Final Summary Report
(01/12/2012 – 26/06/2016)

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1. Executive Summary

The ATTACK project involves two multi-centre clinical trials to treat cancer using a form of cell therapy called Adoptive T-Cell Therapy. Adoptive T-Cell therapy is a promising advance in cancer treatment and involves using a patient's own immune cells.

Trial I: A Phase II Trial to Assess the Activity of NY-ESO-1 Targeted T-Cells in Advanced Oesophagogastric Cancer'. The aim was to determine whether results seen in other trials of this type of cell therapy could be translated into other solid tumour types. The NY-ESO therapy was manufactured by CTL at GMP facilities in Manchester. The first site and sponsor for the clinical trial was the Christie NHS Foundation Trust. The trial opened to recruitment in 2014. To date, two patients have been treated in the trial, one of whom passed away 46 days after initial treatment. The underlying cause of death was investigated by the ATTACK consortium and during the period of investigation, the Christie NHS Foundation Trust suspended enrolment in the trial. The conclusions of the investigation were that the NY-ESO therapy was unlikely to have directly caused the bone marrow failure seen in the applicable patient through on-target toxicity or mispairing. In February 2016, following review of the results, an independent data monitoring committee (IDMC) recommended that recruitment could resume once agreed changes to the protocol were in place. An amendment to the protocol is currently being considered prior to restarting any enrolment in the trial.

Alongside the trial, another work package focused on assay standardization and the efficient monitoring of the trial patients with the aim of determining key markers of therapeutic benefit and potential predictive markers of response. We have established and validated screening assays to assess positivity of expression of NY-ESO-1/LAGE-1 in order to identify patients suitable for the trial. Further assays have also been developed and validated (flow cytometry (FCM) NY-ESO-1 tetramer staining assays and gene-modified T-cell specific real-time PCR assays) to assess the final product and blood post treatment for each patient. The detailed protocols have been made available for participating clinical sites. Screening assays were applied on 39 patients, of which 4 were suitable for treatment. Presence of NY-ESO-1 TCR T cells in patient blood was assessed in two treated patients using both FCM and qPCR methods. Furthermore we have standardised the FCM research assays for quantitation and assessment of differentiation and activation status of NY-ESO-1 TCR T cells prior to infusion and in patient blood, including markers for T cell differentiation, T cell function, and exhaustion. An archive for storage of patient material for future research has been established at EMC. Due to early termination of the project no data have been generated yet with respect to the additional research questions.

In parallel, pre-clinical research has developed novel strategies for the enrichment of effective T- cell subsets and optimized their activation, gene-modification and expansion using newly co-developed soluble activation reagents, culture conditions (i.e. TexMACS medium + IL-7 and IL-15) and a dedicated closed single-use tubing set where all steps were integrated in a stepwise approach. We finally managed to develop a process on the CliniMACS Prodigy which integrates all essential steps for clinical-grade manufacturing of gene-modified T -cells. Automation of the manufacturing process is economically advantageous. This will contribute to the commercial realisation of adoptive cell transfer therapy. Moreover, user interactions and open handling steps for the T cell transduction process were reduced to a minimum to increase safety for each run making a complex procedure more reliable.

Trial II: A randomised phase II trial in Melanoma to investigate an alternative cell manufacturing process with 3 potential improvements compared to the "standard method": a) Selection of $CD62L^+$ cells with greater re-population potential, b) An alternative cell activation step (TransAct) and c) Expansion in IL-7/IL-15 to maintain a less differentiated state with greater repopulation potential. The manufacturing process was qualified pre-clinically and was undergoing GMP validation when the project was terminated.

Unfortunately, due to the delays seen in the progress of ATTACK, the European Union has terminated funding of the ATTACK project with effect from 26th June 2016.

2. Summary Description of Project Context and Objectives

2.1 The ATTACK Project

Targeted nucleic acid delivery by genetically engineering T-cells to target tumour associated antigens expressed on malignant cells demonstrates extraordinary efficacy in pre-clinical models of advanced cancer (Klebanhoff et al., 2011) and recent results confirm the clinical effectiveness of the treatment (Morgan et al., 2006; Robbins et al., 2011; Kochenderfer et al., 2010; Porter et al., 2011). However, the use of engineered T-cells is a challenging field and key clinical and methodological questions remain unanswered. These include:

1. Is the approach active in common epithelial malignancies? - as current trials have focussed on melanoma and B-cell malignancies
2. The production of optimised genetically engineered T-cells is critical and the number and quality of cells can clearly influence the outcome. The choice of the T-cell subset(s) may also be important as they can engraft to different extents.

The aim of ATTACK was to exploit technology for nucleic acid delivery through the clinical testing of adoptive transfer of engineered T-cells to treat cancer and build upon previous EU funded pre-clinical projects (FP5 Chimeric Eurocell; FP6 ATTACK; FP7 ATTRACT). The consortium, which comprises a multidisciplinary and translational research group with wide-ranging relevant expertise, were to initiate two landmark studies to (i) examine activity of engineered T-cells in oesophago-gastric cancer as an example of a hard to treat common epithelial cancer, (ii) undertake a randomised Phase II study to determine whether an optimised cell production system improved the current clinical response rates in patients with metastatic melanoma treated with NY-ESO-1 targeted T-cells.

2.2 Approach and Methodology

The work plan was composed of three principle scientific work packages (WP1-WP3).

WP1: Clinical Trials

WP1 centred upon delivery of the two clinical trials targeting NY-ESO-1. Enrolment onto each trial required patients to be HLA-A*0201 positive and their tumour to be NY-ESO-1 and/or LAGE positive.

Trial I:

A Phase II Clinical Trial to Assess the Activity of NY-ESO-1 Targeted T-cells in Advanced Oesophago-Gastric Cancer

This trial aimed to evaluate the activity of engineered T cells to target NY-ESO-1 in oesophago-gastric cancer for three reasons:
1. NY-ESO-1 is considered one of the best targets for immunotherapy (Cheever et al., 2009).
2. There is preliminary clinical data in patients with both melanoma and synovial sarcoma

(Robbins et al., 2011).
3. There is good evidence of NY-ESO-1 antigen expression in oesophago-gastric cancers.

Success in this trial would indicate substantial activity of adoptive cellular therapy in a common epithelial malignancy that is difficult to treat by conventional means.

Eligible patients (N=28) were to be pre-treated with cyclophosphamide and Fludarabine. Cells would be transduced and expanded using standard protocol (Figure 1). Patients would be given IL-2 after cell infusion. If there were 8 or more RECIST (Response Evaluation Criteria in Solid Tumours) defined responses then the treatment is showing substantial activity in this hard to treat disease and is worthy of future development.

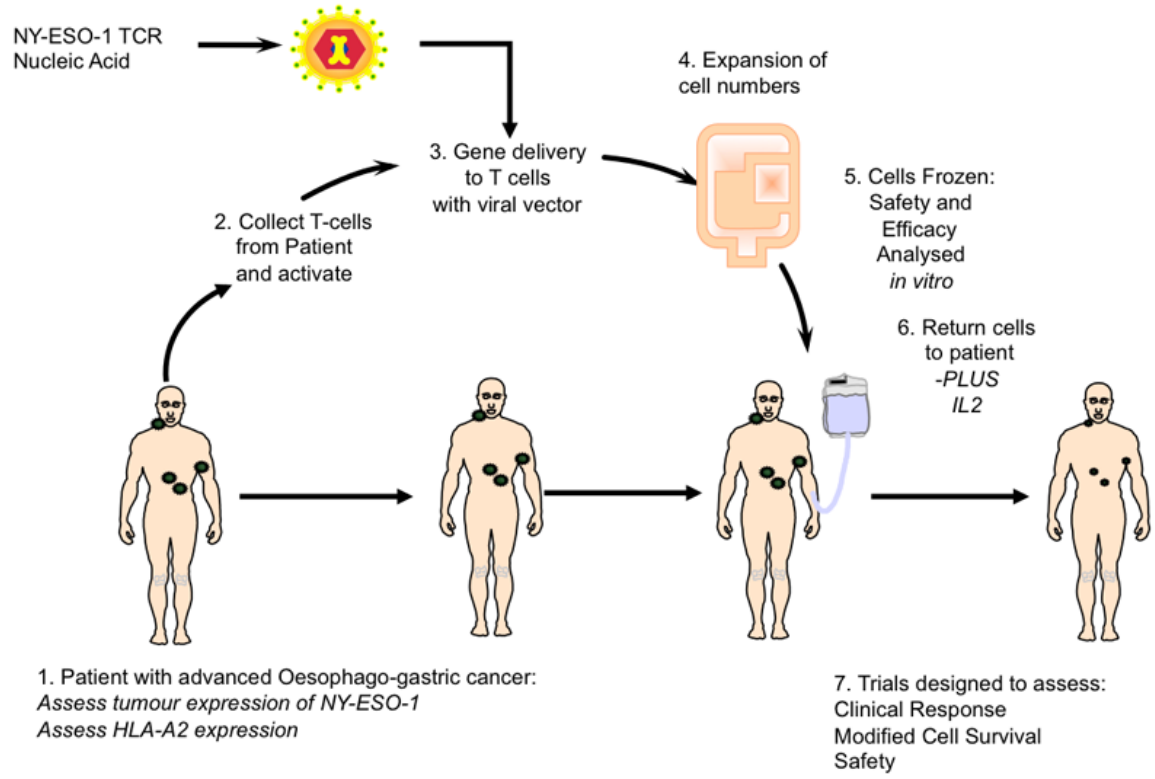


Figure 1: ***Trial I*** was to be a standard Phase II trial in oesophago-gastric cancer patients who have disease after standard therapy. The aim was to assess if this type of therapy is active in a common epithelial malignancy.

Trial II:

A Randomised Phase II Study in Metastatic Melanoma to Evaluate the Efficacy of Optimised Cell Production Protocols

Engineered T-cells targeting NY-ESO-1 have already shown substantial activity in metastatic melanoma (Robbins et al 2011) and we aimed to test this further in a multi-centre phase II study comparing standard methods of cell production (as shown in Figure 1) (Arm 1) with an optimised method of cell production (Arm 2) (Figure 2). The optimised production was expected to produce cells with better engraftment

capability and thereby greater clinical activity. The basic clinical aspects of the trial would be similar to Trial I. A total of 42 patients were to be randomly allocated to either one of these two treatment arms. The arm with the best RECIST defined response rate would be used for future development.

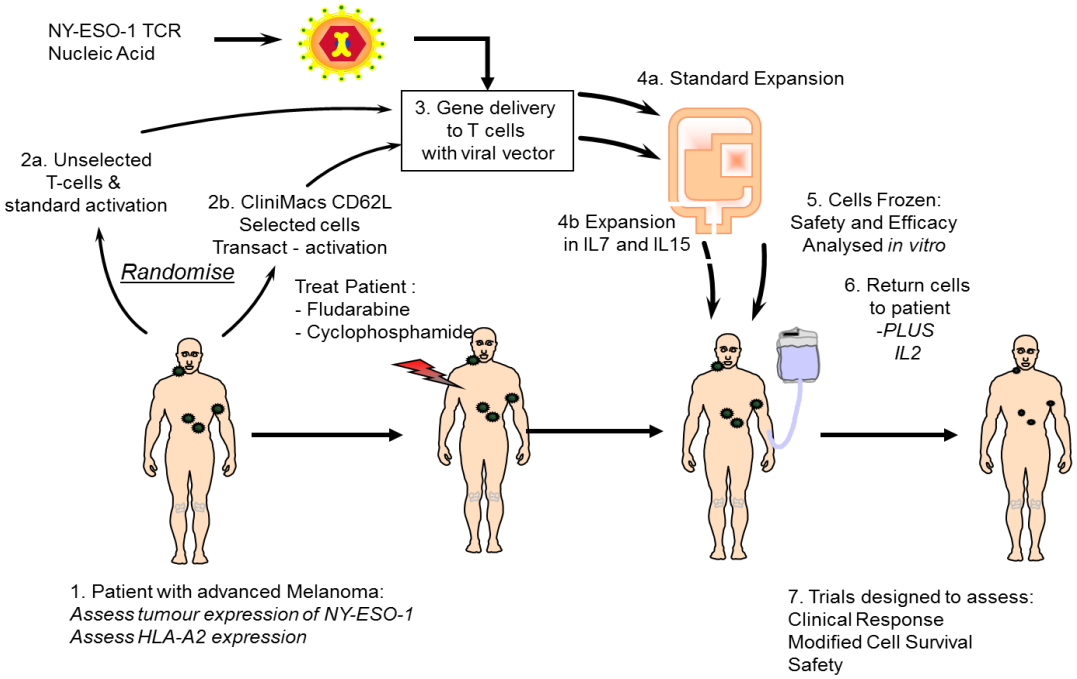


Figure 2: Trial II was a randomised Phase II study in patients with advanced melanoma. The aim was to compare the efficacy of standard engineered T-cell preparation methods using unselected cells and standard expansion protocols with a protocol optimised by members of the consortium, to produce gene-modified T cells that would be assessed for their capacity to provide improved repopulation in the patient.

The basic methods of cell production were to be similar in both trials as was the treatment the patient was to receive. However, in the arms with optimised cell production, the T-cells to be targeted for nucleic acid modification were to be pre-selected (**Figure 3**).

The project was built on strong on-going collaborations and previously successful EU pre-clinical applications (FP5 Chimeric Eurocell; FP6 ATTACK; FP7 ATTRACT (Initial Training Network)). The consortium brings together excellent clinical centres, key established industrial companies in the cell therapy field, an SME with key patents in the field and two service SMEs who aim to provide cell therapy as a service for patients. The project is coordinated by Professor Robert Hawkins (University of Manchester).

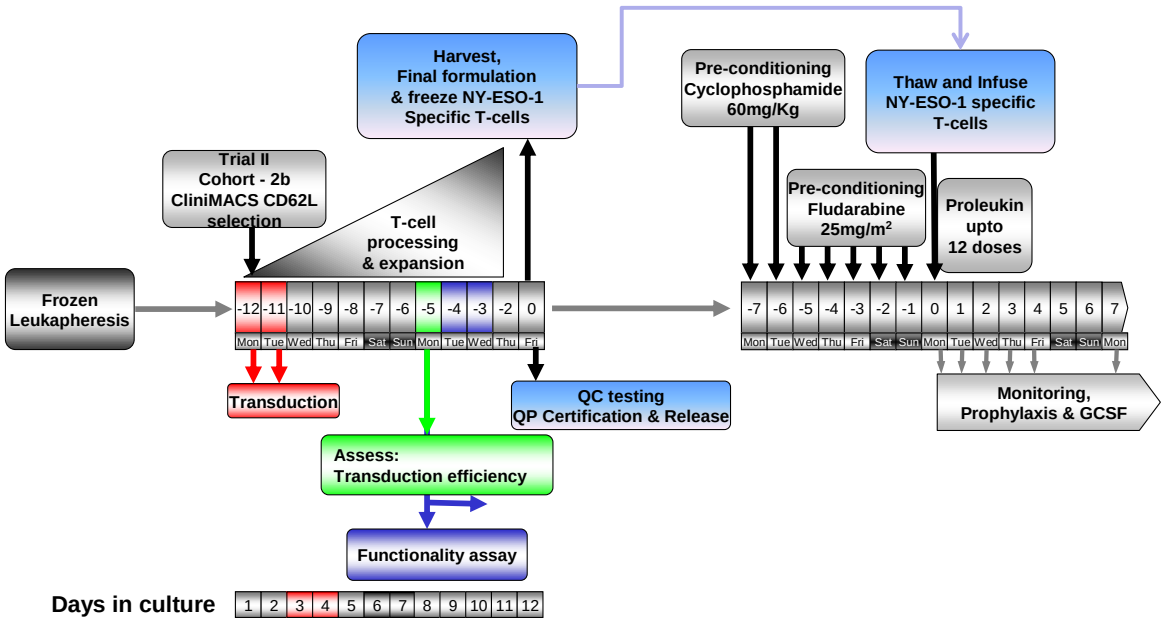


Figure 3: Details of the cell production process and patient treatment.

WP2: Cell Production

WP2 focused on GMP compliant cell production processes for both clinical trials including freezing of cells for use in multi-centre trials. We also aimed to develop a novel automated system for cell production; which would facilitate future larger scale use and testing of adoptive engineered T-cell therapy.

WP3: Laboratory Monitoring

WP3 was concerned with assay standardisation and laboratory monitoring of the two clinical trials with the additional aim of determining markers of therapeutic benefit and potential predictive markers of response.

3. Main Scientific and Technical Results

3.1 WP1 Clinical Trials

Building on encouraging clinical results using T-cells to target NY-ESO-1 in melanoma, synovial sarcoma and myeloma, the consortium were to undertake two ground breaking Phase II trials

Trial 1:

A Phase II Clinical Trial to Assess the Activity of NY-ESO-1 Targeted T-Cells in Advanced Oesophago-Gastric Cancer. Is adoptive engineered cell therapy active in oesophago-gastric cancer (as an example of a common epithelial malignancy) where there is a clear need for more effective therapies?

Trial 2:

A Randomized Phase II Clinical Trial to Assess an Optimised Cell Production Process in Patients with Metastatic Melanoma. Can an optimized cell production protocol enhance clinical outcomes in melanoma where there is clear established activity of engineered T-cell therapy?

Main activities and results:

Trial 1: A Phase II Clinical Trial to Assess the Activity of NY-ESO-1 Targeted T-Cells in Advanced Oesophago-Gastric Cancer

The trial opened to recruitment in September 2014 and 39 patients were pre-screened for HLA status and NY-ESO-1 positive tumours. To date, two patients have been treated in the trial, one of whom passed away 46 days after initial treatment. The underlying cause of death was investigated by the ATTACK consortium and during the period of investigation, the Christie NHS Foundation Trust suspended enrolment in the trial.

The conclusions of the investigation were that the NY-ESO therapy was unlikely to have directly caused the bone marrow failure seen in the applicable patient through on-target toxicity or mispairing. The information was presented to the IDMC (independent data monitoring committee) in February 2016 and following review of the results, recommended that recruitment could resume once agreed changes to the protocol were in place. These included improving safety profile by reducing the chemotherapy (to 3 days of cyclophosphamide and fludarabine at a lower dose), omitting the IL2 and clarifying details within the protocol regarding the management of toxicity.

Protocol amendments were underway when notification was received that the European Union was to terminate funding of the trial due to the delays seen in the progress of the ATTACK program. Notice of intent to terminate was received by the project co-ordinator on 13th May 2016. Termination of funding became effective on 26th June 2016.

Trial I documentation has now been drawn up in preparation for submission to the regulatory authorities if appropriate funding can be secured following withdrawal of EU support.

Trial II: A Randomized Phase II Clinical Trial to Assess an Optimised Cell Production Process in Patients with Metastatic Melanoma.

At the time of receipt of project termination notification, Trial II was in set-up phase and ready for submission to the regulatory authorities pending updated IMPD (Investigational Medicinal Product Dossier) and IB (Investigational Brochure).

After much discussion, the Consortium regretfully concluded that it was not feasible to perform the second trial that was originally planned. Although the issue of cell selection and methods of cell growth remain important and the trial is technically feasible, the Consortium did not feel it would be possible to recruit enough melanoma patients in a reasonable timescale because of other recent new therapies and current large numbers of competing trials. Other possible target diseases were discussed - the only one that is NY-ESO1 positive and sufficiently common to recruit to a randomised phase II study in a timely manner is oesophago-gastric cancer and until we know the outcome of Trial I the consortium felt that this would not be appropriate.

3.2 WP2 GMP Validation and Process Development

WP2 had the following objectives:

1. The manufacture of NY-ESO-1 T-cells using pre-qualified GMP (Good Manufacturing Practice) compliant bioprocessing procedures in Licensed EU manufacturing facilities, providing individual cryopreserved NY-ESO-1 specific autologous T-cell products for the treatment of patients across the consortium of clinical treatment facilities
2. Development of an automated process for cell selection, activation and transduction based on the MILTENYI Prodigy System which will potentially streamline the manufacturing steps during the most labour intensive and process critical procedures and ultimately provide a simplified manufacturing process whilst maintaining an equivalent final product.

Main activities and results:

The two production sites in this consortium are CTL and AmBTU.

CTL successfully validated the NY-ESO-1 specific T-cell manufacturing process once agreements with ADAPTIM for cell manufacturing and virus supply were finalised. Trial I opened to recruitment in 2014. Four batches of cells have been manufactured.

CTL has provided information for amendments to the IMPD and IB for MHRA submission and is ready for new batches to be manufactured, but is awaiting approval of Trial I (ATTACK-OG) to re-open before further batches can be manufactured.

AmBTU is awaiting lentiviral vector from ADPTIM to allow validation of the manufacturing process.

As part of the development of the second arm of Trial II, CTL and MILTENYI have performed development work resulting in 2 successful engineering batches using CD62L selection, IL-7 & IL-15 and TRANSACT activation to produce Naïve/Central memory enriched NY-ESO-1 T-cells (NCM-NY-ESO-1 T-cells).

CTL along with AmBTU and ADAPTIM have developed GMP assays. CTL has performed additional GMP assays of final products to provide stability data. The stability study assessed viability and gene expression of GMP manufactured products with matched cryo-bags and vials stored up to 9 months and vials form the initial validation study with stability data >2 years. In all cases, the bags and vials met the required release criteria demonstrating good long term product stability.

MILTENYI has completed the development of a fully integrated process that allows performance of clinical-grade T-cell selection from blood products, activation of the enriched T-cells and gene-modification via viral vector constructs. Robustness of the automated process using patient material has been verified. Some efforts are still remaining for finalization of the programing of the process (especially protocol generation for batch recording), documentation and definition of QC and IPC tests that are an obligatory part of clinical manufacturing and will have to be completed outside of the project.

Detailed results/achievements

<u>The manufacture of NY-ESO-1 specific T-cells using pre-qualified GMP compliant bioprocessing procedures in Licensed EU manufacturing facilities</u>

During the project CTL implemented an adaptable gene engineered T-cell manufacturing process enabling the incorporation of novel technological improvements in a field which is rapidly developing, ultimately allowing improvements in product quality and reducing manufacturing risk. CTL developed this process and Quality Control methods as part of the engineering batches leading to 3 validation runs, building on work carried out in advance of the project. The validation and QC data was incorporated into the IMPD, CTL developed, and was reviewed and approved by the MHRA as part of the Christie Clinical Trial Application which was approved in June 2013. Once the trial was opened by the Christie in Sep 2014 this resulted in the manufacture of 4 trial batches of which two where infused as part of Trial 1 prior to the trial being put on hold.

Prior to the trial being put on hold, a technology transfer process was underway between CTL and AmBTU enabling the proposed production process and Quality Control of NY-ESO-1 specific T-cells to be transferred from CTL to AmBTU. This work would ultimately enable multicentre manufacture of NYESO-1 T-cells. The lack of available NY-ESO-1 TCR virus held up process validation at the AmBTU site as well as submission of an update to the IMPD to add AmBTU as an additional manufacturer of NY-ESO-1 T-cells.

The process for the second arm of Trial II was developed through two successful engineering batches and was held up after 3 validation runs which subsequently identified stability issues with the new activation reagent called TransAct. While MILTENYI had resolved the stability issue the validation was not completed due to both the Trial 1 hold and ultimately the programme hold.

In summary the work on tasks and deliverables related to the development and validation of the manufacturing process for Trial I were complete, with 4 batches successfully produced for patients within Trial I. The consortium had planned to complete Trial I, with some modifications, following review of patient data from the first two patients treated and the clinical protocol. Development and validation of the manufacturing process for Trial II was also largely complete. The legacy of the work on the manufacturing process and assays completed within the ATTACK project will enable the investigation of NY-ESO-1 in oesophageal-gastric cancer to continue.

The development of an automated process for cell selection, activation and transduction based on the MILTENYI Prodigy system

The development of an automated process for cell selection, activation and transduction based on the MILTENYI Prodigy System can potentially streamline the manufacturing steps during the most labour intensive and process critical procedures and ultimately provide a simplified and therefore more reliable manufacturing process whilst maintaining an equivalent final product.

To assess the impact of current and novel activation systems for potency, expansion, phenotype and transcriptome to identify impact of activation on cell characteristics and function we set up T-cell isolation, -stimulation and -transduction processes from frozen leukapheresis.

Given the published data showing that naïve and central memory T-cells are preferential subsets for adoptive T-cell therapy, we focused on the isolation of CD62L positive cells (containing the indicated T-cell subsets). In order to further automated process steps of the manufacturing of gene-engineered T-cells we also developed a novel soluble activation reagent that can be sterile filtered and biodegradable making it more suitable for automation. We worked on optimized culture conditions as well as dedicated tubing set.

After having developed stand-alone processes for the selection, activation and transduction of T-cells, MILTENYI developed a program integrating T-cell selection from blood products, activation of the enriched T-cells and gene-modification via lentiviral vector in a single device platform. The transduced T- cells can be further expanded for up to 14 days in the Prodigy tubing set. We also developed one dedicated tubing set allowing for the GMP compliant manufacturing of gene-modified T cells in a single use, closed system. Currently the process gives robust results with cells from healthy donors. Further work is required to verify robustness of the automated process using patient material (and potentially implement adaptations if required).

With regard to the development of an automated process for cell selection, activation and transduction and assess comparability of activation methods, all reagents meant to be implemented in the automated clinical manufacturing process of improved gene-

modified T-cells have been tested. Using clinical grade reagents, T-cells (from frozen leukapheresis) can be enriched for CD62L, are efficiently stimulated with a new activation reagent called TransAct and expanded in TexMACS medium, with IL-7 and IL-15. Optimal conditions for T-cell stimulation, expansion and transduction have been assessed and determined. The workflow for the cell manufacturing has been streamlined for transfer to automation development in the prodigy platform. Novel tubing sets, more adapted to the workflow have been designed and produced.

CD62L T-cell selection by the Prodigy was assessed and compared to standard CliniMACS selection. Comparability testing between CliniMACS plus and Prodigy for CD62L enrichment has been performed successfully using a generic enrichment program available on the Prodigy platform. Successful enrichment of CD62L positive cells requires at least labelling and washing steps under cold conditions (using pre-cooled buffer to avoid shedding of CD62L). Therefore we adapted the specific TCT software application regarding automated enrichment on the CliniMACS Prodigy under cold condition and generated comparative performance data for the semi-automated process on the CliniMACS Plus (manual cell preparation prior enrichment). In independent runs it was shown that using the CliniMACS Prodigy in combination with the optimized software application resulted in comparable or better purity and viability of target cells (CD62L+ among CD45+). To test reproducibility of the automated selection process on the CliniMACS Prodigy, enrichment of CD62L cells was then performed using different cell sources: either apheresis or buffy coats were used. Comparable results regarding purity and viability of target cells (CD62L+ among CD45+) were observed with differences in recovery resulting from the heterogeneity of the cell source. Therefore it is recommended to use apheresis product (focused on mononuclear cells) for the enrichment of CD62L+ cells. In summary, overall enrichment on the CliniMACS Prodigy enables an automated process that reduces user interactions to a minimum and increases usability for clinical compliance of cell manufacturing (e.g. no open steps required for cell preparation prior enrichment). Furthermore, the developed software application contains routines to handle possible failures, which will increase the success of target cell enrichment prior activation, transduction and expansion.

Generic formulation and harvest programs for gene-modified and expanded T-cells by the Prodigy have been generated and are functional. A formulation program has also been integrated in the fully automated process so that at the end of the manufacturing run, cells can be reformulated in the desired buffer. All cells are then harvested into a dedicated bag. Specific dose preparation must be done manually.

The novel T-cell stimulatory reagent developed for its implementation in automated platforms has been compared to other stimulatory reagents available for clinical manufacturing. All reagents tested performed equally well and allowed efficient lentiviral and retroviral transduction of enriched T-cells. The Transact Reagent proved to be an efficient tool for the stimulation of enriched T-cells prior transduction especially in closed system thanks to the fact that it is compatible with sterile filtration. Protocols have been optimized e.g. fine-tuning of the time-point for lentiviral transduction to achieve sufficient numbers of transduced T-cells in a maximum of 14 days manufacturing process. Furthermore we can demonstrate the removal of the TransAct Reagent from the cell preparations via simple washing is possible and a complex bead removal process as required for DynaBeads is no longer necessary. As a result, we focused on the automation of the TransAct stimulation on

the CliniMACS Prodigy including the development of optimized protocols regarding stimulation dose or seeding cell density for the enriched CD62L population. In addition, safety aspects for the TransAct Reagent such as dose curve responses were addressed in vitro to ensure safety for clinical applications. Optimization of transduction after polyclonal T-cell stimulation includes determination of best dose and time-point for transduction. The TransAct stimulation reagent was compared with ExpAct or DynaBead (large bead based stimulatory reagents). For all polyclonal stimulation reagents best transduction efficiencies were reached if T-cells were transduced 24h after stimulation when using lentiviral vectors. The given example shows transduction efficiencies (% CD3+ GFP+) of the TransAct stimulated T-cells. Using optimized protocols for the fully-automated enrichment, stimulation and transduction on the CliniMACS Prodigy using the TransActs, we demonstrated that the manufacturing of gene-modified T-cells in the CliniMACS Prodigy resulted in better transduction efficiency compared to the manual process: higher frequency and intensity (MFI) of GFP+ cells were obtained in the CliniMACS Prodigy although cells were treated in a similar way for the small scale (manual process) or TCT (automated Prodigy process). Fully automated manufacture runs of gene-modified T-cells on the CliniMACS Prodigy indicated a comparable T-cell expansion for the full-automated (Prodigy) or manual (small-scale control) manufacturing process of CD62L selected, polyclonal stimulated (TransAct) and lentiviral transduced T-cells. In addition no differences in expansion of lentiviral modified or non-transduced T-cells were observed.

Note: We first focused on the finalization of the lentiviral transduction protocol for the CliniMACS Prodigy before we started to adapt the protocol towards retroviral transduction. First feasibility runs indicate that the process we have developed for lentiviral transduction is suitable for the retroviral modification of selected T-cells although some changes and optimizations are required for the later.

Proof of principle that T-cells can be expanded in the Prodigy has been obtained. Expansion of CD62L enriched T-cells and optimization if the process was assessed. We were able to develop a robust manufacturing process for gene-modified T-cells on the CliniMACS Prodigy. Using this process we demonstrated that no differences in analysed parameter such as absolute cell counts, viability, T-cell phenotype, cell composition or function can be observed comparing transduced to non-transduced T-cells. Moreover, gene-modified T-cells manufactured using the TCT program on the Prodigy displayed convincing on target cytokine secretion and cytotoxicity. Also, we demonstrated that an expansion of CD62L selected cells in the CliniMACS Prodigy resulted in very high final T-cell frequencies independent of genetic modification via lentiviral transduction. Furthermore we were able to develop an expansion protocol which enables manufacturing of a favourable central memory T-cell phenotype. Low frequencies of naïve and effector memory T-cells were determined after polyclonal stimulation, transduction and expansion of gene-modified T-cells. Robustness of manufacturing process of either transduced or non-transduced T-cells was demonstrated and final cell counts with a total of up to 4x1E09 cells with high viability have been obtained.

In summary, we have developed novel strategies for the enrichment of effective T-cell subsets and optimized their activation, gene-modification and expansion using newly co-developed soluble activation reagents, culture conditions (i.e. TexMACS medium + IL-7 and IL-15) and a dedicated closed single-use tubing set where all steps

were integrated in a stepwise approach. We finally managed to develop a process on the CliniMACS Prodigy which integrates all essential steps for clinical-grade manufacturing of gene-modified T -cells. Automation of the manufacturing process is economically advantageous. This will contribute to the commercial realisation of adoptive cell transfer therapy. Moreover, user interactions and open handling steps for the T cell transduction process were reduced to a minimum to increase safety for each run making a complex procedure more reliable.

3.3 WP3 Laboratory Monitoring

WP3 "*Laboratory Monitoring*" focused on assay standardization and the efficient monitoring of the trial patients with the aim of determining key markers of therapeutic benefit and potential predictive markers of response. Within the objective following tasks were defined:

1. The assessment of patient eligibility - HLA-A2 status and NY-ESO-1/LAGE expression;
2. Monitoring of immune status and persistence of infused NY-ESO-1 TCR engineered cells in patients
3. Analysis of general immune and T-cell specific parameters in blood, tumour and pre-infused T-cells

Main activities and results:

The screening assays to assess positivity of expression of NY-ESO-1/LAGE-1 have been established and validated, as has the validation of flow cytometry (FCM) NY-ESO-1 tetramer staining assays and gene-modified T-cell specific real-time PCR assays. The detailed protocols have been made available for participating clinical sites for local implementation.

Screening assays were applied on 39 patients, of which 4 were HLA-A2 positive and had a tumour that expressed NY-ESO-1 and/or LAGE-1. Presence of NY-ESO-1 TCR T-cells in patient blood was assessed in two treated patients using both FCM and qPCR methods.

Furthermore we have standardised the FCM research assays for quantitation and assessment of differentiation and activation status of NY-ESO-1 TCR T-cells prior to infusion and in patient blood, including markers for T-cell differentiation (i.e., CCR7, CD45RA, CD95); T cell function (e.g., CD107, IFNg, GrB) and exhaustion (e.g., PD1, LAG3, TIM3).

An archive for storage of patient material for future research has been established at EMC. Due to early termination of the project no data have been generated yet with respect to the additional research questions.

As per 26 June 2016 (termination of project):

- Task 1: methods are defined, validated (at UNIMAN/CHRIS) and made available for participating clinical sites for local implementation.

The IHC NY-ESO-1 and LAGE antigen screening of patient tumour specimen has only been active at UNIMAN/CHRIS. Thirty nine patients were screened, of which 4 were both HLA-0201A positive and NY-ESO-1 or LAGE-1, and two were actually treated.

- Task 2: flow cytometry (FCM) and quantitative, real time polymerase chain reaction (qPCR) methods for detection of NY-ESO-1 TCR engineered T-cells in patient blood were based on validated methods in place at ADAPTIM and were validated and implemented at EMC (FCM) and UNIMAN (qPCR).

  Blood samples of the 2 patients treated at CHRIS are processed for qPCR detection of NY-ESO-1 TCR T cells at UNIMAN/CHRIS. FCM detection of NY-ESO-1 TCR T cells in these patients was performed at ADAPTIM).

  No products have been sent to EMC yet for detailed analysis of immune parameters.

- Task 3: FCM and IHC method are defined and implemented at EMC.

  No products have been sent to EMC yet for detailed analysis of immune parameters

Detailed results/achievements

Assessment of patient eligibility

Enrolment onto each trial requires patients to be HLA-A2 positive and their tumour to be NY-ESO-1 and/or LAGE positive. HLA-A2 typing was recommended according standard local procedures.

The immunohistochemical (IHC) detection of NY-ESO-1 and LAGE antigen in tumour sections has been set up and validated, including indication of expression score, at UNIMAN/CHRIS.

Assay Development: 102 cases – comprising a mix of gastric and oesophageal specimens, both biopsy and excision specimens fixed in neutral buffered formalin and processed into wax. 3-4 micron thick sections on charged slides prepared for immunohistochemistry. The monoclonal mouse anti-NY-ESO-1 (Invitrogen Cat No 35-6200) Clone E978, was used at a dilution of 1:200 (or 2.5 µg/ml) and the polyclonal rabbit anti-CTAG2 (LAGE) (Biorbyt Cat No orb101530) used at 1:300 (or 1.66µg/ml). Sections of normal testis were used as positive control for both LAGE and NY-ESO-1. Following routine dewaxing and rehydration protocols, antigens for NY-ESO-1 and LAGE (CTAG2), were retrieved using heat mediated antigen retrieval (HMAR), pH 9.0 Leica Bond epitope retrieval solution for NY-ESO-1 and pH 6.0 Citrate buffer for LAGE. Antibody binding was then visualised using Dako EnVision detection kit (Dako, K5007) according to the manufacturer's recommendations. Other detection systems were evaluated but were found to be suboptimal (Ventana Ultra View, Leica Bond Polymer detection kit).

Interpretation: Of the 102 cases employed in the detection study, 97 were evaluable for NY-ESO-1 and 91 for LAGE (Table 1). Failures were due to inability to repeat

(no spare sections), atypical staining or disagreement between two independent scorers. Staining was judged to be negative (score 0), weak (score 1), moderate (score 2), or strong (score3). In cases where interpretation was mixed, an average score was given (for e.g. weak/ moderate score = 1.5).

Score	NY-ESO-1 n = 97	LAGE n = 91
0 (negative)	35	12
1 (weak)	36	25
1.5	5	1
2 (moderate)	10	27
2.5	2	9
3 (strong)	9	17

Table 1: Detection study -NY-ESO-1 and LAGE interpretation

Per July 2014 the detailed protocol was distributed to participating clinical sites for local implementation (EMC, CHU NKI-AVL, and OSR). Local implementation at clinical sites included verification and interpretation of staining by a dedicated pathologist at CHRIS.

Conclusion: The IHC detection of NY-ESO-1 and LAGE antigen in tumour sections has been set up and validated, including indication of expression score. Distribution and mutual expression of both NY-ESO-1 and LAGE are documented. The detailed protocol has been put available for participating clinical sites for local implementation.

The IHC NY-ESO-1 and LAGE antigen screening of patient tumour specimen has only been active at CHRIS.

Peripheral persistence of gene-modified T-cells

Peripheral persistence of gene-modified T-cells was assessed by both FCM (EMC) and qPCR (UNIMAN) on processed and stored peripheral blood mononuclear T-cells (PBMC). Methods for collection, processing and storing PBMC for these assays were defined and implemented at UNIMAN and EMC, and will be communicated to the other participating clinical sites. Standing Operating Procedure (SOPs) include: *SOP-95/1: Laboratory Manual for ATTACK-OG trial (adapted 150401); FMR-001: Clinical Trials Sample Tracking Log*, are in place. The FCM and qPCR assays are validated and implemented at EMC (FCM) and UNIMAN (qPCR).

EMC (FCM): The FMC assay for detection of NY-ESO-1 TCR T-cells in patient blood was validated on 1) fresh peripheral whole blood samples; 2) freshly isolated PBMC and 3) cryopreserved and thawed PBMC. Healthy donor blood samples were spiked with varying numbers of NY-ESO TCR T-cells, processed as described and assessed for detection of the spiked NY-ESO-1 TCR T-cells. From these experimentations we concluded:

1. The FCM assay to quantify NY-ESO1 TCR T-cells is specific, accurate and shows a linear relationship between spiked and detected numbers of NY-ESO-1 TCR T-cells;

2. Recovery of NY-ESO-1 TCR T-cells in whole blood, isolated PBMC and cryopreserved/thawed PBMC varies typically between 60 – 130% in specimen spiked with ≥ 0.1% NY-ESO TCR T-cells (related to % CD3 T-cells);
3. Recovery of the NY-ESO1 TCR+ T-cells is similar in whole blood versus in isolated PBMC -/+ cryopreservation: thus there is no loss of NY-ESO-1 TCR T-cells due to processing and cryopreservation of isolated PBMC;
4. The sensitivity of FCM assay is 0.1% NY-ESO TCR+ T-cells within the CD3+ T-cells.
5. From a concentration of 0.1% NY-ESO TCR+ T-cells (of CD3+ T-cells) onwards the assay generates linear results.

UNIMAN (qPCR): The qPCR assay for detection of NY-ESO-1 TCR T-cells in patient blood was transferred form ADAPTIM and ran on site using controls supplied by ADAPTIM. Healthy donor blood samples were spiked with varying numbers of NY-ESO TCR T-cells and processed as described and assessed for detection of the spiked NY-ESO-1 TCR T-cells.

Quantification and activation status of immune subsets from PBMC

FCM panel for assessment of activation status of immune subsets of PBMC was realized and implemented at EMC, including FCM quantitation and assessment of differentiation and activation status of NY-ESO-1 TCR T-cells prior to infusion and in patient blood was validated on site on 1) fresh peripheral whole blood samples; 2) freshly isolated PBMC and 3) cryopreserved and thawed PBMC. These assays have been set up using different panels to define distinct T-cell subpopulations, including markers for T-cell differentiation (i.e., CCR7, CD45RA, CD95); T-cell function (e.g., CD107, IFNg, GrB) and exhaustion (e.g., PD1, LAG3, TIM3).

Assessment of anti-gene modified T-cell immune responses

Method will be based on the method reported for anti-CAIX CAR modified T-cell immune responses (Lamers et al, Blood, 2011, 117, p72-82). There have been no activities yet to this task.

Archive for future research

EMC: Procedures have been defined for archiving of cryopreserved PBMC and serum / plasma samples. Sample registration, tracking and tracing is performed by the Sample Vision program (Linde gas, Cryoservices inc. Hedel, The Netherlands).

PBMC samples are stored in the gas phase of liquid nitrogen storage tanks; the serum/plasma samples in -80°C freezers. Both liquid nitrogen storage tanks and -80°C freezers are under continuous surveillance of the Xiltrix registration, monitoring and alarm system (IKS, Rosmalen, The Netherlands).

(Standing Operating Procedure SOP-95/1: Laboratory Manual for ATTACK-OG trial (adapted 150401); FMR-001: Clinical Trials Sample Tracking Log are in place).

4. Potential Impact and Main Dissemination Activities

4.1 Potential Impact

The ATTACK clinical trial consortium builds on results from the EU FP6 ATTACK pre-clinical project and EU FP7 ATTRACT training network. Adoptive cell therapy with engineered T-cells is a promising emerging technology with clinical application in the area of nucleic acid delivery for therapeutic purposes.

Before termination of the project, the potential impact of ATTACK was expected to be on several levels, with the most significant impacts being seen in the following area:

Adoptive Cell Therapy Clinical Trials

Success in these trials would enable the ATTACK members and others to carry out larger trials and potentially lead to an approved treatment for multiple cancer types.

The trials could extend evidence of activity to a common epithelial cancer and thereby facilitate testing of the construct in other epithelial cancer which can express NY-ESO-1

The trials would provide data in melanoma where there is already preliminary evidence of extraordinary activity.

Success in these trials would confirm activity in a multi-centre setting which is important as many previous trials have largely been single institutional studies. This would stimulate interest in exploring other TCR and CAR targets.

Clinical Trials Management

Manchester Academic Health Science Centre – Trials Coordination Unit (MAHSC-CTU) has previous experience in managing cell therapy trials, having managed a single centre, CAR T-cell trial and a single centre, TIL trial, both delivered at The Christie NHS Foundation Trust from 2006-2016. However, as a multi-centre and European adoptive T-cell trial, the ATTACK program has enabled each department within MAHSC-CTU to build on this pre-existing trial management expertise.

All departments within the trials coordination unit (data management, statistics, project management, monitoring) have now undergone further training and gained greater experience in setting up and managing complex ATMP trials. As a result, the procedures and processes used within the unit have been improved. ATMP management systems have been refined to improve ATMP handling logistics and traceability. Safety reporting procedures have been developed to expedite the reporting serious events related to the IMP to the relevant committees.

As an integral member of the consortium, the unit has been able to establish good working relationships between both the commercial and non-commercial partners. These relationships will ensure that the expertise within the unit will be utilised in future trials of this nature.

NY-ESO-1 TCR

A key aim of the project as a whole is to see available a licenced engineered T-cell therapy. ADAPTIM has the capability to take this treatment forward commercially.

The NY-ESO TCR therapeutic used in the ATTACK study targets the NY-ESO-1 peptide which is present in multiple cancer types. In addition to the use of the NY-ESO therapy used to treat oesophageal patients in the ATTACK study, the same therapeutic TCR is used within ADAPTIM's NY-ESO SPEAR T-cell which is currently in multi-indication trials in the United States. Pilot studies are on-going in synovial sarcoma, melanoma, multiple myeloma, non-small cell lung cancer ("NSCLC") and ovarian indications and a trial in myxoid round cell lyposarcoma is due to start to late 2016 and early 2017. The trials are on-going in both solid tumours and hematologic cancer types and in cancers where survival rates for patients can be very limited. Patients that are being treated in the N-ESO SPEAR T-cell trials or in the ATTACK study often have limited or no other options for treatment. ADAPTIM's NY-ESO SPEAR T-cell therapies have already shown preliminary evidence of tumour reduction in patients and also show a promising risk/benefit profile. The ATTACK study is the first study in which an NY-ESO TCR therapeutic has been evaluated for the treatment of patients with advanced gastro-oesophageal cancer, another solid tumour.

Cell Processing – Process Development

The successfully developed production protocol used in the ATTACK consortium is of great value for society, contributing to the availability of T-cell therapy for European cancer patients. It can be used in future clinical trials using T-cells modified with other T-Cell Receptors (TCRs) or Chimeric Antigen Receptors (CARs). The tumor types for which T-cell therapy can be useful are nowadays numerous and expanding (e.g. solid tumors like melanoma, NSLC or ovarian cancer and hematological disorders like AML and CML).

Cell Processing – Selection Technology

Successful testing of the optimised method of cell production in trial II, developed by MILTENYI, could open up large markets for this product as the approach could be established as the new international standard.

Cell Processing - The development of an automated process for cell selection, activation and transduction based on the MILTENYI Prodigy system

Automation in conjunction with comprehensive protocols leads to simplification and therefore to a better access of a therapeutic approach while saving time and costs.

Within ATTACK integrated processes were refined to provide results in a shorter period of time and automation reduced manual handling steps. As the CliniMACS Prodigy enables cell processing in a closed system, the clean room requirements will be reduced in comparison to processes with open handling steps, which will also positively impact manufacturing costs significantly. Thereby, reduced personnel costs

and expenses for GMP laboratories will maximize access to this therapeutic approach otherwise available only in few specialized clinical centers worldwide.

Facilitating access to such cell therapy technology is ultimately meant to meet the growing need and demand of patients. Automation with the CliniMACS Prodigy will allow for scale-up of manufacturing processes for commercial use, e.g. when hundreds to thousands of cell therapeutic doses per year are required to perform phase II/III clinical trials with the goal of FDA approval.

To further support such dissemination, technological improvements made within ATTACK were undertaken with cost reduction in mind; as an important success factor for highly individual cellular therapies will be cost, which has to be compatible with health systems.

This project allowed us to address all the fundamental steps required to shift a complex highly manual process towards a robust and fully automated manufacturing of gene-engineered T-cells on a single dedicated platform, the CliniMACS Prodigy.

Biotechnology Industry

The inclusion of a major industrial partner focussed on cell selection technology, along with two SME's who focus on the delivery of cell therapy, as well as major cancer centres with expertise in the field will enhance European expertise and competitiveness in an important emerging market. The research will thus support the European biotechnology industry especially the SME's in this nascent field and ensure the clinical potential is rapidly developed building on excellent scientific expertise in this field in Europe.

Gender Dimensions

Cancer is a common disease that affects both men and women. Our primary targets covered tumours that are common in both men and women. In general, both men and women are keen to take part in this form of research. Whilst we did not anticipate any gender bias, this was monitored. Of the thirty-nine patients pre-screened for HLA status and NY-ESO-1 positive tumours, 28% (11/39) were female.

All of the partner of ATTACK and their legal affiliations work towards gender equality in recruitment. An ATTACK Gender Equality and Action Plan has been developed. This charter sits in a national, European and international context in order to promote good working practice for both men and women in an academic environment. The ATTRACT consortium has set out different objectives to meet the gender action plan and improve gender equality within the field of adoptive cell therapy:

- All of the ATTACK partners and their legal affiliations work towards gender equality in recruitment and promote gender equality awareness

- to encourage all ATTACK members, irrespective of gender, to participate in conferences and workshops

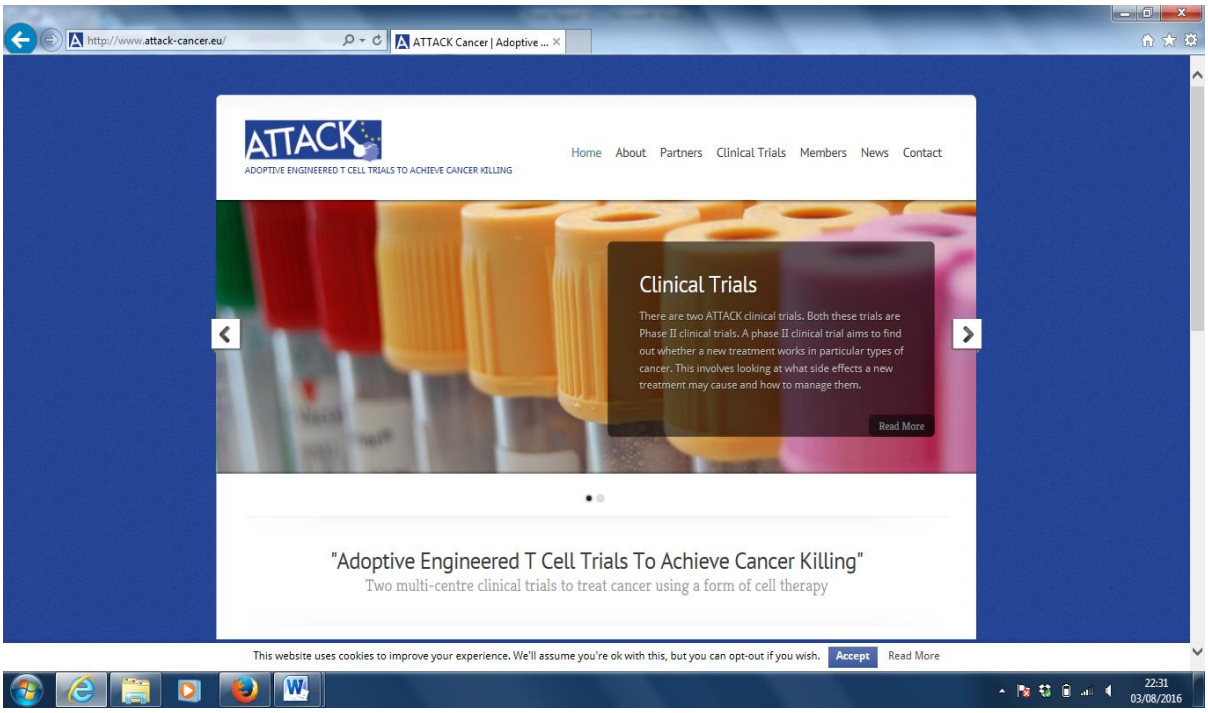
- promotion of participation in committees and working groups for female researchers
- monitor training provision by gender
- provision of family friendly working conditions

4.2 Dissemination Activities

Central to ATTACK is the dissemination of the project to the outside world. Wide dissemination is crucial, promoting knowledge sharing to the oncology community, to patient organisations and to the public as a whole. The FP7 ATTTACK Consortium has developed out of other EU consortia working on related topics. There is thus already a good infrastructure for dissemination in place.

4.2.1 Development of the Project Website

Launched October 2013, the ATTACK website (http://www.attack-cancer.eu/) serves as a central tool for communication and dissemination purposes, providing relevant information on ATTACK activities and objectives, allowing public as well as restricted access.



The structure of the website is designed in a clear and consistent way so that visitors and users can easily navigate around the site.

Public Section

This area provides an insight into the project background in lay terms; details of consortium members and contact points, description of the ATTACK clinical trials and aims of the research activities.

Public queries can be sent via emails to the Project managers. This will then be redirected to appropriate clinical personnel if the matter pertains to a clinical matter or contact details of suitable cancer help organisations can be distributed.

Members Area

A secure password protected portal has been initialised from the ATTACK website folders for internal access. A link on the menu bar to the members section of the ATTACK website is located on the home page of the website.

The intranet fits the following purposes: to convey information and archiving of essential documents for the consortium, the project scientific members to find agendas, minutes of meetings, project projections, quarterly WP progress reports and other resources. This allows the flow of emails directed to the Consortium to be sized down and to be minimised.

4.2.2 Publications

All of the ATTACK personnel have an excellent publication track record and we anticipated that as the project progressed, there would be an increase in joint publications by partners as well as continued high-quality publications by individual groups. During the course of the project, 15 ATTACK related papers have been published by Consortium members.

4.2.3 Presentations

As highlighted, wide dissemination is important for maximum impact. The ATTACK project has been presented at over 100 EU, national and international meetings and conferences, in the fields of immunology, cancer and gene therapy.

4.2.4 Meetings

An important dissemination achievement has been the successful 4th Cellular Therapy of Cancer Symposium which was held in London in March 2013. It was organised by The Society for Cellular Therapy of Cancer, founded by members of the European Commission Framework 6 funded ATTACK Consortium and the European Commission funded Framework 7 consortiums of ATTACK and ATTRACT. The goal of the symposium is to bring together world leaders in basic and translational immunology to present research concepts and clinical studies relevant to the development to the development of T-cell therapy of cancer. The programme included an outstanding group of speakers in cellular therapy research. The meeting highlighted the fast pace at which this field is currently moving and the need for a further understanding of the cellular and molecular basis of the interplay between

tumours and redirected immune cells. The symposia also underscored the importance for strong international collaboration, favoured by EU-like consortia and for standardized GMP procedures and immune-monitoring protocols.

4.2.5 Co-operation with other grants/programmes

There are many valuable partnerships between industry and the academic institutions participating in the project. ATTACK fosters these partnerships, which in turn stimulates significant advancement and collaborative activity both now and into the future.

For example, in May 2014, ADAPTIM entered into a strategic collaboration and licensing agreement with GlaxoSmithKline (GSK – a British company) for the development and commercialisation of its lead clinical cancer programme NY-ESO-1. Under the terms of the agreement, ADAPTIM will co-develop its NY-ESO-1 clinical programme and associated manufacturing optimisation work together with GSK. All efficacy and safety data from trials involving ADAPTIM's NY-ESO-1 T-cell receptors, including data from ATTACK trials, will contribute to the clinical proof of concept to enable GSK to continue development and commercialisation of this therapy.

Other productive ATTACK collaborations are highlighted in Table 2:

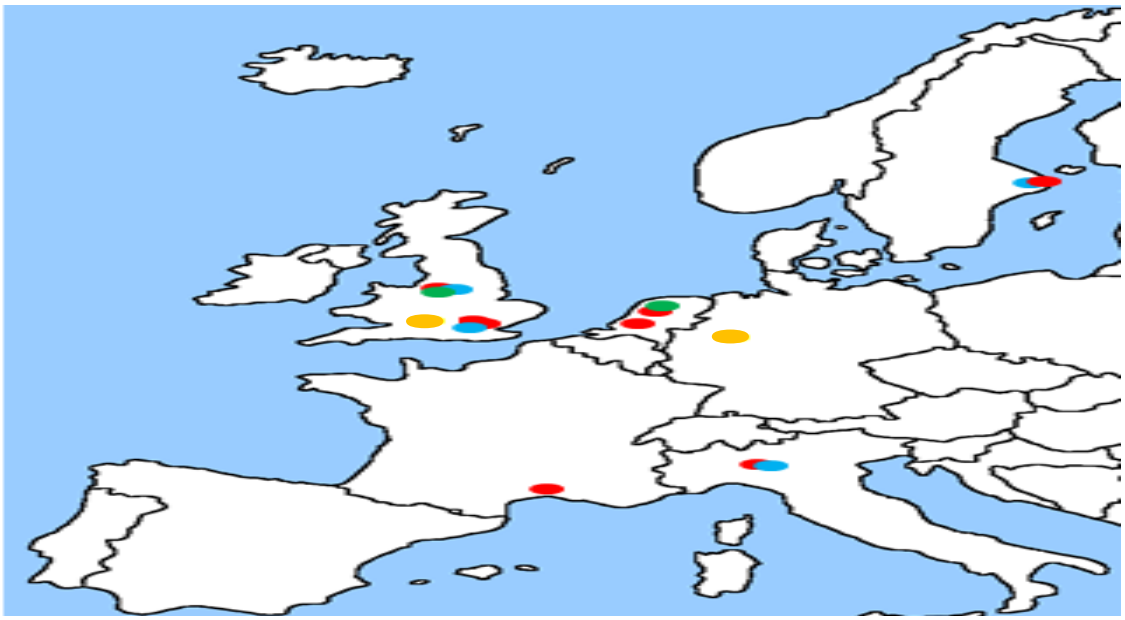
ATTACK Partner	**Collaborator**	**Purpose of Partnership**
EMC	Department of Neurosurgery, EMC	Oncolytic adenovirothearpy in glioblastoma
EMC	Department of Urology, EMC	Oncolytic adenovirotherapy in prostate cancer
EMC	Department of Hematology, EMC	Graft predominance in double umbilical cord blood transplantation
EMC	Department of Pulmonary Diseases, EMC	T-cell activation and co-signaling FCM panels to monitor (immune) therapy strategies in patients with mesothelioma and lung cancer
INSERM	SIRIC, Montpellier, France	Integrated site for clinical trials in cancer research
CTL	ASYMPTOTE	Large volume freezing device
CTL	Sphere Fluidics	Transduction efficiency

Table 2: ATTACK Collaborations with Other Grants/Programmes

5. ATTACK Partners

This project builds upon previous EU funded pre-clinical projects (ATTACK FP6 and ATTRACT FP7) and comprises a multidisciplinary and translational research group with wide-ranging, relevant expertise. Located across 6 countries in the EU, there are 8 hospital sites, 2 cell manufacturing sites, 6 Universities and 4 companies involved in the project.

5.1 Location of ATTACK Partners



Clinical Sites:
Higher education/research organisation:
SME Cell Manufacturing Sites:
Industry:

5. 2 ATTACK Consortium

Partners	Organisation Type	Lead Personnel	Web Address
The University of Manchester UK	University	**Robert Hawkins** **David Gilham** **Helena Kondryn** Cell therapy grants management. Cell therapy translational research with excellent laboratory facilities.	http://www.manchester.ac.uk/
Erasmus Universitair Medisch Centrum Rotterdam Netherlands	University & End User	**Reno Debets** **Stefan Sleijfer** **Cors Lamers** Immunotherapy trial expertise. Major cancer centre with cell therapy trials experience.	http://www.eur.nl/
Karolinska Institutet Sweden	University	**Rolf Kiessling** Immune monitoring.	http://ki.se/start
The Christie NHS Foundation Trust UK	End User	**Paul Lorigan** **Fiona Thistlethwaite** **Was Mansoor** Melanoma/O-G Cancer expertise. Immunotherapy trial expertise. Major cancer centre with cell therapy trials experience. **Ian Emerson** (MAHSC-CTU) **Azad Aziz** (MAHSC-CTU) MAHSC-CTU: Trial setup, conduct and delivery management for Trials I and II	http://www.christie.nhs.uk/ http://www.mahsc-ctu.co.uk/
Institut National de la Sante et de la Recherche Medicale France	University & End User	**Naomi Taylor** Immunotherapy trial centre. Immune monitoring.	http://www.inserm.fr/
Cellular Therapeutics Limited UK	SME	**Ryan Guest** **Nicola Price** A dedicated cell therapy company, GMP and clinical trial experience.	http://www.cellulartherapeutics.co.uk/
Royal Free Hospital, Royal Free Hampstead NHS Trust UK	End User	**David Chow** Immunotherapy trial expertise. Major cancer centre with cell therapy trials experience.	http://www.royalfree.nhs.uk/
Miltenyi Biotec GMBH Germany	Industrial	**Andrew Kaiser** A large company with expertise in cell selection methodology. Focus on development of technology for cell therapy.	http://www.miltenyibiotec.com/en/

Table 5.2 contd ATTACK Consortium

Partners	Organisation Type	Lead Personnel	Web Address
Stichting Het Nederlands Kanker Instituut – Antoni Van Leeuwenhoek Ziekenhuis Netherlands	University & End User	**John Haanen** **Ton Schumacher** T cell therapy translational research and clinical trials. Major clinical centre for immunotherapy of melanoma.	http://www.nki.nl/
Amsterdam Biotherapeutics Unit Netherlands	SME	**Baastian Nuijen** **Joost Van Der Berg** SME focusing on the process development and GMP manufacture of biopharmaceuticals for investigational clinical use	http://www.ambtu.nl/
Adaptimmune Limited UK	SME	**Helen Tayton-Martin** **Dan Williams** Biotechnology company developing engineered T-cell therapy in oncology and infectious disease	http://www.adaptimmune.com/
University College London Hospital NHS Foundation Trust UK	End User	**Daniel Hochhauser** Immunotherapy trial expertise. Major cancer centre with cell therapy trials experience.	http://www.uclh.nhs.uk/
Stockholms Lans Landsting Sweden	End User	**Mats Engström** Clinical trials with immune and cell therapy.	http://www.sll.se/
University College London UK	University	**Hans Stauss** **Emma Morris** Immunotherapy trial expertise. Major cancer centre with cell therapy trials experience.	http://www.ucl.ac.uk/
Ospedale San Raffaele Italy	University & End User	**Anna Mondino** **Chiara Bonini** Immunotherapy and cell therapy trials in melanoma and leukaemia, lymphocyte characterization and manipulation.	http://www.hsr.it/

6. Address of the Project Public Website and Key Contact Details

For further information and access to public project documentation, please see http://www.attack-cancer.eu/

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