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Revolutionizing genome editing with CRISPR/Cas9: patent battles and human embryos

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A new genome editing technology – the CRISPR/Cas9 system – promises to revolutionize the way we modify genetic material in living cells, including how we treat disease. The battle over who owns and controls the technology in this exciting new area is fierce, with numerous patents being filed in multiple jurisdictions. Concerns have, however, been raised over the use of the CRISPR genome editing technology following news in April this year that a team led by Dr Huang Jienjin in Guangzhou, China, had used the technique on human embryos. This article considers the patent landscape for CRISPR, focusing in particular on the two original applications that cover this technology and who may stake a claim over its ownership. It then explores what may be protectable as a patent in Europe in the context of genome editing of human embryos and what regulations are in place to control what might be done, not only by way of research, but also in the clinic, with a focus on the position in the UK.

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BACKGROUND TO THE CRISPR SYSTEM

The CRISPR/Cas9 technology, commonly referred to as simply CRISPR – which stands for clustered, regularly interspaced, short, palindromic repeats – was unearthed from the immune system of bacteria, which use it to identify and fight off invading viral infections. Researchers discovered DNA sequences associated with the bacteria's immune response, termed CRISPR, but until recently, were unable to establish their exact function. A hypothesis for the function of the CRISPR system was formulated once researchers noticed that CRISPR sequences are interspaced with DNA sequences that originate from invading viruses. The CRISPR system was therefore thought to be bacteria's way of integrating short sections of viral DNA into the bacterial genome and protecting the cell from later infections by the same virus.

This underlying technology has now been utilized as a 'programmable' tool to cleave any double-stranded DNA sequence. Whilst studies through loss of function are useful and indeed are the basis for many genetic studies, the accuracy of cleavage using the CRISPR system makes it easier to introduce selected DNA sequences or genes into a target genome using 'donor' DNA. Together with the vast amounts of information researchers have gleaned from mapping different genomes, this tool provides an exciting prospect for editing specific sites in a cell's genome.

The potential use of CRISPR technology is far reaching and has already spread across many sectors within the biotech sphere. In basic biology, the system can be used to study the behavior of cells, engineer model organisms and culture specific cell lines. Biomedical applications could hopefully result in a rise in novel therapies for human disease but it is the developments in human cells which are perhaps the most controversial: the ability to make highly targeted changes in the genome of any living cell, including human stem cells and human embryos.

THE CRISPR PATENT LANDSCAPE

The relative speed, precision and ease of use of the CRISPR system means that the technology is already being utilized in academic and commercial laboratories across the world.

There are two major players in the battle to secure rights to the CRISPR system. The first group is headed by Jennifer Douda, a Professor of Chemistry and Molecular and Cell Biology at the University of California, Berkeley, USA, who, in collaboration with Dr Emmanuelle Charpentier's group, published in August 2012 what is widely regarded as the first characterization of the function of Cas9 within the CRISPR system for introducing site-specific double-stranded breaks in target DNA [1]. Many papers have followed since then describing several applications of the technology, including from Feng Zhang's group at the Broad Institute of Harvard and MIT, USA [2]. Dr Zhang's group reported using several guide sequences to simultaneously edit a genome in eukaryotic cells, promoting the system's easy programmability and wide applicability.

While Doudna's and Charpentier's groups may have published first and have received a number of awards for their work in this field, it is Zhang who has been awarded the first patent on the basic CRISPR technology – US Patent No. 8,697,359, 'CRISPR-Cas systems and methods for altering expression of gene products' (the "Zhang patent") which was granted on 15 April 2015 and is owned by the Broad Institute. Nonetheless, the fight over the rights to control the technology is far from settled.

The waters quickly become muddied if we consider these patent filings in more detail. Despite the Broad Institute being granted the first US patent in relation to the general application of CRISPR, it is Doudna and Charpentier, amongst other inventors, who filed US patent application US 13/842,859 first (the "Doudna/Charpentier patent application"), with the University Of Vienna and The Regents Of The University Of California as the original owners.

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The Zhang patent was filed on 15 October 2013 but it claims priority from four US provisional patent applications, the earliest of which was filed on 12 December 2012. The Doudna/Charpentier patent application was filed on 15 March 2013 and this application also claims priority from four US provisional patent applications, the earliest of which was filed on 25 May 2012, over 6 months before the first priority provisional patent application for the Zhang patent.

So why was Zhang's patent application granted first? Zhang's team opted for the accelerated examination procedure at the United States Patent and Trademark Office (USPTO), known as the Track One program [3], which allowed this patent to be prioritized for examination (and grant).

Despite the fast-track procedure, a more substantive question remains: why was the Doudna/Charpentier patent application not novelty destroying prior art to the Zhang patent which would have led to the patent application being refused? The answer lies in the basic cell types that each group cited in their respective patent applications: prokaryotic vs eukaryotic. The Doudna/Charpentier patent application discloses the use of the CRISPR system in the genome editing of prokaryotic bacteria, whereas Zhang's later application contains claims specifically referencing methods and systems for 'altering expression of at least one gene product comprising introducing into a eukaryotic cell…'.

In a personal declaration submitted to the US patent office on 30 January 2014, Dr Zhang asserted that the CRISPR system was not known to function in eukaryotic cells at the time of the prior art priority applications filed by University of California in May 2012 and October 2012. Zhang's submissions were accepted by the USPTO and the patent was granted.

But the battle does not end there. March 2013 saw the implementation of the Leahy-Smith America Invents Act 2011, which changes the US patent system from a 'first-to-invent' priority regime to a 'first-to-file' regime, bringing it in line with the rest of the world's patent systems. As both CRISPR patent applications were filed prior to March 2013, the earlier 'first-to-invent' regime applies to the ongoing patent battle over CRISPR. This means that Doudna and Charpentier's earlier application could not claim priority over the Zhang patent merely because of the earlier date of the priority provisional patent applications. Instead, priority will be awarded to whoever can demonstrate that they were the first to invent the technology, decided by a formal process of stringent examination under what is known as an 'interference proceeding'.

An interference proceeding can be brought if one patent applicant includes claims that are not 'patentably distinct' from the claims of another, meaning that the subject matter of one set of claims would anticipate, or be obvious in the light of, one or more of the claims in the other application.

Doudna and Charpentier sought interference proceedings in April 2015 by filing a barrage of documentation with the USPTO. Filings included: a set of amended claims which did not limit their application of CRISPR to prokaryotic cells; an explanation as to why Zhang's claims were not 'patentably

distinct' from their own; and a declaration from a recognized expert in genetic engineering that Doudna and Charpentier's earlier application clearly did disclose use of the technology in eukaryotic cells and, in any event, that it contained details of steps that could be taken to apply the system in eukaryotes.

An anonymous third party, who many suspect to be part of Zhang's MIT/Broad Institute group (or their backers), has since filed numerous documents at the USPTO addressing Doudna and Charpentier's amended claims. Further tussles seem inevitable but until the USPTO makes a final decision as to who invented the CRISPR system first, its ownership and control remains uncertain.

It could be 2017 before a final decision is reached by the USPTO but widespread use of the CRISPR system is already underway in laboratories across the world. Meanwhile, a European equivalent to the Zhang patent, EP 2764103 B1, (the "Zhang EP patent") was successfully granted by the European Patent Office (EPO) in July this year and has been validated in numerous European countries including the UK, Germany, France and The Netherlands. Prosecution of this patent application was far from smooth with the application being the subject of six separate anonymous third party observations. The observations disputed patentability on a number of grounds including lack of novelty and inventive step over cited prior art, and lack of clarity of the claims.

Some of the observations included emotive submissions arguing that Doudna and Charpentier should be the primary inventors of the CRISPR system. This probably reflects a perceived unfairness in the industry that recognition should not be given to Zhang's team's work by way of the first granted patent. The patent system does, however, entitle multiple inventions in the same field so long as each subsequent invention meets the patentability criteria. In Europe, as a first-to-file system, assuming the University of California is granted a patent for Doudna's and Charpentier's initial invention concerning CRISPR, this invention would have priority over the Zhang EP patent.

As for the European equivalent to the Doudna/Charpentier patent application (the "Doudna/Charpentier EP application"), this is currently being prosecuted before the EPO. The prosecution history of the Doudna/Charpentier EP application is no less complex with multiple third party observations and prior art citations. Only time will tell whether the patent is granted and what the scope of the granted claims will be.

Depending on the ultimate outcome in this particular patent battle, many who wish to use the system for commercial purposes will have to risk future potential patent infringement proceedings in order to use the technology in the meantime, or seek licences from either or both parties to avoid such risk. Given the tortuous prosecution history for both patent applications and the amount of money at stake, it is likely that any granted patent from either invention will be the subject of further challenges either by way of US post-grant proceedings or EPO oppositions, or revocation actions before the relevant courts in the USA and Europe.

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MODIFICATION OF THE HUMAN GERMLINE USING THE CRISPR SYSTEM

In April this year, a team led by Dr Huang Junji at Sun Yat-sen University in Guangzhou, China, published the results of a CRISPR/Cas9-mediated gene editing experiment conducted in single cell fertilized human embryos [4].

Dr Huang's team used non-viable tripronuclear zygotes, which have one oocyte nucleus and two sperm nuclei, obtained from fertility clinics. They reported that the CRISPR system could effectively cleave the endogenous β-globin gene (*HBB*) – mutations in this gene cause β-thalassemia, a common blood disorder. However, the efficiency of homologous recombination-directed repair of *HBB* was low and the edited embryos were mosaic. Off-target cleavage was also reported in these zygotes and the endogenous δ-globin gene (*HBD*), which is homologous to *HBB*, competed with exogenous donor oligonucleotides to act as the repair template, leading to untoward mutations.

In total, 86 embryos were injected, with 71 surviving after 48 hours and only 54 being genetically tested. Of these 54 embryos, only four (14.3%) had the *HBB* gene cleaved and replaced with the bespoke injected DNA and these edited embryos were mosaic. As a result of these poor results, Dr Huang stopped the experiments believing the technology to still be "too immature".

News of these experiments caused significant uproar in the West [5,6] where certain ethical boundaries are widely accepted, such as the consensus that the modification of the human germline and the cloning of human beings is contrary to public policy and morality. China, by contrast, does not hold the same ethical position, with different cultural influences and traditions, especially where it concerns human life. For example, according to Confucian thinking, someone becomes a person only after they are born. Consequently, from a cultural perspective, experimenting on human embryos is not considered as problematic in China as in the West.

Nonetheless, it was because of ethical concerns that Dr Huang›s team decided to use tripronuclear embryos. Indeed, the publication from Dr Huang›s team confirms that the study conformed to the ethical standards of the Helsinki Declaration (which concerns ethical principles for medical research involving human subjects) and to national legislation, having been approved by a Chinese Medical Ethical Committee.

More recently, on 18 September 2015, the Francis Crick Institute in London released a statement that Dr Kathy Niakan, a group leader at the Crick Institute, had applied to the Human Fertilisation and Embryology Authority (HFEA) for a research licence to use new genome editing techniques based on the CRISPR/Cas9 system on human embryos donated by couples undergoing fertility treatment [7]. The statement confirmed that the work would be for research purposes only and would not have a clinical application. News of the application was quickly picked up by the national press and *Nature News* which provided further background to the application [8–10]. According to these publications, the embryos are to be destroyed once the study is completed and the project is solely aimed at basic research into the genetics of

early human development in order to understand why some women suffer repeated miscarriages.

This proposed research from Dr Niakan's group goes further than the study by Dr Huang's team as the experiments would be conducted on viable human embryos and, to some critics, this represents a slippery slope to genetically-enhanced "designer babies".

RESEARCH ON HUMAN EMBRYOS: THE IP POSITION IN EUROPE

Although there is no global harmonization of intellectual property (IP) rights, some harmonization has been achieved by international treaties, agreements and conventions. The Agreement on Trade-Related Aspects of Intellectual Property Rights (TRIPS) is the most important multilateral instrument for the global harmonization of intellectual property laws. TRIPS was negotiated in 1994 at the end of the Uruguay Round of multilateral trade negotiations conducted within the framework of the General Agreement on Tariffs and Trade (GATT). The agreement is administered by the World Trade Organisation (WTO) and ratification of TRIPS is compulsory for any WTO member.

The objective of TRIPS is to narrow the gaps in the way IP rights are protected around the world and to bring them under common international rules. It establishes minimum levels of protection that each government has to give to the intellectual property of fellow WTO members. In doing so, it attempts to strike a balance between the long term benefits and possible short term costs to society.

TRIPS provides that patent protection must be guaranteed for products and processes in all fields of technology, provided that they are new, involve an inventive step and are capable of industrial application. TRIPS does, however, allow WTO members to exclude from patentability inventions where the prevention of their commercial exploitation is necessary to protect public order or morality, including to protect human life or health, provided that such exclusion is not made merely because the exploitation is prohibited by the law of a WTO member state.

This potential to exclude from patentability inventions that are contrary to public order or morality has already been implemented in the European Union in the context of biotechnology inventions. In 1998, after a 10 year debate in the EU over how best to encourage biotechnology innovation in Europe while, at the same time, addressing ethical concerns, the EU adopted a Directive [11] to harmonize the way in which Member States judge the validity of patents in the biotechnological field (the "Biotech Directive").

The Biotech Directive was not initially popular with all EU Member States – several governments challenged it before the European Court of Justice in an unsuccessful attempt to have it annulled. The Directive came into force in July 2000, and was implemented into UK law as Schedule A2 to the Patents Act 1977.

In order to try to achieve a balance between rewarding innovation (and consequently investment) and addressing ethical concerns, the Biotech Directive distinguishes between:

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1. discoveries, i.e., materials which already exist and which add to or extend scientific knowledge, which cannot be patented; and

2. inventions such as the technical process to isolate or reproduce a natural element, which can be patented.

The Directive specifically excludes a number of inventions from being patentable for ethical reasons. This includes the human body, at the various stages of its formation and development, and the simple discovery of one of its elements, including the sequence or partial sequence of a gene. The Biotech Directive includes a general exclusion to patentability where the commercial exploitation would be contrary to public order or morality and then provides a non-exhaustive illustrative list of inventions that are excluded from patentability so as to provide national courts and patent offices with a general guide to interpreting the general exclusion. This list includes:

- processes for cloning human beings;
- processes for modifying the germline genetic identity of human beings;
- uses of human embryos for industrial or commercial purposes; and
- processes for modifying the genetic identity of animals which are likely to cause them suffering without any substantial medical benefit to man or animal, and animals resulting from such processes.

Thus, in the EU, there is consensus that the following aspects of the technology being developed by Dr Huang's research team and of the research that Dr Niakan wishes to conduct in the UK would not be patentable in the EU under the Biotech Directive:

- anything that amounts to the human body at any stage of its formation or its development;
- any process for modifying the germline genetic identity of human beings; or
- any use of human embryos for industrial or commercial purposes.

Even if a patent is granted that does not fall foul of any of the exclusions under the Biotech Directive, it is important to appreciate what patent law can and cannot protect. This is aptly summarized in recital 14 to the Biotech Directive which we paraphrase here:

"Patent law does not authorise patent holders to implement their inventions but merely entitles them to prohibit third parties from exploiting such inventions for industrial and commercial purposes. Patent law cannot therefore serve to replace or render superfluous national, European or international law which may impose restrictions or prohibitions or which monitor research and the use or commercialisation of research from the point of view of the requirements of public health, safety, environmental protection, the preservation of genetic diversity and compliance with certain ethical standards."

RESEARCH ON HUMAN EMBRYOS: THE REGULATORY POSITION IN THE UK

Regulation of research using human embryos is not harmonized in the EU. The UK, for example, takes a relatively permissive approach with regards to research on human embryos. In contrast, the use of embryos for research is heavily restricted in Germany under the Embryo Protection Act (Embryonenschutzgesetz) 1991 which makes the derivation of embryonic stem cell lines a criminal offence.

Under the UK Human Fertilisation and Embryology (HFE) Act 1990 (as amended), no person may create, keep or use a human embryo without first having a licence or, in some specific circumstances, without there being a third party agreement where one of the parties has such a licence. The HFEA is the UK's independent regulator overseeing the use of gametes and embryos in fertility treatment and research. The HFEA licenses fertility clinics and centers carrying out *in vitro* fertilization (IVF), other assisted conception procedures and human embryo research.

The HFEA can grant research licences for up to 3 years for individual research projects. All licence applications and renewals are evaluated by an HFEA Research Licence Committee. The HFEA aims to process 90% of research licence applications within 3 months of receipt of a properly completed application (which includes the views of the peer reviewers).

In the case of human embryo research, for a licence to be capable of being granted, the research must be deemed to be necessary or desirable and the principle purposes for which a licence may be granted are:

- increasing knowledge about serious disease or other serious medical conditions;
- developing treatments for serious disease or other serious medical conditions;
- increasing knowledge about the causes of any congenital disease or congenital medical condition;
- promoting advances in the treatment of infertility;
- increasing knowledge about the causes of miscarriage;
- developing more effective techniques of contraception;
- developing methods for detecting the presence of gene, chromosome or mitochondrion abnormalities in embryos before implantation; or
- increasing knowledge about the development of embryos.

The HFEA needs to be satisfied that the proposed use of the human embryos is necessary for the purposes of the research. Importantly, any such licences cannot authorize keeping or using a human embryo after the appearance of the primitive streak and this is taken to have appeared in an embryo not later than the end of the period of 14 days beginning with the day on which the process of creating the embryo began, not counting any time during which the embryo is stored. Further, HFEA licences cannot authorize the placing inside a woman of human embryos that have been genetically manipulated in any way.

It is to be assumed that one of the principle purposes advanced in the application from Dr Niakan's group at the Crick Institute is that of increasing knowledge about the causes of miscarriage.

CONCLUSION

The Zhang patent and the Doudna/Charpentier patent application are the first in a complex family of applications, continuation-in-parts and divisionals in many jurisdictions, covering different subject matter and with different priority dates. Each requires careful individual analysis to determine their status and their relative strength.

An invention that covers the use of the CRISPR system in eukaryotic cells is more likely to be of greater commercial value to its owner compared to one that only covers

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prokaryotic cells due to the scope of providing exclusivity in relation to CRISPR genome editing in any type of eukaryotic cell, including any mammalian cells. Therefore even if the Doudna/Charpentier patent application is granted priority over the Zhang Patent, if the claims do not ultimately include eukaryotic cells, this will significantly reduce the value of the invention and place the Broad Institute and MIT in a stronger position to reap the rewards of the technology.

However, the dispute over who owns the first patents covering the CRISPR/Cas9 system may become academic. Whilst these authors were finalizing this article, Dr Zhang's team at the Broad Institute published a paper in *Cell* reporting on the discovery of a protein called Cpf1 that can be used as an alternative to the use of Cas9 [12]. Indeed Cpf1 may prove to be advantageous over the use of Cas9 in terms of ease of use and making site directed insertions more controllable. Assuming the Broad Institute has sought patent protection for this alternative gene editing CRISPR tool, it may become irrelevant whether Dr Zhang's team ultimately loses the battle over its earlier CRISPR/Cas9 patents in the event that this latest technology proves to be significantly superior.

The extent to which CRISPR is being used to genetically modify germ cells and human embryos is being closely monitored by research organizations in the UK. On 2 September 2015, The Academy of Medical Sciences, the AMRC, the BBSRC, the MRC and the Wellcome Trust published a joint statement on the issue of genome editing in human cells [13] in which they confirmed that they would:

"continue to support the use of genome editing in preclinical biomedical research as well as studies that progress and refine these technologies"

and

"that responsibly conducted research of this type, which is scientifically and ethically rigorous and in line with current legal and regulatory frameworks, should be allowed to proceed".

In the clinical context they recognized that

"there may be future potential to apply genome editing in a clinical context using human germ cells or embryos, though this is prohibited by law in the UK and unlikely to be permissible in other European jurisdictions at present".

They added that active early engagement with a wide range of global stakeholders will be needed as this

"raises ethical and regulatory questions, which need to be anticipated and explored in a timely and inclusive manner as the basic research proceeds and prior to any decisions about clinical application".

It is therefore very much a case of 'watch this space' as to how the law and the technology may be allowed to develop in the coming years.

FINANCIAL & COMPETING INTERESTS DISCLOSURE

The authors have no relevant financial involvement with an organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock options or ownership, expert testimony, grants or patents received or pending, or royalties.

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