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Can we harness the power of the immune system to cure childhood cancer?

Chaired by Professor Persis Amrolia and Professor John Anderson

Submitted abstracts selected for poster/oral presentation
(* denotes abstracts selected for oral presentation)

Antibody-based and Bispecific T-cell Dependent Therapies

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Metastatic solid tumors in children have a galloping and devastating tempo and neuroblastoma is a classic example. Dose intensive induction can rapidly induce remission and delay disease progression. However, to improve overall survival, immunotherapy is best applied at the time of minimal residual disease. Myeloid and natural killer (NK) cells are innate immunity effectors that recover early after chemotherapy to guard the blood and bone marrow, the sites at risk of first metastatic recurrence. Through antibody-dependent cytotoxicity, these compartments can be rendered tumor-free. Bispecific antibodies can engage polyclonal T cells to travel into tissues including lymph nodes and tumor masses, to impose a second round of tumor control. Bis-scFv is a tandem joining of two single chain Fv fragments (scFv), one specific for tumor target GD2, and one specific for CD3 on T cells. It is characterized by its small size, monovalency and fast clearance. Both the dimeric form of Bis-scFv and the IgG(anti-GD2)-scFv(anti-CD3) platforms have clinical advantage because of its bivalency, increased size, and prolonged pharmacokinetics. IgG(anti-GD2)-scFv(anti-M^{3+}/BnDOTA) bispecific antibodies exploit pre-targeting radioimmunotherapy (PRIT) to deliver liquid radiation (β and α emitting particles) with exquisite specificity as well as large therapeutic indices for ablating large tumors or single tumor cells. Metastasis in hard to penetrate compartments such as the central nervous system can be eradicated through direct intrathecal or intratumoral injections. Ultimately, antigen-specific idiotype networks or vaccines, invigorated by checkpoint blockades and epitope spreads, can be timed to build an active and permanent protection for long term cancer cure.

The CAR T Cell Revolution in Cancer Therapy

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Chimeric antigen receptors (CARs) combine a binding fragment of an antibody with intracellular signaling domains. We have reported exciting data on CTL019 cell therapy, using engineered T cells expressing an anti-CD19 CAR. Infusion of these cells results in 100 to 100,000x *in vivo* proliferation, durable anti-tumor activity, and prolonged persistence in patients with B cell tumors, including sustained complete responses (CR) in adults and children with acute lymphoblastic leukemia (ALL; see Grupp *et al*., NEJM 2013 and Maude *et al*., NEJM 2014). This talk will update the audience on pediatric engineered cell therapy. Recent updates of our data in ALL show a 93% complete response rate and 79% overall survival at 1 year.

The key to highly active engineered cell therapy appears to be proliferation: the engineered T cells need to be capable of 1000x or more proliferation in the patient to see high responses and significant clinical activity. Without proliferation, there are no responses. In addition, we hope to see cells persist for 6 months or more, providing longer-term disease control allowing some patients to avoid bone marrow or stem cell transplant. Toxicities such as cytokine release syndrome (CRS) can be significant, especially for patients with high disease burden. However, we have shown that the cytokine IL-6 is a key mediator of severe CRS, and that blocking IL-6 using the IL-6 receptor antagonist tocilizumab can rapid and completely resolve life-threatening CRS.

CTL019 cells can undergo robust in-vivo expansion and can persist for over 4 years in patients with relapsed ALL, allowing for the possibility of long-term disease response without subsequent therapy such as stem cell transplant. This approach also has promise as a salvage therapy for patients who relapse after allogeneic stem cell transplant with a low risk of GVHD. CTL019 therapy is associated with a significant CRS that responds rapidly to IL6-targeted anti-cytokine treatment. This therapy has received Breakthrough Therapy designation from the FDA. US and Global phase II multicenter trials are underway and have been completed.

NCI CARs for Childhood Acute Lymphoblastic Leukemia and Neuroblastoma: The Promise and Potential Pitfalls

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CD19 Chimeric Antigen Receptor (CAR) T cell therapy has produced complete response (CR) rates of 70 - 90% in children and young adults with refractory pre-B acute lymphoblastic leukemia (ALL) across multiple institutions. In the Pediatric Oncology Branch (POB) at the National Cancer Institute, 53 children and young adults have been treated with our CD19 CAR T cell therapy (NCT01593696). This is a second generation CAR utilizing the co-stimulatory molecule, CD28. CAR cells are manufactured and reinfused within 2 weeks of apheresis. The CR rate in an intent-to-treat analysis is 60% with 100% of primary refractory ALL patients (n = 12) and those with CNS2/CNS3/ leptomeningeal leukemia (n = 5) achieving a minimal residual disease (MRD) negative CR in both marrow and CSF. The incidence of grade 3 cytokine release syndrome (CRS) was 9% and grade 4 CRS was 7% using the Lee criteria and management algorithm (*Blood* 124:188–95, 2014).

We advocate for a subsequent allogeneic hematopoietic stem cell transplant (HSCT) in all patients achieving MRD-negative CR who have not had a prior transplant and have a suitable donor available as this would be standard-of-care for most with multiply relapsed/refractory disease. Five out of 31 responding patients did not proceed to HSCT as all had at least one prior allogeneic HSCT. Four of these 5 relapsed (2 with CD19+ and 2 with CD19 negative ALL) and one is still in remission at 8 months. Though long-term persistence of the CAR has not been seen, the incidence of combined CD19-negative and CD19-positive relapses in responding patients undergoing a subsequent HSCT is remarkably low at 9% (2/21: one CD19+ at 2 years and one CD19 negative at 100 days). Although not formally addressed head-to-head, this is in stark contrast to other groups where patients do not routinely proceed to HSCT after CD19 CAR T cell therapy.

CAR T cell therapy for most solid tumors has not yet produced meaningful responses and indeed remains a significant challenge to the field. This difference in response is likely multifactorial, and ongoing work by many groups is addressing these issues. The POB is conducting a Phase 1 clinical trial of GD2-targeted CAR T cells in children with neuroblastoma and osteosarcoma (NCT02107963). This is a third-generation CAR with both OX40 and CD28 as co-stimulatory domains Since GD2 is expressed on peripheral neurons at low levels and anti-GD2 antibody in clinical use for neuroblastoma has associated neuropathies, the GD2 CAR also contains an inducible caspase 9 suicide system that can be activated using the small molecule, rimiducid (AP1903). Fourteen patients have been treated to date (12 osteosarcoma, 2 neuroblastoma) with doses up to $1x10^7$ CAR T cells/kg. The best response is stable disease at 1 - 2 months. However, *in vivo* expansion of GD2 CAR T cells has been detected in the blood of patients and no neurotoxicity or > Grade 2 CRS has been observed. These findings to date warrant continued investigation of GD2 CAR T cells at higher doses.

Finally, neurotoxicities and severe cytokine release syndrome (CRS), which can be lethal without timely and appropriate intervention, represent the biggest challenges in exporting this therapy to new centers. Tocilizumab, corticosteroids, and other agents have been used to arrest CRS, but this is not always successful especially when CRS has already become severe or life threatening or there is evidence of end-organ damage. Whether these agents also arrest the cytotoxic function of CAR T cells is unknown. Therefore, balancing patient safety while maximizing tumor cytotoxicity can be a complicated and difficult feat. This presentation will describe these toxicities and outline a user-friendly CRS management algorithm and other strategies for minimizing severe effects while maximizing response.

Future approaches to engineering CAR T-cells

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No abstract available.

Development of CAR-T cell therapy for gliomas

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Introduction

High grade gliomas are aggressive brain tumours both in adults and children. Treatment is highly challenging due to its location within the central nervous system and its capacity to spread within the brain parenchyma, making recurrence common after surgical removal. Among gliomas, Diffuse Intrinsic Pontine Glioma (DIPG) is a rare but particularly aggressive childhood brain cancer for which surgery is not an option (due to its location in the brain stem) and no chemotherapeutic or small molecule regimen has thus far proven beneficial. Therefore, new treatments are urgently needed.

Chimeric antigen receptors (CAR) have shown promising results in haematological malignancies, exhibiting complete and durable responses in patients treated with CD19-redirected T cells. CARs hold the promise to be successful in brain tumours as they can seek and destroy invading tumour cells. Translation of this technology to solid tumours requires, however, further investigation and accurate modelling: to be effective. CAR-modified T cells need to engraft systemically, migrate to the tumour, proliferate in situ and overcome an immunosuppressive microenvironment.

Methods

Here, we used tumour-specific antigen Epidermal Growth Factor Receptor variant III (EGFRvIII) - a common mutation in adult gliomas – as a model antigen to identify the optimal therapeutic settings. We developed an immunocompetent murine model comprising the murine glioma cell line GL261, modified to express EGFRvIII, which was used to establish orthotopic tumours. Tumour bearing mice were treated with syngeneic T cells expressing second generation EGFRvIII targeting CARs with or without concomitant PD1 blockade.

Results

Systemically infused EGFRvIII-specific CAR-T cells efficiently migrated, accumulated at tumour site and were able to control tumour growth and increase survival rate, but did not completely eradicate tumours. Analysis of infiltrates showed the CAR-T cells to be highly activated, to proliferate and to have a pro-inflammatory profile. However, chronic activation and an immunosuppressive microenvironment (PD-L1 expression, T_{reg} infiltration) may be responsible for the failure of CAR-T cells in mediating complete remission. CAR-T cells administration combined with PD1 blockade resulted in a complete and durable response.

Conclusions

This model provides a useful tool to evaluate the best settings for CAR-T cell therapy for gliomas. This data suggest that modulation of the PD1/PD-L1 pathway is necessary in order to make CAR-T cells therapy successful in this setting. Data from this model will provide useful information to translate this approach to DIPGs and other childhood gliomas. Future work will also include the identification of targets specific for these childhood cancers.

Chimeric antigen receptor–engineered gamma-delta T-cells for neuroblastoma immunotherapy

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High-risk neuroblastoma is an aggressive childhood cancer with poor long-term survival rates. Chimeric antigen receptor (CAR) engineered adoptive T-cell therapy shows great promise as it has the potential to specifically kill GD2-expressing neuroblastoma cells whilst sparing healthy tissues. Current approaches have focused almost exclusively on alpha-beta ($\alpha\beta$) T-cells as the effector cell of choice, however gamma-delta ($\gamma\delta$) T-cells (inclusive of vδ1 and vδ2 subtypes), are unconventional T-cells that functionally and phenotypically bridge the gap between both the innate and adaptive immune system. Their combined functional properties are potentially advantageous for cancer immunotherapy, including innate cytotoxicity, professional antigen presenting function, and enhanced tissue tropism.

We report two protocols for the manufacture of $\gamma\delta$ CAR T-cells using a second-generation GD2-specific CAR comprising CD28 and CD3ζ intracellular signalling domains. Zoledronate or Concanavilin A were used to expand $\gamma\delta$ CAR T-cells to clinically relevant number, and vδ1 and vδ2 subtypes showed equivalent cytotoxicity towards GD2+ neuroblastoma cell lines when compared to conventional $\alpha\beta$ CAR T-cells. vδ2 CAR T-cells following 13 day culture were predominantly of 'effector memory' phenotype (CD45RA$^+$/CD27$^-$), however these cells also adopted a professional antigen presenting cell (pAPC) phenotype with high expression of CD86 and HLA-DR. vδ1 CAR T-cells had the highest proportion of 'naïve' cells with lowest expression of exhaustion markers, PD-1 and Tim3, compared to $\alpha\beta$ and vδ2 CAR T-cells .

We conclude that $\gamma\delta$ CAR T-cells can be generated in sufficient number for neuroblastoma immunotherapy. Further study is required to investigate the translational importance including whether 'naïve' vδ1 cells have greater proliferative capacity, tumour homing ability, and survival *in vivo*, and whether $\gamma\delta$ CAR T-cells with a pAPC phenotype are capable of cross-presenting tumour-associated antigens to neighbouring immune cells.

Improving specificity of cancer immunotherapy through novel chimeric antigen receptor design

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Background

Clinical trials of chimeric antigen receptor (CAR) gene modified αβT-cells show unprecedented clinical responses, but with the major limitation of on-target off-tumour toxicity due to expression of most cancer antigens on some normal tissues. This toxicity restricts the repertoire of antigens that can be safely targeted using CAR based cellular immunotherapy. Vδ2+ gamma-delta (γδT) cells engage danger associated molecular patterns in an MHC independent manner and are therefore amenable to more subtle manipulation using CARs containing only co-stimulatory endodomains (CSO-CARs).

Methods

We devised a CSO-CAR construct targeting the myeloid marker CD33 which is brightly expressed on AML blasts and healthy myeloid cells. The construct was expressed in γδT cells which were used in 51Cr cytotoxicity assays using AML cell lines and healthy monocytes as targets. Cytotoxicity of γδT cells expressing anti-CD33 CARs against myeloid progenitors was assessed using co-culture followed by colony formation assay. γδT cells expressing conventional second generation CARs, αβT cells expressing CSO-CARs and non-transduced γδT cells were used as controls. We also generated CSO-CARs targeting the neuroblastoma antigen GD2, and compared cytotoxicity of CSO-CAR γδT cells against γδTCR-engaging GD2+ neuroblastoma cells and GD2+ CT-26, which do not engage the γδTCR. The mechanism of cell activation was demonstrated using latex beads loaded with antibodies engaging either the γδTCR, the CSO-CAR or both, followed by flow cytometric analysis of cytokine production and cell exhaustion. The potential for secondary antigen-specific expansion of CSO-CAR γδT cells was determined using co-culture with irradiated tumour cell lines in the absence of IL-2.

Results

Our novel anti-CD33 CSO-CAR design expressed in γδT cells leads to enhanced killing of AML cell lines with no detectable cytotoxicity against monocytes or myeloid progenitors expressing equivalent levels of CD33. In a solid tumour model, engagement of the CSO-CAR and the γδTCR is required for anti-tumour cytotoxicity. Mechanistic evaluation confirmed that γδT cells expressing these constructs require stimulus of both the γδTCR and the CAR in order to mount a full activatory response. CSO-CAR transduced γδT cells also showed a less exhausted phenotype than those expressing classical second generation CARs and were capable of antigen specific secondary expansion.

Conclusion

γδT cells expressing CSO-CARs offer an opportunity to improve the safety of cellular immunotherapy and to broaden the available repertoire of targets by overcoming on-target off tumour toxicity.

Vaccination to improve the persistence of CD19CAR gene-modified T cells in pediatric acute lymphoblastic leukemia relapsing after SCT

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Introduction / Methods:

Patients with acute lymphoblastic leukaemia (ALL) relapsing after allogeneic stem cell transplantation (SCT) have a dismal prognosis. Whilst unprecedented responses have been seen with second generation CD19 chimeric antigen receptor (CAR) therapy, there is a 30% risk of severe cytokine release syndrome (CRS) resulting from supraphysiological T cell activation. We have investigated an alternative strategy using donor-derived Epstein Barr virus (EBV)-specific T cells (CTL) transduced with a first generation CD19CAR, in which viral reactivation/ vaccination drives physiological proliferation and persistence.

We conducted a multi-centre phase I/II clinical study in paediatric ALL relapsing after SCT. Donor-derived EBV-specific CTL were retrovirally-transduced with a 1st generation CD19CAR. Patients were treated either pre-emptively in the case of molecularly-detectable disease in the bone marrow within the 1st year post-SCT or prophylactically at day 60-70 post-2nd SCT. All patients received lymphodepletion. An initial cohort treated with CD19CAR CTL alone showed poor expansion/ persistence. After a planned interim analysis, the second cohort underwent vaccination with irradiated, donor-derived EBV+ LCLs as a novel strategy to enhance the expansion/persistence of CD19CAR CTL.

Results:

A total of 11 high risk patients (median age 9 yrs) were treated (4 pre-emptive, 7 prophylactic). One patient who had a transient response in cohort 1 was subsequently retreated with vaccination. The median transduction efficiency of CD19CAR CTL was 29% (12 - 59%) and viral copy number 0.37 (0.14 - 1.6). No CRS or graft versus host disease (GVHD) was observed. Eight patients had detectable disease on the day of CD19CAR CTL infusion. At 1 month post CD19CAR CTL infusion, 5 patients were in complete remission (CR), one had a partial response, 3 had stable disease and 3 had no response. However, at a median follow-up of 23 months, 10 of 11 patients have relapsed at 2 weeks-7 months post-infusion. All relapses were CD19+. Currently 3 patients are alive and disease-free, 1 alive with disease and 7 have died from disease progression. The expansion of CD19CAR CTL *in vivo* was poor. However, CD19CAR CTL were detectable by qPCR in a greater proportion of patients and their persistence was improved in the vaccination cohort. CD19CAR CTL had an effector memory phenotype with low expression of exhaustion markers and there was no evidence of immune rejection of CD19CAR CTL.

Conclusion:

Our data demonstrate the feasibility and safety of delivering CD19CAR T-cell therapy for relapse post-SCT in a multi-centre study and illustrate the potential for vaccination to improve CAR T-cell persistence. The limited expansion and efficacy of CD19CAR CTL may reflect the terminally differentiated phenotype of transduced EBV CTL or CAR design either due to absence of a co-stimulatory domain engagement of the IgG-derived Fc hinge domain.

Toward a targeted intravenous delivery platform for paediatric brain tumours by a harmless bacteria virus

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Current treatments for paediatric brain tumours have faced major challenges including toxicity due to lack of tumour selectivity and the blood-brain barrier (BBB). Development of a selective delivery system for paediatric brain tumours would play a major role in the treatment and management of these tumours. For instance, a number of therapeutic targets have been identified; however, their exploitation depends, essentially, on the development of non-invasive DNA delivery platforms that can knock these biological targets. Indeed, gene therapy is promising in this disease, and brain tumours were the first to be treated by clinical gene therapy but success has been limited by the inefficiency of vectors in expressing genes at therapeutic levels within the tumours, and by the BBB, requiring intracranial delivery. Animal viruses are most popular for use in gene delivery; however, they have had limited success systemically because of broad tropism for normal tissues and their targeting is challenging.

We have used bacteriophage (phage), bacteria virus, to develop tumour targeted systemic vectors. Phage is economic and has a historic safety profile as they have been administered to children and adults over many years to treat infectious diseases and approved by the US-FDA to be used as anti-bacterial food additives. Importantly, it has been reported that the filamentous M13 phage is able to traverse the BBB (Frenkel and Solomon, PNAS 2002, 99 5675-79). However, phage has evolved to infect bacteria only with no strategies to deliver genes to human cells. We reported a bacteriophage vector, as hybrid genome between two single-stranded DNA of human adeno-associated virus (AAV) and filamentous M13 phage, termed AAV phage or AAVP, in which gene expression is under the control of AAV genome. We and independent groups reported efficacy of selective intravenous cancer gene therapy in rodents, with the RGD4C-AAVP vector displaying RGD4C ligand to target the tumour specific $\alpha v\beta 3$ integrin receptor. A dog study by the National Cancer Institute-USA demonstrated that RGD4C-AAVP delivered the gene for tumour necrosis factor alpha, TNFα, selectively to naturally occurring cancers while sparing the healthy tissues and repeated therapy resulted in complete tumour eradication in a few dogs with aggressive cancers.

To validate our systemic delivery platform for brain tumours, we first confirmed that RGD4C-AAVP homes selectively to orthotopic intracranial human glioblastoma in mice after intravenous administration and crosses the BBB to target tumour cells, without accumulation in the healthy brain. Next, we expressed therapeutic genes from a temozolomide (TMZ)-induced tumour specific promoter of the glucose-regulated protein, Grp78, and showed enhanced RGD4C-AAVP-guided gene delivery by the BBB-penetrant TMZ and synergistic effect of combination of TMZ and targeted RGD4C-AAVP gene therapy. Then, we showed that the target receptor $\alpha v\beta 3$ is expressed on medulloblastoma and diffused intrinsic pontine glioma (DIPG) cells. Subsequently we proved that our RGD4C-AAVP vector can deliver genes to these cells in a targeted manner mediated by RGD4C binding to $\alpha v\beta 3$, resulting in a targeted medulloblastoma and DIPG tumour cell killing with vector carrying the gene for the membrane associated cytokine TNFα. We further constructed RGD4C-AAVP carrying shRNA candidates to hit therapeutic targets of mammalian target of rapamycin (mTOR) and neuropillin-1 (NRP-1) genes in medulloblastoma cell lines. We found that suppression of either mTOR or NRP-1 gene expression with vector treatment leads to significant inhibition of medulloblastoma cell proliferation and this inhibitory effect was even further enhanced by vector carrying both shRNA for mTOR and NRP-1.

Altogether our data provide evidence that bacteriophage is a promising delivery platform for use in targeted treatment with gene therapy, immunotherapy and personalised therapy for brain tumours in children.

Development of a novel CD19CAR with improved functionality

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No abstract available for IP issues.

Harnessing Cross Reactivity of Cytomegalovirus Reactive Gamma Delta T cells in Paediatric Neuroblastoma

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Background/Objectives

Neuroblastoma is the commonest cancer in infants. Survival in high risk groups is low at 40 - 50%. Newer treatments are needed to improve survival and morbidity. Cytomegalovirus (CMV) is a common viral infection which increases gamma delta (γδ) T cells. We investigated the use of CMV reactive γδ T cells as a potential new immunotherapy.

Methods

Peripheral blood mononuclear cells from 30 paediatric haemato-oncology patients with/without CMV infection were analysed by flow cytometry. γδ T cells were expanded, then co-cultured with CMV infected fibroblasts or neuroblastoma cells. Interferon gamma secretion was measured by ELISA, cytotoxicity by MTT assay and blocking assays identified receptors involved. γδ T cell receptors (TCR) were determined by sequencing.

Results

Paediatric haemato-oncology patients with acute CMV had higher proportions of Vδ1 and non-Vδ1/Vδ2. There was a statistical difference between the frequencies of Vδ1 and Vδ2 ($p = 0.0035$) and non-Vδ1/Vδ2 and Vδ2 ($p = 0.0013$). Vδ1 frequency was higher in CMV infected patients than negative patients but Vδ2 frequency was lower ($p = 0.0312$, $p = 0.0314$ respectively). γδ T cells were expanded to significant numbers for adoptive transfer. γδ T cells from patients with acute CMV infection had statistically significantly higher interferon gamma release in co-cultures with CMV infected fibroblasts and showed cytolytic activity against CMV infected fibroblasts and neuroblastoma cells which was mediated by the γδ TCR and NKG2D receptor. Sequencing showed the dominant chains in CMV infected patients were Vδ1 and Vγ2. Vδ1 CDR3 sequences had minimal diversity but the gamma chain had wide variations.

Conclusion

Acute CMV infection in paediatric haemato-oncology patients leads to an increase in Vδ1 Vγ2 subtype of γδ T cells. They can be expanded for adoptive transfer. They recognise and kill CMV infected targets and neuroblastoma cells via the γδ TCR and NKG2D receptor. CMV reactive γδ T cells are therefore a potential form of immunotherapy.

Generation of single chain antibodies targeting the ectodomain of Anaplastic Lymphoma Kinase (ALK) for childhood cancer immunotherapy

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Anaplastic Lymphoma Kinase (ALK) is an orphan receptor tyrosine kinase known to be aberrantly expressed or mutated in a range of childhood cancers including neuroblastoma, rhabdomyosarcoma and inflammatory myofibroblastic tumour; or to be activated by translocation in anaplastic large cell lymphoma or large cell lung cancer. Interestingly ALK expression is normally restricted to early development identifying it as an attractive potential target for immunotherapy. Thus far, antibodies targeting the ectodomain of ALK have not been developed into biotherapeutics.

To promote the isolation of antibody fragments capable of incorporation within the chimeric antigen receptor format we adopted a phage display approach for selection of single chain variable fragments. RNA isolated from spleens of mice immunised with soluble ectodomain of human ALK served as a template for generation of a single chain variable fragment library. Rounds of panning using recombinant ALK led to enrichment of ALK binding phage. Individual ScFv with specificity for ALK were cloned into ScFv-Fc format antibodies, which attain equivalent specificity and similar brightness of staining of ALK expressing cells as found in a positive control full antibody mAb13.

These ALK specific ScFv are now available for cloning into chimeric antigen receptor format to evaluate ALK specific targeting of neuroblastoma and other childhood cancers.

Development of GD2 and CD3 targeted bispecific T cell engaging antibodies for neuroblastoma immunotherapy

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Introduction

Neuroblastoma is the most common solid cancer in children after brain tumours and despite intensive combination therapies only 40% of patients with high-risk disease survive for five-years. Immunotherapy using bispecific antibodies (bsAb) has shown great promise in haematological malignancies. The T cell engaging form of the bsAb combines the specificity of two monoclonal antibodies in the form of single chain variable fragments (scFvs) to redirect a T cell to recognise and kill a tumour cell. The optimal bsAb format in terms of size, binding affinity, stability and flexibility is critical to achieve maximum efficacy. We have developed and tested a range of formats that have specificity for GD2, which is highly expressed on neuroblastoma, and for CD3, which is expressed on T cells.

Methods

A range of bsAbs with specificity for GD2 and CD3 were generated by retroviral transduction of 293T cells. BsAb proteins secreted in to the culture supernatant were purified by capture of the c-terminal his-tag onto Ni2+ sepharose. Two GD2 scFvs and three different CD3 scFvs were compared for binding affinity by measuring cellular affinity by flow cytometry and Scatchard analysis. To demonstrate bsAb function and compare potency between the different formats, enriched T cells from normal human peripheral blood mononuclear cells were co-cultured with GD2 positive or negative tumour cells at a 5:1 ratio with 0 - 1 µg/ml bsAb. After 16 hours co-culture, tumour cell viability was measured by flow cytometry using annexin and PI.

Results

After co-culture of GD2 expressing tumour cells with T cells in the presence of the various bsAbs, all tested bsAbs demonstrated highly antigen specific T cell activation, and complete elimination of GD2 expressing tumour cells. Work to be presented will demonstrate how affinity and stability biochemical data correlates with relative efficacy *in vitro* and *in vivo*.

Conclusions

We have demonstrated that the anti-GD2 bsAbs show remarkable *in vitro* activity and are a potential therapeutic for GD2 positive tumours including neuroblastoma.

Gene edited T cell therapy for leukaemia

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T-cells engineered using lentiviral vectors to express chimeric antigen receptors against CD19 (CAR19) can mediate sustained leukemic remission, but manufacturing autologous cell products is expensive, time consuming and often problematic in infants. Gene editing of non-HLA matched healthy-donor CAR19 T-cells could allow 'off-the-shelf' therapies to be developed that overcome HLA-barriers. Gene editing with Transcription activator-like effector nucleases (TALENs) can be used to simultaneously disrupt CD52 and T-cell receptor gene expression producing cells that resist conditioning antibodies and have reduced alloreactive potential. A bank of such cells was manufactured to express CAR19 and has been used to secure molecular remission in two infants with refractory B-ALL ahead of a second allogeneic transplantation procedures. Phase 1 trials will now investigate this approach as bridge therapy to secure successful outcomes after transplantation.

Optimizing CAR T-cell therapy for the treatment of neuroblastoma

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Neuroblastoma is the most common solid tumour in children and accounts for 7% of childhood malignancies, affecting mainly children of 5 years or younger. Low risk cases show a 5-year survival rate of 95% with observation only or surgery. Unfortunately, up to 40% of diagnosed neuroblastoma cases are classified as high risk and patients need to undergo intensive treatment including surgery, irradiation and chemotherapy. However, neuroblastoma cells can persist in the bone marrow following chemotherapy which is then associated with a low survival rate. Currently patients cannot be cured and new therapeutic strategies are urgently needed. A new approach for neuroblastoma treatment is to redirect patients' immune system to eradicate tumour cells utilising adoptive cell therapy (ACT). T-cells isolated from patients' blood are engineered to express specific anti-cancer receptors and transferred back into the patients. These chimeric antigen receptors (CARs) are artificial receptors comprising an antibody derived antigen binding domain fused to the T-cell receptor signalling domain CD3 zeta (CD3z). Incorporation of co-stimulatory signalling domains defines the 2nd and 3rd generation of CARs. Co-stimulation supports and enhances long-time survival and persistence of T-cells, which is crucial for the treatment of cancer patients. Clinical trials using 2nd generation CARs have shown promising results, e.g. for leukaemia treatment.

Neural cell adhesion molecule-1 (NCAM-1) is expressed on most neuroblastoma types and represents a possible target for neuroblastoma specific CAR T-cells. However, treating solid tumours harbours an increased toxicity profile due to off-site antigen expression on healthy tissue.

Our hypothesis is that two instead of one CAR will facilitate antigen specific T-cell activation and therefore T-cell mediated cytotoxicity will be controlled and reduced. The rational is to co-express a 1st generation CAR, providing the CD3z signal only, and a co-stimulatory CAR (co-CAR), delivering the co-stimulatory signal. Thus, full T-cell activation will depend on both receptors binding their corresponding targets. NCAM-1 specific co-CARs containing co-stimulatory domains of CD2, CD6, CD28, OX40 or 4-1BB have been generated. However, the impact of each co-stimulator on T-cell activation needed to be assessed beforehand. Consequently we tested each co-stimulatory domain first as a 2nd generation CAR to understand CAR T-cell activation dependencies.

For *in vitro* studies T-cells from healthy donors have been genetically engineered using retroviruses and analysed in co-culture studies with NCAM-1 positive neuroblastoma cell lines. CAR T-cell activation was assessed by measuring the production of interferon gamma (IFN-y) and interleukin-2 (IL-2), as well as tumour cell killing ability. The most promising candidates for co-stimulation are CD2 and CD28, both promoting a strong T-cell activation. The obtained results will now be translated into the co-CAR setting. Our objective is to prove that a 1st generation CAR expressed alongside a co-CAR work as well as the corresponding 2nd generation CAR but shows a reduced toxicity profile. Increasing the safety profile of CAR T-cell therapy to treat solid tumours will have a great impact for future applications.

O-acetylated GD2 as a target for neuroblastoma immunotherapy

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Neuroblastoma is the most common solid extra-cranial cancer in childhood. Despite vigorous combination therapy the majority of children with high risk neuroblastoma still die from their disease. One of the biggest recent advances in neuroblastoma treatment has been using therapeutic monoclonal antibodies (mAb) specific for the ganglioside GD2. GD2 is an antigen highly expressed on the cell surface of neuroblastoma cells with limited expression on selected healthy tissue.

Unfortunately for patients receiving GD2-targeted therapy, presence of this antigen on peripheral nerve cells can result in dose limiting toxicities, including fever and neuropathic pain. New strategies need to be developed to reduce on-target, off-tumour effects. Tumour cells which express GD2 also express their O-acetyl derivative, the ganglioside O-acetyl-GD2 (OAcGD2). It has been shown that OAcGD2 is expressed on tumours of neuroectodermal origin but absent from healthy neuronal tissue. We aim to utilize this highly selective expression pattern of OAcGD2 to deliver more potent, less toxic, targeted therapy for neuroblastoma.

It has been reported that CASD1 is key enzyme in the O-acetylation of GD2. Our reference cell lines include HAP-1 and HAP-1-CasD1 KO lines, which we have transduced with GD2/GD3 synthase to express GD2 and OAcGD2. As part of our target validation methods, we have used the mAb 8B6 to confirm and understand expression patterns of OAcGD2 on our large panel of primary neuroblastoma cells which have been established from fresh tumour tissue. We evaluate this expression using both flow cytometry and immunohistochemistry methods. We are currently performing comparative genomics in OAcGD2-expressing and non-expressing cell lines to gain insight to the regulation of this antigen expression. We also report a set of novel and diverse binders against GD2, some of which are OAcGD2-specific, which we have developed using hybridoma technology to use in novel immunotherapeutics.

Recent advances in immunotherapy detail the use of Chimeric Antigen Receptor (CAR) engineered T-cell based approaches in the treatment of childhood cancer. We propose that OAcGD2 provides a target with enhanced tumour specificity and is potentially a good candidate for mAb and CAR-based immunotherapies for neuroblastoma.

The evaluation of Tumour Infiltrating Lymphocyte Expansion and Characterization for Cell Therapy in Neuroblastoma

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Introduction

Tumour infiltrating lymphocytes (TILs) are found in most solid cancers, while their purpose and function remains mostly unknown. Adult melanoma is an exception and multiple groups showed expansion of these TILs from tumour tissue, to form large numbers of T cells. TIL expansion with the melanoma expansion protocol, has been generally unsuccessful in other solid cancers including paediatric cancers. Recent trials have shown efficacy of checkpoint blockade inhibitors in cancers which did not show any TIL expansion, suggesting there is a naturally occurring T cell response to the tumour. Other research demonstrated successful TIL expansion from renal cell carcinoma (RCC), by incorporating stimulation with CD3/CD28 Dynabeads into to the melanoma protocol. On the basis of these advances, our lab evaluated the possibility to expand TILs from paediatric neuroblastomas using an optimized renal cell carcinoma protocol.

Methods

Between September 2015 and March 2016, 6 neuroblastoma resections and 3 neuroblastoma biopsies were received. Tumour infiltrating lymphocytes (TILs) were expanded from tumour fragments, 2-3 mm in size. Tumour tissue was put in initial expansion for 14 days with high dose IL-2 and CD3/CD28 Dynabeads. After initial expansion, TILs were cultured for another 14 days in the presence of irradiated PMBCs, anti-CD3 antibody and high dose IL-2. Phenotype analysis was done on T cell subtypes and memory phenotype. TILs were co-cultured with tumour fragments of autologous tumour to evaluate tumour reactivity. IFNγ ELISA on supernatant of these co-cultures, was used as a read out.

Results

TILs were expanded from 9 neuroblastoma patients, with very high numbers obtained in 6 out of 9 cases. The TIL population showed high numbers of NKT cells as described previously, but also showed unexpectedly high proportion of $\gamma\delta$ T cells expressing the uncommon Vδ1 and Vδ3-8 chains. Conventional T lymphocytes were a mixture of CD4 and CD8 cells. Co-culture with autologous targets failed to elicit immediate interferon gamma release although cells were capable of producing IFNγ with PMA/Ionomycin stimulation

Conclusion

This study has shown successful and reproducible *ex vivo* expansion of tumour infiltrating lymphocytes in neuroblastoma patients. Future studies need to evaluate tumour reactivity in more depth to validate applicability for therapeutic use.