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Simultaneously Targeted to Tumor and Tumor-Associated Macrophages

PRINCIPAL INVESTIGATOR: Dr. John Maher

CONTRACTING ORGANIZATION: King's College London
London WC2R 2LS

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14. ABSTRACT

Adoptive T-cell immunotherapy represents an exciting advance, pioneered for hematologic malignancies and metastatic melanoma. To translate this technology to epithelial ovarian carcinoma (EOC), chimeric antigen receptors (CAR) may be used to re-target T-cell specificity against native tumor antigens.

The hypothesis underlying this synergistic partnership award is that CAR-based immunotherapy of EOC will be more effective if simultaneous targeting of tumor cells and tumor-associated macrophages (TAM) is achieved.

Initially, we set out to test this hypothesis using T-cells that express CARs with specificity for MUC1 (expressed by tumor cells) and CSF-1R (expressed both by tumor cells and TAM). *In-vitro* experiments demonstrated some efficacy of this therapeutic strategy. We then used a previously established (EOC-like) tumor model based upon MDA-MB-435 cells, engineered to co-express MUC1 and CSF-1 allowing us test therapeutic efficacy *in-vivo*. However, no significant therapeutic activity was observed in this model, prompting us to propose a revised statement of work which has been approved.

As per revised statement of work, we embarked upon a parallel approach in which tumor-associated ErbB receptors were targeted. To achieve this, a CAR named T1E28z was constructed. T1E28z targets T-cells against several ErbB dimer species that are upregulated in EOC. Liposomal clodronate was used to achieve depletion of TAM. To test efficacy, we developed *in-vitro* culture models using patient-derived tumor material. We have shown effective tumor cell killing by autologous T1E28z-transduced T-cells of both patient-derived tumor and EOC cell lines (IGROV-1 and SKOV-3). Next, xenograft EOC models were established using SKOV-3 and IGROV-1 tumor cell lines. Using bioluminescence imaging, we have also shown efficacy of T1E28z⁺ T-cells in controlling established SKOV-3 tumors in SCID Beige mice. Highly efficient depletion of TAM has been achieved using liposomal clodronate but did not influence anti-tumor activity in this model.

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Adoptive Immunotherapy for Epithelial Ovarian Cancer Using T-cells Simultaneously Targeted to Tumor and Tumor-Associated Macrophages

1. Introduction Ovarian cancer remains the most lethal of the gynecologic malignancies, largely owing to its propensity for clinical silence, late presentation and progressive evolution of chemoresistance. In 2009, an estimated 14,600 American women died of this disease, most commonly of the Epithelial Ovarian Carcinoma (EOC) subtype.¹ Despite recent advances in management, it is both striking and depressing that this number exceeds 70% of predicted disease incidence in the same year.¹ Consequently, the need for innovative therapeutic approaches for this devastating tumor is clearly evident.

The objective of the proposed research is to genetically engineer human T-lymphocytes using CAR technology, thereby enabling them to kill epithelial ovarian carcinoma in a safe and effective manner. To achieve this, we have set out to engineer and co-express two distinct CAR-grafted T-cell populations directed against targets expressed by tumor cells (MUC1^{2,3} or ErbB receptors⁴) and associated macrophages (colony-stimulating factor-1 receptor; CSF-1R) or using clodronate⁵ to target TAMs. We hypothesized that these approaches would interact synergistically to achieve enhanced anti-tumor immune effector function.

2. Body

Below, we have presented our research activity over the second 12 months of our project (June 15th 2010 to June 14th 2011) against the agreed Statement of Work.

Task 1 – Finalize Ethical Approval to obtain blood and ascites from patients with EOC, enabling experimental work to be conducted in both research facilities (SGM).

Target	Deliver by initiation of funding
Progress	Achieved in previous report

Task 2 – Obtain a Project License from the United Kingdom Home Office to provide legal authority to conduct controlled procedures on mice, as specified in the accompanying proposal (SGM).

Target	Deliver by 6 months
Progress	Achieved in previous report

Task 3 - Introduce Chimeric Antigen Receptors (CAR) to T-cells derived from patients with EOC (JM).

Peripheral blood mononuclear cells (PBMC) will be activated using CD3+CD28 expander beads. Four CAR will be delivered to separate T-cell populations using the SFG retroviral vector and retronectin-coated tissue culture dishes: (i) HOX – targets MUC1 and contains a fused CD28+OX40+CD3 ζ endodomain;^{2,3} (ii) CSF28z – targets CSF-1R and contains a fused CD28+CD3 ζ endodomain; (iii) HDFTr - targets MUC1 and contains a truncated (inactive) endodomain;^{2,3} (iv) CSFTr targets CSF-1R and contains a truncated (inactive) endodomain. To perform these studies, samples (50ml blood) from a total of 25 patients with EOC will be obtained with informed consent over the duration of the study (3 years).

Target	Deliver first gene transfer experiment within 8 months
Progress	Achieved

Progress since last report:

1. We previously reported that all matched truncated CARs have been constructed, cloned in the SFG onco-retroviral expression vector and used to generate stable PG13 retroviral packaging cell lines.^{2-4,6} We have also constructed a CAR named T1E28z that targets T-cells against ErbB heterodimers. The T1E28z CAR was fully described in the previous annual report submitted. In brief, targeting by this CAR is mediated by a chimeric peptide named T1E which is a fusion of the N-terminus of transforming growth factor- α coupled to the C-terminus of epidermal growth factor.^{7,8} The T1E peptide is a promiscuous ErbB ligand and engages ErbB1 homodimers, all ErbB1 heterodimers and the ErbB2/3 heterodimer. This binding specificity is retained by the T1E28z chimeric antigen receptor.⁹
2. Transgenes from all the mentioned constructs have been successfully delivered to peripheral blood T-cells derived from patients with epithelial ovarian cancer. Patient derived T-cells were activated with CSD3+CD28-coated beads and transduced with retroviral expression vectors. A representative example for one such patient is shown in for **Fig. 1**.

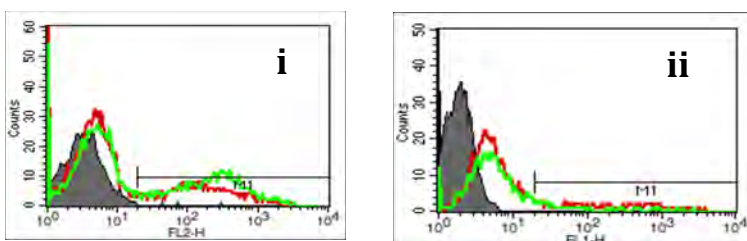


Figure 1. Transduction levels of CAR⁺ T-cells: i) HOX (red) and HDFTr (green) constructs were detected in transduced human T-cells using a biotinylated MUC1 24mer peptide followed by streptavidin-PE. ii) CSF28z (red) and CSFTr (green) constructs were detected in transduced T-cells using polyclonal goat anti-CSF1 antibody followed by anti-goat-Ig-FITC conjugate. The filled histogram shows non-specific staining with goat serum plus secondary reagent.

Task 4 – Separate EOC tumor cells and tumor-associated macrophages from ascites by flow sorting (SGM). Ascites will be removed from a total of 25 patients with EOC with informed consent over the duration of the study (3 years).

Target

Demonstrate feasibility by month 6

Progress

Achieved in previous report

Revised Task 5 - Co-cultivate engineered T-cells with tumor cells/ ascites (JM). T-cells will be mixed at 1:1 ratio in the following combinations: (i) T1E28z (test); (ii) T1NA (truncated control CAR). We will use the $4\alpha\beta$ chimeric cytokine receptor to facilitate expansion and enrichment of gene-modified T-cells. The effect of macrophage depletion cannot reliably be tested *in-vitro* and will be explored *in-vivo*.

Target

Complete by month 24

Progress

Achieved

We have recently described a chimeric cytokine receptor named $4\alpha\beta$ in which the human IL-4 receptor α ectodomain has been fused to the transmembrane and endodomain of the shared β receptor used by IL-2 and IL-15.¹⁰ Although IL-4 is a poor T-cell growth factor, it delivers a potent mitogenic signal when added to cells that express $4\alpha\beta$. Consequently, $4\alpha\beta$ provides a convenient system to expand and enrich for genetically modified T-cells, simply with the addition of IL-4. An example of this is shown in **Figure 2**.

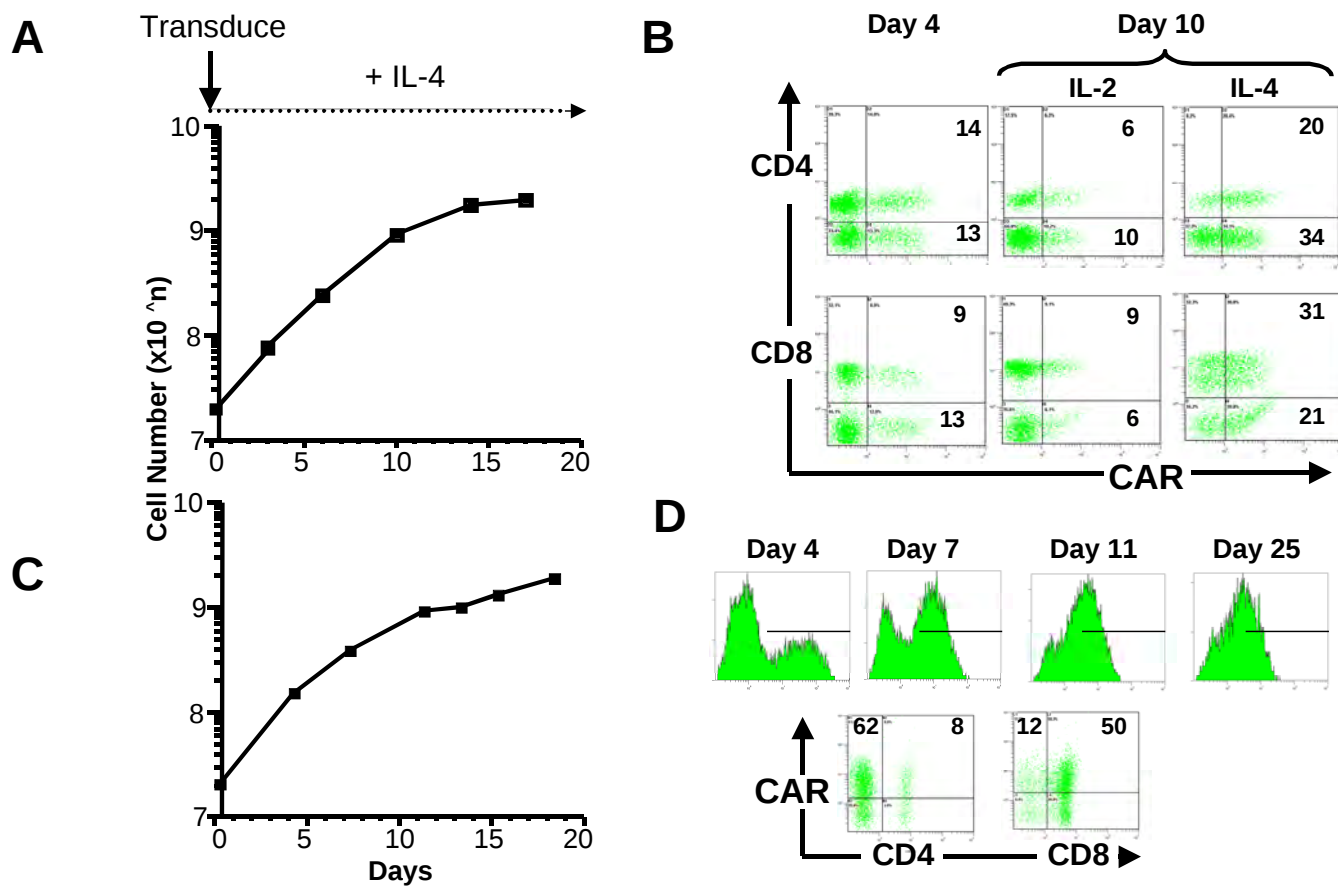


Figure 2. Use of IL-4 to expand T-cells that co-express T1E28z with the $4\alpha\beta$ chimeric cytokine receptor. Panels **A** and **C** show two examples in which T-cells from healthy donors were engineered to co-express T1E28z and $4\alpha\beta$ and were then expanded using IL-4. Enrichment of transduced T-cells in each case is shown in panels **B** (note comparison with IL-2) and **D** respectively. The T1E28z CAR was detected by flow cytometry following staining of cells using anti-human EGF antibody. Markers on histograms and dot plots were set using untransduced control T-cells.

In our previous report, (**Figure 14** – year 1 report), data was presented to show the superior EOC tumor targeting properties of T1E28z compared to the truncated control, T1NA. The T1NA control CAR has only recently been cloned in SFG together with the $4\alpha\beta$ chimeric cytokine receptor. Consequently, in order to expedite completion of this task, comparison has been performed with a second control CAR named P28z.⁶ The P28z CAR is specific for prostate specific membrane antigen, a target that is not expressed in EOC. T-cells from EOC patients were engineered to co-express T1E28z + $4\alpha\beta$ (combination “T4”) or P28z + $4\alpha\beta$ (combination “P4”; **Figure 3A-B**). In each case, transduced T-cells were cultured in human IL-4 to facilitate their selective enrichment and expansion (**Figure 3C**). Note that detection of P28z by FACS is poorly sensitive since it relies upon the ability of polyclonal anti-murine IgG to detect epitopes within the P28z scFv. **Figure 3D**

shows that patient-derived T4⁺ T-cells elicit complete destruction of monolayers derived from (i) autologous tumor ascites; (ii) IGROV-1 cells and (iii) SKOV-3 cells. This was accompanied by production of several cytokines by activated T4⁺ T-cells, including IL-2 and IFN- γ (**Figure 3E**). Note that IL-6 was produced in copious amounts by patient ascites cultures.

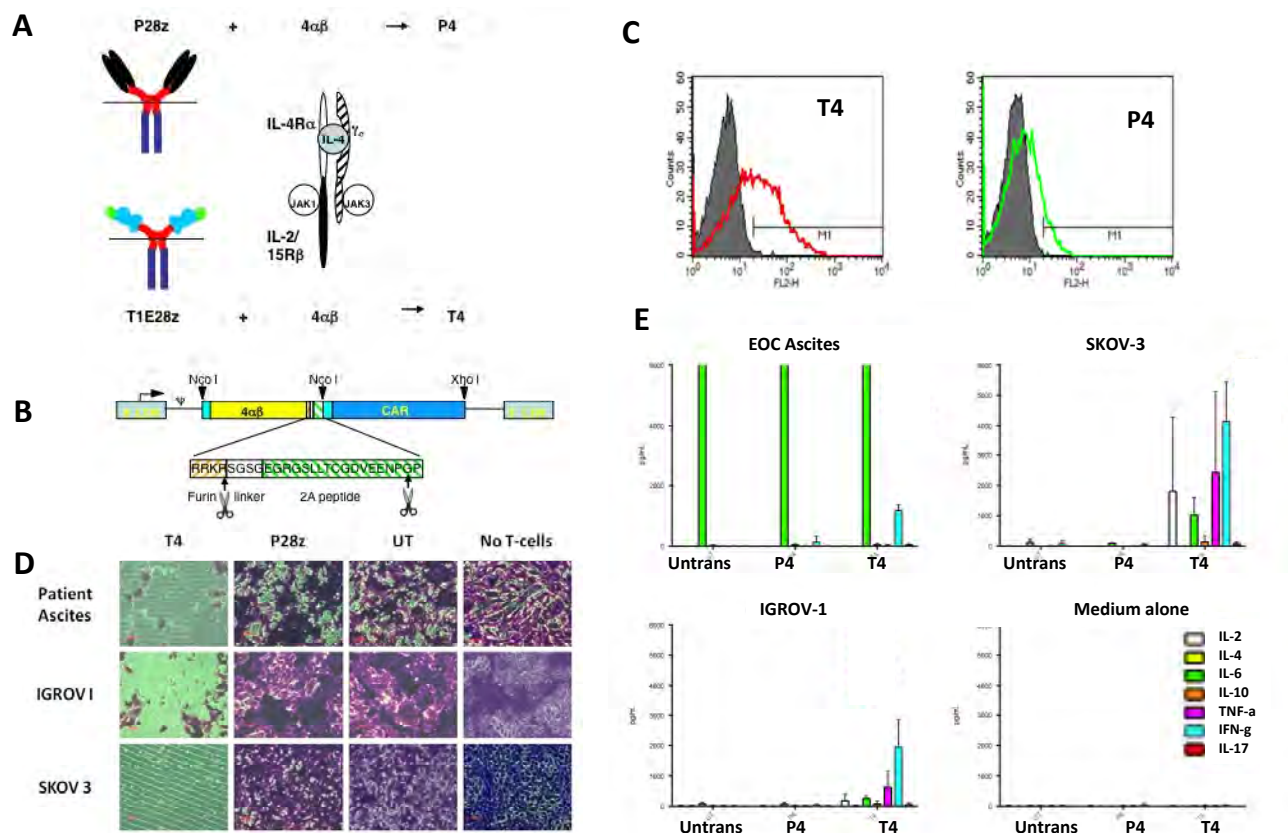


Figure 3. Anti-tumor activity of T4-engrafted patient-derived T-cells. **(A)** Cartoon illustrating the structure of the T4 and P4 constructs. In both cases, a chimeric antigen receptor (ErbB-specific T1E28z or PSMA-specific P28z respectively) has been co-expressed with an IL-4R α / IL-2R β chimeric cytokine receptor (4 $\alpha\beta$). The 4 $\alpha\beta$ chimeric cytokine receptor harnesses the binding of the poorly mitogenic cytokine IL-4 to delivery of a potent IL-2/IL-15-like growth signal to gene-modified T-cells. **(B)** A *Thossea Asigna* 2A peptide placed downstream of a furin cleavage site has been used to achieve stoichiometric co-expression of the 4 $\alpha\beta$ chimeric cytokine receptor with the T1E28z or P28z CAR. Expression was achieved using the SFG retroviral expression vector. **(C)** T4 and P4-transduced patient-derived T-cells were expanded using IL-4 and then analyzed by FACS after staining with anti-human EGF (T4 – red line) or anti-mouse IgG (P4 – green line). In each case, the filled histogram shows similar staining of untransduced T-cells. **(D)** Transduced T-cells (1×10^6 cells) were co-cultivated in a 24 well dish with a confluent monolayer of the indicated tumor cells. After 24 hours, the residual monolayer was fixed and then stained with crystal violet. Darker patches of color indicate presence of tumor and lighter regions show tumor monolayer destruction by the T-cells. **(E)** Cytokine bead array data from the 24h co-culture of patient T-cells with autologous ascites, SKOV-3 cells and IGROV-I cells. Medium alone serves as control (mean $\pm$ SD, n=3).

Task 6 – Generate ffLUC-expressing SKOV-3 cells by retroviral-mediated gene transfer (JM).

Target

Deliver by month 12

SKOV3 and IGROV cells expressing firefly luciferase (ffLUC) have been generated.

Task 7 – Establish tumorigenicity of ffLUC-SKOV-3 and ffLUC-IGROV-1 following intraperitoneal injection in SCID Beige mice, as measured clinically and using bioluminescence imaging (SGM). To permit administration of 3 tumor cell doses to groups of 5 mice, 30 mice will be required for both models.

Target

Deliver by month 18

Progress

Achieved

Intraperitoneal models of ovarian cancer were set up using two serous ovarian cancer cell lines, IGROV-1 and SKOV-3. Cells were engineered to express ffLUC in order to facilitate non-invasive and serial tracking of tumor progression using bioluminescence imaging. To test tumorigenicity, SKOV-3 and IGROV-1 cell lines were injected IP at doses of 0.5×10^6 , 1×10^6 or 3×10^6 cells in groups of 3 mice each. Results are shown in **Figure 4**. Both cell lines produced satisfactory IP models at all doses. We selected 1×10^6 cells as the most suitable tumor cell inoculum for future studies on the basis that tumor progression occurred at an intermediate rate, but was nonetheless robust.

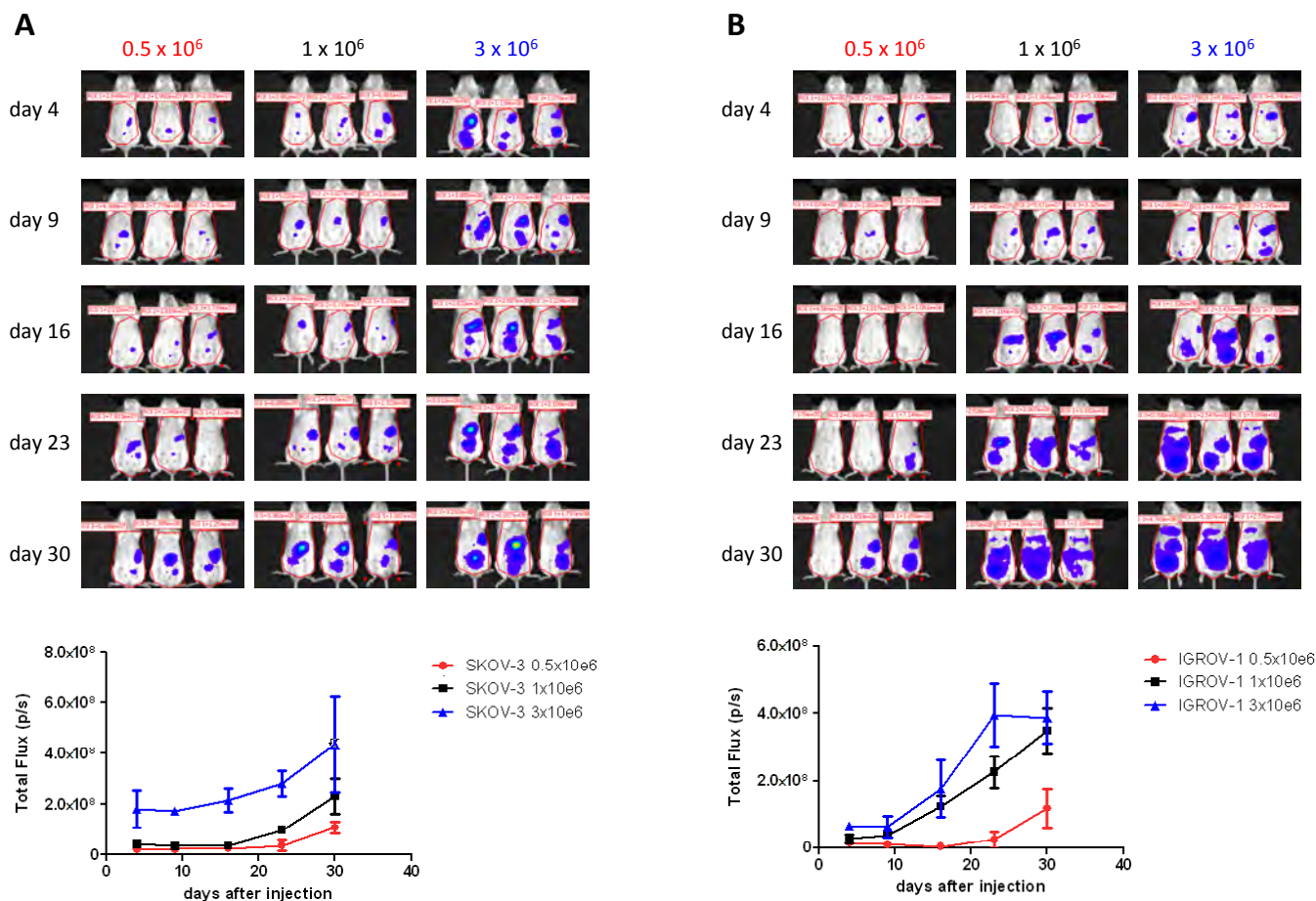


Figure 4. Establishment of EOC xenograft models. Firefly luciferase-expressing SKOV-3 (**A**) or IGROV-1 cells (**B**) were inoculated by intraperitoneal injection at doses of 0.5×10^6 , 1×10^6 or 3×10^6 cells. Tumor progression was tracked using bioluminescence imaging at the indicated intervals. Upper panels show images of mice at all timepoints. Lower panels show pooled total light emission (mean $\pm$ SD, n=3 mice per dose level).

Task 8 – Generate sufficient CAR/ rrLUC⁺ T-cells to treat tumor-bearing mice by intraperitoneal injection (JM). From 50ml blood, we can generally isolate 5×10^7 PBMC. Will need to achieve approximately 10-fold T-cell expansion *in-vitro* to allow the administration of 2×10^7 T-cells per mouse (described in Task 9). This level of T-cell expansion is generally achievable using CD3+CD28 expander beads in 7 – 10 days.

Target
Progress

Deliver by month 20
Achieved in part

Initially, our intention had been to conduct this work package using T-cells engineered to express the MUC1-specific CAR, HOX (targeting tumor) and the CSF28z CAR (targeting TAM). We set out to test the efficacy of this approach using an EOC-like ascitic tumor model in which firefly luciferase-expressing MDA-MB-435

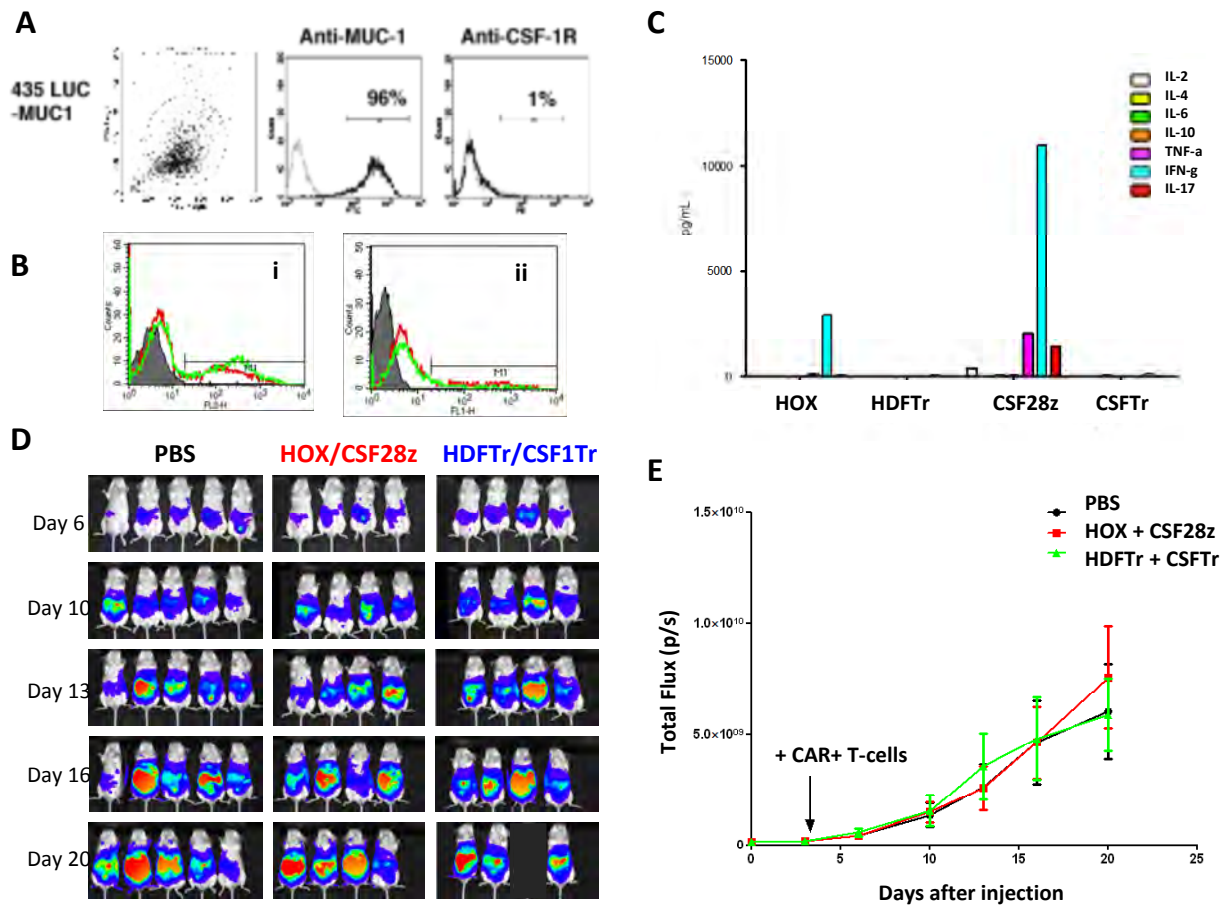


Figure 5. Testing of therapeutic efficacy of MUC1/ CSF-1R-targeted T-cells *in-vivo*. (A) MDA-MB-435 breast cancer cells were engineered to co-express firefly luciferase and MUC-1 (“MUC LUC”). Expression of MUC1 was detected using the HMFG2 monoclonal antibody. Tumor cells do not express CSF-1R. (Bi) Expression of HOX (red) and HDFTr (green) was detected in transduced T-cells using a biotinylated MUC1 24mer peptide followed by streptavidin-PE. (Bii) Expression of CSF28z (red) and CSFTr (green) were detected using a monoclonal anti-CSF1 antibody followed by anti-rat-IgG-PE. (C) Cytokine production by the indicated T-cell populations (1×10^6 cells) when co-cultured with T47D FMS tumor cells. Supernatants were harvested after 24 hours and analyzed by cytokine bead array. (D) MUC LUC tumor (2×10^6 cells) was injected IP in SCID beige mice (n=5 per group). After 3 days, animals received the indicated T-cell combinations (8×10^6 of each T-cell population). Bioluminescence imaging was performed at the indicated time points. Where necessary animals were culled if clinically unwell. Pooled bioluminescence data for each group is shown in E (mean $\pm$ SD).

breast cancer cells were engineered to co-express MUC-1 (**Figure 5A**). Flow cytometric analysis of the transduced T-cell populations used in this experiment is shown in **Figure 5B**. To validate function of transduced T-cells *in-vitro*, they were co-cultivated with breast cancer cell line T47D FMS, which expresses both MUC-1 and CSF-1R. Cytokines produced by T-cells following 72 hour co-culture with T47D FMS cells were measured using a Th1/Th2/Th17 cytokine bead array (**Figure 5C**). These data confirm that both CAR-transduced T-cell populations were capable of recognizing their target antigen. To test therapeutic efficacy *in-vivo*, MUC LUC tumor was established in the peritoneal cavity. After 3 days, CAR-transduced T-cells were injected IP in the following combinations: (i) HOX and CSF28z-transduced (the test combination; (ii) HDFTr and CSFTr (the control combination). PBS served as an additional control treatment. Tumor status was evaluated thereafter using bioluminescence imaging (**Figure 5D-E**). These data indicate that this combination of engineered T-cells achieved no therapeutic effect when tested in this aggressive tumor model and prompted us to submit a revised statement of work in which we proposed to target ErbB receptors (tumor) and TAM (using liposomal clodronate). This revision to the statement of work has been accepted.

Task 9 – Monitor tumor-progression in mice following T-cell therapy, using dual bioluminescence imaging (SGM). Five groups of mice will be used in these experiments:

Group 1 receive liposomal clodronate + T1E-28z T-cells.

Group 2 receive liposomal clodronate + T1NA T-cells.

Group 3 receive T1E-28z T-cells alone.

Group 4 receive T1NA T-cells alone.

Group 5 receive PBS.

These experiments will require a total of 140 mice. This number will allow 2 experiments per tumor model, each requiring 35 mice as follows: Five groups of 7 mice which will be treated as indicated above. Numbers have been calculated to permit meaningful statistical analysis while allowing for principles of reduction, refinement and replacement. In all cases, animals will be sacrificed if any symptoms develop, or in the event of progressive tumor growth (indicated by increasing bioluminescence signal intensity). If tumor rejection occurs, animals will be maintained in the facility for their natural lifespan.

Target

Deliver first therapeutic experiment by month 24

Progress

Achieved in part

The data presented above indicate that dual targeting with HOX and CSF28z is ineffective in targeting a strongly MUC1⁺ tumor xenograft, at the T-cell doses used. This disappointing result prompted us to consider alternative targets expressed by EOC tumor cells and associated TAM. To target tumor cells, we now propose to use the T1E28z CAR as detailed in Task 5 above. In our last annual report, we showed that TAM found in association with intraperitoneal tumor xenografts in SCID Beige mice express very low levels of CSF-1R on the cell surface. This finding may explain the lack of effect of CSF28z⁺ T-cells in the *in-vivo* study described above. Consequently, we set out to achieve TAM depletion using an alternative strategy, using liposomal clodronate.

First, we set out to test the efficiency of liposomal clodronate to achieve TAM depletion in SCID Beige mice that bear an EOC xenograft. Firefly luciferase-expressing SKOV-3 cells were inoculated IP in SCID Beige mice. On day 4 and weekly thereafter, mice received IP liposomal clodronate. Liposomal PBS served as negative control. **Figure 6A** shows that liposomal clodronate exerted no significant effect on tumor progression despite achieving complete depletion of intraperitoneal (F4/80⁺) macrophages in these mice (**Figure 6B-C**).

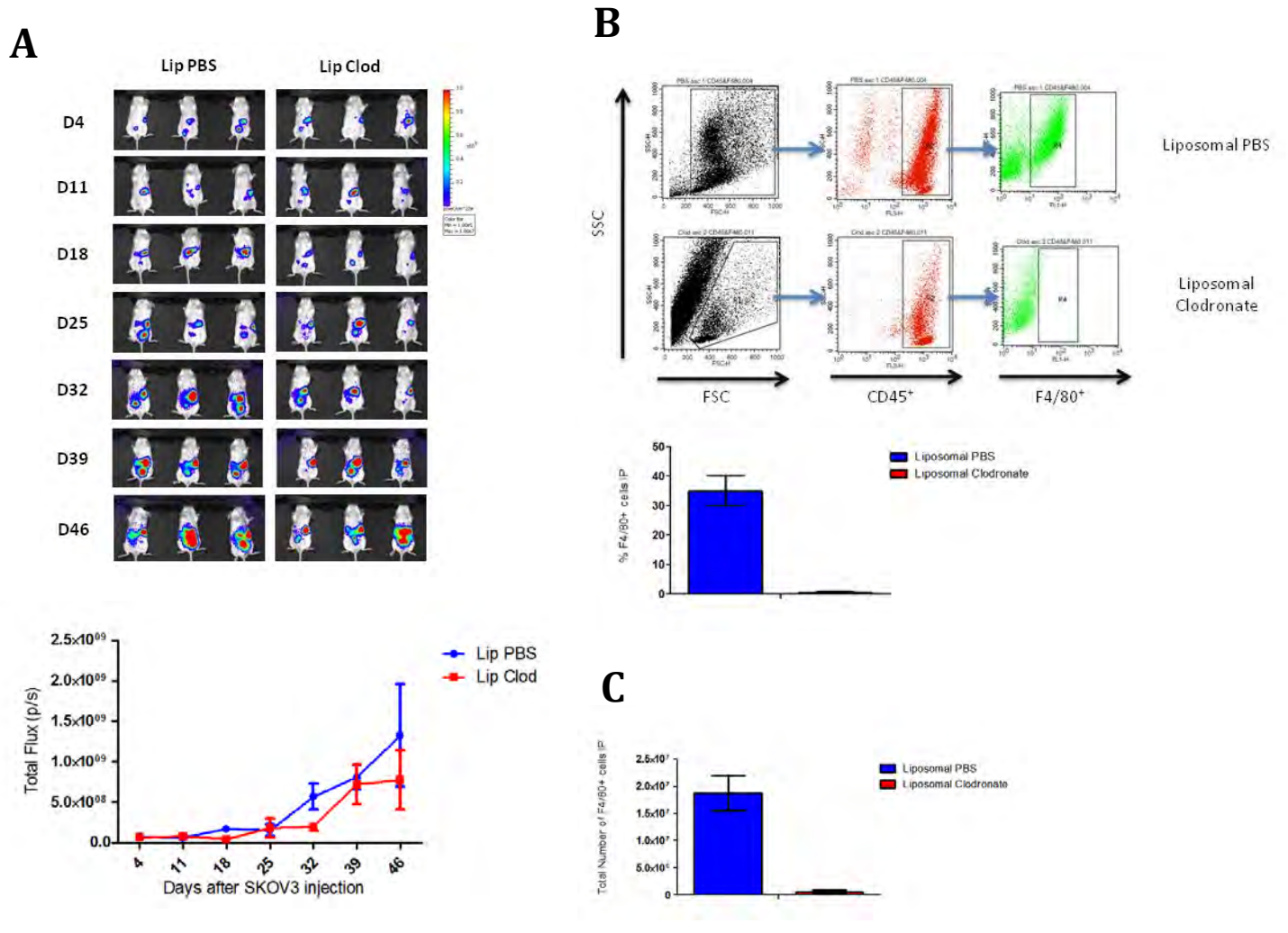


Figure 6. Effect of liposomal clodronate on intraperitoneal F4/80⁺ macrophages in mice bearing SKOV-3 xenografts.

(A) Mice were injected IP with 1×10^6 firefly luciferase-expressing SKOV-3 cells. From day 4 onwards, the mice received a weekly IP dosage of liposomal PBS or liposomal clodronate (62.5 μ l). Tumor growth was monitored, using bioluminescence imaging, at the indicated times. No significant changes were found between liposomal PBS and liposomal clodronate treatment ($n=3$, tested by two way ANOVA).

(B) Percentage of F4/80⁺ macrophages in the peritoneal cavity of mice following treatment with liposomal clodronate or liposomal PBS as control. The upper panel shows the gating strategy used to analyse these cells by FACS. Note the large number of dead cells in the clodronate treated mice (population on the left of the gate in the FSC v SSC plot). When gating on CD45⁺ hematopoietic cells, the SSC^{LO} (lymphoid population) remains intact whereas the SSC^{HI} population (containing TAM) is reduced. The graph shows the percentage of F4/80⁺ cells present in the peritoneal cavity (mean $\pm$ SD, $n=3$). Data were collected on day 46 post-SKOV3 inoculation and after 7 weekly treatments with liposomal PBS or liposomal clodronate.

(C) Total number of macrophages (F4/80⁺ cells) in the peritoneal cavity of mice following treatment with liposomal clodronate or PBS. Data were collected on day 46 post-SKOV3 inoculation and after 7 weekly treatments with liposomal PBS or liposomal clodronate.

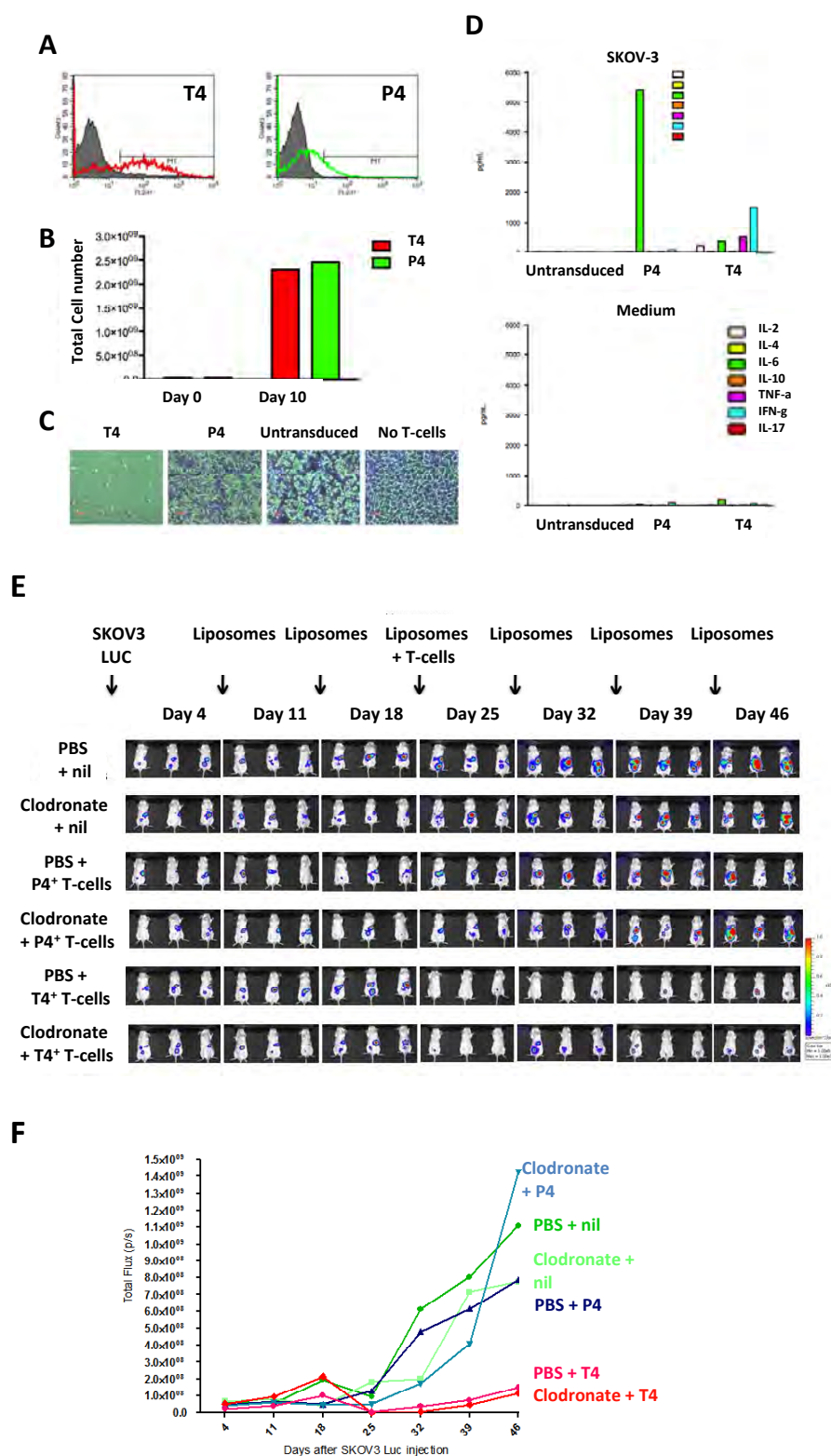


Figure 7. Testing of therapeutic efficacy of T4⁺ T-cells alone or with liposomal clodronate to treat established SKOV-3 tumor xenografts. **(A)** FACS analysis of T4⁺ and P4⁺ cells immediately prior to adoptive transfer into mice. T1E28z was detected using anti-human EGF antibody while P28z was detected using anti-mouse IgG. **(B)** *In-vitro* expansion of T4⁺ and P4⁺ T-cells using IL-4 over a 10 day period. **(C)** Prior to adoptive transfer into mice, T4⁺ and P4⁺ T-cells (1 × 10⁶ cells) were co-cultivated with SKOV-3 cells *in-vitro*. After 24 hours, nonadherent T-cells and supernatant were removed and residual monolayer was fixed and stained using crystal violet. **(D)** Supernatants in C were analyzed for the indicated cytokines using a bead array. The peak of IL-6 in the P4⁺ T-cell sample is likely to be spurious. **(E)** Mice were inoculated IP with 1 × 10⁶ SKOV-LUC cells. Animals were treated with liposomal clodronate or PBS on day 4 and weekly thereafter. After 18 days, when tumors had become established, animals were treated with 2 × 10⁶ T-cells that express T4 or P4. Bioluminescence imaging was used to track tumor status at the indicated intervals. The graph represents the mean BLI emission from each of the indicated groups of 3 mice over time.

Having confirmed that liposomal clodronate is highly effective in achieving TAM depletion, we next proceeded to evaluate therapeutic efficacy of the combination of T4⁺ T-cells (**Figure 7A**) with liposomal clodronate in mice bearing an established SKOV-3 tumor xenograft. Control T-cells were engineered to express P4 (the

combination of 4 α β co-expressed with P28z, a CAR that is specific for PSMA; **Figure 7A**). To deplete TAM, animals received liposomal clodronate (or liposomal PBS) on day 4 and weekly thereafter. Gene-modified T-cells were expanded with IL-4 for 10 days (**Figure 7B**) and the function of T4⁺ T-cells was confirmed using assays of monolayer destruction (**Figure 7C**) and cytokine production (**Figure 7D**) prior to adoptive transfer into mice. The bioluminescence images shown in **Figure 7E** indicate that on the day of T-cell infusion, discrete tumors were visible in all mice. Within one week, marked tumor regression was observed in all mice treated with T4⁺ T-cells. Tumors have gradually increased since that time point. Depletion of macrophages by clodronate has not influenced the therapeutic activity of T4⁺ T-cells significantly. This experiment is currently still ongoing and will be repeated and data pooled to give 6 mice per group. A similar study will then be performed in mice bearing IGROV-1 tumor xenografts.

Efforts to clone a renilla luciferase-T1E28z construct have been initiated in order to establish T-cell bioluminescence imaging in future experiments.

Task 10 – Engineer vector to co-express CAR with human sodium iodide symporter (hNIS) using 2A cleavage system and deliver/ validate expression in human T-cells (JM).

Target
Progress

Deliver by month 24
Not achieved by month 22

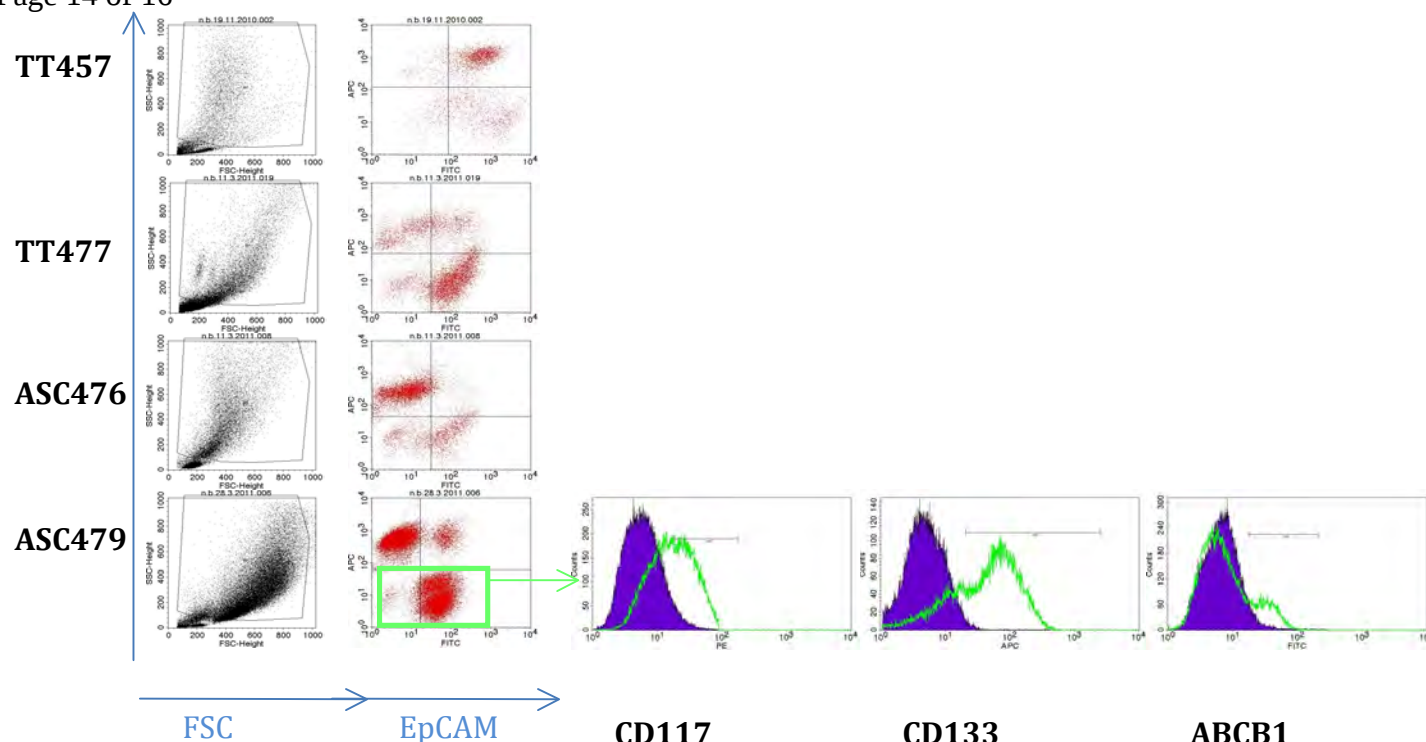
We have not achieved this target yet, this was primarily due to unanticipated resignation of the postdoctoral research assistant at the Imperial site. The new post holder will assume responsibility for developing this and other T-cell imaging strategies.

Task 14 – Establish primary EOC ascites-derived tumor cells in SCID Beige mice (+/- stem cell enrichment *ex-vivo*; SGM). We anticipate the use of 60 SCID Beige mice for these studies to facilitate dosing/ optimization.

Target
Progress

Deliver by month 30
Experimental work initiated

We have initiated work in this task by purification of patient-derived tumor cells from ascites and tumor tissue using antibody markers associated with tumor cell ‘stemness’. **Figure 8** shows an example of cells from ascites and tumor tissue stained from a patient with ovarian cancer. Tumor cells were separated using magnetic beads coated with antibodies against EpCAM on tumor cell surface. Flow cytometry was used to confirm enrichment of samples for tumor cells and for identification of CD45 negative cells that expressed CD133, CD117 and ABCB1.

**Figure 8**

FACS analysis of EOC cells purified from patient ascites (ASC) or tumor tissue (TT) samples. Both sample types can be separated into CD45⁺ cells of hematopoietic origin (stained with APC) and EpCAM⁺ cells of tumor origin (stained with FITC). The lower row shows a representative analysis of the EOC tumor stem cell markers CD133, CD117 and ABCB1 expressed by CD45⁺ cells isolated from the ascites sample ASC479

3. Key Research Accomplishments

- All necessary approvals have been secured to allow the initiation of this project.
- All chimeric antigen receptors have been engineered, cloned and used to generate viral vector.
- Feasibility of transduction of patient T-cells has been repeatedly demonstrated, with efficiency even in patients with 'bulky' ovarian cancer.
- Use of the model based upon the MDA-MB-435 tumor cell line, allowing testing of our hypothesis with the preferred target pair (MUC1 and CSF-1R) has shown that this dual targeting approach is ineffective.
- By contrast, experimental data gathered using the ErbB-specific T1E28z CAR indicate that it achieves significant activity against EOC, both *in-vitro* and in SCID Beige mice bearing established tumor xenografts.
- Our results suggest that depletion of TAMs in the *in-vivo* SKOV-3 EOC model using clodronate does not have a beneficial effect. This finding contrasts with the accepted dogma concerning the deleterious role of TAMs in ovarian cancer.
- Systems to identify and purify putative EOC stem cells have been put in place.

4. Reportable Outcomes

Abstracts describing our work have been presented at the British Society of Immunology Meeting 2010.

1. Brewig N, Parente-Pereira AC, Maher J, Ghaem-Maghamsi S (2010) An in-vivo xenograft model to study simultaneous targeting of cancer cells and immunosuppressive tumour-infiltrating myeloid cells. British Society of Immunology (2010). **Immunology** 131 S1 Abstract 589.

2. Parente-Pereira AC, Brewig N, Ghaem-Maghami S, Maher J (2010) Immunotherapy of epithelial ovarian cancer using CAR engrafted T-cells: in vitro development. British Society of Immunology (2010). **Immunology** 131 S1 Abstract 601.

5. Conclusions

The data presented in this report demonstrate that T-cells from patients with epithelial ovarian cancer can be genetically targeted against ErbB receptor dimers that are aberrantly upregulated on tumor cells. Importantly, we have demonstrated efficacy against established tumor xenografts *in-vivo* and using matched patient T-cell/tumor cell co-cultivations *in-vitro*. Data gathered to date would not support a clinical rationale for targeting of TAM. Imaging studies to be performed in the next year will be very informative in addressing the question of whether *in-vivo* T-cell longevity is a limiting factor in the efficacy of ErbB re-targeted T-cells. If this is the case, we may be able to exploit the IL-4-regulated growth system we have developed¹⁰ to achieve selective support of ErbB re-targeted T-cells *in-vivo*, particularly in the tumor microenvironment. The implications of this approach for patients will depend upon development and optimization of the approach described here. This will also depend upon clinical experience that we hope to gather when T4⁺ T-cells are tested in a first in man phase 1 study, which we hope to initiate soon in patients with head and neck cancer. In that study, a regional delivery approach will be used whereby T-cells are injected into locally recurrent tumor masses. Intraperitoneal administration of ErbB re-targeted T-cells can be envisioned as a potential therapeutic application of this approach in patients with EOC.

6. References

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