

CLAIMS:

1. A method of designing an immunoglobulin library for optimisation of a biological property of a first lead immunoglobulin, the method comprising:

a) identifying one or more related immunoglobulins of common lineage with and that bind the same target antigen as the first lead immunoglobulin, said one or more related immunoglobulins being related to the first lead immunoglobulin, each immunoglobulin having been raised against a target antigen by immunisation of a transgenic non-human mammal comprising human immunoglobulin genes with the target antigen and said one or more related immunoglobulins being derived from the same germline sequence by somatic hypermutation of a germline sequence in the transgenic non-human mammal;

b) comparing amino acid sequences of the first lead immunoglobulin and the one or more related immunoglobulins;

c) identifying, based on the sequence comparison, one or more sites at which there are variant amino acid residues between:

(i) the first lead immunoglobulin and the one or more related immunoglobulins, and/or

(ii) where the one or more related immunoglobulins is a plurality of immunoglobulins, between the plurality of immunoglobulins,

wherein the one or more sites at which there are variant amino acid residues comprise somatic hypermutation hot spots targeted during the immune response that are potential sites for modification of the first lead immunoglobulin;

d) selecting one or more sites for modification to replace an amino acid of the first lead immunoglobulin with the corresponding variant amino acid of one or more of the related immunoglobulins, based on the sequence comparison; and

e) generating immunoglobulin sequences for the library based on the sequence of the first lead immunoglobulin, modified at one or more of the selected sites for modification.

2. The method of claim 1, wherein the one or more related immunoglobulins have at least 70%, 80%, 85%, 90%, 95% homology in at least one CDR region to the lead immunoglobulin.

3. The method of claim 1 or claim 2, wherein the one or more related immunoglobulins have at least 70% homology in CDR3 to the lead immunoglobulin and / or at least 70% homology in CDR1 and/or CDR2 to the lead immunoglobulin and / or at least 70% homology in the framework regions to the lead immunoglobulin.

4. The method of any preceding claim wherein the plurality of related immunoglobulins comprises at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, or 25 immunoglobulins.
5. The method of any preceding claim wherein step c) comprises identifying sites for modification within the CDRs of the immunoglobulin sequences, wherein a site within the CDRs is considered a site for modification if there is a variant amino acid residue present in at least one, two, three, four, or five of the related immunoglobulins.
6. The method of any preceding claim wherein step c) further comprises identifying sites for modification outside the CDRs of the immunoglobulin sequences, wherein a site outside the CDRs is considered a site for modification if there is a variant amino acid residue present in at least 20% of the related immunoglobulins.
7. The method of claim 6 wherein a site outside the CDRs which would otherwise be identified as a site for modification is not identified as a site for modification if modifying the site would lead to the introduction of one or more of the following features into the modified immunoglobulin: (i) unpaired cysteines, (ii) oxidation sites (free methionines), (iii) glycosylation sites, (iv) deamidation sites, and (v) isomerisation sites.
8. The method of any preceding claim wherein the sequences selected in step d) include variant sequences reflecting each possible combination of modifications at the sites for modification.
9. The method of any preceding claim wherein the modifications at the sites for modification include only conservative amino acid substitutions.
10. The method of any preceding claim wherein the variant immunoglobulins include no modifications outside the sites for modification.
11. The method of any preceding claim,
 - (i) wherein step e) further comprises generating sequences of additional variant immunoglobulins, wherein the sequences are further modified at one or more of the selected sites for modification to replace an amino acid of the first lead immunoglobulin with a conservative amino acid replacement for the corresponding variant amino acid, and / or ,

(ii) wherein step e) further comprises generating sequences of additional variant immunoglobulins, wherein the sequences are further modified at one or more of the selected sites for modification to replace an amino acid of the first lead immunoglobulin with an amino acid not found at the corresponding residue of the related immunoglobulins, and / or,
(iii) further comprising step f) generating an immunoglobulin library comprising immunoglobulins having the sequences generated in step e), optionally further comprising step g) screening the immunoglobulin library to identify one or more immunoglobulins having desired biological properties.

12. The method of any preceding claim, comprising, prior to step a), the step α) of generating and sequencing a plurality of immunoglobulins, including a first lead immunoglobulin and one or more related immunoglobulins, optionally wherein the plurality of immunoglobulins is generated by immunising a non-human mammal, preferably a mouse or rat, preferably a transgenic mouse or rat expressing human immunoglobulin genes, with a target antigen.

13. The method of any preceding claim, comprising, prior to step a), the step β) of identifying a first lead immunoglobulin.

14. The method of any preceding claim wherein the immunoglobulins are antibodies, or antigen-binding fragments of an antibody or wherein the immunoglobulins comprise or consist of heavy chain only antibodies or wherein the immunoglobulins comprise or consist of V_H domains of antibodies.

15. A method of optimising a lead immunoglobulin, the method comprising:

- A) performing the method of any preceding claim; and
- B) selecting one or more optimised immunoglobulins from the library based on a desired property of the optimised immunoglobulin.