What is the most important advance in dermatology in the last 25 years?

By Basu Dawar

The criteria used to determine the winner of a Nobel Prize in Physiology or Medicine is released fifty years after the original pronouncement.[1] After all, the criteria used in determining any verdict, be that of a Nobel Prize or an essay on advances in dermatology, underpins the outcome. Therefore, I begin by presenting my criteria:

1. The components of an advance should be closely-related where possible

Advances can often be divided into components, for example biologics into etanercept, ustekinumab, ipilimumab etc. The broader the advance, the more loosely-related its components can be; broader advances can lay claim to more components. This gives advances with wide definitions an unwarranted advantage in being named the most important. Thus the term 'biologics' for example, is perhaps not best suitable for this essay, as it has been previously criticised for its broadness.[2] Using terms for advances which have components that are more closely-related, can alleviate this issue.

2. The advance should have significantly increased dermatological knowledge

The British Association of Dermatologists' mission-statement is 'healthy skin for all', which indicates that this is their ultimate aim. Increasing the knowledge-base of dermatology is essential to achieving 'healthy skin for all'; it is highly important for the development of better diagnostics and treatments (preventative and therapeutic) for instance.

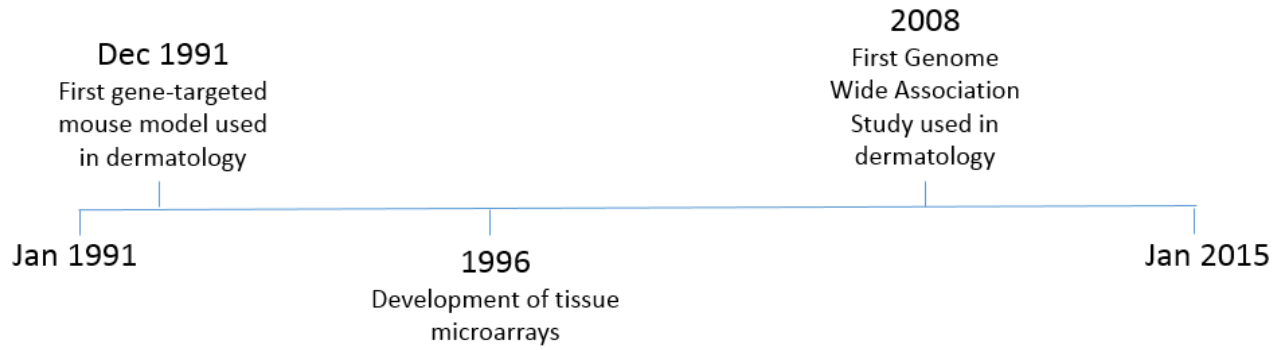
The confines of current clinical interventions in dermatology is demonstrated by the fact that skin diseases within the UK accounted for 394,000 Disability Associated Life Years in 2010 alone, despite the relatively high provision of dermatology services that are free at the point-of-use.[3] The limitations of current treatments help to explain why this is. Many dermatological diseases either lack therapies, or have therapies of a limited effectiveness. Furthermore, treatments often bring with them difficulties in administration or side-effects. This affects patient adherence; essentially decreasing effectiveness.[4] Hence many people suffer from a reduced quality of life, despite using the recommended therapeutics for their dermatological condition(s).[5]

Therefore, considering that better clinical interventions are required to achieve 'healthy skin for all', it is legitimate to propose that the 'most important advance' should have significantly increased dermatological knowledge within the last 25 years (and will continue to do so), as this is a catalyst for the development of future clinical interventions.

3. Knowledge which has aided in the development of existing clinical interventions will be granted a higher value than knowledge which has not

Differentiating between the many advances that have increased dermatological knowledge is necessary in determining the most important advance. A key reason for increasing dermatological knowledge is to assist in the development of clinical interventions, thus the value/benefits of dermatological knowledge can be assessed in these terms. Therefore, advances which have produced knowledge that has aided in the development of clinical interventions will be given a higher value than knowledge which has not.

Advances which meet the criteria



The timeline illustrates the advances which have best met the criteria. Out of these, I believe gene-targeted mice have been the most successful.[6] I will offer my reasons for this, and then compare gene-targeted mice to the other advances on the timeline.[7,8]

To clarify, although '*the first reports in which homologous recombination in ES cells was used to generate gene-targeted mice were published in 1989*', gene-targeted mice have only been applied to dermatology (and thus existed in dermatology) since 1991.[9,10] As we are focusing on advances in dermatology, gene-targeted mice therefore fit the 25 year timeframe.

Gene-targeted mice in dermatology

Background

Twenty-five years ago, gene-modification studies were overwhelmingly dependent on cell culture and transgenic mice (produced through non gene-targeting methods). These methods have significant limitations regarding their use in understanding gene function. *In vitro* studies are limited in their generalisability, as they do not represent the complexity of

highly-developed organisms.[11] Regarding transgenic (non gene-targeted) mice, their use is restricted to the addition of genes at random loci within the genome.[12] These constraints meant that there were previously substantial barriers to understanding the workings of the integumentary system in health and disease. Gene-targeted mice have helped to alleviate this problem, by allowing for a greater level of genetic modification in complex organisms. This profound achievement was recognised by the Nobel Prize in Physiology or Medicine being awarded to the creators of gene-targeted mice in 2007.[6]

Components

Gene-targeted mice can be described as either knockout or knockin. In knockout mice, the gene of interest has essentially been deleted.[13] In knockin mice, the gene has been inserted at a specific locus, replacing an existing DNA sequence.[14] The effects of the knocked-out/knocked-in gene do not necessarily have to be present from an embryonic stage. Gene-targeted mice can be created so that one can choose to activate the knockout/knockin of a gene at a particular point in the mouse's lifespan (controlled via drug administration). These mice are known as conditional knockout/knockins, and can be useful when studying genes crucial to embryogenesis for instance. Also of note, gene-targeted mice can be generated so that the gene modification either applies to a specific tissue, or the whole organism. The methods that produce different gene-targeted mice are very similar, since the general principles are the same, as illustrated below.[6]

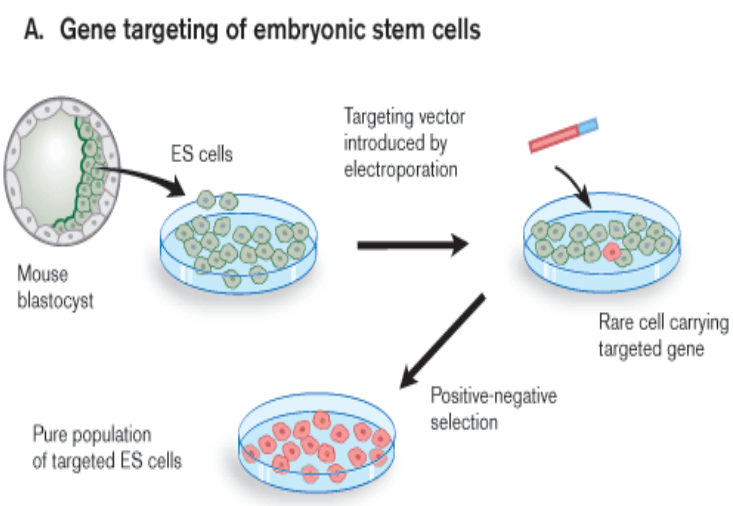
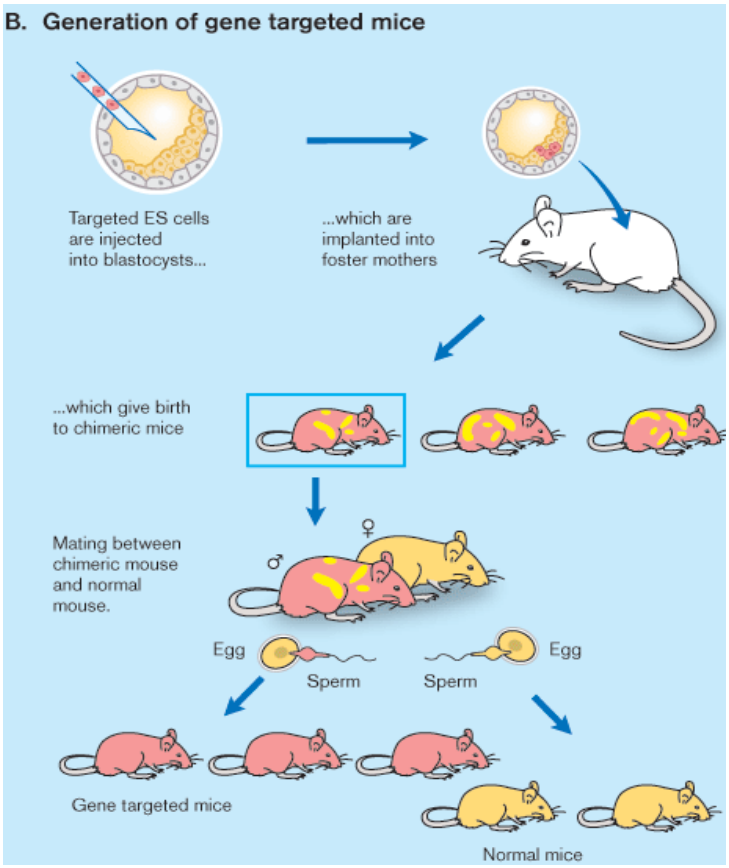


Figure A: Note that homologous recombination is what allows the targeting vector to modify the target gene



How have gene-targeted mice benefited dermatology?

Genomic sequencing has identified ~25000 genes in the mouse and human genome, of which only 1% are unique to either species.[15] It is not surprising, therefore, that many important molecular processes are strongly conserved, affording insights into our own skin in health and disease. A large number of studies using gene-targeted mice have contributed to dermatology, below are a small proportion of them.

Identification of therapeutic targets

Gene-targeted mice have identified new therapeutic targets, by characterising the functions of molecules in the skin, as demonstrated below.

Mice with a knockout of aquaporin-3 (encodes a transmembrane protein facilitating in water transport) helped demonstrate that aquaporin-3 is important for keratinocyte proliferation.[16] Aquaporin-3 knockout mice have also been shown to be resistant to skin carcinogenesis.[17] Thus the aquaporin-3 signalling pathway could be a potential therapeutic target in non-melanoma skin cancer.[17]

MicroRNAs play an essential role in the skin; illustrated by the severe cutaneous phenotype of mice with a knockout of Dicer (required for microRNA synthesis).[18] Knockout mice have been used to identify the specific functions of microRNAs in the skin.[19] These discoveries point to new therapeutic targets, and provide insight into the microRNA inhibitors currently in development. [19,20]

Current Treatments

Gene-targeted mice have been especially beneficial in developing new treatments for metastatic melanoma.

Cytotoxic T-lymphocyte associated protein-4 (CTLA-4) knockout mice were required to demonstrate that CTLA-4 is involved in the negative regulation of the immune response, as the function of CTLA-4 was previously unclear.[21,22] This led to the hypothesis that CTLA-4 inhibitors could be used to augment the immune response in cancer, which resulted in the

development of ipilimumab.[22] Ipilimumab has significantly improved the prognosis of metastatic melanoma.

Similarly, studies of programmed cell death receptor 1 (PD-1) knockout mice provided the first major evidence that PD-1 is a negative regulator of the immune response.[23,24] This led to the development of pembrolizumab (a PD-1 inhibitor), which was recently approved for the treatment of metastatic melanoma. Pembrolizumab may have reduced side-effects compared to ipilimumab.[25] Remarkably, this was first suggested on realisation that PD-1 knockout mice experience less deleterious effects than CTLA-4 knockout mice.[25]

Treatments in clinical trials

Gene-targeted mice offer much hope for future potential treatments.

Recessive dystrophic epidermolysis bullosa (RDEB) is a blistering skin disease due to loss-of-function mutations in the *COL7A1* gene, which encodes for the alpha-chain of collagen type VII. One potential method of treating RDEB is to perform a bone-marrow transplant, as it provides a supply of 'healthy' stem-cells (without the defective *COL7A1* mutation) to the skin, resulting in the production of 'healthy' keratinocytes. This therapeutic approach was validated in a study which demonstrated that the survival of col7a1 knockout mice is prolonged after bone-marrow transplantation.[26] Following on from this, the authors commenced a phase 1-2 trial in patients with RDEB, it found that bone-marrow replacement could be beneficial in treating particularly severe forms of epidermolysis bullosa.[27]

The utility of gene-targeted mice has also been shown in pseudoxanthoma elasticum (PXE); a heritable metabolic disease that causes mineral deposition in peripheral connective-tissue. The development of abcc6 knockout mice was important in understanding the pathogenesis of PXE; this mouse-model demonstrated that circulating factors in the bloodstream are critical to the pathological mineralisation process of PXE.[28] Subsequent studies found that magnesium supplementation reduced mineral deposition in abcc6 knockout mice, and also vice versa, the dietary restriction of magnesium increased the pathological mineralisation process in abcc6 knockout mice.[29,30] On this basis, a phase 2 trial is investigating the use of magnesium supplements in PXE, which could lead to the first effective therapy for PXE.[31]

Current diagnostics

Previously, the significance of tight-junctions within the epidermis was unclear, as evidence suggested that epidermal tight-junctions were poorly developed.[32] Mice with a knockout for the gene encoding claudin-1 (a protein component of tight-junctions) produced a phenotype demonstrating a severely defective skin barrier, thus illustrating the importance of tight-junctions within the epidermis, as well as validating the function of claudin-1.[32] This resulted in *CLDN1* (encodes for claudin-1) being labelled as a candidate-gene in skin diseases where barrier dysfunction is implicated. It also led to *CLDN1* mutations being identified as the cause of NISCH syndrome, an autosomal recessive disorder characterised by hypotrichosis, scarring alopecia, icthyosis, and sclerosing cholangitis.[33] As a result of this, prenatal testing and confirmatory diagnosis of NISCH syndrome is now available.[34]

The discovery that col5a2 (encodes for a key component of collagen type V) knockout mice have a phenotype resembling Ehlers-Danlos syndrome (EDS), pointed out for the first time

that *COL5A2* gene mutations could cause EDS.[35] This insight led to the identification of *COL5A2* mutations in patients with EDS, allowing the current use of genetic sequencing for confirmatory diagnosis, as well as the pre-implantation diagnosis of EDS in specific cases, via *COL5A2* mutation detection.[36,.37]

Fully-humanised biologics

Gene-targeted mice have been used to develop fully-humanised monoclonal antibodies (although often referred to as being produced by transgenic mice, gene-targeting is used as part of the process to knockout mice genes).[38] The advantage is that these fully-humanised antibodies have no mouse-components unlike chimeric antibodies. This removes a source of immunogenicity, with the aim of reducing potential side-effects.[39] Ustekinumab and ipilimumab are examples of biologics developed via this method. [40]

Limitations

As with other disease models, the findings discovered using gene-targeted mice cannot immediately be assumed to be applicable to humans. This is due to differences in human and mouse physiology, and also because the investigation of gene function through genetic-modification leaves the possibility of findings being influenced by potential compensatory mechanisms, via redundancy of related gene products.[41] Other limitations include gene-targeted mice often being more expensive and time-consuming to produce than other types of disease models.

Future

The ongoing Knockout Mouse Project aims to generate a public resource of mouse embryonic stem cells, with a null-mutation in every gene of the mouse genome.[42] Mice produced from this project have already identified genes previously unrecognised as being involved in the pathogenesis of dermatoses; more are likely to be discovered.[43] Furthermore, existing knockout mice lines can cost as much as $26,200 for four mice, this project will be expected to reduce the cost to $115 per a knockout mouse, encouraging greater use in research.[44]

Other Advances

Tissue microarrays

Tissue microarrays are a high-throughput technique that allow the assaying of hundreds of small quantities of patients' tissues at a time. This has been useful in understanding the molecular pathogenesis of particular dermatoses, and has identified potential therapeutic targets.[7] For example, a study used tissue microarrays to identify that MERTK (a receptor tyrosine kinase) expression was correlated with melanoma progression, thus presenting MERTK as a potential therapeutic target.[45] Tissue microarrays have also been used to identify potential melanoma biomarkers.[46]

So far, clinical applications of knowledge from this source have not been evident, whereas information discovered using gene-targeted mice has already been applied to develop clinical applications. Thus the criteria favour gene-targeted mice over tissue microarrays as being the most important advance.

Genome wide association studies (GWAS)

GWAS assay single nucleotide polymorphisms in large populations comprising of healthy individuals and those who have the disease of interest, in order to identify disease-associated loci.[8] Associations between particular genes and a disease can then be determined from this, which can help lead to novel biological insights, allowing for the development of clinical interventions.

In dermatology, GWAS have validated many existing therapeutic targets, and identified novel disease-associated loci, providing new candidate disease-susceptibility genes. For example, a GWA-study associated psoriasis with a (previously unidentified) locus containing the gene *IL28RA*, which encodes for the alpha-subunit of the IL-28 receptor.[47]

To date, there does not appear to have been any clinical interventions derived from GWAS in dermatology. Additionally, GWAS has identified fewer novel therapeutic targets than gene-targeted mice. This prevents GWAS from being considered the most important advance. Also, it is important to note that GWAS may be replaced by next-generation sequencing in the future, as this provides greater information and reduces the difficulty of identifying disease-susceptibility genes.[48]

Conclusion

'Healthy skin for all' is a goal of enormous ambitions, which will require the development of better diagnostics and treatments if it is to be achieved. The advances discussed are catalysts for this. Of these, gene-targeted mice have demonstrated the most success: by providing us with powerful new insights into the skin in health and disease, current as well as potential future clinical interventions, and procedures important in the creation of biologics.

The prominence of gene-targeted mice in dermatology is illustrated further by the Journal of Investigative Dermatology, which has the highest impact factor out of all journals dedicated to dermatology. In 2014, every issue included at least one original article utilising gene-targeted mice.[49-60] The Knockout Mouse Project will likely lead to even greater use of gene-targeted mice in the future.

In concluding that gene-targeted mice has been the most important advance, it is necessary to acknowledge that its success in meeting the criteria has likely been influenced by it having existed longer than the other advances discussed, which does generate some bias. However, this issue is inherent when comparing almost any advance, and adjusting for it would generate greater limitations. Therefore, on consideration of the criteria, it is legitimate to state that the applications of gene-targeted mice make it the most important advance in dermatology of the last quarter-century.

Word Count: 2496

References

1. Nobel media AB. *Nomination and selection of Medicine Laureates*. http://www.nobelprize.org/nomination/medicine. (accessed 23 November 2014)

2. Katz KA. "Biologics": a clinically meaningless term. *Br J Dermatology* 2006: 154: 809-812

3. CJ Murray, MA Richards, JN Newton, et al. UK health performance: findings of the Global Burden of Disease Study 2010. *Lancet* 2013; 381; 997–1020

4. Levender M, Balkrishnan R, Feldman S. Chapter 72: adherence to treatment. In: Maibach M, Gorouhi F. *Evidence Based Dermatology.* Second Edition. McGraw-Hill Medical; 2012. p1076

5. Beattie PE, Lewis-Jones MS. A comparative study of impairment of quality of life in children with skin disease and children with other chronic childhood diseases. *Br J Dermatol* 2006; 155: 145–151

6. Nobel media AB. *Genetic Modification in Mice.* http://www.nobelprize.org/nobel_prizes/medicine/laureates/2007/advanced.html (accessed 23 November 2014)

7. Barrette K *et al.* Research Techniques Made Simple; Tissue Microarray. *Journal of Investigative Dermatology* 2014: 134; e24

8. Zhang, X. Genome-wide association study of skin complex diseases. *J. Dermatol Sci* 2012; 66; 89–97

9. Baribault H, Oshima RG. Polarized and functional epithelia can form after the targeted inactivation of both mouse keratin 8 alleles. *J Cell Biol* 1991; 115:1675–1684

10. Nobel Media AB. *Press Release.* http://www.nobelprize.org/nobel_prizes/medicine/laureates/2007/press.html (accessed 23 November 2014)

11. Oh JW, Hsi T, Guerrero-Juarez CF et al. Organotypic Skin Culture. *Journal of Investigative Dermatology* 2013; 133, e14. http://www.nature.com/jid/journal/v133/n11/full/jid2013387a.html (accessed 23 November 2014)

12. Case Western Reserve University. *Transgenic or Knockout Mice?* http://ko.cwru.edu/info/strategies.html. (accessed 23 November 2014)

13. Manis JP. Knock out, knock in, knock down--genetically manipulated mice and the Nobel Prize. *N Engl J Med* 2007: 357; 2426–2429

14. Koch Institute for integrative cancer research at MIT. *Knockins and Knockouts.* http://ki.mit.edu/sbc/escell/models/knock (accessed 23 November 2014)

15. Medical Research Council. *Use of animals in the MRC*. http://www.mrc.ac.uk/research/research-policy-ethics/use-of-animals/use-of-animals-in-the-mrc/ (accessed 17 December 2014)

16. Hara-Chikuma M, Verkman AS. Aquaporin-3 facilitates epidermal cell migration and proliferation during wound healing. *J Mol Med* 2008; 86: 221–231

17. Hara-Chikuma M, Verkman AS. Prevention of skin tumorigenesis and impairment of epidermal cell proliferation by epidermal cell proliferation by targeted aquaporin-3 gene disruption. *Mol Cell Biol* 2008; 28:326–332

18. Ning MS, Andl T. Control by a hair's breadth: the role of microRNAs in the skin. *Cell Mol Life Sci* 2012; *1-21*

19. Mi QS, Xu YP, Wang H, Qi RQ, Dong Z, Zhou L. Deletion of microRNA miR-223 increases Langerhans cell cross-presentation. *Int J Biochem Cell Biol* 2013; 45: 395–400

20. Guinea-Viniegra J, Jimenez M, Schonthaler HB, Navarro R, Delgado Y, Jose Concha-Garzon M, Tschachler E, Obad S, Dauden E, Wagner EF. Targeting miR-21 to Treat Psoriasis. *Sci Transl Med* 2014; 6: 225re221

21. Waterhouse, P., J. Penninger, E. Timms, A. Wakeham, A. Shahinian, K. Lee, C. Thompson, H. Griesser, and T. Mak. Lymphoproliferative disorders with early lethality in mice deficient in CTLA-4. *Science* 1995; 270:985–988

22. Lipson EJ, Drake CG. Ipilimumab: an anti-CTLA-4 antibody for metastatic melanoma. *Clin. Cancer Res* 2011; 17: 6958–6962

23. Nishimura H, Minato N, Nakano T, Honjo T. Immunological studies on PD-1 deficient mice: implication of PD-1 as a negative regulator for B cell responses. *Int. Immunol* 1998; 10; 1563–1572

24. Nishimura, H. , Nose, M. , Hiai, H. , Minato, N. & Honjo, T. Development of lupus-like autoimmune diseases by disruption of the PD-1 gene encoding an ITIM motif-carrying immunoreceptor. *Immunity* 1999; 11; 141–151

25. Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. *Nature Rev. Cancer* 2012; 12; 252–264

26. Tolar J, Ishida-Yamamoto A, *et al*. Amelioration of epidermolysis bullosa by transfer of wild-type bone marrow cells. *Blood* 2009: 113: 1167-1174

27. Wagner JE, Ishida-Yamamoto A, *et al*. Bone marrow transplantation for recessive dystrophic epidermolysis bullosa. *N Engl J Med* 2010: 363: 629-639

28. Jiang Q, Endo M, Dibra F *et al*. Pseudoxanthoma elasticum is a metabolic disease. *J Invest Dermatol* 2009; 129; 348–354

29. LaRusso J, Li Q, Uitto J. Elevated dietary magnesium prevents connective tissue mineralization in a mouse model of pseudoxanthoma elasticum (Abcc6−/−). *J Invest Dermatol* 2009; 129: 1388–1394

30. Jiang Q, Uitto J. Restricting dietary magnesium accelerates ectopic connective tissue mineralization in a mouse model of pseudoxanthoma elasticum *(Abcc6$^{-/-}$)*. *Exp Dermatol* 2012: 21:694–699

31. Lebwohl M. Magnesium Supplements in the treatment of pseudoxanthoma elasticum. http://clinicaltrials.gov/show/NCT01525875 (accessed 23 November 2014)

32. Furuse M, Hata M, Furuse K *et al.* Claudin-based tight junctions are crucial for the mammalian epidermal barrier: a lesson from claudin-1-deficient mice. *J Cell Biol* 2002: 156: 1099–1111

33. Hadj-Rabia S, Baala L, Vabres P *et al.* Claudin-1 gene mutations in neonatal sclerosing cholangitis associated with ichthyosis: a tight junction disease. *Gastroenterology* 2004: 127: 1386–1390

34. Gene Dx. *NISCH Syndrome.* http://www.genedx.com/test-catalog/disorders/nisch-syndrome (accessed 3 Jan 2015)

35. K. Andrikopoulos, X. Liu, D.R. Keene, R. Jaenisch, F. Ramirez. Targeted mutation in the col5a2 gene reveals a regulatory role for type V collagen during matrix assembly. *Nat. Genet* 1995; pp. 31–36

36. Michalickova K, Susic M, Willing MC, Wenstrup RJ, Cole WG. Mutations of the alpha2(V) chain of type V collagen impair matrix assembly and produce Ehlers-Danlos syndrome type I. *Hum Mol Genet* 1998; 7; 249–255

37. Malfait F, Wenstrup R, Paepe AD. *Ehlers-Danlos Syndrome, Classic Type.* http://www.ncbi.nlm.nih.gov/books/NBK1244/ (accessed 23 November 2014)

38. Strachan T, Read A. *Human Molecular Genetics 4th Edition.* Chapter 21: Genetic Approaches to Treating Disease; p685. Garland Science; 2011

39. Hwang WY, Foote J. Immunogenicity of engineered antibodies. *Methods* 2005; 36; 3–10

40. Nelson AL, et al. Development trends for human monoclonal antibody therapeutics. *Nature Rev Drug Discov* 2010; 9, 767–774

41. Scharfenberger L, Hennerici T, Kiraly G *et al*. Transgenic mouse technology in skin biology: generation of complete or tissue-specific knockout mice. *J Invest Dermatol* 2014; 134; e16

42. National Institutes of Health. NIH Knockout Mouse Project (KOMP). http://www.nih.gov/science/models/mouse/knockout/komp.html (accessed 23 November 2014)

43. Liakath-Ali K, Vancollie VE, Heath E, Smedley DP, Estabel J, et al. Novel skin phenotypes revealed by a genome-wide mouse reverse genetic screen. *Nat Commun* 2014; 5; 3540. doi: 10.1038/ncomms4540

44. Waltz E. Price of mice to plummet under NIH's new scheme. *Nat Med* 2005; 11:1261

45. Schlegel J, Sambade MJ *et al*. MERTK receptor tyrosine kinase is a therapeutic target in melanoma. *The Journal of Clinical Investigation* 2013: 123(5): 2257-2267

46. Nazarian RM, Prieto VG *et al*. melanoma biomarker expression in melanocytic tumour progression: a tissue microarray study. *J Cutan Pathol* 2010: 37: Suppl 1:41-7

47. Strange A *et al*. A genome-wide association study identifies new psoriasis susceptibility loci and an interaction between HLA-C and ERAP-1. *Nature Genetics* 2010: 42: 985-990

48. Bush WS, Moore JH. Chapter 11: Genome-wide association studies. PLoS Comput Biol 2012; 8; e1002822

49. Mao X, Li H, Sano Y, Gaestel M, Mo Park J, et al. MAPKAP Kinase 2 (MK2)-Dependent and -Independent Models of Blister Formation in Pemphigus Vulgaris. *J Invest Dermatol* 2013; 134; 68-76

50. Matsui S, Murota H, Takahashi A *et al*. Dynamic analysis of histamine-mediated attenuation of acetylcholine-induced sweating via GSK3β activation. *J Invest Dermatol* 2014; 134: 326–334

51. Cowburn AS, Alexander LEC, Southwood M et al. Epidermal deletion of HIF-2α stimulates wound closure. *Journal of Investigative Dermatology* 2014; 134; 801-808

52. J. Bar, V. Kumar, W. Roth, N. Schwarz, M. Richter, R.E. Leube, T.M. Magin Skin fragility and impaired desmosomal adhesion in mice lacking all keratins. *J Invest Dermatol* 2014; 134; 1012–1022

53. Wang XQ, Lee S, Wilson H et al. Ganglioside GM3 depletion reverses impaired wound healing in diabetic mice by activating IGF-1 and insulin receptors. *Journal of Investigative Dermatology* 2014; 134; 1446-1455

54. Adeyo O, Allan BB et al. Palmarplantar keratoderma along with neuromuscular and metabolic phenotypes in Slurp1-deficient mice. *J Investigative Dermatology* 2014; 134; 1589-1598

55. Leiva AG, Chen AL et al. Loss of mpzl3 function causes various skin abnormalities and greatly reduced adipose depots. *J Investigative Dermatology* 2014; 134; 1817-1827

56. Sano Y, Park JM. Loss of epidermal p38α signal prevents UVR-induced inflammation via acute and chronic mechanisms. *J Investigative Dermatology* 2014; 134; 2231-2240

57. Campbell L, Emmerson E et al. Estrogen receptor-alpha promotes alternative macrophage activation during cutaneous repair. *J Investigative Dermatology* 2014; 134; 2447-2457

58. Fischer H, Langbein L et al. Loss of keratin k2 expression causes aberrant aggregation of k10, hyperkeratosis and inflammation. *J Investigative Dermatology* 2014; 134; 2579-2588.

59. Chung JS, Tamura K et al. DC-HIL expressing myelomonocytic cells are critical promoters of melanocytic growth. *J Investigative Dermatology* 2014; 134; 2784-2794

60. Elishmereni M, Fyhrquist N et al. Complex 2B4 regulation of mast cells and eosinophils in murine allergic inflammation. *J Investigative Dermatology* 2014; 134; 2928-2937