Case No: HP-2013-000001/HP-2014-000001

Neutral Citation Number: [2016] EWHC 87 (Pat)

IN THE HIGH COURT OF JUSTICE
CHANCERY DIVISION
PATENTS COURT

Royal Courts of Justice
Rolls Building
Fetter Lane
London
EC4A 1NL

Date: 01/02/2016

Before :

THE HON MR JUSTICE HENRY CARR

- -

Between :

Regeneron Pharmaceuticals Inc

Claimant

- and -

(1) Kymab Limited
(2) Novo Nordisk A/S

Defendants

- -
- -

Justin Turner QC, Joe Delaney and William Duncan (instructed by **Allen & Overy LLP**) for the **Claimant**
Michael Tappin QC & James Whyte (instructed by **Powell Gilbert LLP**) for the **First Defendant**
Piers Acland QC (instructed by **DLA Piper UK LLP**) for the **Second Defendant**

Hearing dates: 18-20, 23- 27, 30 November and 7 & 8 December 2015

- -

Approved Judgment

I direct that pursuant to CPR PD 39A para 6.1 no official shorthand note shall be taken of this Judgment and that copies of this version as handed down may be treated as authentic.

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Mr Justice Henry Carr:

Introduction

1. This is a claim brought by the Claimant ("Regeneron") for infringement of European Patent (UK) No. 1 360 287 ("the 287 Patent") and European Patent (UK) No. 2 264 163 ("the 163 Patent"). The 163 Patent is a Divisional of the 287 Patent. The specifications are the same in all material respects, although the claims are different. The claimed priority date of the patents is 16 February 2001. The patents generally relate to transgenic mice that can be used as platforms for therapeutic antibody discovery. More specifically, they concern the replacement of mouse variable (VDJ/VJ) gene with human variable genes to produce immunoglobulin loci that will undergo the natural process of rearrangement during B cell development to produce hybrid antibodies. A locus comprising a combination of human variable gene segments and endogenous mouse constant gene segments is known as a "reverse chimeric locus". Subsequently, fully human antibodies can be made by replacing the mouse constant regions with the desired human counterparts.

2. The first Defendant ("Kymab") is offering to the pharmaceutical industry various strains of transgenic mice that are alleged, either *per se* or through the process by which they are produced, to infringe the patents. Kymab denies infringement and counterclaims for revocation. The second Defendant ("Novo") was alleged to be jointly liable with Kymab for infringement in the United Kingdom. However the claim against Novo in these proceedings was abandoned shortly before the start of the trial. Novo nonetheless maintained its challenge to the validity of both patents. Novo adopted Kymab's Grounds of Invalidity and its opening and closing submissions.

3. There have been concurrent opposition proceedings before the EPO in respect of the 287 Patent, in which Kymab and Novo were opponents. The 163 Patent was granted on 14 October 2015 and will be subject to opposition at the EPO. The parties agreed to its late introduction into these proceedings as the issues in respect of both patents are similar. By a decision dated 28 November 2014 the Opposition Division revoked the 287 Patent. Shortly before the UK trial the Technical Board of Appeal allowed an amended claim set in respect of the 287 Patent and the trial has proceeded on the basis of those amended claims. Consequential amendments to the specification have not yet been made by Regeneron. However, I was told that they are unlikely to be significant. Although the result of the hearing before the TBA is known, the written reasons for its decision have not yet been handed down.

4. Only claims 5 and 6 of the 287 Patent and claim 1 of the 163 Patent are alleged to be infringed. Claim 1 of the 287 Patent is relevant to the construction of claims 5 and 6 and to the issues of validity that I need to consider. The parties did not suggest that I needed to consider any other claims in order to resolve the issues raised in these proceedings.

5. Kymab challenges validity on the basis of insufficiency. Kymab also claims "cross-anticipation" based on alleged loss of priority (i.e. that the patents are anticipated by matter that retains priority in the application for the other patent). It alleges lack of novelty and lack of inventive step in the light of PCT/US91/00245 published as WO 91/10741 on 25 July 1991 ("Kucherlapati"); and obviousness in the light of publication entitled "*The Preparation of Human Antibodies from Mice Harbouring*

Immunoglobulin Loci" published in 1997 in the textbook "*Transgenic animals; generation and use*" ("Brüggemann 1997"). It also pursued an attack of lack of inventive step in the light of US Patent 5,770,429 ("Lonberg"). However, Lonberg was abandoned by Kymab in its written closing speech following conclusion of the evidence. Finally, it pursues an attack of added matter against claim 1 of the 287 Patent, which, if successful, would lead also to invalidity of claims 5 and 6 of that patent; and against the 163 Patent.

Technical Background

6. The subject matter of the patents and the prior art is of great technical complexity, and there are lengthy textbooks concerning genetic engineering and immunology, which are the relevant areas of expertise. The parties prepared an agreed Technical Primer. Although I found it very helpful as an introduction to the technology, it is too long to reproduce in this judgment. Justin Turner QC, who appeared with Joe Delaney and William Duncan for Regeneron, attached an Annex to his written closing speech which set out key aspects of the technology and which did not, as a general matter, appear to be controversial. After conclusion of the oral hearing, and at my request, Michael Tappin QC and James Whyte, who appeared for Kymab, informed me of two points of substance in relation to the Annex which were in issue, and I have altered it to take account of them. The result is a relatively short summary, some parts of which I include as technical background, and which has the virtue of being agreed between the parties. This approach might be considered in other patent cases involving very complex technology. It requires co-operation between the parties' representatives, who deserve considerable credit for achieving this in the present case. I have added some diagrams from the Technical Primer to illustrate certain aspects of the text.

7. The following was common general knowledge at the priority date. Later in this judgment I will deal with matters where there is a dispute as to common general knowledge.

Monoclonal antibodies

8. By the priority date the potential uses of antibodies for use in treating human disease had been well recognised, and a number of different antibodies had been developed and approved for use, including:

   (a) Muromonab-CD3 (anti CD3) approved in 1986, which was one of the earliest examples of a mouse monoclonal antibody;

   (b) Abciximab (glycoprotein IIb/IIIa) approved in 1994, a chimeric antibody;

   (c) Daclizumab (anti CD25) approved in 1997, a humanised antibody;

   (d) Rituximab (anti CD20) approved in 1997, which was one of the best known examples of a chimeric antibody;

   (e) Palivizumab (anti respiratory syncytial virus glycoprotein) approved in 1998, a humanised antibody;

   (f) Infliximab (anti TNF) approved in 1998, a chimeric antibody;

   (g) Basiliximab (anti CD25) approved in 1998, a chimeric antibody; and

(h) Alemtuzumab (anti CD52) approved in 2001, which was one of the best known examples of a humanised antibody.

9. It was known by the priority date that in order to avoid an immune response against the therapeutic antibody (known as a HAMA response or human anti mouse antibody response) it would be preferable for that antibody to be a human antibody, as opposed to a murine antibody or a chimeric antibody with mouse variable regions and human constant regions. Humanised antibodies, where the complementary determining regions of a murine antibody had been grafted onto a human antibody, were better than chimeric antibodies.

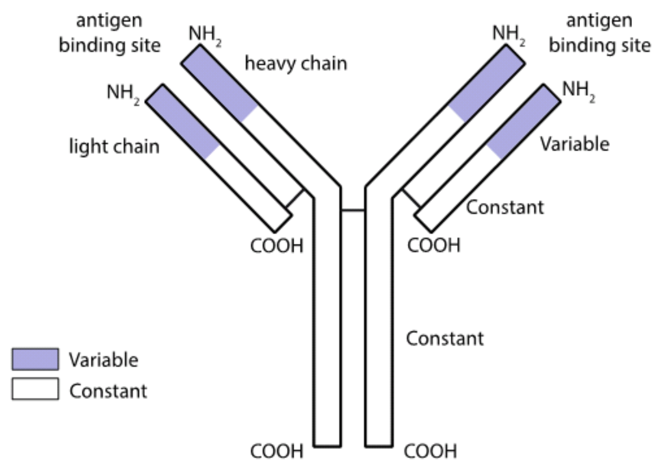
10. The antibodies that were used for therapy were monoclonal antibodies. Monoclonal antibodies were first described in Köhler and Milstein's 1975 Nature paper where they proposed a technique whereby a transformed (cancerous) B cell (i.e. myeloma) was fused with a normal antibody producing B cell to create a "hybridoma" that grows freely in vitro while continuing to produce an antibody. The hybridoma thus carried a single rearranged IgH gene, and a single rearranged IgK or IgL gene, and secreted antibody molecules of a single specificity into the culture supernatant.

VDJ recombination / somatic hypermutation

11. Antibodies are initially encoded for by a number of discrete coding segments in the germline DNA known as variable (V), diversity (D) and joining (J) gene segments, and constant (C) gene segments. The heavy chain has VDJ and C and the light chains have just V, J and C gene segments. During B-cell development the VDJ and VJ segments are joined together at the DNA level in a process known as VDJ recombination or somatic gene rearrangement. The rearrangement ensures that by combinatorial use of a specific (but limited) number of different gene segments a huge variety of antibodies can be generated eventually. The initial rearrangement of the heavy chain always uses the μ constant chain, but as discussed below, class switching to another constant chain can occur following B-cell activation. The heavy chain constant regions in humans are Cμ, Cδ, Cγ3, Cγ1, Cα1, Cγ2, Cγ4, Cε and Cα (in order from 5' to 3'). The heavy chain will re-arrange before the light chain, and is used to make the B cell receptor as discussed below.

12. The light chains are found in two isotypes, kappa (κ) and lambda (λ). No functional difference has been found between antibodies having λ or κ light chains. The κ and λ light chains are encoded by genes on different chromosomes, and there is a small difference in their gene structure prior to rearrangement in that in the λ locus the J segments (and, in the case of mouse, a V segment) occur interspersed amongst the constant region exons.

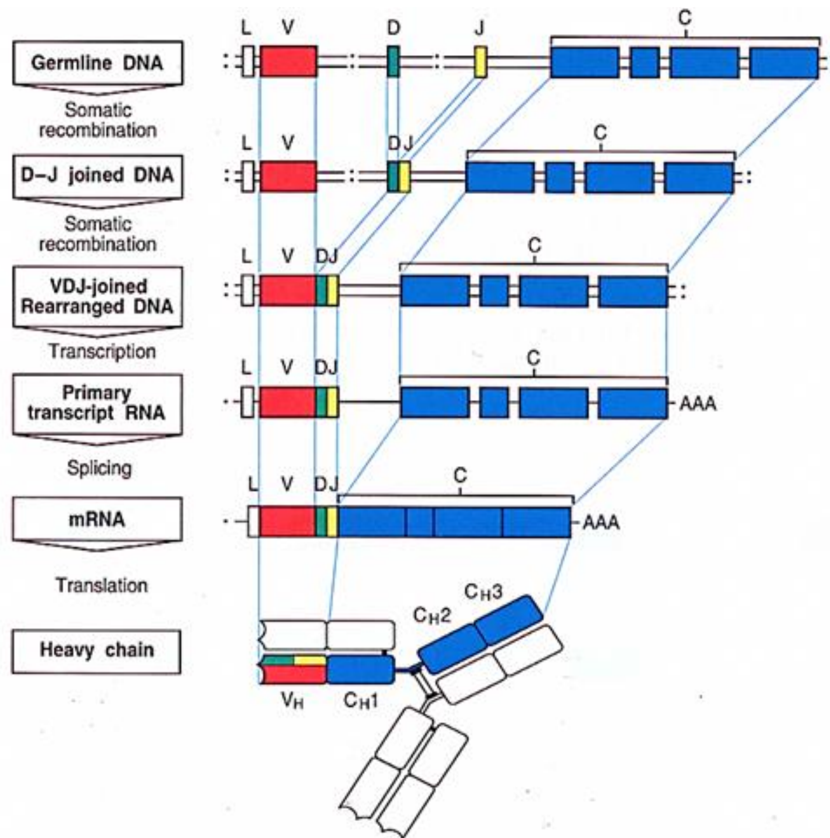
13. The immunoglobulin genes, which are responsible for encoding the heavy and light chains of an antibody, are not present in germline B cells in the form to be transcribed as a complete functional unit that encodes an antibody. Instead, the loci contain a series of segments which recombine during the maturation process of the B cell to form unique immunoglobulin heavy and light chain loci.



The typical organisation of an immunoglobulin molecule composed of two identical heavy chains and two identical light chains. The lines that connect the two heavy chains and that connect the light chain to the heavy chain are disulphide bonds.

14. In principle, a given B cell will express a single type of B cell receptor, composed of two identical heavy chains and two identical light chains. B cell receptors have the same form and structure as an immunoglobulin molecule, with the distinction that the B cell receptor is permanently anchored in the plasma membrane.

15. The process of rearrangement, and then transcription and translation of the antibody heavy chain is shown in the diagram below:



16. Following VDJ rearrangement, if a B cell is engaged by a fully mature and activated T cell of appropriate specificity, the B cell turns on activation induced cytidine deaminase ("AID"). The activity of AID results in a wide spectrum of mutations in the antibody genes. A given V segment may sustain anywhere from zero to dozens of such mutations, leading to numerous amino acid substitutions in the V regions. Favourable mutations confer an increase in affinity for the antigen, and B cells harbouring such mutations have a selective advantage. Deleterious mutations that decrease affinity or introduce a stop codon eventually ensure that the irrelevant B cells are eliminated. This process of mutation of the antibody genes to develop antibodies of increased affinity is known as somatic hypermutation, and serves to further improve the strength of the antibody response.

17. The rearrangement process brings the V segment promoter into proximity with the intronic enhancer and initiates transcription of the now functional "gene". A second enhancer 3' of the constant regions of heavy and light chains (known as the 3' enhancer) also exists and was known at the priority date.

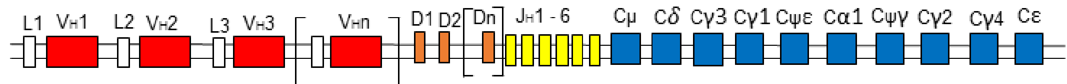
18. An antibody can be cleaved to release two fragments – the antigen binding fragment containing both antigen binding sites (known as the Fab fragment), and the parts of the heavy chain constant region which have the regions that interact with the other components of the immune system (known as the Fc fragment). The parts of the antibody making up the Fc fragment, when still attached to the rest of the antibody, is referred to as the Fc portion of the antibody.

Immunoglobulin Locus Size

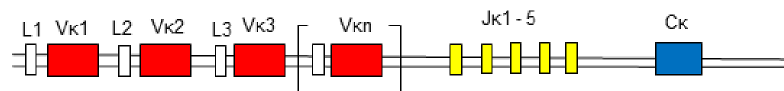
19. The mouse and human Ig loci are of different sizes and contain different numbers of V, (D) and J genes. There is not a one-to-one relationship between V genes in the human and mouse locus. Below are diagrams showing the structures of the human and mouse Ig loci:

Human Loci

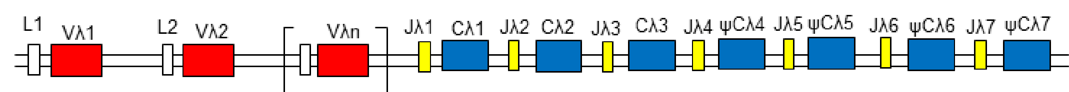
Heavy chain



κ light chain

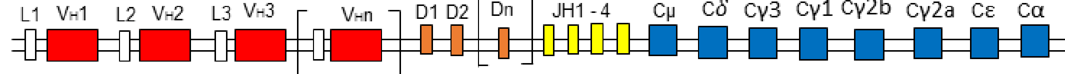


λ light chain

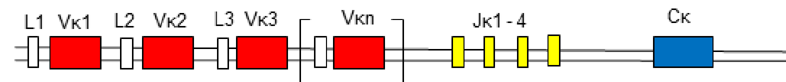


Mouse Loci

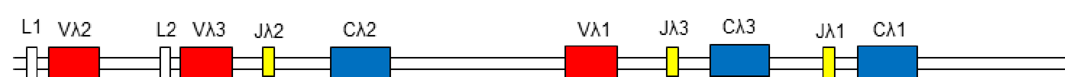
Heavy chain



κ light chain



λ light chain



20. The human IgH locus is approximately 1250 kb long and is located on chromosome 14. The human kappa locus is approximately 1820 kb long and is located on chromosome 2, and the lambda locus is approximately 1050 kb long and is located on chromosome 22.

21. The murine IgH locus is approximately 3 Mb in length and is located on chromosome 12. The murine kappa locus is approximately 3.2 Mb in length, and the murine lambda locus is smaller than the other loci at just 240 kb in length.

Transgenics

22. Transgenics is the term used to describe the introduction of a DNA fragment encoding a functional gene product which is inserted into the germ line of a different species. Transgenics can be done broadly by two techniques. The first is *random integration* into the target genome, and the second is by *targeted integration*. Targeted integrations can be achieved by two methods, *homologous recombination* and *site-specific recombination*.

23. Most methods of gene targeting involve rare events and so it is very desirable to be able to select or screen for the desired gene alteration and to discriminate against others. This is where embryonic stem cells ("ES cells") are used. DNA from a targeting construct can be introduced into ES cells by lipofection or electroporation to incorporate the DNA into the ES cell genome. Selectable markers can be used to select for ES cells into which the targeting construct has integrated by using the presence of a selection agent to remove ES cells in which the construct has not been integrated into the genome. Selection markers can either be positive or negative selection markers.

24. Well known positive selectable markers include the neomycin resistance gene. This gives resistance to the antibiotic neomycin in bacteria and also in mammalian cells to a compound which inhibits growth, G418. Its presence thus allows ES cells that incorporate the Neo gene to inactivate the otherwise growth inhibitory compound, G418. Consequently, only cells that carry the Neo cassette will survive in media that contains G418. Herpes virus thymidine kinase ("TK") gene was a marker gene that was commonly used for negative selection. Herpes virus thymidine kinase, the product of the TK gene, transforms the drug gancyclovir into a toxic compound that will kill cells. Therefore, any cell that retains the TK gene can be eliminated through exposure to gancyclovir. A combination of positive-negative selection may be useful in providing enrichment of homologous integrants, but the actual event of homologous integration must be assessed at the level of clonal descendants, obtained from cells resistant to for example, both G418 and gancyclovir.

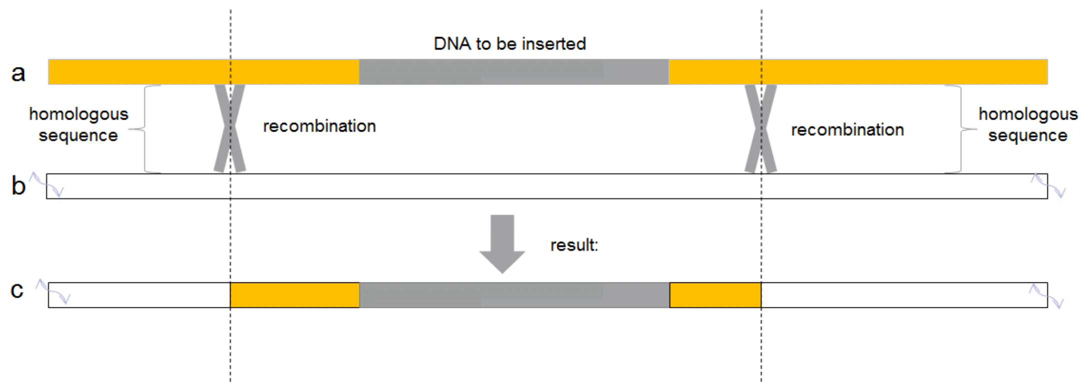
25. ES cells in which correct integration has occurred are injected into blastocysts and transferred to the uterus of a foster mother. The resulting offspring are chimaeras, i.e. they are composed of genetically distinct cells derived from the modified ES cells and from the blastocyst. Those mice displaying a high degree of chimaerism are then selected, and bred with a wild-type mouse to produce a transgenic mouse that is heterozygous for the transgene. Two heterozygous transgenic mice are then bred to produce a homozygous transgenic mouse.

Targeting Vectors

26. Vectors to carry the DNA fragment to be incorporated into the host genome need to be constructed for introduction into the host genome. Depending on the intended method of integration (homologous recombination or site-specific recombination) the targeting vector will be different.

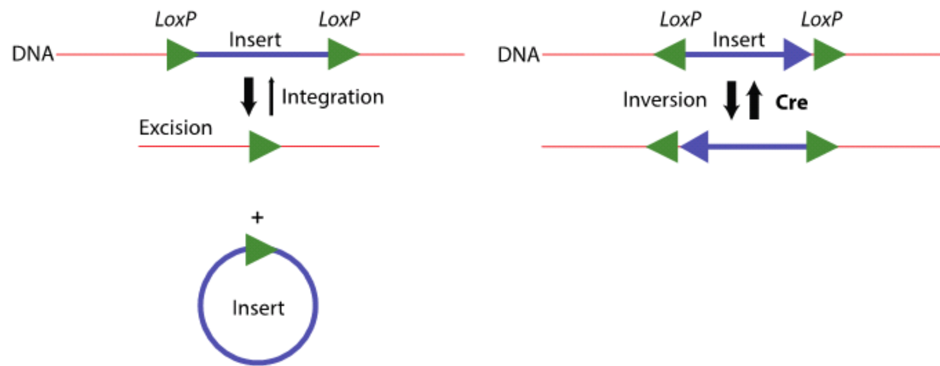
27. A targeting vector for homologous recombination will include flanking "homology arms" to promote homologous recombination. These homology arms are DNA fragments that are selected such that they are identical in DNA sequence to the

sequences that flank the target sequence being modified or replaced in the genome. The design of the homology arms should be such that they are highly related, preferably completely identical, to the DNA sequences they are intended to target. The length and degree of sequence homology of the homology arms is an important factor in determining the efficiency of incorporation of the targeting construct.



General scheme for gene targeting by homologous recombination: (a) the targeting vector consists of a left and right homology arm that flanks the payload to be inserted; (b) the locus to be targeted; and (c) homologous recombination may occur anywhere within the arm of homology resulting in insertion of the payload as well as portions of the homology arms. The position of recombination is indicated by the hatched vertical line.

28. A targeting vector for site-specific recombination involves the use of a *site-specific recombinase* enzyme and its target sequence, such as the Cre-*loxP* system from the bacteriophage P1 and/or the Flp-*FRT* system from the budding yeast *S. cerevisiae*. Both the Cre and Flp recombinase enzymes cleave DNA at a distinct target sequence, the *loxP* and *FRT* site respectively, and ligate it to the cleaved DNA of a second target site of the same nature, to generate a specific recombination event. The site-specific recombination sequence is first introduced into the host by homologous recombination, followed by the use of the site for site-specific recombination. Site-specific recombination can be used for targeted insertion or replacement of an endogenous gene in whole or in part.



Cre/Lox recombination. Left: The green triangles denote the sequence elements that constitute the LoxP site. If two LoxP sites in the same orientation flank a stretch of DNA (insert), then expression of active Cre recombinase will result in excision of that DNA insert. This reaction is in principle reversible, as indicated, however the intramolecular reactions are favoured over intermolecular reactions (left half of diagram). For inversions (right) the reactions in either direction are intramolecular and thus have more or less equal probability. Right: If the LoxP sites are in opposite orientation, then the insert is inverted upon expression of active Cre recombinase, as indicated by the blue arrowhead.

29. Site directed integration by site-specific recombination can be achieved by recombinase-mediated cassette exchange ("RMCE"). RMCE involves flanking the insert with two different recombination sequences and using these to exchange the insert with the sequence flanked by the sites in the host genome. The site-specific recombination system was particularly efficient at making deletions from the host genome, by flanking the sequences to be deleted with site-specific recombination sites, and using the recombinase enzyme to excise the intervening DNA.

Screening for Integration

30. Only approximately 0.1-1% of ES cells which survive selection using a selection agent will contain the targeting construct at the correct location, and the remainder will be likely to have the construct randomly integrated into the genome. Although the targeting frequency in ES cells is higher than in somatic cells, it is still therefore necessary to screen large numbers of ES cells in order to identify ES cells in which correct targeting has occurred.

31. The two screening methods which were used at the Priority Date were PCR and Southern blotting:

a) PCR involves amplification of a DNA fragment spanning the integration site, using a primer specific to a sequence in the exogenous DNA and a primer specific to a sequence in the endogenous chromosome, adjacent to the homology arm or site-specific recombination site.

b) Southern blotting involves using restriction enzymes to cut the chromosome at two sites, one within the exogenous DNA and one within the endogenous chromosome, outside of the homology arm or site-specific recombination site. The fragments are separated by size using gel electrophoresis, transferred to a membrane, and sequences of interest are identified by hybridization with a labelled homologous DNA probe. The restriction enzyme(s) and probe(s) are

chosen so that a change in fragment size will be detected when there is a correct integration event.

32. Both of these methods encounter problems when the homology arms of the targeting construct are greater than a certain length, due to the difficulties in amplifying fragments greater than 1.5 kb in length, in the case of PCR; and in identifying unique restriction enzyme sites for Southern blotting in sequences greater than approximately 8 kb in length. Because of these limitations in screening, the use of homology arms longer than about 8 kb was similarly limited.

Making Targeting Vectors

33. Traditional cloning techniques involved the use of PCR to generate DNA fragments and the use of restriction enzymes and DNA ligase to join the DNA fragments together. These techniques were largely restricted to the generation of constructs up to approximately 20 kb in size. The traditional vectors used for this type of construct were bacterial plasmid vectors.

34. By the priority date *recombineering* was a well-known alternative method for constructing targeting vectors. Recombineering is a form of bacterial homologous recombination pioneered by Professor Francis Stewart's laboratory. Prof. Stewart gave evidence in this case (see below). Recombineering is based on homologous recombination in *E. coli* mediated by the bacteriophage proteins RecE/RecT or Redα/Redβ.

35. In recombineering, a linear piece of DNA with short homology arms is used to replace the section of a circular target DNA molecule with the corresponding homology arm sequences in an *E. coli* host cell. As restriction enzymes are not used, the limits on the size limitation of vectors due to a possible lack of suitable restriction sites are no longer an issue. The *E. coli* containing the correct modification are screened for using selection markers, and by standard junction PCR screening techniques.

36. The method allows for the engineering of long complex DNA structures in bacterial vectors including modifications such as point mutation, sequence insertions and/or deletions to be carried out at any position on a target DNA molecule. Once the vector has been made in *E. coli* it can be isolated, and used for making modifications to ES cells.

BACs and BAC Libraries

37. Bacterial artificial chromosomes ("BACs") are single-copy high capacity plasmids, propagated in *E. coli*. BAC clones can be used to clone large segments of genomic material, up to at least 300 kb. BACs are easy to handle and the clones are stable. DNA purification and sequencing from BACs is, in general terms simple and straightforward. However, Kymab contends that repetitive elements in the DNA, and in particular long repetitive elements, can make sequencing difficult. BAC libraries provide valuable resources for, amongst other things, storing, accessing and cloning various genes of interest.

38. By partially digesting the genome of an organism (using restriction enzymes), a BAC library of large segments can be created representing some, or all, of the genome of that particular organism. BAC libraries for the human and mouse genome were available from commercial entities by the priority date. In order to identify and select an appropriate BAC clone of interest, the library must be "screened" using primers corresponding to sequences of interest. Alternatively, where the BAC library has been sequenced and characterised, the BAC clones may have been "mapped" to the genome by the provider of the custom BAC library.

The Expert Witnesses

Sir Martin Evans

39. Professor Sir Martin Evans FRS gave expert evidence on behalf of Regeneron in the field of genetic engineering. He became Professor of Mammalian Genetics at Cardiff University in 1999 and is now Chancellor of that University. He was awarded the Albert Lasker basic medical research award in 2001 together with Mario Capecchi and Oliver Smithies:

> "For the development of a powerful technology for manipulating the mouse genome with exquisite precision, which allows the creation of animal models of human disease."

In 2007 he shared the Nobel Prize for Physiology and Medicine (with Mario Capecchi and Oliver Smithies) for the "Discovery of principles for introducing specific gene modifications in mice by the use of embryonic stem cells". He is a pre-eminent mouse embryologist, and a leader in his field.

40. Kymab submits that although Prof. Evans had some pre-priority experience of homologous recombination in ES cells using conventional small targeting vectors, and some teaching experience relating to site-specific recombination, he lacked practical experience which would enable him fully to assist the court. It was suggested that, through no fault of his own, his evidence was not based on the realities of performing relevant techniques in the laboratory. It was submitted that he was "gazing at these issues from afar"

41. I reject these criticisms of Prof. Evans' evidence. He was highly experienced in modifying and handling embryonic stem cells and had a broad knowledge of molecular biology techniques, in particular homologous recombination and site-specific recombination. He had extensive teaching experience of the relevant techniques. He explained during his cross-examination that he had terminated his work in active targeting and other lab processes close to the priority date in about 2001/2002. Therefore he considered that it was easy for him to take his mind back to the priority date. In my judgment he was an exemplary expert witness who gave his evidence with clarity and fairness.

Professor Stewart

42. Prof. Francis Stewart is currently Professor of Genomics in the Biotechnology Centre at the Technische Universität Dresden and Director of the Centre. Previously (from 1991 until 2001) he was a group leader of the Gene Expression Program of the

European Molecular Biology Laboratories (EMBL) in Heidelberg. Prof. Stewart started working with Cre and Flp recombinases in 1991. In the mid-1990s, he became interested in homologous recombination in ES cells, and began to think about using homologous recombination for recombinant DNA technology, which led to his discovery of recombineering.

43. Recombineering has had a great impact on the field; it has been used, for instance, in the EUCOMM mouse project in order to achieve 16,000 gene targeting events in mouse ES cells. Prof. Stewart was also an advisor to Regeneron in its application of recombineering to its VelociGene project, and was an author of the Valenzuela paper, which I consider further below.

44. Prof. Stewart has had very considerable experience of ES cell engineering. His laboratory has performed close to 1000 ES cell homologous recombinations. He also has experience of developing or trying to develop protocols for BAC stitching by recombineering and by meganuclease digestion and ligation and of attempting to achieve large-scale RMCE.

45. No personal criticism of Prof. Stewart was made on behalf of Regeneron. However, it was suggested that, when addressing sufficiency, he was unduly influenced by an internal Regeneron document called, for the purposes of these proceedings, the "VelocImmune history document", which would not have been available to the skilled person. Further, it was submitted that he was unduly influenced by accounts that he had been given by Kymab's legal team of Regeneron's internal work on the VelocImmune Project, when he had not personally reviewed the notebooks.

46. I consider that it is evident from Prof. Stewart's first report that he reached his own conclusions about sufficiency and made reference to the VelocImmune history document only to corroborate views that he had formed. As to the second point made by Regeneron, it does have some force. In particular, for the purposes of its insufficiency attack, Kymab alleges that in order to put the invention into effect the skilled person would need to identify the 5' end of the murine V gene, which would have presented an undue burden at the priority date. During cross-examination it was put to Prof. Stewart that the relevant BACs could have been pulled out of a BAC library. It appeared that Prof. Stewart did not consider that this issue raised a great problem, but changed his mind as a result of discussions with lawyers about the difficulties that Regeneron are alleged to have experienced. This does affect the weight that I will attach to particular aspects of Prof. Stewart's evidence, which I identify below. I do not consider that this affects the weight to be attached to Prof. Stewart's evidence in general. I consider that he gave his evidence in a manner that was instructive and engaging, and I found it of great assistance.

Professor Ploegh

47. Prof. Ploegh gave expert evidence on behalf of Regeneron. He is an immunologist and Professor of Biology at the Massachusetts Institute of Technology ("MIT"), where he is a member of the Whitehead Institute for Biomedical Research. He was a member of the Department of Pathology at Harvard Medical School (between 1997-2005 and was an incumbent of the Edward J Mallinckrodt, Jr. Professorship of Immunopathology. He was Director of the Graduate Program in Immunology at Harvard Medical School from 1997 to 2005. He has been teaching undergraduate and

graduate immunology classes in the USA since 1992, and taught similar classes in the Netherlands from 1984 to 1992.

48. It was submitted on behalf of Kymab that Prof. Ploegh was not a specialist in B cell biology and lacked practical knowledge as he was unfamiliar with the work of companies producing transgenic mice at the priority date. I do not consider that there was anything in these points. Prof. Ploegh explained in his first report that his research interests at Harvard Medical School in the late 1990s and early 2000s included experiments resulting in the creation of mice with no circulating immunoglobulins, which nonetheless maintained a normal B cell compartment. These mice were created by inter-crossing animals deficient in AID with mice that had lost the ability to secrete IgM. He continued these research interests at MIT, with particular emphasis on the creation of mouse models by somatic cell nuclear transfer. While his experiments did not require the manipulation of cloned pieces of DNA, they made use of ES cell and mouse technology in a manner similar to the construction of knock-out mice. Since joining the Whitehead Institute in 2005, his laboratory has also made use of targeted insertion of DNA into ES cells for the construction of transgenic animals.

49. Prof. Ploegh's evidence was criticised on the basis that he had been steeped in Regeneron's case since he was first instructed in 2013, having been involved in and having attended the Opposition proceedings before both the Opposition Division and the TBA. I reject this criticism. I do not consider that Prof. Ploegh was biased in favour of Regeneron, which this submission appears to suggest. Similarly I reject the criticisms of Prof. Ploegh that he was on the lookout for any possible ambiguity or defect in the prior art and took unmeritorious points during his cross-examination. On the contrary, I consider that he expressed points of disagreement with Prof. Howard in a calm and courteous manner, in respect of issues on which reasonable experts might differ.

Professor Howard

50. Prof. Howard gave expert evidence in the field of Immunology on behalf of Kymab. He joined the Department of Immunology at The Babraham Institute, Cambridge, and in 1986 he was appointed head of the department. For several years during this period he was involved in the production and study of monoclonal antibodies. He left the Babraham Institute in 1994 to take up the position of Professor of Cell Genetics at the Institute of Genetics at the University of Cologne, which he held until 2011, when he became a Professor Emeritus. In 2012 he was appointed as Director of the Instituto Gulbenkian de Ciência in Lisbon, a position that he still holds. In 1995 he was elected as a Fellow of the Royal Society and in 2007 he was made a Member of the Academia Europaea.

51. Obviously, Prof. Howard is a leader in the field of immunology. However, he was clear during his cross-examination that he had not worked on producing antibodies for use in therapy and was not a B cell specialist. I gained the impression that he was not involved in the relevant field at the priority date and had had to read the literature from 1994 onwards to reconstruct what was the common general knowledge at the priority date (see in particular T8/1288/6 – 1290/3). That does not mean that he was unable to assist the Court in respect of the immunology issues in this case. As a T cell specialist he knew a great deal about B cells, and was able to explain the complex

disclosures of the prior art and other scientific literature with remarkable clarity and simplicity. As with the other expert witnesses, I found his evidence of great assistance. I will need to bear in mind, however, when considering the issue of inventive step, that Prof. Howard's evidence was based on hindsight reasoning.

The Witnesses of Fact

Dr Andrew Murphy

52. Dr Murphy was the only one of Regeneron's witnesses of fact to be cross-examined. He is currently Senior Vice President of Research at Regeneron. He joined Regeneron in 1999 as Director of Genomics and Bioinformatics, and was promoted to Vice President of Gene Discovery and Bioinformatics in 2001. His work at Regeneron has encompassed a wide range of research, including the development of the VelocImmune transgenic mouse. Dr Murphy is one of the named inventors of the patents and is the author of the VelocImmune history document which is relied on by Kymab. I consider that he gave his evidence very fairly.

Dr Yancopoulos, Professor DeFranco and Professor Ishida

53. The evidence of a number of other witnesses of fact was relied on by Renegeron, which was not challenged in cross-examination. In my judgment, this evidence has some significance in relation to inventive step and I will deal with it in detail when considering that question.

Dr Friedrich

54. Dr Friedrich is Chief Operating Officer of Kymab who confirmed that the contents of the Product and Process Descriptions ("PPDs") were accurate. He was briefly cross-examined and no criticism is made of his evidence. I shall consider it further when dealing with the issue of infringement.

The Skilled Team

55. It is common ground that the patents are addressed to a skilled team comprising an immunologist with an interest in the production of therapeutic antibodies and a genetic engineer with an interest in the creation and development of transgenic animals. Regeneron submits that the genetic manipulations required to produce a transgenic mouse in 2001 (and today) are difficult. Any skilled team working in this area has to be highly skilled, although, by definition, unimaginative and not inventive. I accept this submission.

56. In addition, Profs. Evans and Stewart agreed that the skilled team would not expect its experiments to work first time and would know that trial and error would be required in order to get such experiments to work. Further the skilled team would need to use controls and troubleshoot in order to optimise experimental conditions. Prof. Evans said that optimising conditions is the very skill of those involved in this field. Accordingly, a certain amount of persistence and willingness to repeat experiments is a part of the skill in this art. I will bear those facts in mind when considering sufficiency.

57. Kymab submits that persons with the skills to attempt to put the teaching of the patents into effect would have about three years' post-doctoral experience. Prof. Stewart explained that such people are at the height of their experimental powers, before they move away from the bench and their practical, hands-on ability lessens. Kymab suggests that such persons could follow a protocol and exercise ordinary skill in making it work, but could not develop a new protocol, which would require creative problem-solving and inventiveness.

58. I do not accept that the abilities of the unimaginative skilled team would be so limited. Because of the nature of the art, the skilled team would include a genetic engineer with considerably more than three years post-doctoral experience, who would act as team leader. It would include an experienced immunologist and a number of team members with experience in performing the necessary experiments. Further, they would use common general knowledge, and routine trial and error, in an attempt to adapt an experimental protocol if it did not work. Prof. Evans expressed the position as follows, and I accept his evidence:

> "Q. If you hit a problem in this field, it is often unclear what the cause of the problem is.
>
> A. Absolutely.
>
> Q. Let alone how to fix it.
>
> A. Absolutely, and the standard protocol would be to try not to change more than one parameter at a time. Things really go wrong when you have got two faults at the same time. So you try to get back to the point where there is one fault which you can then test, and that would be the standard scientific technique in this sort of field."

59. However, I do accept Kymab's submission that the skilled team does not have the imagination or creative ability to solve problems that cannot be tackled by routine, iterative trial and error. It lacks any inventive capacity. It is important to bear this firmly in mind, even in an art in which, by definition, success may follow after a number of initial failures.

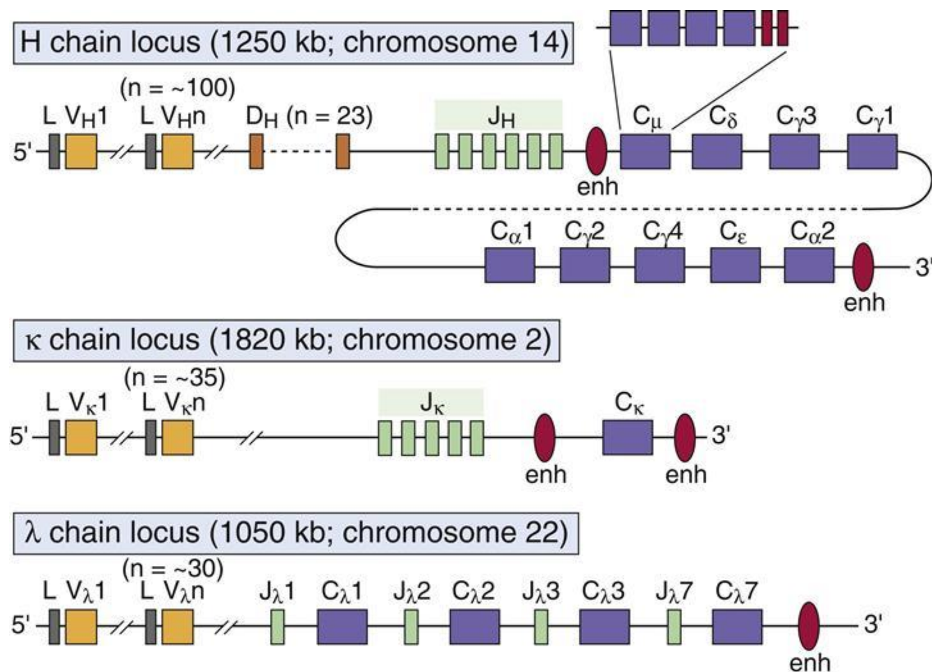
Common General Knowledge

60. I shall now consider areas of common general knowledge which are, or may be, in dispute between the parties. I shall apply the legal principles in respect of identification of common general knowledge as set out by Arnold J in *KCI Licensing Inc v Smith & Nephew Plc* [2010] FSR 31 at [105]–[115], which were approved by the Court of Appeal at [2011] FSR 8 at [6]. There are a significant number of disputes, and when considering each issue I will identify the area of the case to which it is relevant.

An undiscovered 5' enhancer (construction and infringement)

61. As discussed in the Technical Background, the human heavy chain (IgH) locus is about 1.25 Mb long. It consists of a large number of V (variable) gene segments (each

preceded by its own leader sequence L and its own promoter). These are followed by a number of D (diversity) gene segments and a smaller number of J (joining) gene segments. There is then a series of constant regions. There is also an enhancer in the intron between the J gene segments and the first (Cμ) constant region and a further enhancer at the 3' end (beyond the most proximal constant region). These enhancers are shown in red marked "enh" in the figure below:



62. These enhancers, positioned at the proximal end of the immunoglobulin locus, are transcriptional control elements that encourage the process of antibody protein production. The existence of the 3' enhancer was first published in March 1990 and the existence of the intronic enhancer was known well before 1990. Their existence was common general knowledge at the priority date.

63. Kymab contends that although it was not known whether there was a regulatory element distal (5') of the V gene segments, the skilled person would have been aware at the priority date that there *could* be such a regulatory element. In other words, it was common general knowledge that a 5' enhancer might exist. In support of this contention, Kymab relies upon a short passage in a 1996 review by Brüggemann and Neuberger "*Strategies for expressing human antibody repertoires in transgenic mice"* [L2/38] at page 4:

> "An attractive alternative would be to replace the mouse Ig loci with the human Ig loci; in this way it would be possible to retain and exploit any possible regulatory sequences in the mouse loci that are located distal to protein coding regions."

64. It also relies on a similar suggestion in Brüggemann 1997 [B/3]:

> "Although the human genes [in existing transgenic mice] can utilise expression, selection and mutation mechanisms provided by the mouse B-cells, the efficiency with which they do so is clearly reduced. It will be interesting to find out whether the

> provision of V or C region genes or as yet unidentified regulatory sequences - in short, driving the generation of antibody repertoires from large almost locus size regions - provides a transgenic construct with authentic expression capabilities."

65. I do not accept that it was common general knowledge at the priority date that an unidentified 5' enhancer might exist. I do not consider that these brief suggestions, made some years before the priority date in publications which addressed much wider issues, were sufficient to make the theory common general knowledge. I note that the theory does not appear to have been accepted in subsequent publications between 1996/7 and the priority date. Prof. Ploegh explained during his cross-examination that there was no evidence in the published literature (as distinct from the speculations referred to above) that there were regulatory sequences at the 5' end of the locus, distal to any protein coding regions. He also explained that even if the thought had occurred to the skilled person that there might be such an enhancer, its likely impact would be minimal, and the skilled person building a construct would not take account of every possible speculation. I accept his evidence.

Size of insertions and deletions by homologous recombination (sufficiency)

66. Kymab submits that it was common general knowledge at the priority date that insertions and deletions of only modest size had been achieved. I accept this submission. The largest insertion that had been achieved by homologous recombination by 2001 was under 15 kb, being a 13.8 kb insertion reported in a publication by Cvetkovic *et al* in *The Journal of Biological Chemistry* (2000) 275 [L3/59]. Prof. Evans explained that that the skilled person would not know what to expect in terms of the reduction in efficiency with an insertion above 15 kb, but that "the sensible expectation would be that it would have been harder as you go bigger" (T3/498/21-499/21).

67. As to deletions, at the priority date, Profs. Evans and Stewart agreed that only rarely had deletions of greater than 20 kb been successful by homologous recombination. In 2001, there was a general view that deletions of over 20 kb were very large and that trying to achieve them by homologous recombination was very inefficient. The removal of 20 kb of sequence in 2001 would have been regarded as a very large deletion. The skilled genetic engineer would not have any experience of achieving deletions over 20 kb by 2001 (e.g. Evans XX T3/498/12-20). I find that it was common general knowledge at the priority date that deletions of over 20 kb could not be routinely obtained.

68. As to combining insertions and deletions, the largest that had been achieved by homologous recombination by 2001 or 2002 was of modest size, perhaps below 10 kb, and this was common general knowledge (Evans XX T3/499/22-500/7).

Homology arms (sufficiency)

69. Kymab submits that it was common general knowledge at the priority date that standard homology arms were in the range of about 3 to 8 kb and that longer arms could not be checked by junction PCR or Southern blotting. I accept this. It was a limitation inherent in the screening methods that were used at the priority date. There

was also a common understanding that increasing the total length of homology arms beyond about 15 kb was of little benefit for the frequency of combination (Evans XX T3/504-505). Although Prof. Evans did not agree that this view was soundly based, I find that it was widely accepted at the priority date.

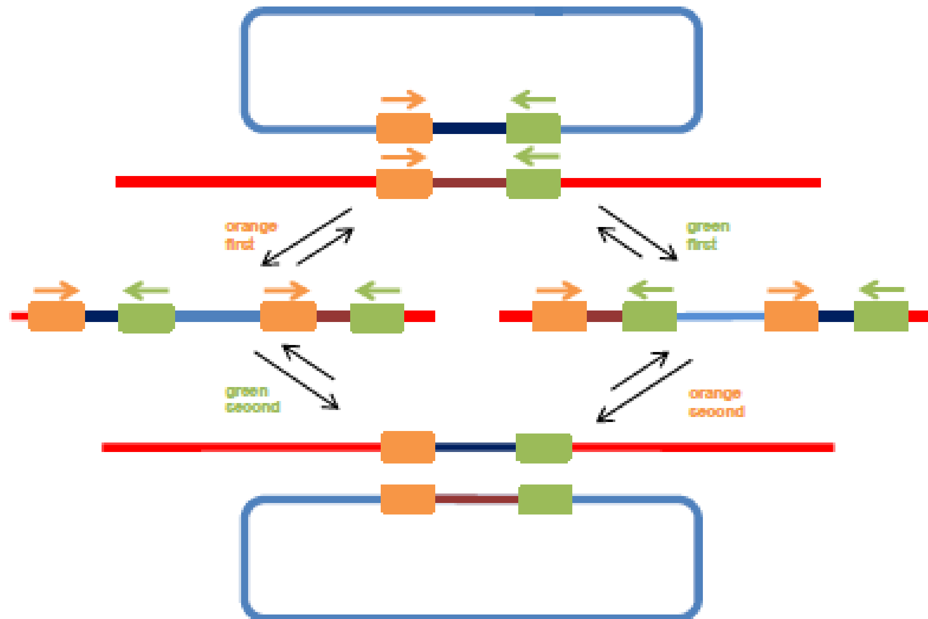
Site-specific recombination (sufficiency)

70. It was common general knowledge at the priority date that large deletions were possible using the Cre-*Lox* system, discussed above. However, Prof. Stewart explained that insertions were more difficult, as there is a natural tendency for excision to be favoured over insertion. Prof. Evans considered that it was common general knowledge that large insertions were feasible using single site-specific recombination sites. He based this on a paper by Call *et al* published in *Human Molecular Genetics* (2000) 9 (12); 1745-1751 [L3/65]. However, Prof. Stewart, who had been actively working in the Cre-*Lox* field at the priority date, considered that the Call paper was not part of the common general knowledge and in any event would be treated with considerable scepticism. I accept the evidence of Prof. Stewart on this issue.

RMCE

71. Prof. Stewart explained that variations on *lox*P sites were also common general knowledge at the priority date, including *lox*511. These sites were incompatible with each other. *Lox*511 sites would only recombine with each other, and not with *lox*P sites (and vice versa).

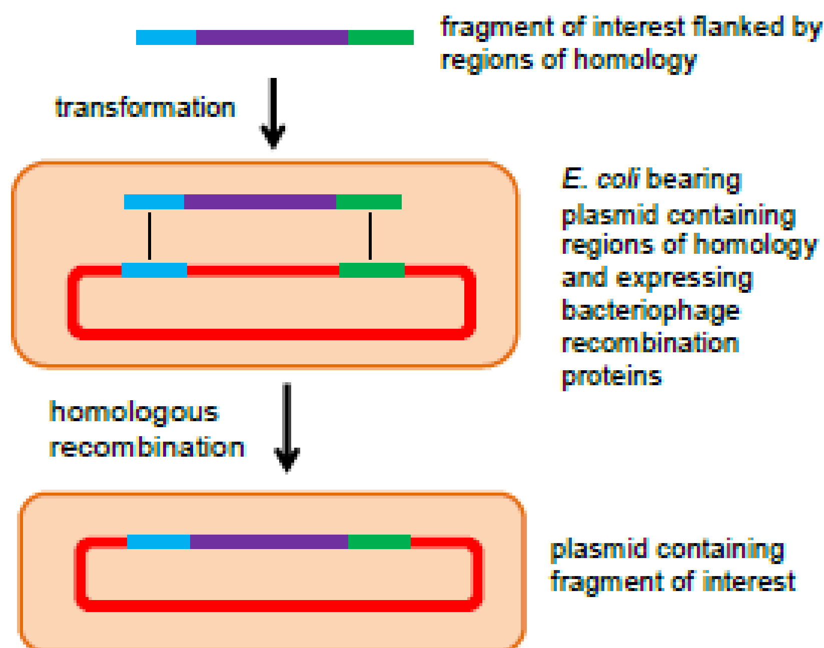
72. The use of such incompatible (heterotypic) *lox* sites led to the development of the technique of RMCE. This could be used to replace genomic DNA (flanked with heterotypic *lox* sites) with exogenous DNA (flanked with the same heterotypic *lox* sites) carried on a circular vector. This is shown in Prof. Stewart's first report (where the green and orange boxes represent different types of *lox* site):



73. Prof. Stewart explained that the largest exchanges achieved by the priority date were less than 10 kb for less than 10 kb, and that it was not until 2007 that large RMCE was achieved, using stringent selection strategies. I accept his evidence on this issue.

Recombineering

74. The technique of recombineering was common general knowledge at the priority date. It did not require the use of restriction enzymes and allowed modifications to be made to large vectors such as BACs. Recombineering utilised *E. coli* in which the endogenous homologous recombination machinery had been inactivated and replaced with that derived from certain bacteriophages. In such bacteria, homologous recombination occurred even with very short homology arms (about 50 bp). Short homology arms could be added to the DNA of interest and the homologous recombination machinery of the mutant *E. coli* used to create a targeting vector, as shown in Prof. Stewart's first report:



75. Prof. Stewart explained at [142] of his first report that donor fragments of 200-300 kb (as contemplated in the patents – see below) were considerably longer, by an order of magnitude, than any which had been used for recombineering at the priority date. I accept his evidence on this issue.

BACs and BAC libraries (sufficiency)

76. Kymab acknowledges that by the priority date, vectors were available which could hold more DNA than traditional plasmids. For example, BACs were circular DNA molecules that could hold up to about 300 kb of DNA. It also acknowledges that BACs could be used to create libraries of segments of the genome of an organism. A good BAC library would contain over 100,000 clones corresponding to various fragments of the genome. Unless the library had been mapped to the genome already, to find a BAC containing a sequence of interest it was necessary to screen the library using probes or primers corresponding to the sequence of interest. Kymab submits that this was a problem for mouse BAC libraries, given the incomplete knowledge of the mouse genome at the priority date.

77. Prof. Evans did not agree with Prof. Stewart about the existence or extent of this alleged problem. He said at [25] of his second report:

> "I agree with Prof. Stewart at paragraph 64 of his report that DNA purification and sequencing from BACs was considered simple and straightforward at the Priority Date. However I disagree with him at paragraph 66 that the incomplete knowledge of the mouse genome did not allow ready identification of BACs. Whilst it is true that the entire nucleotide sequence of the mouse genome had not been

> published at the Priority Date, extensive work had already been carried out to characterise the mouse genome. By the Priority Date unique sequence markers had been identified along the entirety of the mouse gene at an interval of around 300 kb… In addition many gene specific markers have also been identified… Using the information available at Priority Date the Skilled Genetic Engineer would have been able to design a probe from almost any region of the mouse gene and this would have been standard laboratory work."

78. When Prof. Stewart was cross-examined about this issue, he said:

> "A. I do agree with that. I have indeed done these sorts of exercises myself and when I approached this case, when in the first time I was thinking about this I thought, okay, it is fine, doing chromosomal walking, fishing out BAC clones, that is obviously straightforward and I was very surprised to then find out about the troubles that Regeneron had to isolate the V regions from the mouse genome.
>
> Q. So let us leave Regeneron on one side for a moment. When you say "the troubles", did you get that from the VelocImmune History document or did you go to the notebooks to find this?
>
> A. No, in the course of discussions about the case I became aware of ----
>
> Q. Discussions with?
>
> A. With my legal colleagues.
>
> Q. Right, but you have not been through the notebooks yourself.
>
> A. No, I have not."

79. I prefer Prof. Evans' evidence on this issue. In my judgment, the experts would have been in agreement, but Prof. Stewart was swayed by accounts that he had been given of the difficulties that Regeneron were alleged to have experienced in "fishing out BAC clones".

Modifying the endogenous mouse Ig loci (inventive step)

80. Kymab submits that it was common general knowledge at the priority date that it was potentially advantageous to use a targeted approach to make modifications to the endogenous mouse Ig. The starting point for this submission is the work of the Rajewsky group published in 1993/94 in which targeted replacements at the mouse heavy chain locus had been made while retaining the mouse expression control elements of the locus, as reflected in Taki et al *Science* (1993), 262; 1268-1271 [L1/26] ("Taki"); and Zou et al *Current Biology* (1994), 4; 1099-1103 [L2/33] ("Zou"). Kymab contends that the Rajewsky work indicated that partial replacements

of the endogenous mouse locus could be made, and the introduced genes would function in the context of the remaining endogenous protein-coding and regulatory regions.

81. This was the view of Prof. Howard, who said in [15] of his second report:

> "From the work described in the Rajewsky publications, the Skilled Immunologist was aware that one of the implications of engineering the endogenous locus (as opposed to using random insertions) was that the introduced DNA need not provide all the sequences necessary for antibody production. This applied both to regulatory sequences and protein coding sequences. … This is in contrast to random insertions, for which the introduced DNA has to provide all of the regulatory and protein coding sequences necessary for antibody rearrangement and expression. The Skilled Immunologist therefore knew that targeting to the endogenous locus brought with it a flexibility to retain certain mouse sequences instead of having to provide them all."

82. Kymab contends that the advantages of the targeted approach were set out in a series of reviews by Dr Brüggemann (on occasion with Prof. Neuberger) over several years. In particular, it relies upon a review by Brüggemann et al *Immunology Today* (1996), 17 (8); 391-397 at 394 ("the Brüggemann and Neuberger 1996 Review"). According to Kymab, the possible advantages of the approach were common general knowledge, even though it was not regarded as the only way forward. It therefore contends that a "reverse chimeric locus", comprising a combination of human variable genes and endogenous mouse constant genes, was an obviously desirable goal.

83. Regeneron, by contrast, contends that at the priority date, the focus was on attempts to produce a fully human transgene. The prior art mice comprised both human variable genes *and* human constant genes, because the mice were being used to produce fully human antibodies. The benefits of the reverse chimeric locus, with human variable regions and mouse constant regions, which produced hybrid antibodies, were not appreciated until years after the priority date, although it has now been widely acknowledged. It contends that this is clear from the unchallenged evidence of its witnesses of fact, as well as a very considerable amount of published literature, published both before and after the priority date.

84. As to witnesses of fact, Regeneron relies on the evidence of Professor DeFranco, who was fairly described by Prof. Howard as "eminent in this field". Together with other eminent immunologists, he served on the scientific advisory board of Abgenix between 1998 and 2004. Prof. DeFranco describes the production of XenoMouse I (with 5 V segments) and subsequent production of XenoMouse II (with 66 V segments). The idea of a reverse chimeric locus never occurred to him or his colleagues. He said in his witness statement:

> "At no time during my involvement with Abgenix did it occur to me that deficiencies in B cell development and antibody responses could be addressed by creating a hybrid locus at the endogenous immunoglobulin heavy locus with human variable

> regions and mouse constant regions. Such an approach was not obvious to me."
>
> "Nor at any time before 16 February 2001 did anyone associated with Abgenix or its SAB (or anyone else) suggest in my presence that this was a strategy of interest or raise the potential benefits of such an approach as disclosed in paragraphs [0116] and [0117] of the Patent."

85. Secondly, Regeneron relies on the evidence of Professor Ishida, who was working at Kirin, and, from 1992, produced transgenic antibodies in mice by chromosomal transfer. Rather than integrating the relevant sequence by random integration into the murine genome, this involved introducing a human chromosomal fragment into the mouse. The objective remained the same, to create a mouse expressing human immunoglobulin genes.

86. Kirin entered into a collaboration with Medarex and this resulted in the production of the Kirin Medarex mouse in November 2001 which comprised both transgenic and transchromosomal components. The idea of a reverse chimeric locus never occurred to him or his colleagues. Prof. Ishida stated:

> "I have a recollection of discussing this matter with Nils Lonberg during our collaboration and his suggesting it could perhaps be addressed by altering the cytokine environment in the mouse by, for example, administering interleukin 6. Neither he, nor anyone else, suggested this had anything to do with the nature of the human constant region and its interaction with the mouse B cell, and this did not occur to me either."

87. Thirdly, Regeneron relies on the evidence of Dr Yancopoulos, who is President, Chief Scientific Officer and Founding Scientist at Regeneron Pharmaceuticals and is a co-inventor of the patents. Dr Yancopoulos explained that before the priority date, Regeneron were customers of Medarex, but were dissatisfied with the performance of Medarex's fully human Ig transgene mice, which were failing efficiently to produce antibodies to those targets which were known to induce antibody responses in wild type mice. He describes how he arrived at the concept of a reverse chimeric locus, which had not occurred to anyone else at Medarex:

> "Having experienced the issues associated with the Medarex mice, I became very excited about an idea that I had which I thought had a very significant chance of fixing all of the problems of the Kirin-Medarex mice, without requiring new adjuvant solutions, or the breeding of excessive numbers of animals. My idea was that the problem could be addressed by retaining the mouse constant regions and creating a hybrid locus, as we have described in the Patent. Such a mouse would theoretically not only be much more efficient and optimised in its immune function but also simultaneously avoid all the inherent breeding problems seen in the Medarex and Kirin mice, in terms of having to account for multiple transgenic loci

> at multiple non-endogenous locations, not to mention the instability problems with the Kirin mini-chromosome.
>
> The solution seemed to me to be simple and elegant. Until I introduced the possibility to them under confidentiality (see paragraph 45 below), at no time did any Medarex scientist even raise the possibility of any solution remotely akin to what we now refer to as VelocImmune®. They never raised the possibility that putting variable segments into the endogenous mouse locus would have any advantage. Nor did they raise the possibility that retaining mouse constant regions would have any utility or advantages."

88. Kymab submits that all of this evidence is of little value as companies were wedded to the fully-human approach for commercial reasons, having invested time and money in it. Therefore, according to Kymab, although there was a repeated recognition that changing the mouse constant region to human was the cause of some of the remaining difficulties with existing transgenic mice, the commercial imperative was to press on. I reject this submission. None of these witnesses were challenged in cross-examination, and this suggestion was not put to them. I consider that their evidence provides a helpful insight into the thought processes of leaders in the field at the priority date.

89. As to publications, Regeneron submits that the common theme was a desire to make transgenic mice producing 'fully human' antibodies. All of the transgenic prior art mice that were used to make antibodies incorporated human constant regions in the constructs. For example, in Nicolson et al. *The Journal of Immunology* (1999), 6898-6906 (L3/57) the authors describe the transgenic mouse made by the Neuberger and Brüggemann group. Page 1 describes the use of YAC-based human IgH, IgK and IgL transloci in a background in which endogenous mouse IgH and IgK chain expression has been inactivated. The heavy chain construct was made using a 240 kb YAC comprising 5 human Vs, all the human Ds and all the Js, and C mu and C delta. Prof. Howard was asked about this paper during cross-examination:

> "Q. Now, these are leaders in the field and nowhere do they mention the potential benefits of the reverse chimeric locus, and by that I mean retaining mouse constant genes in their transgenic loci."
>
> A. No, that is correct."

90. Regeneron has cited a number of papers that show that this same thinking continued well after the priority date. It was not until 2013 and 2014 that the benefits of the reverse chimeric locus were recognised in the published literature. For example, Neuberger and Brüggemann moved from a fully human transgene to a reverse chimeric transgene, as reported in Osborn et al *J Immunol* (2013), 190:1481-1490 (L5/95):

> "In most of the published mouse strains currently under use, segments of the human IgH and IgL loci comprising differing numbers of human V, D, and J segments linked to human C

regions have been integrated into the mouse germline (16,17) with the endogenous mouse Ig loci having been rendered non-functional through targeted gene disruption (18).

…Because the transgenic mice can essentially be viewed as a source of Ag-specific IgV genes (with the desired IgC region provided at a later stage during the creation of cell lines for bulk Ab production), we wondered whether the transgenic approach could be improved if the germline configuration human IgV_H-D-J_H segments were linked to endogenous (rather than human) IgC_H regions."

91. As to Taki and Zou, it is Regeneron's case that they describe early work carried out in 1993 and 1994 by the Rajewsky group in which they investigated small targeted insertions into the mouse Ig locus. Taki describes the targeted replacement (using homologous recombination with short homology arms) of the mouse J_H locus with a single rearranged mouse VDJ transgene. The purpose was to create a mouse useful to researchers studying the process of isotype switching and somatic hypermutation of the encoded transgene. The mouse described in Taki produced only fully mouse antibodies. Zou describes the targeted replacement (using Cre-*Lox*) of a mouse constant region gene (the C gamma gene) with its human counterpart. The resulting transgenic mice expressed chimeric antibodies with mouse variable regions and human IgG1 constant regions.

92. Prof. Ploegh explained that the work carried out by this group related to fundamental aspects of mouse B-cell biology. They were not interested in producing human antibodies in the same way as the Brüggemann and Neuberger laboratories, but rather were demonstrating certain proofs of concept. He said:

"the early Zou papers were following on very shortly on the discovery of gene targeting by homologous recombination. The Rajewsky lab was one of the very first to adapt Cre-lox based strategies to engineer the genome in a specific manner. I view these papers as demonstrating proof of concept. Yes, one can do these things. As we discussed yesterday, the number of academic laboratories that were engaged in producing fully human antibodies can be counted on the fingers of one hand. Brüggemann, Neuberger, I could not think of many others, they were narrowly focused on producing fully human immunoglobulins. As I pointed out, the Rajewsky lab is focused on studying B-cell physiology, the regulation of a normal B-cell response, elucidating mechanisms of hypermutation class switching and so forth."

93. Prof. Howard accepted that neither of these early papers were teaching or refer to the benefits of the reverse chimeric locus (Howard XX D9/1371/18-21).

94. As to the 1996 Brüggemann & Neuberger Review, Regeneron submits that the short passage on page 394 relied on by Kymab needs to be read in context. It is a teaching to target the endogenous locus but it is not a teaching to use a reverse chimeric locus. At the end of this passage the authors explicitly state:

> "Furthermore technologies for direct gene replacement (e.g. using the Cre-LoxP system) might allow the generation of animals in which much of the DNA of the mouse Ig loci is substituted by human Ig-gene DNA."

The focus of the publication is on producing human antibodies by substituting as much as possible of the mouse Ig loci with human Ig-gene DNA. In the conclusions (page 6) the authors note that:

> "Human Ig genes introduced in germline configuaration can be re-arranged, expressed and hypermutated in the mouse background. While it is not clear that all these processes operate with optimal efficiency, they certainly operate at a level sufficient to ensure that the transgenic mice can be used to obtain hybridomas secreting high-affinity human antibodies against a variety of antigens. "

They explain that it now technically feasible to contemplate making a mouse having the whole human Ig loci but that it might be better to be judicious in the selection of certain human V segments.

95. Prof. Howard accepted that there is no suggestion anywhere in the paper to retain mouse constant regions.

96. Kymab also relies on a review by Brüggemann in *Molecular Biology of B Cells,* edited by Honjo, Alt and Neuberger (2004) 547-561 (L5/88), which Prof. Ploegh indicated was broadly representative of common general knowledge at the priority date, and which referred to the work of the Rajewsky group in 1993-1994. This post-priority review summarises the position before the priority date as follows:

> "the various strategies that had been used to introduce and express human Ig heavy and light chain genes in transgenic animals and show that a diverse human antibody repertoire can be obtained in a mouse background with silenced endogenous IgH and IgL chain loci…I also draw attention to present shortcomings and speculate what the future may provide."

97. The review refers to the early work done by Taki and Zou and comments that "the success in targeted replacement of mouse for human genes favourably supports more extensive alterations that may allow the expression of fully human antibody repertoires controlled by the endogenous Ig loci". The conclusions are set out on page 558, where it is observed that:

> "several new technologies will be used to produce high-affinity fully human antibodies. Whole Ig loci will be routinely transferred into different species of animals."

98. I find that in 2004, the Brüggemann group was advocating the production of fully human antibodies in transgenic systems, and that the early work of Taki and Zou had been superseded.

99. Having regard to all of the above, I accept Prof. Ploegh's evidence during his cross-examination that the thinking of those skilled in the art

> "was unidirectional, narrowly focussed, myopically obsessed, one might say, with the production of human antibodies."

By the priority date, the art had moved on from early, tentative suggestions by the Rajewsky group, and it was thought that progress would be made by the introduction of more human Vs into the mouse locus. There was no suggestion to use a reverse chimeric locus to produce chimeric antibodies, or that such a locus would offer any benefits over the "fully-human" approach.

Inactivating the endogenous murine locus (construction)

100. In my judgment, it was common general knowledge at the priority date that in the well known transgenic mice produced by Brüggemann, Abgenix and Medarex, the endogenous loci were inactivated by the introduction of a stop codon or, insofar as there was any deletion, only J segments were removed. Inactivation is referred to, for example, in the Nicolson (1999) paper of which Profs. Brüggemann and Neuberger were co-authors. The abstract explains that:

> "We have produced mice that carry the human Ig heavy (IgH) and both κ and λ light chain transloci in a background in which the endogenous IgH and κ loci have been inactivated."

This is elaborated on page 2 which refers to the insertion of a stop codon and Neo cassette and explains that:

> "Mice with their endogenous H chain or κ L chain loci rendered non-functional have been described previously."

The Patents

101. The patents are very complex disclosures that are not possible to understand without the assistance of expert evidence. The experts concentrated on the 287 specification, as the 163 Patent is essentially the same, other than changes necessary to reflect differences in the claims. In this judgment, I refer to paragraphs in the description of the 287 Patent. I will consider specific disputes between the parties after explaining the general disclosure of the 287 Patent.

LTVECs and the MOA assay

102. Whilst a feature of all of the claims of the 287 Patent is *in situ* replacement of mouse variable region Ig genes with human variable region Ig gene segments, much of the description relates to broader applications of certain molecular biology methods which it discloses and claims. As explained in [0002], the 287 Patent describes methods which can be used to:

> "target, via homologous recombination, and modify, in any desirable fashion, endogenous genes and chromosomal loci in eukaryotic cells."

103. As set out in [0010] these methods involve:

(i) bacterial homologous recombination (i.e. recombineering) to engineer a desired genetic modification within a large cloned genomic fragment to create large targeting vectors ("LTVECs") for use in eukaryotic cells;

(ii) introducing LTVECs into eukaryotic cells to modify the endogenous chromosomal locus of interest; and

(iii) using an assay for modification of allele ("MOA") of the parental allele "that does not require sequence information outside of the targeting sequence, such as, for example, quantitative PCR" to determine those eukaryotic cells in which the targeted locus has been modified as desired.

104. Thus, the 287 Patent may be thought of as comprising two distinct yet related disclosures. In addition to the reverse chimeric locus, the 287 Patent discloses an approach to homologous recombination which contemplates the use of large genomic fragments from BACs with long homology arms coupled with the quantitative modification of allele assay to detect successful targeting events. This combination is alleged to permit the successful insertion, deletion and detection of larger genomic DNA fragments than was possible using prior art methods, as explained in [0002]:

> "The field of this invention is a method for engineering and utilizing large DNA vectors to target, via homologous recombination, and modify, in any desirable fashion, endogenous genes and chromosomal loci in eukaryotic cells. These large DNA targeting vectors for eukaryotic cells, termed LTVECs, are derived from fragments of cloned genomic DNA larger than those typically used by other approaches intended to perform homologous targeting in eukaryotic cells. The field of the invention further provides for a rapid and convenient method of detecting eukaryotic cells in which the LTVEC has correctly targeted and modified the desired endogenous gene(s) or chromosomal locus (loci). The field also encompasses the use of these cells to generate organisms bearing the genetic modification, the organisms, themselves, and methods of use thereof."

105. Paragraph [0003] sets out the advantages of LTVECs:

> "The use of LTVECs provides substantial advantages over current methods. For example, since these are derived from DNA fragments larger than those currently used to generate targeting vectors, LTVECs can be more rapidly and conveniently generated from available libraries of large genomic DNA fragments (such as BAC and PAC libraries) than targeting vectors made using current technologies. In addition, larger modifications as well as modifications spanning larger genomic regions can be more conveniently generated than using current technologies. Furthermore, the present invention takes advantage of long regions of homology to

> increase the targeting frequency of "hard to target" loci, and also diminishes the benefit, if any, of using isogenic DNA in these targeting vectors.”

106. At [0005] the Patent explains that prior art methods involve the use of targeting vectors having regions of homology of less than 10-20 kb. The limitations of the prior art assays are then explained:

> “…Assays for successful targeting involve standard Southern blotting or long PCR …from sequences outside the targeting vector and spanning an entire homology arm (see Definitions); thus, because of size considerations that limit these methods, the size of the homology arms are restricted to less than 10-20 kb in total.”

107. As explained above, both of the prior art methods encountered problems when the homology arms of the targeting construct were greater than a certain length, due to the difficulties in amplifying fragments greater than 1.5 kb in length when using PCR, and in identifying unique restriction enzyme sites for Southern blotting in sequences greater than approximately 8 kb in length.

108. Paragraph [0007] explains that one of the benefits associated with being able to use long homology arms in a targeting vector is that it opens up a convenient and rapid way to use BAC libraries to make targeting vectors:

> “The ability to utilize targeting vectors with homology arms larger than those used in current methods would be extremely valuable. For example, such targeting vectors could be more rapidly and conveniently generated from available libraries containing large genomic inserts (e.g. BAC or PAC libraries) than targeting vectors made using current technologies, in which such genomic inserts have to be extensively characterized and trimmed prior to use.”

109. [0007] also asserts other potential advantages of LTVECs, including the ability to make larger modifications; the ability to make modifications spanning larger genomic regions in fewer steps; increasing the targeting frequency of "hard to target" loci; and avoiding the need to use isogenic DNA in these targeting vectors.

110. The 287 Patent explains that prior art assays cannot be used to detect successful targeting in the case of LTVECs because of their long homology arms:

> “[0078] It should be emphasized that previous methods to detect successful homologous recombination in eukaryotic cells cannot be utilized in conjunction with the LTVECs of Applicants' invention because of the long homology arms present in the LTVECs. Utilizing a LTVEC to deliberately modify endogenous genes or chromosomal loci in eukaryotic cells via homologous recombination is made possible by the novel application of an assay to determine the rare eukaryotic cells in which the targeted allele has been modified as desired,

> such assay involving a quantitative assay for modification of allele (MOA) of a parental allele, by employing, for example, quantitative PCR or other suitable quantitative assays for MOA."

111. MOA is defined in [0073] of the 287 Patent:

> "“Modification of allele” (MOA) refers to the modification of the exact DNA sequence of one allele of a gene(s) or chromosomal locus (loci) in a genome. This modification of allele (MOA) includes, but is not limited to, deletions, substitutions, or insertions of as little as a single nucleotide or deletions of many kilobases spanning a gene(s) or chromosomal locus (loci) of interest, as well as any and all possible modifications between these two extremes."

112. In summary, the idea is to detect unmodified alleles, so that a cell in which one allele has been modified can be distinguished from a cell in which both alleles remain unmodified. Accordingly, a quantitative MOA assay may be used to detect cells in which the target locus has been modified. This allows the use of LTVECs with long homology arms, because the assay detects the level of umodified alleles, as described in detail at [0090]-[0092] of the 287 Patent. In particular, [0091] explains that:

> "In contrast to traditional methods, in which a difference in restriction fragment length spanning the entire homology arm or arms indicates the modification of one of two alleles, the quantitative TaqMan® method [an example of the MOA assay] will detect the modification of one allele by measuring the reduction in copy number (by half) of the unmodified allele. Specifically, the probe detects the unmodified allele and not the modified allele."

113. Prof. Evans explained the MOA assay, as described in the 287 Patent in his first report at [63]-[64]:

> "Taking these passages together, and the document as a whole, it would be clear to the person skilled in the art that the MOA assay requires the detection by an appropriate probe (using various techniques) to detect the unmodified parental allele. If the gene has been correctly targeted there will only be one modified allele and one unmodified allele. Modifying both alleles in a single manipulation would be unexpected. The assay measures this reduction in the frequency of the unmodified allele from two to one.
>
> The proposal in the '287 Patent is therefore not to detect the targeted ES cell clone by detailed analysis of the targeted allele but just its alteration or loss. The detection of a targeted ES cell clone is seen by concentrating on the intact endogenous alleles and seeing a change from two copies to one in the correctly targeted cell clone (MOA), whereas an incorrectly targeted cell

> clone retains two copies. This is an elegant and clever approach that was not generally known or used by those skilled in the art. It obviates interference in the analysis from a randomly integrated targeting vector. It also facilitates using larger targeting vectors because the size of arms is immaterial and detailed restriction maps (or sequence information) are no longer required. It particularly lends itself to replacement and/or deletion events. It may perhaps be particularly powerful coupled with the use of bacterial recombination to construct the targeting vectors because here, too, detailed knowledge of the restriction maps is not needed.

114. Prof. Evans explained the benefits of the MOA assay further during his cross-examination:

> "Let us just refresh our minds a little bit of what this very clever assay is. You established in our first meeting, I am of the old school. We used to try very hard to use homologous recombination to make a deletion knock-out change in a desired locus and we always had to find that not only by things, as we have discussed, like PCR ending or Southern blot but also looking to make sure we have actually made the change we want, so we are always looking at our changed locus. One is always completely forgetting about the fact that there is an unchanged locus in the background except that we have to discriminate against that when we are doing our tests. What the MOA has done is to invert that logic."

115. The benefit of using LTVECs in combination with the MOA assay is indicated in [0094] of the 287 Patent, which concludes:

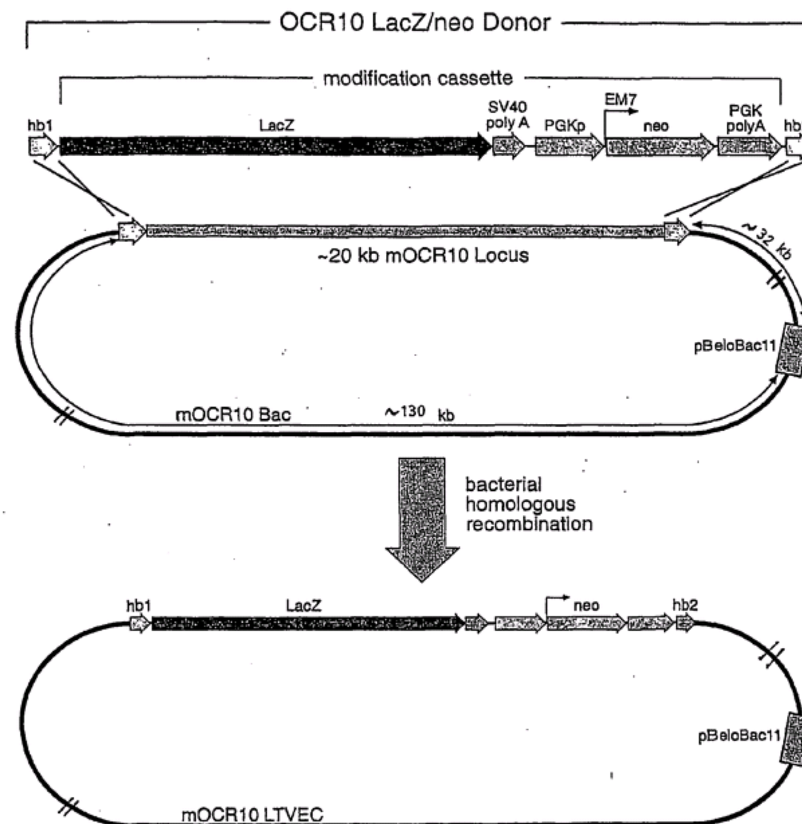
> "While many of the techniques used in practicing the individual steps of the methods of the invention are familiar to the skilled artisan, Applicants contend that the novelty of the method of the invention lies in the unique combination of those steps and techniques coupled with the never-before-described method of introducing an LTVEC directly into eukaryotic cells to modify a chromosomal locus, and the use of quantitative MOA assays to identify eukaryotic cells which have been appropriately modified. This novel combination represents a significant improvement over previous technologies for creating organisms possessing modifications of endogenous genes or chromosomal loci."

Example 1

116. Example 1 at [0095]-[0107] is a specific illustration of the general approach explained above, in respect of a mouse ES cell in which a gene of interest (the OCR10 gene) has been deleted. The 287 Patent asserts a significant improvement in speed and cost effectiveness of vector construction and screening. It also suggests that the use of this method has improved targeting frequency of the mOCR10 gene compared to prior

attempts with previous technologies. [0097] describes the identification of a BAC clone containing the mouse OCR10 (mOCR10) gene. This was done by screening a mouse BAC library using identified PCR primers derived from the sequence of the mOCR10 gene. [0098] explains that the BAC clone was used to generate an LTVEC which also included a modification cassette including a reporter gene (LacZ) and a selectable marker (Neo). The LTVEC is of the type shown in general terms in Figure 1 and when introduced into an ES cell it will lead to replacement of the mOCR10 coding sequence with the modification cassette.

117. It is important to be aware of the size of the LTVEC that was introduced into the ES cells, and the size of the mOCR10 deletion from those cells in Example 1. Construction of the donor fragment (the modification cassette and homology boxes) is described in [0098]-[0099]. The donor fragment is about 6 kb in length and so can be created using traditional cloning techniques. Production of the LTVEC is described in [0100] and shown in Figure 2:



As can be seen from this figure, this LTVEC, when introduced into ES cells, is intended to lead to the replacement of 20 kb of mOCR10 sequence with the modification cassette of about 6 kb in length. The removal of 20 kb of sequence is described in [0102] as “a very large deletion”. Profs. Evans and Stewart agreed that this reflects the view of the skilled person in 2001/2. The experts also agreed that the skilled genetic engineer, given Example 1, would have been able to reproduce it.

Example 2

118. Example 2 is said to provide additional data that supports the use of the long homology arms when targeting ES cells [see in particular Table 1 at 0108]. The experiments in Table 1 all involve the introduction of small cassettes (under 10 kb) and the largest deletion is 30 kb. It was clear from the cross-examination of Prof. Evans that the 287 patent provides no evidence, by way of example or other data, that the techniques employed can achieve any more extensive replacement than 10 kb for 30 kb.

> " Q. Just looking back at Table 1 again if you could with me, these are all cases in which targeting constructs of a similar nature to that in Example 1 have been used, in that they are LTVECs containing fairly short modification cassettes, are they not? For example, if you look at the data in the first line, we see that the LTVEC is 147 kb, the two arms are together 140 kb and so the cassette is 7 kb -- cassette plus vector backbone is 7 kb.
>
> A. Yes, and they also have a figure for the deletion there.
>
> Q. That is right, but in terms of what is going into the genome they are all fairly short cassettes. The largest deletion here is 30 kb. Professor, there is no evidence here that replacements can be made which consists of more than replacing 30 kb with a few kbs?
>
> A. That is what this table showed."

Example 3

119. Although characterised as an example, this section of the Patent discloses the concept of the reverse chimeric locus, as well as purporting to set out materials and methods to put this concept into practice. Paragraph [0113] refers to prior art approaches that used transgenic mice to produce fully human antibodies. It states:

> "More recently, endogenous genes have been knocked out of mice, and the genes replaced with their human counterparts to produce entirely human antibodies. Unfortunately, the use of these constructs has highlighted the importance of an endogenous constant region in the development and optimization of antibodies in B cells. Mice producing fully human antibodies have reduced immune responses. This may because human antibodies produced by transgenic mice with entirely human constructs have reduced affinity as compared to their mouse counterparts. Reduced affinity could effect B-cell maturation and survival. Accordingly, the much acclaimed methods of producing humanized antibodies in mice and other organisms, wherein endogenous variable and constant regions of the mice are knocked out and replaced with their human counterparts, has not resulted in optimal antibodies."

120. Prof. Howard accepted that the person skilled in the art would appreciate that this is a reference to the prior art (fully human Ig locus) transgenic mice developed by Brüggemann, Lonberg and Abgenix.

121. The purpose of making a mouse with a reverse chimeric locus is explained in paragraphs [0115]-[0121]. [0115] states that:

> "A transgenic mouse is created that produces hybrid antibodies containing human variable regions (VDJ/VJ) and mouse constant regions. This is accomplished by a direct, in situ replacement of the mouse variable region genes with their human counterparts. The resultant hybrid immunoglobulin loci will undergo the natural process of rearrangements during B-cell development to produce the hybrid antibodies."

122. [0116] explains that these hybrid antibodies are not the final therapeutic products because they contain immunogenic mouse constant regions, but they can subsequently be engineered to make fully human antibodies for use in therapy by replacing the mouse constant region with an appropriate human constant region. Thus the specificity of the variable regions generated in the mouse is retained and the issue of immunogenicity avoided by producing a final antibody product that is fully human.

123. Advantages of the reverse chimeric locus approach are set out at [0116]-[0118]. Paragraph [0116] states that:

> "…Antibodies generated by the new mouse will retain murine Fc regions which will interact more efficiently with the other components of the mouse B cell receptor complex, including the signaling components required for appropriate B cell differentiation (such as Iga and Igb). Additionally, the murine Fc regions will be more specific than human Fc regions in their interactions with Fc receptors on mouse cells, complement molecules, etc. These interactions are important for a strong and specific immune response, for the proliferation and maturation of B cells, and for the affinity maturation of antibodies."

124. Prof. Ploegh explained this passage at [71]-[72] of his first report. The B cell receptor ("BCR") includes two accessory subunits, Igα and Igβ. Engagement of these signaling proteins is essential for B cell survival, activation and the internalisation of the BCR upon binding of an antigen. The 287 Patent teaches that because the Fc regions of human and mouse antibodies are not conserved between the species, the mouse Igα and Igβ subunits will interact less effectively with human Fc regions than with mouse Fc regions. This will impair B cell development and in addition may affect signal transduction via the BCR. Prof. Howard agreed with this explanation during his cross-examination.

125. Paragraph [0117] states that :

> "Because there is a direct substitution of the human V-D-J/V-J regions for the equivalent regions of the mouse loci all of the sequences necessary for proper transcription, recombination, and/or class switching will remain intact. For example, the murine immunoglobulin heavy chain intronic enhancer, Em, has been shown to be critical for V-D-J recombination as well

> as heavy chain gene expression during the early stages of B cell development … whereas the immunoglobulin heavy chain 3' enhancer region appears to be critical for class switching … as well as heavy chain gene expression at later stages of B cell differentiation…Given these various, yet crucial, functions of the transcriptional control elements, it is desirable to maintain these sequences intact."

126. This explains why the endogenous locus is targeted. The transcriptional and other control elements present in the wild-type mouse will be present including in particular the murine immunoglobulin heavy chain intronic enhancer and 3' enhancer.

Materials and Methods of Example 3

127. In the section entitled 'Materials and Methods', the 287 Patent describe a number of approaches that it alleges could be used to create transgenic mice having reverse chimeric loci using LTVECs and the quantitative MOA assay.

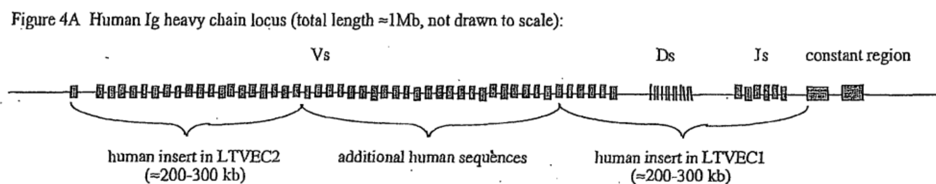
128. Paragraph [0123] states that

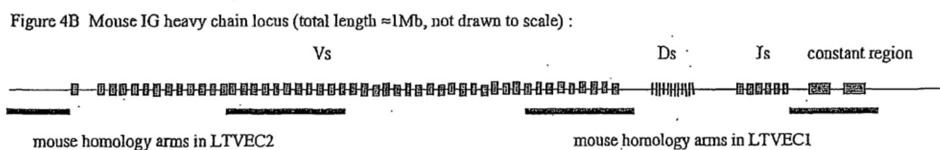
> "Precise replacement of the mouse heavy chain locus variable region (VDJ) with its human counterpart is exemplified using a combination of homologous and site-specific recombination in the following example, which utilizes a two-step process. One skilled in the art will recognize that replacement of the mouse locus with the homologous or orthologous human locus may be accomplished in one or more steps. Accordingly, the invention contemplates replacement of the murine locus, in whole or in part, with each integration via homologous recombination."

129. Regeneron points out that this passage specifically contemplates that replacement of the mouse locus with the human locus may be accomplished in more than one step. Kymab points out that this passage contemplates replacement of a part or *the whole* of the mouse locus with the human locus. Both are correct. The 287 Patent then proposes two approaches for putting this into practice. I will consider the feasibility of these approaches when addressing the insufficiency objection.

The first proposed approach

130. [0124]-[0129] describe a first proposed approach to achieving the objective of replacing the mouse IgH variable locus with the human IgH variable locus. This uses a number of human BAC clones containing the entire variable region of the human IgH locus, as shown in Figure 4A, and a number of mouse BAC clones covering sections of the mouse IgH locus to provide homology arms for the LTVECs (as shown in Figure 4B). [0124] states that "large insert (BAC) clones spanning the entire VDJ region of the human heavy chain locus are isolated (Figure 4A)." Some sizes are indicated on those figures, in kb and Mb.

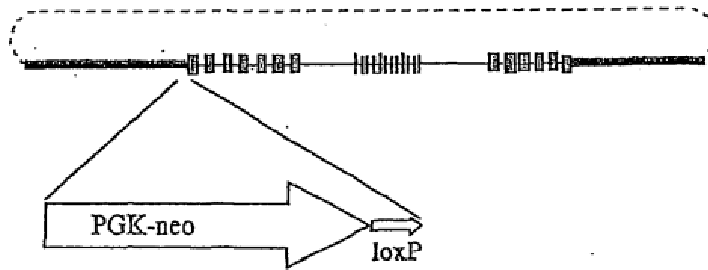




131. The first step in a method to achieve this is described in [0125]:

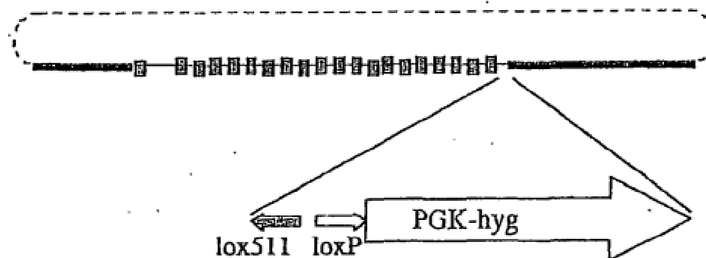
> "[0125] In the first step, LTVEC1 (Figure 4D) is constructed by bacterial homologous recombination in E. coli. LTVEC1contains, in order: a large mouse homology arm derived from the region upstream from the mouse DJ region but whose absolute endpoints are not important; a cassette encoding a selectable marker functional in ES cells (PGK-neomycinR in this example); a loxP site; a large human insert spanning from several V gene segments through the entire DJ region;and a mouse homology arm containing the region immediately adjacent to, but not including, the mouse J segments. The 5' end of the downstream arm and the placement of the loxP sites define the 3' end of the region to be replaced in the locus. Mouse ES cells will be transformed by standard techniques, for example, electroporation, with linearized LTVEC1. Because direct introduction of LTVEC1 results in a modification of the endogenous variable gene locus, neomycin resistant colonies can be screened for correct targeting using a MOA assay. These targeted ES cells can give rise to mice that produce antibodies with hybrid heavy chains. However, it will be preferable to proceed with subsequent steps that will eliminate the remainder of the mouse variable segments."

132. Accordingly the first step is the construction of LTVEC1 from BAC clones and its introduction into ES cells to replace (at least) all the mouse DJ gene segments with several human V gene segments and the human DJ gene segments. This LTVEC is shown in Figure 4D:



133. The human insert in LTVEC1 is about 200-300 kb in length (as shown on Figure 4A). Prof. Stewart explained (based on information from Prof. Howard) that the minimum amount of mouse sequence to be replaced is about 100 kb in length. That was on the assumption that no mouse V gene segments are replaced. If mouse V gene segments are replaced, (as the claims of 287 require) then the relevant mouse sequence will be considerably larger.

134. The second step in this method is described in [0126]. It contemplates the construction of LTVEC2 from the BAC clones (as shown in Figure 4C of the 287 patent):



135. LTVEC2 is introduced into ES cells to replace the most distal mouse V gene segments with the most distal human V gene segments (about 200-300 kb). After the second step, a large number of mouse V gene segments will remain in the modified locus. [0127] asserts that those segments can be deleted (by transient expression of Cre in the ES cells or by the production of mice which are then bred with mice expressing Cre).

136. [0128] suggests a first way to replace remaining mouse V gene segments with additional human V gene segments. It suggests the use of bacterial homologous recombination to produce a clone containing additional human V gene segments flanked by heterotypic *lox* sites, followed by the use of RMCE. [0129] suggests an alternative approach for the replacement of remaining mouse V gene segments by additional human V gene segments, using a technique known as TAMERE.

The second proposed approach

137. [0130]-[0139] set out the second proposed approach. It discloses the use of LTVECs which contain only mouse, rather than human, sequence to introduce heterotypic *lox* sites at the proximal and distal ends of the mouse variable region locus, followed by RMCE to replace the mouse sequence with the orthologous human locus.

Homologous recombination is used only to introduce *lox* sites. Otherwise, RMCE is used.

138. Having inserted the *lox* sites, the scheme of the second proposed approach requires the intervening mouse locus to be replaced by the whole or part of the human locus. Prof. Stewart explained at [169] of his first report that the human sequence needs to be at least 75 kb long in order to include V, D and J gene segments. [0133] suggests that this sequence can be obtained from a single human BAC and flanked with *lox* sites, followed by RMCE in the mouse ES cells ([0136]).

139. Paragraphs [0134]-[0136] propose an alternative way to carry out the replacement of the entire locus using RMCE that does not involve obtaining the sequence from a single human BAC. The suggestion is to obtain two or three human BACs which overlap, to add heterotypic *lox* sites to the most distal and most proximal BACs and to introduce them all into the mouse ES cells. The theory is that the BACs will undergo homologous recombination with each other, followed by RMCE. [0137] suggests using a further RMCE step, while [0138]-[0139] describe making larger human sequences for insertion by RMCE from multiple BACs using either meganucleases and ligation or recombineering.

The light chains

140. Paragraph [0140] contemplates replacement of the light chain variable gene loci in the mouse, so that the resultant transgenic mouse will have a genome comprising an entirely human variable region and an entirely endogenous mouse constant region:

> "The final steps in creating the human variable/mouse constant monoclonal antibody producing mouse will be performing the equivalent variable region substitutions on the lambda and kappa light chain loci and breeding all three hybrid loci to homozygo[s]ity in the same mouse. The resultant transgenic mouse will have a genome comprising entirely human heavy and light chain variable gene loci operably linked to entirely endogenous mouse constant region such that the mouse produces a serum containing an antibody comprising a human variable region and a mouse constant region in response to antigenic stimulation. …"

The Claims in Issue

141. Claim 1 of the 287 Patent is a process claim in the following terms:

> "A method of modifying an endogenous immunoglobulin heavy chain variable region gene locus in an isolated mouse embryonic stem (ES) by an *in situ* replacement of V, D, and J gene segments of the endogenous locus with orthologous human V, D and J gene segments, to create a modified immunoglobulin locus that produces hybrid antibodies containing human variable regions and mouse constant regions, said method comprising:
>
> a) obtaining a large cloned genomic fragment greater than 20 kb containing orthologous human V, D, and J gene segments;

b) using bacterial homologous recombination to genetically modify the cloned genomic fragment of (a) to create a large targeting vector for use in a mouse ES cell (LTVEC);

c) introducing the LTVEC of (b) into a mouse ES cell to replace said V, D, and J segments *in situ* with the orthologous human V, D and J gene segments; and

d) using a quantitative assay to detect modification of allele (MOA) in the mouse ES cell of (c) to identify a mouse ES cell in which said V, D and J segments have been replaced *in situ* with the orthologous human V, D and J gene segments."

142. The following issues of construction appeared to be common ground between the parties, but in any event I find as follows from a consideration of claim 1 in context:

143. First, the claim is confined to a method of modifying the Ig heavy chain locus and does not concern the light chain locus. The preamble to the claim identifies the product to be produced by the method, namely a heavy chain variable region gene locus which has been modified in an ES cell so that V, D, and J gene segments of the endogenous locus have been replaced with orthologous human V, D and J gene segments, to create a modified immunoglobulin locus that produces hybrid antibodies containing human variable regions and mouse constant regions (i.e. a reverse chimeric locus).

144. Secondly, steps (a) to (d) reflect the first approach of Example 3 and require the introduction of human V, D and J segments at the endogenous locus. Thus they require use of a large cloned genomic fragment greater than 20 kb (for example, a BAC), the use of bacterial homologous recombination (recombineering) to create an LTVEC, introduction of the LTVEC in ES cells to replace V, D and J segments *in situ*, and use of a quantitative MOA assay to detect correctly targeted cells.

145. Thirdly, the claim requires at least one endogenous V gene segment (as well as D and J gene segments), i.e. at least about 150 kb of mouse sequence, to be replaced with at least one orthologous V human gene segment (as well as D and J gene segments), i.e. at least about 75 kb of human genomic sequence. There is no doubt that at least about 75 kb of human genomic sequence is required to be inserted. Whether, in addition, at least about 150 kb of mouse sequence is required to be deleted depends upon the meaning of "*in situ* replacement" which I shall consider below. I will also consider whether claim 1 covers the case where the mouse sequence is deleted.

146. Fourthly, the parties agreed that step (b) imposes a practical limitation on the size of the cloned genomic fragment which could be used (and hence on the amount of human sequence which replaces the endogenous sequence), because an LTVEC produced by recombineering (hence a BAC) could not be larger than about 300 kb.

147. Fifthly, although I do not consider that the history of amendments to the claims affects their construction, I was told that claim 1 was amended before the TBA (amongst other amendments) to exclude the case where the entire endogenous immunoglobulin variable gene locus was replaced with the entire human gene locus in

a single step (although Regeneron did not accept that this was the correct construction of claim 1 as granted).

In situ replacement

148. This is the principal issue of construction between the parties. Kymab contends that "*in situ* replacement" requires deletion of the murine variable gene segments and insertion of the human variable human segments in the same place. Otherwise, Kymab submits, a replacement *in situ* has not been made. Regeneron submits that *in situ* replacement simply means replacing "in the position of" and does not require deletion. So the relevant murine variable segments must at least be moved (and inactivated) so that the human variable segments can be inserted in their position, but they do not have to be deleted. This issue is of primary relevance to infringement. In the Kymouse constructs, the murine variable gene segments are rotated and re-inserted in a different place in the genome, so that, according to Regeneron, they cease to function. They are not physically deleted.

149. In considering this issue, I shall apply the principles of construction as set out in *Virgin Atlantic Airways Ltd v Premium Aircraft Interiors UK Ltd* [2010] RPC 8 at [5]. The task for the Court is to determine what the person skilled in the art would have understood the patentee to have been using the language of the claim to mean. Furthermore, the claim must be construed without knowledge of the alleged infringement, as it does not change its meaning depending on the acts of the defendant. However, it is necessary to have in mind the issue(s) on infringement in order to focus on the points that are material. In *Technip France SA's Patent* [2004] RPC 46 Jacob LJ said:

> "Although it has often been said that the question of construction does not depend on the alleged infringement ('as if we had to construe it before the defendant was born'…) questions of construction seldom arise in the abstract. That is why most sensible discussion of the meaning of language runs on the general lines 'does it mean this, or that, or the other?' rather than the open-ended 'what does it mean?'"

150. I shall also bear in mind the observations of Floyd LJ in *Adaptive Spectrum v British Telecommunications* [2014] EWCA Civ 1462:

> "It must be remembered, however, that the specification and claims of the patent serve different purposes. The specification describes and illustrates the invention, the claims set out the limits of the monopoly which the patentee claims. As with the interpretation of any document, it is conceivable that a certain, limited, meaning may be implicit in the language of a claim, if that is the meaning that it would convey to a skilled person, even if that meaning is not spelled out expressly in the language. However it is not appropriate to read limitations into the claim *solely* on the ground that examples in the body of the specification have this or that feature. The reason is that the patentee may have deliberately chosen to claim more broadly than the specific examples, as he is fully entitled to do."

151. The parties agree that the phrase '*in situ* replacement' is not a term of art. Therefore, it is for the court to determine its meaning, but it needs to be educated in order to do so.

In *Ultraframe UK Limited v Eurocell Building Plastics Ltd* [2005] RPC 36 at [5] –[7] Jacob LJ considered the principles of claim construction and in particular, what a person skilled in the art would have understood the patentee to have used the language of the claim to mean. At [7] he said:

> "Before the court gets to the examination room it has to do some swotting: to get into its mind the relevant knowledge of the skilled man. For how a document will be understood depends on the reader."

152. Kymab relies on the following arguments in support of its interpretation of the phrase '*in situ* replacement'. First, it submits that the 287 Patent uses the word "replace" (or "replacement") in contradistinction to "insert" (or "insertion"). It relies in particular on [0012], [0073] and [0084]. For example, [0012] states:

> "Another embodiment of the invention is a method wherein the genetic modification to the endogenous gene or chromosomal locus comprises deletion of a coding sequence, gene segment, or regulatory element; alteration of a coding sequence, gene segment, or regulatory element; insertion of a new coding sequence, gene segment, or regulatory element; creation of a conditional allele; or replacement of a coding sequence or gene segment from one species with an homologous or orthologous coding sequence from a different species."

153. I do not accept this submission. It is true that this paragraph deals separately with deletion, alteration, insertion and replacement. However, "replacement" is the only case where it discusses inter-species substitution and so a direct comparison between these terms is over-simplistic.

154. Secondly, it observes that methods described to achieve replacement (and in particular Example 3) all contemplate deletion of the mouse variable genes. Subject to an issue that arises in relation to paragraph [0125], I accept this and it lends some support to Kymab's construction. However this submission depends on analysis of a non-limiting example in the specification, and of preferred embodiments, and it may be that the patentee has chosen to claim more broadly than this.

155. Thirdly, Kymab relies upon particular paragraphs of the 287 Patent which use the adjectives "direct" or "precise" to describe the replacement, and other passages which use the noun "substitution" to describe *in situ* replacement. In particular, [0115] refers to "a direct, *in situ* replacement of the mouse variable region (VDJ/VJ) genes with their human counterparts". [0117] refers to "a direct substitution of the human V-D-J/V-J regions for the equivalent regions of the mouse loci". [0123] refers to "precise replacement of the mouse heavy chain locus variable region (VDJ) with its human counterpart". At [0119] the 287 Patent explains that the human VDJ / VJ regions have been substituted, and at [0140] that "the resultant transgenic mouse will have a genome comprising entirely human heavy and light chain variable gene loci". Again, these paragraphs are all discussing preferred embodiments. They lend some support to Kymab's construction although they are not inconsistent with a construction which includes the case where the murine variable gene segments are moved to a different place in the genome and inactivated, and where the human variable gene segments are put in the same place as they previously occupied.

156. Kymab's argument assumes that the word "replacement" is used throughout the specification exclusively to refer to deletion and insertion. In my judgment, this is not the case. In particular, [0113], when describing the prior art, states that:

> "Accordingly, the much acclaimed methods of producing humanized antibodies in mice and other organisms, wherein endogenous variable and constant regions of the mice are knocked out and replaced with their human counterparts, has not resulted in optimal antibodies."

[0063] defines a "gene knockout" as "a genetic modification resulting from the disruption of the genetic information encoded in a chromosome locus." So in knockout mice, the endogenous locus is disrupted and not removed. I have found that the skilled person would be aware from his common general that the endogenous loci in the transgenic mice produced by Brüggemann, Abgenix and Medarex, were inactivated. This is reflected in the text of [0113] which refers to the methods of producing humanised antibodies in such mice as "much acclaimed".

157. [0113] refers to "variable and constant regions of the mice that are knocked out and replaced with their human counterparts". Prof. Howard was cross-examined about this paragraph:

> "Q. …the person skilled in the art would have no doubt that this was a reference to the mice made by Brüggemann, Lonberg, Abgenix. Yes?
>
> A. I believe that is what it is referred to, yes."

So, in my judgment, the 287 Patent refers to the variable and constant regions of mice as having been "replaced" with their human counterparts, when the corresponding mouse segments have been inactivated but not removed. Kymab accepts this, but submits that this is a reference to the prior art and not the invention. However, Kymab's construction requires that the word "replacement" be interpreted in a more narrow sense in the claims than it is used in paragraph [0113] of the description. Whilst this conclusion could conceivably be right, it would need a powerful reason to support it.

158. Furthermore, [0125] indicates that removal of murine V, D and J segments is not required. It contemplates removal of D and J segments only when inserting human V, D and J. This paragraph concludes:

> "These targeted ES cells can give rise to mice that produce antibodies with hybrid heavy chains. However, it will be preferable to proceed with subsequent steps that will eliminate the remainder of the mouse variable segments."

159. [0125] is undoubtedly describing *in situ* replacement. It suggests that Kymab's interpretation of integer (c) which requires at least one mouse V to be deleted, is incorrect. Such deletion is preferable, so claim 1 includes, but is not limited to, this case.

160. Regeneron submits that the term "*in situ* replacement" must be construed in context. It submits that it is apt to describe a positional replacement. The human sequences are to be inserted in the original position of the mouse segments juxtaposed with the mouse constant regions. The skilled person would appreciate that the patentee is using this language to distinguish targeted replacement from random insertion into the genome. Had the patentee intended to require removal of mouse V, D and J sequences as part of the process steps he would have stated this. Further, Regeneron suggests that it would make no technical sense for the patentee to have excluded embodiments where the murine sequence had been moved to a different location and rendered inactive, given that this was a well-known alternative to deletion at the priority date.

161. Kymab responds that the patentee did have a purpose in limiting his claim to the case where the relevant murine sequence had been deleted, which, it submits, is disclosed in paragraphs [0117] and [0119] of the 287 Patent. [0117] states that:

> "Because there is a direct substitution of the human V-D-J/V-J regions for the equivalent regions of the mouse loci all of the sequences necessary for proper transcription, recombination and/or class switching will remain intact."

162. Reference is then made to the intronic enhancer and the 3' enhancer, and the paragraph concludes: "Given these various, yet crucial, functions of the transcriptional control elements, it is desirable to maintain these sequences intact." Kymab submits that the skilled person would understand the patent to be explaining that a benefit of the "direct substitution" of the human VDJ/VJ regions for the equivalent regions of the mouse loci would be that all the mouse regulatory elements would remain intact and in place to regulate the human variable locus which had replaced the mouse variable locus.

163. The next step in the argument is the contention that in 2001/2 it was not known whether there were regulatory sequences 5' (distal) to the variable genes in the mouse Ig loci, but the skilled person would be aware that such regulatory sequences might be present. According to Kymab, the skilled person reading [0117] would appreciate that the patentee was identifying a benefit of "direct substitution" that applied to all mouse regulatory sequences, not limited to the intronic and 3' enhancers, but also to any 5' regulatory sequences. The benefit is that when the mouse variable region genes are removed and replaced by the human variable region genes, the various regulatory sequences will sit as close to the human genes as they did to the mouse genes and can carry out their regulatory function. However, if the human variable genes were to be inserted between the mouse variable genes and the mouse constant genes, then any 5' regulatory sequences would be displaced upstream relative to the human variable genes – they would end up about 2.5 Mb away from the human variable genes. Kymab submits that the skilled person would appreciate that in such circumstances it could not be assumed that they would perform their regulatory function with respect to the inserted human variable genes.

164. So according to this argument, the patentee has chosen to limit his claim on the basis of a hypothesis that 5' regulatory sequences might have a significant effect, and therefore needed to stay where they were. Kymab submits that, although it is now known that that this is incorrect, this does not meet the point, as the skilled person (and the patentee) believed that it might be the case at the priority date.

165. Kymab relies on [0119] in support of this argument. This paragraph states that:

> "The substitutions of the human V-D-J or V-J regions into the genuine murine chromosomal immunoglobulin loci should be substantially more stable, with increased transmission rates to progeny and decreased mosaicism of B cell genotypes compared with the currently available mice (Tomizuka, K., Shinohara, T., Yoshida, H., Uejima, H., Ohguma, A., Tanaka, S., Sato, K., Oshimura, M., and Ishida, I. Proc Natl Acad Sci (USA) 97:722-727 (2000)). Furthermore, introduction of the human variable regions (VDJ/VJ) at the genuine murine loci in vivo will maintain the appropriate global regulation of chromatin accessibility previously shown to be important for appropriately timed recombination events (Haines, B. B., and Brodeur, P. H. Eur J Immunol. 28:4228-4235 (1998))."

166. The Haines and Brodeur paper, referred to at the end of [0119,] contains a suggestion that there might be a locus regulatory element at the 5' end of the mouse lgH locus:

> "In conclusion, our data and others' indicate that D-distal VH gene segments exist in a distinct regulatory environment relative to the D-proximal segments. It has been suggested that conserved regulatory elements such as an enhancer or locus control region (LCR) may reside upstream of the Igh locus [1, 15]. These elements could function in the propagation of chromatin changes as well as mediate transcriptional and recombinational regulation at D-distal V regions."

Therefore, Kymab submits that the skilled person is expressly referred to a paper which suggests that a 5' regulatory sequence could be significant and would appreciate that there might be a benefit, contemplated by the patentee, to keeping it in place, rather than displacing it upstream, away from the human variable genes.

167. I reject Kymab's submissions on this issue for the following reasons. First, [0117] only refers to the intronic and the 3' enhancers, and makes no reference to the possibility of a 5' enhancer. As explained by Prof. Howard, the intronic and the 3' enhancers were known to be positioned in the proximal end of the immunoglobulin locus, and were known to be transcriptional control elements that encourage the process of antibody protein production. They would not be displaced irrespective of whether mouse sequences are deleted as part of the *in situ* replacement. If the patentee had been concerned about the unproven possibility that a 5' enhancer might have a significant regulatory effect, he would have made this clear in the 287 Patent.

168. Secondly, I have concluded that it was not common general knowledge at the priority date that an unidentified 5' enhancer might exist. I have accepted Prof. Ploegh's explanation that there was no evidence (as distinct from speculation) in the published literature that there were regulatory sequences at the 5' end of the locus, distal to any protein coding regions. I have also accepted his evidence that even if the thought had occurred to the skilled person that there might be such an enhancer, its likely impact would be minimal, and the skilled person building a construct would have to be practical and would not take account of every possible assumption. It follows that the

skilled person would not consider the possibility of a 5' enhancer when reading the 287 Patent, or if he did, would not consider it to be of importance. In this context, it is relevant that Prof. Howard did not suggest in his reports that there was a common general knowledge belief that there may be a 5' enhancer or that such an enhancer was of any importance, or that any putative enhancer would affect the skilled person's interpretation of the Patent (see Howard 1 [59] (b); Howard 2 [47]; and D9/1458/13-25).

169. Thirdly, as to the Haines and Brodeur paper, it is only at the end of the paper that the authors speculate that elements may be located upstream of the IgH locus. In its abstract, the paper suggests merely that there may be additional undiscovered long-range regulatory elements. I agree with Regeneron that this provides insufficient basis for construing the Patents as requiring deletion of the entire murine variable gene locus.

170. Kymab also relies on an indication in [0118] that if there is only one chimeric locus, events which could otherwise result in therapeutically irrelevant antibodies could be avoided. It suggests that if human variable genes are inserted and mouse variable genes are retained, then therapeutically irrelevant antibodies will be produced. It therefore contends that one of the benefits of the 287 Patent will only be obtained if the mouse variable genes are deleted. I reject this submission. I did not understand Prof. Ploegh to accept that therapeutically irrelevant antibodies would be produced where the retained mouse genes were inactivated. In any event, I gained the clear impression from Prof. Ploegh that this was a trivial issue which was most unlikely to occur and would be of little concern if it did occur. Therefore I do not consider that it provides a technical reason for the patentee to have limited his monopoly in the manner suggested by Kymab.

171. Finally, Kymab contends that the MOA assay cannot detect a case in which the mouse V, D and J gene segments in question have been retained but inactivated. All it can do is to detect that there has been a change in the genomic DNA of one allele. Kymab submits that step (d) of claim 1 could not be performed unless the mouse V, D and J segments have been deleted, and therefore this must be what the patentee intended by "*in situ* replacement". I do not accept this argument, as I consider that the evidence establishes that the MOA assay could be used to detect insertions where no deletion had been made. This was explained by Prof. Evans in Annex 1 to his first report at figure 29 and [8(c)] to [10]. When Prof. Stewart was cross-examined about this issue, he accepted that the MOA assay could be used to detect insertions where no deletion had been made. He suggested that the skilled person might prefer to use regular junction PCR, but whether another method might work better is not the point. In particular, when asked about use of the MOA assay to detect insertions where no deletion had been made, he said:

> "A. Yes, that is absolutely fine.
>
> Q. This is perfectly straightforward, is it not? There is nothing at all inventive or unusual or surprising about this which would not be plain as a pikestaff in the light of the patent in suit, the '287 patent?

> A. It is fine. However, I think the skilled genetic engineer would prefer to do a junction PCR."

Assessment

172. In my judgment, *in situ* replacement means replacing 'in the position of'. The phrase is apt to describe a positional replacement and this is how it is used in context. The human sequences are to be inserted in the original position of the mouse segments juxtaposed with the mouse constant regions. The skilled person would appreciate that the patentee is using this language to distinguish targeted replacement from random insertion into the genome. This includes the case where the relevant murine sequence is deleted, and also the case where it is moved to a different location and inactivated.

173. The word "replacement" is used in the specification to describe both deletion and displacement/inactivation. The invention is concerned with the use of LTVECs and the MOA assay to enable the targeted insertion and detection of human variable V, D and J gene segments, in place of mouse variable V, D and J gene segments, whilst retaining the mouse constant segments. Given that inactivation was a well-known alternative to deletion at the priority date, I do not consider that it would make technical sense for the patentee to have excluded embodiments where the murine sequence had been moved to a different location and rendered inactive.

Scope of Claim 1 of 287

174. I have concluded that claim 1 includes the case where the relevant murine sequence is deleted, and also the case where it is moved to a different location and inactivated. Deletion of the relevant mouse gene segments is undoubtedly within the scope of the claim, and repeatedly referred to in the specification.

175. [0125] describes (amongst other things) deletion of 100 kb of mouse sequence (being the DJ region, which is flanked by the inner ends of the arms) and insertion of 200-300 kb of human sequence. This is what is illustrated in Figure 4. Furthermore, in the case of replacement by deletion and insertion, the minimum required by claim 1 is a deletion of 150 kb and insertion of 75 kb, although the claim is not limited to these amounts. This was clear from the cross-examination of Prof. Evans:

> "Q. Now, we have looked at what [0125] is about, what it is describing. Now, we have talked about the amount of mouse sequence that is being deleted in the replacement step. It is 100 kb of mouse sequence at least and I think you would agree that what [0125] is envisaging is putting in its place about 200-300 kb of human sequence?
>
> A. I believe it is, yes.
>
> Q. I think, and we looked at these numbers earlier, you say, well, if you wanted to replace at least one mouse V, as well as the Ds and Js, you would have to replace 150 kb of mouse sequence.
>
> A. Yes, I think that is probably about right.

> Q. And if you wanted to replace it with one human V, as well as the human Ds and Js, that is about 75 kb.
>
> A. Yes."

176. Accordingly, claim 1 squarely includes replacements that were summarised as '100 kb out, 200-300 kb in' and '150 kb out, 75 kb in'. Furthermore, as I have explained, the parties agreed that an LTVEC produced by recombineering (hence a BAC) could not be larger than about 300 kb. Accordingly claim l covers introduction of up to about 300 kb of human sequence. However, there is no upper limit imposed by the claim on the amount of mouse sequence that is to be replaced.

Claims 5 and 6 of the 287 Patent

177. Claims 5 and 6 are product by process claims, which use the phrase "obtainable by": They are in the following terms:

> "5. A genetically modified eukaryotic cell or a mouse comprising a genetically modified immunoglobulin heavy chain variable region locus obtainable by the method of any one of the preceding claims *in situ* in place of the endogenous immunoglobulin heavy chain variable region gene locus."
>
> "6. A mouse embryonic stem (ES) cell containing a genetically modified immunoglobulin heavy chain variable region gene locus obtainable by the method of any one of claims 1 to 4 in situ in place of the endogenous immunoglobulin heavy chain variable region gene locus."

178. The history and nature of product by process claims was considered by Birss J in *Hospira UK v Genentech Inc* 2014 EWHC 3587 at [125]-[147] and [154]. I derive the following principles from this judgment in respect of "obtainable by" claims:

   i) their purpose is to claim a product irrespective of how it was made but with a shared characteristic which results from using a given process [132];

   ii) the claim has to specify the characteristic being referred to [133];

   iii) "obtainable by" claims present clarity problems and should only be permitted if there is no alternative way of defining the product in question [135]; and

   iv) for a product to be "obtainable by" a process it must have every characteristic which is the inevitable consequence of that process [154].

179. The products of claim 5 and 6 are, in the case of claim 5, a genetically modified eukaryotic cell or a mouse, and in the case of claim 6, a mouse ES cell where the required genetic modification has been made to the Ig heavy chain variable gene locus. Regeneron contends that claims 5 and 6 specify the characteristic that results from using the process of claim 1. In the case of all products within claims 5 and 6, the characteristic is "a genetically modified immunoglobulin heavy chain variable region gene locus…*in situ* in place of the endogenous immunoglobulin heavy chain variable locus." Therefore, the scope of the claims extends to products (cells and mice) which contain human heavy chain variable region genes in the endogenous position of the mouse IgH locus. I agree.

180. Kymab contends that claims 5 and 6 are limited to the case where the endogenous V, D and J segments have been deleted, as on its construction this is a requirement of the process of claim 1. Unless this occurs, on Kymab's case, the products are not obtainable by the process. However, I have rejected the argument that claim 1 requires deletion of those murine segments. The same reasoning applies to claims 5 and 6.

181. Kymab also contends (and it is common ground that) the process of claim 1 is limited to a method in which up to about 300 kb of orthologous sequence is introduced. Accordingly, Kymab submits that a characteristic of a product of the method of claim 1 is that it contains no more than 300 kb of orthologous sequence. A product is not "obtainable by" the method of claim 1 if it contains more orthologous sequence than could have been carried on a single BAC.

182. I do not accept that construction. It is true that a characteristic of a product of the method of claim 1 is that no more than 300 kb of orthologous sequence can be introduced in one step. However, if the process has been repeated on a number of occasions, so that more than 300 kb of orthologous sequence have been inserted, the resulting product, in my judgment, still falls within claims 5 and 6. I say nothing, for the moment, about whether the 287 Patent enables, without undue burden, such repeated insertions to be made, as clearly claims may extend to products or processes which they do not enable. In my view, claims 5 and 6 extend to products where, for example, there have been three insertions of 300 kb or less of orthologous sequence.

183. It follows that claims 5 and 6 are of wide scope, a matter which is of relevance, in particular, to infringement and sufficiency:

i) As with claim 1, they include products in which 100 kb of endogenous sequence has been deleted and 200-300 kb of orthologous sequence has been inserted and products in which 150 kb of endogenous sequence has been deleted and 75 kb of orthologous sequence has been inserted.

ii) Unlike claim 1, they contain no limitation as to the use of LTVECs or the MOA assay. Provided that the product is obtainable by the process of claim 1, it does not matter how it is produced.

iii) They contain no limitation as to the amount of endogenous sequence which may have been deleted and include products in which the entire endogenous heavy chain sequence has been deleted.

iv) They contain no limitation as to the amount of orthologous sequence which may have been inserted and include products in which the entire orthologous heavy chain sequence has been inserted.

Accordingly, claims 5 and 6 extend to cells and mice in which the entire mouse variable IgH locus has been replaced by the entire human variable IgH locus.

Claim 1 of the 163 Patent

184. Claim 1 of the 163 Patent is a product claim in the following terms:

> "A transgenic mouse that produces hybrid antibodies containing human variable regions and mouse constant regions, wherein said mouse comprises an *in situ* replacement of mouse VDJ regions with human VDJ regions at a murine chromosomal immunoglobulin heavy chain locus and an *in situ* replacement of mouse VJ regions with human VJ regions at a murine chromosomal immunoglobulin light chain locus."

185. Kymab submits that, as with claim 1 of the 287 Patent, "*in situ* replacement" limits the claim to a transgenic mouse where mouse VDJ and VJ regions have been deleted. However, I have rejected this construction in respect of claim 1 of the 287 Patent. The same reasoning applies to claim 1 of the 163 Patent.

186. As with claims 5 and 6 of the 287 Patent, claim 1 of the 163 Patent is of wide scope. In particular:

    i) The claim is not confined to a single product. It includes mice in which different amounts of VDJ and VJ regions have been replaced with human VDJ and VJ regions, for example a mouse in which one VDJ and one VJ region have been replaced, and mice in which several such regions have been replaced.

    ii) As with claim 1 of 287 Patent, it includes products in which 100 kb of endogenous sequence has been deleted and 200-300 kb of orthologous sequence has been inserted; and products in which 150 kb of endogenous sequence has been deleted and 75 kb of orthologous sequence has been inserted.

    iii) Unlike claim 1 of the 287 Patent, it contains no limitation as to the use of LTVECs or the MOA assay.

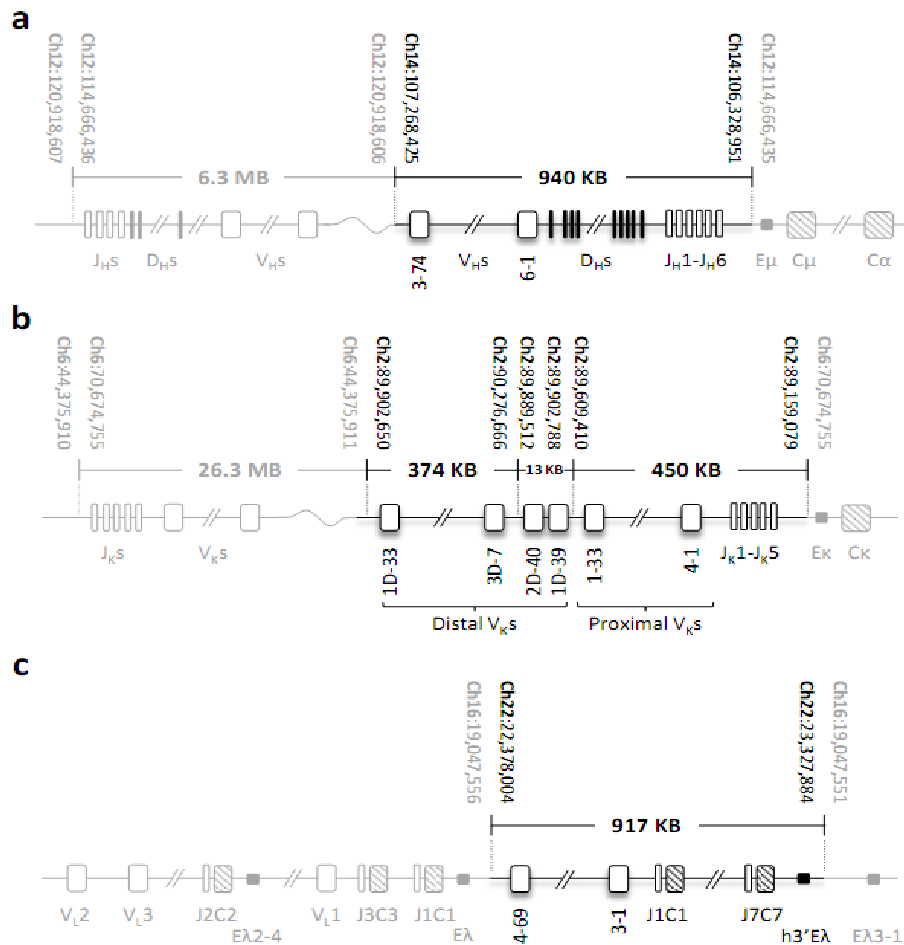
    iv) The terms "VDJ regions" and "VJ regions" as used in claim 1 of the 163 Patent, encompass the whole mouse and human variable gene loci. Accordingly the claim extends to a mouse in which the entire murine variable gene loci have been replaced with the entire human variable gene loci.

Infringement

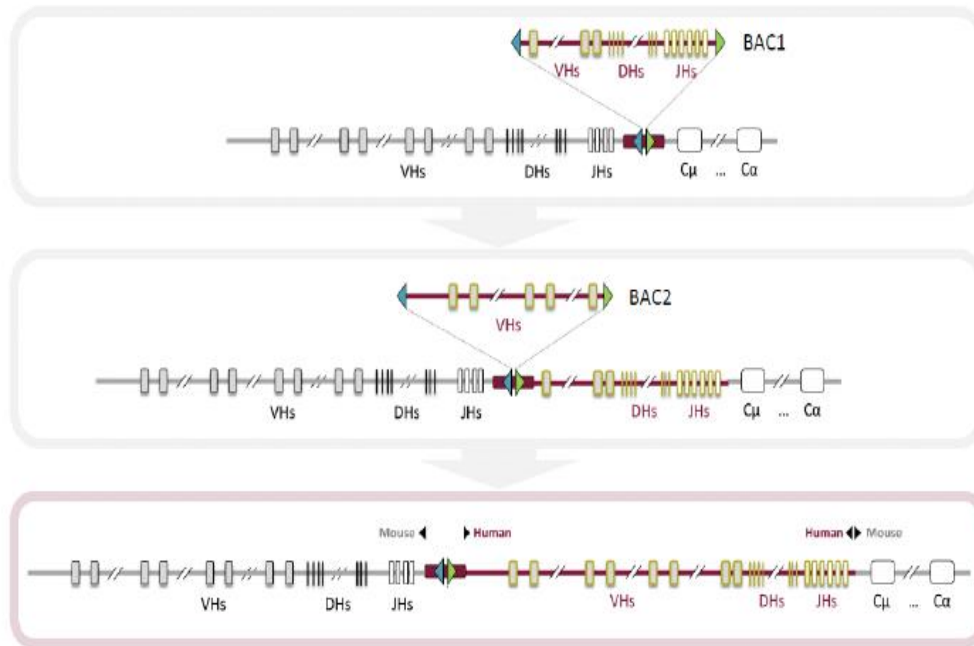
187. Three strains of mice which have already been manufactured are alleged to infringe: HK, HL and HKL. In HK, the IgH and IgK loci have been modified; in HL, the IgH and IgL loci have been modified; in HKL all three loci have been modified. The mouse IgH locus is modified by insertion of part or all of the human IgH variable region between the mouse variable region and the mouse C region, followed by an inversion of the mouse variable region, which is displaced upstream. The mouse constant region is retained. The modification to the mouse IgK locus is similar – an insertion of part or all of the human IgK variable region between the mouse variable region and the mouse C region, followed by an inversion of the mouse variable region which is displaced upstream.

188. The modification to the mouse IgL locus is different – all or part of the human IgL variable locus and the human IgL constant regions have been inserted downstream of the mouse IgL locus (which has not been inverted).

189. These modifications (in the form where the whole human loci have been inserted) are shown below (figure taken from page 3 of the PPD):



190. Kymab inserts human sequence into the mouse locus using a technique called sequential RMCE, which it claims to have devised. This involves the introduction of a series of so-called "landing pads" into which human sequence is inserted using a series of BACs. The following figure (figure 2 from the PPD) shows the process for the heavy chain locus:



191. The process used by Kymab is not alleged to infringe claim 1 of the 287 Patent as it does not involve the use of LTVECs, nor the use of an MOA assay.

192. The infringement case is set out at [289] of Regeneron's Opening skeleton (which is somewhat narrower than the pleadings). Infringement is alleged of the following claims:

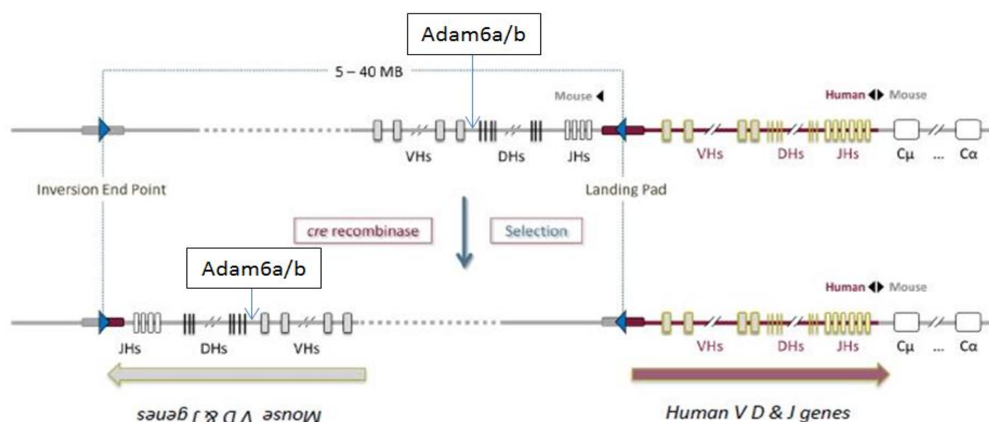
i) Product claims 5 and 6 of the 287 Patent. Regeneron claims that every strain of Kymouse is and was generated from a product within the scope of those claims;

ii) Product claim 1 of the 163 Patent. Regeneron's case is that Kymouse strains having both modified IgH and IgK loci (namely, the HK and HKL strains) are mice within the scope of claim 1; and

iii) Process claims 2 and 3 of the 163 Patent. However, these claims stand or fall with claim 1 of 163, both in respect of infringement and validity. Neither party addressed them separately in their closing speeches, and no separate issue arises in respect of these claims which I am required to determine.

Inversion and displacement upstream of mouse sequences

193. Figure 3 of the PPD shows an inversion of mouse sequence upstream of inserted human sequence in the IgH locus:



194. The effect is not only to reverse the order of the mouse VDJ sequences, but also to move these inverted sequences many mega-bases upstream of their original position. Dr Friedrich explained in cross-examination that for the IgH locus the mouse VDJ sequence is moved upstream such that the (now) most proximal mouse V is 3.8 Mb away from the 5' distal human V. The PPD (Figure 1) shows that the IgK locus will be moved even further away, approximately another 20 Mb upstream.

Infringement of the claims in issue

195. Kymab's primary submission in support of non-infringement of all claims is based on its construction of "*in situ* replacement". Because, in all strains of the Kymab mice, the endogenous Ig sequence has not been deleted, it alleges that none of the claims are infringed. As I have rejected this construction of "*in situ* replacement", I do not accept this submission.

196. However, Kymab denies that the inversion and displacement of the mouse V genes causes them to be substantially inactivated. It submits that leaving the mouse V genes in the genome, in an inverted state, is a trade-off. Prof. Howard's opinion in his oral evidence was that, unless they were moved to a distant location, the mouse V genes could still be used to some extent. This usage is undesirable. However, Kymab claims that there is a counter-balancing advantage in that the function of interstitial, non-Ig, genes that are located within the locus (for instance the Adam 6a/b genes which are involved in male fertility) is retained. If the locus had been deleted, so too would have the interstitial genes. During re-examination, Dr Friedrich suggested that Kymab accepted the possibility of a certain background level of fully mouse antibodies in the product, in order to maintain the function of any known or unknown interstitial genes.

197. I reject Kymab's submission, for the following reasons: First, I have not been referred to any part of the PPD, the expert reports, Dr Friedrich's statement, or the cross-examination of Regeneron's witnesses, which sets out the relevant facts on which Kymab now seeks to rely. As it is advanced as a substantive defence to infringement, it should at least have been put to Regeneron's witnesses.

198. Secondly, Kymab's own publication indicates that the inverted, displaced locus is functionally inactivated. Kymab's paper "Complete humanisation of the mouse

immunoglobulin loci enables efficient antibody discovery" published in *Nature Biotechnology* (2014) , 32.4; 356-363 (L5/97) explains that prior to inversion, some usage of the retained mouse V segments was observed. It then explains how inversion and displacement was utilised to prevent that effect from occurring: This is shown in figure 2a and 2b and explained on page 3:

> "We analyzed the species origin of V, D and J segment usage in mRNA transcripts of antibody genes in mice with human BACs inserted via S-RMCE. No mouse D or J segments were found in heavy-chain or kappa light-chain transcripts. However, we detected both human and mouse V-segment sequences in IGH and IGK transcripts. The frequency of mouse V-segment usage diminished as more BACs were inserted (Fig. 2a). Nevertheless, to prevent mouse V-segments from being used to generate antibodies in the transgenic mice, we created inversions in the heavy-chain and kappa light-chain loci. The inversions moved the entire mouse variable gene clusters several megabases away on the same chromosome and inverted them relative to the mouse constant regions."

199. Thirdly, Dr Friedrich explained in cross-examination that "Our intention for inversion was to silence or reduce the use of mouse Vs". This is consistent with the Kymab *Nature Biotechnology* paper referred to above. Assessment of infringement is, of course, objective. However, it is relevant that the technical purpose of inversion and displacement was to functionally inactivate the retained mouse V segments.

200. Finally, Kymab contends that all strains of the Kymab mice contained more than 300 kb of orthologous sequence, and that a product is not "obtainable by" the method of claim 1 if it contains more orthologous sequence than could have been carried on a single BAC. However, I have concluded that if a process has been repeated on a number of occasions, so that more than 300 kb of orthologous sequence have been inserted, the resulting product still falls within claims 5 and 6. In this regard, there is no evidence that each insertion by Kymab is of an orthologous sequence greater than 300 kb. On the contrary, Figure 7 of the PPD identifies BAC 1 as 167 kb. Furthermore, Figure 2 of Kymab's *Nature Biotechnology* paper indicates that all of the inserted human BACs are considerably less than 300 kb.

201. For these reasons, and based on my construction of the claims in issue, I conclude that all strains of Kymouse mice described in the PPDs were or would be generated from a product within the scope of claims 5 and 6 of the 287 Patent; and that Kymouse strains having both modified IgH and IgK loci (namely, the HK and HKL strains) are mice within the scope of claim 1 of the 163 Patent.

Sufficiency

Legal principles

202. Both parties referred me to the comprehensive summary of the law of "classical insufficiency" and "excessive claim breadth" set out by Arnold J in *Sandvik v Kennametal* [2012] RPC 23 at [106]-[124]. I shall apply those principles. I set out below certain aspects of the law which are of particular relevance to the present case.

Ordinary methods of trial and error/undue burden

203. In *Mentor Corp v Hollister Inc* [1993] RPC 7 at [14] the Court of Appeal approved the following statement by Aldous J at first instance, which still represents the law:

> "The section requires the skilled man to be able to perform the invention, but does not lay down the limits as to the time and energy that the skilled man must spend seeking to perform the invention before it is insufficient. Clearly there must be a limit. The sub-section, by using the words clearly enough and completely enough, contemplates that patent specifications need not set out every detail necessary for performance, but can leave the skilled man to use his skill to perform the invention. In so doing he must seek success. He should not be required to carry out any prolonged research, enquiry or experiment. He may need to carry out the ordinary methods of trial and error, which involve no inventive step and generally are necessary in applying the particular discovery to produce a practical result. In each case, it is a question of fact, depending on the nature of the invention, as to whether the steps needed to perform the invention are ordinary steps of trial and error which a skilled man would realise would be necessary and normal to produce a practical result."

204. This also represents the settled approach of the Technical Board of Appeal of the EPO, as expressed in *T 226/85 Unilever/stable bleaches* at [8] and referred to by the Court of Appeal in *Mentor v Hollister* (supra):

> "Even though a reasonable amount of trial and error is permissible when it comes to the sufficiency of disclosure in an unexplored field or – as in this case – where there are many technical difficulties, there must then be available adequate instructions in the specification or on the basis of common general knowledge which would lead the skilled person necessarily and directly towards success through the evaluation of initial failures or through an acceptable statistical expectation rate in case of random experiments."

205. I derive the following from these passages:

i) The patent need not set out every detail necessary for performance of the invention, as the skilled person applies his common general knowledge.

ii) The skilled person is seeking success when trying to perform the invention.

iii) There is a balance to be struck between ordinary steps of trial and error on the one hand, and prolonged research, enquiry or experiment on the other.

iv) Ordinary methods of trial and error involve no inventive step.

v) The enquiry is fact-sensitive and depends on the nature of the invention.

206. This latter point was emphasised by Pumfrey J (as he then was) in *Halliburton Energy Services Inc v Smith International (North Sea) Ltd and others* in a passage approved by Jacob LJ [2006] EWHC Civ 1715 at [13]:

> "All the same, one must be on one's guard against formulations that gloss the statutory requirement as there is always a risk that they will end up being substituted for it. This is a particular risk where the subject of the specification is very complex and its development would anyway be expected to be accompanied by a great amount of work. What is 'prolonged' in this context? It is always necessary to keep a balance between the interests of the public and the interests of the patentees in the sense that it is necessary to guard against imposing too high a standard of disclosure merely because the subject matter is inherently complex."

207. The subject matter of the 287 Patent is highly complex and a significant amount of work would be expected to be required in order to develop it. The policy of encouraging innovation in highly technical fields needs to be balanced against the importance of guarding against patents which require invention on the part of the skilled person to implement and where the scope of claims exceeds the technical contribution.

Excessive claim breadth

208. The legal principles are important to the result in the present case, and so I will consider them in some detail. The following passage from the judgment of Arnold J. in *Sandvik v Kennametal* (supra) sets out the relevant principles:

> "122. The main points which I drew from Lord Hoffmann's opinion in *Biogen v Medeva* were as follows:
>
> i) A claim will be invalid for insufficiency if the breadth of the claim exceeds the technical contribution to the art made by the invention. It follows that it is not necessarily enough to disclose one way of performing the invention in the specification.
>
> ii) The breadth of the claim will exceed the technical contribution if the claim covers ways of achieving the desired result which owe nothing to the patent or any principle it discloses. Two classes of this are where the patent claims results which it does not enable, such as making a wider class of products when it enables only one and discloses no principle to enable the others to be made, and where the patent claims every way of achieving a result when it enables only one way and it is possible to envisage other ways of achieving that result which make no use of the invention.
>
> iii) The patent in *Biogen v Medeva* was invalid because it was an example of the second class of objectionable claim.

> 123. The key point which emerges from Lord Hoffmann's opinion in *Kirin-Amgen v Hoechst* is his explanation at [112] of what he had meant by "a principle of general application" in *Biogen v Medeva*:
>
> "In my opinion there is nothing difficult or mysterious about it. It simply means an element of the claim which is stated in general terms. Such a claim is sufficiently enabled if one can reasonably expect the invention to work with anything which falls within the general term."
>
> 124. I summarised the reasoning of the House in *Generics v Lundbeck* as follows:
>
> i) The House agreed with Lord Hoffmann in *Biogen v Medeva* that it was important for United Kingdom patent law to be aligned, so far as possible, with the jurisprudence of the EPO. Furthermore, the House also agreed with Lord Hoffmann that the statement of principle which he quoted from *Exxon/Fuel oils* correctly stated the law.
>
> ii) The House considered that the instant case was to be distinguished from *Biogen v Medeva* because it was concerned with a claim to a single chemical compound whereas *Biogen v Medeva* concerned a product-by-process claim of broad scope.
>
> iii) It was a mistake to equate the technical contribution of the claim with its inventive concept. In the instant case, the technical contribution made by claims 1 and 3 was the product, and not the process by which it was made, even though the inventive step lay in finding a way to make the product. It followed that the breadth of the claim did not exceed the technical contribution which the invention made to the art."

209. Mr Turner asked me to consider the example of a claim to an aerofoil of a particular shape, which could potentially be used in many different types of aircraft. He submitted that it did not have to work in every type of aircraft in order for the patent to be sufficient. If the claim is to a single product, without limitation as to its use, then it is enough to enable one way of making the product. If, on the other hand, the claim is to a class of products, that class of products is enabled only if the skilled man can work the invention in respect of all members of the class (*Lundbeck v Generics* [2008] RPC 19 per Lord Hoffmann at para 34).

210. The principle is expressed in *Terrell on the Law of Patents* (17th edition) at paragraph 13-11 as follows:

> "It is therefore now settled law that the disclosure must be sufficient to enable the whole width of the claimed invention to be performed … The EPO applies the same principle that all members of a claimed class must be enabled …

> The principle was elegantly illustrated in Nokia v IPCom where the claim required "coarse frequency synchronization at least if the accuracy of the carrier frequencies is not adequate"; the patentee contended that if the former feature could not be made to work it did not matter because adequate accurate oscillators were available. The contention was rejected, because:
>
> "It is not permissible in law expressly to claim product features or process steps which are not enabled. … The claim can be thought of as split notionally into two parts: one where the coarse frequency step is not needed and the other where it is. If the part where coarse frequency synchronisation is needed is not enabled, the claim will be insufficient."
>
> Accordingly, in order to be sufficient, the description must enable the invention to be performed across the full width of the claim."

211. As discussed in Terrell, it is the settled approach of the TBA to require that the whole subject matter defined in the claims must be capable of being performed without undue burden and without invention. This appears from the following decisions, cited by Jacob LJ in *Novartis AG v Johnson & Johnson Medical Ltd* [2010] EWCA Civ 1036 at [72]-[77]:

> "…the requirement of sufficient disclosure can only mean that the whole subject-matter that is defined in the claims, and not only a part of it, must be capable of being carried out by the skilled person without the burden of an undue amount of experimentation or the application of inventive ingenuity." (*T 435/91 Unilever / detergents* at [2.2])
>
> "…claims may not be considered allowable if they encompass subject-matter which in the light of the disclosure provided by the description can only be performed with undue burden or with application of inventive skill." (*T 694/92 Mycogen / modifying plant cells* at [5])
>
> "Even though a reasonable amount of trial and error is permissible when it comes to assessing sufficiency of disclosure, there must still be adequate instructions in the specification, or on the basis of common general knowledge, leading the skilled person necessarily and directly towards success, through evaluation of initial failures. … the principle underlying Article 83 [is] that the skilled person should be given sufficient guidance for performing the invention without undue burden over the whole range claimed." (*T 1743/06 Ineos / amorphous silica* at [1.9]-[1.10])

212. Jacob LJ encapsulated this principle in *Novartis* at [74]:

> "The heart of the test is: "Can the skilled person readily perform the invention over the whole area claimed without undue burden and without needing inventive skill?""

213. In *Regeneron Pharmaceuticals Inc v Genentech Inc* [2013] EWCA Civ 93 at [100]-[101] Kitchin LJ said, when considering plausibility in the context of sufficiency:

> "It must therefore be possible to make a reasonable prediction the invention will work with substantially everything falling within the scope of the claim or, put another way, the assertion that the invention will work across the scope of the claim must be plausible or credible. The products and methods within the claim are then tied together by a unifying characteristic or a common principle. If it is possible to make such a prediction then it cannot be said the claim is insufficient simply because the patentee has not demonstrated the invention works in every case.
>
> On the other hand, if it is not possible to make such a prediction or if it is shown the prediction is wrong and the invention does not work with substantially all the products or methods falling within the scope of the claim then the scope of the monopoly will exceed the technical contribution the patentee has made to the art and the claim will be insufficient. It may also be invalid for obviousness, there being no invention in simply providing a class of products or methods which have no technically useful properties or purpose."

214. That passage emphasises the fact that if the invention does not work with substantially all of the products or methods falling within the scope of the claim then the claim will be insufficient. As Kitchin LJ said at [96] of *Regeneron*:

> "It is now well established that the scope of the monopoly, as defined in the claims, must correspond to the technical contribution the patentee has made to the art. An aspect of this requirement is that the specification must enable the invention to be performed to the full extent of the monopoly claimed."

Insufficiency: the facts

215. Mr Tappin submits that the teaching of the patents does not enable the skilled person to perform the claimed inventions over the whole area claimed without undue burden or the exercise of inventive skill. I shall consider first the objection of excessive claim breadth in respect of claim 1 of the 287 Patent.

Claim 1 of 287 – breadth of claim

216. As discussed above, the first approach of the 287 Patent at [0125]-[0129] discloses: homologous recombination to make a replacement of mouse sequence with human sequence at the proximal end of the variable gene IgH locus [0125]; a similar replacement by homologous recombination at the distal end [0126]; and dealing with

the remaining, intervening, mouse V segments and the introduction of further human V gene segments by one or more of Cre-mediated deletion [0127], RMCE [0128] or TAMERE [0129].

217. I have concluded that:

i) the minimum replacement by LTVEC1 that is described in [0125] is a deletion of 100 kb of mouse sequence (being the DJ region, which is flanked by the inner ends of the arms as described in [0125]) and an insertion of 200-300 kb of human sequence (as shown in Fig.4);

ii) the minimum replacement that is required by claim 1 of the 287 patent involves a larger deletion (150 kb) but a smaller insertion (75 kb); and

iii) The claim also includes the case where the mouse sequence is displaced and deactivated, but it is not limited to exclude deletion of the mouse sequence, which is specifically described in the specification.

218. In my judgment, it was clear from the evidence of Profs. Evans and Stewart that insertions and deletions of this size could not be performed without undue burden in 2001/2, and that the likelihood was that neither of the insertions and deletions referred to in (i)-(ii) above would have worked.

219. In particular, Prof. Evans agreed that insertion of 75 kb by homologous recombination was considerably larger than what had been achieved by 2001/2. As to deletion, he agreed that there is no publication even now of a deletion by homologous recombination of 100 kb or more of the mouse genome. It follows that a replacement of 200-300 kb of human sequence for 100 kb of mouse (or of 75 kb human for 150 kb mouse) was considerably larger than any that had been achieved by homologous recombination at the priority date.

220. Mr Tappin submits, and I agree, that the 287 Patent instructs the skilled person to perform a replacement that combines an unprecedented insertion with an unprecedented deletion. That is the task set for a skilled person who would not have had any experience of using BACs as targeting vectors. Given that the Patent discloses new techniques to enable larger insertions and deletions than had hitherto been achieved, the fact that it was unprecedented does not necessarily mean that it could not be performed without undue burden. However, as Prof. Evans agreed, it is highly ambitious. Prof. Evans also agreed that there was nothing to make it credible that either of these replacements by homologous recombination would work, and he said it would be too strong to call the patents' techniques 'feasible'.

221. Prof. Evans fairly acknowledged that the likelihood was that neither the replacement of 100 kb of mouse sequence with 200-300 kb of human sequence, nor the replacement of 150 kb mouse with 75 kb human, would have worked in 2001/2 (T4/604/2-605/9). In my judgment he was right to do so. Prof. Stewart's evidence, which I accept, was that the '100 kb out, 200-300 kb in' / '150 kb out, 75 kb in' replacements by homologous recombination are not technically feasible even today, and that they would certainly not have been seen as feasible at the priority date (Stewart 1 [147], 2 [55], 3 [12]).

222. Since both sides' experts agree (i) that neither of these replacements would have been considered feasible in 2001/2 and (ii) the likelihood is that neither would have worked, Kymab submits that the death knell has sounded for the first approach of Example 3.

223. The same applies to the minimum replacement by deletion and insertion required by claim 1 of the 287 Patent. A '150 kb out, 75 kb in' replacement was not enabled. Even though the claim is not limited to deletion as well as insertion, it is still necessary to enable this without undue burden, since on any view it is within the scope of the claim.

Regeneron's work

224. In my view, Prof. Stewart's opinion is corroborated by Regeneron's own attempts to put the 287 Patent into practice. When considering this area of the case I bear in mind that this is, inherently, a very difficult art in which trial and error, and perseverance following initial failure, should be expected. Nonetheless, Regeneron's internal history confirms that deletions and insertions of the sizes referred to above were not technically feasible at the priority date, and could not have been achieved without undue burden.

225. In summary, Regeneron's "VelociGene" work is described, in early form, in examples 1 and 2 of the Patent, which enables the creation of longer homology arms. During the course of the "VelocImmune Mouse" project described by Dr Murphy in his evidence, Regeneron gained considerable experience, which the skilled team would have lacked at the priority date, in the use of its VelociGene technology. Furthermore, it had available to it during this project the MOA assay, which David Valenzuela had conceived in about September 1999.

226. Regeneron began developing VelocImmune mice in early 2000. Dr Murphy explained that before the start of the VelocImmune project, Lynn Macdonald of Regeneron had acquired a great deal of experience with bacterial homologous recombination as well as BAC library screening and the construction of BAC-based targeting vectors. As recorded in the "VelocImmune plans" presentation that was made to the chairman of the Regeneron board on 28 April 2002 [DX-AM/21/2], the VelociGene experience up to that point had involved a BAC targeting vector that made an average deletion of 8 kb and used a replacement cassette of 4.5 kb, very considerably smaller than the size of deletions and insertions contemplated in the 287 Patent.

227. Dr Murphy gave evidence about the VelocImmune History document which he had prepared in order to explain his contribution to the invention. He explained that it was a document prepared from memory, but following his cross-examination, I do not consider that it is materially inaccurate. Dr Murphy discussed three ideas in that document for replacing the variable regions of the Ig loci. His second method essentially reflects the first approach of Example 3 of the 287 Patent. Regeneron itself was doubtful about whether this approach was feasible. Dr Murphy commented in the VelocImmune History Document:

> "Construct BACs with human inserts, selection cassettes and mouse homology boxes. The initial idea … was to do three

> steps of recombination in ES cells to sequentially replace the proximal, distal and middle third of the IgH VDJ region using very large hybrid BACs. This method required the capability to stitch together BACs to add the long mouse homology arms onto human BAC segments and to combine multiple human inserts – typically BACs contain 100-200 kb of sequence with ends "randomly" located. This capability had not been established at the time. In addition, we were not sure that even with long (>20 kb, but up to >100 kb) mouse homology arms we could insert long sequences (100s of kb) delete long sequences (100s of kb) in ES cells."

228. Furthermore, Regeneron did not try to perform a deletion and insertion by homologous recombination of anything like the size of sequences proposed in the 287 Patent. After several failures, it managed to delete 16.6 kb of mouse sequence and replace it with an insertion of about 144 kb of human sequence. Kymab submits that even this was done using improvements which were not disclosed in the 287 Patent, and which would not have been obvious to the person skilled in the art. I will consider whether this submission is correct later in this judgment. However, in my view, this history confirms that replacements on the scale of '150 kb out / 75 kb in' or '100 kb out / 200-300 kb' could not have been performed, and certainly not have been performed without undue burden by the skilled team, by homologous recombination, at the priority date.

The second proposed approach of Example 3

229. As discussed above, the second proposed approach relies on RMCE. I will deal with it briefly, since, in my judgment, the experts were agreed that it would not have worked. The difficulty with this proposed method would have arisen when trying to perform the RMCE in ES cells, to exchange the mouse locus for the BAC-borne human sequence (as shown in fig.13 of Stewart 1). The 287 Patent discloses that the whole 3 Mb should be replaced in one step by RMCE. Prof. Stewart explained why this would not work in his second report at [39]-[46] and I accept his evidence. Prof. Evans confirmed that it was right to conclude from his first report at [120] that he did not think that this was a feasible approach.

The importance of Example 3

230. So far, I have concluded that neither of the approaches of Example 3 would have enabled the skilled person to perform deletions and insertions of mouse and human sequences on the scale disclosed in Example 3 and included within the scope of the claims in issue. Furthermore, the deletions and insertions are of unprecedented size and no other method of achieving this is disclosed in the description.

231. Kymab submits that this must lead to a finding of insufficiency. Essentially, it argues that where a patent sets out a protocol for performing an ambitious and unprecedented task, and gives no hint that it should be departed from, then the most that the unimaginative skilled team could do is to perform routine trials to put it into practice. It could not develop a new protocol if the two methods in the patent fail, as that either constitutes an undue burden or requires invention.

232. Whilst I consider that there is much force in this submission, it is, in my judgment, too generalised. The skilled team is entitled to apply its common general knowledge, in the event of failure of the methods disclosed by the 287 Patent, when trying to achieve success in putting the invention into practice. If an obvious, standard approach would occur to it, which would necessarily and directly lead to success, then, even though not disclosed in the Patent, this would be an answer to the objection of insufficiency. I will therefore consider the various alternative approaches that have been discussed during the trial. I agree with Kymab, however, that the fact that neither of the proposed methods of Example 3 would enable the claimed method to be performed across the full width of the claim without undue burden, is not a promising start for the patentee.

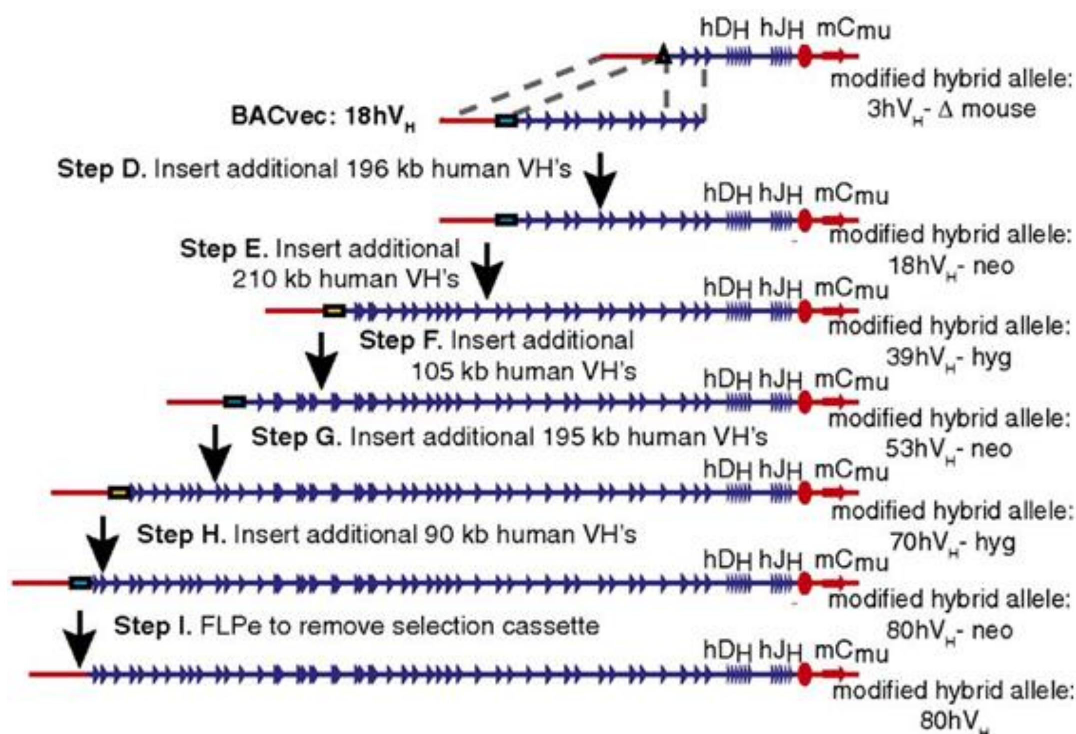
The Macdonald paper

233. In 2014 Lynn Macdonald and others (including Dr Yancopoulos and Dr Murphy) published a paper in *Proceedings of the National Academy of Sciences* (2014), 111; 5147 describing Regeneron's work in replacing six megabases of mice immune genes in a precise manner and *in situ* (in the orthologous position) with the corresponding human immune genes (L5/99).

234. This was developed from an idea of Dr Murphy, conceived in mid-2003 and described in the VelocImmune History document:

> "we eventually were able to target the distal end of the mouse IgH locus and delete the 3 megabases of mouse sequence in between as we had predicted. At around this point (I think), I had the idea of doing sequential targeting using overlapping human sequences (from overlapping BACs) and repeatedly using the same distal mouse arm, but alternating selection (neoR hygS to neoS hygR etc)."

235. This approach is shown, for example, in Fig. 1C of Macdonald, for the heavy chain:



236. Prof. Stewart explained that the Macdonald approach of step-wise addition of overlapping human sequence is not disclosed in the 287 Patent and is, in his view, highly inventive. It employs a series of homologous recombination steps, each of which uses the same mouse homology arm, which was 20 kb long, at the distal end. Specifically, the upstream homology arm from step B (the insertion of the distal *lox* site) is used again as the red mouse homology arm in each of steps D, E, F, G, and H. This becomes possible only upon deletion of the mouse variable gene locus. The proximal end of each targeting construct is different in each step, being derived from the end of the human BAC which overlaps the distal end of the human sequence inserted by the previous step. This involves alternating selection markers and using the MOA assay for the loss of the previous selection marker.

237. Prof. Stewart described the Macdonald paper in the following terms:

> "an original, and elegant approach…one that I had not previously come across before reading Macdonald" (Stewart 2 [14](d));"
>
> " a beautiful piece of science…an outstanding solution to the problem" [T5/715$_{6-12}$]"
>
> " I think you are doing Drew Murphy a great disservice by saying that is pretty all obvious. The solution he came up with I think is elegant. It was a really outstanding piece of thinking and very creative." [T6/913$_{14-18}$]"

238. This evidence accords with Prof. Stewart's assessment when he reviewed the Macdonald paper for purposes unrelated to his evidence in this case. I accept his evidence and I conclude that the Macdonald approach is not one that would have occurred to the unimaginative skilled person.

Multiple insertions without deletion

239. During the course of cross-examination of Prof. Stewart, Mr Turner handed up a simple drawing which, he suggested, showed an obvious approach that the skilled team could adopt, if it found that the amount of human sequence that it was attempting to insert was too large. He suggested the use of a targeting vector to make a modest insertion by homologous recombination at the proximal end of the locus. On this approach the insertion (without deletion) is of a section of human sequence that is as small as it needs to be in order to be achievable. Following that, the suggestion is to use a series of further sequential insertions by homologous recombination, working along from the proximal and towards the distal end. Again, the idea is to make each step small enough so that it can be achieved on its own.

240. I do not accept that this approach would occur to the unimaginative skilled person when trying to perform the invention of the 287 Patent. Such an approach occurred to Prof. Evans when he was asked, during preparation of his expert reports, "to say how it could be done by shunting the mouse up and never deleting them". However, he did not put this suggestion into his expert reports because he considered that such a scheme was inventive and not something that the skilled person would have done. When explaining why this had not been included in any of his reports, he said:

> "Well, that was my personal take. That was using my inventiveness, if you like. I do not want to say that the person skilled in the art would have chosen any other way of doing this."

241. He confirmed this later in his cross-examination (Evans XX [T7/1013]):

> Mr. Tappin: "As I understood it, what this diagram [X2/35] was being suggested for was the idea of doing sequential insertions.
>
> A. I think it may have been, but it is a very useful diagram for this ----
>
> Q. And of course sequential insertions were what you were being asked about at the outset and which you did not put in your report because you said that was not what the skilled person would have done.
>
> A. That is right. So shunting it up, as I think we have heard."

242. It is entirely understandable why Prof. Evans considered that this approach would not have occurred to the skilled person when seeking to implement the 287 Patent. As discussed above, the 287 Patent discloses homologous recombination to make a replacement of mouse sequence with human sequence at the proximal end of the variable gene IgH locus [0125]; a similar replacement by homologous recombination at the distal end [0126]; and dealing with the remaining, intervening, mouse V segments and the introduction of further human V gene segments by one or more of Cre-mediated deletion [0127], RMCE [0128] or TAMERE [0129]. This is entirely different from an approach which employs small sequential insertions building up from the proximal end, and therefore effectively ignores the distal end. In agreement

with Prof. Evans, Prof. Stewart did not think that this was an obvious approach. He regarded it as a simplified illustration of Regeneron's work as described in the MacDonald paper, which, far from being routine, he considered to be "hugely impressive" [T6/923$_{4-20}$]. I accept Prof. Stewart's evidence on this issue.

243. Furthermore, Mr Tappin submitted, and I accept, that the drawing at [X2/35] is deceptively simple, in that its basic nature omits all complexities that the skilled person would be likely to have encountered, even if this approach had been obvious when implementing the 287 Patent, which it was not. In his first report, Prof. Evans considered an alternative way of using homologous recombination to that disclosed in the 287 Patent, in order to carry out its invention. This was explained at [10]-[11] and Figures 31-34 of Annex 2 to that report. That proposal involves doubling the number of ES cell homologous recombination steps, by interposing a step in between each of the steps that introduces human sequence. The purpose of the extra steps is to recycle a junction point that can be used for MOA and to check for integration using a negative selection marker (Evans XX [T7/1036$_{24}$-1037$_{12}$]). Neither Prof. Evans nor Prof. Stewart considered that this scheme is one that would have occurred to the skilled person (Evans XX [T7/1038$_{18-23}$]; Stewart 2 [71]). It is far more complex than the drawing at [X2/35] and shows, in my view, that the latter is an oversimplification.

Repetition of the homologous recombination process

244. Regeneron contends that if faced with a difficulty in deleting, for example, 150 kb of mouse sequence and inserting 75 kb of human sequence, the skilled person would follow directions in the 287 Patent simply to perform a small deletion and insertion using homologous recombination, and then repeat a second step of homologous recombination using a second BAC and a third step using a third BAC etc. It contends that there is no minimum size of insertion, and that there is no impediment to doing the same thing more than once. It contends that this approach is expressly disclosed in the 287 Patent

245. In particular, [0036] concerns a preferred embodiment which differs from the claims as amended in the TBA, in that it contains no limitation in respect of V, D and J gene segments:

> "Yet another preferred embodiment is a method of replacing, in whole or in part, in a mouse embryonic stem cell, an endogenous immunoglobulin variable region gene locus with its homologous or orthologous human gene locus comprising:
>
> a) obtaining a large cloned genomic fragment containing, in whole or in part, the homologous or orthologous human gene locus;
>
> b) using bacterial homologous recombination to genetically modify the large cloned genomic fragment of (a) to create a large targeting vector for use in the embryonic stem cells;
>
> c) introducing the large targeting vector of (b) into mouse embryonic stem cells to replace, in whole or in part, the

> endogenous immunoglobulin variable gene locus in the cells; and
>
> d) using a quantitative PCR assay to detect modification of allele (MOA) in the mouse embryonic stem cells of (d) to identify those mouse embryonic stem cells in which the endogenous variable gene locus has been replaced, in whole or in part, with the homologous or orthologous human gene locus."

246. [0037] discloses another embodiment which adds steps (e)-(h) to the method of [0036]:

> "In another embodiment, the method further comprises:
>
> e) obtaining a large cloned genomic fragment containing a part of the homologous or orthologous human gene locus that differs from the fragment of (a);
>
> f) using bacterial homologous recombination to genetically modify the cloned genomic fragment of (e) to create a large targeting vector for use in the embryonic stem cells;
>
> g) introducing the large targeting vector of (f) into the mouse embryonic stem cells identified in step (d) to replace, in whole or in part, the endogenous immunoglobulin variable gene locus; and
>
> h) using a quantitative assay to detect modification of allele (MOA) in the mouse embryonic stem cells of (g) to identify those mouse embryonic stem cells in which the endogenous immunoglobulin variable region gene locus has been replaced, in whole or in part, with the homologous or orthologous human gene locus."

247. [0038] contemplates the repetition of steps (e)-(h) in a further preferred embodiment:

> "Still another preferred embodiment is a method of wherein steps (e) through (h) above are repeated until the endogenous immunoglobulin variable region gene locus is replaced in whole with an homologous or orthologous human gene locus."

248. [0123] includes the following sentence. "One skilled in the art will recognise that replacement of the mouse locus with the human locus may be accomplished in one or more steps".

249. Relying on these passages, Regeneron submits that building up the replacement by repeated BAC insertions is specifically disclosed in the 287 Patent. Therefore, the skilled person could begin by inserting a BAC with a small amount of human sequence (say, for example 10 kb), and repeat this again and again until the claim was put into practice.

250. I reject this submission for the following reasons. First, I do not accept that repeated BAC insertions of, for example 10 kb would carry out the method of claim 1 of the 287 Patent. The claim requires at least one V segment (as well as D and J gene segments) to be replaced with at least one orthologous V human gene segment (as well as D and J segments) i.e. at least about 75 kb of human genomic sequence. Step (b) of claim 1 requires the creation of a large targeting vector for use in a mouse ES cell (LTVEC). Step (c) specifies that the method comprises:

> "introducing the LTVEC of (b) into a mouse ES cell to replace said V, D and J segments *in situ* with the orthologous human V, D and J gene segments."

251. Therefore, it is the introduction of the LTVEC (which is a single BAC) in step (c) which effects the *in situ* replacement of the V, D and J gene segments. Subsequent steps cannot be relied on as effecting the *in situ* replacement. Accordingly, the claim does contain a minimum requirement for insertion of at least 75 kb of human genomic sequence in step (c).

252. Secondly, even if the claim included the case where the minimum replacement was built up by a series of small BAC insertions, it is not limited to that case. It includes within its scope replacements in a single step of (a) '100 kb out, 200-300 kb in'; (b) '150 kb out, 75 kb in'. Therefore, the full extent of the claimed monopoly is not enabled.

253. Thirdly, Mr Tappin (in a short rejoinder speech) pointed out that until Regeneron's reply speech, [0036]-[0038] had barely been referred to. Prof. Evans did not suggest in his reports that the simple solution was to repeat small, repeated insertions of BACs. His proposed solution in Annex 2 of his first report was much more complex than that. Nor were [0036] –[0038] (as distinct from the drawing at X2/35 which I have considered above) suggested to Prof. Stewart during his cross-examination as a solution to difficulties in implementing the Patent.

254. Mr Tappin further submitted that [0036]–[0038] did not contemplate a replacement at the proximal end, followed by further, sequential insertions. Rather, they were generalised disclosures of the method explained in more detail at [0125]–[0126] where there is a targeted replacement at the proximal end, followed by targeted replacement at the distal end, followed by steps in the middle. Yet further, [0038] and [0123] said no more than that steps could be repeated to replace the whole of the mouse locus with the whole of the human locus. This was quite different from the series of small sequential BACs insertions now contemplated by Regeneron to achieve even the minimum single V (plus Ds and Js) replacement required by the claim.

255. This led to short post-trial written submissions on this issue by the parties, which I have carefully considered. I agree with Mr Tappin. I am not satisfied that either of the experts contemplated the repeated sequential BACs approach that was suggested by Regeneron in its reply speech. Paragraphs [0036] – [0038] are, in essence, consistory clauses, which are then described in more detail in the methods and materials section. I do not accept that they are disclosing a different method of a series of small sequential insertions. Accordingly, I prefer the interpretation of [0036] – [0038] and [0123] advanced by Kymab.

256. Fourthly, if the solution were as simple as is now suggested by Regeneron, then it would have been at the forefront of Regeneron's expert reports. It was not. The explanation advanced by Mr Tappin is that, again, this apparently simple solution ignores all the complications that the skilled person would have encountered in practice. I agree with this. It is corroborated by the great difficulties that Regeneron experienced when seeking to put the invention into practice, and by the ingenuity of the Regeneron approach disclosed in the MacDonald paper. If the solution had been to sequentially insert small amounts of human gene sequence on BACs, no ingenuity would have been required at all.

Assessment of insufficiency

257. For these reasons, I have concluded that the whole subject matter defined in the claim 1 of the 287 Patent was not capable of being performed at the priority date without undue burden and without invention. The difficulty does not relate to some hypothetical puzzle at the edge of the claim, but rather to the central disclosure of the specification, and the amounts of genetic sequence of which it contemplates the deletion and insertion. None of the methods of the 287 Patent for achieving this, as disclosed in Example 3 would have worked. The task contemplated was unprecedented and could not have been achieved, if at all, without a great deal of creative thinking at the priority date. I do not accept that all embodiments within the claim are unified by a single principle of a reverse chimeric locus. This is not a principle that enables the method to be performed, rather it is the result of successfully carrying out the method. Accordingly, the insufficiency objection succeeds in respect of claim 1 of the 287 Patent.

258. It follows that claims 5 and 6 of the 287 Patent and claim 1 of the 163 Patent are also invalid for insufficiency. I have concluded that they are of considerably wider scope than claim 1 of the 287 Patent. Even if I had concluded that claim 1 of the 287 Patent was not of excessive breadth, I would still have concluded, for the reasons set out above, that these wider claims were insufficient.

Further insufficiency objections

259. In the light of my conclusions, it is unnecessary for me to consider in detail the further insufficiency objections that were advanced on behalf of Kymab, only some of which were pursued by the end of the trial. Nonetheless, given that they were the subject of evidence and argument, I will set out my conclusions about the main points.

Effect of long homology arms

260. Kymab submits that the evidence was that long homology arms, and in particular the LTVECs disclosed in the 287 Patent, would make matters worse and would decrease the likelihood that homologous recombinations in ES cells on the scale contemplated in the 287 Patent would have worked. I do not accept this submission. The Patents describe a quantitative MOA assay which creates the possibility of using longer homology arms. This improves the frequency of homologous recombination events. Prof. Stewart had published this advantage, being an author named on the Valenzuela paper published in *Nature Biotechnology* (2003) 21; 447-451 [L5/86B]; he repeated this in Glaser *et al. Nature Genetics* 37.11 (2005); 1187-1193, citing the Valenzuela

paper (CX-AFS/14). When he was cross examined on this issue, Prof. Stewart accepted that longer homology arms were beneficial:

> "Q. But you accept that there are benefits to having long homology arms. There is nothing between us on that.
>
> A. Yes."

261. However, I do accept Kymab's submission that this improvement came nowhere near to enabling deletions and insertions on the scale which I have discussed above, and which are clearly within the scope of all the claims which I have addressed.

The reduced amount of DNA

262. Kymab submits that even the '16.6 kb out, 144 kb in' replacement, that was eventually achieved by Regeneron, used improvements made internally during development work, which are not disclosed in any of the patents. In particular, it argues that this limited success was achieved by targeting with the 3hVH construct by using the counterintuitive step of *reducing* the mass of DNA used during electroporation.

263. Kymab points out that the standard electroporation procedure for plasmids was 10-30 µg per 10^6-10^7 cells, according to the Sambrook textbook [CX-AFS/9/19]; Evans XX [T6/963_{10}-964_{23}]. It claims that Regeneron discovered an important way of obtaining a clear improvement to targeting efficiency, both in terms of increasing true positives and decreasing false positives. This was by lowering the amount of DNA used to electroporate ES cells. It refers to Example 5 of Regeneron's US Patent Application 10/415440 with an international filing date of 31st October 2001 [DX-AM1/4/37]:

> "Standard methods for targeted modification of genes in mouse embryonic stem (ES) cells typically employ 20 to 40 µg of targeting vector in the electroporation procedure. Applicants have discovered that with LTVECs, electroporation with much lower amounts of DNA—in the range of about 1 to 5 µg per $1x10^7$ cells—doubles the frequency of correctly targeted homologous recombination events while greatly reducing the number of secondary, non-homologous insertion events. This clear improvement in targeting efficiency is important because it significantly reduces the number of ES cells clones that need to be screened to find several positive clones with a correctly targeted, single-copy modification."

264. Kymab points out that this non-standard technique was used in VelocImmune, including in all the attempted integrations of the MAID1116 vector, which was used for the first step in Figure 1B of Macdonald 2014, where the vector is called 3hVH (Murphy 2 [51](b); Murphy XX [T2/219_{19}-221_{23}]). Those electroporations used 2 µg per 10^7 cells, i.e. within the advantageous range of Example 5. The same Example 5 is in Regeneron's equivalent granted EP 1 399 575 [DX-ME/11/15], and is claimed in claim 14.

265. In answer, Regeneron claims that this point was not pleaded and was not dealt with in the expert reports. It alleges that lowering the amount of DNA was standard laboratory practice. It claims it was never suggested to Prof. Evans that the result of using more DNA might be that the skilled person would not achieve the correctly integrated LTVEC.

266. I reject the pleading point, as difficulties in achieving deletions and insertions are clearly raised in the particulars to paragraph 3 of the Re-Amended Grounds of Invalidity. Whilst the solution of lowering the amount of DNA is not referred to, the general issue is clearly raised. I attach more weight to the objection that this issue was not raised in the expert reports, and the relevant patent application and patent first appeared in the cross-examination bundles. However, I did not sense that Prof. Evans had any difficulty with understanding the issue or dealing with the questions that were asked of him. Furthermore, the relevant cross-examination took place on Day 4, and Prof. Stewart was called on Day 5, and so there was an opportunity for Regeneron to cross-examine Prof. Stewart. However, I was not referred by Regeneron in its closing to cross-examination of Prof. Stewart on this issue.

267. So I must form a judgment based upon what Prof. Evans said. As with the rest of his evidence, his answers were clear, concise and entirely frank. His evidence on Example 5 was as follows:

> "Q. All right, but what they are saying here is that, despite the increased molecular weight of the LTVECs, they are using a very low amount of DNA.
>
> A. Yes, they do.
>
> Q. And they [say] that has two benefits. "[It] doubles the frequency of correctly targeted homologous recombination events while greatly reducing the number of secondary, non-homologous insertion events", and that is clear improvement of targeting efficiency. They do not tell us exactly what greatly reducing means, but obviously the overall benefit is more than double. Yes? So they are doubling the frequency of the correct event and greatly using [reducing] the number of others.
>
> A. Yes, it is more than double.
>
> Q. More than double. The targeting frequency is more than double. We do not know quite how much. What we know, professor, is that when Regeneron managed to get their 3VH targeting vector to target, they used a level of DNA, which of course the patent does not suggest you should use this special low level of DNA, does it?
>
> A. No.
>
> Q. And if they had used standard levels of DNA, we have no way of knowing that they would have been successful?

> A. No, we do not, but I think we should comment on this, my Lord, that this is a very interesting claim here. Once again I do not think this is a claim that would have been known about or available to the standard person skilled in the art."

268. I consider that Prof. Evans confirmed that use of this very low amount of DNA was not something that would have been known or considered by the unimaginative skilled person at the priority date, and that there is no way of knowing whether Regeneron would have been successful in deleting 16 kb and inserting 144 kb without it. Therefore I accept Kymab's submission that the '16.6 kb out, 144 kb in' replacement was achieved using an improvement not disclosed in the 287 Patent and which would not have occurred to the uninventive skilled person at the priority date. This is an additional reason in support of my conclusion that the claims of the patents with which I am concerned are insufficient.

MOA Improvements

269. Kymab submits that the use of two reference probes, as disclosed at [0091] of the 287 Patent, was found by Regeneron to be unreliable. It refers to the VelocImmune History Document, where Dr Murphy recorded that "initial attempts to get LOA to work for autosomal, two copy genes were dicey at best." Kymab claims that the MOA assay had to be considerably refined by the use of a primary MOA screen with four reference genes and a secondary MOA screen with two further reference genes (and also Neo and LacZ genes), which made the assay more reliable.

270. It submits that these refinements to the MOA screen were adopted in the VelocImmune project, and four reference genes were used in a primary screen by August 2001, and the materials & methods of the Valenzuela 2003 paper reflect the more refined assay. Further, the Macdonald paper confirms that the MOA assay used for the engineering of the mouse Ig loci which it describes (i.e. including the 3hVH targeting step) was as set out in the Valenzuela paper, i.e. using six reference genes; [Evans T7/1014$_4$-1015$_{14}$].

271. Thus, Kymab argues that Regeneron had a benefit, not available to the skilled person, when seeking to carry out homologous recombination, of having developed the MOA assay from one that was "dicey at best" to one that worked reliably.

272. Whilst there is force in Kymab's submission in this regard, I have concluded that the use of additional probes is the kind of routine development that the skilled team would make without inventiveness, in order to improve the reliability of the assay. Dr Murphy explained that the extra reference probes helped to prevent false positives (D2/213-214). The presence of false positives would require some further checking and elimination, but it was not established that their presence would have led to failure of the MOA, which would require the skilled person to miss the positives entirely (as may occur upon a false negative). Furthermore, Prof. Evans expected that the skilled person may well use multiple controls in any event (D4/598/12-16) and the worked example in the 287 Patent already demonstrates the use of two probes. It seems a routine step from this to increase the number of probes to improve reliability.

Identifying the 5' end

273. Kymab submits that the skilled person would not have been able to identify the 5' end of the murine V segments without undue burden. I reject this as a ground of insufficiency. In my view, the 5' end could have been identified by the standard technique of chromosome walking. BACs associated with the 5' end could have been identified by standard bioinformatics or biological approaches. Alternatively the skilled person could have searched GenBank for sequence information associated with the 5' end of the mouse IgH locus and screened a BAC library with that. I accept that none of this would have been easy at the priority date, but I do not accept that, in an art of this nature, it would have amounted to an undue burden or would have required anything other than routine work. This conclusion is, in my view, supported by the evidence of Prof. Evans, and by the initial reaction of Prof. Stewart, which I find to be more reliable than his subsequent view, influenced by what he had been told about Regeneron's internal difficulties.

Submissions following circulation of the draft judgment

274. After I had sent the draft judgment to the parties' legal representatives for correction of obvious errors, I received a further written submission on behalf of Regeneron, which alleged that there were certain material omissions from the draft judgment which I was asked to consider before handing it down ("Regeneron's post-judgment submissions'). It was suggested that I should consider these issues in order to provide further findings of fact for the Court of Appeal. Counsel for Regeneron drew attention to the guidance issued by the Court of Appeal in *Re M (A Child) (Non-accidental injury: burden of proof)* [2008] EWCA Civ 1261 at [36]-[40]. This explains that it is the duty of an advocate who considers that there is a material omission from the judgment to draw this to the attention of the judge so that he or she may deal with it.

275. Two issues are raised: First, Regeneron submits that, following the teaching in the Patents, the person skilled in the art could insert human DNA comprising at least one V segment, and D and J segments, of about 75 Kb. Secondly, Regeneron submits that the skilled person could perform additional insertion steps following the initial insertion of one V, Ds and Js.

276. I invited written submissions from Kymab and Novo in response. Mr Tappin submits that, by raising these points, Regeneron is seeking to re-open the argument about whether the claimed inventions are enabled, by claiming that the skilled person could make an initial insertion, with a small deletion, followed by further insertions to build up the locus. In my judgment, this is the effect of Regeneron's post-judgment submissions.

277. I have rejected all of the ways in which Regeneron argued at trial that the claim was enabled across its full width, based on the Macdonald approach (judgment [233]-[238]); multiple insertions without deletion based on X2/35 (judgment [239]-[243]); and repetition of the homologous recombination process based on [0036]-[0038] and [0123] of the 287 Patent (judgment [244]-[256]). This is another attempt to re-argue the same issue. Nonetheless I will address the substance of Regeneron's post-judgment submissions.

278. At [4] of its post-judgment submissions Regeneron claims that Kymab did not run a positive case that an insertion of 75kb with a small deletion was not enabled, and that any such submission would have been inconsistent with the evidence. I am asked to

consider Regeneron's closing submissions on this point at [294]-[299] and the alleged absence of a contrary argument from Kymab. I reject this contention. In fact, Kymab strongly disputed this issue at [371]-[374] of its written closing and in its oral closing at [T10 1672/21-1675/3]. For example, [374] of Kymab's closing explicitly addressed the feasibility of a replacement by a small deletion of 17 Kb and an insertion of 75 Kb:

> "Such a replacement involving a deletion of 17 kb and an insertion of 75 kb was put to Prof. Stewart (Stewart XX [T6/92417-9263]), on the assumption that the targeting construct could be made and everything else was working perfectly. He said that in 2001 he would have considered that it was very ambitious and would have had no idea if it would work. Naturally he agreed that today, it could be made to work – a fact that we only know from the Regeneron work and the Macdonald 2014 publication, achieved only with the application of VelociGene advances not described in the patents. The evidence does not support the making of such a replacement without undue burden, or indeed making it at all without the VelociGene advances – indeed, even with them, the targeting efficiency was only 0.2% (Macdonald Table 1)."

279. Furthermore, the evidence that an insertion could be made following a small deletion comes from Regeneron's success in achieving this using the 3hVH vector. In this judgment, I have accepted Kymab's case that this '16.6 kb out, 144 kb in' replacement, eventually made by Regeneron, was achieved using an improvement not disclosed in the 287 Patent, which would not have occurred to the uninventive skilled person at the priority date (judgment [262]-[268]). If this is to be challenged, it must be by way of an appeal.

280. At [5] of its post-judgment submissions, Regeneron cites certain passages from the cross-examination of Prof. Stewart. I have already considered Prof. Stewart's evidence on this issue, and the parties' submissions in their written and oral closings. In my judgment, this evidence did not establish that a '17 Kb out, 75 Kb in' replacement could have been achieved by the skilled person in 2001 using the methods disclosed in the patent or other standard techniques. I have reached the opposite conclusion, for the reasons set out in detail in this judgment.

281. This deals with the substance of the first finding that Regeneron invites me to make in its post-judgment submissions. I decline to do so.

282. As to the second issue, at [14] of Regeneron's post-judgment submissions, it requests me to confirm that I have not made a finding that the person skilled in the art having inserted human sequence into the murine locus (such as V, D and J sequences of 75 Kb) could not thereafter without undue effort insert further sequences at all. In the alternative, in the event that I have made (or do make) such a finding, I am requested to address Regeneron's submission that it is not open to the Defendants to take this point given that (allegedly) it did not plead this point or address it in evidence or cross-examine about it.

283. In its post-judgment submissions, Regeneron relies on [0123] of the 287 Patent. [0123] was a part of Regeneron's argument based on [0036]-[0038] of the 287 Patent, which was raised in Kymab's closing speech and its supplemental note submitted after conclusion of the trial, and in particular in the section headed "the locus could be built up in a series of steps." I have dealt with this at [244]-[256] of this judgment. In summary, I have rejected Regeneron's case on this issue.

284. As to the pleading point, paragraph 3 of the Re-Re-Amended Grounds of Invalidity raises excessive claim breadth and sub-paragraph (g) expressly pleads that "Example 3 of the 287 Patent purportedly enables the insertion and/or deletion of sequence lengths which were longer than could be inserted and/or deleted by the skilled person without undue burden." Regeneron attempted to answer this allegation in a number of ways, including a contention (which I have rejected) that after a small deletion, the locus could be built up in a series of steps without undue burden. The pleading raises the issue, and it is not required that it anticipates all possible responses to it.

285. Furthermore, Kymab draws attention to the way in which this allegation was raised by Regeneron. Regeneron argued that, even if the methods of Example 3 did not enable the inventions across the breadth of the claims, the locus could be built up by the techniques shown in the Macdonald paper, which was addressed by Prof. Stewart and was the subject of cross-examination of Prof. Evans. Alternatively, it was suggested that the locus could be built up in a series of steps, as shown in [X2/35]. This was not addressed in the expert reports as it emerged during the cross examination of Prof. Stewart and Prof. Evans was subsequently cross-examined about it. The case based on [0036]-[0038] and [0123] emerged in Regeneron's closing speech and so Kymab cannot be criticised for not addressing it in evidence. Indeed, Kymab submitted that Regeneron should not be permitted to rely on this argument as it was raised too late. Nonetheless, I considered it and rejected Regeneron's case at [244]-[256]. Specifically, I have rejected Regeneron's case that the locus could be built up in a series of steps without undue burden, and I do not consider it necessary to repeat or expand on these reasons.

286. In its post-judgment submissions at [13] Regeneron has relied on a short passage from the cross-examination of Prof. Stewart at T5/673/21-25. This was referred to by Regeneron at [41](c) of its written closing, and I considered it when reaching my conclusions on this issue. In this part of his cross-examination, Prof. Stewart was asked to forget about the disclosure of the 287 Patent and to assume that he had the Valenzuela paper in front of him. This was published in 2003 and its disclosure is different from that of the 287 Patent.

General observations about submissions on draft judgments

287. It is very important that Counsel should draw to the attention of the court any material omissions in a judgment, rather attempting to save up such points for the Court of Appeal. In *Re T (Contact: Alienation: Permission to Appeal)* [2002] EWCA Civ 1736; [2003] 1 FLR 531, Arden LJ said at [50]:

> "In a complex case, it might well be prudent, and certainly not out of place, for the judge, having handed down or delivered judgment, to ask the advocates whether there are any matters which he has not covered. Even if he does not do this, an

> advocate ought immediately, as a matter of courtesy at least, to draw the judge's attention to any material omission of which he is then aware or then believes exists. It is well-established that it is open to a judge to amend his judgment, if he thinks fit, at any time up to the drawing of the order. In many cases, the advocate ought to raise the matter with the judge in pursuance of his duty to assist the court to achieve the overriding objective (CPR 1.3, which does not as such apply to these proceedings); and in some cases, it may follow from the advocate's duty not to mislead the court that he should raise the matter rather than allow the order to be drawn. It would be unsatisfactory to use an omission by a judge to deal with a point in a judgment as grounds for an application for appeal if the matter has not been brought to the judge's attention when there was a ready opportunity so to do. Unnecessary costs and delay may result. I should make it clear that there are general observations for assistance in future cases, and that I make no criticisms of Counsel in this case."

288. I should make clear that I make no criticism of Counsel for Regeneron for complying with what they consider to be their obligations to the Court in drawing attention to what they perceive to be omissions in the judgment in a very complex case. However, as a general observation for assistance in future cases, it should be possible to do this very simply, as the omission will be obvious. I would hope that this will rarely be necessary, particularly where the parties have provided a list of issues on which judgment is required. Although in complex, high value cases, there is always a temptation to make just one more point after the trial has concluded, this should be resisted. The primary purpose of circulating a draft judgment is to allow typographical or other obvious errors to be corrected, and it is not an opportunity to re-argue the case. In *R (Mohamed) v Secretary of State for Foreign and Commonwealth Affairs (No 2)* [2011] Q.B. 218 Lord Judge CJ said at 315 [3]-[4]:

> "3…Just because any draft is a draft judgment the opportunity for correction is available, and from time to time it is taken, not only on the application of one of the parties, but also on the judge's personal initiative if, on re-reading his draft, he thinks it appropriate to do so. In short, the judge is not bound by the terms of the draft judgment which has been circulated in confidence…
>
> 4 The primary purpose of this practice is to enable any typographical or similar errors in the judgments to be notified to the court. The circulation of the draft judgment in this way is not intended to provide an opportunity to any party (and in particular the unsuccessful party) to reopen or re-argue the case, or to repeat submissions made at the hearing, or to deploy fresh ones. However, on rare occasions, and in exceptional circumstances, the court may properly be invited to reconsider part of the terms of its draft…"

Cross-anticipation

289. This is a complex argument advanced by Kymab, which depends on (a) the proposition that the patents were insufficient at the priority date and (b) the results of a reference to the Enlarged Board of Appeal in T 557/13, which is not yet available. I do not consider that it is necessary for me to decide this issue, given that I have concluded that the claims of the patents to which I have been referred are insufficient, and therefore invalid, and I do not see how this argument advances Kymab beyond its insufficiency and added matter contentions.

The Prior Art

Kucherlapati

290. This citation is relied on by Kymab both in support of lack of novelty and lack of inventive step. It is necessary to consider the whole of the document to evaluate these claims. Kucherlapati concerns the making of fully human antibodies in preference to chimeric antibodies. Therefore, it follows the common general knowledge and is a quite different idea to the reverse chimeric locus of the 287 Patent. That appears from pages 1-3 of Kucherlapati.

291. Two strategies are advanced to achieve this. The first is to introduce the construct randomly into the genome followed by inactivation of the endogenous gene. The other is direct replacement of the mouse loci by insertion of a human sequence. This is discussed generally on pages 5-6.

292. V_H is defined on page 6 line 14. It means V region genes, as opposed to D region genes, which are "D", and J region genes, which are "J_H". Hence, Kucherlapati states at page 6 line 20:

> "In order for a functional heavy chain Ig polypeptide to be produced, three discontinuous DNA segments, from the V_H, D and J_H regions must be joined."

293. The passage relied on by Kymab is at page 13:

> "As already indicated, the target locus may be substituted with the analogous human locus. In this way, the human locus will be placed substantially in the same region as the analogous host locus, so that any regulation associated with the position of the locus will be substantially the same for the human immunoglobulin locus. For example, by isolating the entire V_H gene locus (including V, D, and J sequences), or portion thereof, and flanking the human locus with sequences from the mouse locus, preferably sequences separated by at least about 5 kbp, in the host locus, preferably at least about 10 kbp in the host locus, one may insert the human fragment into this region in a recombinational event(s), substituting the human immunoglobulin locus for the variable region of the host

> immunoglobulin locus. In this manner, one may disrupt the ability of the host to produce an endogenous immunoglobulin subunit, while allowing for the promoter of the human immunoglobulin locus to be activated by the host enhancer and regulated by the regulatory system of the host."

294. On its face, this passage does not make sense. The reference to "the entire V_H gene locus (including V, D, and J sequences)" is inconsistent with the definition of V_H as meaning V regions, and not D or J regions. Therefore, the reference V_H appears to be a mistake. The passage referring to is a locus which is broader than, but which includes, V, D and J sequences.

295. I accept Regeneron's submission that the person skilled in the art would either not understand this passage or alternatively understand it to mean "by isolating the entire <u>heavy chain locus</u> (including V, D and J sequences), or portions thereof, and flanking the human locus". That latter interpretation is most likely, as it makes the passage internally consistent, and consistent with the single example disclosed in Kucherlapati (see below).

296. If Kucherlapati was disclosing a reverse chimeric locus, it would be expected to discuss its benefits, which it does not. Prof. Howard considered that Kucherlapati was disclosing a positional replacement i.e. targeting the endogenous locus, but was not teaching the potential benefits of the reverse chimeric locus:

> "Q. You would agree that Kucherlapati is teaching nothing about the benefits of the reverse chimeric locus in terms of B-cell development. Yes?
>
> A. Nothing about B-cell development at all.
>
> Q. It provides no motivation to the person reading the document in 2001 to engineer a mouse with a reverse chimeric locus at the endogenous position. It provides no motivation to do that.
>
> A. The motivation of course has more to do with the position effects -- the preservation of the intronic enhancer, which is explicit that that should be an advantage of a construction of this kind."

297. The remainder of the document is consistent with the interpretation that I have reached, namely that the reference to V_H is an error. Page 13 continues:

> "Once the human loci have been introduced into the host genome, either by homologous recombination or random integration, and host animals have been produced with the endogenous immunoglobulin loci inactivated by appropriate breeding of the various transgenic or mutated animals, one can produce a host which lacks the native capability to produce endogenous immunoglobulin subunits, but has the capacity to

> produce human immunoglobulins with at least a significant portion of the human repertoire."

298. This makes clear that the constructs referred to in the preceding paragraphs do not have the capacity to produce endogenous immunoglobulin sub-units.

299. Kucherlapati then continues:

> "The functional inactivation of the two copies of each of the three host Ig loci, where the host contains the human Ig kappa and/or lambda loci would allow for the production of purely human antibody molecules without the production of host or host/human chimeric antibodies."

300. So the disclosure is aimed at the production of purely human antibodies, and not the production of host/human chimeric antibodies (and consequently the reverse chimeric locus).

301. It is necessary to consider the sole example in Kucherlapati, in order to understand the disclosure of page 13 in context. It is headed "Production of human Ig by chimeric mice". It shows a human locus flanked with sequences from the mouse locus which form the homology arms. This is consistent with the rest of the document. The object is to produce fully human antibodies in preference to chimeric antibodies.

302. Kymab relies upon the reference to "the host" in the cited passage on page 13. It submits that this is a reference to the intronic enhancer. Kymab points out that the construct in the example deletes the host enhancer which lies between the variable region and the constant region. However, I do not accept that this would be understood as a reference to the 5' enhancer as Kymab contends, as opposed to the 3' enhancer. I have concluded that the 3' enhancer had been part of the common general knowledge from March 1990. By contrast, I have rejected Kymab's case that the existence of a 5' enhancer was common general knowledge at the priority date.

303. As to the attack of anticipation, this is plainly unsustainable in respect of claim 1 of the 287 Patent, as there is no disclosure of LTVECs or the MOA. Furthermore, I have found that there is no clear and unambiguous disclosure of a reverse chimeric locus. As to inventive step, I have considered the person skilled in the art and the common general knowledge earlier in this judgment. There is no need to paraphrase the claims to understand their inventive concepts. When properly understood, I consider that Kucherlapati is teaching away from the production of a reverse chimeric locus, and following the common general knowledge in seeking to create fully humanised antibodies. This was the explanation provided by Prof. Ploegh in his reports and oral evidence, which I accept. Furthermore, Kucherlapati plainly does not render obvious LTVECs or MOA.

Brüggemann 1997

304. I have already considered Brüggemann 1997 when discussing common general knowledge. It is a short chapter from a textbook reviewing work done by 1997 to produce fully human antibodies in mice.

305. The chapter begins by discussing the Ig segments that had been used for the construction of gene constructs and transfers into mouse cells. The chapter describes that these experiments consisted of various V-D-J segments together with C region insertions, and that whilst initially there had been discussion of whether mouse regulatory and enhancer sequences were needed, it had been shown that they were not necessary, provided the human equivalents were included. So the general teaching is against the inclusion of mouse sequences.

306. The section relied on by Kymab starts at the top of page 400, where the authors describe how "further improvements of the human antibody production in transgenic mice could be achieved". Reference is made to previous attempts to replace mouse V and C elements by Zou and Taki. Prof. Ploegh accepted that in this section Brüggemann 1997 was recognising the importance of employing the mouse endogenous regulatory sequences by reference to the earlier work of Taki and Zou. However, he rejected the suggestion that, in light of this chapter, the skilled team would have wanted to carry out the same exercise that Taki carried out (replacement of mouse J region by a rearranged mouse VDJ gene) but using a rearranged human VDJ gene. He explained that the skilled team would know that the efforts of the Brüggemann/Neuberger group were directed to expanding the size of the human loci that were introduced into mice and rejected the idea that it would conclude from this chapter that it should focus on making a targeted replacement of variable regions only (T8/1180). He emphasised that nowhere in Brüggemann 1997 is it suggested that it would be important to retain the mouse constant regions. I accept his evidence on this issue.

307. Furthermore I consider that during his cross-examination Prof. Howard made clear that he did not support an obviousness case based on Brüggemann 1997:

> "Q. Now, having looked at at least parts of this article, it is correct that it is not teaching the reverse chimeric locus or the benefits to be achieved by retaining mouse constant regions, is it?
>
> A. No.
>
> Q. And the skilled team reading this in 2001, it provides no motivation to them to make the reverse chimeric locus or to retain the mouse constant regions, does it?
>
> A. No, I do not think I would wish to say that; no. I believe there is a subliminal motivation throughout the entire story, but I agree that this paper does not stress it.
>
> Q. And it offers no practical teaching on how to make a reverse chimeric locus of the endogenous position, does it?
>
> A. No.
>
> Q. Can I suggest to you that in 2001 the person skilled in the art who was interested in using transgenes to produce human antibodies and who was aware of how this was being done in

> the prior art with the non-targeted transgenes and the fully human locus who picked up Brüggemann would not see any reason for making a reverse chimeric locus. It is just not there in Brüggemann, is it?
>
> A. No, you are right from that point of view, but on the other hand I believe that the person to whom you refer would also pick up quite a lot of other documentation about this problem."

308. Therefore, I do not consider that Brüggemann 1997 made it obvious to make a reverse chimeric locus. Further, there is no case of obviousness in respect of LTVECs and MOA, nor in respect of the requirement to replace both the heavy and light chain loci in the 163 Patent.

309. I consider that Brüggemann 1997 reflects the common general knowledge at the priority date. Those working in the field were focussed on the production of fully human antibodies, as shown by the papers to which I have referred when considering common general knowledge, and by the evidence of Profs. DeFranco and Ishida and Dr Yancopoulos.

Added Matter

Legal Principles

310. I shall apply the principles recently set out by the Court of Appeal in *Nokia v IPCom* [2013] R.P.C. 5 at [46]-[60] and in *AP Racing Ltd v Alcon Components Ltd* [2014] EWCA Civ 40 at [7]-[11].

311. In summary:

(i) An intermediate generalisation occurs when "a feature is taken from a specific embodiment, stripped of its context and then introduced into the claim in circumstances where it would not be apparent to the skilled person that it has any general applicability to the invention" (*Nokia v IPCom* at [56]).

(ii) The question is whether the feature in question would be seen by the skilled person as being generally applicable or only of significance in the context in which it was specifically disclosed (*Nokia v IPCom* at [59]-[60]).

(iii) An amendment does not require literal support in the application: The test is whether the skilled person is presented in the patent with new information relating to the invention in the patent as compared to the application. This depends on whether the combination of claimed features in the patent derives directly and unambiguously from the application, read as a whole (T 667/08 of 20 April 2012, and the EPO Guidelines for Examination Part H, Chapter IV, §2.2).

(iv) There is a distinction between what a claim covers and what it discloses. Not everything falling within the scope of a claim is necessarily disclosed (*Nokia v IPCom* at [50]).

The 287 Patent

312. Kymab alleges that claim 1 of the 287 Patent, as allowed by the EPO, is a classic intermediate generalisation, as no method having all the features of claim 1 is disclosed in the 287 Patent as filed (which was in all respects identical to the text of the PCT Application). Further, it claims that if, as I have found, *in situ* replacement is construed as including cases in which the mouse sequence is not replaced, but rather displaced and deactivated, then this is not disclosed in the Application, and it must amount to added matter.

313. The objection of intermediate generalisation has been rejected by the TBA, and, in substance the same points were rejected by the Opposition Division. In my view, the EPO was correct to dismiss this objection.

314. It is convenient to begin with a comparison of the disclosure of page 9 lines 18-33 of the PCT Application, with the amended claim as allowed by the TBA. This passage of the PCT Application contains the following disclosure:

> "One embodiment of the invention is a method of replacing, in whole or in part, in a non-human eukaryotic cell, an endogenous immunoglobulin variable region gene locus with a homologous or orthologous human gene locus comprising:
>
> > a) obtaining a large cloned genomic fragment containing, in whole or in part, the homologous or orthologous human gene locus;
> >
> > b) using bacterial homologous recombination to genetically modify the large cloned genomic fragment of (a) to create a large targeting vector for use in the eukaryotic cells (LTVEC);
> >
> > c) introducing the LTVEC of (b) into the eukaryotic cells to replace, in whole or in part, the endogenous immunoglobulin variable gene locus; and
> >
> > d) using a quantitative assay to detect modification of allele (MOA) in the eukaryotic cells of (c) to identify those eukaryotic cells in which the endogenous immunoglobulin variable region gene locus has been replaced, in whole or in part, with the homologous or orthologous human gene locus."

315. I shall now consider the differences between this general disclosure of the method and claim 1 of the 287 Patent. Claim 1 is limited to carrying out the method on ES cells wherein the mouse heavy chain is replaced, in whole or in part, whereas the cited passage from the PCT Application is not so limited. However, page 12 lines 20-22 discloses a preferred embodiment produced by the method disclosed in the PCT Application, namely "an embryonic stem cell wherein the mouse heavy chain variable region locus is replaced, in whole or in part, with a human heavy chain variable locus". To limit the claim to include these features is not, in my judgment, an intermediate generalisation, as the disclosure of the PCT is general, rather than limited to a specific context.

316. Furthermore, claim 1 contains limitations to in situ replacement, to V, D and J gene segments, and to the cloned genomic fragment being greater than 20 kb. All of these

points were considered by the Opposition Division, which rejected the argument that the limitations added subject matter. I agree with their reasoning. In particular, the Opposition Division concluded in relation to *in situ* replacement on page 5:

> "… '*in situ*' replacement is not specifically linked to a method of replacement exemplified in the application but is a general feature of the claimed invention, i.e. modifying an endogenous locus in place. The term '*in situ'* is used in the first paragraph of the section "brief description" of example 3. Said section is not associated with specific features of the example but is a general introduction to the application of the method of the invention to the replacement of mouse VDJ/VJ genes with their human counterparts. Said method is generally disclosed on pages 9-11 of the application as originally filed. Therefore the term '*in situ*' is not inextricably linked to the specific methods used to replace the murine immunoglobulin locus in example 3 but refers to the general method subject-matter of claims 1 and 9 and the products of claims 14-16 obtained by said method. Thus the OD comes to the conclusion that the introduction of the terms '*in situ* replacement', '*in situ* in place' or 'replaced *in situ*' in claims 1, 9, 14, 15 and 16 does not violate Article 123(2) EPC."

317. As to the added matter objection based on the limitation to V,D and J segments, the Opposition Division considered this in the context of an auxiliary request, at page 11 of the Decision. I agree with their reasoning.

> "As indicated with regard to the Main Request, the OD finds on pages 46-48 a basis for the replacement of V and J or V, D and J gene segments. Moreover if page 43 refers to VDJ/VJ genes, the person skilled in the art knows that the variable region is formed by V(D)J gene segments as shown in Figure 4 and disclosed for example on page 46, line 26 or page 48, line 9. Therefore the person skilled in the art would directly and unambiguously derive from the content of the application as filed the replacement of V and J or V, D and J gene segments. Claim 1 is directed to the generic replacement of V and J or V, D and J gene segments and not to the specific replacement of one V and J, i.e. of a specific part of the V(D)J region. Therefore claim 1, and as a consequence the AR 5 fulfills the requirements of Article 123(2) EPC."

318. As to the added matter based on the limitation to "cloned genomic fragments greater than 20 kb" this point was rejected by the Opposition Division at page 5 of their Decision. I agree with the reasoning of the Opposition Division:

> "The OD notes that the background and summary of the invention refer extensively to the use of large vectors and in particular to vectors that can accommodate large DNA fragments. On page 6, lines 4-6, the application specifically discloses that the LTVEC vectors of the invention are capable of accommodating large DNA fragments greater than 20 kb. Even though the term "capable of" does not require that the vectors indeed accommodate fragments greater than 20 kb, the person skilled in the art will find on page 8 of the description a specific embodiment disclosing the cloning of a fragment greater than 20 kb. Moreover all the cloned DNA fragments of example 3 fall within the range of greater than 20 kb (see for

> example figure 4 disclosing human fragments of 200 to 300 kb). The application as filed discloses the lower end of the range greater than 20 kb in the context of the LTVEC vector as well as examples comprising cloned fragments falling within the range of greater than 20 kb. Therefore the OD considers that the feature of claims 1 and 2 "cloned DNA fragment greater than 20 kb" does not add subject-matter."

319. For these reasons I reject the allegation of intermediate generalisation. I also reject the allegation that if, as I have found, *in situ* replacement is construed as including cases in which the mouse sequence is not replaced, but rather displaced and deactivated, then this is not disclosed in the Application, and it must amount to added matter. This confuses what is covered by claim 1 of the 287 Patent with what is disclosed in that Patent. Displacement and deactivation is within the scope of claim 1, but is not disclosed by it.

The 163 Patent

320. Whilst a number of added matter points are pleaded, only two were pursued in Kymab's Closing, in addition to those which I have already dealt with in relation to claim 1 of the 287 Patent.

321. First, Kymab submits that there is no disclosure in the Application of *in situ* replacement of mouse VJ regions at a *single* light chain locus as opposed to both light chain loci.

322. In my judgment, this, again, confuses the scope of claim 1 with its disclosure: claim 1 of 163 covers, but does not disclose, a mouse having replacement at a single light chain locus. Furthermore, claim 33 as dependent on claim 30 of the application expressly discloses a mouse having a replacement of mouse VJ regions at "one or more loci selected from heavy, kappa and lambda".

323. Secondly, Kymab submits that there is no disclosure in the Application of a mouse which has *in situ* replacement at more than one locus and is heterozygous for one or more such replaced loci (as opposed to a mouse bred to homozygosity for all three replaced loci).

324. This again confuses the scope of claim 1 with its disclosure: claim 1 of 163 does not disclose anything about homo or heterozygosity. It does not disclose new subject matter, beyond that disclosed in the Application, for example at page 14 lines 1-6. This disclosure is at the same level of generality, in this respect, as claim 1 of the 287 Patent.

325. In any event, I find that it would be apparent to the skilled team, from the disclosure of the Application when read in the light of common general knowledge, that in creating the final reverse chimeric mice it would be necessary to pass through the heterozygote to reach the homozygote, and that this is, impliedly, unambiguously disclosed. This is reflected in the agreed technical background, which was common general knowledge at the priority date (see [25] of this judgment). The section at page 43 lines 14-19 of the Application explicitly discloses a mouse producing hybrid antibodies with human variable regions (VDJ/VJ) and mouse constant regions

produced by *in situ* replacement. The mouse produced by claim 33 of the Application, as dependent on claim 31, would be heterozygous for whatever modification was made to its endogenous loci.

Conclusion

326. I have concluded as follows:

i) All strains of Kymouse mice addressed in the PPDs were or would be generated from a product within the scope of claims 5 and 6 of the 287 Patent; and Kymouse strains having both modified IgH and IgK loci (namely, the HK and HKL strains) are mice within the scope of claim 1 of the 163 Patent

ii) None of the claims which I have been asked to consider, namely claims 1, 5 and 6 of the 287 Patent and claim 1 of the 163 Patent is anticipated or rendered obvious by the prior art.

iii) There is no added matter in respect of the 287 or 163 Patents..

iv) However, all of the claims of the 287 and 163 Patents which I have been asked to consider are invalid for insufficiency, on the basis that, at the priority date, the skilled person would not have been able to perform the invention over the whole area claimed without undue burden and without needing inventive skill.

327. Accordingly, I shall dismiss Regeneron's claim for infringement and allow Kymab's and Novo's counterclaim for revocation.