approaches to treat pancreatic cancer are currently pursued. The NCI is currently carrying out a clinical study of IL-2-stimulated TIL-infusion in patients with metastatic pancreatic cancer (ClinicalTrials.gov identifier: NCT01174121). Another study is evaluating the safety and efficacy of EGFR-directed bispecific antibody-expressing T-cells (BATs) in patients with locally advanced or metastatic pancreatic cancer who have already undergone 1-2 rounds of chemotherapy (ClinicalTrials.gov identifier: NCT03269526), although the T-cells themselves will be harvested from blood. Specialized T-cell-based therapies targeting private mutations in patients with metastatic cancers have resulted in remarkable clinical responses (Tran *et al*, 2015; Tran *et al*, 2016; Tran *et al*, 2014). In line with this, future clinical studies are likely to benefit translational data linking anti-tumor T-cells in pancreatic cancer to recognition of specific private mutations to improve survival as it was recently shown for a patient with metastatic breast cancer (Zacharakis *et al*, 2018). The activation of T-cell populations with T-cell receptors (TCRs), which specifically recognize mutated host molecules, i.e. neoepitopes, represent a clinically significant step towards refining T-cell-based immunotherapies and cancer vaccines.

Thus, it is an object of the present invention to improve and further develop personal ACTs for treatment of tumor diseases.

SUMMARY

The inventors identified the concept of TURICs having improved tumor reactive properties. Populations of unstimulated T-cells that reside in a precursor T-cell pool exist in body samples, which are used as common T-cell sources. These unstimulated T-cells are so low in frequency that they have not been able to proliferate in large numbers and do not exhibit classical anti-tumor activity, e.g. cytokine production, in detectable amounts after stimulation of the freshly isolated cells with tumor specific peptides or alternatively, with synthetic peptides representing the tumor mutations. The inventors have further identified that these unstimulated precursor T-cells can readily cultured and selected by the method of the disclosure. As shown in the Examples, when co-cultured in the presence of tumor specific peptides or autologous tumor cells, highly focused immune cells (TURICs) are generated that exhibit strong anti-tumor activity directed to these particular type of tumor cells or tumor specific peptides. The so produced TURICs exhibit a highly

specific tumor reactivity resulting in anti-tumor responses above the average of T-cells, which can be isolated and proliferated with known methods.

Thus, in one aspect, the present disclosure refers to a method for producing a T-cell product containing tumor uber reactive immune cells (TURICS) from a body sample obtained from a patient, the method comprising the steps of

a) optionally isolating the T-cells from the body sample;

b) stimulating the T-cells in vitro in the presence of a cytokine cocktail of the cytokines interleukin 2 (IL-2), interleukin 15 (IL-15) and interleukin 21 (IL-21) and a stimulating peptide or a group of stimulating peptides;

c) determining a reactivity factor in the T-cell sample, wherein said reactivity factor is indicative for the presence of T-cells targeting the stimulating peptide or at least one peptide of the group of stimulating peptides;

d) in case the reactivity factor is positive, identifying the T-cell sample as a tumor reactive T-cell sample; otherwise identifying the T-cell sample as a non-reactive T-cell sample;

e) culturing the non-reactive sample in vitro in the presence of the cytokine cocktail of IL-2, IL-15 and IL-21 and either one of autologous tumor cells or the stimulating peptide or the group of stimulating peptides to form a T-cell product;

f) optionally stimulating the T-cell product in vitro in the presence of the cytokine cocktail of IL-2, IL-15 and IL-21 and the stimulating peptide or the group of stimulating peptides;

g) determining the reactivity factor in the T-cell product; and

h) in case the reactivity factor is positive selecting the T-cell product as a T-cell product containing TURICS

In another aspect, the present disclosure refers to use of a composition containing at least one T-cell product with TURICs obtained according to the method as disclosed herein in the manufacture of a medicament for treating cancer.

Thus, in a first example, the present disclosure provides a method for producing a T-cell product containing tumor uber reactive immune cells (TURICs) comprising the steps of

a) providing a body sample containing T-cells of a patient;
b) optionally isolating the T-cells from the body sample;
c) stimulating the T-cells *in vitro* in the presence of a cytokine cocktail of the cytokines interleukin 2 (IL-2), interleukin 15 (IL-15) and interleukin 21 (IL-21) and a stimulating peptide or a group of stimulating peptides;
d) determining a reactivity factor in the T-cell sample, wherein said reactivity factor is indicative for the presence of T-cells targeting the stimulating peptide or at least one peptide of the group of stimulating peptides;
e) in case the reactivity factor is positive, identifying the T-cell sample as a tumor reactive T-cell sample; otherwise identifying the T-cell sample as a non-reactive T-cell sample;
f) culturing the non-reactive sample *in vitro* in the presence of the cytokine cocktail of IL-2, IL-15 and IL-21 and either one of autologous tumor cells or the stimulating peptide or the group of stimulating peptides to form a T-cell product;
g) optionally stimulating the T-cell product *in vitro* in the presence of the cytokine cocktail of IL-2, IL-15 and IL-21 and the stimulating peptide or the group of stimulating peptides;
h) determining the reactivity factor in the T-cell product; and
i) in case the reactivity factor is positive selecting the T-cell product as a T-cell product containing TURICs.

In a second example, the present disclosure also provides a composition containing at least one T-cell product with TURICs for use in treatment of a cancer patient, comprising performing the method according to the first example of the disclosure to obtain a T-cell product with TURICs and administering the T-cell product with TURICs to the patient.

Claims

1. A method for producing a T-cell product containing tumor uber reactive immune cells (TURICS) from a body sample obtained from a patient, the method comprising the steps of
   a) optionally isolating the T-cells from the body sample;
   b) stimulating the T-cells *in vitro* in the presence of a cytokine cocktail of the cytokines interleukin 2 (IL-2), interleukin 15 (IL-15) and interleukin 21 (IL-21) and a stimulating peptide or a group of stimulating peptides;
   c) determining a reactivity factor in the T-cell sample, wherein said reactivity factor is indicative for the presence of T-cells targeting the stimulating peptide or at least one peptide of the group of stimulating peptides;
   d) in case the reactivity factor is positive, identifying the T-cell sample as a tumor reactive T-cell sample; otherwise identifying the T-cell sample as a non-reactive T-cell sample;
   e) culturing the non-reactive sample *in vitro* in the presence of the cytokine cocktail of IL-2, IL-15 and IL-21 and either one of autologous tumor cells or the stimulating peptide or the group of stimulating peptides to form a T-cell product;
   f) optionally stimulating the T-cell product *in vitro* in the presence of the cytokine cocktail of IL-2, IL-15 and IL-21 and the stimulating peptide or the group of stimulating peptides;
   g) determining the reactivity factor in the T-cell product; and
   h) in case the reactivity factor is positive selecting the T-cell product as a T-cell product containing TURICS.

2. The method according to claim 1, wherein the group of stimulating peptides consists of up to 20 different stimulating peptides, preferably up to 10 different stimulating peptides, more preferably up to five different stimulating peptides.

3. The method according to claim 1 or 2, wherein the stimulating peptides are mutated or non-mutated tumor-specific peptides, wherein the mutated tumor specific peptides contain an amino acid sequence with a mutation found in tumor cells of the patient but not in cells of healthy tissue of the patient.

4. The method according to claim 3, wherein the mutation is located in the middle of the peptide.

5. The method according to any of the previous claims, wherein the stimulating peptides have a length in the range from 5 to 31 amino acids, preferably 7 to 25 amino acids, more preferably 9 to 21 amino acids.

6. The method according to any of the previous claims, wherein the stimulation of the T-cells and/or the T-cell product is performed on 10^2 to 10^8 cells, preferably on 10^3 to 10^6 cells, more preferably on 10^4 to 10^5 cells.

7. The method according to any of the previous claims, wherein the T-cells and/or the T-cell product are stimulated for 1 hour to 10 days, preferably for 3 hours to 5 days, more preferably 1 day to 3 days.

8. The method of any of the previous claims, wherein the non-reactive T-cell sample is cultured for 1 to 10 days, preferably for 3 to 9 days, more preferably for 6 to 8 days.

9. The method according to any of the previous claims, wherein in steps c) and g) the stimulating peptide or each peptide of the group of stimulating peptides is present in a concentration of from 1 pg/10^5 cells to 1 mg/10^5 cells, preferably in a concentration of 1 ng/10^5 cells to 100 µg/10^5 cells, more preferably in a concentration of from 1 µg/10^5 cells to 10 µg/10^5 cells.

10. The method of any of the previous claims, wherein the non-reactive T-cell sample and the autologous tumor cells are cultured in a ratio ranging from of 1000:1 to 1:1000, preferably in a ratio ranging from of 10:1 to 1:10, more preferably in a ratio of 7:1 to 3:1.

11. The method according to any of the previous claims, wherein the reactivity factor is selected from T-cell proliferation, cytokine production, cytotoxicity, e.g. killing of the cells of the diseased body sample, degranulation, in particular defined by CD107a positivity, maturation and or differentiation, in particular defined by the combination of CD45RA and CCR7, expression of a T-cell activation marker in particular selected from CD25, CD56, CD69 of MHC class II molecules; an exhaustion and/or activation markers selected from Foxp3, LAG-3, TIM-3, 4-1BB, PD-1, CD127 (IL-7R), the IL-21 receptor or T-cell signaling, in particular selected from the zeta chain phosphorylation, preferably, the reactivity factor is the IFNγ concentration and the reactivity factor is positive if the concentration of IFNγ is above a predefined IFNγ threshold.

12. The method according to any of the previous claims, wherein
    - steps c) and d) are additionally carried out with a comparative peptide or a group of comparative peptides as the stimulating peptide or the group of stimulating peptides, wherein the comparative peptide(s) contain(s) the non-mutated sequence corresponding to the mutated tumor specific peptide sequence, when a mutated tumor specific peptides was used as stimulating peptide, and/or
    - steps g) and h) are additionally carried out with a comparative peptide or a group of comparative peptides as the stimulating peptide or the group of stimulating peptides, wherein the comparative peptide(s) contain(s) the non-mutated sequence corresponding to the mutated tumor specific peptide sequence, when a mutated tumor specific peptides was used as stimulating peptide, and wherein the T-cell product is deselected in case the reactivity factor for stimulating the T-cell product with the comparative peptide or the group of comparative peptides is equal to or higher than the reactivity factor for stimulating the T-cell product with the mutated tumor specific peptide or the group of tumor specific peptides.

13. The method according to any of the previous claims, wherein the body sample is not a tumor a sample, preferably the tumor sample is selected from whole blood, serum, plasma, urine, tears, sperm, saliva, synovial fluid, umbilical cord, placenta tissue, bone marrow, exhaled air, lavage material, such as bronchoalveolar lavage, cerebrospinal fluid (CSF), primary, secondary lymphoid tissues, samples from the gut lumen, samples from peritoneal cavity, transplanted material, transplanted cells, transplanted tissue(s) or organ(s).

14. The method according to claim 12, wherein each comparative peptide is applied in a concentration similar to that of the corresponding tumor-specific peptide.

15. Use of a composition containing at least one T-cell product with TURICs obtained according to the method of any one of claims 1 to 14 in the manufacture of a medicament for treating cancer.