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Alcyomics®

Assessing the safety and Graft versus Host Disease (GvHD) reactivity of ATMPs

Funded by

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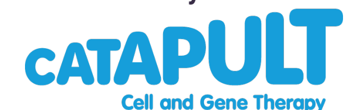


1.0 COVID-19 Project in Collaboration with SNBTS

1.1 Introduction

Pre-emptive therapy with antiviral drugs, for viral infections especially in the immunosuppressed, including post-Haematopoietic Stem Cell Transplant patients, is associated with drug resistance (1) and toxicity (2,3). Adoptive immunotherapy to restore antiviral immunity, pioneered by Riddell and colleagues (4), is an alternative treatment for patients with refractory viral infections after failure of standard therapies. Adoptive virus-specific cytotoxic T lymphocytes (CTLs) can successfully reconstitute cellular antiviral immunity by reducing the viral load and the severity of virus disease in HLA-matched and HLA-partially-matched recipients (5,6). Despite these promising results, *de novo* or exacerbated GvHD remains a potential risk associated with the adoptive cellular therapy in unrelated HLA mismatched recipients. In the last 2 decades, the purity of isolated virus-specific CTLs has significantly improved (7,8) minimizing the risk of alloreactive GvHD. Most clinical trials have reported the reconstitution of antiviral immunity after adoptive transfer of, for example, cytomegalovirus (CMV)-CTLs without major complications, although small subgroups of patients had onset or aggravation of pre-existing GvHD (9-14).

No strong correlation between the cell dose and the success of antiviral immune-reconstitution has been observed (15). Very low doses of anti–Epstein-Barr virus (EBV)–specific T cells, (range, 150 to 53,796) $CD3^+$ T cell/kg, have been reported to efficiently clear the viremia (15).

These results suggest that increasing the purity of antiviral T cells and reducing the cell-dose might reduce the risk of GvHD while restoring the antiviral immunity. Alcyomics has previously used their *in vitro* human skin explant test (Skimune®) to predict GvHD prior to clinical trial (15,17) and to confirm the safety and specificity of anti CMV T cell products, showing only minimal GvH type lesions in recipient skin biopsies and reduced cytokine levels, especially interferon gamma (IFNγ) (17). The Scottish National Blood Transfusion Service (SNBTS) has a track record in developing allogeneic cellular therapies including mesenchymal stem cells (MSC) and virus-specific T cells. These products are made from starting materials from healthy donations and are matched to the recipient at least the blood group level (as for organ transplantation), or at the HLA level for T cells. These products are commonly only partially HLA-matched to the recipient, and it is important to determine whether the HLA disparity carries a risk of GVHD and/or that the partially matched cells will be rejected by the recipient, reducing the efficacy of the cellular product. Alcyomics technology is well suited to testing donor/recipient mis-matches.

SNBTS is currently developing donor-derived T cell therapies for COVID-19 from third party allogeneic donors (18). Anti-SARS-Cov-2 T cell products can be used in the treatment of COVID-19 by boosting the immune system in those patients unable to respond adequately to the disease. Alcyomics technology is being used to rapidly assess potential safety of the cells before use in a first in man clinical trial. The data from the study will form part of the scientific information dossier as supporting evidence for the safety profile of the cellular product. An additional aim will be to submit a research publication on the outcome of the study. Here we report initial results from the study.

1.0 COVID-19 Project in Collaboration with SNBTS

1.2 Methodology

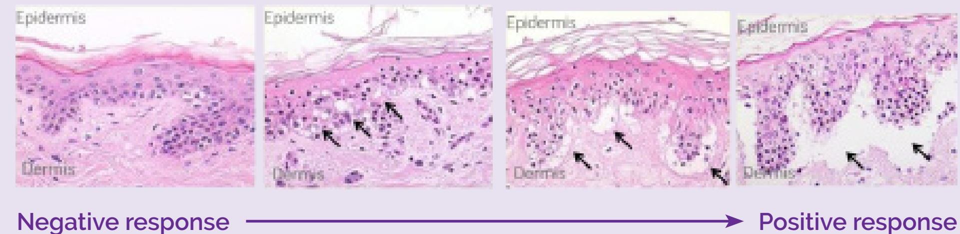
Alcyomics Skimune® assays, used 3rd party healthy volunteer blood samples and skin biopsies to compare the reactivity of the SARS-Cov-2- specific T cells, supplied by SNBTS, with non-specific 3rd party cells using the same healthy volunteer skin biopsies. Mixed lymphocytes reactions (MLR) were performed between the COVID -19 specific T cells and unmanipulated donor cells (as a positive control) and the non-specific 3rd party cells. The responder cells, after 7 days mixed lymphocyte culture (MLC) were co-cultured with 3rd party skin biopsies. MLC cell proliferation was assessed by [^{3}H]-Thymidine uptake and supernatants were collected for cytokine release. Cells were harvested and counted using a β–scintillation counter.

A minimum of 3 different donors were used to test each batch of COVID- 19 specific T cell product and a total of 6 batches were tested. The cell culture supernatants were analysed using MSD Multi-Spot Proinflammatory panel 1 V-Plex kit (Meso Scale Diagnostics) following manufacturer's instructions. The biomarkers included in this panel were IFNγ -, IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12p70, IL-13 and TNF-α. Human leukocyte antigen (HLA) typing and ABO blood group typing was performed on all donor samples.

Data comparison for the T cell proliferation assay was made between unmanipulated peripheral blood mononuclear cells (PBMCs) and the COVID-19 specific T cells (virus specific T cells -VST). Statistical analysis was carried out using repeated measures one-way ANOVA.

For the Skimune® assays, negative controls consisted of co-culture of PBMCs and autologous skin alone and co-culture of the skin in medium only. The positive control was skin incubated with unmanipulated non-COVID-19 T cells (PBMCs). The endpoint of the assay was the histopathological damage observed in the skin tissue caused by exposure to the PBMCs or VST material. This output was measured as skin grades (grades I to IV) according to the severity of the skin tissue damage observed, where; Grade I is considered negative, with intact upper keratinocyte layer. Grade II refers to vacuolisation of the epidermis. Grade III shows severe damage of the epidermal layer, with initial separation of the epidermal and dermal layers. Grade IV refers to severe damage, with complete separation of the epidermis and dermal layer (Figure 1). Grade II or higher is regarded as a positive response. Statistical analysis was performed using a one-way repeated measures ANOVA with a Bonferroni post-test. All statistical tests were carried out using Prism GraphPad software (version 5). Statistical differences were considered significant if p value was <0.05.

Figure 1: Grading the reactivity the response in Skimune® skin explants

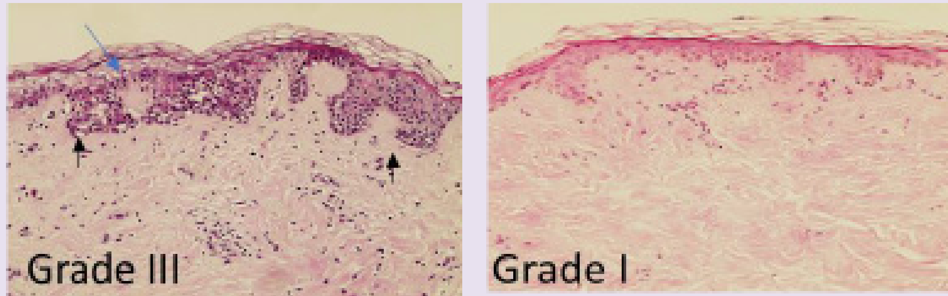


Negative response → Positive response

Skin is observed for histopathological damage (Grades I-IV, left to right in Figure 1) due to an immune response to the cellular product. The reactivity of the cellular product is performed using a clinical grading system as described by Lerner et al. Transplant Proceedings, 1974: p367. The grading process is performed blind by two members of the Alcyomics team trained in the grading process.

1.0 COVID-19 Project in Collaboration with SNBTS

Figure 2: Representative Skimune® histology where C19L4/PBMC shows the grade III positive response with unmanipulated cells and C19L4/VST shows the grade I negative response with COVID 19 specific T cells.



C19L4/PBMC **C19L4/ VST**

1.3 Results Summary

Exemplar COVID 19 specific T cells were tested in the Skimune® assays and T cell proliferation assays, with the addition of cytokine profiling. Six batches of unmanipulated cells (PBMCs) and manipulated cells (COVID-19 specific cells (VST)) were tested in n=3 experiments using 3 separate donors.

Five of six evaluable batches showed an overall negative response in the Skimune® assays and did not induce Graft versus Host type reactivity in the skin of HLA mis-matched third-party donors, i.e., gave rise to only background levels of minimal skin damage compared to unmanipulated non-COVID 19 specific T cells. One batch showed a positive response in the skin biopsies and also increased levels of T cell proliferation and a rise in cytokine release as measured in the cell culture supernatants were observed. These results were probably due to underlying non-permissible HLA mis-matches between the COVID specific T cells and skin biopsy donor. We are seeking to repeat these investigations with donors of known HLA-types in order to generate more data to support this observation. This will give key information about permissible and non-permissable HLA matches.

1.4 References

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2.0 Testing peptides loaded on tolerogenic dendritic cells for Safety prior to AuToDeCRA 2 clinical trial for patients with Rheumatoid Arthritis - in collaboration with Newcastle University

2.1 Introduction

Rheumatoid arthritis (RA) is an autoinflammatory disease, which manifests with joint pain and reduced mobility. Among the established risk factors are genetic predisposition and loss of immune self-tolerance, leading to increased inflammatory responses to autoantigens. A cure is not yet available, therefore this disease is managed with general immunosuppressants (i.e. methotrexate or biological medicines). Recent advances have seen immunotherapies at the forefront of novel treatment options for autoimmune diseases, that could bypass the harmful effects of general immunosuppressants. In this context, tolerogenic dendritic cells (tolDC) generated *ex vivo* have been assessed and approved for safety, in RA patients receiving autologous tolDC injected in the knee (AuToDeCRA 1 clinical trial, Phase 1 (1). The protocol for the generation of autologous tolDC has been previously developed at Newcastle University (2,3).

2.2 Purpose of current study

During AuToDeCRA 1, tolDC were loaded with synovial fluid as a relevant source of autoantigens. The trial showed that injection of these tolDC into an inflamed joint of RA patients was safe. However, because the identity of the autoantigens present in the synovial fluid was unknown, questions regarding the modulatory effects of tolDC on autoantigen-specific T cells in these patients could not be addressed. Given the encouraging results, an efficacy study will follow (AuToDeCRA 2). RA has a strong immune component and is characterised by the presence of autoreactive T cells, which are specific to autoantigens. Due to increased knowledge on autoantigens in RA since AuToDeCRA 1 was completed, for the next trial (AuToDeCRA 2) a cocktail of four previously characterised citrullinated peptides (cit-peptides) antigens will be used to load autologous tolDC during AuToDeCRA 2 (4). This will allow for immunomonitoring of cit-peptide-specific T cell responses before and after tolDC administration. The aim of the current study was to test the hypothesis that the cit-rullinated peptides were safe to use and cause no adverse reaction when loaded onto tolDC compared to loading onto mature (immunogenic) DC. It is hypothesized that the administered cells tolDC in the clinical trial, will down regulate inflammatory responses in a cit-antigen peptide- specific manner, with the aim of improving outcome in RA patients.

2.0 Testing peptides loaded on tolerogenic dendritic cells for Safety prior to AuToDeCRA 2 clinical trial for patients with Rheumatoid Arthritis - in collaboration with Newcastle University

2.3 Methodology

A full protocol to include RA patients was approved by the Local Ethics Committee prior to commencement of the study. A total of 7 patients were enrolled in the study. Whole blood was collected and a skin biopsy was obtained 14 days after blood withdrawal. A serum tube was also collected. Peripheral blood mononuclear cells, isolated from the blood sample were used to generate the mature (matDC) and tolerogenic dendritic cells (tolDC), as previously described (2), which were then left unloaded, or loaded with a cocktail of 4 cit-rullinated peptides. All cit-peptides were the same as those to be used in the AuToDecCTRA 2 clinical trial. All patients were HLA typed for HLA-DRB*0401 prior to commencement of the study.

The generated matDC and tolDC were harvested and characterised by flow cytometry prior to use in autologous mixed lymphocyte/DC co-cultures, T cell proliferation and Skimune® assays (5,6). All DCs were tested unloaded or loaded with the cocktail of cit-peptides. Supernatants were collected from Skimune® assays for cytokine analyses, prior to being processed for histopathological analysis. A total of 10 cytokines were measured with the V-PLEX Proinflammatory Panel 1 Human Kit (MSD).

Skin was fixed in formalin and routinely stained with haematoxylin and eosin. Tissue sections were then imaged and graded according to Lerner's classification grading system (7) from grade I to grade IV as previously described in Section 1.2.

2.4 Results Summary

The tolDC showed characteristic phenotypes, including decreased expression of the co-stimulatory molecule CD83 compared to matDC. In addition, tolDC showed lower levels of pro-inflammatory cytokines (e.g. especially IL-12p70 and IFNγ). In addition, upon co-culture with autologous lymphocytes, tolDC induced significantly lower levels of IFNγ than matDC.

5 RA patients were included in the study and peripheral blood lymphocytes pre-cultured with matDC (unloaded or loaded with cit-rullinated peptides) showed an increase of 1 or 2 skin grades when compared to the medium control. In comparison the majority of skin sections pre-cultured with tolDC (unloaded or loaded with citrullinated peptides) showed no change compared to the medium control. The results demonstrated that tolDC loaded with citrullinated peptides did not cause an adverse immune response, as shown by low levels of skin histopathological damage and low levels of cytokine release.

2.5 Acknowledgments

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3.0 Overall Summary and Conclusions

The Skimune® assay was used to assess both the potential GvHD activity of anti-SARS-Cov-2 T cell products and the safety of citrullinated peptides for use in the AuToDecTRA 2 clinical trial. Both exemplars are valuable contributions to novel ATMP development and this NAATTC project has allowed the safety testing of these products during their development and prior to clinical trial using unique technologies. Further exploitation of the use of the Skimune® assay will be an important step forward ensuring the safety of ATMPs in the future. The results of this project will be published in peer reviewed journals. The results generated by the project on the safety of the anti-SARS-Cov-2 specific T cells were presented at the Safety Pharmaceutical Society and Immunology and Immunogenetic conferences in 2021. An abstract has also been accepted for the European Bone Marrow Transplant Group meeting in Prague, March 2022.