CLAIMS

1. An in vitro method for assessing the ~~functionality~~target antigen binding and activation capacity of chimeric antigen receptor (CAR) expressing cells, comprising:

- Labelling target antigen expressing cells and CAR expressing cells with different labels, wherein the target antigen expressing cells and the CAR expressing cells are prepared from the same cell line;
- Co-incubating the labelled target cells and the labelled CAR expressing cells,
- Analyzing the cells in order to assess membrane acquisition by the CAR expressing cells from the target antigen expressing cells, the membrane acquisition being indicative of the binding and/or activation capacity of the CAR expressing cells to the target antigen, thereby assessing the functionality of the CAR expressing cells; and

wherein the cells are immune cells.

2. The method of claim 1, wherein the co-incubation is performed at least 1 hour~~, preferably during 1 to 5 hours, even more preferably during 1 to 3 hours~~.

3. The method of claim 2, wherein the co-incubation is performed during 1 to 5 hours.

4. The method of claim 3, wherein the co-incubation is performed during 1 to 3 hours.

~~3~~5. The method of any of ~~claim 1 or 2~~claims 1-4, wherein the co-incubation is performed in a 1:0.5 to a 1:10 target antigen expressing cells to CAR expressing cells ratio.

~~4~~6. The method of any of claims 1-~~3~~5, wherein the cells analysis is performed by cell sorting analysis~~, preferably flow-cytometry analysis~~.

7. The method of claim 6, wherein the cell sorting analysis is flow-cytometry analysis.

~~5~~8. The method of any of claims 1-~~4~~7, further comprising a step of incubating target antigen expressing cells with an antibody that recognizes the same or an overlapping epitope compared to the antibody from which the CAR is derived before co-incubating the labelled target antigen cells and the labelled CAR expressing cells.

~~6~~9. The method of any of claims 1-~~5~~8, wherein the antibody is a monoclonal antibody~~, preferably a monoclonal antibody that comprises CDRs of the monoclonal antibody from which the CAR is derived~~.

10. The method of claim 9, wherein the monoclonal antibody comprises CDRs of the monoclonal antibody from which the CAR is derived.

~~7~~11. The method of any of claims 1-~~6~~10, wherein the method further comprises testing control cells that do not express the targeted antigen.

~~8~~12. The method of any of claims 1-~~7~~11, wherein the method further comprises testing control CAR expressing cells that do not recognize the targeted antigen.

~~9~~13. The method of any of claims 1-~~8~~12, further comprising the selection of the functional CAR expressing cells and/or CAR construct.

~~10~~14. The method of any of claims 1-~~9~~13, wherein the labels are membrane markers~~, preferably lipophilic tracers or dyes, even more preferably selected from the group consisting of MINI26, PKH26-PCL, PKH67, MINI67, PKH67-PCL, PKH26, PKH26-PCL, Vybrant CM-DiI, DiI and DiO~~.

15. The method of claim 14, wherein the membrane markers are lipophilic tracers or dyes.

16. The method of claim 14, wherein the membrane markers are selected from the group consisting of MINI26, PKH26-PCL, PKH67, MINI67, PKH67-PCL, PKH26, PKH26-PCL, Vybrant CM-DiI, DiI and DiO.

~~11~~17. The method of any of claims 1-~~10~~16, wherein the immune cells are selected from the group consisting of T lymphocytes, B lymphocytes, natural killer cells, natural killer T cells, monocytes and antigen presenting cells.

~~12~~18. An *in vitro* method for selecting functional CAR expressing cells for adoptive cell therapy, which method comprises~~-~~:

(i) transducing the population of immune cells with a nucleic acid sequence encoding a CAR, ~~preferably wherein the population of immune cells has been isolated from a biological sample from a subject to be treated,~~

(ii) selecting a subpopulation of said isolated cells expressing the CAR and,

(iii) assessing ~~their~~the functionality of the CAR expressing cells by the method according to any of the preceding claims~~, preferably towards target antigen expressing cells that expresses the antigen for which the CAR has been generated~~.

~~(iv) optionally selecting the functional CAR expressing cells and/or the CAR nucleic acid construct from the CAR expressing cells that experienced the membrane acquisition form the antigen expressing cells.~~

19. The *in vitro* method of claim 18, further comprising

(iv) selecting the functional CAR expressing cells and/or the CAR nucleic acid construct from the CAR expressing cells that experienced the membrane acquisition form the antigen expressing cells.

20. The *in vitro* method of claim 18, wherein the population of immune cells has been isolated from a biological sample from a subject to be treated.

21. The *in vitro* method of claim 18, wherein assessing the functionality of the CAR expressing cells is towards target antigen expressing cells that expresses the antigen for which the CAR has been generated.

~~13~~22. The method of any of claims 1-~~12~~21, wherein the antigen is HLA-G~~, preferably HLA-G1~~.

23. The method of claim 22, wherein the HLA-G is HLA-G1.