successful therapies in heterogeneous tumours (and their heterogeneous metastases).

Drug administration in heterogeneous tumours will seldom kill all tumour cells. The initial heterogeneous tumour population may bottleneck, such that few drug resistant cells will survive. This allows resistant tumour populations to replicate and re-grow the tumour through a branching evolution mechanism. The resulting repopulated tumour may also be heterogeneous and will be resistant to the initial drug therapy used. The repopulated tumour may also return in a more aggressive manner.

The administration of cytotoxic drugs often results in initial tumour shrinkage. This represents the destruction of initial non-resistant subclonal populations within a heterogeneous tumour, leaving only resistant clones. These resistant clones now contain a selective advantage in the presence of chemotherapy and can replicate to repopulate the tumour. Replication will likely occur through branching evolution, contributing to tumour heterogeneity. The repopulated tumour may appear to be more aggressive. This is attributed to the drug-resistant selective advantage of the tumour cells and additional genetic changes that occur during therapy and the disease course.

WO 2014/168874 describes a method for making a personalized neoplastic vaccine for a subject diagnosed as having a neoplasia, which includes identifying a plurality of mutations in the neoplasia, analysing the plurality of mutations to identify a subset of at least five neo-antigenic mutations predicted to encode neo-antigenic peptides and producing, based on the identified subset, a personalised neoplasia vaccine.

Thus there is a need for alternative methods for treating cancer, in particular heterogeneous tumours.

SUMMARY

The present inventors have determined that truncal mutations, that is mutations present in essentially all tumour cells in a heterogeneous tumour, can be identified through multi-region sampling of the tumour or through approaches to identify clonal mutations in single biopsies. For example, the cancer cell fraction, describing the fraction of cancer cells harbouring a mutation, can be determined in order to distinguish neo-antigens likely to be present in every cancer cell in the tumour (truncal neo-antigens) from neo-antigens only present in a subset of tumour cells (branch neo-

antigens). As used herein, the term "truncal mutations" is synonymous with the term "clonal mutations". They are both intended to define mutations present in essentially all tumour cells in a heterogeneous tumour. As used herein, the term "branched mutations" is synonymous with the term "sub-clonal mutations". They are both intended to define mutations present in a subset of tumour cells. The administration of therapeutic T cells which target truncal neo-antigens, rather than branch neo-antigens, or the administration of vaccines as described herein, enables an effective immune response to be mounted against the entire tumour and thus reduces the risk of resistant cells repopulating the tumour.

Thus in one embodiment the present disclosure provides a method for identifying a truncal neo-antigen in a tumour from a subject which comprises the steps of:
i) determining mutations present in a sample isolated from the tumour; and
ii) identifying a truncal mutation which is a mutation present in essentially all tumour cells; and
iii) identifying a truncal neo-antigen, which is an antigen encoded by a sequence which comprises the truncal mutation.

In one embodiment, the present disclosure provides a method for identifying a truncal neo-antigen in a tumour from a subject which comprises the steps of:
i) determining mutations present in a plurality of samples isolated from the tumour; and
ii) identifying a truncal mutation which is a mutation present in all samples; and iii) identifying a truncal neo-antigen, which is an antigen encoded by a sequence which comprises the truncal mutation.

The truncal mutation may be a single nucleotide variant or an insertion/deletion or a splice site mutation resulting in a change in the amino acid sequence (coding mutation).

The mutations may be identified by Exome sequencing, RNA-seq, whole genome sequencing and/or targeted gene panel sequencing. Suitable methods are known in the art. Descriptions of Exome sequencing and RNA-seq are provided by Boa et al. (Cancer Informatics. 2014; 13(Suppl 2):67-82.) and Ares et al. (Cold Spring Harb Protoc. 2014 Nov 3;2014(1 1):1 139-48); respectively. Descriptions of targeted gene panel sequencing can be found in, for example, Kammermeier et al. (J Med Genet.

2014 Nov;51 (1 1):748-55) and Yap KL et al. (Clin Cancer Res. 2014. 20:6605). See also Meyerson et al., Nat. Rev. Genetics, 2010 and Mardis, Annu Rev Anal Chem, 2013. Targeted gene sequencing panels are also commercially available (e.g. as summarised by Biocompare (http://www.biocompare.com/Editorial-Articles/161 194-Build-Your-Own-Gene-Panels-with-These-Custom-NGS-Targeting-Tools/).

The method may comprise the step of assessing the subject's HLA allele profile to determine if a truncal neo-antigen peptide will bind to a MHC molecule of the subject. Suitable methods are known in the art, e.g. OptiType, Szolek et al., 2014.

In one embodiment the present disclosure provides a method for providing a T cell population which targets a truncal neo-antigen in a tumour from a subject which comprises the steps of:
i) identifying a T cell from a sample isolated from the subject, which is capable of specifically recognising a truncal neo-antigen peptide; and
ii) expanding the T cell to provide a T cell population which targets the truncal neo-antigen.

One skilled in the art will appreciate that references herein to a T cell "recognising" a truncal neo-antigen or truncal neo-antigen peptide include recognition in the form of a truncal neo-antigen peptide:MHC complex.

The disclosure also provides a method for identifying a truncal neo-antigen specific T cell which comprises the following steps:
i) determining mutations present in a sample isolated from the tumour; and
ii) identifying a truncal mutation which is a mutation present in essentially all tumour cells; and
iii) identifying a truncal neo-antigen, which is an antigen encoded by a sequence which comprises the truncal mutation;
or
i) determining the mutations present in a plurality of samples isolated from a tumour;
ii) identifying a truncal mutation which is a mutation present in all samples;
iii) identifying a truncal neo-antigen, which is an antigen encoded by a sequence which comprises the truncal mutation; and
iv) identifying from a sample from said subject a T cell capable of specifically recognising the truncal neo-antigen as a truncal neo-antigen specific T cell.

The sample from which the T cell is identified may be a blood sample, a tumour sample, a tumour-associated lymph node sample or sample from a metastatic site.

The disclosure also provides a method for providing a T cell population which targets a truncal neo-antigen in a tumour which comprises the steps of:

(a) identifying a truncal neo-antigen in a tumour from a subject which comprises the steps of:

i) determining mutations present in a sample isolated from the tumour; and

ii) identifying a truncal mutation which is a mutation present in essentially all tumour cells; and

iii) identifying a truncal neo-antigen, which is an antigen encoded by a sequence which comprises the truncal mutation;

or

i) determining the mutations present in a plurality of samples isolated from a tumour;

ii) identifying a truncal mutation which is a mutation present in all samples; iii) identifying a truncal neo-antigen, which is an antigen encoded by a sequence which comprises the truncal mutation; and

b) identifying a T cell from a sample isolated from a subject which is capable of specifically recognising said truncal neo-antigen; and

c) expanding the T cell to provide a T cell population which targets the truncal neo-antigen.

The disclosure also provides a T cell population which is obtained or obtainable by a method which comprises the steps of:

(a) identifying a truncal neo-antigen in a tumour from a subject which comprises the steps of:

i) determining mutations present in a sample isolated from the tumour; and

ii) identifying a truncal mutation which is a mutation present in essentially all tumour cells; and

iii) identifying a truncal neo-antigen, which is an antigen encoded by a sequence which comprises the truncal mutation;

or

i) determining the mutations present in a plurality of samples isolated from a tumour;

ii) identifying a truncal mutation which is a mutation present in all samples;

iii) identifying a truncal neo-antigen, which is an antigen encoded by a sequence which comprises the truncal mutation; and

b) identifying a T cell from a sample isolated from a subject which is capable of specifically recognising said truncal neo-antigen; and

c) expanding the T cell to provide a T cell population which targets the truncal neo-antigen.

Thus, the resulting T cell population is enriched with an increased number of T cells which target truncal neo-antigens (for example, compared with the sample isolated from the subject).

The sample may be a tumour, blood, tissue or peripheral blood mononuclear cells from the subject.

The truncal neo-antigen may be generated by a truncal mutation identified according to the method as defined herein.

The population of T cells may comprise $CD8^+$ T cells, $CD4^+$ T cells or $CD8^+$ and $CD4^+$ T cells.

The method may comprise providing at least a first and a second T cell, wherein the first T cell targets a first truncal neo-antigen generated by a first truncal mutation and the second T cell targets a second truncal neo-antigen generated by a second truncal mutation.

In one embodiment the present disclosure provides a T cell composition which comprises a truncal neo-antigen specific T cell or a population of T cells as described herein.

The truncal neo-antigen may be identified by the method as defined herein.

The truncal neo-antigen specific T cell may be a T cell as defined herein.

The truncal neo-antigen specific T cell may express a chimeric antigen receptor (CAR) or a T cell receptor (TCR) or an affinity-enhanced T cell receptor (TCR) which

specifically binds a truncal neo-antigen or truncal neo-antigen peptide (i.e. a peptide derived from the truncal neo-antigen), as discussed further hereinbelow.

Methods for generating TCRs and affinity enhanced TCRs are known in the art. Affinity enhanced TCRs are TCRs with enhanced affinity for a peptide-MHC complex. Methods include e.g. the isolation of TCR genes that encode TCRs from patient samples (e.g. patient peripheral blood or TILs), and the improvement of TCR affinity for a peptide-MHC complex via modification of TCR sequences (e.g. by in vitro mutagenesis and selection of enhanced affinity (or affinity matured) TCRs). Methods of introducing such TCR genes into T cells are known in the art. Methods of identifying optimal-affinity TCRs involving the immunisation of antigen-negative humanised transgenic mice which have a diverse human TCR repertoire (e.g. TCR/MHC humanised mice such as ABabDII mice) with antigen, and isolation of antigen-specific TCRs from such immunised transgenic mice are also known in the art (see e.g. Obenaus M et al., Nat Biotechnol. 33(4):402-7, 2015).

In one embodiment the present disclosure provides an MHC multimer comprising a truncal neo-antigen peptide, wherein the truncal neo-antigen is identified by the method as defined herein. MHC multimers and methods of using them to isolate T cells are known in the art, for example as described in Hadrup, Nature Methods 6:520-526 2009; and Andersen, Nature Protocol 7:891-902, 2012.

MHC multimers as described herein may be used in methods of the disclosure, for example in methods of identifying NES T cells. The MHC multimers may be used to identify, expand or enrich NES T cells in methods as described herein, for example methods for producing a T cell or T cell population or composition as described herein.

In one embodiment the present disclosure provides a vaccine comprising a truncal neo-antigen peptide from a truncal neo-antigen identified by the method as defined herein. As discussed herein, a truncal neo-antigen vaccine according to the disclosure may be delivered as a dendritic cell vaccine pulsed or loaded with the truncal neo-antigen, or genetically modified (via DNA or RNA transfer) to express one, two or more truncal neo-antigens.

In one embodiment the present disclosure provides a T cell composition as defined herein for use in treating cancer.

Disclosed herein is the use of a T cell composition in the manufacture of a medicament for the treatment of a cancer in a subject, wherein the T cell composition comprises T cells selectively expanded to target clonal neo-antigens characteristic of the subject's cancer, wherein a clonal neoantigen has a cancer cell fraction (CCF) 95% confidence interval of greater than or equal to 0.75.

In one embodiment the present disclosure provides a T cell as defined herein for use in the manufacture of a medicament for the treatment of cancer.

In one embodiment the present disclosure relates to a method for treating cancer in a subject which comprises administering a T cell composition as defined herein to the subject.

The method may comprise the following steps:

(i) isolation of a T cell containing sample from the subject;

(ii) identification and expansion of a T cell population which targets the truncal neo-antigen; and

(iii) administering the cells from (ii) to the subject.

The method may comprise the following steps:

(i) isolation of a T cell containing sample;

(ii) engineering the T cell to express a CAR or TCR which recognises said truncal neo-antigen as described herein to provide a T cell population which targets the truncal neo-antigen; and

(iii) administering the cells from (ii) to the subject.

In one embodiment the method also encompasses the step of identifying a truncal neo-antigen as described herein, i.e. the disclosure provides a method for treating cancer in a subject, wherein said method comprises;

(a) identifying a truncal neo-antigen in a tumour from a subject which comprises the steps of:

i) determining mutations present in a sample isolated from the tumour; and

ii) identifying a truncal mutation which is a mutation present in essentially all tumour cells; and

iii) identifying a truncal neo-antigen, which is an antigen encoded by a sequence which comprises the truncal mutation;

or i) determining the mutations present in a plurality of samples isolated from a tumour;

ii) identifying a truncal mutation which is a mutation present in all samples;

iii) identifying a truncal neo-antigen, which is an antigen encoded by a sequence which comprises the truncal mutation; and

b) identifying a T cell from a sample isolated from a subject which is capable of specifically recognising said truncal neo-antigen;

c) expanding the T cell to provide a T cell population which targets the truncal neo-antigen; and

d) administering said T cell population to said subject.

The method may comprise the steps of;

(a) identifying a truncal neo-antigen in a tumour from a subject which comprises the steps of:

i) determining mutations present in a sample isolated from the tumour; and

ii) identifying a truncal mutation which is a mutation present in essentially all tumour cells; and

iii) identifying a truncal neo-antigen, which is an antigen encoded by a sequence which comprises the truncal mutation;

or

i) determining the mutations present in a plurality of samples isolated from a tumour;

ii) identifying a truncal mutation which is a mutation present in all samples; iii) identifying a truncal neo-antigen, which is an antigen encoded by a sequence which comprises the truncal mutation; and

(b) providing a T cell containing sample;

(c) engineering a T cell to express a CAR or TCR which recognises said truncal neo-antigen to provide a T cell population which targets the truncal neo- antigen; and

(d) administering said T cell population to the subject.

In one embodiment the T cell is engineered to express a CAR or affinity-enhanced TCR as described herein.

The disclosure also provides a method of treating a patient who has cancer comprising:

(i) identifying a patient who has cancer; and

(ii) administering to said patient a T cell or T cell population as defined herein.

The truncal neo-antigen, T cell or T cell population may have been identified or produced according to embodiments of the disclosure as described herein.

The sample may be a tumour sample, a blood sample or a tissue sample or a peripheral blood mononuclear cells sample from the subject.

In one embodiment the present disclosure relates to a method for treating cancer in a subject which comprises administering a vaccine as defined herein to the subject.

DESCRIPTION OF THE FIGURES

Figure 1 - Pipeline for prediction and identification of neo-antigen reactive T cells in NSCLC samples. Exome seq and RNAseq are used to define neo-epitopes from truncal and branch mutations. The binding of mutant or wild type peptides to the patient's HLA is predicted and peptides with predicted high affinity (green) to HLA (low IC_{50}) and those with high affinity as mutant and low as a wild type (blue) are selected to generate fluorescent MHC multimers to be used in the identification of NES T cells in tumour samples.

Figure 2 - Illustration of the difference between truncal mutations and branch mutations in sample isolated from lung cancer, brain cancer and normal lung and brain tissue.

Figure 3 - Illustration of the pipeline from identification of truncal mutations in a tumour to the identification of neo-antigen-specific T cells.

Figure 4 - (A) Identification of neo-antigen specific T cells in early stage lung cancer. Predicted affinities of mutant (Y axis) versus wild type (X axis) obtained from exon sequencing data are shown. Red dots indicate predictive peptides with a high score (low affinity) for the patient's HLA in the WT form and low score (high affinity) in the mutant form. (B) In vitro expanded TILs were stained with fluorescent tetramers loaded with the predicted mutated peptides or control Cytomegalovirus (CMV) peptides and analyzed by flow cytometry. CMV reactive T cells are found at equal frequencies (0.2-0.3%) in tumour and normal lung.

CLAIMS

1. Use of a T cell composition in the manufacture of a medicament for the treatment of a cancer in a subject, wherein the T cell composition comprises T cells selectively expanded to target clonal neo-antigens characteristic of the subject's cancer, wherein a clonal neoantigen has a cancer cell fraction (CCF) 95% confidence interval of greater than or equal to 0.75.

2. A use according to claim 1, wherein the T cell composition is enriched with T cells which are specific to clonal neo-antigens.

3. A use according to claim 1 or 2, wherein the expanded population of clonal neo-antigen-reactive T cells has a higher activity than the population of T cells which have not been expanded, as measured by the response of the T cell population to restimulation with a clonal neo-antigen peptide.

4. A use according to claim 3, wherein the activity is measured by cytokine production, and wherein a higher activity is a 5-10-fold or greater increase in activity.

5. A use according to claim 1, wherein said T cells have been selectively expanded by a method comprising the steps of:

a) providing a sample comprising a T cell population which is capable of specifically recognising said neo-antigen; and

b) co-culturing the T cell population with a composition comprising the neo-antigen, or neo-antigen peptide, and an antigen presenting cell;

wherein the neo-antigen has been identified in a sample of a tumour from said subject as being a clonal neo-antigen.

6. A use according to claim 5, wherein said T cells are selectively expanded using a plurality of clonal neo-antigens or clonal neo-antigen peptides, wherein each of said peptides comprises a different clonal mutation.

7. A use according to claim 6, wherein said plurality of peptides comprises between 2 and 100 peptides.

8. A use according to any one of claims 5 to 7 wherein said antigen presenting cell has been loaded or pulsed with a peptide derived from said clonal neo-antigen.

9. A use according to any one of claims 5 to 8 wherein said antigen presenting cell is a dendritic cell.

10. A use according to any one of claims 5 to 9 wherein the T cell population is cultured with IL-2 or anti-CD3 and/or CD28 antibodies.

11. A use according to any one of claims 5 to 10 wherein the T cell population is isolated from a sample of the tumour of the patient to be treated.

12. A use according to claim 11, wherein the T cell population comprises tumour infiltrating lymphocytes (TILs).

13. A use according to any one of claims 5 to 12 wherein the population of T cells comprises CD8+ T cells, CD4+ T cells or CD8+ and CD4 T+ cells.

14. A use according to any one of claims 5 to 13 wherein at least a first and a second T cell is provided, wherein the first T cell targets a first clonal neo-antigen generated by a first clonal mutation and the second T cell targets a second clonal neo-antigen generated by a second clonal mutation.

15. A use according to any one of claims 5 to 14 wherein said expanded T cell population is enriched with T cells which target clonal neo- antigens relative to the starting sample.

16. A use according to any preceding claim, wherein the T cell composition is to be used in combination with a checkpoint inhibitor.

17. A use according to any preceding claim wherein the cancer is non-small cell lung cancer (NSCLC) or melanoma.