

Claims

1. A method for obtaining tumour nucleic acid for sequencing, comprising providing a medium containing tumour cells shed from a solid tumour sample and/or released during mechanical disruption of at least part of the solid tumour sample, and extracting nucleic acid from the shed and/or released tumour cells.
2. A method for sequencing nucleic acid from a solid tumour sample, wherein said method comprises the steps of:
- 10 (i) providing a medium containing tumour cells shed from said solid tumour sample and/or released during mechanical disruption of at least part of said tumour sample; and
- (ii) extracting nucleic acid from the shed and/or released tumour cells.
3. The method according to claim 1 or claim 2 wherein neither said tumour sample nor said shed or released tumour cells are cultured *ex vivo* prior to extraction of said nucleic acid.
4. The method according to any preceding claim wherein there is no disruption of the solid tumour sample by enzymatic or other non-mechanical means prior to nucleic acid extraction.
- 20 5. The method according to any preceding claim, wherein the method comprises the following steps:
- (a) providing a medium containing tumour cells shed from a solid tumour sample and/or released during mechanical disruption of at least part of the solid tumour sample;
- 25 (b) isolating the shed and/or released tumour cells from the medium; and
- (c) extracting nucleic acid from the shed and/or released tumour cells.
6. The method according to any preceding claim wherein the medium contains tumour cells which have been shed from the solid tumour sample directly into the medium *ex vivo*.
- 30 7. The method according to any preceding claim wherein the solid tumour sample has been retained in the medium for a period of at least about 5 minutes, 10 minutes, 15 minutes, 30 minutes, 60 minutes/1 hour, 1.5 hours, 2 hours, 2.5 hours, 3 hours, 3.5 hours, 4 hours, 4.5 hours, 5 hours, 5.5 hours, 6 hours, 6.5 hours, 7 hours, 7.5 hours, 8 hours, 8.5 hours, 9 hours, 9.5 hours, 10 hours, 10.5 hours, 11 hours, 11.5 hours or 12 hours or more prior to a step of extracting nucleic acid from the shed tumour cells.
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8. The method according to any preceding claim wherein the cells have been shed into the medium during storage or transport of the solid tumour sample.
9. The method according to any preceding claim wherein the method does not comprise
5 a step of mechanically disrupting the solid tumour sample prior to the extraction of nucleic acid for sequencing.
10. The method according to any preceding claim wherein the solid tumour is selected from non-small cell lung cancer (NSCLC), melanoma, renal cancer, bladder cancer, head
10 and neck cancer, and breast cancer.
11. The method according to any preceding claim wherein the solid tumour is selected from NSCLC, melanoma and head and neck cancer.
12. The method according to any preceding claim which further comprises the step of
15 sequencing nucleic acid extracted from the shed and/or released tumour cells.
13. The method according to claim 12 wherein the sequence information generated is suitable for the identification of a clonal neoantigen(s) from the tumour.
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14. The method according to claim 13 further comprising the step of identifying a clonal neoantigen(s) from the tumour.
15. The method according to any preceding claim further comprising isolating tumour
25 infiltrating lymphocytes (TIL) from at least part of the solid tumour sample.
16. The method according to claim 15 wherein the TIL are selectively expanded to produce a population of clonal neoantigen-specific T cells (cNeT).
17. The method according to any preceding claim further comprising the step of
30 removing non-tumour cells by negative selection prior to the extraction of nucleic acid for sequencing.
18. The method according to claim 17 wherein the negative selection comprises
35 immunomagnetic negative selection.

19. The method according to claim 18 wherein the immunomagnetic negative selection comprises depletion of CD45+ cells, red blood cells, platelets, granulocytes, heterogeneous lymphocyte populations, fibroblasts, endothelial cells and/or hematopoietic cells.

5 20. The method according to any preceding claim wherein the medium is selected from a group consisting of HypoThermosol, Dulbecco's Modified Eagle's Medium (DMEM), Ham's F10 medium, Ham's F12 medium, Advanced DMEM, Advanced DMEM/F12, minimal essential medium, DMEM/F-12, DMEM/F-15, Liebovitz L-15, RPMI 1640, Iscove's modified Dulbecco's media (IMDM), OPTI-MEM SFM, N2B27, MEF-CM, PBS or a combination
10 thereof, wherein preferably the medium is HypoThermosol.

21. Use of tumour cells which have been shed from a solid tumour sample into a medium *ex vivo* and/or released during mechanical disruption of at least part of the tumour sample to provide tumour nucleic acid for sequencing.

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22. A method for selectively expanding a T cell population for use in the treatment of cancer in a subject, the method comprising the steps of:

(a) providing medium containing tumour cells shed from a solid tumour sample and/or released during mechanical disruption of at least part of the tumour sample;

20 (b) extracting nucleic acid from the shed and/or released tumour cells;

(c) sequencing nucleic acid extracted from the shed and/or released tumour cells;

(d) identifying a neoantigen from the tumour using the sequence information obtained in step (c);

25 (e) isolating tumour infiltrating lymphocytes (TIL) from at least part of said solid tumour sample; and

(f) co-culturing the TIL with an antigen presenting cell which presents the neoantigen identified in step (d).

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