

The question, whether “fully human” mAbs such as derived from phage display libraries or transgenic mice are less immunogenic than humanized mAbs cannot be answered yet, because full immunogenicity data are available for just two human mAbs. An anti-tumor necrosis factor mAb, developed from phage-displayed human libraries induced antibody responses in 12% of patients - at the higher end of the incidence of anti-antibody responses of the humanized antibodies (Hwang, W. Y., et al. (2005). Methods, 36, 3-10).

Evaluation of the immunogenicity of the first registered human mAb generated by the transgenic approach demonstrated that mAb treatment resulted in the generation of antibodies in approximately 5.5% of treated cancer patients (Jakobovits, A., et al. (2007). Nat. Biotechnol. 25, 1134., Lofgren, J. A., et al. (2007). J. Immunol. 178, 7467).

A need therefore remains for a method and means for producing antibodies that are specific for their targets, but are less immunogenic. According to the invention the reduction of immunogenicity is at least partially achieved by providing a transgenic non-human mammal comprising, at least in its B cell lineage, a nucleic acid encoding at least an immunoglobulin light chain or heavy chain, wherein the heavy- or light chain encoding sequence is provided with a means that renders it resistant to DNA rearrangements and/or somatic hypermutations, preferably such a non-human animal is a rodent, more specifically a mouse. The nucleic acid preferably encodes a human, human-like or humanized immunoglobulin chain.

Summary

In one aspect, the present disclosure provides a transgenic non-human animal that has been immunized with an antigen wherein the genome of said animal comprises a transgene encoding a rearranged human immunoglobulin light chain variable region, wherein the rearranged human immunoglobulin light chain variable region is a germline sequence; and wherein the transgenic animal

comprises a population of B cells that secrete a repertoire of antibodies that bind said antigen, said repertoire of antibodies comprising said rearranged human immunoglobulin light chain variable region and a heavy chain variable region resulting from somatic hypermutation in response to said antigen.

5 In another aspect, the present disclosure provides a method for the production of a transgenic non-human animal, comprising the step of introducing a transgene comprising a rearranged nucleic acid encoding a rearranged human immunoglobulin light chain variable region into the non-human animal germline, wherein the rearranged nucleic acid encodes a rearranged human
10 immunoglobulin light chain variable region comprising a germline sequence; and wherein said rearranged human immunoglobulin light chain variable region is capable of pairing with at least two different heavy chain variable regions.

15 In yet another aspect, the present disclosure provides a method for obtaining immunoglobulin heavy chain variable regions, characterized in that B cells obtained from the transgenic non-human animal as described herein after immunization of said transgenic non-human animal with a specific antigen are used as a source of immunoglobulin heavy chain variable regions.

20 In yet another aspect, the present disclosure provides a method for producing a desired antibody comprising exposing a transgenic non-human animal as described herein to an antigen such that an antibody response is induced and isolating antibodies specific for the antigen.

25 In yet another aspect, the present disclosure provides a method for producing a desired antibody comprising exposing a transgenic non-human animal as described herein to an antigen such that an antibody response is induced and isolating cells producing such antibodies, culturing and harvesting said antibodies.

30 In yet another aspect, the present disclosure provides a method for producing a desired antibody comprising exposing a transgenic non-human animal as described herein to an antigen such that an antibody response is induced and isolating a nucleic acid encoding at least part of such an antibody, inserting said nucleic acid or a copy or a derivative thereof in an expression cassette and expressing said antibody in a host cell.

In yet another aspect, the present disclosure provides a method for producing a mixture of recombinant antibodies, the method comprising: - exposing a transgenic non-human animal as described herein, to an antigen such that an antibody response is induced;

5 - isolating nucleic acids encoding such antibodies, or encoding a part of such antibodies wherein said part comprises the heavy chain variable region of said antibodies; and

10 - inserting said nucleic acids or copies thereof in an expression cassette and expressing a mixture of antibodies that all share the same identical rearranged human variable region in a host cell.

15 In yet another aspect, the present disclosure provides progeny of a transgenic non-human animal as described herein, the progeny comprising, integrated in its genome, a nucleic acid encoding a rearranged human immunoglobulin light chain variable region, wherein the rearranged human immunoglobulin light chain variable region is a germline sequence; and wherein the progeny comprises a population of B cells that secrete a repertoire of antibodies that bind said antigen, said repertoire of antibodies comprising said rearranged human immunoglobulin light chain variable region and a heavy chain variable region resulting from somatic hypermutation in response to said
20 antigen.

25 In yet another aspect, the present disclosure provides a cell that is isolated from a transgenic non-human animal as described herein or the progeny cell that is isolated from a transgenic non-human animal as described herein or the progeny as described herein, the cell comprising integrated in its genome a nucleic acid encoding a rearranged human immunoglobulin light chain variable region, wherein the rearranged human immunoglobulin light chain variable region is a germline sequence.

30 In the remainder of the specification mice are typically used as examples of the non-human mammals. The transgenic non-human mammalian hosts are capable of mounting an immune response to an antigen, where the response produces antibodies having primate, particularly human, variable regions. Various transgenic hosts may be

Claims

1. A transgenic non-human animal that has been immunized with an antigen wherein the genome of said animal comprises a transgene encoding a rearranged human immunoglobulin light chain variable region,

wherein the rearranged human immunoglobulin light chain variable region is a germline sequence; and

wherein the transgenic animal comprises a population of B cells that secrete a repertoire of antibodies that bind said antigen, said repertoire of antibodies comprising said rearranged human immunoglobulin light chain variable region and a heavy chain variable region resulting from somatic hypermutation in response to said antigen.

2. The transgenic non-human animal according to claim 1, wherein the transgene encoding a rearranged human immunoglobulin light chain variable region comprises a human germline light chain V gene segment joined to a human germline light chain J gene segment, such that there is no mutation due to said joining.

3. The transgenic non-human animal according to claim 1 or 2, wherein the transgene encoding a rearranged human immunoglobulin light chain variable region further comprises an immunoglobulin constant light chain region.

4. The transgenic non-human animal according to claim 1 or 2, wherein the transgene is operatively linked to an endogenous immunoglobulin constant light chain region.

5. The transgenic non-human animal according to any one of claims 1-4, which is a mammal.

6. The transgenic non-human animal according to any one of claims 1-5, which is a rodent or mouse.

7. A method for the production of a transgenic non-human animal, comprising the step of introducing a transgene comprising a rearranged nucleic acid encoding a rearranged human immunoglobulin light chain variable region into the non-human animal germline.

wherein the rearranged nucleic acid encodes a rearranged human immunoglobulin light chain variable region comprising a germline sequence; and

wherein said rearranged human immunoglobulin light chain variable region is capable of pairing with at least two different heavy chain variable regions.

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8. The method according to claim 7, wherein the transgenic non-human animal further harbors a non-rearranged human heavy chain variable region locus.

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9. A method for obtaining immunoglobulin heavy chain variable regions, characterized in that B cells obtained from the transgenic non-human animal according to any one of claims 1-6 after immunization of said transgenic non-human animal with a specific antigen are used as a source of immunoglobulin heavy chain variable regions.

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10. A method for producing a desired antibody comprising exposing a transgenic non-human animal according to any one of claims 1-6 to an antigen such that an antibody response is induced and isolating antibodies specific for the antigen.

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11. A method for producing a desired antibody comprising exposing a transgenic non-human animal according to any one of claims 1-6 to an antigen such that an antibody response is induced and isolating cells producing such antibodies, culturing and harvesting said antibodies.

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12. The method according to claim 11, further comprising immortalizing said cells after the culturing step and prior to harvesting said antibodies.

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13. A method for producing a desired antibody comprising exposing a transgenic non-human animal according to any one of claims 1-6 to an antigen such that an antibody response is induced and isolating a nucleic acid encoding at least part of such an antibody, inserting said nucleic acid or a copy or a derivative thereof in an expression cassette and expressing said antibody in a host cell.

14. A method for producing a mixture of recombinant antibodies, the method comprising:

- exposing a transgenic non-human animal according to any one of claims 1-6, to an antigen such that an antibody response is induced;

- isolating nucleic acids encoding such antibodies, or encoding a part of such antibodies wherein said part comprises the heavy chain variable region of said antibodies; and

- inserting said nucleic acids or copies thereof in an expression cassette and expressing a mixture of antibodies that all share the same identical rearranged human variable region in a host cell.

15. Progeny of a transgenic non-human animal according to any one of claims 1-6, the progeny comprising, integrated in its genome, a nucleic acid encoding a rearranged human immunoglobulin light chain variable region,

wherein the rearranged human immunoglobulin light chain variable region is a germline sequence; and

wherein the progeny comprises a population of B cells that secrete a repertoire of antibodies that bind said antigen, said repertoire of antibodies comprising said rearranged human immunoglobulin light chain variable region and a heavy chain variable region resulting from somatic hypermutation in response to said antigen.

16. A cell that is isolated from a transgenic non-human animal according to any one of claims 1-6 or the progeny according to claim 15, the cell comprising integrated in its genome a nucleic acid encoding a rearranged human immunoglobulin light chain variable region,

wherein the rearranged human immunoglobulin light chain variable region is a germline sequence.

17. The cell according to claim 16, wherein the cell further comprises integrated in its genome a nucleic acid encoding an immunoglobulin light chain constant region, or wherein said rearranged human immunoglobulin light chain variable region is operatively linked to an endogenous immunoglobulin light chain constant region.

1.A transgenic non-human mammal comprising, at least in its B-cell lineage, a nucleic acid encoding at least an immunoglobulin light chain or heavy chain, wherein the heavy or light chain encoding sequence is provided with a means that renders it resistant to DNA rearrangements and/or somatic hypermutations.

~~2.A transgenic non-human mammal according to claim 1, which is a rodent, preferably a mouse.~~

~~3.A transgenic non-human mammal according to any one of claims 1-2, wherein the light/heavy chain-encoding sequence is integrated in the genome of the non-human mammal.~~

~~4.A transgenic non-human mammal according to claim 3, wherein the integration is in a locus that is resistant to silencing.~~

~~5.A transgenic non-human mammal according to claim 4, wherein the integration is in the Rosa-locus.~~

~~6.A transgenic non-human mammal according to any one of claims 1-5, wherein the light chain-encoding nucleic acid is provided with a means that allows expression of said nucleic acid essentially limited to cells of B-cell lineage.~~

~~7.A transgenic non-human mammal according to claim 6, wherein the light chain-encoding nucleic acid is provided with a means that allows expression of the light chain-encoding nucleic acid predominantly during a certain stage of the development of B-cells.~~

~~8.A transgenic non-human mammal according to claim 7, wherein said means comprises a promoter selected from the group of CD19, CD20, μ HC, VpreB1, VpreB2, VpreB3, λ 5, Ig α , Ig β , κ LC, λ LC, and BSAP (Pax5).~~

~~9.A transgenic non-human mammal according to claim 7 or 8, wherein said means comprises a cre-lox system or the like.~~

~~10.A transgenic non-human mammal according to any one of claims 1-9, wherein the light chain-encoding sequence encodes a light chain capable of pairing with at least two different heavy chains encoded by the non-human mammal.~~

~~11.A transgenic non-human mammal according to any one of claims 1-10, wherein at least one of the endogenous loci encoding an endogenous light chain is functionally silenced.~~

~~12.A transgenic non-human mammal according to anyone of claims 1-11, wherein the endogenous κ light chain locus is functionally silenced.~~

~~13.A transgenic non-human mammal according to any one of claims 1-12, wherein the sequence of the light chain-encoding sequence is a human $\nu\kappa$ sequence.~~

~~14.A transgenic non-human mammal according to claim 13 wherein the light chain-encoding sequence is a germline-like sequence.~~

~~15.A transgenic non-human mammal according to claim 14, wherein the light chain encoding sequence is a germline sequence.~~

~~16.A transgenic non-human mammal according to claim 15, wherein the germline sequence is based on O12.~~

~~17.A transgenic non-human mammal according to claim 1 wherein the light chain encoding nucleic acid comprises in 5'-3' direction: a $\nu\kappa$ promoter, a human leader, a human V gene, optionally a MoE κ i enhancer, a rat constant region (κ) and optionally a (truncated) MoE κ 3' enhancer.~~

~~18.A transgenic non-human animal which has been provided with an expression cassette for the expression of a desired proteinaceous molecule in cells during a certain stage of development in cells developing into mature B cells, said cassette comprising: means for preventing silencing of expression of the desired proteinaceous molecule after introduction into a host cell, and means for timing expression of the desired proteinaceous molecule with the desired developmental stage of the host cell.~~

~~19.A transgenic non-human animal which has been provided with an expression cassette according to claim 18, wherein said means for timing expression is a promoter of which the activity is essentially limited to the certain stage of development.~~

~~20.A transgenic non-human animal which has been provided with an expression cassette according to claim 18 or 19 wherein said certain stage starts at a stage immediately preceding or coinciding with the onset of the expression of light chain molecules by said cells at a certain stage of development into a mature B cell.~~

~~21.A transgenic non-human animal which has been provided with an expression cassette according to claim 19 or 20, wherein said promoter is selected from the group consisting of the CD19 promoter, the Ig- α promoter, the Ig- β promoter, the μ hc promoter, the $\nu\kappa$ promoter, or analogues and/or homologues thereof.~~

~~22.A transgenic non-human animal which has been provided with an expression cassette according to any one of claims 19-21, wherein said promoter drives the expression of a cre-like protein.~~

~~23.A transgenic non-human animal which has been provided with an expression cassette according to claim 22 whereby the sequence encoding the desired proteinaceous molecule is activated by the action of said cre-like protein.~~

~~24.A transgenic non-human animal which has been provided with a set of nucleic acids that are expression cassettes, wherein one nucleic acid comprises an~~

~~expression cassette according to claim 22 and the second nucleic acid comprises a sequence encoding a desired proteinaceous molecule under control of a constitutive promoter which can be activated by the action of a cre-like protein.~~

5 ~~25. A transgenic non-human animal which has been provided with an expression cassette according to claim 23 or 24, wherein said activation is achieved by removal of a “stop”.~~

~~26. A transgenic non-human animal which has been provided with an expression cassette according to anyone of claims 18-25, wherein said desired proteinaceous molecule is a polypeptide chain of an immunoglobulin.~~

10 ~~27. A transgenic non-human animal which has been provided with an expression cassette according to claim 26, wherein said polypeptide chain is a light chain.~~

~~28. A transgenic non-human animal which has been provided with an expression cassette according to claim 27, wherein the sequence encoding said polypeptide chain is rendered essentially incapable of rearrangement.~~

15 ~~29. A transgenic non-human animal which has been provided with an expression cassette according to claim 27 or 28, wherein said light chain is a germ line like light chain.~~

~~30. A transgenic non-human animal which has been provided with an expression cassette according to claim 29, wherein said germ line like light chain is O12.~~

20 ~~31. A transgenic non-human animal which has been provided with an expression cassette according to claim 28-30, wherein said rearrangement is prevented by the absence of elements at least partially responsible for somatic hypermutation such as the MoEki enhancer.~~

25 ~~32. A transgenic non-human animal which has been provided with an expression cassette according to anyone of claims 18-31, wherein said means for prevention of silencing are means for insertion into a locus in the genome of the host cell that is resistant to silencing.~~

30 ~~33. A transgenic non-human animal which has been provided with an expression cassette according to claim 32, wherein said means for insertion are means for homologous recombination into said site resistant to silencing.~~

~~34. — A transgenic non-human animal which has been provided with an expression cassette according to claim 32 or 33 wherein said locus is the rosa locus.~~

~~35. A transgenic non-human animal which has been provided with an expression cassette according to any one of claims 1-16, which comprises in 5'-3' direction: a vk~~

~~promoter, a human leader, a human V gene, optionally a MoEκi enhancer, a rat constant region (κ) and optionally a (truncated) MoEκ3' enhancer.~~

~~36. A method for producing a desired antibody comprising exposing a non-human mammal according to any one of claims 1-35 to an antigen such that an antibody response is induced and isolating the antibodies specific for the antigen~~

~~37. A method for producing a desired antibody comprising exposing a non-human mammal according to any one of claims 1-36 to an antigen such that an antibody response is induced and isolating cells producing such antibodies, culturing and optionally immortalizing said cells and harvesting said antibodies.~~

~~38. A method for producing a desired antibody comprising exposing a non-human mammal according to any one of claims 1-37 to an antigen such that an antibody response is induced and isolating a nucleic acid encoding at least part of such an antibody, inserting said nucleic acid or a copy or a derivative thereof in an expression cassette and expressing said antibody in a host cell.~~

~~39. Progeny of a transgenic non-human animal according to any one of claims 1-17, the progeny comprising, at least in its B-cell lineage, a heavy or light chain encoding sequence together with a means that renders the sequence resistant to DNA rearrangements and/or somatic hypermutations.~~

~~40. — Progeny of a transgenic non-human animal according to any one of claims 18-35, the progeny comprising an expression cassette for the expression of a desired proteinaceous molecule in cells during a certain stage of development in cells developing into mature B-cells.~~

~~41. A cell that is isolated from a transgenic non-human animal according to any one of claims 1-17, the cell comprising a heavy or light chain encoding sequence together with a means that renders the sequence resistant to DNA rearrangements and/or somatic hypermutations.~~

~~42. A cell that is isolated from a transgenic non-human animal according to any one of claims 18-35, the cell comprising an expression cassette for the expression of a desired proteinaceous molecule in cells during a certain stage of development in cells developing into mature B-cells.~~