered NK cells hypersensitive to IL-15, as evidenced by superior proliferation, survival, IFN-γ production and cytotoxicity towards tumours. CIS was shown to interact with JAK1, inhibiting JAK1 kinase activity and targeting it for proteasomal degradation. *Cish*$^{-/-}$ mice were resistant to experimental melanoma, prostate and breast cancer metastasis *in vivo*, and this was intrinsic to NK cell activity (Delconte *et al.,* Nat Immunol, 2016). This study uncovered a potent checkpoint in NK cell-mediated tumour immunity.

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Emerging Advances in Cellular Therapies for Virus-Associated Cancers

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Persistent viral infections are associated with the majority of human cancers where infectious agents have been recognized as the primary etiological agent. These viruses contribute to the malignant transformation of human cells either through the expression of oncogenic proteins or chronic inflammation. In spite of the high prevalence of these viral infections in humans, only a small proportion of these individuals who may have an underlying immune defect develop malignant disease. Furthermore, many of these viruses have evolved unique mechanisms to avoid the host immune system to successfully establish latent infection with limited gene expression. Technological advances in delineating the role of cellular immune responses in the control of viral infections and ability to rapidly expand these effector cells in vitro have provided an important platform for the development of novel immunotherapeutic strategies to treat virus-associated cancers. While autologous T cell therapies have provided promising results, development of “off-the-shelf” third-party allogeneic virus-specific T cell therapies have emerged as powerful tools to treat many of the virus-associated diseases. It is anticipated that adoptive T cell therapy in combination with newly emerging immune checkpoint inhibitors and therapeutic vaccines will provide opportunities to successfully treat advanced metastatic virus-associated cancers which are currently not amenable to standard therapeutic strategies.

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Predicting and enhancing benefit of immunotherapies in triple negative breast cancer

Belinda Parker

Not available at time of printing

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The importance of Immune-modulation in the management of cancer

Angus Dalgleish, wai liu

We have recently reported the ability of a heat killed injectable mycobacterium product known as IMM 101 to induce clinical remissions in advanced melanoma and to enhance survival in a randomised study in advanced pancreatic cancer patients when added to Gemcitabine versus Gemcitabine alone. IMM 101 induces activation of the innate immune response especially NK and gdT cell activation. In addition it boosts TH-1 cell immunity responses and appears to enhance the response to other modalities

including chemo and Radiotherapy and especially to Ipilimumab and Pembrolizumab. Unlike other combinations there is no added toxicity.

Other Immune-modulators include the IMiDs such as Lenalidomide which greatly enhances cell mediated and antibody responses to vaccines in both murine models and humans, and in addition it enhances ADCC and responses to antibodies such as Rituximab. The favourable effect of immune modulation would appear to involve the ability to inhibit T reg and Myeloid derived suppressor cells. Lenalidomide is very effective at inhibiting T reg cells. However, a number of drugs such as cyclophosphamide can also enhance this effect as and are most effective at low and hence relatively non toxic doses.

Naltrexone which is an anti opiate receptor blocker also induces marked immune modulation only at low doses (LDN). In addition to its effect on opiate receptors we have recently reported that it is a strong antagonist of TLR 9 which could explain its marked anti inflammatory effects on Crohns disease. Its effect on immune-modulation may be explained by its differential effect on gene expression at low and high doses. At low doses LDN is non toxic and enhances other immunotherapy modalities.

CBD and THC are cannabinoids which have no cytotoxic activity yet we have reported that they enhance responses to radiotherapy in the mouse glioma model and that the effect is more marked when given metronomically in a number of cell line assays. We will report on combinations of these agents as well as new clinical studies.

In addition it will be stressed that IMM 101, the IMiDs and LDN all require high vitamin D3 (A hormone!) levels to be effective.

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Modulation of innate immunity for cancer therapy

Bryan Williams

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Targeting cross-presenting dendritic cells with nanoparticulate vaccines for antigen-specific cancer immunotherapy.

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Non-antigen-specific stimulatory cancer immunotherapies are commonly complicated by off-target effects. Antigen-specific immunotherapy, combining viral tumor antigen or personalised neo-epitopes with immune targeting, offers a solution. However, the lack of flexible systems targeting tumor antigens to cross-presenting dendritic cells (DCs) limits clinical development. Although antigen-anti-CLEC-9A mAb conjugates target cross-presenting DCs, adjuvant must be co-delivered for cytotoxic T-cell (CTL) induction. We functionalized tailored nanoemulsions encapsulating tumor antigens to target Clec9A (Clec9A-TNE). Clec9A-TNE encapsulating ovalbumin (OVA) antigen targeted and activated cross-presenting DCs without additional adjuvant, promoting antigen-specific CD4+ and CD8+ T cell proliferation, CTL and antibody responses. OVA-Clec9A-TNE-induced DC activation required CD4 and CD8 epitopes, CD40 and IFN-α. Clec9A-TNE encapsulating human papillomavirus (HPV) E6-E7 significantly suppressed HPV-associated tumor growth while E6-E7-CpG did not. Clec9A-TNE loaded with pooled B16F10 melanoma neo-epitopes induced epitope-specific CD4+ and CD8+ T cell responses, permitting selection of immunogenic neo-epitopes. Clec9A-TNE encapsulating six neo-epitopes significantly suppressed B16-F10 melanoma growth in a CD4 T cell-dependent manner. Thus, cross-presenting DCs targeted with antigen-Clec9A-TNE stimulate therapeutically-effective tumor-specific immunity, dependent on T cell help.

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Targeting human CD141+ DC for cancer immunotherapy

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DC are a heterogeneous cell population, with specialist subtypes driving specific immune responses. In mice, the cDC1 subset (also referred to as Batf3-dependent DC, XCR1+ DC, CD8+ DC in lymphoid tissues and CD103+ DC in peripheral tissues) is essential for the induction of tumour immune responses and for the efficacy of checkpoint inhibitor blockade and adoptive T cell immunotherapies. Vaccines that can deliver antigens (Ag) directly to dendritic cells (DCs) in vivo are more effective than cell-based therapies in mouse models and are promising approaches to translate to humans. CD141+ DC are the human cDC1 equivalent and specifically express the C-type lectin-like receptor CLEC9A that facilitates cross-presentation of dead cell Ag. NYESO1 and WT1 are well characterised, highly immunogenic tumour associated Ag (TAA) expressed by a broad array of tumour types. We developed a recombinant human chimaeric IgG4 antibody (Ab) specific for human CLEC9A genetically fused to

NYESO1 or WT1. For comparison we developed TAA fusions with chimaeric IgG4 Ab specific for human DEC-205, which is expressed by many human leukocytes, and β-galactosidase as an irrelevant isotype control. CLEC9A-NYESO1 and CLEC9A-WT1 Abs retained their binding specificity for CD141+ DC. Following uptake of CLEC9A-WT1, CD141+ DC cross-presented a WT-1 HLA-A24-restricted epitope for recognition by specific cytotoxic T cells. Likewise, a HLA-A2-restricted NYESO1 epitope was cross-presented by CD141+ DC following uptake of CLEC9A-NYESO1. For both TAA, the CLEC9A Abs were more efficient at delivery of Ag for cross-presentation than DEC-205 or isotype control Abs. Targeting TAA to human CD141+ DC using CLEC9A Ab is therefore an attractive strategy to induce or boost tumour immune responses.

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A preclinical platform for evaluating targeted therapy and immunotherapy combinations in BRAF^{V600E} melanoma

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Immunotherapy and targeted therapy are standard of care in melanoma and there is great interest in combining these approaches. Evaluating the efficacy of such combinations requires preclinical models that are both immunogenic and sensitive to targeted inhibitors. Several syngeneic models have recently been developed using mouse melanoma cell lines with clinically-relevant genetic backgrounds, including the YUMM1.1 line (*Braf^{V600E}Cdkn2a$^{-/-}$Pten$^{-/-}$)*. However, the suitability of this model as a preclinical tool in the context of standard-of-care clinical therapies has not been well characterised. We also found the line to be lowly immunogenic, limiting it's potential as a platform for preclinical immunotherapy studies. Specifically, we found that YUMM1.1 tumours grown in immunocompetent C57BL/6 mice had low T cell infiltrate and grew at a similar rate to tumours in immunocompromised mice, indicating the line is poorly recognized by the immune system. We found the line expresses MHC Class I but likely lacks sufficient neoantigen expression to stimulate adaptive immunity. Hence we derived a new model, YOVAL1.1, by stably transducing YUMM1.1 to express ovalbumin (OVA) to enhance immunogenicity and allow tumour-specific T cell tracking *in vivo*. YOVAL1.1 showed high sensitivity to T cell killing *in vitro* and we observed a significant delay in tumour growth in C57BL/6 mice compared to immunocompromised mice. YOVAL1.1 tumours express high levels of MHC Class I, PD-L1 and CD80 and have significantly increased CD3+ immune infiltrate compared to vector transduced tumours. We also found that YOVAL1.1 is sensitive to targeted therapies currently used in the clinic for melanoma, including BRAF^{V600E} and MEK inhibitors. Importantly, the response and resistance of this model to targeted therapy recapitulates observations in human models and in the clinic. YOVAL1.1 is a transplantable model, which is both immunogenic and sensitive to clinical targeted therapies, and in which tumour-specific immune responses can be tracked *in vivo*. Together, this makes YOVAL1.1 a valuable and cost-effective platform to guide strategic development of combined targeted and immunotherapy approaches in BRAF^{V600E} melanoma.

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Restoring immuno-competency in Chronic Lymphocytic Leukemia

Fabienne Mackay

Not available at time of printing

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Thymic function in T cell reconstitution after cell-ablative therapy

Georg Holländer

Not available at time of printing

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Harnessing natural mechanisms of tissue regeneration to boost immune function in cancer patients

Jarrod Dudakov

Not available at time of printing

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In vitro generation of T cells for therapeutic application

JC Zuniga-Pflucker

Not available at time of printing

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Generation of CTLs from iPSCs transduced with TCR genes: toward the development of "off-the-shelf T cells"

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We have proposed a strategy to use the iPSC technology for expansion of tumor antigen specific CTLs; iPSCs produced from T cells (T-iPSCs) inherit rearranged TCR genes, and thus all regenerated T cells from T-iPSCs express the same TCR. Based on this idea, we have succeeded in regenerating MART1-specific CTLs from a melanoma patient (Vizcardo et al, Cell Stem Cell, 2013). Recently we have developed a culture method by which CTLs expressing CD8ab heterodimer with high antigen specific cytotoxicity can be regenerated (Maeda et al, Cancer Research, 2016).

Currently, we are trying to apply this strategy to allogeneic setting. To this end, we thought of a method in which non-T cell derived iPSCs are transduced with exogenous TCR genes (TCR-iPSCs). As a source of such iPSCs, we are going to use HLA-haplotype homo iPSCs established by iPSC stock project conducted by Shinya Yamanaka. In this project, HLA-homo iPSCs will be transplanted to HLA-haplotype heterozygous recipients, expecting that allo-reaction could be reduced. At present, top two frequent HLA-homo iPSC are available, covering 24% of the Japanese population. To test the idea of TCR-iPSCs, we lentivirally transduced non-T cell derived iPSCs with WT1-specific TCR gene cloned by our group. Regenerated CTLs from TCR-iPSCs were found to exhibit cytotoxicity comparable to those from T-iPSCs carrying the same WT1-TCR, indicating that this strategy works well. We are planning to apply this method clinically for acute myeloid leukemia by targeting WT1 antigen. In the future, we plan to establish “TCR-iPSC bank” that covers various tumor antigens and HLA haplotypes, aiming to realize “off-the-shelf T cell therapy” against cancer.

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Development of functional, mature cytotoxic T-cells from cord HSC via a molecularly-defined, scalable and clinically translatable culture system

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Chimeric Antigen Receptor (CAR-) T cell immunotherapy is revolutionising cancer treatment. However, the vast majority of CAR-T products in development are autologous therapies; inevitably they will struggle to reach mass adoption given the cost and complexity – two recent FDA-approved products have price tags of US$373,000 and US$475,000 per treatment. Other major issues include: time to manufacture the product, complexity of release specifications for each product, and starting cell variability as a result of compromised immune systems following chemotherapy prior to creating autologous CAR-T cells. Logically, a precisely defined, consistent, ‘off-the-shelf’ CAR-T product with broad histocompatibility is the future of this technology.

In vitro directed differentiation of T-cells from stem cells sources, such as induced pluripotent stem cells (iPSCs), provides a platform to generate a ‘limitless’ supply of CAR-T cells. Indeed, it has been shown that co-culture of iPSCs with genetically-engineered mouse support cells can be used to direct differentiation into hematopoietic lineage then into T-cells. However, the current manufacturing system is inherently inconsistent and not suitable for clinical use, given the support lines are of mouse origin. Human cell line analogues have been attempted with limited success. Small molecule patterning has been shown to create HSCs from iPSCs, although conversion of these HSCs to T-cells remains challenging.

Accordingly, we have established a molecularly defined, xeno-free, stroma-free, serum-free T cell differentiation culture system suitable for upscale manufacture. For the first time we demonstrate the generation of mature CD8αβ+ CD4- TCRαβ cytotoxic T-cells from cord blood HSCs without an animal stromal cell component. When activated via CD3/CD28 co-stimulation, the *in vitro* generated T-cells have strong dose-dependent cytotoxic function. Using this system we can create ~100 CD3+TCRαβ+CD8αβ+ cytotoxic T-cells per cord HSC, over 47 days of differentiation. With the prospect of post T-cell expansion, using our system 1 cord blood sample can yield up to 2.5 x 10^{11} T-cells. We have, however, observed donor cord variability, appearing to affect the end-state of T-cell maturity. This work potentially unlocks a pivotal step in generating unlimited CAR-T cells from iPSC, along with new avenues for allogenic CAR-T products from HLA matched cord blood tissue. Furthermore, this manufacture system serves as a stand-alone technique to enable immune reconstitution for a variety of diseases.

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The origins of thymic NK cells and ILC1

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Innate immune cells are found in the thymus and have been thought to arise from bone marrow progenitors but definitive evidence of this origin has been lacking. By examining T cell factor 1 (TCF-1), a transcription factor critical for the development of both adaptive T cells and innate lymphoid cells (ILC), we uncover alternate origins for thymic NK cells and ILC1. Surprisingly, while the development of all ILC subsets, including NK cells, is blocked in the absence of TCF-1 in the bone marrow, NK cell numbers are normal in peripheral tissues. We found that TCF-1 acted in thymic progenitors to limit the expression of NK cell signature genes normally allowing the formation of T cells. Instead, ILC1 arise from bone marrow progenitors. We demonstrate that TCF-1 globally regulates chromatin accessibility which significantly impacts the suppression of genes critical for determining the fate in thymic precursor cells. Derepression of this thymic checkpoint resulted in the development of thymic-derived NK cells that reconstitute the entire peripheral compartment to prevent tumour establishment. Collectively, our data reveal the pivotal role of TCF-1 in the thymus as a molecular switch to suppress alternative fates allowing T cell development and restricting NK cell development.

POSTER ABSTRACTS

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Melt electrospun 3 D lattices for the clinical scale expansion of immunomodulatory cells including human regulatory T cells (Tregs)

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One of the most significant hurdles to the affordable, accessible delivery of cell therapy is the cost and difficulty of expanding cells to clinically relevant numbers. Immunotherapy to prevent autoimmune disease, tolerate organ transplants or target cancer critically relies on the expansion of specialised T cell populations. We have designed 3D-printed cell culture lattices with highly organized micron-scale architectures to bind monoclonal antibodies that trigger cell proliferation. This 3D technology platform facilitate the expansion of therapeutic human T cell subsets, including regulatory, effector, and cytotoxic T cells while maintaining the correct phenotype. This cell expansion platform is user-friendly and expedites cell recovery and scale-up, making it ideal for translating T cell therapies from bench to bedside. Using an optimized cell handling protocol we are able to achieve over 200 fold expansion of human T cells in 14 days, and this yield is compatible with clinical dose numbers for cGMP manufacturing. In addition when the 3D lattice is used to grow regulatory T cells the activation and expansion environment favors FOXP3 expression compared with donor matched cultures with bead based expansion technology. The in vitro suppressor assay of these regulatory cells suggests that this enhanced FOXP3 expression is linked to increased potency. These data suggest that the quality of therapeutic T cells manufactured using 3D lattices may be enhanced, and this could result in more doses per batch, or fewer cells required per does. Both of which would have a positive impact on cots of goods.

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TCR silencing with shRNA as a foundation for a bank of allogeneic CAR19 T-cells generated by single-step genetic modification using the piggyBac transposase

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Aim: Autologous CD19-specific CAR (CAR19) T-cells have shown remarkable efficacy against B-cell malignancies, but manufacture of individualised products is expensive, delays patient treatment and is unfeasible in patients with insufficient healthy T-cells. We sought to evaluate TCR suppression by short hairpin RNA (shRNA) in low-cost piggyBac-generated CAR T-cells, in order to generate a ready-made allogeneic product devoid of alloreactivity.

Methods: PiggyBac transposon plasmids were generated for expression of CAR19-28z alone, or additionally with shRNA against either TCRα-chain or TCRβ-chain (shRNA designed by Takara Bio Inc). T-cells isolated by immunomagnetic separation were electroporated with piggyBac transposon and transposase plasmids (n=3 donors per construct), and expanded over 22 days via CD19 stimulation with IL-15 support. TCR^{neg} cells were enriched by immunomagnetic CD3 depletion in CAR T-cell cultures that had been transfected with shRNA, to create a final product. Final products were further cultured to ensure stability of TCR suppression.

Results: CAR T-cell final products had expanded over 100-fold. Overall, the proportion of T cells with the desired $TCR^{neg}CAR^{+}$ phenotype varied significantly (5% for CAR19-28z alone, 41% with additional TCRα-chain shRNA and 70% with additional TCRβ-chain shRNA). CAR expression on T-cells was robust with CAR19-28z alone or with additional TCRβ-chain shRNA (96% and 98%, respectively), but was significantly lower with additional TCRα-chain shRNA (56%). The proportion of CAR T-cells that were TCR^{neg} was significantly greater with additional TCRα-chain and TCRβ-chain shRNA (74% and 71%, respectively) compared to CAR19-28z alone (5%). TCR silencing was stable over a further 42 days with TCRβ-chain shRNA, but not TCRα-chain shRNA (CAR T-cells lacking TCR: 71% vs 1%, respectively).

Conclusion: CAR19 T-cells with stably suppressed TCR were successfully generated by introducing genes for both CAR and shRNA against TCRβ-chain into T-cells via single-step genetic modification with the piggyBac transposase system. This platform could form the foundation for a bank of inexpensive allogeneic TCR^{neg} CAR T-cells with low GVHD potential, suitable for rapid treatment of many partially HLA-matched recipients.

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Working towards personalized immunotherapy in breast cancer

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Immunotherapy has revolutionized cancer treatment, with sustained responses to immune checkpoint inhibitors reported in a number of malignancies. Such therapeutics are now being trialed in aggressive or advanced cancers that are heavily reliant on untargeted therapies, such as triple negative breast cancer. However, responses have been underwhelming to date and are very difficult to predict, leading to an inability to accurately weigh up the benefit-to-risk ratio for their implementation. The tumor immune microenvironment has been closely linked to immunotherapeutic response, with superior responses observed in patients with T cell-inflamed or 'hot' tumors. Therefore, there is a need to characterize tumour infiltrating lymphocytes (TILs) in patients to give us a deeper understanding of tumour behaviour and expected response to therapy. Utilizing multiplexed immunohistochemistry on tissues from breast cancer patients pre, mid and post chemotherapy we have demonstrated the superior prognostic information that can be gathered from TIL characterisation. Our findings demonstrate that analysis of immune cell types and their activation status can be used to predict response to chemotherapy. Furthermore, we have demonstrated the benefit of sequential biopsies throughout neoadjuvant chemotherapy administration, where specific cell populations at mid and post chemo can predict patient survival. Similar characterization techniques have now been applied for pre-treatment mesothelioma tissues, where baseline immune status can predict response to therapy, and most importantly, response to immunotherapy. These studies offer rationale for pre- and post-tumour biopsies to monitor benefit and response to immunotherapeutics.

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Expression of cancer stem cell CD44 in patients with chronic gastritis, pre-cancerous and gastric cancer

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Background and Aim

Gastric cancer is another marker of CD44 cancer stem cell disease in gastrointestinal cancer, including gastric cancer.

The aim of this study was to investigate the expression of CD44 cancer stem cells in tissues from the gastrointestinal or surgical patients, including the association of CD44 cancer stem cell expression and clinical symptoms in patients with chronic gastritis, precancerous and gastric cancer.

Materials and Methods

Biopsy specimens were obtained from Esophagogastroduodenoscopy (EGD) and surgery. The gastric lesions were divided into 3 groups according to the stage of the disease. The first group is the chronic gastritis. The second group is the precancerous gastric lesion, including gastric atrophy and intestinal metaplasia. The third group is gastric cancer. The CD44 cancer stem cell expression was done by using immunohistochemistry technique.

Results

A total 106 patients were included in this study. 68 male and 38 female. Mean age 57±2, including 33 in group 1, 31 in group2 and 42 in group3.Patients baseline characteristic was shown in table 1. CD44 cancer stem cell were expression 2/33(6.06%) in group 1, 7/31 (22.58%) in group2 and 31/42 (73.81%) in group3.significant high expression in gastric cancer patients (OR = 2.98; 95% CI=1.21-4.86; p= 0.024).

Conclusion

Studies show that the expression of CD44 cancer stem cells is associated with the severity of the inflammation and changes of the gastric tissue to gastric cancer. Some patients in group 1 and group 2 who expression of the CD44 cancer stem cell were at risk or high risk for developing gastric cancer. The information above can be used to develop effective patient care. Close follow up should be recommended for high risk patients.

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New strategies to improve the efficacy of personalized cancer immunotherapy

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To overcome the limitations current cancer vaccines and improve their efficacy, we have developed a versatile antigen delivery platform targeting cross-presenting dendritic cells (DCs) *in vivo*. By preparing of a solid-in-oil-in-water double emulsion through sequential reagent addition, we developed a tailored nanoemulsion (TNE), which was functionalized to target Clec9A (Clec9A-TNE), a DC-specific endocytic receptor expressed by cross-presenting $CD103^+$ and $CD8^+$ DCs and plasmacytoid (p)DCs in mice and $BDCA3^+/CD141^+$ DCs in humans. Clec9A-targeting TNEs are stable in physiological environments and after i.v. injection in mice, they are selectively taken up by $CD8^+$ DCs and pDCs in spleen and tumor bed. TNEs traffic to both early endosomes and lysosomes, essential prerequisite for an efficient antigen processing and presentation through MHC class I and II pathways. Clec9A-targeting TNE encapsulating a reference antigen (ovalbumin, OVA) *without adjuvant* targeted and activated cross-presenting DCs and promoted antigen-specific $CD4^+$ and $CD8^+$ T-cell proliferation, cytotoxic T-cell activity and antibody responses *in vivo*. To exploit the tumour "mutanome" with our TNE platform, we have developed a functional assay to rank immunogenicity of individual neo-epitopes using the murine B16-F10 melanoma as a model. Four weekly i.v. injections of Clec9A-targeting TNEs loaded with a functionally selected pool of neo-epitopes strongly inhibited the *in vivo* growth of the highly aggressive and poorly immunogenic B16F10 melanoma cells and induced strong epitope-specific IFN-g T-cell immunity. Similar findings were also observed in a mouse model of HPV-driven carcinoma in which Clec9A-targeting TNEs loaded with recombinant E6/E7 viral oncoproteins showed a markedly superior efficacy as compared to a standard vaccination protocol.

Versatile, personalized, antigen-specific cancer vaccines are a long-sought therapeutic strategy in cancer immunotherapy. Clec9A-targeting nanoemulsions represent such a platform to deliver recombinant tumour protein or neo-epitope antigens specifically to cross-presenting DCs *in vivo*. This platform can fully exploit the neo-epitope target repertoire of individual tumours thereby improving the feasibility and efficacy of personalized cancer immunotherapy.

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Constitutive activation of YAP/TAZ disrupts pancreatic homeostasis

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Publish consent withheld

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Rational design of cancer vaccines by targeting dendritic cells

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As dendritic cells (DCs) are potent at inducing T cell responses, they have been studied for the development of immunotherapies to combat cancer. Several DC surface molecules have been successfully targeted *in vivo* using monoclonal antibodies to deliver antigen and induce T cell responses that confer tumour protection. One candidate is the C-type lectin-like receptor Clec9A, which shunts antigen efficiently into the cross-presentation pathway, facilitating MHC class I presentation to $CD8^+$ T cells. Tumour vaccines often utilise tumour-associated peptides, and more recently, tumour-specific mutated peptides, so called tumour neo-antigens. A recent report identified immunogenic B16 melanoma neo-antigens. Here, we investigated whether these tumour antigens can be harnessed for vaccinations using Clec9A-targeting antibodies. We demonstrated that the *in vivo* delivery of these neo-antigens to Clec9A does not induce antigen-specific T cell responses that can be detected by ELISpot. However, vaccination with some of these neo-antigens did induce cytotoxic T lymphocytes that killed peptide-coated target cells *in vivo*. Furthermore, when a pool of these neo-antigens were delivered to Clec9A, significant anti-tumour protection was induced in the B16-metastatic melanoma model. These data suggest that mutant epitopes are poorly immunogenic and that vaccines will require multiple mutant epitopes to induce effective anti-tumour protection. Interestingly, we have also shown that B16 melanoma-bearing mice treated with a new adjuvant and checkpoint inhibitors develop a protective T cell-dependent anti-tumour response, yet these protective T cells did not recognise the described neo-antigens. These findings highlight that only a small subset of neo-antigens are actively involved in tumour rejection and that these are yet to be identified. From a vaccine perspective, it may become critically important to identify bona fide rejection antigens and delineate these from poorly immunogenic mutant epitopes. In that vein, we have recently discovered spliced antigens that may prove to be more immunogenic.

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SAR131675 a VEGFR-3 specific inhibitor reduces tumour growth in a mouse model of colorectal cancer liver metastases by modulating the immune system.

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Background and Aims:

Colorectal liver metastases (CRLM) is mainly derived from haematogenous dissemination however, lymphatic dissemination may also contribute to its spread. SAR131675 is a potent and selective VEGFR-3 inhibitor. This study aims to analyse the SAR131675 effects on tumour dissemination in a mouse model of CRLM.

Methods.

CRLM were induced in CBA mice by intra-splenic tumour injection of a mouse colorectal tumour cell line (MoCR). Animals were allocated into SAR131675 treatment or vehicle control groups. SAR131675 (100 mg/kg) was given daily from day 10 post-tumour induction until day 21, when tissues were collected. Tumour stereology Tumour volume and burden were assessed by sterealogy. Immunostaining was used to determine lymphatic and blood vessel density (podoplanin/ CD31 and CD34), proliferation (Ki67), apoptosis (caspase3), VEGFR3 expression (VEGFR3, macrophages (F4-80) and T cells (CD3). Serum cytokines were evaluated using.

Results.

Treatment with SAR131675 significantly reduced the number of metastases and tumour burden compared to control ($p<0.02$). No significant differences were seen in lymphatic and blood vessel density and in VEGFR3, ki-67 and caspase expression between groups. However significant reduction in macrophage numbers were seen in the treated group (in the liver and around the tumour vascular beds). Additionally there was a decrease in serum IL-10 and an increase in IFNγ in SAR131675 treated mice ($p<0.05$), denoting a T helper 1(Th1) immune response.

Conclusion.

SAR131675 treatment inhibits tumour progression at least in part through the modulation of the immune system possibly by reversing the immunosuppressive tumour microenvironment and inducing a tumouricidal immune response.

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Defining the Scheduling and Immunological Parameters underlying the Efficacy of Neoadjuvant Immunotherapy

Jing Liu, Jake S O'Donnell, Stacey Allen, Mark J Smyth, Michele W Teng

Cancer immunotherapies targeting CTLA-4 and or PD-1/PD-L1 alone or in combination have elicited remarkable clinical responses in some advanced cancers. Currently, their efficacy in the treatment of earlier stages of disease is being assessed in an adjuvant setting. We previously demonstrated in two orthotopic mouse models of spontaneously metastatic breast cancer (4T1.2 and E0771), the significantly greater therapeutic power of neoadjuvant compared with adjuvant immunotherapies in the context of cancer surgery[1]. Elevated and sustained peripheral tumor-specific $CD8^+$ T cells after neoadjuvant immunotherapy underpinned the outcome. In this study, we demonstrated that $Batf3^+$ DCs and type I IFN were critical for the efficacy of neoadjuvant anti-PD-1+anti-CD137 immunotherapy. Loss of $Batf3^+$ DCs or blocking type I IFN receptor in neoadjuvant treated tumor-bearing mice significantly reduced tumor-specific activated $CD8^+$ T cells in the blood and primary tumor. We next assessed how varying the scheduling and duration between neoadjuvant immunotherapies and surgery impacts on long-term survival. Importantly, maintaining the duration between resection of the primary 4T1.2 tumor and neoadjuvant anti-PD-1+anti-CD137 short (4 days) compared to long (10 days) was critical generating long-term survivors. Interestingly, the addition of adjuvant combination immunotherapies (anti-PD-1+anti-CD137 or anti-CTLA-4+anti-PD-1) following their respective neoadjuvant treatment did not generally increase the proportion of mice cured. In contrast, biochemical immune-related adverse events (irAEs) increased in these tumor-bearing mice compared to similar groups that received only two doses of neoadjuvant combination immunotherapy. Overall, our data suggest that a short dose of neoadjuvant combination immunotherapy may suffice to induce effective anti-tumor immunity while reducing the development of severe irAEs.

1. **Liu J**, Blake SJ, Yong MC, Harjunpää H, Ngiow SF, Takeda K, Young A, O'Donnell JS, Allen S, Smyth MJ, Teng MW. Improved Efficacy of Neoadjuvant Compared to Adjuvant Immunotherapy to Eradicate Metastatic Disease. **Cancer Discov.** 2016 Dec;6(12):1382-1399.

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Is Australia "cell and gene therapy ready"? A vision for a world class cell and gene therapy industry in Australia

Tim Oldham

FDA approved the first two CAR-T therapies in 2017, offering new hope for cancer patients with treatment refractory disease and confirming the maturation of the cellular immunotherapy modality from research to commercial/clinical readiness.

Transformational therapeutic outcomes will however require transformation in manufacturing and supply chains, research translation and funding. The centralised, big batch, make-to-stock, sell-and-forget pharmaceutical supply chains of today are inadequate. Supply chains must accommodate just in time production initiated only when a clinician identifies an eligible patient *and* manufacturing confirms an available production slot. Clinical sites become part of the regulated supply chain. Manufacturing is labour intensive and in many cases each production run is for a single patient treatment. New translational research approaches are emerging to accommodate these new supply chains, and a highly variable, living therapeutic product with a very long duration of action. Production costs are significantly higher than traditional pharmaceuticals and prices are extremely high, frequently US$250,000+ per treatment before hospital costs. Potentially curative outcomes are driving pay-for-performance models.

Is Australia ready to take full advantage of this therapeutic and technical revolution? Australia has a proud history in cell therapy and cellular immunotherapy research, however lacks the commitment to succeed being shown by other countries and lacks critical pieces of supply chain capability essential to ensure early adoption of late stage and commercial products here.

By making a commitment to a “cell and gene therapy ready” Australia, and investing a modest $100m in three initiatives, Australia can reap a triple bottom line benefit from this revolution in the form of:

1. >500 direct advanced manufacturing jobs in the near term, potentially double this in the mid-term and significant spill-over jobs, all difficult to move off-shore;
2. Improved, earlier access to cost effective, breakthrough therapies delivering transformational health outcomes for >3,000 Australian terminal blood cancer and single genetic defect patients in the near term alone; and
3. Improved translational research efficiency and company formation providing greater economic return on the world-class science and substantial dollars invested in R&D over previous decades.

The three initiatives are:

1. Establishing commercial scale cell and gene therapy manufacturing and supply chain capability (cell processing, viral vectors, clinical centres of excellence, critical materials security)
2. Establishing a national “venture catalyst” organisation as the focal point for research translation and commercialisation
3. Market access (regulatory and reimbursement) innovation

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Tissue-specific differences in the tumour microenvironment and immunotherapeutic responses

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Immunotherapies that harness the immune system against cancer are an attractive proposition for cancer treatment. While there have been some promising successes, only a small fraction of patients obtain clinical benefit. It has become clear that the immunosuppressive tumour microenvironment (TME) is a major obstacle for immunotherapies, because the TME suppresses immune responses leading to reduced efficacy. We previously demonstrated that the site of tumour growth is a major determinate in sculpting the organ-specific TME, and thus predisposes treatment efficacy[1]. In this project, we hypothesise that the TME of visceral tumours is more immunosuppressive than the ones of the tumours growing elsewhere. We investigated in murine models the difference in the TME in breast cancer growing orthotopically and the same breast cancer growing in the common metastatic sites, such as the lungs. Our findings showed that the breast cancer growing in the lungs were resistant to immunotherapies whereas the breast cancer growing orthotopically could be completely eradicated even when the cancer burden was greater. Through an institutional prospective community-based rapid autopsy program (CASCADE), we obtained genetically matched metastases from several sites in the same breast cancer patients and investigated the TME from these tumours using novel technologies such as RNAseq and multiplex immunohistochemistry. Strikingly, the TMEs from the same organs in different patients have similar immune gene expression profiles and in contrast, TMEs from the same patient differ greatly in different organs. Together, our research demonstrates an organ-specific difference between TMEs that leads to different responses to therapies. We anticipate that further study of how cancer cells sculpt the TME at different sites will greatly enhance our understanding of the TME and provide promising targets to enhance current immunotherapies, especially for patients that do not respond to existing therapies.

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Expression and polymorphisms of MDM2 in gastric cancer correlate with clinicopathological characteristics

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Background and Aims: The MDM2 SNP309 polymorphism has been suggested as translating increased intracellular MDM2 levels and limits the cellular availability of functional p53. Thus, the aim of this study was to determine whether the MDM2 SNP309 polymorphism and MDM2 expression are associated with clinicopathological outcome and gastric cancer risk.

Methods: This cross-sectional study identified the MDM2 SNP309 polymorphism in a total of 68 patients by using Taq Man SNPs Genotyping assay. Immunohistochemistry was performed to evaluate MDM2 expression levels among patients with chronic gastritis, precancerous lesions, and gastric cancer. Logistic regression was used to estimate associations between polymorphisms, MDM2 expression, clinicopathological outcomes, and gastric cancer risk. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated by multivariate Cox proportional hazards regression modelling, to determine the strength of any association with outcome.

Results: There was a significant link between the MDM2 SNP309 G/G homozygous polymorphism and MDM2 expression, and gastric cancer risk (OR=1.57, 95% CI=1.39–2.03, *p*=0.039). MDM2 expression was also suggested to be associated with gastric cancer risk. Poor clinicopathological outcome of gastric cancer indicated an association with MDM2 expression, including tumor location in the upper gastric region (OR=1.48, 95% CI=1.25–3.54, *p*=0.037), undifferentiated type (OR=2.47, 95% CI=1.38–4.14, *p*=0.016), presence of lymphatic invasion (OR=1.96, 95% CI=1.22–3.19, *p*=0.014), and unresectable tumor (OR=3.39, 95% CI=1.61–4.94 *p*=0.017).

Conclusion:Our study indicates an association between the MDM2 SNP309 G/G homozygous polymorphism, MDM2 protein expression, and clinicopathological outcome. Understanding genetic polymorphisms and expression of *MDM2* may help explain gastric cancer risk in Thai populations.

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Mechanisms of resistance of tumors to adoptive cell transfer and vaccination

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Dual-specific T cells, expressing a Her2-specific chimeric antigen receptor (CAR) and a gp100-specific TCR, were generated using genetic modification. Adoptive transfer of dual-specific T cells, combined with an injection of a gp100-expressing vaccinia virus vaccine, eradicated a variety of large syngeneic mouse tumours, including E0771-Her2 breast tumors (Slaney *et al*, 2017). However, some tumors, including AT3-Her2 breast tumors, do not respond completely. Therefore, E0771-Her2 and AT3-Her2

provided an opportunity to define elements of susceptibility and resistance to therapy. Using immunohistochemistry, we demonstrated a mixoid nature of AT3-Her2 tumors composed of islands of round tumor cells amidst streams of spindle-shaped tumor cells and stroma. Whereas infiltration of adoptively transferred dual-specific T cells was robust and evenly distributed in E0771-Her2 tumors, T cells localized to areas of spindle-shaped cells and were largely excluded from AT3-Her2 tumor islands. In addition, using in vitro cytotoxicity assays, AT3-Her2 cells were demonstrated to be relatively resistant to both perforin- and TNF-mediated killing. In ongoing experiments, we seek to enhance the abilities of dual-specific T cells to infiltrate tumors and kill malignant cells. Addressing these parameters may enable the extension of this therapy to a broader range of tumours and lead to the design of new immunotherapy regimens for the treatment of cancer patients.

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Targeting of multiple determinants on solid tumors using chimeric antigen receptor T cells

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The generation of chimeric antigen receptor (CAR) T cells, an immunotherapy for the treatment of cancers, has showed high efficacy in anti-CD19 CAR-T cells in B cell malignancies, with up to a 90% complete remission rate. However, the clinical efficacy of CAR T cells in hematological malignancies has not been achieved in solid tumors. CAR T-cell therapy for solid tumors faces multiple hurdles, including inefficient CAR-T trafficking and nonhomogeneous tumor antigen expression. In order to boost the trafficking of CAR-T cell and avoid antigen escape for solid tumors, we have designed CAR-T cells that target more than one tumor antigen. Our key focus is TAG-72 is a glycoprotein specifically found on the surface of many adenocarcinoma cells, including ovary, prostate, breast, colon, lung and pancreatic cancers. We are combining this with another transmembrane protein which is also highly expressed in many tumors. Accordingly we have generated a second generation anti-TAG72 CAR which co-stimulates CAR-T cells to become cytolytic but also induces T-cell expansion upon repeated exposure to the antigen. The synergistic combination of the two markedly enhances the binding affinity of the CAR-T cell to the cancer cells and results in more complete cytotoxicity of cancer cells in vitro, as demonstrated using the real-time xCelligence platform. We also found that the dual combination did not lead to destruction of normal cells in our in vitro cytotoxicity assay.

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Association of MDM2 Protein Expression and Histopathological Grading of Cholangiocarcinoma in Northeast Thailand

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Background: Cholangiocarcinoma (CCA) is one of the most common cancers in Thailand, particularly in north eastern part, where the prevalence of liver fluke infection is high. Mdm2 protein has been characterized as a negative regulator of the tumor suppressor protein p53, which contributes to an increase cancer risk.

Objective: To investigate the expression levels of MDM2 protein and evaluated its association with histopathological grading of CCA patients.

Methods: We performed the immunohistochemistry in 64 formalin-fixed paraffin-embedded (FFPE) tissues of CCA specimens from three hospital medical center in Northeast Thailand. MDM2 protein expression was evaluated by five independent pathologists. Statistical analyses were using the SPSS used for Windows (version 20.0; SPSS, Chicago, IL, USA).The association between MDM2 protein expression and histopathological grading of CCA specimens were evaluated by using logistic regression model. A *p*-value less than 0.05 were considered as significant.

Results: Histopathologically diagnosed 16 cases of well differentiated adenocarcinoma, 20 cases of moderately differentiated adenocarcinoma and 28 cases of poorly differentiated adenocarcinoma were identified from CCA specimens. MDM2 protein was found to be expressed at high levels in 34 cases of the tumor, while 30 cases were expressed MDM2 protein at low levels. Expression of MDM2 protein at high levels associated with poorly differentiated adenocarcinoma ($p<0.01$, OR=1.59, 95% CI 1.472-1.713).

Conclusions: Our results indicated that high expression of MDM2 protein in CCA patients is associated with higher histopathological grading of CCA patients in Northeast Thailand. The expression of MDM2 protein characterizes the invasive CCA, which may be of a prognostic value.

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Expression of CD44 cancer stem cell and MDM2 in Cholangiocarcinoma Correlates with Poor Clinicopathological characteristics

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Background

CD44 cancer stem cell and MDM2 plays major roles in survival signaling in many cancer cells including cholangiocarcinoma. This study aimed to investigate the distribution of clinicopathological characteristics of cholangiocarcinoma related to the combination between expressions of CD44 cancer stem cell and MDM2 protein by using immunohistochemistry in Thai population.

Methods

A total 64 cholangiocarcinoma tissues were enrolled in this study during June 2012-June 2017. The immunohistochemical staining results were evaluated by two independent pathologists, who was blinded to the clinicopathological details of the patients. The assessment of CD44 and MDM2 staining (score) was based on the percentage of positive cells [lack of staining was scored as negative, 1–50% were classified as 1+, >10 and ≤50% as "2+" and >50% as "3+"]. The cases classified as 0 were considered negative, whereas 1+, 2+ and 3+ were established as positive cases. Significant parameters from the univariate analysis were then assessed in the final by using multivariate analysis using Cox proportional hazards regression modeling with step-wise forward selection, $p < 0.05$ considered as statistically significant.

Results

In total, 64 cholangiocarcinoma cases (78.1% males and 21.9% females), 35.9% intrahepatic type and 64.1% extrahepatic type. The distribution of clinicopathological characteristics of cholangiocarcinoma related to the combination between expressions of CD44 cancer stem cell and MDM2 protein (multivariate analysis) are high histologic grade [$p = 0.016$, OR = 2.48 (1.26-4.28)],large tumor size [$p = 0.042$,OR = 2.59 (1.19-3.28)], lymph node metastasis [$p = 0.013$,OR= 2.91(1.82-4.09)] , distant metastasis [$p = 0.001$,OR = 3.38(1.59-5.21)] and poor 5 year survival [$p = 0.041$,OR 1.52 (1.04-2.26)] were all found to be significantly related to a combination of CD44 and MDM2 expression.

Conclusions

CD44 and MDM2 were significant to poor the clinicopathological outcome of cholangiocarcinoma patients.Co-expression of CD44 and MDM2 in cholangiocarcinoma tissue may indicate an unfavorable patient outcome and may serve as a useful practical adjunct to conventional prognostic indicators for cholangiocarcinoma.

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Optimising T-cell transduction and expansion conditions for CAR T-cell therapy in solid cancers

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The use of immunotherapy for treating advanced cancer has developed dramatically in recent years. Transferring T cells encoding tumour-specific chimeric antigen receptors (CAR) has shown great promise in treating haematological cancers but has been less successful in patients with solid tumours. Although partial remissions were observed in several clinical trials for solid cancer, most patients were stable or in disease progression at follow up. Furthermore, CAR T cells have been reported to expand less well and fail to persist after infusion to these patients. Therefore, the optimal production of long-lived and highly functional CAR T cells is clearly required for the successful application of this immunotherapy to solid tumours. Here, we investigate the ability of T lymphocytes derived from solid cancer patients receiving chemotherapy to result in the generation of satisfactory CAR T-cell products. we hypothesize that on-treatment blood specimens of solid cancer patients may contain a high proportion of central memory T cells and thus represent the optimal starting population for manufacturing highly functional and effective CAR T cells. To test this hypothesis, CAR T cells are produced using leukocyte populations isolated from glioblastoma, advanced pancreatic cancer and metastatic colorectal cancer patients at three-time points: before chemotherapy, during chemotherapy and at relapse. Two types of starting population,unsorted PBMCs and magnetically sorted CD4+ and CD8+ T cells, are compared for their transduction efficiency, expansion, and immune phenotype. Initial results have shown that on- treatment CAR T cells proliferate strongly and sorted T cells have a higher transduction efficiency. This study will determine of T cells harvested from cancer patientsat different stages of their disease can generate therapeutically useful yields of CAR T cells.

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Inhibition of the renin-angiotensin system reduces tumour growth and modulates macrophage activation in a mouse model of colorectal cancer liver metastases.

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Background and Aims:

Over 50% of colorectal cancer (CRC) patients develop liver metastases. Surgical resection is the only potentially curative treatment for these patients, but 40-80% of them develop disease recurrence (1,2). Liver regeneration, following metastatic resection, induces upregulation of growth factors/cytokines that impact on micrometastases present in the remnant liver leading to tumour recurrence. Macrophages are a major part of the tumour microenvironment and depending on local stimuli macrophages can adopt different phenotypes (M1/M2) influencing tumour progression. Inhibition of the Renin Angiotensin System

(RAS) has shown to inhibit tumour growth. This study aims to determine how macrophages are effected by Captopril (RAS inhibitor) treatment on tumour development.

Methods:

Changes in macrophage receptors and secreted factors are investigated in murine CRC liver metastases, following RAS inhibition. Mice were treated with Captopril (250mg/kg) or with saline. Livers were harvested on days 16 or 21 after tumour induction and archived as formalin fixed paraffin embedded (FFPE). Double immunohistochemistry was performed for F4/80 (macrophage marker) and activation markers (Arginase, IL-10, VEGF) to determine macrophage phenotype.

Macrophage cell lines (J774 and P388D1) and a mouse CRC cell line (MoCR) were cultured with Captopril. Cytokines/growth factors released in response to treatment were evaluated with ELISA.

Results

Captopril reduced tumour infiltrating macrophages and particularly VEGF expressing macrophages *in vivo*. Captopril reduced the secretion of TNF-α, a pro-inflammatory cytokine, by macrophages in *in vitro* cultures.

Conclusion

Captopril treatment during and following liver resection inhibits infiltration of macrophages including VEGF-secreting macrophages and *in vitro* alters macrophages towards an anti-inflammatory phenotype.

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Imaging Activated Platelets in Cancer- An Avenue For Early Cancer Detection and Targeted Therapy.

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The early detection of primary tumours and metastatic disease is vital for successful therapy and is contingent upon highly specific molecular markers and sensitive, non-invasive imaging techniques. The relationship between platelets and cancer, and the abundance of activated platelets in the tumour microenvironment has been well described. Here we investigate a unique single-chain antibody (scFv), which we generated to specifically target activated platelets, as a novel biotechnological tool for molecular imaging of cancer.

The $scFv_{GPIIb/IIIa}$, which binds specifically to the activated form of the platelet integrin receptor GPIIb/IIIa present on activated platelets, was conjugated to either Cy7, ^{64}Cu or ultrasound-enhancing microbubbles. Molecular imaging via fluorescence imaging, PET and ultrasound was performed in mice bearing human cancer xenografts to confirm specific targeting of $scFv_{GPIIb/IIIa}$ to activated platelets in the tumour stroma.

Using the $scFv_{GPIIb/IIIa}$ we successfully showed specific targeting of activated platelets within the microenvironment of human breast adenocarcinoma, triple negative breast adenocarcinoma, fibrosarcoma and B cell lymphoma xenograft models via three different molecular imaging modalities. The presence of platelets within the tumour microenvironment, and as such their relevance as a molecular target epitope in cancer was further confirmed via immunofluorescence of human tumour sections of various cancer types, thus validating the translational importance of our novel approach to human disease.

Our study provides proof of concept for the early diagnosis and localisation of tumours by molecular targeting of activated platelets. These findings warrant further investigation of this activated platelet specific $scFv_{GPIIb/IIIa}$, as a universal marker for cancer diagnosis and further on as a possible theranostic agent, by utilizing a single probe for cancer diagnosis, disease monitoring and targeted therapy.

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PD-L1 expression is a prognostic factor in subgroups of gastric cancer patients stratified according to their levels of CD8 and FOXP3 immune markers

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Current studies aiming at identifying single immune markers with prognostic value have limitations in the context of complex antitumor immunity and cancer immune evasion. Here, we show how the integration of several immune markers influences the predictions of prognosis of gastric cancer (GC) patients. We analyzed Tissue Microarray (TMA) by multiplex immunohistochemistry and measured the expression of immune checkpoint molecule PD-L1 together with antitumor CD8 T cells and immune suppressive FOXP3 Treg cells in a cohort of GC patients. Unsupervised hierarchical clustering analysis of these markers was used to define correlations between CD8 T, FOXP3 Treg, and PD-L1 cell densities. We found that FOXP3 and PD-L1 densities were elevated while CD8 T cells were decreased in tumor tissues compared to their adjacent normal tissues. However, patient stratification based on each one of these markers individually did not show significant prognostic value on patient survival. Conversely, the combination of the ratios of CD8/FOXP3 and CD8/PD-L1 enabled the identification of patient subgroups with different survival outcomes. As such, high densities of PD-L1 in patients with high CD8/FOXP3 and low CD8/PD-L1 ratios correlated with increased survival. Collectively, this work demonstrates the need for the integration of several immune markers to obtain more meaningful survival prognosis and patient stratification. In addition, our work provides insights into the complex tumor immune evasion and immune regulation by the tumor-infiltrating effector and suppressor cells, informing on the best use of immunotherapy options for treating patients.

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Fibroblast activation protein (FAP) is overexpressed in glioblastoma brain tumours and represents a potential target for immunotherapy

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Glioblastoma is the most lethal form of primary brain tumour. Standard treatment combines debulking surgery, radiotherapy and temozolomide chemotherapy but is of limited efficacy, producing a median survival time of only ~15 months. Glioblastoma is not responsive to checkpoint inhibitors and there have been no meaningful improvements to treatment in decades. Accordingly, there is much interest in developing novel therapies based on immune targeting of glioblastoma. The success of these approaches, however, relies on identification of a target antigen expressed on tumour cells but not healthy tissues. We show here that fibroblast activation protein (FAP) is a candidate for such approaches. FAP is a membrane-bound extracellular protease with limited expression in healthy tissues but elevated expression on fibroblasts involved in tissue remodelling, such as during wound healing or tumour formation. Here, immunohistochemical staining of glioblastoma patient tissue specimens revealed expression of FAP in the majority of tumours examined, whereas adjacent normal brain tissue uniformly lacked expression. FAP was detected in both the main tumour parenchyma and in a distinct perivascular pattern. These results are supported by analysis of RNA sequencing and microarray data from The Cancer Genome Atlas (TCGA), which showed significantly elevated levels of *FAP* gene expression in glioblastoma specimens compared to normal brain tissue. A panel of 15 patient-derived glioma neural stem cell cultures was examined for FAP expression by flow cytometry, revealing that the majority (10/15) expressed some level of cell surface FAP. This finding reveals that, in contrast to epithelial cancers where FAP expression is limited to stromal fibroblasts, the cancer cells themselves may express FAP in glioblastoma. Together, these data reveal FAP as a potential candidate for targeted immunotherapy approaches in glioblastoma.

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Smart, Closed & Connected Solutions for Cell Therapy Production

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As patient specific, autologous cell therapies are advancing through clinical trials and are moving quickly toward commercialization, there continues to be a need for robust, closed, automated and scalable manufacturing solutions that can accommodate many patient samples, simultaneously. What will be required is a means to process patient material in a fashion which maximizes the efficiency of the processing workflow while meeting safety, quality and regulatory requirements. Any production approach should retain the fundamental principles of preventing contamination, patient sample mixing, loss of identity, and other events which may compromise the physical properties and integrity of the patient sample and/or final product.

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Rational Development for Cell and Gene Therapy Manufacturing

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Translating open, manual manufacturing processes toward closed, automated and integrated workflows can offer significant benefits for developing cell and gene therapies. This presentation will review key considerations in the planning and execution of process development toward these goals and explore a case study for the scale-up of pluripotent stem cells.

Eradicate Cancer 2018

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