Final Progress Report form

<table>
<thead>
<tr>
<th>Grant reference number:</th>
<th>MESOUK18-2</th>
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<tbody>
<tr>
<td>Scientific project title:</td>
<td>Immunotherapy for malignant mesothelioma using IL-6-neutralising CAR T-cells.</td>
</tr>
<tr>
<td>Lay project title:</td>
<td>Improving the power and safety of white blood cells that have been taught to destroy mesothelioma.</td>
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<tr>
<td>Grant holder(s):</td>
<td>Dr John Maher</td>
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<tr>
<td>Researcher(s):</td>
<td>Mr Daniel Larcombe-Young</td>
</tr>
<tr>
<td>Host institution:</td>
<td>King’s College London</td>
</tr>
<tr>
<td>Project start date:</td>
<td>February 2019</td>
</tr>
<tr>
<td>Project end date:</td>
<td>June 2021</td>
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</tbody>
</table>

Host institution’s press or publicity office details

| Name: | Victoria Fenton |
| Email: | Victoria.fenton@kcl.ac.uk |
| Address: | |
| Telephone: | |

Guidelines for completion:

- Supporting information (e.g. manuscripts, tables, charts, images) should be attached at the end of this form.
- Lay sections must be written in language that can be easily understood by a non-scientific audience. This is particularly important as lay sections may be:
  a. Placed on the AUK-BLF website to inform members of the public/supporters/potential donors about the research we fund and its achievements
  b. Used by lay members of the AUK-BLF Research Review Panel or Board of Trustees to help assess the outcomes of the grant

Please provide a glossary where scientific terms cannot be avoided. AUK-BLF reserves the right to request a redraft of lay sections if they are not deemed acceptable.
(Tip: It may be helpful for an individual with no scientific/research background to review your lay sections prior to submitting this form).
- Please contact AUK-BLF’s Research and Innovation team if you require further guidance on completing this form.
Section A – Project Information

This information will be used by AUK-BLF to:

- Evaluate the progress of your research in relation to your original proposal
- Understand the conclusions arising from the final outcomes of your research
- Communicate your findings and successes to AUK-BLF supporters and potential donors

1. What lung disease(s) is/are relevant to the research?
   Malignant Mesothelioma

2. In lay terms, what problem/need does this research aim to address? (max. 200 words).
   Malignant pleural mesothelioma (MPM) remains an incurable disease, with increasing global incidence. Immunotherapy is emerging as a potent new modality for the treatment of otherwise incurable malignant disease. One ground-breaking approach involves genetic manipulation of patient T-cells to express a Chimeric Antigen Receptor (CAR).

Scientific abstract (from the original research proposal)

Malignant pleural mesothelioma (MPM) remains an incurable disease, with increasing global incidence. Immunotherapy is emerging as a potent new modality for the treatment of otherwise refractory malignant disease. One ground-breaking approach involves genetic manipulation of patient T-cells to express a Chimeric Antigen Receptor (CAR). CARs are fusion molecules in which a tumour specific binding domain is linked to a bespoke T-cell activating endodomain. Recent clinical trials have demonstrated unprecedented efficacy of CAR T-cell immunotherapy in B-cell and plasma cell malignancy, leading to US licensing of two therapeutic products in 2017. However, efficacy against solid tumours such as mesothelioma remains elusive. Furthermore, this therapy has high toxic potential, notably due to the occurrence of cytokine release syndrome (CRS).

We have developed a CAR T-cell approach for solid tumours, named T4 immunotherapy, which comprises a panErbB-specific CAR (T28ζ) that is co-expressed with a chimeric cytokine receptor (4ab). T28ζ, engages 8 of 9 possible ErbB homo- and heterodimers, several of which are over-expressed in mesothelioma. In 4ab, the ectodomain of interleukin 4 receptor (IL-4R) a has been fused to the signalling domain of IL-2Rb. Addition of IL-4 to T4+ CAR T-cells causes their exponential expansion and selective enrichment. This enables the manufacture of therapeutic CAR T-cell products from a blood draw in 2 weeks, obviating the need for leukapheresis. Furthermore, use of 4ab favours the selective persistence of the CAR T-cells in IL-4-producing tumours, such as mesothelioma.

We have demonstrated anti-tumour activity of T4 immunotherapy in several preclinical solid tumour models. Growth of established mesothelioma xenografts was significantly delayed and sometimes eradicated following intra-cavitary administration of T4+ T-cells. Furthermore, an ongoing Phase I clinical trial of intratumoural T4 immunotherapy for squamous cell carcinoma of head and neck (SCCHN) has achieved a disease control rate of 70% (9/13 patients), in the absence of dose-limiting toxicities. A key factor in reducing toxicity is the use of local T-cell delivery, either directly into a tumour (SCCHN) or into a cavity within which tumour growth occurs (mesothelioma). In this setting, we have not observed systemic leakage/absorption of CAR T-cells to sites where on-target/off-tumour toxicity could occur. However, we have shown that if large numbers of CAR T-cells encounter high tumour burden within a body cavity, IL-6-dependent CRS results because of absorption of cytokine into the circulation.

The primary cause of IL-6-mediated toxicity is a process known as trans-signalling. This results from binding of IL-6 to soluble (s)IL-6Ra, forming a complex that can then binds to the ubiquitously expressed membrane (m) receptor, mgp130. This mode of IL-6 signalling promotes vascular leakage and contrasts with ‘classic’
signalling, where IL-6 binds to cells that co-express membrane-bound (m)IL-6Ra and mgp130. The most effective treatment for CRS is Tocilizumab, a monoclonal antibody that binds both sIL-6Ra and mIL-6Ra, thereby inhibiting both classic and trans-signalling by IL-6. However, it would be preferable to prevent CRS in the first instance, by selective inhibition of IL-6 trans-signalling alone. To address this, we have engineered T4+ CAR T-cells to co-express a soluble isoform of gp130 (sgp130), termed gp130_RAPS (rheumatoid arthritis antigenic peptide-bearing soluble form). At 50kD, gp130_RAPS is the smallest isoform of gp130 and is a potent and selective antagonist of IL-6 trans-signalling. Intriguingly, mesothelioma provides a secondary rationale for therapeutic antagonism of IL-6 trans-signalling. IL-6 is markedly elevated in the tumour microenvironment where it exerts pleiotropic actions, several of which are protumourigenic. These include chemoresistance, enhanced angiogenesis, autocrine tumour cell growth, induction of immunosuppressive myeloid derived suppressor cells and impaired dendritic cell function. Most of these malevolent effects of IL-6 are mediated by trans-signalling. IL-6 does exert some stimulatory effects on T-cells, but these appear to be mediated by classic signalling, which is not inhibited by sgp130 isoforms.

3. Lay abstract (from the original research proposal)

More effective treatments are required for patients with mesothelioma. One approach involves teaching a particular type of white blood cell, called a T-cell, to recognise and destroy tumour cells. This is achieved by equipping the T-cells with a radar-like system, called a ‘CAR’ that can detect specific ‘flags’ produced by tumour cells. In blood cancer, CAR T-cells have achieved dramatic responses (90% remission) in otherwise untreatable patients. However, the treatment can cause severe flu-like side effects (CRS) because a protein known as IL-6 is over-produced when the CAR T-cells engage the cancer. Sometimes such reactions can be fatal.

IL-6 also plays a role in favouring mesothelioma progression, by increasing tumour growth and creating an environment that ‘switches off’ the CAR T-cells so that they cannot attack the cancer. Consequently, we propose to engineer CAR T-cells that can target mesothelioma cells whilst simultaneously neutralising IL-6. We predict that this approach will preserve anti-tumour activity in mesothelioma, whilst reducing side effects.

In this project, we will use a CAR named ‘T4’ that we previously engineered, which is currently under evaluation in a clinical trial in head and neck cancer patients. Importantly, mesothelioma tumours also produce the flags that are recognised by T4. Alongside T4, we will also engineer the T-cells to release a protein that blocks IL-6. T4 immunotherapy detects a series of flags that are commonly produced by many cancers, including mesothelioma. This makes it very difficult for the cancer to ‘hide’, since many different flags would need to be shut down by the tumour simultaneously. However, T4 immunotherapy also has potential to cause side effects since these flags are produced at low levels in normal tissues. Therefore, we need to progress cautiously in developing this treatment. When injected directly into tumours, T4 immunotherapy has never caused side effects in mice. Based on this finding, we obtained approval to test this treatment in patients with advanced head and neck cancer. Sixteen patients have now been safely treated with increasing doses, achieving disease control in 10 cases. This experience provides an important stepping-stone to testing of a related strategy in mesothelioma, since a similar set of flags are produced by these cancers. However, injection of high doses of T4 immunotherapy into body cavities (such as the chest or abdominal space in which mesothelioma occurs) has induced side effects in mice. This side effect resembles a severe form of flu and may require intensive care treatment.

Our research will build on our previous work demonstrating that T4 immunotherapy can shrink mesothelioma tumours in mice. Whilst a higher T4 dose would likely provide greater benefit, the risks of side effects are also increased. Clinical trials in blood cancer patients have implicated IL-6 in causing these severe flu-like side effects. To overcome this, we propose to engineer T-cells that express both T4 and a protein that neutralises IL-6. We believe that this will provide a potent, yet safer, treatment for mesothelioma.
4. **Scientific summary of your progress and achievements to date and your conclusions arising from the final outcomes of your research. Please state whether the overall objectives as set out in the original application have been achieved (max. 500 words).**

The malignant pleural mesothelioma (MPM) cell lines REN, Ju77, Lo68, H28, H226 and H2052 were characterised for the expression of membrane bound gp130 (mgp130) and IL-6Ra (flow cytometry), soluble IL-6Ra and IL-6 (ELISA). All cell lines expressed high levels of mgp130, with little or no expression of mIL-6Ra. The Panel of 6 cell lines produce varying amounts of IL-6, with REN and H2052 producing the highest levels. sIL-6Ra was only detected in the supernatant of H2052 and Lo68. Increased proliferation of H226 and H2052 was observed in response to the addition exogenous IL-6 and sIL-6Ra at 10ng/ml over 7 days, suggesting these cells lines are responsive to IL-6 trans signalling.

T-cells from healthy donors were stably transduced using the SFG retrovirus to express T4, RT4 (co-expresses the RAPs antagonist of IL-6 trans-signalling) or a CAR truncated control (RT1N4 – also co-expresses RAPs). MTT assays were used to demonstrate that in cocultures of CAR T-cells with the panel of 6 mesothelioma cells lines, RT4 can kill and re-stimulate over multiple cycles with comparable efficacy to T4. No increased efficacy was seen between RT4 and T4, likely due to the modest levels of sIL-6Ra produced by the tumour cell lines in culture. T-cell activation was confirmed by measuring T-cell proliferation and IL-2 and IFNγ release.

Co-cultivation of CAR T-cells with MPM cell lines was repeated in the presence of exogenous IL-6 or IL-6/sIL-6Ra to determine the effect of IL-6 trans-signaling upon anti-tumour activity and whether this is modulated by release of gp130_RAPS by RT4 CAR T-cells. No decrease in tumour cell proliferation was seen between T4 and RT4.

An in vivo experiment using SCID beige mice with an intraperitoneal (i.p.) injection of tumour cells and i.p. administration of CAR+ RT4, T4, RT1N4 or PBS was performed to determine if RT4 exhibits greater safety and efficacy than T4. Intrapertioneal administration of T4 immunotherapy can induce CRS in SCID Beige mice due to macrophage activation causing high levels of IL-6 release. Human sgp130_RAPS crosses the species barrier should be capable of neutralising IL-6 mediated trans signalling and reducing toxicity. Tumours cells were given an extended engraftment period prior to the administration of CAR T-cells to increase CRS intensity. A clear reduction in weight loss was seen in the 7 days post CAR T-cell administration in the RT4 group when compared to T4. However, although there was strong efficacy for both RT4 and T4 groups, no increased efficacy was seen for RT4.

To assess the effect of blockade of IL-6 trans-signaling on the tumour microenvironment, an immunocompetent model or RT4 immunotherapy using Balb/C mice was created. Mouse T cells isolated from the spleens of Balb/C mice were transduced to express a murine version of T4. Unlike their human equivalents, mouse T cells do not survive for long in culture and are notoriously difficult to work with. Because of the reduced culture period, the 4ab enrichment system was not practicable.

Ectropic packaging cell were used to produce retrovirus capable of transducing mouse T-cells to express mT1E29z_mRAPS or mT1E298z (the murine versions of RT4 and T4, minus 4ab). However, fully murine versions of these constructs have been challenging to develop. The transduction of mouse CAR T cells proved difficult to achieve and caused several delays in the project. Once this system was optimised, it was demonstrated that mouse CAR T-cells were able to kill the murine mesothelioma cell line AB22 both in culture and in vivo. AB22 tumour cells were transduced to express firefly luciferase so that they could be detected using bioluminescent imaging. However, when injected into immunocompetent Balb/C mice, they were rejected rapidly because of the immunogenicity of the luciferase tag. To overcome this, mice were lymphodepleted using two doses of 100mg/kg cyclophosphamide prior to tumour cell injections, which resulted in 100% engraftment rate. With the immunocompetent model of mesothelioma set up, several in vivo experiments were performed to assess whether mT1E28z_RAPS was able to out compete mT1E28z alone with regards to tumour eradication and reduction in CRS. These experiments showed mT1E28z_RAPS T cells were able to control tumour growth, and there were signs that they could also reduce acute weight loss. In one experiment, two mice from the control mT1E28z group were culled due to unacceptable toxicity, whereas none in the mT1E28z_RAPS group experienced similar toxicity. This could indicate that inhibition IL-6 trans-signaling is reducing CRS.
In addition to the main project, to advance more sophisticated models of mesothelioma, the post holder developed six patient derived xenograft (PD) models using patient tumour material from surgeries performed at Guy's Hospital, London. Tumour specimens received from the surgeon were dissected into 2mm fragments and freshly implanted into the flank of immunocompromised mice immediately after surgery. Once tumours from the first cohort of mice grew to 10x10mm, they were harvested and both reimplemented into a second cohort, and viably frozen for cryogenic storage for future implantation. After three passages, experiments using the PDX model can be performed in larger cohorts of mice. Unlike traditional mesothelioma cell lines, PDX models retain heterogeneous cell populations and are a more clinically relevant model of mesothelioma. During the post holders’ two-year tenure, he was able to establish six stable PDX models of mesothelioma which can be used for future experiments at King’s. Very few labs globally have established this kind of model using mesothelioma tumour material, making it a valuable resource for mesothelioma research.

6. Lay summary of your progress and achievements to date and your conclusions arising from the final outcomes of your research. Please state whether the overall objectives as set out in the original application have been achieved (max. 500 words).

We have shown that mesothelioma cancer cells produce IL-6 and related proteins which increase cancer growth and survival. When additional IL-6 and its related proteins were added to the cancer cells, they responded by dividing more rapidly than those left untreated.

Human T-cells from healthy donors were transformed into T4 CAR T-cells which target and kill mesothelioma cancer cells. In addition to this, a protein which neutralises IL-6 was added to the T4 T-cells (creating RT4). We demonstrated that RT4 CAR T-cells can kill a range of mesothelioma cells in a laboratory setting.

To test whether these RT4 T-cells can be used to treat solid mesothelioma tumours in a setting more comparable to the clinic, a mouse experiment was designed to test the safety and power of the therapy. RT4 CAR T-cells were able to efficiently reduce tumour size in mice and showed signs of reducing toxicity when compared to T-cells that do not neutralise IL-6.

To prevent the human T-cells and tumour cells being rejected by the mice, it is necessary to use mice which do not have an immune system. However, this may cause complex interactions CAR T-cells have with a fully functioning immune system to be overlooked. Therefore, developed a system of making mouse versions of the CAR T cells tested them in mice with full immune systems. This allowed us to look at some crucial interactions between the immune system and RT4 CAR T-cells which may reduce the toxicity sometimes seen in this kind of therapy.

The data produced from this project support rationale for using these CAR T cells to treat patients with mesothelioma in a clinical trial.

7. In lay terms, how do your results contribute to or change the diagnosis, prevention, treatment and/or outcome of lung disease? What are the likely short- or long-term benefits of your findings to patients with a lung condition? Are there any impacts on quality of life? (max. 500 words).

In the short term, this project has helped understand the importance of IL-6 signaling in mesothelioma. As a longer-term benefit, a great deal of data has been generated to support the argument for using RT4 immunotherapy to treat patients with mesothelioma in a clinical trial (currently in a phase I clinical trial to treat head and neck cancer).

8. In lay terms, please summarise the outcomes of your work in 3 bullet pointed ‘take home’ messages for our supporters and donors. This should focus on why your work has been beneficial to people with lung disease.
• Mesothelioma is a disease with a very poor prognosis. Targeted CAR T cell therapy could be a viable therapeutic intervention for this type of cancer.
• Demonstrating how IL-6 plays a role in the progression of mesothelioma could lead to a better understanding of the development of this disease.
• Armouring T4 CAR T cells with IL-6 inhibiting capabilities could improve the effectiveness of these T cells when targeting mesothelioma.

9. In lay terms, please indicate any new directions for further research suggested by this work. Does the knowledge gained from this project lead to any further research opportunities for yourself or for the scientific community? (max. 200 words).

IL-6 signalling plays a role both tumour development and immune responses against cancer. Armouring CAR T cells can be an effective way of increasing the potency of the therapy, this project showed that by enabling the T cells to inhibit IL-6 trans-signaling, increased efficacy can be achieved.

10. In lay terms, have you encountered any problems or difficulties which have impacted on the progress of your project (e.g. logistical, financial, scientific issues)? Yes/No

If yes, please provide details.

There were some initial difficulties encountered when attempting to transduce mouse T-cells to express the CAR. Our protocols were altered to reduce the time between harvest of T-cells, activation, and transduction, which has resolved the issue. Transient virus is now produced rather than using stable packaging cell lines to produce the retrovirus used for gene transfer.

11. In lay terms, have there been any deviations from the original research plan? AUK-BLF appreciates the nature of research and that deviations may be necessary. This section is intended to enable the Fundraising Team to explain these changes.

No

Section B – Publications

In the table below:
• List any publications resulting from this project to date, including any that are currently in preparation, submitted or in press. Please attach electronic copies of draft or published manuscripts.
• List any high-profile presentations resulting from this project to date (e.g. invited keynote speeches or oral presentations at national or international conferences), including any that are currently submitted. If possible, please send a copy of the abstracts with this report.

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<tr>
<th>Publication type (original research article/review/published abstract/book chapter/oral pres./poster pres.)</th>
<th>Title</th>
<th>Authors</th>
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<td>Details (For journal pubs, include the journal name, volume, page and date of pub).</td>
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Section C – Public engagement

In accordance with the AUK-BLF Terms and Conditions, grant holders are expected to be involved in a number of public engagement activities throughout their projects (at least one lay article to an AUK-BLF publication and give at least one lay presentation on behalf of AUK-BLF each year).

1. Please provide details of public engagement activities undertaken in the previous year and plans for further activities in the next year.

AUK-BLF is a charity funded through voluntary donations and is always in need of support. If you are unsure of ways that you can get involved and engaged with the public, please contact the Research and Innovation team.

We were scheduled to host a lab visit which was unfortunately cancelled due to social distancing restrictions.

Section D – Outputs and outcomes

We are keen to record all the different outcomes that result from AUK-BLF funded work to help us assess the impact of our funded research. Please answer the following questions for us to evaluate our portfolio of work and inform our research strategy.

1. Have you applied for any further funding (either from AUK-BLF or from another funder) as a result of this work?

   Not as yet

   If yes, please provide the following details:
   a. The funding organisation
   b. The type of award
   c. The value of the award
   d. The outcome (success/unsuccessful)
2. Have any important **collaborations** resulted from this AUK-BLF funded project?

   Yes

   If yes, please provide the following details:
   a. The type of collaboration and what is involved
   b. The name of the individuals/group/company you are collaborating with

   We continue to collaborate with Mr Andrea Bille in the development of mesothelioma PDX models.

3. Has the grant holder or researcher received a **promotion** as a result of this AUK-BLF funded project?

   Yes

   If yes, please provide the following details:
   a. New title/role
   b. When the promotion is effective from
   c. Reason for promotion

   The researcher was granted funding to start his PhD which commenced at the end of this project (June 2021).

4. Has the grant holder or researcher received an **award** or other form of recognition (e.g. research prize/medal/appointed to editorial board/funding committee) as a result of this AUK-BLF funded research?

   No

   If yes, please provide the following details:
   a. Title of award or recognition scheme
   b. Level of award (e.g. regional, national, international)
   c. When the award was made
   d. Reason for award

5. Has there been any **impact on clinical practice, policy or education** resulting from this AUK-BLF funded grant?

   No

   If yes, please provide details, for example:
   a. Citation in clinical guidelines (give title and organisation issuing guideline)
   b. Citation in clinical reviews; for example, those published in the *BMJ/Lancet*
   c. Citation in other policy documents at any level
   d. Citation in systematic reviews e.g. Cochrane
   e. Membership of a guideline committee
   f. Invited to take part in any national consultations e.g. led by the Department for Health
   g. Involvement/participation in an advisory committee with a national remit
Section E – Intellectual Property

Information provided in this section will assist AUK-BLF to identifying any possible intellectual property that may arise from this research and that may be exploitable ultimately for the benefit of patients with lung conditions.

1. Outline any collaborations or associations with commercial partners that have occurred or are planned as a result of this AUK-BLF funded research.
   
   N/A

2. List any active patents and details of prospective filings under review that have arisen in whole or in part from this AUK-BLF funded research.
   
   N/A

3. Are you currently, or have you previously been in contact with your host institute’s technology transfer office in relation to a commercial opportunity arising from this AUK-BLF funded research?
   
   **No**
   
   If yes, please provide details.

4. Briefly outline any potential translational and/or commercial application which you believe may have arisen or may arise from this AUK-BLF funded research.
   
   Data generated from this project could potentially be used to support a Phase I/II clinical trial using T4 immunotherapy in mesothelioma.

5. Has this AUK-BLF funded research led to the generation of any research reagents, e.g. antibodies, cell lines, transgenic mouse models, which could be of use to other researchers and potentially of commercial value?
   
   **Yes**
   
   If yes, please provide details.
   
   PDX models of mesothelioma have been developed as indicated above.
6. Is there any information included in this report that could compromise your intellectual property or confidentiality if used externally?

Yes

If yes, please provide details.

We are considering whether a patent filing may be appropriate to protect the RAPS technology.

Section F - Declaration

- All information given in this report is correct
- AUK-BLF will continue to be acknowledged and kept informed about future publications and developments arising from this work
- This report may be distributed to AUK-BLF’s Research Review Panel
- This report may be distributed to AUK-BLF’s intellectual property representatives
- This report, or a summary of it, may be distributed to the individuals, trusts or companies that have supported this grant
- This report, or a summary of it, may be used to raise funds that will support more future research in lung conditions, in marketing campaigns and communications, and on the research pages of the AUK-BLF website

I agree to the above conditions and confirm that the content of this report has been approved by the grant holder.

<table>
<thead>
<tr>
<th>Name: John Maher</th>
<th>Signature: John Maher</th>
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<tbody>
<tr>
<td>Position: Consultant and Senior Lecturer in Immunology</td>
<td>Date: 29/07/2021</td>
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Section G - Attachments

Please attach any relevant information in this section (e.g. manuscripts, tables, charts, images).

Figure 1 Analysis of human mesothelioma susceptibility to IL-6 trans-signaling. Analysis of expression of mgp130 (blue histogram) on a panel of six mesothelioma cell lines. Cells were probed using an anti-human APC conjugated antibody, with THP-1 as a negative control. The level of staining was compared to isotype controls (red histogram) (A). Analysis of expression of human membrane bound IL-6Ra (mIL-6Ra) (blue histogram) on a panel of six mesothelioma cell lines. Cells were probed using an anti-human IL-6Ra APC conjugated antibody, with THP-1 as a positive control. The level of staining was compared to isotype controls (red histogram) (B). H226 and H2052 human mesothelioma cells lines were cultured in the presence or absence of 10ng/ml sIL-6Ra and 10ng/ml IL-6 alone or in combination. 1x10^5 cells were plated in triplicate for each condition in a 24 well plate for three days. MTT assays were performed to determine cell proliferation compared to tumour alone wells (C).
Figure 2 Assessing the efficacy of CAR T cells transduced to express T4, RT4, RT1NA or non-transduced (UT) against mesothelioma cell lines. Dose response cocultures performed using a panel of six mesothelioma cell lines. 1x10^4 tumour cells were cultured with either T4, RT4, RT1NA CAR+ T cells, or UT T cells, at a decreasing effector to target ratio for 72h. Cytotoxicity was measured using an MTT assay and tumour cell viability was plotted as a percentage of tumour alone. Supernatant was analysed for IL-6 and IFNγ using ELISAs (A and B). 1x10^5 H2052 mesothelioma cells were cultured in triplicate in the presence and absence of 10ng/ml IL-6/sIL-6Ra for 72 hours (C). Dose responses were repeated using T4, RT4 or RT1NA CAR+ T cells, or UT T cells, on 1x10^4 H2052 mesothelioma cells in the presence and absence of 10ng/ml IL-6/sIL-6Ra for 72 hours (D).

Figure 3 Efficacy of CAR T cells in mice with SKOV-3 intraperitoneal tumours. Mice were inoculated with 1x10^6 SKOV-3_Luc tumours injected into the peritoneal cavity. Serial bioluminescent imaging was performed to measure tumour burden. On day 28, mice were injected i.p. with either 10x10^6 RT4 (n=5), 10x10^6 T4 (n=5), 10x10^6 RT1NA (n=5), 20x10^6 RT4 (n=3), 20x10^6 T4 (n=3), or PBS (n=5) (A and B). Body weight change from day of T cell injection is presented as percentage change from baseline (C).
Figure 4 Transduction and efficacy of mouse CAR T cells expressing mT1E28z or mT1E28z_RAPS. Murine T cells were extracted from spleens of Balb/C mice and isolated using a negative selection bead system. The T cells were activated using anti mCD3/mCD28 dynabeads and transduced 24 hours later using transient retro virus produced using ecotropic Phoenix ECO cells. Transduction efficiency of these T cells was determined using flow cytometry after staining with anti-mouse CD3 APC conjugated antibody and biotinylated anti-mouse EGF primary and streptavidin PE secondary (A and B). Dose response cocultures were performed the mouse mesothelioma cell line AB22 and human cell lines H226 and H2052. 1x10^4 tumour cells were cultured with either mT1E28z or mT1E28z_RAPS CAR+ T cells, or UT T cells, at a decreasing effector to target ratio for 72h. Cytotoxicity was measured using an MTT assay and tumour cell viability was plotted as a percentage of tumour alone (C). Schematic illustrating in vivo engraftment strategy for AB22_Luc mouse tumour cells in Balb/C mice (D). 4x10^6 AB22_Luc (AB22_LT) tumour cells were injected i.p. into Balb/C mice. Three doses of 100mg/kg cyclophosphamide were administered on day -2, 3 and 8 to improve engraftment of the immunogenic tumour cells. Serial bioluminescent imaging was performed to measure tumour burden (E and F).
Figure 5 Efficacy of murine CAR T cells in an immunocompetent Balb/c mouse model. Twenty Balb/C mice were pre-treated with 100mg/kg cyclophosphamide i.p. on day -2 prior to a 4x10^6 AB22 LT tumour cell inoculation on day 0. A second 100mg/kg cyclophosphamide dose was administered i.p. on day 5. 5x10^6 CAR+ mT1E28z (n=6), mT1E28z_RAPS (n=4) or UT (n=5) T cells or PBS were injected i.p. on day 6. Serial bioluminescent imaging was performed to measure tumour burden (A and C). Body weight change from day of T cell injection is presented as percentage change from baseline (B).