CLAIMS

1. A method for editing the genome of an isolated B lymphocyte comprising the following steps:
   (i) activating endogenous activation-induced cytidine deaminase of the B lymphocyte; and
   (ii) introducing a DNA molecule comprising a nucleotide sequence encoding a (poly)peptide of interest into the B lymphocyte.

2. The method according to claim 1, wherein the method does not involve an exogenous nuclease and/or an engineered nuclease, such as a CRISPR nuclease, a zinc finger nuclease, a transcription activator-like nuclease or a meganuclease.

3. The method according to claim 1 or 2, wherein the DNA molecule is a linear or linearized DNA molecule.

4. The method according to any one of claims 1 – 3, wherein the DNA molecule is a single strand DNA molecule (ssDNA) or a double strand DNA molecule (dsDNA).

5. The method according to claim 4, wherein the DNA molecule is dsDNA molecule.

6. The method according to claim 5, wherein the DNA molecule has blunt ends or overhangs.

7. The method according to any one of claims 1 – 6, wherein the nucleotide sequence of the DNA molecule encoding the (poly)peptide of interest is codon-optimized.

8. The method according to any one of claims 1 – 7, wherein the DNA molecule comprises an intronic sequence upstream and/or downstream of the nucleotide sequence encoding the (poly)peptide of interest.

9. The method according to claim 8, wherein the intronic sequence comprises a splice recognition site.

~~10. The method according to claim 8 or 9, wherein the intronic sequence contains Ig-locus intronic sequences.~~

~~11. The method according to claim 10, wherein the intronic sequence comprises an intronic sequence of a J-segment downstream intron and/or an intronic sequence of a CH-upstream intron.~~

~~12. The method according to any one of claims 1 – 11, wherein the DNA molecule comprises a splicing enhancer.~~

~~13. The method according to any one of claims 1 – 12, wherein the genome of the B lymphocyte is edited to express a modified immunoglobulin chain comprising in N- to C-terminal direction: a variable domain, the (poly)peptide of interest and a constant domain.~~

~~14. The method according to any one of claims 1 – 13, wherein the genome of the B lymphocyte is edited to express a modified immunoglobulin chain, wherein an endogenous variable domain is replaced by the (poly)peptide of interest.~~

~~15. The method according to any one of claims 1 – 14, wherein the DNA molecule comprises a nucleotide sequence encoding a cleavage site upstream and/or downstream of the nucleotide sequence encoding the (poly)peptide of interest.~~

~~16. The method according to claim 15, wherein the cleavage site is a T2A cleavage site.~~

~~17. The method according to any one of claims 1 – 16, wherein the (poly)peptide of interest comprises or consists of a pathogen binding domain, a V_H domain, or a V_H– V_L domain.~~

~~18. The method according to any one of claims 1 – 17, wherein the (poly)peptide of interest comprises or consists of CD4, dipeptidyl peptidase 4, CD9, or angiotensin-converting enzyme 2 or a fragment or sequence variant thereof.~~

19. The method according to any one of claims 1 – 18, wherein the isolated B lymphocyte is a primary B lymphocyte.

20. The method according to any one of claims 1 – 19, wherein the method comprises obtaining an engineered B lymphocyte, wherein the genome of the B lymphocyte comprises the nucleotide sequence encoding the (poly)peptide of interest.

21. The method according to any one of claims 1 – 20, wherein the method further comprises a step (iii) of confirming integration of the nucleotide sequence encoding the (poly)peptide of interest into the genome of the B lymphocyte.

22. The method according to any one of claims 1 – 21, wherein the isolated B lymphocyte is cultured in RPMI or IMDM with 10% FBS, 1% NEAA, 1% sodium pyruvate, 1% beta-mercaptoethanol, 1% Glutamax, 1% penicillin/streptomycin, 1% kanamycin, and 1% transferrin.

23. The method according to any one of claims 1 – 22, wherein the isolated B lymphocyte is cultured at 1 x 10^5 to 1x 10^6 cells/ml, preferably at 2 x 10^5 cells/ml.

24. The method according to any one of claims 1 – 23, wherein the B lymphocyte is cultured in a culture medium comprising an activator of activation-induced cytidine deaminase.

25. The method according to claim 24, wherein the activator of activation-induced cytidine deaminase is selected from the group consisting of: a cytokine, an anti-B cell receptor antibody or fragments thereof, a TLR agonist, a CpG-B agonist, an imidazoquinoline compound or a combination of any of said activators.

26. The method according to claim 25, wherein the cytokine is selected from the group consisting of CD40L, IL4, IL2, IL21, BAFF, APRIL, CD30L, TGF-β1, 4-1BBL, IL6, IL7, IL10, IL13, c-Kit, FLT-3, IFNα or any combination thereof.

~~27. The method according to any one of claims 24 – 26, wherein the cytokine is administered at a concentrations of 0.01-20ng/ml.~~

~~28. The method according to any one of claims 1 – 27, wherein the B lymphocyte is cultured in a medium comprising IL4 and/or CD40L.~~

~~29. The method according to claim 28, wherein activating of the activation-induced cytidine deaminase is performed by co-culture with a CD40L expressing cell line and addition of IL-4.~~

~~30. The method according to claim 29, wherein the concentration of IL-4 (in the final culture medium) is 0.005 – 0.03 ng/ml, preferably 0.01 – 0.025 ng/ml, more preferably 0.015 – 0.02 ng/ml and most preferably 0.16 ng/ml.~~

~~31. The method according to claim 29 or 30, wherein the CD40L expressing cell line is K562L.~~

~~32. The method according to any one of claims 1 – 31, wherein introducing the DNA molecule into the B lymphocyte is performed up to 10 days after activating the activation-induced cytidine deaminase, preferably up to 7 days after activating the activation-induced cytidine deaminase, more preferably up to 5 days after activating the activation-induced cytidine deaminase, even more preferably up to 2 days after activating the activation-induced cytidine deaminase and most preferably about 1 day after activating the activation-induced cytidine deaminase.~~

~~33. The method according to any one of claims 1 – 32, wherein the method does not comprise transducing the B lymphocyte with a retrovirus.~~

~~34. The method according to any one of claims 1 – 33, wherein the DNA molecule is introduced by nucleofection.~~

~~35. The method according to any one of claims 1 – 34, wherein the B lymphocyte is reactivated after introducing the DNA molecule into the B lymphocyte.~~

~~36. The method according to any one of claims 1 – 35, wherein B-cell stimulating agents, for example as defined in claims 25 – 31, are applied to the B lymphocyte after introducing the DNA molecule into the B lymphocyte.~~

~~37. The method according to any one of claims 1 – 36, wherein the B lymphocyte is treated with a DNA inhibitor capable of blocking alternative-end joining before introducing the DNA molecule into the B lymphocyte.~~

~~38. The method according to any one of claims 1 – 37, wherein the DNA molecule comprising a nucleotide sequence encoding the (poly)peptide of interest is incubated with a Ku protein, such as Ku70/Ku80, before introducing the DNA molecule into the B lymphocyte.~~

~~39. The method according to any one of claims 1 – 38, wherein the DNA molecule comprises a nuclear localization signal, such as SV40 nuclear localization signal.~~

~~40. The method according to any one of claims 1 – 39, wherein the DNA molecule does not comprise a nucleotide sequence encoding GFP or RFP.~~

~~41. The method according to any one of claims 1 – 40, wherein the DNA molecule comprises a promoter.~~

~~42. The method according to any one of claims 1 – 41, wherein the DNA molecule comprises a transcription unit.~~

~~43. The method according to any one of claims 1 – 42, wherein about 24 hours after activation of the activation-induced cytidine deaminase of the B lymphocyte, the B lymphocyte is treated with a nuclease inhibitor, such as Mirin.~~

~~44. The method according to any one of claims 1 – 43, wherein the B lymphocyte is a human B lymphocyte.~~

~~45. An engineered B lymphocyte obtainable by the method according to any one of claims 1 – 44.~~

~~46. An engineered B lymphocyte comprising an edited immunoglobulin gene locus comprising a heterologous insert comprising a nucleotide sequence encoding a (poly)peptide of interest inserted in its switch region.~~

~~47. The B lymphocytes according to claim 45 or 46, wherein the B lymphocyte is human.~~

~~48. The B lymphocyte according to any one of claims 45 – 47, wherein the switch region of an immunoglobulin gene locus of the B lymphocyte comprises a cleavage site, in particular a T2A cleavage site.~~

~~49. The B lymphocyte according to any one of claims 45 – 48, wherein the switch region of an immunoglobulin gene locus of the B lymphocyte comprises a nucleotide sequence encoding a pathogen binding domain, a V_H domain, or a V_H – V_L domain.~~

~~50. The B lymphocyte according to any one of claims 45 – 49, wherein the switch region of an immunoglobulin gene locus of the B lymphocyte comprises a nucleotide sequence encoding CD4, dipeptidyl peptidase 4, CD9, or angiotensin-converting enzyme 2 or a fragment or sequence variant thereof.~~

~~51. The B lymphocyte according to any one of claims 45 – 50, wherein the B lymphocyte does not express a fluorescent reporter protein.~~

~~52. The B lymphocyte according to any one of claims 45 – 51 for use in medicine.~~

~~53. The B lymphocyte for use according to claim 52, wherein the B lymphocyte is engineered according to any one of claims 1 – 44.~~

~~54. The B lymphocyte for use according to claim 52 or 53, wherein the engineered B lymphocyte is administered to a patient.~~

~~55. The B lymphocyte for use according to claim 54, wherein the patient receiving the engineered B lymphocyte is the same patient from whom the B lymphocyte was isolated prior to engineering.~~

~~56. Method for B cell therapy comprising the following steps:~~

~~(a) isolating a B lymphocyte from a patient;~~

~~(b) engineering the B lymphocyte according to any one of claims 1 – 44; and~~

~~(c) administering the engineered B lymphocyte to the patient.~~

~~57. A cell line of B lymphocytes according to any one of claims 45 – 51.~~

~~58. A method for generating an antibody or a fragment thereof comprising a (heterologous) (poly)peptide of interest, the method comprising the following steps:~~

~~(1) providing an engineered B lymphocyte or a B cell line according to any one of claims 45 – 51 and 57, wherein the B lymphocyte comprises an edited immunoglobulin gene locus comprising a heterologous insert comprising a nucleotide sequence encoding the (poly)peptide of interest inserted in its switch region;~~

~~(2) culturing the engineered B lymphocyte or the B cell line; and~~

~~(3) isolating the antibody or the fragment thereof comprising the (heterologous) (poly)peptide of interest from the B cell culture.~~

~~59. The method according to claim 58, wherein the engineered B lymphocyte is obtained by a method according to any one of claims 1 – 44.~~

~~60. The method according to claim 58 or 59 further comprising characterization of the antibody or antibody fragment, wherein characterization comprises~~

~~Performing functional assays to determine the function of the antibody or antibody fragment;~~

~~— Performing binding assays to determine the binding specificity of the antibody or antibody fragment and/or the binding partner/epitope recognized by the antibody or antibody fragment; and/or~~

~~— Performing neutralization assays to determine the ability of the antibody or antibody fragment to neutralize a toxin or a pathogen.~~

~~61. Antibody obtainable by the method according to any one of claims 58 – 60.~~

~~62. A composition comprising the B lymphocyte according to any one of claims 45 – 51 or the antibody according to claim 61.~~

~~63. The composition according to claim 62 further comprising a pharmaceutically acceptable carrier.~~

~~64. The composition according to claim 62 or 63 for use in medicine.~~

~~65.~~1. A method for immunotherapy comprising administration of ~~the~~ an antibody ~~according to claim 61, the engineered B cell according to any one of claims 45 – 51 or the composition according to claim 62 or 63~~ to a subject in need thereof,

wherein the antibody is obtainable by a method comprising the following steps:

(1) providing an engineered B lymphocyte obtainable by a method for editing the genome of an isolated B lymphocyte comprising the following steps:

(i) activating endogenous activation-induced cytidine deaminase of the B lymphocyte; and

(ii) introducing a DNA molecule comprising a nucleotide sequence encoding a (poly)peptide of interest into the B lymphocyte,

wherein the B lymphocyte comprises an edited immunoglobulin gene locus comprising a heterologous insert comprising a nucleotide sequence encoding the (poly)peptide of interest inserted in its switch region;

(2) culturing the engineered B lymphocyte or the B cell line; and

(3) isolating the antibody or the fragment thereof comprising the (heterologous) (poly)peptide of interest from the B cell culture.