Adoptive transfer of ImmPACT expanded Multiple Antigen Specific Endogenously derived T cells (MASE-T) in combination with Lymphodepletion and anti-PD-1 to patients with metastatic melanoma

A phase I study

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The study will be conducted as described in this protocol and according to Good Clinical Practice (GCP) guidelines and regulatory requirements. The investigator allows direct access to data sources/documents (including patient charts) for monitoring, audit and/or inspection from the Danish Medicines Agency, GCP-units or other national health authorities.

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1 Synopsis

1.1 Rationale

This protocol describes the application of Adoptive Cell Transfer (ACT) of Multiple Antigen (Ag) Specific Endogenously derived T cells (MASE-T) alone or in combination with Pembrolizumab in patients with metastatic melanoma.

There are around 350-400 new cases of patients with metastatic melanoma (MM) per year in Denmark. MM is a very aggressive cancer with a poor prognosis. Traditional oncological treatments such as surgery, chemotherapy and radiation therapy have a poor effect, and the 5-year overall survival has hitherto been less than 10 %.[1]
Substantial improvements have been made in the treatment of MM; especially immunotherapy is showing promising results with checkpoint inhibitors (CPI) such as programmed cell death protein 1 (PD-1) and Cytotoxic T Lymphocyte–associated Antigen 4 (CTLA-4) blocking antibodies administered as standard treatment in the frontline.[2,3,4] The 5-year overall survival has now reached 52 %, 44 % and 26 % in nivolumab/ipilimumab, nivolumab, and ipilimumab respectively.[5]

However, a subset of patients – approximately 50 % experience no response to therapy, with clear primary resistance. The reasons for primary resistance include inadequate T-cell infiltration into the tumour, as well as immunosuppressive factors within the tumour microenvironment.[6,7] Emerging data also demonstrates that patients who fail responding to CPI, do not mount a sufficient T cell response towards the tumour.
One of the strategies to overcome these obstacles have been ACT with tumour infiltrating lymphocytes (TILs).[8]
A crucial condition for optimal ACT based on TILs is the generation of sufficient numbers of tumour-reactive T cells.[9,10]
However, the expansion of TILs requires extensive *ex vivo* culturing often at the cost of T cell differentiation and functional activity. Most TIL based ACT products are non-specifically expanded providing growth preference to co-infiltrated virus specific T cells, and it is currently challenging to expand T cells in an antigen-specific manner, while at the same time obtaining the ideal functional characteristics for specific and strong tumour-killing capacity with sufficient persistence.[11,12,13]

Recent data suggest that the majority of tumour specific T cells responsible for tumour rejection under CPI are recruited from peripheral blood and lymph system, while not present in the tumour prior to treatment.[14] This is supported by the finding that most tumour resident T cells are dysfunctional.[15,16]

1.1.1 Expansion of Multiple Ag specific T cells (MASE-T) with ImmPACT technology

To overcome the mentioned limitations, we have designed artificial antigen-presenting scaffolds for antigen-driven T cell expansion, generating a MASE-T cell product enriched for selected specificities towards antigens known to be expressed by melanoma cells. The antigen-scaffolds will ensuring optimal T cell stimulation by mimicking the *in vivo* stimulation of T cells by dendritic cells in the lymph nodes. The scaffolds contain both the antigen specific element – in the form of a peptide-MHC

molecule and cytokine (IL2 and IL21), to provide growth and functional signals to the antigen specific T cell. As a result of this T cell expansion strategy, we can obtain a T cell product enriched for tumour-antigen specific T cells. Superior functional activity towards tumor cells and antigen recognition compared to conventional T cell expansion strategies has been demonstrated *in-vitro*. Importantly, antigen-specific T cells in the MASE-T cell product possess a 'younger' phenotype, which has previously been described to correlate with improved *in vivo* persistence.[17] Similar antigen presenting scaffolds have been successful in the expansion of both antigen-specific T cells and chimeric antigen receptor T cells (CAR-Ts).[18]

1.1.2 Infusion of Multiple Ag Specific T cells (MASE-T)

Hence, we aim to infuse a T cell product for ACT derived from peripheral blood T cells. The melanoma-antigen specific T cells in the peripheral blood sample will engage with the Ag-scaffold to receive preferential expansion signals. The Ag-scaffold will ensure the presence of all required T cell stimulation factors to form an immunological synapse for efficient T cell stimulation.

The MASE-T cell product will be administered alone in the first six patients. If tolerated, it will be combined with the standard treatment Pembrolizumab, a PD-1 blocking antibody, with the rationale to enhance the MASE-T cell product anti-tumour activity by blocking inhibitory signals of T-cell activation in the tumour microenvironment. .

1.2 Objectives and endpoints

The primary objective is to evaluate the safety and feasibility of the MASE-T treatment alone or in combination with Pembrolizumab in patients with stage IV metastatic melanoma according to Common Terminology Criteria for Adverse Events (CTCAE version 5.0).

The secondary objectives are to evaluate T cell profile and persistence *in vivo* from tumor biopsies and blood samples as well as evaluation of the clinical efficacy of the treatment according to RECIST 1.1 and iRECIST. In addition, best overall response (BOR), duration of response (DOR), overall survival (OS), progression-free survival (PFS) will be monitored.

1.3 Study design

The study is a phase 1, non-randomized study. The trial will be conducted in two parts, six patients are enrolled in part A. If the production of the MASE-T cell product was feasible for the majority (≥50%) of patients intended to treat in Arm A and the toxicity was acceptable, six patients will further be included in part B.

Patients will be included and treated at the Department of Oncology at Herlev Hospital. Patients can also be referred from other oncology centres in Denmark. The inclusion period is expected to run over approximately 2 years, starting in Spring 2021. We expect all patients to finish treatment within 3 years.

At the time of inclusion peripheral mononuclear cells (PBMCs) will be collected from the patient in order to produce the MASE-T product. Production is expected to take 2 weeks. The cell product will be cultivated with 30 pre-selected melanoma tumour antigens for HLA-A2 positive patients.

Patients will be treated as followed:
Part A (6 patients): Lymphodepleting chemotherapy (cyclophosphamide 500 mg/m^2/day i.v. on day -4, -3, -2 and fludarabine Phosphate 30 mg/m2/day i.v. on day -4, -3) followed by i.v. infusion of the MASE-T product on day 0.
Part B (6 patients): Lymphodepleting chemotherapy (cyclophosphamide 500 mg/m^2/day i.v. on day -4, -3, -2 and fludarabine Phosphate 30 mg/m2/day i.v on day -4, -3) followed by i.v infusion of the MASE-T product on day 0. Pembrolizumab 2 mg/kg will be administered on day -1 and day +21.

Blood samples for research purposes will be collected at baseline, during treatment, and at time of progression. Tumour tissue will be collected if possible, at baseline and after the first evaluation scan and at progression.

Patients will be closely monitored and upon clinical or radiological signs of progression, patients will be transferred to standard of care and followed for overall survival by medical journal entry.
Patients who respond to the treatment will be followed with clinical evaluation and blood samples for 5 years. With the treatment period (expected 3 years) and follow-up period (5 years) we expect end of study 8 years after initiation.

The study is monitored by the Good Clinical Practice (GCP) unit and will be reported to the Danish Medicines Agency, the Research Ethics Committee and the Danish Data Protection Agency.

1.4 Population

HLA-A2 positive patients with stage IV metastatic melanoma are eligible for inclusion. Other inclusion criteria are performance status (PS) 0-1, at least one measurable lesion according to RECIST 1.1, acceptable kidney- and liver functions and absence of major co-morbidities. Patients with brain metastasis may be excluded.
Importantly, the treatment will only be completed on patients, where we can manufacture an acceptable MASE-T product (see IMPD). A total of 12 patients will be treated with the MASE-T cell product this study. It is only the patients who receive the MASE-T infusion that counts in the trial.

1.5 Toxicity

The MASE-T ACT product has never been tested in humans but multiple clinical trials with adoptive cell transfer of autologous ex vivo expanded TILs have been carried out and is reasonably well tolerated.[19]
The MASE-T ACT product is derived from the autologous PBMCs, and though it is enriched with tumour reactive T cells, the MASE-T product share a similar phenotype with conventional TIL-derived cell products, and we expect a comparable safety and toxicity profile. Additionally, in comparison to conventional ACT, the patients in this trial are treated with a milder regime with less lymphodepleting

chemotherapy and without Interleukin 2 (IL-2) stimulation. The National Center for Cancer Immune Therapy (CCIT-DK) at the oncology department at Herlev Hospital have treated over 100 patients with TIL-derived ACT and is experienced in managing side effects.

Pembrolizumab is a standard treatment in patients with MM. For patients treated with checkpoint inhibitor monotherapy (PD-1 or PD-L1 antibodies) CTC grade 3-4 adverse events (AE) occur at frequencies of about 10% and include rashes, gastrointestinal problems, hepatitis and hypophysitis. The oncology department at Herlev Hospital treat patients with Pembrolizumab daily and are experienced in managing side effects. Patients included in the study have already been treated with standard pembrolizumab or nivolumab. If the patients experienced grade 3-4 adverse events on the PD-1 treatment they are excluded from this trial (see exclusion criteria).

Combining ACT with lymphodepletion and checkpoint inhibitors
Several clinical studies have already combined ACT with lymphodepletion and checkpoint inhibitors and has been reported as reasonably well tolerated. (NCT01993719, NCT01174121, NCT02500576, NCT02621021)

1.6 Evaluation of toxicity and clinical response

Toxicity and safety are assessed by CTCAE v. 5.0 at fixed time points. Baseline imaging is performed at baseline, 6 and 12 weeks after MASE-T infusion, and thereafter every third or sixth month according to the follow-up schedule and will be evaluated according to RECIST 1.1. The follow-up period will continue for up to 5 years with regular blood tests and diagnostic imaging (RECIST 1.1).

1.7 Translational research and immunological response evaluation

To evaluate the MASE-T treatments and the immunological response, blood samples for experimental analyses are drawn at fixed time points. Blood samples will be collected at: 1) baseline 2) 1 week after MASE-T infusion 3) 3 weeks after MASE-T infusion and at the clinical evaluations.
Core needle biopsy will be collected if possible, at baseline and at the first evaluation scan and will be analysed for T cell infiltration and signs of tumour cell killing. At signs of progression a new tumour biopsy will be performed for further analyses.

2 Introduction and rationale

2.1 Current treatment landscape for MM patients in Denmark

In Denmark there are approximately 2300 new cases of melanoma each year and approximately 350 patients are diagnosed with metastatic melanoma (MM).[20,21]
In the metastatic setting, the most widely used first line therapy is immunotherapy comprising the checkpoint inhibitors (CPIs) pembrolizumab or nivolumab for patients with tumours expressing PD-L1 >1 %, while ipilimumab (also a CPI) in combination with nivolumab are mostly used in patients with tumours expressing PD-L1 < 1%.[22] This is based on data demonstrating that PD-1 inhibitor monotherapy is superior to ipilimumab and is equivalent in objective response rates and survival

compared to the combination therapy for patients with tumours expressing PD-L1 >1 %, but is significantly less toxic.[23]
Second line therapy consists of ipilimumab, if PD-1 inhibitor monotherapy was administered in first line. If the combination was administered in first line, second line treatment for patients with BRAF wildtype tumours is IL-2 infusion or oral temozolomide, or entry into a clinical trial.[24,25]
Another treatment option exists for the roughly 40% of MM patients with BRAF mutated tumours; combination treatment with BRAF inhibitor dabrafenib in combination with the MEK inhibitor trametinib.[26]
Due to huge advances in the treatment of MM, OS has increased from median 9-10 months in 2009 to multiple trials reporting 2-years survival rates in the range of 50 %. [22,26,27]However, the patients enrolled in these trials are highly selected, and the benefit of these advances in the real-world population remains to be prospectively validated.[21]
Immunotherapy is therefore showing promising results, but new and improved treatment strategies are still needed.

2.2 Tumour immunology and cancer immunotherapy

In recent years there has been immense progress in the understanding of the interplay between the immune system and cancer cells. It has become clear that the immune system recognizes and reacts against cancers and that an effective immune response against cancer cells is associated with a better prognosis.[28] The presence of an immune response can be clearly illustrated by TILs that often exist in an inactivated state or at an immunological equilibrium with the cancer cells in the tumour microenvironment. The inactive state of the TILs is characterized by abnormal intracellular signaling, apoptosis and reduced proliferative capabilities.[29] It is widely accepted that cancer cells at some point escape the immunological equilibrium in a process called *immuno-editing* that is an evolution-like process where cancer cells mutate and progressively find ways to avoid immune recognition or activation.[30] The escape results in a progressive cancer disease that requires external intervention.
Cancer immunotherapy is defined as the approach to combat cancer by generating or augmenting an immune response against cancer cells. Over the past decade, two types of immunotherapy have emerged as particularly effective in cancer treatment; the use of CPIs to enhance natural anti-tumour activity and the administration of specific anti-tumour immune cells via ACT.
The treatment with CPIs relies on boosting a pre-existing population of potentially tumour-reactive T cells in the patient. Thus, in poorly immunogenic patients, CPIs alone are likely to fail.[31] In this regard the administration of tumour-recognizing T cells via ACT would enable immune-based therapies for poorly immunogenic tumours and potentially augment responses in tumours that are unresponsive to CPI.

2.3 Adoptive cell therapy (ACT) in patients with Metastatic Melanoma

The goal of ACT is to generate a robust immune mediated anti-tumour response via infusion *of ex vivo* manipulated T cells. ACT-based strategies utilizing T cells to destroy tumours can be divided into a) isolation of naturally occurring tumour specific T cells from existing tumour masses (TILs) and b) the genetic modification of blood-derived T cells to allow specific recognition of tumour cells. In both settings, T cells are manipulated ex vivo followed by expansion and later reinfusion into the lymphodepleted patient. ACT with TIL is visualized in figure 1.

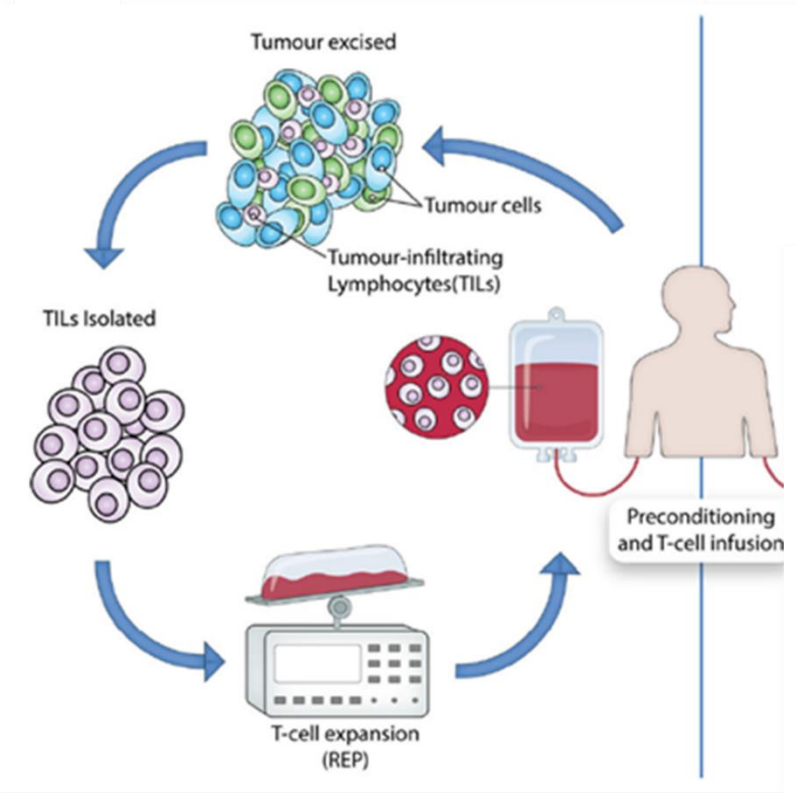

Figure 1. Adoptive T cell transfer approach to harness the immune system to treat cancer: Adoptive cell therapy (ACT) with TILs (tumor infiltrating lymphocytes)

2.3.1 ACT with TILs

ACT with TILs has been tested in multiple trials world-wide and also at CCIT, Herlev Hospital, reporting objective response rates of 40-50% in MM patients, including complete tumour regression in 10 to 25 % of the patients.[8] It has also been shown that patients refractory on anti-PD-1 still respond to an infusion of TILs.[32] Treatment with TILs is only applicable when a tumour biopsy of at least 2 cm can be removed. Unfortunately, a large group of patients does not have surgically respectable tumors and cannot be offered treatment.

2.3 Tumour antigens

T cells recognise cancer cells by binding to cancer-related peptides presented on a MHC molecule. Some tumour antigens are highly tumour specific while others are with lower tumour specificity:
Antigens of high tumour specificity:

Three types of tumour antigens have the potential to elicit immune responses that are strictly tumour specific:

- *Neo-antigens* are derived from spontaneous mutations acquired during carcinogenesis and are present in most tumour types. The contribution of these antigens to tumour immunogenicity is expected to vary according to the mutation rate (tumour mutational burden (TMB)).[33] The contribution is expected to be higher in melanomas due to a high MTB induced by UV radiation and in lung carcinomas that arise from tobacco smokers.[34]
- *Cancer-germline genes* are not expressed in normal tissue but are expressed in male and female germline cells, in trophoblastic cells and in a substantial fraction of tumours. The mechanism of activation involves demethylation of the promotor of these genes.[35,36] Cancer-germline genes (MAGE, BAGE, GAGE, LAGE-1, NY-ESO-1) are frequently expressed in melanoma, bladder, head and neck and lung carcinomas.
- *Viral antigens* have been shown for a subset of human tumours including cervical carcinoma, nasopharyngeal carcinoma, adult T cell leukaemia and hepatocarcinoma and has been useful for cancer prevention.

Antigens of low tumour specificity

Two types of tumour antigens a known to have low tumour specificity:

- *Differentiation antigens* (tissue-specific expression) is found in melanoma patients where T cells recognize both tumour cells and normal melanocytes. The antigens are melanocyte-specific proteins such as tyrosinase, Melan-A (MART1) and GP100.[37]
- *Overexpression of proteins* in tumours provide an opportunity for a specific T cell response, that is if tumour cells present an amount of peptide-HLA complexes that is above the threshold of normal cells, a specific antitumoral T cell response could occur. Examples of overexpressed proteins are HER2 (in breast and ovarian cancer) and WT1 (in leukaemia).[38,39]

2.4 ImmPACT scaffolds for expansion of MASE-T product

To expand MASE-T we have established ImmPACT scaffolds. ImmPACT scaffolds are antigen-presenting scaffolds based on dextran polymers with bound biotinylated peptide-MHC monomer, IL-2 and IL-21 in an optimized stoichiometric ration (figure 2B). Expansion of antigen-specific cells are based on the ability of the T cells to recognize and bind peptide-MHC complexes on the dextran polymer, which is the main driver of the interaction. Upon interaction of the ImmPACT scaffold to the TCR, the T cell is further stimulated with IL-2 and IL-21, both of which are important for T cell expansion, survival and memory. The interaction between ImmPACT scaffolds and T cells leads to specific expansion of T cells, with a favorable phenotype and ability to recognize and eradicate melanoma tumours (figire 2A). Using a library of 30 antigens (both cancer germline genes and melanocyt specific antigens) for different ImmPACT scaffolds we can create a multitargeted and polyclonal T cell product. (Table 1)

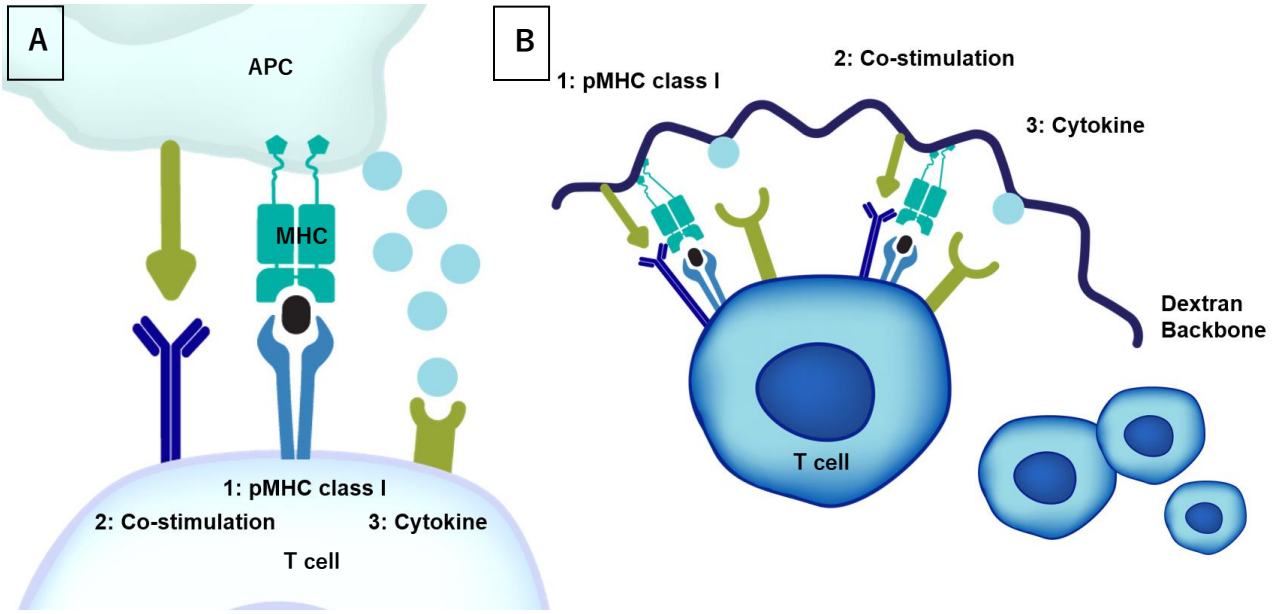

Figure 2. **A**) T cell activation and proliferation (expansion) in humans requires presentation of an antigen through the MHC molecule on an antigen presenting cell (APC), co-stimulation and cytokine release is also mandatory. **B**) In vitro T cell activation and expansion of Ag specific T cells for ACT through ImmPACT scaffolds consisting of a dextran backbone with bound peptide MHC monomer, IL-2 and IL21 (cytokines).

Table 1: Top 30 shared melanoma antigens and HLA-A2 sequences

No.	Protein	Sequence	No.	Protein	Sequence
1	MAGE-A2	LVHFLLLKY	**16**	MC1R	TILLGIFFL
2	gp100 / Pmel17	IMDQVPFSV	**17**	Melan-A / MART-1	ILTVILGVL
3	CDKN1A	GLGLPKLYL	**18**	TRP-2	FVWLHYYSV
4	gp100 / Pmel17	YLEPGPVTA	**19**	KIF20A	AQPDTAPLPV
5	Melan-A / MART-1 (WT)	EAAGIGILTV	**20**	MAGE-A10	GLYDGMEHL
6	MAGE-C2	KVLEFLAKL	**21**	p53	RMPEAAPPV
7	MAGE-A10	SLLKFLAKV	**22**	SSX-2	KASEKIFYV
8	gp100 / Pmel17	KTWGQYWQV	**23**	STEAP1	FLYTLLREV
9	STEAP1	MIAVFLPIV	**24**	TRP-2	SVYDFFVWL
10	Telomerase	RLFFYRKSV	**25**	GnTV	VLPDVFIRCV
11	LAGE-1	MLMAQEALAFL	**26**	Livin (ML-IAP)	SLGSPVLGL
12	MAGE-A2	YLQLVFGIEV	**27**	MAGE-A1	YLEYRQVPV
13	MAGE-C2	LLFGLALIEV	**28**	Meloe-1	TLNDECWPA
14	STAT1-alpha/ß	KLQELNYNL	**29**	NY-ESO-1 / LAGE-2	SLLMWITQC
15	TAG-1	SLGWLFLLL	**30**	TRAG-3	ILLRDAGLV

2.5 ACT with Multiple Ag specific T cells (MASE-T)

Adoptive cell therapy with the MASE-T product has never been tested in patients but is anticipated to have a great potential. The technique is simpler than with TIL; with expansion of T cells from blood (no need of tumour biopsy for production of T cells) and only requires one step expansion. The MASE-T product targets multiple tumour specific antigens and is therefore anticipated to be more tumour directed than ACT with TILs.

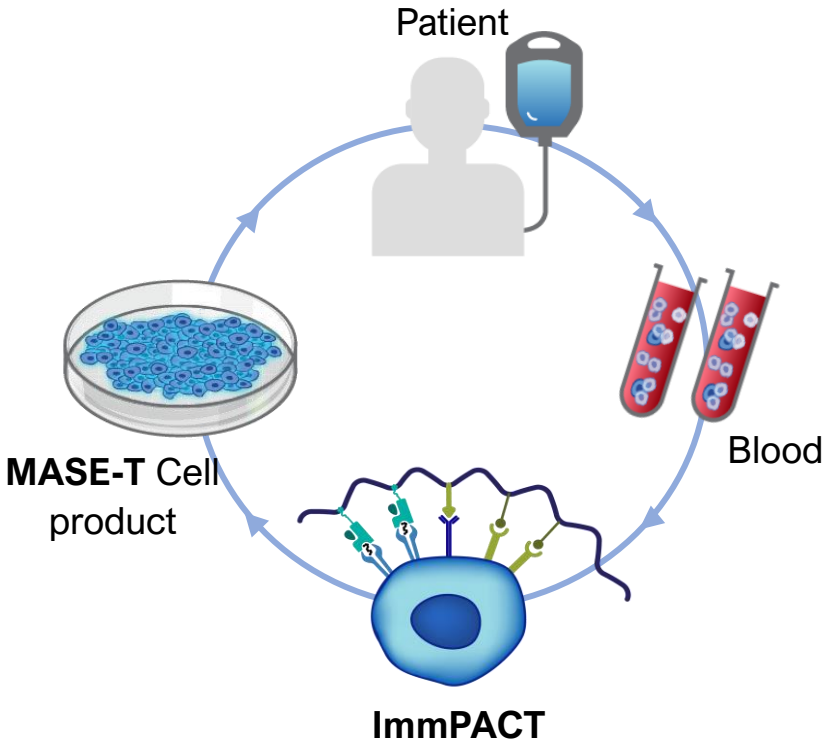

Figure 3 ACT with MASE-T.

2.6 The supportive regimen

Traditionally, the ACT is assisted by a supporting regime. Lymphodepleting regimens are applied in most trials with ACT. The lymphodepletion provides an *immunological window* where sufficient stimulatory cytokines and growth factors are readily available for the infused TILs while also depleting regulatory and suppressive cells. [40]

Following MASE-T infusion, patients also receive a single dose pegfilgrastim to help reconstitute bone marrow function after the lymphodepleting chemotherapy.

Besides the well-established supportive regime of lymphodepletion, the efficacy of ACT may be further supported by immune *checkpoint inhibition*. Immune checkpoints are surface receptors on immune cells that serve as regulatory brakes in the immune system to avoid autoimmunity. The checkpoints are exploited by cancer cells to avoid immune recognition and activation. In recent years checkpoint inhibitors (especially anti-PD-1) have yielded impressive results in many cancer forms

and have now become standard therapy in malignant melanoma.[41] Emerging studies show that immunotherapy in contrast to traditional cancer medicines might be more dependent on the mutational load of the individual patient rather than the cancer diagnosis. [42,43] This could mean that a more personalized approach to cancer therapy like ACT is needed.
Both ACT and checkpoint inhibition work by tipping the immunological balance in favor of activation and away from suppression or avoidance by the cancer cells. Due to the increasing success of checkpoint inhibitors, it is speculated that combination with ACT would provide a synergistic immunological benefit to cancer treatments - much like combining different checkpoint inhibitors has been - and scientific evidence is aligning with this theory.[44]

It has been shown repeatedly that tumour-specific TILs frequently express PD-1 and that PD-1 inhibition increase their tumour killing capacities.[45,46,47] This fits the general perception that PD-1 predominantly potentiates the effector functions of T cells[48]. The results are so promising that several clinical studies combining PD-1 blockade with ACT are already ongoing (NCT01993719, NCT01174121, NCT02500576, NCT02621021).

Based on the available evidence and in order to optimize the anti-tumour activity of the infused MASE-T product, ACT is combined with pembrolizumab in patient in group B. To maximize the *in vivo efficacy*, we administer pembrolizumab just before and three weeks following the MASE-T infusion.

Two trials at CCIT-DK, Herlev Hospital has been conducted where combination of ACT with TILs has been combined with both lymphodepleting chemotherapy, IL-2 injections and CPIs, which was tolerable and feasible for all 30 patients. (NCT03296137, NCT03287674)

2.7 CCIT-DK is a leading center for ACT

Center of Cancer Immune therapy (CCIT-DK) at Herlev Hospital is a leading European center of TIL-based ACT. Despite the promising results of this therapy, widespread application and testing of ACT is limited by the considerable expertise, facilities and resources required for the TIL expansion, infusion and supportive regime. Due to this complexity, only a handful of centers in the world are currently able to perform ACT. At CCIT we have many years of experience, and we have over the years built up the required expertise and effectiveness in all steps of the process.

3. Hypothesis, objectives and endpoints

3.1 Hypothesis

Our hypothesis is that the treatment with lymphodepleting therapy and MASE-T alone or in combination with Pembrolizumab is well tolerated and a feasible treatment option in patients with MM.

3.2 Primary objective and endpoint

The primary objective is to asses **tolerability and feasibility** of the treatment. The primary endpoint is adverse events assessed by CTCAE 5.0

3.3 Secondary objectives and endpoints

To **characterize T cell profile and persistence *in vivo*** from tumor biopsies and blood samples and to characterize biomarkers for immunological response to the treatment.
To evaluate the **clinical efficacy**. Endpoints are Best Overall Response (BOR) according to RECIST 1.1 and iRECIST, Duration of response (DOF), Progression Free Survival (PFS) and Overall Survival (OS).

RECIST 1.1 Guidelines:

- Complete response (CR): All lesions disappear.
- Partial response (PR): Defined as a ≥ 30 % reduction in the sum of all measurable parameters longest diameter.
- Stabile disease (SD): Defined as a < 30 % reduction in the sum of all measurable parameters diameter or a < 20 % increase in the sum of all measurable parameters longest diameter.
- Progressive disease (PD): Defined as a > 20 % increase in the sum of all measurable parameters longest diameter *or* the appearance of new lesions.
- CR and PR are to be verified by a subsequent evaluation after no less than 4 weeks.

4. Trial design

The trial is designed as a phase I, single-centre, non-randomized clinical trial. The trial will be conducted in two parts, six patients are enrolled in group A. If the treatment is possible to produce in 3 out of 6 patients and is feasible for all patients, six patients will be treated in group B.
Part A: Lymphodepleting chemotherapy and MASE-T infusion
Part B: Lymphodepleting chemotherapy and MASE-T infusion combined with Pembrolizumab

4.1 Trial period

The trial is expected to start Spring 2021 and patients are expected to be enrolled during the course of 2 years. All 12 patients are expected to have ended treatment during the course of 3 years. Patients will be followed for up to five years. End of trial is defined as the date of last patients' last visit which is expected 8 years after initiation.

4.2 Statistical consideration

The study is non-blinded and non-comparative. Descriptive statistics will be used to estimate the immunological response and clinical response rate. Descriptive statistics will also be used to sum up the duration of response and the patient characteristics. A power calculation for the required sample size to determine of primary and secondary endpoints cannot be formally calculated. A samples size of 12 participants is chosen due to practical considerations that include a realistic time frame as well as the limited available facilities and trained personal.

4.3 Study population

HLA-A2 positive patients with histologically confirmed metastatic or inoperable melanoma who have progressed on standard therapy. Importantly, the treatment can only be completed in patients, where we are able to produce an acceptable cell product from the drawn blood sample.

4.4 Inclusion criteria

1. Age ≥ 18 ≤ 75
2. Progressive disease on or after anti-PD-1/anti-PD-L1 monotherapy or progressive disease on or after anti PD-1 plus anti-CTLA-4 therapy
3. The patient has histologically confirmed metastatic cutaneous melanoma. Patients with metastatic ocular/mucosal or other non-cutaneous melanoma cannot be included
4. The patient is HLA-A2 positive
5. ≥ 10 % of lymphocytes in peripheral blood are CD8+
6. At least one measurable parameter according to RECIST version 1.1 guidelines
7. ECOG performance status of 0 or 1
8. No significant toxicity from previous cancer treatments (CTC ≤ 1)
9. Women of childbearing potential: Negative serum pregnancy test and must use effective contraception. This applies from screening and until 6 months after treatment. Birth control pills, spiral, depot injection with gestagen, subdermal implantation, hormonal vaginal ring and transdermal depot patch are all considered effective contraceptives
10. Men with female partner of childbearing potential must use effective contraception from screening and until 6 months after treatment. Effective contraceptives are as described above for the female partner. In addition, documented vasectomy and sterility or double barrier contraception are considered effective contraceptives
11. Signed statement of consent after receiving oral and written study information
12. Willingness to participate in the planned treatment and follow-up and capable of handling
13. The patient has met the following haematological and biochemical criteria:
    a) AST and ALT ≤2,5 X ULN or ≤5 X ULN with liver metastases
    b) Serum total bilirubin ≤1,5 X ULN or direct bilirubin ≤ ULN for patient with total bilirubin level > 1,5 ULN
    c) Serum creatinine ≤1,5 X ULN
    d) ANC (Absolute Neutrophil Count) ≥1,000/mcL
    e) Platelets ≥ 75,000 /mcL
    f) Hemoglobin ≥ 9 g/dL or ≥ 5.6 mmol/L

4. 5 Exclusion criteria

1. Another malignancy or concurrent malignancy unless disease-free for 3 years
2. Requirement for immunosuppressive doses of systemic corticosteroids (>10 mg/day prednisone or equivalent) or other immunosuppressive drugs within the last 3 weeks prior to screening
3. Prior treatment with adoptive transfer of Tumor Infiltrating T cells (TIL)

4. Grade 3-4 adverse events upon treatment with PD-1 checkpoint inhibitors (only phase B)
5. Patients who have any CNS lesion that is symptomatic, greater than 1 cm in diameter or show significant surrounding edema on MRI scan will not be eligible until they have been treated and demonstrated no clinical or radiologic CNS progression for at least 2 months. However, patients with subclinical brain metastasis with a maximum of 4 metastasis < 1 cm can be included.
6. The patient has any condition that will interfere with patient compliance or safety (including but not limited to psychiatric or substance abuse disorders)
7. The patient is pregnant or breastfeeding
8. The patient has an active infection requiring systemic therapy
9. The patient has received a live virus vaccine within 30 days of planned start of therapy
10. Significant medical disorder according to investigator; e.g severe asthma or chronic obstructive lung disease, dysregulated heart disease or dysregulated diabetes mellitus.
11. Concurrent treatment with other experimental drugs
12. Any significant active autoimmune disease
13. Severe allergy or anaphylactic reactions earlier in life
14. Known hypersensitivity to one of the active drugs or one or more of the excipients.
15. Unrelieved lower urinary tract obstruction

5. Conduct of study

5.1 Overview

The study is a phase I clinical trial enrolling patients with metastatic melanoma who are refractory to PD-1 antibody treatment. All patients will be included and treated at the Department of Oncology, Herlev Hospital. Patients can be referred to treatment from other centers in Denmark. The study is monitored by the Good Clinical Practice unit (GCP-unit) at the Capital Region (RegionH) and will be reported to the Danish Medicines Agency, the Research Ethics Committee and the Danish Data Protection Agency.

Referral of patients is to be made to the visitation office of clinic 5at the department of oncology, Herlev Hospital.

We aim to include 12 patients. 6 in part A and 6 in part B. The inclusion is estimated to start in Q2 of 2021 and be completed within three years. Primary feasibility and toxicity are thus scheduled within 3 years of trial initiation.

Patients are screened, treated and entering follow-up as described in the following sections.

5.2 Screening

After signing informed consent according patients are screened for eligibility.

The screening is divided in two phases:

Phase 1: Patients will be screened for HLA-A2 specificity by flowcytometry. HLA-A2 specificity test by flowcytometry is completed within two days. Further the frequency of CD8+ lymphocytes in peripheral blood will be tested by flowcytometry. If the patient is HLA-A2 positive and have ≥ 10 % CD8+ lymphocytes in peripheral blood they will enter the second phase of screening. If the patient

is HLA-A2 negative or have < 10 % CD8+ lymphocytes in peripheral blood they will be excluded. Furthermore, the first six patients will additionally be screened for the presence of tumor-antigen specific CD8 T cells, corresponding to the 30 tumor-antigen-derived peptides included in the ImmPACT scaffold. Only patients who are HLA-A2 positive, have ≥ 10 % CD8+ lymphocytes and have >0.002% (with a limit of 10 detectable events) tumor-antigen tetramer positive CD8 T cells will continue for phase 2 screening.

Phase 2: Patients will further be screened for the following parameters and HLA-A2 specificity will be validated.

- Complete physical examination including height, weight, performance status, vital signs including blood pressure, heart rate, saturation and temperature.
- Pathologic confirmation of metastatic melanoma (archival tissue may be used)
- HLA-A2 specificity
- PET-CT of chest, abdomen and pelvis
- MR cerebrum
- ECG
- Pregnancy test: Women in the fertile age must take a pregnancy test. This includes women who are not surgically sterilized, who are not postmenopausal and who have not used safe contraceptions regularly within the last 6 months
- Comprehensive blood test panel including:
    - Hematology: Hemoglobin, leukocytes with diff-count and platelets
    - Liver: ASAT, ALAT, albumin, alkaline phosphatase, bilirubin, LDH, INR, APPT.
    - Kidney: Sodium, potassium, creatinine, carbamide, phosphate, Ca-ion
    - Other: CRP
    - Chronic infections: HIV, Hepatitis B surface antigen, Hepatitis B surface antibody, Hepatitis B core antibody, Hepatitis C IgG, HTLV IgG, EBV IgG, Trepone,
    - Endocrine: TSH, T4, T3, Cortisol, Oestradiol (female), prolactin (female), Testosterone (male)
- Kidney function (clearence)
- Reviewing the checklist for inclusion/exclusion for treatment.

5.3 Treatment plan

12 patients will be included in total. The first six patients will be included in group A and the following 6 in group B. Enrollment of the first three patients in group A will be staggered; the second and third patient will not be included until the previous patient completes day 7 of the treatment without intolerable toxicity

If the following criteria are met six additional patients can be included in part B: 1. production of the MASE-T cell product was feasible for at least 50% of patients intended to treat in part A. 2. Acceptable toxicity observed in part A patients defined as MASE-T related grade III/IV toxicity in no more than 1 of 6 patients.

For both groups a peripheral blood sample of 300 mL will be drawn right after inclusion to manufacture the MASE-T product. Around ten days after, patients will be hospitalized and treated with 3 days of lymphodepleting chemotherapy (day -4, -3 and -2). On day 0 the MASE-T product will

be administered (figure 4). Two hours after MASE-T infusion, pegfilgrastim is given to help reconstitute bone marrow function.
If the treatment is feasible for all six patients in group A, pembrolizumab will be administered at day -1 and day 21 in group B. (table 2)

Treatment schedule (Table 2)	Hospital					Outpatient clinic
Day	-4	-3	-2	-1	0	21
Cyclophosphamide 500 mg/m² IV	x	x	x			
Fludarabine Phosphate 30 mg/m² IV	x	x				
MASE-T IV infusion					x	
Pegfilgrastim 6 mg s.c					x	
Pembrolizumab 2 mg/kg ONLY PART B				x		x

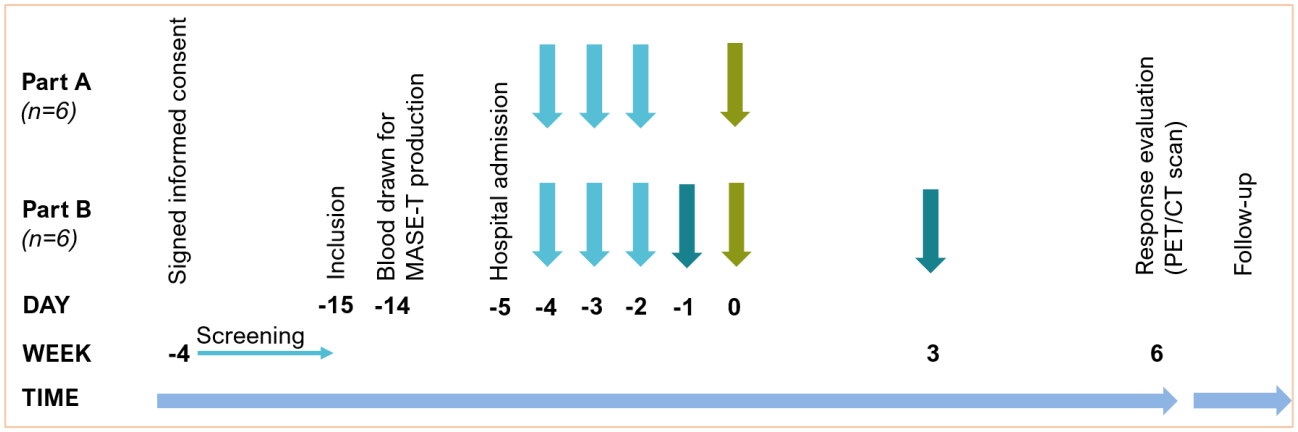

Figure 4 ***Flow chart treatment plan***

Light Blue arrows indicate conditioning with cyclophosphamide 500 mg/m² /dose on days -4, -3, and -2 and Fludarabine Phosphate 30 mg/m²/dose on day -4 and -3.
Dark blue arrows indicate PD-1 inhibitor, pembrolizumab, given at 2 mg/kg/dose on days -1 and 21.
Green arrows indicate infusion of MASE-T product
Respons to therapy evaluation is completed with PET/CT scans.

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5.4 Overview of blood tests and evaluation during the treatment course

The patients are continuously monitored on several parameters before, during and after MASE-T infusion. Group B are also monitored before every treatment with Pembrolizumab.

Time point	Screening	Peripheral blood drawn for MASE-T production	At admission	Before Lymphodepleting chemotherapy	Group A: No treatment Group B: 1st of 2 Pembrolizumab	MASE-T infusion	After MASE-T infusion	Group A: No treatment Group B: Pembrolizumab 2nd of 2	First clinical evaluation	Second clinical evaluation	Third clinical evaluation
Day	**-21**	**-15**	**-5**	**-4-2**	**-1**	**0**	**+6**	**+21**	**+42**	**+84**	**+168**
BP, P, Tp	x		x	x	x	x	x	x	x	x	x
Toxicity assessment	x		x		x	x	x	x	x	x	x
PS, clinical assessment	x		x	x	x	x	x	x	x	x	x
Weight	x		x	x	x						
Screening blood tests	x										
Research blood samples*			x			x	x	x	x	x	x
Routine blood tests			x	x	x	x	x	x	x	x	x
ECG	x						x				
Kidney function	x										
Urine sample			x								
Tumour biopsy		x							x		
PET-CT	x								x	x	x
MR cerebrum	x										

* See section 8.1, “Research blood tests”

5.5 Medicinal products used in the protocol

5.5.1 Cyclophosphamide (sendoxan®)

Cyclophosphamide (sendoxan®) is an alkylating drug that works by creating covalent bindings with biologically important macromolecules. In cancer therapy, it binds and links DNA strings preventing cell division. The effect can be opposed by cell DNA repair systems that are usually impaired in cancer cells. The binding also damages important cellular functions and leads to cell death. Cyclophosphamide is given as an intraveneous infusion for three consecutive days in a dosage of 500 mg/m^2. Patients are treated according to local guidelines.

5.5.2 Fludarabine phosphate ("Actavis" or "Ebewe")

Fludarabine phosphate (Fludarabinphosphat "Actavis"® or Fludarabinphosphat "Ebewe"®) is a pro-drug that is concerted to the active triphosphate 2-fluoroara-ATP. It is an anti-metabolite that inhibits DNA synthesis while simultaneously reducing RNA and protein synthesis. Fludarabine phosphate is used in the treatment of hematological diseases as chronic lymphatic leukemia. Fludarabine phosphate is given as an intravenous infusion for 2 consecutive days in parallel with cyclophosphamide infusion in a dosage of 30 mg/m^2 body surface. Patients are treated according to local guidelines.

The lymphodepleting regimen is milder than normally administered when we treat patients with TIL therapy. Heczey et al have treated patients with neuroblastoma with this regimen and found it safe and manegable, also in combination with CAR T therapy and anti-PD-1 therapy.[49]

5.5.3 Pembrolizumab

Pembrolizumab (Keytruda®) is an anti-PD-1 antibody that inhibits the PD-1/PD-L1 pathway on T cells. PD-1 is a surface receptor on activated T cells and when it binds PD-L1 on target cells, it induces the cell death of the activated T cell. Cancer cells use PD-L1 expression to avoid immune recognition. Pembrolizumab is approved by the EMA for treatment of metastatic melanoma. Patients in group B will be treated with 2 mg/kg Pembrolizumab the day before infusion of MASE-T (day -1) and on day +21. Patients are treated according to local guidelines.

5.5.4 The MASE-T product

The MASE-T product contains melanoma tumour antigen specific T cells. They are infused IV into the patient on day 0. The number of cells in the product varies between patients. It usually consist of 20-200 million cells. See the Investigational Product Medicinal Dossier (IMPD) for more information.

5.5.5 Pegfilgratim (Neulasta)

Pegfilgratim (Neulasta) is an analog human granulocyte colony stimulatory factor (GM-CSF). It stimulates the bone marrow into producing white blood cells and increasing the peripheral blood count. It is usually given to cancer patients who suffer from low blood counts following chemotherapy. Pegfilgrastim helps the patients recover from the lymphodepleting chemotherapy by a single dose of 6 mg s.c. at 2 hours after MASE-T infusion.

Medicinal products are only administered by trained medical staff and are labelled according to Annex 13 in the GMP regulations.

5.6 Guidelines for supportive and concomitant therapy

Supportive treatment is given on ordinary medical indications estimated by the clinician responsible for the treatment. Any measures should be specified in the patient chart and flow sheet. The following is meant as a guideline and other medications can be administered if appropriate.
During the lymphodepleting chemotherapy the following are given to protect mucosa of the bladder and relieve dehydration, nausea or vomiting:
Supplementary fluid therapy during cyclophosphamide treatment.
Fixed:

- 1 L isotonic saline on day -5, -4, -3, -2. The patient should additionally drink 2 liters fluid a day during chemotherapy.
- Inj. Mesna, (25% of cyclophosphamide dosage) IV x 4 on day -4 and -3 and -2.
- Inj. Aloxi 250 µg IV on day -4 and -2.
- Tabl. Emend, 125 mg x 1 on day -4 and 80 mg on day -3 and-2.

If needed:

Tabl. Motilium, 20 mg if needed max x 3.
Tabl. Temesta, 1-2 mg if needed, max x 4.
Tabl. Pantoloc, 40 mg if needed max x 2.

To prevent opportunistic infections:

Tabl. Sulfamethizole with Trimethoprime, 400/80 mg x 1 daily for 6 months from day -4.
Tabl. Aciclovir, 400 mg x 2 for 6 months from day 0.
Tabl. Diflucan, 100 mg x 1 on day 0 and until the neutrophile count is > 1000/µl.

After MASE-T infusion:

Inj. Pegfilgrastim, 6 mg x 1 s.c. on day 0, two hours after TIL infusion.
Tabl. Pethidin, 25 mg if needed max x 4.

Oxygen on nasal catheter if needed.
In case of fever during neutropenia

- Patients are treated with antibiotics according to local guidelines for febrile neutropenia described in appendix 4.

In case of anemia or trombocytopenia

- Transfusions with filtered and radiated blood should be administered in line with institutional guidelines
- Transfusion with platelets is indicated if platelets < 20/µl or if it is otherwise clinically indicated.

In case of diarrhea:

- Patients are treated according to local guidelines that include loperamide and fecal samples.
- Loss of fluid and electrolytes are corrected orally or intravenously and evaluated daily.

In case of immune related adverse events (irAEs) to checkpoint inhibitors local guidelines are followed and e.g. corticosteroid can be administered:

- IrAEs are managed according to national guidelines that are available at https://immuntox.dk.

In some cases, e.g. localized bone pain, local radiotherapy can be prescribed.

- Radiotherapy is preferably to be avoided within the 3 weeks period of hospitalization
- Radiated areas cannot be used as parameters in the assessment of treatment response.
- If possible, not all evaluable areas should be included in the radiated area. If all evaluable areas are treated, the patient is withdrawn from the study to ensure correct evaluation.

5.7 Follow-up

After completion of treatment patients enter the follow-up phase. At follow-up visits patients are evaluated clinically, with blood samples, project blood samples and PET/CT scans. Follow-up visits will be scheduled after 6 weeks, 12 weeks and thereafter every third month for the first two years and every six months thereafter, or as clinically indicated, for a total of 5 years.

	Follow-up (5 years)														
Months from MASE-T infusion	**1.5**	**3**	**6**	**9**	**12**	**15**	**18**	**21**	**24**	**30**	**36**	**42**	**48**	**52**	**60**
PS, clinical assessment	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
CTCAE 5.0	0	0	0												
Routine blood tests	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Research blood sample	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PET/CT	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tumour biopsy	0	0[1]													

[1] Tumour biopsy only at progression

5.8 End of study

Criteria for end of treatment are:

- Completion of treatment
- Unacceptable toxicity
- Patients request
- Progressive disease
- Stopping treatment for other reasons at the discretion of the treating physician

In case patients stop treatment but are not in progression they will enter the follow-up phase unless they request otherwise. In case of progression patients are excluded from the trial and offered standard of care treatment according to local guidelines.

5.9 Early termination of the treatment

Several reasons can lead to early termination of the planned treatment:

- Failure to manufacture the MASE-T product from peripheral blood sample
- Patient's request
- Unexpected medical conditions if the principal investigator finds it to be in the patient's best interest
- Patients will end the study treatment if they start treatment with another experimental drug or other systemic anticancer treatment
- Treatment is terminated in case of serious adverse events that make completion of the treatment impossible
- Patients who are for any reason excluded after initiating study treatment will still be offered treatment of any adverse events that occurs in relation to their participation in the protocol.

5.10 Criteria for treatment modifications

At the discretion of the treating physician, treatment with Pembrolizumab (group B) might be delayed or stopped if the patient experiences toxicity. Treatment modifications are performed following standard of care guidelines, which are accessible at https://free.mymedcards.dk/?q=immuntox.

5.11 Concurrent treatment

Supportive treatment can be administered based on clinical judgement and should be noted in the patient chart according to existing practice.
Local radiotherapy or surgery is allowed if the lesion was present at baseline and if not involving a target lesion.

6. Manufacturing of the MASE-T product with ImmPACT technology

Acquisition of blood sample for PBMCs two weeks prior to treatment. 300 mL blood is drawn from the patient to manufacture the MASE-T product. Blood will be drawn at the outpatient clinic (Clinic 5 at Herlev Hospital) by experienced nurses and collected in a 300 mL blood bag. The blood bag is immediately transported to the GMP lab.

6.1 Expansion of PBMC cultures to MASE-T product

The ImmPACT Ag-scaffold strategy has been designed to enable *ex vivo* expansion of ag-specific T cells directly from patient blood and without the need for tumor biopsy. Utilizing the ImmPACT Ag-scaffolds presenting the top 30 shared antigens, we can successfully expand ag-specific CD8+ T cells from patient PBMCs and generate higher numbers of ag-specific CD8+ T cells after day 14 of culture compared to culture initiation.

The MASE-T product consists of expanded antigen specific T cells from the patients own blood. Importantly, no residual Ag-scaffold is detected in the final T cell product . See IMPD for more details.

6.2 Handling and transportation of the MASE-T product

The infusion bag with the MASE-T product is labelled with patient ID and a patient specific transport form is filled out. The infusion bag and transport form are placed in a secure hatch for immediate pickup by a trained clinician, who validates the information on the infusion bag and the transport form. The transport form is signed, and the infusion bag is transported directly to the patient. Before administration, the information on the infusion bag is revalidated by the treatment staff and matched to the ID of the patient through patient identification.

7 Adverse events, toxicity and precautions

7.1 Adverse events and reactions

Adverse events (AE) are defined as an undesirable event in a participant during a clinical trial – regardless of whether or not the event is considered related to the investigational treatment. All AEs reported spontaneously by the subject or observed by the investigator or staff will be recorded and described in the patient chart and the electronic Case Report Form (eCRF). The severity and consequences will be recorded for each AE and the severity and relation to the study medication will be assessed in accordance with the guidelines described here.

Relation to the treatment will be determined by investigator in accordance with the following guideline:

0: Unrelated– no temporal relation, other etiologies very likely the cause

1: Related - clear temporal relation with laboratory confirmation or a positive retreatment test

Grading of Adverse Events refers to the intensity and severity of the reaction. Events are graded using CTCAE version 5.0 - see appendix 1. The following scale can be used if this CTCAE is not applicable:

1: light
2: moderate
3: severe
4: life threatening
5: lethal

Patients who experience AEs will be monitored by relevant clinical and paraclinical evaluation assessed by the attending clinician. All AEs must be monitored until satisfactory restitution or stabilization. Results of the monitoring must be recorded in the patient chart and eCRF.

Abnormal laboratory tests are not recorded in the eCRF unless they have caused an AE, resulted in termination of the treatment or meet the criteria of a serious adverse event (see the following).

Serious Adverse Events (SAEs) are defined as any medical events or affects that:

- results in death
- is life threatening (at the time of the event)
- requires hospitalization or prolongation of existing patients' hospitalization
- results in persistent or significant disability or incapacity

- leads to a congenital anomaly or birth defect
- is an otherwise significant medical event

Adverse Reactions (ARs) can be either expected if they are described in the IMPD or in the relevant product summary, or unexpected if the grade or severity does not fit the available product information. If an event is assessed to be caused by the investigational treatment, it is classified as an adverse reaction (AR). An AR can be classified as a Serious Adverse Reaction (SAR) by the same criteria as a SAE.
Suspected unexpected serious adverse reaction (SUSAR) is an unexpected adverse reaction that is deemed related to the investigational treatment. The section describing known side effects and their frequencies in the prescribing information of the used medicinal products will be used as reference for classifying an AE as unexpected. Side effects from the MASE-T product will be classified as unexpected using "Reference Safety information" section in the IMPD.

7.2 Reporting of Adverse Events and Adverse Reactions

The investigator reports SAEs andSARs to the sponsor within 24 hours. Sponsor judges whether a SAR fulfills the criteria of being a SUSAR and reports SUSARs to the Danish Medicines Agency within 7 days if fatal or considered life threatening, and otherwise within 15 days. Consequences for the study must be reported. Sponsor submits a yearly list that summarizes any SARs and SUSARs as well as a report regarding the safety of study participants to the Danish Medicines Agency and the Research Ethics Committee (investigator can report to the Research Ethics Committee as well).
Sponsor submits a final report to the Danish Medicines Agency at the end of the study with a description of all SARs and SUSARs.
The following is not to be reported:

- deaths caused by the malignant disease or progression
- hospitalizations or prolongation of current hospitalization caused by the malignant disease including:
  - weight loss
  - fatigue
  - electrolyte derangement
  - pain management
  - anxiety
  - palliative hospitalization
  - stay at hospice or terminal care
  - progression of the underlying disease
  - hospitalizations or prolongation of current hospitalization if the sole reason for hospitalization or prolongation is one or more of the following:
  - fluid treatment or treatment of nausea
  - blood transfusion
  - platelet transfusion
  - febrile leucopenia/neutropenia
  - administration of investigational procedures
  - placement of a permanent intravenous catheter

These events are registered in the eCRF (RedCAP).

7.3 Toxicity related to lymphodepleting chemotherapy

7.3.1 Cyclophosphamide

Cyclophosphamide is a classical chemotherapeutic drug and its usual limiting toxicities are myelosuppression (neutropenia, thrombocytopenia and anemia) and urotoxicity (cystitis, haematuria and hemorrhagic cystitis). Concomitant Mesna therapy along with sufficient rehydration markedly reduces the frequency and severity of the urotoxicity. The most common adverse reactions are alopecia, nausea and vomiting. Further information is available from the Danish Medicines Agency.

7.3.2 Fludarabine phosphates

Fludarabine phosphates most common side effects are myelosuppression (neutropenia, thrombocytopenia and anemia), infections including pneumonia, cough, fatigue, limpness, nausea, vomiting and diarrhea. Other adverse reactions are shivering, edema, malaise, peripheral neuropathy, visual disturbances, anorexia, mucositis, stomatitis and rash. Severe opportunistic infections and death have also been reported. Further information is available from the Danish Medicines Agency.

7.4 Toxicity related to MASE-T infusion

Toxicity related to ACT with MASE-T product are theoretically comparable to treatment with ACT with tumour infiltrating lymphocytes (TILs). The most common toxicities during TIL therapy are due to the effects of the lymphodepleting preparative regimens and the subsequent IL-2 after TIL infusion. TIL-related toxicity is less common, but patients may develop, mostly transient, hypoxia, dyspnoea, hypotension, fatigue, nausea and chills and fever shortly after infusion of TILs.[8]
Other signs of toxicity develop later after infusion may consist of melanoma associated autoimmune disease such as vitiligo, diffuse erythematous full-body rash, hearing loss or uveitis, of which latter promptly responds to topical corticosteroids. See reference safety information in IMPD.

7.5 Toxicity related to Pegfilgastrim

The most common side effects are bone and muscle pain, which are generally mild to moderate in severity and can be treated with standard painkiller.

7.6 Toxicity related to Pembrolizumab

Pembrolizumab is a checkpoint inhibitor and can cause toxicities by an *overactivation* of the immune system called immune related adverse events (irAEs). They include skin toxicities (erythema, eczema or exanthema with generalized itching and urticarial, worsening of existing autoimmune skin disorders (such as psoriasis and roseaca), gastrointestinal toxicities (diarrhea/colitis), endocrinopathies (hypophysitis, thyroiditis etc.) hepatotoxicity, pneumonitis, ocular toxicity, myocarditis, neuropathy and immune related nephrotoxicity. The most common side effects are

fatigue, diarrhea, skin rash and itching affecting about 10 % of patients. Further information is available from the EMA.

Heczey et al have treated patients with neuroblastoma with the same lymphodepleting regimen in combination with CAR T cells and anti-PD-1 therapy and have found it manageable and feasible.[49]

7.8 Risks and disadvantages regarding invasive procedures

Tumour biopsies have a slight risk of infection and bleeding. Pain and bruising might also occur in the area. Other risks are highly dependent on anatomical location. Biopsies will only be performed if deemed safe by relevant specialist.

Blood tests can cause local pain and bruising. Blood testing will involve frequent hospital visits.

7.9 Monitoring of organ function

Hematological monitoring of blood counts is indicated for all patients throughout treatment. Leukocyte count, platelet count and hemoglobin values will be checked daily at fixed intervals. Blood cell counts will be performed on day -5 before start of chemotherapy and every day during hospitalization until neutrophil counts is >500/µl and leukocyte count is >1.000/µl. Chemotherapy will not be given to patients with a leukocyte count < 500/µl and/or platelet number <50.000/ µl before the start of chemotherapy.

Kidney- and urine functions are closely monitored. Any obstruction of the urinary tracts, cystitis or infection will be resolved before start of treatment. Patients will be treated with Mesna and fluid therapy to decrease the frequency and severity of bladder toxicity from chemotherapy. The patients' urine will be tested for microscopic hematuria before start of cyclophosphamide and this drug will be discontinued if cystitis associated with micro- or macroscopic hematuria occurs during treatment.

7.10 Risks and precautions

Infertility can occur during chemotherapeutic treatment and there is also a risk of a permanent affection of the fertility.

Vaccination with live vaccines should be avoided prior to- and 6 months after treatment with Pembrolizumab (Group B).

Depolarizing muscle relaxants that inhibits the cholinesterase activity interact with cyclophosphamide and in concomitant treatment could modulate the effect of drugs such as *suxamethonium*. This can result in prolonged apnea when anesthetized and the combination should be avoided. The anesthesiologist should be informed if the patient has received treatment with cyclophosphamide within 10 days before putting into general anesthesia.

Grapefruit or grapefruit juice should be avoided since grapefruit contains a substance that can impair the activation of cyclophosphamide and thereby hinder its effect.

Transfusion related graft versus-host reactions have been observed in patients receiving treatment with fludarabine phosphate after transfusion with non-radiated and non-filtered blood. Participants in need of blood transfusions within 6 months after receiving with fludarabine phosphate should only receive radiated and filtered blood. To this end, the blood bank will be informed of the patients' enrolment in the protocol and the patient will then only receive radiated blood for 6 months after treatment. All blood in the Capital Region is filtered.

8 Translational research

Blood samples and tumour tissue are collected for research purposes.

8.1 Research blood tests

In total 350 ml of blood will be collected for research purposes in the period from initiation of treatment until the patient meets for the first evaluation after treatment. At each evaluation 78 mL will be collected and potentially another 78 mL at progression. These blood samples are collected to assess the effect of treatment on the immune system and persistence of infused T cells. The amount of blood taken during the course of the study does not exceed what the body itself is capable of producing between each test.

Immune monitoring is performed on heparinized blood by flow cytometry.
These will be collected at:

- 100 mL at baseline (day -5)
- 60 ml on day +5
- 60 ml on day +21
- 60 ml on day +42
- 60 ml on day +84
- and thereafter 60 ml every third month at time of clinical evaluation and at progression.

Mononuclear cells from the heparinized blood (PBMCs) are isolated using Lymfoprep/Leucosep density gradient technique. The mononuclear cells are washed and resuspended in a freezing media consisting of 90% heat inactivated humane type AB serum and 10% DMSO. The cells are frozen at -150°C until analysis. Analyses will primarily focus on evaluation of treatment induced quantitative changes in T-cells and will include quantitative and qualitative analyses of blood T cells measuring concentration and phenotypic stage of T-cells by flow cytometry using surface marker CD3, CD4, CD8, CD28, CD95, CCR7, and CD45RA amongst others. Furthermore, regulatory T cells, myeloid derived suppressor cells (MDSC), NK cells, NKT cells, dendritic cells, and monocytes will be quantitated by flow cytometry. Specific attention is given to the number and phenotype of the antigens specific T cells coming from the infusion product. These will be traced based on their antigenicity to determine the kinetics and persistence over time. Furthermore, selected subgroups of cell may be subjected to single-cell analyses.

ELISpot IFN-γ analyses and new advanced technologies including combinatorial coding flow cytometry will be employed for quantification of tumour antigen specific T-cells where feasible and otherwise to asses general immune status.
Serum samples (9 mL) **and plasma samples** (9 mL) will be collected at the same time points as the heparinizied blood and also on day 0, 1 and 2. Serum and plasma samples are centrifuged and frozen for later use.

8.2 Tumour samples

Biopsies are performed from accessible tumour lesions at baseline, at the first clinical evaluation (after 6 weeks) and in case of progression (only performed for patients treated at Herlev University Hospital). Biopsies will only be performed if deemed safe and without unreasonable risk for the patient. Biopsies will be paraffin embedded, tumour cell lines and TILs will be grown and used for later immune analyses.
Avaliable tumor samples in the clinical biobank "Patobank" can be used. These samples will only be used if enough material is available that this usage will have no consequence for the future cancer therapy of the patient

8.2.1 Gene-based arrays

The aim of the gene analyses is to learn about:
Differences among patients in expression level of a panel of relevant normal genes in the tumour microenvironment which could influence the chance of benefit from treatment, and expression of tumour/patient specific mutated genes which could influence the chance of benefit from treatment, including specific tumour gene expression signatures[50] and mutations in the tumour cells and patient-specific neo-antigens derived from these mutations[51].
These analyses will contribute to the identification of patients who are most likely to respond to treatment.
Tumour tissue gene expression profiles will be analyzed on either cryopreserved or FFPE-preserved tumour tissue. We will retrieve gene expression data on approximately 500 cancer/immunity related genes using *Illumina targeted RNA* sequencing applicable to degraded RNA. Targeted sequencing on a limited number of defined genes will be performed on the tumour tissue to obtain an *immune profile* to determine which genes are expressed in the tumour tissue.
Next Generation Sequencing (NGS) with extensive mapping and whole genome sequencing of tumours and normal cells of the individual patient will be used to obtain information on tumour-specific mutations. Gene sequencing will be performed on tumour cells (tumour gene profile) and leukocytes (normal gene profile) and by subtracting the two the tumour specific gene expression (neo-antigens) is found.

8.2.2 Cell lines and cell cultures

Establishment of autologous short term *in vitro* cultured tumor cell lines (as in ref. [52]and[53], or equivalent methods) and lymphocyte cultures (as in ref. [54]or equivalent methods) will be attempted using these samples. These cultures will be primarily used to perform correlates of tumor-recognition *in vitro* (as in ref.[54]) with immune parameters and clinical outcomes following therapy on an exploratory basis. The informed consent sheet will contain information about the possibility to

generate cell lines and cell cultures, that under pseudonymized or anonymized form can be used for health research purposes in multiple countries, according to the Danish National Ethics Committee guidelines

9 Data handling and reporting

The participants are allocated a study number at inclusion to ensure that they cannot be directly identified. The PI will create an allocation key with study numbers and social security numbers that will be securely stored in an access-restricted folder created by the Capital Regions IT department "CIMT". Selected collaborators at CCIT will be able to gain access to patient personal information to secure proper treatment and evaluation. All handling of personal data will be reported and approved by the Danish Data Protection Agency "Videnscenter for dataanmeldelser" through Herlev Hospital and according to Danish law. The PI has access to the patient chart including medical records and medication list before inclusion in the study in order to obtain relevant information and ensure patient safety.

9.1 Electronic case report form

All relevant data is registered in the eCRF (electronic Case Report Form) developed in cooperation with the Clinical Research Unit (KFE). The PI is responsible for creating the eCRFs and the subsequent recording and reporting of data. The sponsor and PI are both responsible for data analysis on all included patients.
Patient data and eCRF will be stored for 25 years after study completion in accordance with current guidelines for storage of personal information in clinical trials. A final report will be drafted in collaboration between the members of the study group.
The eCRF will include:

- Toxicity (CTC registration)
- Immunological response
- Clinical effect parameters.

9.2 End of study

The study sponsor will inform the Danish Medicines Agency and Research Ethics Committee within 90 days of the study completion. If the study is prematurely terminated, the Danish Medicines Agency and the Research Ethics Committee will be informed of the reason for the termination.

10 Research partner institutions

Laboratory analyses are performed at DTU or at CCIT or other locations within RegionH. Other analyses on either tumour tissue or blood samples can be performed at partner institutions but only after establishing a specific written agreement for transfer and handling of materials and data.
In cases where patient samples are to be sent abroad, they will be handled according to national laws and regulations in the receiving country. All personal information will be pseudononymized, and

a data processing agreement will be signed between the sponsor or PI and the data processors abroad. If any gene analysis is performed abroad, the data processing agreement will include the five criteria described later in "Ethical considerations regarding gene-based research" in the section "Ethical aspects". If the data processing is performed in another country or location within Denmark, permission will be applied for at the Danish Data Protection Agency.

11 Ethical considerations

11.1 Recruitment of patients and informed consent

In Denmark, metastatic melanoma patients are treated exclusively at the three participating Departments of Oncology. Referred patients will be screened for eligibility and invited to an informative consultation with the project manager or a collaborator.

The patients will receive oral and written information and signed consent of participation will be obtained before inclusion. The oral information includes information and counseling about the planned genetic analyses to enable patients to decide on whether or not to receive information about unexpected findings. The study will adhere strictly to the Helsinki declaration. The patient information follows the guidelines listed in appendix 2. Any contact to eligible patients will be done in accordance with the Danish Health Act, § 46, paragraph 3.

11.2 Patient identification

Patients are allocated a study number at inclusion. The number is given sequentially and is not based on the patients name or birthday. This number will be used to identify the patient and will be used in the eCRFs. Data and patient material will be treated pseudo anonymized and confidential.

11.3 Insurance

Participants of the study are covered by insurance from the Danish Patient Compensation Association "Patienterstatningen".

11.4 General ethical aspects

Despite many treatment advances the majority of patients with MM are still incurable. Most patients will at some point find themselves without viable treatment options. The purpose of this study is to investigate whether treatment with MASE-T infusion in combination with lymphodepleting chemotherapy with or without pembrolizumab could benefit these patients.
Based on the current knowledge and when the described precautions are taken, we find that the potential benefit of participating in this study outweighs the potential risks and downsides. At the individual level, eligible patients have advanced disease and suffered progression on traditional treatments. MASE-T may represent an additional treatment option and that may for many patients outweigh a potential risk of toxicity. Participation is always voluntary and is preceded by oral and

written information of the study. The treatment will be stopped at any time in case of unacceptable adverse reactions or at the patients request.

11.5 Ethical considerations regarding gene-based research

Data regarding potential disease-causing genes may be generated as a byproduct of certain modern laboratory techniques. Byproduct data will not be used or explored further since only data regarding tumour specific genes are processed more closely. Therefore, we do not expect to obtain explicit knowledge regarding disease-causing genes. If by chance these analyses will discover known mutations with potential significant impact on patient's health, the case will be discussed in an independent expert workgroup in collaboration with the Clinical Genetics Department at Rigshospitalet, unless the patient has chosen not to be informed as stated in the patient information. The following criteria will determine if further actions are indicated:

- A reasonable degree of possibility that a genetic disposition is present.
- Solid documentation linking the genetic disposition and the development of disease.
- The tests used to determine the genetic disposition are well established.
- The disease in question can be prevented or treated.
- The link between the genetic disposition and the development of disease has considerable importance for the patient.

If indicated, the patient will be contacted for referral to the relevant Genetics Department for additional information and testing. In cases where a patient is deceased, or do not want information regarding significant health issues, a medical assessment using the 5 above mentioned criteria will determine whether relatives to the patient is to be informed in accordance with Danish law (*Sundhedsloven* § 43, stk. 2, nr.2.).

11.6 Approvals

The PI is to obtain permission from the Danish Medicines Agency "Lægemiddelstyrelsen", the National Research Ethics Committee "National Videnskabsetisk komité" and the Danish Data Protection Agency "Videnscenter for dataanmeldelser" before starting the study.
The law dealing with personal data will be respected. Information concerning study participants is protected according to the law concerning personal data and the Act on Research Ethics Review of Health Research Projects.

11.7 Amendments

An application to the Danish Medicines Agency and the Research Ethics Committee will be made if substantial changes to the protocol are to be made. These changes can then be implemented after approval. Changes to the protocol are considered substantial in accordance with guidelines described in "Vejledning om ændringer af et godkendt forskningsprojekt" on www.nvk.dk/forsker/forskervejledning and "Skema om ændringer til kliniske forsøg" on www.sundhedsstyrelsen.dk.

12 Biobank and handling of biological material

12.1 Research biobank

In connection with the current study, blood samples and tumour tissue will be stored at -80 or -140 °C in a research biobank at the CCIT in room PA102. They will be stored until all analyses concerning the study are finished. In case of surplus samples after all planned analyses are finished, these samples will be transferred to a biobank for future research.

12.2 Biobank for future research

Surplus samples are stored in a biobank for future research at CCIT. For research in other areas with material in the biobank, new approvals from the Research Ethics Committee and Danish Data Protection Agency will be needed.

12.3 Disposal of biological material

When approval for storage of biological samples expires, remaining material will be fully anonymized or disposed of according to the local guidelines for disposal of biohazardous waste.
If a patient withdraws his/her informed consent, all biological material will be destroyed at the patients' request.

13 Administrative aspects and publications

13.1 Publications

Inge Marie Svane, Sine Reker Hadrup and Tine Juul Monberg constitute the group responsible for the project. Under the circumstance that the Vancouver rules are met, the group responsible for the project holds equal rights to the achieved results. The use and presentation of the data in any form, orally or written, can only take place with accept from everybody in the group. Positive, negative and inconclusive results will be reported in scientific journals. Author succession will be determined based on individual contributions and the investigators will be co-authors on further publications derived from this study. The main analysis will be done when all patients have been treated. Additional interim analyses can be done after treatment of respectively the first 3 and the first 6 subjects. These results might be published.
No later than a year after the trial has ended, the trial results must be entered in EudraCT and subsequently, data will be published on www.clinicaltrialsregister.eu. Further, the results will be reported to the Danish Medicines Agency.

13.2 Economy

This project has been initiated by Inge Marie Svane from the Center for Cancer Immune Therapy, Denmark, in collaboration with Sine Reker Hadrup from the Technical University of Denmark, dept of Health technology. The project is supported via a grant from 'immunterapi-puljen på finansloven' with the project title: Empowering cancer immunotherapy in Denmark, applied by Professor, MD Inge Marie Svane, j.nr. 4-1612-236/8. From this grant the budget for DTU is 960.681 dkr (incl. 44% overhead to DTU) and the budget for CCIT-DK is 1.141.208 dKr (incl 3.1% overhead to hospital)

The Research Ethics Committee will be informed in case further funding is obtained. The technology behind ImmPACT is developed in Sine Reker Hadrups research group and patent protected by DTU. DTU has made a licence agreement with the spin-out PokeAcell to make use of this technology for development of a cell therapy product. This licence agreement has been signed by president of DTU Anders Bjarklev and CEO at PokeAcell Anne Cordt. Sine Reker Hadrup is co-founder of PokeAcell. PokeAcell will also financially support the production of the Ag scaffold as part of their development process towards commercially viable cell product. The financial contribution from PokeAcell is 1.5-2 mio dkr. IMS is associated with PokeAcell as an independent advisor and provide consultancy services in connection with clinical development of the ImmPACT technology. The collaboration between the three parties in the study is regulated by a contract.

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