THE EUROPEAN LANDSCAPE FOR HUMAN GENOME EDITING

A review of the current state of the regulations and ongoing debates in the EU

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1. Introduction

This scoping paper has been prepared in the context of the forthcoming workshop on the European landscape for human genome editing that is being jointly organised by the Federation of European Academies of Medicine (FEAM - www.feam-site.eu), the UK Academy of Medical Sciences (AMS - www.acmedsci.ac.uk) and the French Academy of Medicine (www.academie-medicine.fr); and supported by the InterAcademy Partnership for Health (www.iamp-online.org) and the French Academy Foundation.

The workshop, involving high-level representation from academia, research funding agencies, patient representative groups, ethical review bodies, industry, regulatory and other key European authorities, will provide an opportunity to facilitate international discussions and explore the landscape for human genome editing across the EU.

The aims of the workshop will be to:

- Understand current scientific activities in the EU with respect to genome editing – focussing on human applications.
- Understand the current regulatory landscape for human genome editing research and clinical applications across the EU.
- Understand the ongoing debate on genome editing across the EU.
- Identify any areas where there are significant differences, e.g. between countries, and if possible consider the driving forces for these differences (e.g. ethics, public opinion).
- Discuss the need for a European regulatory framework to govern the safe and acceptable use of human genome editing.

Nothing in this briefing paper, although it has been prepared with input from experts in the various national academies of FEAM, from publicly available information sources, and from personal communications with key stakeholders, should be considered as the formal positions of any of the organisations supporting the workshop. All errors of commission and omission in this rapidly changing field are the responsibility of the independent author commissioned to collate the material.

2. Overview of key issues

Rapid advances in the science and application of genome editing have taken place over the last few years, aided by the development of new technologies such as CRISPR-Cas9. These advances appear to be outstripping the current regulatory oversight mechanisms that are in place, particularly relating to human genome editing. There are significant differences across Europe on how the technology is being applied in basic and clinical research and how it is being regulated. There is much discussion going on in some countries, particularly in Germany, France and the UK on this matter.

Such a variation in the regulatory oversight, and the controversial nature of the national debates that led to such legislation (as in the use of embryos in research), might indicate the

future challenges around seeking the establishment of any new supportive pan-European framework for the further application of genome editing.

2.1 The regulation of research using genome editing in early human embryos and germline cells

The regulatory context worldwide is highly varied, ranging from no or blurred regulations through to very restrictive laws, and with some countries having both. Within Europe there is a mix of Europe-wide conventions, which are more or less adopted by different countries, as well as country-specific regulations and/or guidelines, which vary widely in the type of research that can be done.

This mix is confusing; it complicates or restricts the ability of scientists to work together across national boundaries and potentially leads to discrimination. It may also discourage research by restricting funds and making boundaries uncertain, together making it difficult to share reagents and data, and develop infrastructure that could be international.

The funding of frontier research and the support of collaborative research programmes within Europe in the application of genome editing will become increasingly important as the technology is developed further. Whilst the number of applications to the European Research Council for the funding of genome-editing related basic research is increasing, the focus of the research is only on the use of human embryonic stem cells or human induced pluripotent stem cells at present in view of the restrictions within Horizon 2020 concerning the use of human embryos. With the increased availability for research of donated embryos from IVF programmes across the EU it would seem timely for clarification of the EU's position on this matter. The restrictions on funding of research at a European level is inhibiting collaborations between the more "permissive" countries, in the context of embryo research, and those with more prohibitive legislation in place.

2.2 The regulation of research and applications relevant to human somatic genome editing, within Europe.

There is a reasonable degree of harmonisation across Europe in the regulatory environment relating to somatic cell based genome editing and an expectation that existing laws and guidelines relating to gene therapy will be adequate to regulate future genome editing applications. However there is an appreciation of the possible need to reconsider some details regarding the regulatory oversight in the clinical aspects of human somatic cell-based therapy, especially where the methods and hence safety related issues differ from those of conventional gene therapy.

2.3 The regulation of germline genome editing for clinical applications.

The regulatory context worldwide is highly varied, ranging from no regulations through to very restrictive laws, and with some countries having both. Objections to the development of germline genome editing for clinical applications is embodied in the regulations adopted by most, but not all countries. However, the confused nature of the regulatory approaches to relevant research makes this consensus fragile. Some countries want to keep the door open, others want it firmly shut. Calls that have been made for a moratorium on germline genome research have been somewhat vague in their demands and could inadvertently lead to researchers feeling vilified and not as open as they might be about their research at a time when transparency and global cooperation is essential.

Within the European context there is a general consensus that at present such human germline applications should not be permitted, and this is likely to be enforced for some time to come through the imminent implementation of the EU Clinical Trials Regulation No 536/2014 that will prevent the carrying out of gene therapy trials which may result in modifications to the subject's germline genetic identity. Most European countries have ratified the Oviedo Convention and thus have formally stated their positions on prohibiting intentional human germline modification, but there does appear to be a lack of clarity on how the Convention's provisions for clinical application affects basic research.

2.4 The importance of public engagement

Public opinion can change, and sometimes does so rapidly, and it can support or even drive changes in regulation, which can be in either direction - becoming less supportive as with genetically modified (GM) crops or more supportive as with techniques such as pre-implantation genetic diagnosis (PGD) for example, which used to be banned in Switzerland until the public vote in 2015.

Having such a dialogue amongst all parties involved, including civil society, is critical. However, support for such an approach, and the acceptance of the role of public engagement and opinion in driving regulatory changes, also varies widely across Europe. The need for a well-informed dialogue is also critical, but the wide variation in the regulatory environment and the lack of clarity on key issues does not encourage public understanding, acceptance or support of the science and its application.

2.5 Developing ethical positions

The developments of ethical perspectives that will influence the regulatory oversight of such biomedical developments are a national responsibility within the EU. Such ethical viewpoints vary considerably across Europe, from utilitarian and pragmatic approaches (the UK being an example), to those that sanctify all human life including early embryos (as in Italy and Germany).

2.6 Addressing the wider healthcare applications of genome editing

Much of the current focus across Europe on the scientific and regulatory developments concerning the application of genome editing for improvements to human health has focused on issues concerning human embryo use and human gene therapy etc. Genome editing does however have significant potential in other fields of human health including the enhancement of xeno-transplantation therapy and the use of gene drives and the modification of wild insect populations to reduce the impact of pathogens and disease vectors (malaria, dengue, Zika virus etc.).

There clearly will be an ongoing need for further study on the research applications and the regulatory, societal, and policy challenges of these wider applications of genome editing.

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3. An introduction to genome editing

In its broadest sense, the term genome editing comprises a range of molecular techniques that allow targeted changes to be made to the genomes of organisms. Genome editing can:

- Modify genetic information within an organism's genome to correct disease causing mutations, change one naturally occurring genetic trait into another, or even create new characteristics.
- Remove specific regions from genomes, such as those which lead to a disease or confer disease susceptibility.
- Add transgenes (including genes from other organisms) to specific locations in genomes.
- Create disease models (in animals or human cells in vitro).
- Allow the development of screens (for genes, pathways, etc), to understand gene function and mechanisms, to understand how genes are regulated.

Genome editing precisely modifies nucleotides (A, T, G, C) in the genetic code, and is carried out by using specifically engineered "molecular scissors" to create precise breaks in the genome, and deleting, inserting or replacing a given stretch of DNA by harnessing the DNA repair mechanisms of cells.

3.1 Techniques for creating breaks in the genome: sequence-specific nucleases

Nucleases are enzymes that cut nucleic acids. They can be engineered to target specific sites within genes and create breaks in the genome. There are four kinds of sequence-specific nucleases currently used in genome editing.

a) Meganucleases: Unlike the other methods below, which have separate DNA recognition and nuclease components, meganucleases (homing endonucleases) have DNA recognition built in. Meganucleases occur naturally and can be engineered, to some extent, to target specific sequences. They were important as they were used to understand the mechanisms and parameters of DNA target site recognition and of DNA repair that underlie and helped in the development of the other methods.

b) Zinc-Finger Nucleases (ZFNs): Zinc-fingers, protein structures containing zinc ions, can be designed to recognise and bind to unique sequences in a genome (ZF domains were first recognised in a large class of transcription factors that bind DNA to control the activity of genes). The specific combinations of zinc-fingers are then fused to a nuclease that will cut a single DNA strand (a "nickase"; usually Fok1). To create a complete break in the DNA (a double strand break, or DSB), which is required for genome editing, a pair of ZFNs are designed to recognise opposite strands of DNA at the same location. This has the advantage in that it makes the system very specific, but it is less efficient than using a nuclease that cuts both strands at once (such as Cas9, see below).

c) Transcriptor Activator-Like Effector Nucleases (TALENs): Transcriptor activator-like (TAL) effectors are proteins produced by *Xanthomonas* bacteria (a family of plant pathogens). TAL effectors can be engineered to target desired DNA sequences and when fused to nucleases can be used to create breaks in a similar way to ZFNs.

d) Clustered Regularly Interspersed Short Palindromic Repeats (CRISPRs): CRISPRs are an immune defence system found in bacteria to protect against viruses; the system exploits short stretches of viral DNA incorporated into the bacterial genome that, when expressed as RNA and matched to the DNA sequence of an invading virus, trigger CRISPR associated (Cas) nucleases to make a double strand break in the viral DNA. CRISPRs can be easily engineered to specify where a break should be made in the genome. A synthetic RNA

molecule is developed which contains a short region (of 20 bases) that is designed to recognise the target DNA sequence, and the rest contains a region that interacts with the nuclease (most often Cas9) that is introduced into the cell at the same time. In this way the RNA guides the nuclease to the desired location. Because it is simple to make the CRISPR part, the guide RNA (or gRNA), and it does not need to be physically linked to the nuclease, it easier to implement CRISPR-Cas9 than the three other systems above.[1]

3.2 Techniques for repairing and adjusting the genome

Once a break is made in the genome at the desired position the DNA repair mechanisms of a cell are triggered. These can be harnessed to make the desired changes via two main mechanisms. Homology Directed Repair or Homologous Recombination involves the use of a DNA fragment as a template for repair, which also contains the genetic sequence to be introduced, and can be used to replace or insert nucleotides or full genes or to make precise deletions, even of very large sizes. Non-Homologous End Joining (NHEJ) doesn't require a template and simply repairs the break, but most often introduces small deletions or insertions in doing so. Slight changes made to the genome, even a single nucleotide change, will often stop a gene from functioning; creating a "knock out", but repair mechanisms can also be purposefully harnessed to make insertions and deletions of full genes.[2] For example, deletions can be made by using two guide RNAs flanking a gene, where the intervening DNA sequence will often be lost during the repair process.

3.3 A note on terminology

The terms "gene editing" and "genome editing" are often used interchangeably. However, it is considered that the term 'genome editing' better describes the process whereby each guide RNA (or Zinc-finger or TALEN) effectively searches the whole genome for its specific target(s). Further, the methods can also be used to edit non-gene sequences. Genome editing is also distinct from alternative (older) methods of creating genetic alterations, such as transgenic mice where DNA integrates into the genome at random or gene targeting via homologous recombination (referred to above). Genome editing leads to genetic alteration or genetic manipulation – but as it is possible to use it to make very subtle changes in a gene; the altered sequence may be indistinguishable from a naturally occurring variant of the gene.

4. The impact upon science of targeted genome editing using CRISPR/ Cas9

Research into genome editing is not a new development. What has been a significant stimulus for the current international debates is the growing appreciation of the "game-changing" nature of the CRISP/Cas9 technology. Compared to the use of other engineered nuclease techniques used to insert, delete or replace DNA in the genome of an organism CRISPR is much quicker, easier to use and cheaper, and may be more precise in its application, and is thus having a significant impact on research. CRISPR/Cas9 can also be used to edit multiple genes simultaneously.

Appendix 2 of this paper provides a recent timeline on the development of CRISPR/Cas9 based genome editing, identifying a number of the key publications in this field. In reviewing current developments in the development of genome editing, and in particular whether the current legislative/regulatory systems in place in Europe (and globally) for human genome editing are fit for purpose, it is important that a clear distinction is made between the

[1] Ran F.A. *et al* Genome engineering using the CRISPR-Cas9 system. Nature Protocols 8, 281–2308 (2013)http://www.nature.com/nprot/journal/v8/n11/full/nprot.2013.143.html
[2] Source: UK Science Media Centre Factsheet http://www.sciencemediacentre.org/

application of the technology in basic research, pre-clinical research, and in clinical studies, and between its use in the development of somatic cell-based therapies (gene, cell and regenerative) and in particular its potential for reproductive/germline changes.

4.1 Developments in basic and preclinical research applications of genome editing.

The new tools of genome editing have already demonstrated their significant potential in basic research, including the elucidation of poorly understood genetic functions. CRISPR has been used to identify essential genes in human cells and tumour-specific vulnerabilities.[3][4] CRISPR has also been used to re-programme adult somatic cells into stem cells [5] and to study the influence of epigenetics.[6]

The application of CRISPR-based genome editing is already leading to new opportunities in the development of improved research animal models of human disease, with the efficiency in the development of mouse or non-human primate models of disease being improved considerably.

Improved genome editing technology may play a key role in the field of xeno-transplantation and the use of animals as organ donors e.g. pigs in kidney and lung transplantation.[7] The editing of pig genes which could lead to rejection or infection in human recipients is being studied, including work on the possible genome-wide inactivation of porcine endogenous retroviruses (PERVs).[8]

The genetic modification of wild populations is considered a potentially very effective approach for reducing the impact of disease vectors and pathogens. The possibility of the development of a Cas9 mediated gene drive has, for example, been proposed for the population modification of the malaria vector, potentially blocking either the development of the insect vector or the reproductive capability of the mosquito.[9] Concern has been raised over the implications of the simplicity of such procedures for creating a CRISPR-Cas9-mediated gene drive, and the inherent risks for the wild population of an accidental or deliberate release.[10,11]

4.2 Clinical research and applications in somatic cells

Despite their promise and early success, the currently used genetic therapeutic technologies - gene therapy (which enables restoration of missing gene function by viral transgene expression) and RNA interference (RNAi) (which mediates repression of defective genes by knockdown of the target mRNA) - have a number of limitations that preclude their utility for a large number of diseases. Genome editing techniques based on programmable nucleases-ZFNs, TALENS and CRISPR/Cas9 are opening up the possibility of achieving therapeutic

[3] J.Osario.The genetic essence of human cells. Nature Reviews Genetics. 2015 Oct 27; 16, 683 doi:10.1038/nrg4037
[4] T.Hart *et al* High-Resolution CRISPR Screens Reveal Fitness Genes and Genotype-Specific Cancer Liabilities. Cell 2015 Dec 3; 163(6):1515-26. http://www.ncbi.nlm.nih.gov/pubmed/26627737
[5] S.E.Howden *et al*. Simultaneous Reprogramming and Gene Correction of Patient Fibroblasts. Stem Cell Reports. 2015 Dec 8; 5(6): 1109–1118. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4682122/
[6] H Ledford. Epigenetics: The genome unwrapped. Nature 528, S12–S13 (03 December 2015) http://www.nature.com/nature/journal/v528/n7580_supp/full/528S12a.html
[7] *Reardon S. New life for pig-to-human transplants.2015 [09/12/15]; http://www.nature.com/news/new-life-for-pig-to-human-transplants-1.18768.*
[8] Yang *et al.* Genome-wide inactivation of porcine endogenous retroviruses (PERVs) *Science 27 Nov 2015: Vol. 350, Issue 6264, pp. 1101-1104 http://science.sciencemag.org/content/350/6264/1101.full*
[9] Gantz V.M *et al. Highly efficient Cas9-mediated gene drive for population modification of the malaria vector mosquito Anopheles stephensi. December 8, 2015 vol. 112 no. 49 http://www.pnas.org/content/112/49/E6736.abstract*
[10] Oye K.A. *et al Science 17 Jul 2014 .Regulating gene drives http://science.sciencemag.org/content/early/2014/07/16/science.1254287*
[11] *DeFrancesco. L. (2015) Nature Biotechnology 33 1019-1021 , Gene drive overdrive*

genome editing in diseased tissues and cells, leading to the removal or correction of deleterious mutations or the insertion of protective mutations.[12]

The overview by Sangamo Biosciences of a number of the ongoing (commercially led) programmes for human somatic gene therapy using ZFN targeting illustrate that a wide range of diseases are being studied in humans at the research and pre-clinical stage. In addition to the studies for HIV/AIDS which are now at Phase II, other lead indications include Hunter Syndrome, Haemophilia, Gaucher Disease, Fabry Disease, Beta-thalassemia and sickle-cell disease.[13]

The FDA authorised the world's first human clinical trial for an *in vivo* genome editing application from Sangamo in December 2015. The Phase I/II open-label, dose escalation study will be in nine adult males with severe haemophilia.[14]

Following FDA approval early in 2015, the company is using its technology to disrupt the CCR5 gene in cells of an AIDS patient's immune system to make these cells permanently resistant to HIV infection. The aim is to provide a population of HIV-resistant cells that can fight HIV and opportunistic infections thereby mimicking the characteristics of individuals that carry the natural CCR5 delta-32 mutation. The study by Tebas *et al* demonstrated the safety and feasibility of inducing acquired genetic resistance to HIV infection in humans through the infusion of autologous CD4 T cells in which the CCR5 receptor had been rendered dysfunctional by ZFNs targeting.[15]

In November 2015, researchers at Great Ormond Street Hospital for Children in London treated a one-year old child with acute lymphoblastic leukaemia (ALL) using TALEN-modified T cells.[16] This *ex vivo* approach to gene editing was developed by researchers from the Paris-based company, Cellectis.

Despite the successes of ZFN and TALENS-based approaches, it is felt that CRISPR will prove to be an easier and more cost-effective way forward.

An alternative to using somatic cells is genome editing of autologous induced pluripotent cells (iPS cells) as a source of genetically corrected cells for transplantation. The potential advantage of this approach is that single edited iPS clones can be identified and sequenced fully to identify clones that have no off-target effects. Genomes of embryonic stem cells and reprogrammed human pluripotent stem cells (iPS) have been modified by CRISPR/Cas9. For example, the amplification of CGG triplets of the gene encoding for the protein FMR1 and responsible for the fragile X syndrome has been corrected in iPS cells derived from patients, then differentiated into neurons, thus demonstrating that amplification induces the methylation of the gene promoter and thus the gene silencing, whereas correction allows for its re-expression.[17]

Many *in vivo* gene editing studies are currently underway in mouse models of Duchenne muscular dystrophy using CRISPR. Work by Long *et al (*2016)*[18],*Tabebordar *et al* (2016)

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[12] Cox D.B.T *et al*. "Therapeutic genome editing: prospects and challenges." Nature Medicine 21, 121–131 (2015)http://www.nature.com/nm/journal/v21/n2/abs/nm.3793.html

[13] www.sangamo.com/pipeline/index.html

[14] http://investor.sangamo.com/releasedetail.cfm?ReleaseID=944828

[15] Tebas, P.*et al*. "The New England Journal of Medicine". 370: 901-910 (2014). "Gene Editing of CCR5 in Autologous CD4 T Cells of Persons Infected with HIV."
http://www.nejm.org/doi/full/10.1056/NEJMoa1300662#t=articleTop

[16] Leukaemia success heralds wave of gene-editing therapies
http://www.nature.com/news/leukaemia-success-heralds-wave-of-gene-editing-therapies-1.18737

[17] C.Y Park *et al* .Reversion of FMR1 Methylation and Silencing by Editing the Triplet Repeats in Fragile X iPSC-Derived Neurons. Volume 13, Issue 2, p234–241, 13 October 2015

[18] Long et al (2016). Postnatal genome editing partially restores dystrophin expression in a mouse model of muscular dystrophy. Science. 2016 Jan 22;351(6271):400-3 http://www.ncbi.nlm.nih.gov/pubmed/26721683

and Nelson *et al*[19] demonstrated restored dystrophin expression and improved muscle function in the animal model. This marks the first time that CRISPR has successfully treated a genetic disease inside a fully developed living mammal with a strategy that has the potential to be translated to human therapy.

Future developments in somatic cell-based therapy were discussed at the International Summit on human gene editing held in Washington in December 2015, which concluded that because such proposed clinical uses are intended to affect only the individual who receives them, they can be appropriately and rigorously evaluated within existing and evolving regulatory frameworks for gene therapy, and regulators can weigh risks and potential benefits in approving clinical trials and therapies.[20] Whether the existing regulatory framework for gene therapy (via the EMA's Advanced Therapy Medicinal Products assessment (ATMP) in Europe) will be fit for purpose for genome editing applications is unclear. Genome editing is somewhat different from traditional gene therapy, which employs viral vectors, suggesting that different approaches to safety assessment may be required.

4.3 European Regulatory Oversight of Advanced Therapy Medicinal Products

European Commission Regulation EC/1304/2007 on Advanced Therapy Medicinal Products - ATMP (gene, cellular and tissue-based) sets out the EU requirements for such therapies and standards for clinical trials. A single, centralised assessment procedure run by the European Medicines Agency covers safety, efficacy and quality.[21] The EMA's Committee on Advanced Therapies (CAT) addresses regulatory issues concerning gene therapy, regenerative medicine and somatic cell therapy. It also interacts with the EC DG Research, Science & Innovation on the inclusion of ATMP-related topics in future EU research programmes.

Ethical aspects are the responsibility of individual EU Member States (at a national and local level) but the EU Clinical Trials Directive 2001/20/EC and the Clinical Trials Regulation EU No 536/2014 (which comes into effect in May 2016) states that "… no gene therapy trials may be carried out which result in modification to the subject's germline genetic identity". It is not clear whether this restriction is solely focussed on intentionality. It has also been suggested that this restriction will also depend on the definition of a "clinical trial", for it is unlikely that any changes made to the human germline would or could be done as part of a conventional trial with controls, etc.

4.4 Clinical research and applications in germline cells

The possibility of genome editing being used to make genetic alterations in gametes or embryos, which will be carried by all of the cells of a resulting child and which will be passed on to subsequent generations as part of the human gene pool. Examples that have been proposed range from avoidance of severe inherited diseases to "enhancement" of human capabilities. Such modifications of human genomes might include the introduction of naturally occurring variants or totally novel genetic changes thought to be beneficial.

The publication, in April 2015, by Chinese scientists Junjiu Huang and colleagues that they had used CRISPR gene editing technique in human (non-viable) embryos to modify the mutated gene of β-globin responsible for the blood disorder β-thalassemia, stimulated much discussion on what research was acceptable and what should be appropriate jurisdiction in

[19] Nelson C.E *et al* In vivo genome editing improves muscle function in a mouse model of Duchenne muscular dystrophy.Science. 2016 Jan 22;351(6271):403-7
[20] http://www8.nationalacademies.org/onpinews/newsitem.aspx?RecordID=12032015a
[21] http://www.ema.europa.eu/ema/index.jsp?curl=pages/regulation/general/general_content_000296.jsp

this field.[22] The purpose of the research, using triploid and therefore non-transferable embryos, was to address a knowledge gap in the understanding of DNA repair mechanisms in human early embryos, and to determine to what extent the CRISP/Cas9 system would allow the replacement of the mutated gene of β-globin responsible for thalassemia.

The UK Human Fertilisation and Embryology Authority (HFEA)[23] recently approved a research application from the Francis Crick Institute to use genome editing techniques on human embryos.[24] The aim of the research, led by Dr Kathy Niakan, a group leader at the Institute, is to understand the genes human embryos need to develop successfully. The work carried out at the Crick will be for research purposes and will look at the first seven days of a fertilised egg's development (from a single cell to around 250 cells). This knowledge may improve embryo development after in vitro fertilisation (IVF) and might provide better clinical treatments for infertility, using conventional medical methods.

In April 2016, it was reported in Nature News that Professor Fredrik Lanner at the Karolinska Institute in Stockholm has also received ethical approval for planned research involving the use of CRISPR-Cas9 in human embryos to explore early human development.[25]

Professor Azim Surani, Director of Germline and Epigenomics Research at the Gurdon Institute, University of Cambridge, is using genome editing methodology to study human primordial germ cell development.[26]

In view of the difficulties and practical limitations of embryo editing, a number of groups have studied the potential utility of editing spermatogonial stem cells.[27,28]

5. The current international environment for genome editing research

The ongoing international debate on whether the current legislative/regulatory systems in place for human genome editing are fit for purpose has sought to make a clear distinction between the application of the technology in basic (pre-clinical) research and in clinical studies, and between its use in the development of somatic cell-based therapies (gene, cell and regenerative) and in particular its potential for reproductive/germline changes. (It has been argued that the future focus should be on whether there is a reproductive purpose or not, and not between somatic versus germ line, in view of the implications of advances in iPS technologies).[29]

5.1 Regulations on the use of embryos in research

One-cell-stage embryos play a key role in research into genome editing. However, for ethical reasons, many countries have strict regulations regarding the creation of human embryos for research. Most Member States of the EU have ratified the Convention for the Protection of Human Rights and Dignity of the Human Being with regard to the Application of Biology and Medicine: Convention on Human Rights and Biomedicine (The Oviedo Convention), which addresses research on embryos and *inter alia* prohibits the creation of human embryos for

[22] Liang P. *et al.* Protein Cell. 2015 May; 6(5):363-72. CRISPR/Cas9-mediated gene editing in human tripronuclear zygotes. http://www.ncbi.nlm.nih.gov/pubmed/25894090
[23] http://www.hfea.gov.uk/
[24] https://www.crick.ac.uk/news/science-news/2016/02/01/hfea-decision/
[25] http://www.nature.com/news/gene-editing-research-in-human-embryos-gains-momentum-1.19767?WT.mc_id=FBK_NatureNews
[26] http://www.gurdon.cam.ac.uk/research/surani
[27] D.A Fanslow et al 2014 PLOS ONE 9: e 112652. Genome Editing in Mouse Spermatogonial Stem/Progenitor Cells Using Engineered Nucleases. http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0112652
[28] Matthew H Porteus and Christina T Dann. Molecular Therapy 23, 980-982 (June 2015) "Genome Editing of the Germline: Broadening the Discussion" http://www.nature.com/mt/journal/v23/n6/full/mt201583a.html
[29] *Discussion at INSERM Ethics Committee Workshop on Ethics of CRISPR-Cas9. Paris 16 March 2016*

research purposes. As can be seen in the feedback from various European Academies of Medical Sciences (Appendix 1) and from the work of the Euro Stem Cell initiative, there is considerable variation across Europe in the national regulatory framework for the sourcing and use of embryos (and human embryonic stem cells).[30]

Countries including Lithuania, Slovakia and Poland have strict prohibitions against the creation of embryos for research purposes or cloning embryos for research purposes, structure their laws in a manner that classifies the embryo as a potential research subject (with the research having to benefit the embryo) and contain provisions for legal violations in their penal and/or medical codes.

Italy bans research on embryos, including the use of embryos to derive stem cell lines, and prohibits the creation of embryos for research purposes but researchers are permitted to use imported embryonic stem cell lines for research.

Through its Embryo Protection Act[31], Germany bans the importation, utilisation and derivation of stem cells in the country, but allows the importation of stem cell lines created from surplus IVF embryos before 2008, subject to various conditions and ethical guidelines. In Austria, although research on embryos, including the derivation of embryonic stem cell lines, is banned, as the use of imported embryonic stem cell lines was not addressed by the legislation, this is therefore permissible.

French legislation allows the use of surplus IVF embryos but prohibits the creation of human embryos for research and more specifically the creation of transgenic human embryos.

UK legislation allows the use of surplus IVF embryos for research and the creation of embryos for research purposes by IVF or cloning. Research on embryos that are older than 14 days is however prohibited. In a number of other countries embryonic stem cells can be derived legally from surplus embryos donated for (and no longer needed) for IVF treatment, e.g. Bulgaria, Czech Republic, Finland, France, Greece, Portugal and Sweden. In Finland, as in the UK, the use of such IVF- derived embryos is allowed for up to 14 days after fertilisation, whereas this is limited to seven days in Switzerland.

Such variations as summarised above, and the controversial nature of the national debates that led to such regulatory oversight on embryo use in research, would indicate the challenges around seeking any new pan-European framework. Legislative changes are possible – as demonstrated in France, where, following the changes to its legal and regulatory framework in 2013 it is now allowed to use spare human embryos from IVF laboratories in research.[32]

5.2 The regulatory environment for germline gene modification

As demonstrated in the surveys by Motoko Araki and Tetsuya Ishii[33] and by Rosario Isasi *et al*[34] into the genetic technology regulatory environment, the international legislative oversight of genome editing is somewhat complex and diverse. Although many countries (29 of the 39 surveyed) do ban human germline gene modification, it does not appear to be totally

[30] http://www.eurostemcell.org/stem-cell-regulations
[31] http://www.auswaertiges-amt.de/cae/servlet/contentblob/480804/publicationFile/5162/EmbryoProtectionAct.pdf
[32] Katherine Drabiak-Syed: New President, New Human Embryonic Stem Cell Research Policy: Comparative International Perspectives and Embryonic Stem Cell Research Laws in France. Biotechnology Law Rep. 2013 Dec 1; 32(6): 349–356. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3869533/
[33] M. Araki & T. Ishii (2014) International regulatory landscape and integration of corrective genome editing into in vitro fertilization. Reproductive Biology and Endocrinology (2014) 12:108 http://rbej.biomedcentral.com/articles/10.1186/1477-7827-12-108
[34] R. Isasi, E. Kleiderman and B. M. Knoppers. "Genetic Technology Regulation: Editing policy to fit the genome?" Science 22 Jan 2016: Vol. 351, Issue 6271, pp. 337-339

prohibited worldwide. The remaining 10 countries include the USA and those countries which were ambiguous about the legal status of the modification, including South Africa, Peru, Argentina, Russia, and from Europe - Iceland, Slovakia, and Greece.[35] . There is no formal legislation in place in the USA regulating the clinical application of germline gene editing, but with a temporary moratorium in place, no applications for funding of clinical research proposals for germline alterations will be accepted by the National Institutes of Health, nor the clinical trial accepted by the FDA. These constraints currently do not formally apply to organisations not seeking federal funding e.g. commercial organisations, charities, foundations or individuals, although it is unlikely that FDA endorsement would be forthcoming.

China, India, Ireland, and Japan forbid germline gene editing based on guidelines that are less enforceable than laws, and may be subject to amendment. The temporary legislation in Israel, which currently bans germline gene modification, may change. There are possible exemptions in the relevant law which may permit it upon the recommendation of an advisory committee.

The use of genome editing for reproductive purposes is illegal in the UK, and would require Parliamentary approval before it would be possible. However, although there is no legal ban on modifying the human germline in research *per se,* nothing is allowed without a licence from the Human Fertilisation and Embryology Authority (HFEA).

Belgium, Bulgaria, Canada, Denmark, Sweden, and the Czech Republic ban germline gene modification on the grounds that a modified gene may be inherited by offspring or that the gene modification may impair the human embryo. It is unclear whether genome editing-mediated germline gene correction would remain illegal in those countries if it were to be demonstrated that genome editing could more efficiently correct a mutation in the germline.[36]

5.3 The Council of Europe: EU Member States Ratification of the Oviedo Convention

The Convention for the Protection of Human Rights and Dignity of the Human Being with regard to the Application of Biology and Medicine Convention on Human Rights and Biomedicine (ETS No 164) was opened for signature in Oviedo (Spain) on 4 April 1997.[37] Article 13 of the Oviedo Convention prohibits intentional human germline modification but permits human genome modification only for preventative, diagnostic or therapeutic purposes. Thirty five Member States have signed the Convention, but only 29 of these have also ratified it and implemented the principles into their national laws. Six of those ratifying Member States have reservations limiting the extent to which they are bound to certain provisions (Croatia, Denmark, France, Norway, Switzerland and Turkey). Neither the UK nor Germany have either signed or ratified the Convention.

It is understood that the Convention was mostly designed to address developments in gene therapy and that it was drafted before iPS cells were first derived. This is of significance in the context of the Convention's relevance to genome editing, for such cells blur the distinction between somatic and germ cells, since it is now possible to derive germ cells from iPS cells that have been derived from somatic cells. Whilst the Convention is clear on some issues, for example around the creation of embryos for research and the deliberate passing on of genetic changes, there is a lack of clarity on a number of its definitions (e.g. germline) and on its relevance for basic research versus clinical research.

[35] https://static-content.springer.com/esm/art%3A10.1186%2F1477-7827-12-108/MediaObjects/12958_2014_1276_MOESM1_ESM.xlsx
[36] M. Araki & T. Ishii (2014) International regulatory landscape and integration of corrective genome editing into in vitro fertilization. Reproductive Biology and Endocrinology (2014) 12:108
[37] http://www.coe.int/t/dg3/healthbioethic/Activities/01_Oviedo%20Convention/

6. Relevance of calls for a moratorium on aspects of human germline genome editing

Although the publication of the paper by Huang *et al* in April 2015 on the use of CRISPR genome editing in human embryos added to concerns over the development of human germline engineering that could have an impact on the genome of offspring, a number of research groups had already recognised the potential (and anticipated ethical challenges) of using such editing in humans. There have subsequently been various proposals for a moratorium on such an application.

6.1 A call for "Asilomar 2"

A number of US-based scientists, some of whom were involved in the drafting of the 1975 Asilomar "moratorium" statement on recombinant DNA techniques[38], met early in 2015 to discuss the future potential for genome editing. Whilst acknowledging that there was a clear distinction between basic research and the clinical application of genome editing, the group suggested that there may be a need for a similar Asilomar moratorium in this space. They recommended that researchers should "Refrain from any modification of the germinal nuclear genome for therapeutic purposes until uncertainty regarding risks is clearly assessed and a broader consultation of this scenario has occurred, even if a therapeutic benefit appears to be expected....."[39] Throughout 2015 a number of other organisations either made similar calls for a "moratorium" on germline gene editing, or argued that in time there might be morally acceptable uses of this technology, and therefore such bans would not be beneficial.

6.2 A view from the gene therapy industry

Edward Lanphier, CEO of the gene therapy company Sangamo Biosciences, argued in "Nature Comment" in March 2015[40] that as "heritable human genetic modification poses serious risks, and the therapeutic benefits are tenuous", at this early stage scientists should agree not to modify the DNA of human reproductive cells. Whilst expressing concern over the potential public outcry over such research being exploited for non-therapeutic modifications, thus harming the development of non-heritable therapeutic approaches (including those being developed by Sangamo, and other regenerative medicine organisations), the paper was somewhat unclear over the need to distinguish research versus clinical applications.

6.3 Endorsement for a moratorium by the German Academies

The September 2015 statement by the German Academies on "The opportunities and limits of genome editing" endorsed calls for an international moratorium on "all forms of germline engineering that could have an impact on the genome of offspring". The paper argued that such a moratorium would provide an opportunity to discuss unresolved questions, develop recommendations for regulation, but should not lead to a restriction on methodological developments or limit any promising new genome editing approaches.[41]

6.4 The Statement of the Hinxton Group on genome editing technologies

The Hinxton Group is an International Interdisciplinary consortium on stem cells, ethics and law, established to explore the ethical and policy challenges of transnational scientific

[38] http://www.ncbi.nlm.nih.gov/pmc/articles/PMC432675/pdf/pnas00049-0007.pdf
[39] Baltimore.D. *et al*. "A prudent path forward for genomic engineering and germline gene modification." Science 03 Apr 2015: Vol. 348, Issue 6230, pp. 36-38 http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4394183/
[40] http://www.nature.com/news/don-t-edit-the-human-germ-line-1.17111
[41] www.leopoldina.org/uploads/tx_leopublication/2015_3Akad_Stellungnahme_Genome_Editing.pdf.

collaboration in human embryo and stem cell research. Membership includes expert representation from a wide range of countries including the UK, USA, Mexico, Germany, Israel, Spain, the Netherlands and Italy. In September 2015 the Hinxton Group issued a *"Consensus statement on Genome Editing Technologies and Human Germline Modification'*[42] which emphasised that "modern genome editing technologies and CRISPR-Cas9 in particular, are not only very precise, but also easy, inexpensive and critically, very efficient". The statement noted that while much of the focus of public discussion on genome editing has been on potential clinical applications, it is in basic scientific research where many of the more immediate and exciting developments will take place.

The signatories to the Hinxton Group Statement did not believe that sufficient knowledge was currently available to consider the use of genome editing for clinical reproductive purposes, but that "when all safety, efficacy and governance needs are met, there may be morally acceptable uses of this technology in human reproduction …" The Statement emphasised the importance to scientific and societal discussions on the use of genome editing of appropriate governance and oversight, and meaningful and substantial public engagement. It also called for a roadmap for research to establish the safety of genome editing for use in humans. Safety research is important both to clarify the extent and impact of off-target events (unintended genetic alterations) and mosaicism (variation across cells).

6.5 UK biomedical research funders joint statement on human genome editing

The Academy of Medical Sciences and a number of other medical research funders (including the Wellcome Trust, MRC, BBSRC and Cancer Research UK) published an initial supportive statement on genome editing in September 2015, in response to the calls for a moratorium on such research and its potential human applications.[43] The signatories consider that genome editing has a huge potential to improve health and that these advances should therefore be welcomed. The signatories support the continued use of genome editing in pre-clinical biomedical research and that (in the UK, within the confines of the Human Fertilisation and Embryology Act, 2008) this research may involve the use of somatic or germ cells, including human embryos up to 14 days old. The UK research funders recognise the longer-term potential of germ-line genome editing and the associated ethical and regulatory questions that will need to be considered.

6.6 The Wellcome Trust

Based in the UK, but global in focus, the Wellcome Trust (www.wellcome.ac.uk) is one of the leading charitable foundations and provides more than £800 million a year to support science, the humanities as well as education, public engagement and the application of research to medicine. The Trust was one of the key signatories of the initial statement made by UK biomedical research organisations in support of genome editing in human cells. The Trust has expressed concern over the impact on the research endeavour in this field of calls for an increasingly wide-ranging and "vague" moratorium on genome editing research, suggesting that this could lead to some researchers becoming vilified, and therefore not as open as they might be about their research, at a time when transparency and global cooperation is essential.[44]

The Trust considers that genome editing has huge potential to improve health and that advances in this field should be welcomed and encouraged. It is open to the longer-term therapeutic potential of germ-line genome editing and would like to see this possibility

[42] http://www.hinxtongroup.org/Hinxton2015_Statement.pdf
[43] http://www.acmedsci.ac.uk/more/news/human-genome-editing-research-should-proceed-say-leading-uk-science-bodies/
[44] Presentation at INSERM Ethics Committee meeting on CRISPR-Cas9 April 2016

explored as research and policy develops. The Trust's current perspective is that nothing should be ruled in or out at this stage.

6.7 The UNESCO International Bioethics Committee call for a moratorium on gene editing of the human germline

The UNESCO Universal Declaration on the Human Genome (Art 24) proposed that human germline interventions "could be contrary to human dignity" (and so therefore should be prohibited).[45]

In October 2015, at its 22nd Session, UNESCO's International Bioethics Committee (IBC) published a report 'Updating its Reflection on the Human Genome and Human Rights'.[46] This was done in the light of genome editing and other developments. The report, written in the light of developments in *inter alia* genome editing acknowledged that "gene therapy could be a watershed in the history of medicine and that genome editing is unquestionably one of the most promising undertakings of science for the sake of all humankind". The IBC report cautioned that "this development seems to require particular cautions and raises serious concerns, especially if the editing of the human genome should be applied to the germline and therefore introduce hereditary modifications, which could be transmitted to future generations".

The IBC called for a moratorium on gene editing of the human germline until the safety and efficacy of the procedures were adequately proven as treatments, and called on states and governments to:

1. produce an international, legally binding instrument to ban human cloning for reproductive purposes
2. renounce the possibility of acting alone in relation to engineering the human genome and accept to cooperate on establishing a shared, global standard for this purpose
3. encourage, through the means of national legislation as well as international regulations, the adoption of rules, procedures and solutions, which can be as non-controversial as possible, especially with regard to the issues of modifying the human genome and producing and destroying human embryos.

The IBC report is somewhat unclear about using genome editing for research on human embryos and germline cells. Furthermore, on publishing its updated report the IBC did not take the opportunity to address the differences between basic research on human embryos and clinical applications.

6.7 The view from the International Summit on Human Gene Editing

Included in the final conclusions arising from the International Summit held in Washington in December 2015 (organised under the auspices of the (US) National Academy of Sciences, the National Academy of Medicine, the Chinese Academy of Sciences, and the Royal Society of the UK) was the statement that (i) it would be irresponsible to proceed with any clinical use of germline editing unless and until the relevant safety and efficacy issues have been resolved, based on appropriate understanding and balancing of risks, potential benefits, and alternatives, and (ii) there is broad societal consensus about the appropriateness of the proposed application. It was considered that any clinical use should proceed only under appropriate regulatory oversight. In the statement from the Summit it

[45] http://www.unesco.org/new/en/social-and-human-sciences/themes/bioethics/human-genome-and-human-rights/
[46] https://en.unesco.org/news/unesco-panel-experts-calls-ban-editing-human-dna-avoid-unethical-tampering-hereditary-traits?language=en

was acknowledged that as scientific knowledge advances and societal views evolve, the clinical use of germline editing should be revisited on a regular basis.[47]

6.8 The European Group on Ethics in Science

The European Group on Ethics in Science and Technologies (EGE)[48] was established originally as an independent advisory body of the President of the European Commission. Its work was to have been finished in January 2016 but it has since moved to the EC's DG Research and reports to the Research Commissioner. On 11 January EGE issued a statement on gene editing in which the group cautioned against reducing the debate to safety issues and the potential health risks or health benefits of gene editing technologies. There was also a need for a consideration of 'human dignity, justice and proportionality'. EGE noted that gene editing of somatic cells was currently in clinical development for a variety of conditions, and that this was different and distinct from germline gene modification. EGE's paper did not support a clear-cut distinction between a possible moratorium on research with a clinical application as opposed to related basic research. Some EGE members considered that all research into human germline gene modification for reproductive purposes cannot be ethically justified and endorse the need for a moratorium on any basic research involving human germline gene modification until a regulatory framework is in place. Other EGE members consider that some aspects of such research are currently justified.

7. Cross-sector European perspectives of human genome editing

7.1 The funding of genome editing research by Horizon 2020 and the European Research Council

The European Commission will acknowledge that it has no formal competence to harmonise the legal situation in Member States concerning genome editing, or the use of embryos or stem cells in research, as this is a matter for individual Member States, but it is able to restrict how the EU research funds are used.

All applicants for funding by the European Research Council (ERC) to carry out research using the CRISPR-Cas9 technology will be considered by the Ethics Review process of the ERC Executive Agency (ERCEA). In addition to having to comply with the additional restrictions raised by national legislation, applicants to ERC will be aware that the EC's Horizon 2020 funding regulations[49] prohibit:

- research activities aimed at human cloning for reproductive purposes
- research activities intended to modify the genetics of human beings that could make such changes heritable
- research activities intended to create human embryos solely for the purposes of research or stem cell procurement, including the technique of somatic cell nuclear transfer See Article 19(3) of the Horizon 2020 Framework Programme Regulation (EU) No 1291/2013.
- research that may "kill embryos".

A recent assessment by Professor Maria Filipa Ferraz de Oliveira (DG Research, ERC Executive Agency) on research programmes involving CRISPR-Cas9 [50] clearly

[47] http://www8.nationalacademies.org/onpinews/newsitem.aspx?RecordID=12032015a
[48] https://ec.europa.eu/research/ege/index.cfm
[49] Article 19(4) of the Horizon 2020 Framework Programme Regulation (EU) No 1291/2013.
[50] Reported at the INSERM Ethics Committee workshop on genome editing (16 March)

demonstrates the rapid growth in the research endeavour in this space in the EU. The first projects involving the technology only went for ethical review by the ERC less than two years ago, with just one application to the Starting Grant Call 2014. However, during last year, 26 projects involving the use of CRISPR-Cas9 sought funding under the ERC's 2015 Advanced Grant call. An examination of the types of cells involved in such recent applications showed that 17 projects will be using animal cells, 16 human somatic cells, but six projects planned to use either human embryonic stem cells or human induced pluripotent stem cells or animal embryonic stem cells.

Any research seeking EC funding to use gene-editing in human cells would, if via human stem cells (both adult and embryonic), have to comply with the specific national legislation in place in individual EU Member States, as well as general EU Directives that govern the acquisition, storage, and use of human tissues and cells. No activity will be funded in a Member State where such activity is forbidden, and no funding will be granted for research activities that are prohibited in all Member States.

In addition, research involving animals in genome editing studies will need to comply with Directive 2010/63/EU on the Protection of Animals Used for Scientific Purposes, as well the various Directives and Regulations on the Contained Use, Deliberate Release and Transboundary Movement of GMOs (2001/18/EC, 2009/41/EC and Reg EC 1946/2003. Under the EU's Precautionary Principle policy, researchers may also be required to have conducted an assessment of predictable risks and potential benefits before the start of any project and implement any necessary safety measures.

7.2 The provision of advice to the European Parliament on life sciences: STOA

The Office for Scientific and Technology Option Assessment (STOA) was established to provide the European Parliament's Committees and other parliamentary bodies concerned with independent, high-quality and scientifically impartial studies and information for the assessment of the impact of possibly introducing or promoting new technologies and identifying, from the technological point of view, the options for the best courses of action to take. Assessing new developments in health and new technologies in the life sciences is a current priority for STOA, and whilst it is understood that the Office is aware of the ongoing international discussions concerning genome editing, it does not appear that there are any immediate plans to advise Parliamentarians on the matter.

7.3 Current activities of the European Academies

7.3.1The position of the German Academies on genome editing in Europe

In September 2015 the Leopoldina (The National Academy of Sciences), acatech (The National Academy of Science of Science and Engineering), the Union of the German Academies of Sciences and Humanities, and the German Research Foundation (DFG) released a joint statement on this topic which describes how genome editing works, its current stage of development, its field of application and its advantages over conventional gene modification.[51] In addition to the statement by the Leopoldina *et al*, a more detailed review of the German perspective on human genome editing was carried out in the recent analysis by an expert group of German scientists from the Berlin-Brandenburg Academy of Sciences and Humanities (BBAW). The paper analyses the 'loopholes' and inconsistencies in the current German Embryo Protection Act with regard to germline interventions.[52]

[51] 'The Opportunities and Limits of Genome Editing'. www.leopoldina.org/uploads/tx_leopublication/2015_3Akad_Stellungnahme_Genome_Editing.pdf.
[52] 'Human genome surgery – towards a responsible evaluation of a new technology' www.gentechnologiebericht.de/bilder/BBAW_Human-Genome-Surgery_PDF-A1b-1.pdf).

7.3.2 Development of an EASAC position on the wider aspects of genome editing

The European Academies Science Advisory Council (EASAC) formed by the national science academies of the EU Member States (www.easac.eu) is in the process of establishing a Working Group on Genome Editing, with representation from its academies. FEAM representation on this initiative will be from the Belgian Royal Academy of Medicine. The proposed scope of the EASAC review will be quite broad, and in addition to assessing the contribution of genome editing to fundamental research will examine issues for a range of applications relevant to human health and other societal priorities.

7.3.3 Review by the French National Academy of Medicine

A Working Party of the French National Academy of Medicine has been established to review the full range of issues concerning the genetic modification of human germinal cells and embryos in the light of recent technological developments (CRISPR etc). The group's work on: the identification of potential medical indications of the new molecular genetics techniques; the possible risks and uncertainties in their use; and the ethical issues they raise, is ongoing. Some of the main conclusions that have been raised to date include the importance of continued support for scientifically relevant basic and pre-clinical research in this field, including that on germ cells and human embryos, particularly for a better knowledge of mechanisms regulating gametogenesis and any anomalies affecting the early development of the embryo.

Changes to the French legislation to allow the modification of the genome of germ cells or an embryo leading to the birth of a child would not be supported at this time.

7.4 Developing European perspectives on the ethical issues

7.4.1 The Nuffield Council on Bioethics

The UK based Nuffield Council on Bioethics has established a Working Group to oversee its review of the impact of new developments in genome editing.[53] The review is in two parts, the first of which (due to report in summer 2016) will address conceptual and descriptive issues relating to the impact of genome editing technology. The first part is broad, taking in microorganism, plant, animal and human applications. It will explore the basis and scope of public interest in different uses of genome editing and the grounding of distinctions between morally unacceptable, morally acceptable and morally desirable uses. From among the wide range of applications for genome editing the initial report will identify priority areas for further consideration. The second part of the project will then address practical, normative questions for a priority field of application identified in the first part, looking at the how genome editing technologies should be used to respond to a defined set of challenges and the way their use might transform the set of challenges in return.

7.4.2 Development of advice by the Ethics Committee of INSERM

The Ethics Committee of INSERM - the French National Institute for Health and Medical Research - was tasked by INSERM to review developments in the application of CRISPR technology and to identify:

- What were the new ethical issues raised by such technology?
- Did the speed of its development raise any special problems?
- Did the ease of its application require the supervision of its implementation in the laboratory?

[53] http://nuffieldbioethics.org/project/genome-editing/#sthash.gID3e6uS

In providing its recommendations on the matter in February 2016[54], the Committee considered that the establishment of any international moratorium of the use of genome engineering at this time was implausible, but that the prohibition of all germinal nuclear genome modifications for reproductive purposes in humans should continue to be respected at present. In its response to INSERM, the Committee recommended that any calls to change the French legislation on this matter should be refused until uncertainties concerning all risks are clearly evaluated and only when there has been a broader consultation with multiple partners from civil society. The INSERM Ethics Committee hosted a workshop for European-based experts on this topic on 16 March and it is planning further external activities in Europe to raise awareness of the issues.

7.5 The importance of the European patients' perspectives on genome editing

The Patients Network for Medical Research and Health EGAN (www.egan.eu) is an alliance of both National Genetic Alliances and European disease specific patient organisations with a special interest in genetics, genomics and biotechnology. Especially, but not only, genetic disorders are represented within EGAN.

The UK-based patient group – Genetic Alliance UK – has been following developments in this field very closely and has sought to explain CRISPR-Cas9 genome editing to its members. It is currently carrying out a survey to gather views on gene editing techniques from those affected by genetic conditions.[55] This is being funded under a European Commission funded project (NERRI) investigating the public perception of innovative health-related technologies. The results of the survey will be available in the near future.

7.6 Ongoing commercial development of genome editing technologies

Most of the commercial developments concerning genome editing appear to be taking place in the USA - with a few exceptions in Europe.The US-based Sangamo Biosciences has a number of potential therapeutic approaches in the pipeline based on its zinc finger nuclease (ZFN)-mediated genome editing technology.[56] Following FDA approval early in 2015, the company is using its technology to disrupt the CCR5 gene in cells of an AIDS patient's immune system to make these cells permanently resistant to HIV infection. The aim is to provide a population of HIV-resistant cells that can fight HIV and opportunistic infections thereby mimicking the characteristics of individuals that carry the natural CCR5 delta-32 mutation. The FDA authorised the world's first human clinical trial for an *in vivo* genome editing application from Sangamo in December 2015. The Phase I/II open-label, dose escalation study will be in nine adult males with severe haemophilia.[57]

Recent developments in Europe in this field include the treatment (under a special licence from the UK MHRA) of a young leukaemia patient with the French-based company Cellectis' TALEN gene-edited allogeneic UCART product candidate.[58]

Collaborative research agreements have been established between the French-based gene therapy company Genethon and CRISPR Therapeutics headquartered in Basel, Switzerland to accelerate gene therapy research programmes.[59] German-based Bayer have also recently announced a joint venture with CRISPR Therapeutics in order to develop and commercialise therapeutics for blood disorders, blindness, and congenital heart disease.[60]

[54] http://www.inserm.fr/mediatheque/infr-grand-public/fichiers/l-ethique-a-l-inserm/crispr-saisine-cei-fr-fevrier-2016
[55] http://www.geneticalliance.org.uk/genome-editing-.htm
[56] www.sangamo.com/pipeline/index.html)
[57] http://investor.sangamo.com/releasedetail.cfm?ReleaseID=944828
[58] http://www.cellectis.com/sites/default/files/cellectis_pr_151105_0.pdf
[59] http://crisprtx.com/news-events/news-events-press-releases-2015-12-18.php
[60] http://www.albanydailystar.com/science/dna-editing-revolution-bayer-will-invest-325-million-euros-to-crispr-therapeutics-chula-vista-daily-science-14615.html

Larger well-established pharmaceutical companies are adapting the technology to their own in-house research programmes as well as seeking external collaborations. Most of these collaborations are with US-based genome editing based start-up companies established by the early pioneers of CRISPR-Cas9 such as Editas (co-founded by Feng Zhang (MIT) and Jennifer Doudna (University of California)); Caribou (co-founders Jennifer Doudna and Martin Jinek (University of Zurich)), and CRISPR Therapeutics.

Some examples of company activity in Europe include those of Novartis and Astra Zeneca. It has been reported that Novartis, with its main research activities in Cambridge, Massachusetts and Switzerland, has adopted CRISPR to research potential gene therapies and to identify drug targets. CRISPR is being used to investigate thousands of genes related to cancer as potential drug targets. CRISPR is enabling this to be done more quickly, precisely and relatively cheaper than other methods, and this is aiding decisions on which drug targets should advance to drug discovery projects. Collaborative agreements have been established with Intellia Therapeutics and Caribou Biosciences using CRISPR genome editing technology for the discovery and development of new medicines, and for the development of new drug discovery tools.[61]

AstraZeneca announced in January 2015 that it had established four external research collaborations in the application of CRISPR for the identification and validation of new drug targets in pre-clinical models that closely resemble human diseases. These collaborations are with the Wellcome Trust Sanger Institute, Cambridge, UK, the Innovative Genomics Initiative, California and the Broad Institute/Whitehead Institute.Astra Zeneca and the Sanger Institute co-hosted an international conference on CRISPR in the UK in January 2016 on the application of CRISPR technology to the understanding and treatment of human disease – addressing key themes such as recent advances in genome editing technology, challenges to progressing disease models and CRISPR-based genome-wide screening.The conference also showed many ways in which CRISPR was already making a difference in pre-clinical studies in traditional drug development.[62]

[61] https://www.novartis.com/news/media-releases/novartis-collaborates-intellia-therapeutics-and-caribou-biosciences-explore
[62] https://www.genomeweb.com/gene-silencinggene-editing/sanger-astrazeneca-host-crispr-conference-focus-human-biology-public

APPENDIX 1

A REVIEW OF REGULATORY GOVERNANCE FOR GENOME EDITING IN EUROPE

In preparation for the Paris workshop on human genome editing, members of FEAM and other Academies of Medical Science within Europe were asked for their expert views on the regulatory and policy environment for the development of genome editing for human therapeutic applications in their country. Feedback on this matter was based around the proposed main themes of the workshop:

- Basic and pre-clinical research
- Clinical use of genome editing in somatic cells
- Clinical use of germline genome editing.

Some of the information of current legislation summarised here may not be directly relevant to genome editing *per se* but it has been included as it helps to illustrate a number of the internal inconsistencies in countries that seek to regulate such new technologies.

(In summarising such comprehensive feedback from the Academies of Medical some factual errors may have been introduced. These will be corrected upon notification.)

A.1 BASIC AND PRE-CLINICAL RESEARCH APPLICATIONS

The United Kingdom

The Human Fertilisation and Embryology Authority (HFEA) is the UK's statutory regulator of assisted conception and human embryo research. The HFEA was established by Parliament through the Human Fertilisation and Embryology Act of 1990. HFEA regulates and (for certain issues) allows the creation and use of use of embryos in research (under the auspices of a specific HFEA Licence) only up to 14 days after fertilisation, or before the establishment of the primitive streak should this occur before 14 days.[63,64] Within these confines, genome editing can only be done in a research context. Any genetically modified embryos would be classed as "non-permitted" which means they could not be implanted into a woman.

In the UK the regulatory oversight for stem cell use is provided by the Human Tissue Authority (HTA), the HFEA (as they are obtained from embryos), the Medical Research Council's UK Stem Cell Bank and its steering group, and the Medicines and Healthcare Products Regulatory Agency (MHRA). The UK Stem Cell Toolkit is a regulatory tool for those seeking to use human stem cells for research.[65]

In September 2015 the HFEA received an application from researchers at the Francis Crick Institute (Dr Kathy Niaken) to use genome editing (CRISPR) in a research project using human embryos to understand the genes human embryos need to develop successfully, and to examine causes of reproductive failure.

It was proposed that the knowledge gained from the research might ultimately benefit patients without necessarily requiring treatment using genome editing. The research licence

[63] http://www.hfea.gov.uk/161.html
[64] http://www.hfea.gov.uk/docs/07032016_Currently_licenced_research_projects.pdf
[65] http://www.sc-toolkit.ac.uk/home.cfm

was approved on 14 January 2016, subject to the project obtaining appropriate research ethics committee approval.

The UK regulatory environment for such research is carefully constructed to ensure cautious progression of complex and sensitive issues. The importance of the science and any potential clinical benefits is critical to the regulation of such research. Ethical considerations and public opinion (including that of patients) are important to provide incentives for any change in regulations.

Spain

It is possible to carry out research with stem cells, gametes and embryos within the limits of the Assisted Human Reproduction Techniques Act (14/2006) and the Biomedical Research Act (14/2007). These laws do not allow the creation of human embryos for research, but do allow the use of donated embryos whose development is not more than 14 days. Experimental embryos are not allowed to be implanted. There will be gaps in the legislation, for being over 10 years old, the laws did not foresee such technologies as genome editing. The limits in both laws (Assisted MRTA and BRA) are somewhat unclear – there are no guidelines *per se*. All research must be approved by local ethics committees or the National Commission for Assisted Reproduction of the National Bioethics Committee. The main driving force for decisions on regulations include scientific societies, hospitals and research centres, ethics committees and public opinion.

Germany

There is currently no research planned in Germany that will use genome editing techniques in human embryos. Genetic engineering in general is regulated by the German Genetic Engineering Act which *inter alia* seeks to protect the life and health of people, environment, plants and animals against harmful effects resulting from genetic manipulations. It also seeks to create the legal framework for the exploration, development, use and promotion of the scientific, technical and economic aspects of genetic technology.[66]

The use of embryos for research is prohibited in Germany as outlined in the Embryo Protection Act 1991[67], which makes the derivation of embryonic stem cell lines a criminal offence. The 2002 Stem Cell Act does not allow the generation of embryos for research. The embryo is also protected under the German Constitution. The Basic Law also protects the freedom to pursue science and research. German law gives priority to adult stem cells under the 2002 Stem Cell Act, but the importation of embryonic stem cell lines into Germany is permitted under strict conditions, including the fact that the imported ES lines must have been derived before May 2007. Embryonic stem cell lines can only be used for research if they are vital in developing new medical and scientific knowledge.

The Central Ethics Commission for Stem Cell Research provides the necessary approval for the importation of stem cell lines. The German National Ethics Council provides advice and issues opinions on the wider medical and scientific issues affecting society and human health. Pre-implantation genetic diagnosis became officially legal in 2011 after a judgement of the Federal Court of Justice demonstrated that the law did not prevent doctors from the genetic screening of artificially produced embryos before transferring them to a woman.

[66] http://www.bvl.bund.de/EN/06_Genetic_Engineering/genetic_engineering_node.html;jsessionid=3833FE261B0A5C5A1EC81ADF3AC6B134.2_cid350#doc1414308bodyText1

[67] http://www.eurostemcell.org/regulations/regulation-stem-cell-research-germany

France

There have been a number of significant changes to the legislative framework for genome research in France over the last 20 years, particularly that involving the use of human embryos and human embryonic stem cells.[68] In 1994 France adopted comprehensive bioethics legislation for the first time which banned both the creation of embryos for research and the experimentation on embryos. Subsequent changes to the legislation (2004, 2011) maintained this general prohibition, but provided an exception that would permit research on embryos under an approved set of conditions, including the use of unneeded cryopreserved embryos from IVF laboratories.[69] In August 2013 the Senate and National Assembly adopted Law 2013-715 which now permits embryo and hESC research by general authorisation, rather than via an exemption process.

The Agence de la Biomédecine (ABM) is the French statutory regulator of assisted reproduction and human embryo research. The ABM was established by Parliament through the Act of 2004. All research protocols including human embryos or human embryonic stem cells must be authorized by ABM.

The French legislation is now considered to be generally supportive of basic research in this field and does appear to distinguish between any interventions aimed at modifying the genetic characteristics of the offspring, and research which does not directly lead to the birth of a child whose genetic characteristics would have been modified. It is felt though that there is still some ambiguity between the provisions of the Public Health Code and the French Civil Code.

- Article L2151-5 of the Public Health Code authorises embryo research with authorisation and under conditions.
- Article L 2151-2 forbids the creation of transgenic embryos.
- Article 16-4 of the French Civil Code (paragraph 3) stipulates that "without prejudice to research aimed at preventing and treating genetic diseases, no alteration may be made to genetic characteristics for the purposes of modifying the person's descendants".

Sweden

The Law on Genetic Integrity (2006: 351) regulates research on human eggs. Such research requires consent from both egg and sperm donors. Experiments on fertilised eggs and on eggs submitted to nuclear transfer are only allowed for up to 14 days after fertilisation or nuclear transfer. After this the eggs have to be destroyed. A fertilised egg may be stored frozen for a maximum of five years. No fertilised egg submitted for such research may be introduced into a woman's body.

In its July 2015 statement (a "SMER Comment") on the subject of CRISPR-Cas9 and the possibility to edit the human genome, the Swedish National Council on Medical Ethics stated that research on embryos with the technique may also provide useful results, e.g. regarding infertility or stem cell therapy. According to the Council, the current regulation in Sweden is well balanced.[70] In April 2016, it was reported in Nature News that Professor Fredrik Lanner

[68] Katherine Drabiak-Syed "New President, New Human Embryonic Stem Cell Research Policy: Comparative International Perspectives and Embryonic Stem Cell Research Laws in France" http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3869533/
[69] Research on embryos and embryonic stem cells. French Code of Public Health ; Articles L2151-1 to L2151-8 (2011)
[70] http://www.smer.se/news/smer-comments-the-technique-crispr-cas9-and-possibilities-to-edit-the-human-genome/

at the Karolinska Institute in Stockholm has received ethical approval for research involving the use of CRISPR-Cas9 in human embryos to explore early human development.[71]

Switzerland

Article 119/a of the Constitution of the Swiss Federation (overseen by the Federal Office of Public Health – BundesamtfűrGesundheit) strictly prohibits cloning and genetic modifications of human germline cells and embryos for any purpose. The creation of human embryos for research is not allowed.

The National Ethics Committee provides opinion, but Switzerland practices a form of direct democracy through which it is inevitable that the public decides. The main driving force in Switzerland for decisions on regulations of this type of research is public opinion. The constitutional ban on genetic modifications was enacted as a consequence of a popular referendum on 12 March 2000. The use of Pre-implantation Genetic Diagnosis, which was not allowed in Switzerland, was put to a public vote in 2015 and it was finally approved.[72]

Estonia

In Estonia unused embryos from IVF may be used for research with the consent of the germ cell donors. It is not allowed to transfer embryos used for scientific research to female recipients.

The research is regulated by the Act on Artificial Fertilisation and Embryo Protection. The Estonian Parliament has ratified the Oviedo Convention and thus the creation of human embryos for research is not allowed. The regulatory framework is overseen by bodies such as the Estonian Council on Bioethics and the Tallinn Medical Research Ethics Committee.

Czech Republic

Ethics approval and public opinion are the main driving forces in the regulatory oversight of such research. The creation of and use of human embryos in any experimental work is absolutely forbidden by law. Contrary to any experimental work on human embryos, embryos of animal origin can be used experimentally without any legal restrictions, and the genetic manipulation of whole animal embryos is allowed. The legislation states that embryonic stem cells of human origin (possibly originating from unused embryos from IVF centres) may be used for appropriate experimental work, and this is not prohibited- but controlled and strictly registered. Genetic modifications have to be reported to the local GMO authority. The Committee for the Supervision of GMO work is affiliated with the Ministry of the Environment.

There is a list of requirements regulating who can work with human stem cells. Nevertheless, human embryonic stem cells can only be used as cell lines (either established or short-term). Human embryonic cells cannot be used for further cloning or cultivation of embryos. This is under strong legislative control with severe penalties for trespassing. There is no research planned on genome editing techniques in embryos.

There are technological developments taking place in basic research on both somatic and stable embryonic stem cell lines.

[71] http://www.nature.com/news/gene-editing-research-in-human-embryos-gains-momentum-1.19767?WT.mc_id=FBK_NatureNews

[72] http://www.swissinfo.ch/directdemocracy/genetic-screening_pre-implantation-diagnosis-to-be-allowed/41485136

Lithuania

The Law on Ethics of Biomedical Research (Article 3) prohibits:

- The creation of human embryos for the purposes of biomedical research
- Research on human embryos or human foetuses during which or after which a human embryo or human foetus is destroyed or a human embryo is not placed into a woman's uterus
- The import and export of tissues of a human embryo, embryonic stem cells and lines or tissues of a foetus and the stem cells taken from them (but this does not apply to stem cells taken from umbilical cord of placenta after the birth of a child, and the samples taken for genetic research)

Biomedical research involving modifications of the human genome may only be carried out for preventive, diagnostic or therapeutic purposes and only in the cases when it is not intended to modify the genome of descendants.

The supervision of such biomedical research is a responsibility of research ethics committees – either the Lithuanian Bioethics Committee (the main driving force for all decisions) or regional biomedical research ethics committees.

Italy

In 2001 the Italian Parliament ratified the Oviedo Convention. The derivation of embryonic stem cell lines is banned in Italy, as well as therapeutic and reproductive cloning**,** but it is permitted to use imported embryonic stem cell lines for research.

Law No. 40/2004 on standards in the field of assisted procreation prohibits any kind of research on embryos, as the latter must be considered as an activity contrary to the embryo's interests, with the sole exception of research that might (potentially) have a benefit for its health. Therefore, it prohibits embryo manipulation, germline modification and human cloning for reproductive or therapeutic research purposes.

Whilst Article 13 (experimentation on human embryos) of the Italian law on assisted reproductive technologies clearly prohibits any experimentation and research on human embryos, there is one exception to this ban in that research aimed at pursuing therapeutic and diagnostic purposes, intended to protect the health and development of the embryo on which it is performed, is permitted when no other alternative methods are available.
In June 2015 the Constitutional Court of Italy, lifted the ban preventing fertile couples, known to be carriers of severe genetic diseases, from accessing pre-implantation genetic diagnoses (PGD). There is however still no regulation with respect to specific methods to access PGD.
In 2015 the European Court of Human Rights acknowledged that banning a woman (in Italy) from donating embryos obtained from *in vitro* fertilisation to scientific research was not contrary to respect for her private life. The Court noted that there was no European consensus regarding the donation of embryos not destined for implantation.

A 2009 Ministerial Degree that confined research funding to tissue (adult) stem cell research, thus excluding embryonic stem cell research, has so far been unsuccessfully challenged by a number of Italian scientists.

Greece

The Oviedo Convention has been ratified by Greece (Law 2619/1996) and hence genetic research is governed by such legislation and by other laws on medically-assisted reproduction (3305/2005). Genetic manipulation of germinal cells is explicitly prohibited by

Article 13 of the Oviedo Convention. Research on embryos *in vitro* (including the use of embryonic stem cells) is allowed if they are surplus embryos, not used for reproductive purposes, and consent has been obtained from the gamete donors. If no consent exists the embryos can be used after five years in storage. The creation of human embryos for research is not allowed.

The regulation is overseen by the Independent National Authority on Medically Assisted Reproduction. The Hellenic National Bioethics Commission has adopted an opinion on stem cell research. In addition to the two organisations above, the other driving forces for decisions on the regulation framework are the National Organisation for Medicines and the Ministry of Health.

Norway

Ethics and politics are the main driving forces for decisions about regulation of this kind of research. These questions are on the political agenda with different political parties being liberal or restrictive in their general attitude. Ethical discussions regarding biotechnology have been “institutionalized” through a separate Biotechnology Advisory Board (an independent advisory body to the Norwegian Government), with a broad involvement of lay people that are supposed to provide recommendations for the government on such issues.

It is understood that at present there is no research planned in Norway that will use genome editing techniques in human germinal cells or embryos. The creation of human embryos for research purposes is illegal. The use or genetic manipulation of germinal cells, stem cells, or embryos for research is regulated by the Norwegian Law on Biotechnology (LOV-2003-12-05-100), and the Norwegian Law on Gene technology (LOV-1993-04-02-38). In general, research on fertilized eggs is prohibited, unless it is aimed at improving IVF success rates or technologies for prenatal diagnostics or research to gain knowledge that can lead to future treatments for serious diseases. However, this may change as the law currently is being revised.

Current Norwegian legislation (The Biotechnology Act) prohibits the introduction of genetic changes that can be inherited in humans. This has been interpreted as a ban on any genetic modification of human germ cells or embryos, even for basic research.The Norwegian Biotechnology Advisory Board voted on 12 January 2016 to allow ‘germline editing for basic research’. According to the statement this will only be permitted using supernumerary embryos donated by couples having IVF treatment, and only using embryos up to 14 days old. The Advisory Board recommended that the current ban should not include basic research where the germ cells or embryos are not used to establish a pregnancy. The Advisory Board recommended that the same restrictions should apply to research involving germline editing. Such research should not involve the development of methods for the clinical application of germline editing.

A.2 THE CLINICAL USE OF GENOME EDITING IN SOMATIC CELLS

The United Kingdom

The editing of somatic cells for basic research or in a clinical context is overseen by the Human Tissue Authority (HTA), created by the Human Tissue Act 2004, which regulates matters relating to human bodies, organs and tissues for research and transplantation. The clinical application of somatic cell therapies, including those based on genome editing is regulated by the HTA and licensed by the Medicines and Healthcare Products Regulatory Agency (MHRA), under the scope of the Advanced Therapy Medicinal Products legislation. The Gene Therapy Advisory Committee (GTAC) is a research ethics committee that is

overseen by the UK Health Research Authority and considers proposals for clinical studies involving gene therapy and stem cells.

It is not thought that any of these organisations are developing any specific CRISPR-related gene therapy policies.

The treatment of a young patient with acute lymphoblastic leukaemia (ALL) using TALEN-modified T cells at the Great Ormond Street Hospital for Children, in London, was allowed after 'compassionate use' (approval under the MHRA special licence arrangements) had been obtained. This enabled the child to be treated with this unlicensed investigational medicinal product in which all other treatments had failed.

France

The different categories of cell therapies are governed by different regulatory statuses. Clinical research protocols are authorised by national (The National Agency for the Safety of Medicine and Health Products (ANSM), and European bodies.[73]

Switzerland

Clinical trials of somatic cell therapy have been conducted in Switzerland since 1994, subject to authorisation. One of the main driving forces for decisions on regulation is public opinion. The regulation of somatic gene therapy is divided into *in vivo* and *ex vivo* gene therapies:

In vivo gene therapy trials, i.e. trials in which the genes to be transferred are directly inserted in the patient's body by means of vectors, are governed by the Federal Law on Therapeutic Products which came into force on 1 January 2002, and specifically by Section 5 of the Ordinance on Clinical Trials of Therapeutic Products. The Swiss Agency for Therapeutic Products (Swissmedic) is responsible for approving experimental gene therapy and also submits the dossier for consideration to the SECB, the Federal Office for the Environment (FOEN) and the Federal Office of Public Health (FOPH). The SECB issues Statements on the biological safety of the preparation for the proband as well as for human beings and the environment in general.

Ex vivo gene therapy trials, i.e. trials in which the therapeutic gene is transferred in vitro to cells or tissue before insertion in the patient's body, are governed by the Federal Ordinance on Transplantations, and require approval by the FOPH. The SECB is also requested to issue Statements in this regard.In addition, approval for *ex vivo* and *in vivo* gene therapy trials requires the consent of the local ethics committee.The guidelines of Good Manufacturing Practice (GMP) in accordance with the European Guide to GMP (1997) must be observed for the manufacture of gene therapy products.

Czech Republic

There does not appear to be any national regulatory framework for gene therapy in humans in the Czech Republic. It is considered fully experimental and out of the scope of insurance companies (who are not willing to participate) thus limiting its usage considerably. Only very few individuals – usually supported by private resources – are referred to international clinical trials.Gene therapy is considered an interesting and beneficial option for experimental treatment approaches to monogenic diseases. There are no serious ethical or

[73] Cecile Remuzat *et al*, Market access pathways for cell therapies in France, Journal of Market Access & Health Policy 2015, 3: 29094 http://www.jmahp.net/index.php/jmahp/article/view/29094

public opinion constraints. There is a long history of GMO research on somatic cells - mainly in academia.

Lithuania

It is understood that at present there is no formal regulatory framework or oversight body in place for overseeing gene therapy applications in humans. There does not appear to be any plans to prepare genome editing for somatic (non-heritable) clinical applications. The Lithuanian Bioethics Committee would be the main driving force for regulations in this field.

Spain

The Drug Use Law (10/2013) and the Biomedical Research Act (14/2007) provide the regulatory framework for gene therapy in humans. It is considered that this would cover gene therapy carried out by genome editing. The National Bioethics Committee and the Research Ethics Committee of hospitals/centres of research provide the relevant oversight of these regulations.

Greece

The only relevant legal framework is that arising from Article 13 of the Oviedo Convention. There is no specific body in place to regulate this research, but the Independent National Authority in Medically Assisted Reproduction, the Hellenic National Bioethics Commission and the Ministry of Health have general competence in this field.Genetic manipulation of somatic cells is allowed and gene therapy is permitted. A number of early proof-of-principle studies in somatic cell therapy are in progress in academic and research institutions.

Sweden

All experiments on human somatic cells have to be approved by an ethical review board, according to the legislation (Etikprovningslagen 2003:460). The Swedish Medical Products Agency would need to approve any therapeutic applications in humans. Such legislation would address gene therapy carried out by genome editing.

The Swedish National Council on Medical Ethics held a conference on gene editing and CRISPR-Cas9 in the Swedish Parliament. The Council had previously published a brief review on the international debate in which it expressed optimism about the possibilities that the technique might provide, particularly regarding somatic gene therapy against serious diseases.[74]

Estonia

Gene therapy in Estonia is not permitted outside laboratory research. It is not thought that any plans are in place to prepare genome editing for somatic clinical applications. Estonia's regulatory framework for gene therapy in humans is based on its ratification of the Oviedo Convention – with Articles 11-14 of the Convention being most relevant.

Norway

Gene therapy is regulated by law via The Biotechnology Act. Gene therapy on fertilized eggs and foetuses, and gene therapies that introduces genetic changes in sperms and eggs are

[74] http://www.smer.se/news/smer-comments-the-technique-crispr-cas9-and-possibilities-to-edit-the-human-genome/

illegal. Gene therapy in humans is only allowed to treat or prevent severe medical conditions, and the government needs to approve such treatments. Before approval, members of the Norwegian Advisory Board for Biotechnology are allowed to voice their opinion. It is considered that the current rules would also cover gene therapy carried out by genome editing, but there do not appear to be any plans in place at present to prepare genome editing for somatic (non-heritable) clinical applications.

A.3 THE CLINICAL USE OF GERMLINE GENOME EDITING

The United Kingdom

There is no current or planned research in the UK investigating the use of genome editing for heritable clinical applications.

Numerous discussions on the regulatory and ethical considerations associated with such potential applications are taking place in the UK, stimulated mainly by groups such as the Hinxton Group, the Nuffield Council on Bioethics (and its ongoing Genome Editing Project Working Group) and the Wellcome Trust.

Any changes to the legislation that would impact on the clinical use of genome editing techniques in embryos or germline cells would be under the control of the Human Fertilisation and Embryology Act which would need to be changed by primary legislation. The UK Government's position on this matter is that the use of genetically modified sperm, eggs or embryos in treatment is illegal. It does not appear there are any plans to bring forward any new legislation on this matter in the current parliament. The recently introduced Mitochondrial Donation Regulations, which do allow germline modification but only in the context of replacing mtDNA, have yet to authorise any applications.

Switzerland

In Switzerland germline engineering is forbidden. The rules are set in the Swiss Constitution as well as subordinate legislation e.g. Federal Laws on IVF and genetic technologies in humans, on transplantation medicine; research on human subjects, medicinal products and medical devices. Article 119 of the Swiss Constitution specifically mentions reproductive cloning and genetic engineering: "all forms of cloning and interference with the genetic material of human reproductive cells and embryos are unlawful".

Research with human embryonic stem cells is allowed and regulated by the Stem Cell Research Act, but it is not allowed to genetically modify the totipotent embryo. The Stem Cell Research Act does not allow the cloning of embryos or the production of chimera or hybrids.

There has been much discussion in the lay press about genome editing and its implications. There are ongoing discussions on the ethical considerations of such studies, but as the Swiss law prohibits germline modification this means that there is unlikely to be any imminent changes to the *status quo* in Switzerland. Various institutions in Switzerland will continue to assess the current legal framework in the light of ongoing scientific developments.

France

France ratified the Oviedo Convention in 2011 and thus formally accepted the principles of Article 13 of the Convention, regarding the prevention of the introduction of any modification of the human genome into the genome of descendants.

Discussions on the regulatory and ethical considerations associated with germline genome editing are taking place in many institutions: INSERM Ethics Committee, scientific societies including the French Society of Human Genetics and the French Society of Cell and Gene Therapy), the National Academy of Medicine, in Parliament (Parliamentary Office for Evaluation of Scientific and Technological Options - OPECST, and in the National Consultative Ethics Committee.

Estonia

There are no planned or current research activities in Estonia. There appears to be little, if any, discussion on the regulatory or ethical considerations associated with such possible developments. The Act on Artificial Fertilisation and Embryo Protection would be the focus of any such legislation on this matter.

Czech Republic

It is forbidden by Czech law to carry out any such genetic modification research on human embryos. Also, embryos cannot be created nor manipulated for IVF projects.

However, a comprehensive large-scale pre-implantation or pre-natal genetic diagnostic is a diagnostic standard in the country. If genetic mutation associated with a syndrome in an index family is molecularly identified, then blastomere biopsy of embryos is an allowable option to embryo-transfer only index-mutation negative embryos. But no genetic manipulation in terms of correction work is allowed.

Greece

There is no research in progress or planned investigating the use of genome editing for heritable clinical applications in Greece.The Hellenic National Bioethics Commission (www.bioethics.gr) is in the process of drafting an opinion on genome editing and which will be published soon.

Lithuania

There does not appear to be any plans for the carrying out of research investigating genome editing for heritable clinical applications. There are however ongoing discussions about regulatory and ethical considerations of such applications. There are currently no legal and/or ethical rules that would impact on the clinical use of genome editing techniques or embryos or germline cells.

Spain

There is no research currently planned in Spain investigating genome editing for heritable clinical applications. There does not appear to be any key regulatory or ethical discussions on these issues at present.

The Assisted Human Reproduction Techniques Act (14/2006) and the Biomedical Research Act (14/2007) would impact on the clinical use of genome editing techniques.

Sweden

There is no planned or current research investigating the use of genome editing for heritable clinical applications. Genome editing of the germline is explicitly forbidden by law (the Law on Genetic Integrity, 2006:351). Attempts to carry out research or clinical purposes that result in genetic changes that are transmitted through the germline in humans are not allowed. Treatments aimed at introducing heritable genetic changes in humans are not allowed.

Germany

There is no planned or current research investigating the use of genome editing for heritable clinical applications in Germany. This kind of research is banned by the German Embryo Protection Act. It is the understanding of the National Academy of Sciences Leopoldina that there are strong reservations against such applications in Germany. The topic is subject to ongoing discussions by the public, among scientists and politicians. As suggested in the recent analysis of a German scientist expert group from the Berlin-Brandenburg Academy of Sciences and Humanities, it is considered that there are potential loopholes in the German Embryo Protection Act, which might eventually allow germline therapies that would support the life and integrity of an embryo.[75]

Norway

Research investigating the use of genome editing for heritable clinical conditions would be illegal under present legislation.

There are regulations regulating the use of surplus fertilized eggs which would limit the possibilities of such studies. Also pre-implantation genetic diagnosis (PGD) is restricted to severe genetic diseases, which currently do not allow PGS for instance. However, the Norwegian Act on Biotechnology is being revised and some of these restrictions may be removed.

The Norwegian Biotechnology Advisory Board voted on 12 January 2016 to allow ‘germline editing for basic research’. According to the statement this will only be permitted using supernumerary embryos donated by couples having IVF treatment, and only using embryos up to 14 days old. The Advisory Board recommended that the current ban should not include basic research where the germ cells or embryos are not used to establish a pregnancy. The Advisory Board recommended that the same restrictions should apply to research involving germline editing. Such research should not involve the development of methods for the clinical application of germline editing.

It is understood that the Norwegian Government is expected to issue a White Paper on the current Biotechnology Act later in 2016 or 2017, which may be followed by a proposal for legislative change.

APPENDIX 2

THE DEVELOPMENT OF CRISPR-Cas9 GENOME EDITING: A RECENT TIMELINE ON RESEARCH AND APPLICATIONS

As Eric Landler (Broad Institute, MIT and Harvard) pointed out in his "Perspective" review on the history of the development of CRISPR-Cas9, although it is only been three years since researchers first reported that the technology could enable efficient genome editing in living eukaryotic cells, many scientists from a wide field of activity have played a key role over the last 20 years in helping to move the science to that pivotal point.[76]

Landler's review is also important for emphasising that most of this early seminal work was carried out by younger researchers near the very start of their career, and that much of it was performed in institutions somewhat removed from the traditional mainstream focus of such research, such as Alicante (Spain), France's Ministry of Defence, Danisco's corporate laboratories and the University of Vilnius, Lithuania.

More than 1,000 articles on CRISPR-Cas9 indexed in "Pubmed" had been published before the end of 2015, and the research output continues.

The timeline below (and associated references) - collated by the UK Academy of Medical Sciences - is by no means exhaustive, but provides a good overview of the more recent application of CRISPR-Cas9.

- In 2012, American scientists Jennifer Doudna and Emmanuelle Charpentier *et al* publish the first account of the CRISPR/Cas9 system in Science.[77]
- In **January 2013**, South Korean scientists Seung Woo Cho *et al* (PI: Jin-Soo Kim) investigate the use of CRISPR in human cell lines.[78]
- In **February 2013**, American scientists Le Cong *et al* (PI: Feng Zhang) apply CRISPR/Cas9 to mouse and human cells *in vitro*, and also publish their results in Science.[79]
- In the same **February 2013** edition, American scientists Prashant Mali *et al* (PI: George Church) published a similar study using CRISPR/Cas9 to edit genes in human stem cells.[80]
- In December 2013, Dutch scientists Gerald Schwank *et al* (PI: Hans Clevers) repair the CFTR gene, mutations in which cause cystic fibrosis, by CRISPR/Cas9 in intestinal stem cell organoids.[81]
- In **February 2014**, Chinese scientists use CRISPR to create a double-mutation in a one-cell cynomolgus monkey embryo.[82]
- In **March 2014**, American scientists Fu *et al* (PI: Keith Joung) report that truncated guide RNAs can reduce off-target events.[83]

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[76] Eric Landler *"The Heroes of CRISPR". Cell **164**, January 14 2016 18-24*)
[77] Charpentier E *et al* (2012). *A programmable dual RNA guided DNA endonuclease in adaptive bacterial immunity*. Science **337**, 816-821. http://science.sciencemag.org/content/337/6096/816.long%20
[78] Cho SW *et al*. (2013). *Targeted genome engineering in human cells with the Cas9 RNA guided-endonuclease.* Nature Biotechnology **31**, 230-232.http://www.nature.com/nbt/journal/v31/n3/full/nbt.2507.html
[79] Cong L *et al* (2013). *Multiplex Genome Engineering using CRISPR/Cas systems.* Science **6121**, 819-823.http://science.sciencemag.org/content/339/6121/819
[80] Mali P, *et al.* (2013). RNA-guided Human Genome Engineering via Cas9. **6121**, 823-826. http://science.sciencemag.org/content/339/6121/823.long
[81] Schwank G, *et al*. (2013). *Functional Repair of CFTR by CRISPR/Cas9 in Intestinal Stem Cell Organoids of Cystic Fibrosis Patients.*Cell Stem Cell **6**, 653-658. http://www.cell.com/cell-stem-cell/abstract/S1934-5909%2813%2900493-1
[82] Niu Y, *et al.* (2014). *Generation of Gene-Modified Cynomolgus Monkey via Cas9/RNA-Mediated Gene Targeting in One-Cell Embryos.* Cell **4**, 836-843.http://www.sciencedirect.com.libproxy.ucl.ac.uk/science/article/pii/S0092867414000798
[83] Fu Y, *et al*. (2014). *Improving CRISPR-Cas nuclease specificity using truncated guide RNAs*. Nature Biotechnol**32**, 279–284. http://www.ncbi.nlm.nih.gov/pubmed/24463574

- In **March 2014**, an American clinical trial shows that zinc-finger nucleases (ZFNs) can be safe and effective in humans. For the first time, researchers used ZFNs to target and destroy a gene in the immune cells of 12 people with HIV (*ex vivo* therapy), increasing their resistance to the virus.[84, 85]
- In **February 2015**, German scientists Van Trung Chu *et al* (PI: Ralf Kuhn) publish a paper detailing a method to increase the efficiency of HDR for CRISPR in mammalian cells.[86]
- In **April 2015**, Chinese scientists Puping Liang *et al* (PI: Junjiu Huang) use CRISPR in human non-viable embryos to modify the gene responsible for β-thalassaemia.[87] The paper is the first to use CRISPR in human embryos and receives a lot of media attention
- In **June 2015**, Researchers in the USA and Israel - Ayal Hendel *et al* (PI: Matthew Porteus) report that chemical alterations to synthesized single guide RNAs (sgRNAs) enhance genome editing efficiency in human primary T cells and $CD34^+$ hematopoietic stem and progenitor cells.[88]
- In **July 2015,** American scientistsTakeshi Maruyama *et al* (PI: Hidde L Ploegh) publish a paper detailing how to inhibit NHEJ to increase the efficiency of CRISPR.[89]
- In **September 2015,** Dr Kathy Niakan, a group leader at the Francis Crick Institute in the UK, applies to the Human Fertilisation and Embryology Authority (HFEA) to use CRISPR on human embryos in order to understand the genes human embryos need to develop successfully.[90,91]
- In **October 2015**, American scientists Bernd Zetsche *et al* (PI: Feng Zhang) publish a paper in Cell detailing an alternative Crispr/CAS9 system based on a different, smaller enzyme called Cpf1. Cas9 requires two RNA molecules to cut DNA; Cpf1 needs only one. Cpf1 also creates tickly ends, whereas Cas9 creates blunt ends.[92, 93]
- In **November 2015**, Great Ormond Street Children's Hospital in UK successfully used TALENs (*ex vivo*) to engineer T cells to treat acute lymphoblastic leukaemia (ALL) in one patient.[94]
- In **November 2015**, The company Editas report that they plan to use CRISPR to treat Leber congenital amaurosis (retinal disease) by 2017. Editas was founded in part by Jennifer Doudna and Feng Zhang (2 of 5 founders), two of the first developers of the CRISPR technology.[95, 96]

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[84] Tebas P *et al.* (2014). *Gene Editing of CCR5 in Autologous CD4 T Cells of Persons Infected with HIV. NEJM***.** http://www.nejm.org/doi/full/10.1056/NEJMoa1300662

[85] Nature News. (2014). *Gene-editing method tackles HIV in first clinical trial.*http://www.nature.com/news/gene-editing-method-tackles-hiv-in-first-clinical-test-1.14813#/b1

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[90] Francis Crick statement on the HFEA application.https://www.crick.ac.uk/news/news-archive/2015/09/18/statement-on-hfea-application

[91] Nature News. (2015). UK scientists apply for licence to edit genes in human embryos. http://www.nature.com/news/uk-scientists-apply-for-licence-to-edit-genes-in-human-embryos-1.18394?WT.mc_id=TWT_NatureNews

[92] Zetsche B, *et al.* (2015). *Cpf1 is a single RNA-guided endonuclease of a class 2 CRISPR-Cas system*. Cell. http://www.cell.com/cell/abstract/S0092-8674%2815%2901200-3?_returnURL=http%3A%2F%2Flinkinghub.elsevier.com%2Fretrieve%2Fpii%2FS0092867415012003%3Fshowall%3Dtrue

[93] Nature News. (2015). *Alternative CRISPR system could improve genome editing.* http://www.nature.com/news/alternative-crispr-system-could-improve-genome-editing-1.18432

[94] http://www.gosh.nhs.uk/news/press-releases/2015-press-release-archive/world-first-use-gene-edited-immune-cells-treat-incurable-leukaemia

[95] Editas announce plan to use CRISPR to treat retinal disease. (2015). http://uk.businessinsider.com/editas-wants-to-use-crispr-by-2017-2015-11?r=US&IR=T

[96] Video about Editas' use of CRISPR. (2015). http://www.technologyreview.com/emtech/15/video/watch/katrine-bosley-coding-better-health/

- In **November 2015**, American scientists Renee Cottle *et al* (PI: Gang Bao) improve CRISPR delivery, and demonstrate that a high level of targeted gene modification can be achieved using glass-needle microinjection to deliver the reagents into human cells.[97]
- In **December 2015**, the FDA authorized the world's first human clinical trial for an *in vivo* genome editing application. Allows a Phase 1/2 open-label, dose-escalation study in up to nine male adults with severe haemophilia B to start in 2016.[98]
- In **January 2016**, American scientists Ian Slaymaker *et al* (PI: Feng Zhang) publish a paper where they have engineered Cas9 so that it is less error prone.[99]
- In **January 2016**, American scientists Benjamin Kleinstiver (PI: Keith Joung *et al*) altered the part of Cas9 which contacts the DNA, further improving fidelity and reducing the error rate.[100]
- In **January 2016**, three proof of principle studies are published in the same issue of Science regarding the use of CRISPR to treat mice models of Duchenne muscular dystrophy: American scientists Chengzu Long *et al* (PI: Eric Olson) restore dystrophin expression using CRISPR[101]; American scientists Christopher Nelson *et al* (PI: Charles Gersbach) and Mohammadsharif Tabebordbar *et al* (PI: Amy Wagers) use *in vivo* genome editing (CRISPR//Cas9) to improve muscle function[102, 103]
- In **January 2016**, researchers at the Cedars-Sinai Board of Governors Regenerative Medicine Institute in the USA use CRISPR to prevent retinal degeneration in a rat model of retinitis pigmentosa.[104]
- On **14 January 2016**, the UK HFEA's licence committee considered the application of Kathy Niaken (The Francis Crick Institute) to use CRISPR-Cas9 in her research project on the causes of reproductive failure.
- In **January 2016**, American scientists Alexander Bassuk *et al* (PI: Vinit Mahaian) publish a paper showing they have used CRISPR to repair an *RPGR* point mutation that causes X-linked retinitis pigmentosa (XLRP) in patient derived iPSCs.[105]
- On **1 February 2016**, the UK HFEA agrees to renew Kathy Niaken's application to use CRISPR in human embryos for three years.[106] However, the project still requires approval from the Research Ethics Committee (REC) before it can begin.
- In **February 2016**, Yang *et al* in the USA use an AAV vector to introduce CRISPR/Cas9 into the liver of newborn to correct the OTC gene, mutations in which cause a urea cycle disorder. They found that when performed in adult mice, it was less successful and in some cases lethally exacerbated the condition.[107]
- In **February 2016**, Sangamo Biosciences Inc.,the US-based gene therapy company announces that the U.S. Food and Drug Administration (FDA) has cleared the Company's Investigational New Drug (IND) application for SB-318, a single treatment

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[97] Cottle RN, *et al*. (2015). *Controlled Delivery of B-Globin-Targeting TALENs and CRISPR/Cas9 into Mammalian Cells for Genome Editing Using Microinjection*. Scientific Reports**5**, 16031.http://www.nature.com/articles/srep16031

[98] http://www.raps.org/Regulatory-Focus/News/2015/12/08/23734/FDA-Authorizes-1st-Human-Study-to-Use-In-Vivo-Genome-Editing-Application/

[99] Slaymaker I, *et al*. (2016).*Rationally engineered Cas9 nucleases with improved specificity.* Science. **351**, 84-88. http://science.sciencemag.org/content/351/6268/84

[100] Kleinstiver BP, *et al.* (2016). *High-fidelity CRISPr-Cas9 nucleases with no detectable genome-wide off-target effects*. Nature. http://www.nature.com/articles/nature16526.epdf?referrer_access_token=N30oov4y3qAWLggfaPDrsdRgN0jAjWel9jnR3ZoTv0NhMOr9FO19ILKW2b5xEDRGy-38DiKNGS9tmO1aScuXVIX0DUae3DQ1-Oi5vLN6ecFrr1mMD9T1Bjtoni_09gQLEQkG7wkNnF1S54BPowZv32QqXFxiR_rN54d1VtMyz2cl_zviAyxMazE1FWVRx0H8DiYQYLMvjDlY56Z0hqHlrBbO9KgLgawyzgI9tadHnYA%3D&tracking_referrer=www.nature.com

[101] Long C *et al*. (2016).*Postnatal genome editing partially restores dystrophin expression in a mouse model of muscular dystrophy*. Science **351**, 400-403. http://science.sciencemag.org/content/351/6271/400

[102] Nelson C, *et al.* (2016). *In vivo genome editing improves muscle function in a mouse model of Duchenne muscular dystrophy.* Science. **351**, 403-407. http://science.sciencemag.org/content/351/6271/403

[103] Tabebordar M *et al*. (2016*). In vivo gene editing in dystrophic mouse muscle and muscle stem cells*. Science **351**, 407-411. http://www.ncbi.nlm.nih.gov/pubmed/26721686

[104] http://www.sciencecodex.com/gene_editing_technique_improves_vision_in_rats_with_inherited_blindness-173014

[105] Bassuk A, *et al.* (2016*). Precision Medicine: Genetic Repair of Retinitis Pigmentosa in Patient-Derived Stem Cells.*Scientific Reports **6**.http://www.nature.com/articles/srep19969

[106] http://guide.hfea.gov.uk/guide/ShowPDF.aspx?ID=5966

[107] Yang Y, *et al.* (2016).*A dual AAV system enables the Cas9-mediated correction of a metabolic liver disease in newborn mice.* Nature Biotech http://www.ncbi.nlm.nih.gov/pubmed/26829317

strategy intended to provide a life-long therapy for Mucopolysaccharidosis Type I (MPS I).[108]

- In **March 2016**, work is published by Kamel Khalili and colleagues at the Lewis Katz School of Medicine, Philadelphia on the elimination of HIV-1 genomes from human T-lymphoid cells by CRISP/Cas9 gene editing.[109]
- In **April 2016**, Kang *et al* also report using CRISPR/Cas9 to successfully introduce the naturally occurring *CCR5Δ32* allele, which confers resistance to HIV infection, into the genome of non-viable human embryos.[110]
- In **April 2016**, it becomes known that Fredrik Lanner at the Karolinska Institute in Stockholm is also preparing to apply CRISPR-Cas9 in human embryos to explore early human development. [111]

[108] http://www.prnewswire.com/news-releases/sangamo-biosciences-announces-fda-clearance-of-investigational-new-drug-application-for-zfn-mediated-genome-editing-treatment-of-mps-i-300216423.html
[109] Kaminski R, *et al*. (2016) Elimination *of HIV-1 Genomes from Human T-lymphoid cells by CRISP/Cas9 Gene Editing.* Nature Scientific Reports **6***Article 22555*
[110] Kang X, *et al*. (2016). *Introducing precise genetic modifications into human 3PN embryos by CRISPR/Cas-mediated genome editing.* J Assist Reprod Genet
[111] http://www.nature.com/news/gene-editing-research-in-human-embryos-gains-momentum-1.19767?WT.mc_id=FBK_NatureNews