12 September 2018
EMA/47066/2018
Human Medicines Research and Development Support Division
Inspections, Human Medicines, Pharmacovigilance and Committees Division

Report of the EMA expert meeting on genome editing technologies used in medicinal product development

18 October 2017
European Medicines Agency, London, United Kingdom

30 Churchill Place ● Canary Wharf ● London E14 5EU ● United Kingdom
Telephone +44 (0)20 3660 6000 **Facsimile** +44 (0)20 3660 5520
Send a question via our website www.ema.europa.eu/contact

Genome Editing Technologies in Drug Development – EMA Expert Meeting provides State of the Art and Outlook

Meeting report

The EMA Committee for Advanced Therapies (CAT) and the Committee for Human Medicinal Products' (CHMP) Pharmacogenomics Working Party (PGWP) organised an expert meeting on genome editing.

Leading academic institutions as well as industry stakeholder with the most advanced drug development programs using genome editing discussed current state of the art, challenges and opportunities linked with genome editing used in drug development and provided case studies of the most advanced developments.

Professor Guido Rasi, EMA Executive Director, provided the introduction stating that genome editing is an example of new development paradigms that we see progressing with unprecedented speed. It is not a new concept but recent advancement in science and technologies have generated a wealth of new research that needs to be translated into real treatments for patients.

The development of new approaches, involving the use of meganucleases, zinc finger nucleases, TALENs, and CRISPR/Cas9 have made editing of the genome less expensive, more precise and efficient compared to previous methods.

These therapies may potentially ameliorate or cure genetic diseases. However these innovative products come with complexity and challenges to develop, manufacture, evaluate them and ultimately make them available to patients.

Professor Rasi highlighted that it is the time for Regulators to reflect on those challenges but also exploit opportunities and explore collaboration with stakeholders to facilitate and move towards treatments for patients.

The first session provided an academic state of the art and future outlook on genome editing and technologies.

Shengdar Tsai from the St Jude Children's Research Hospital, Memphis, US, concurred that it is an exciting time for genome editing therapies which enables a careful, rigorous, and empirical approach to developing transformative, potentially curative, clinical genome editing strategies. Dr. Tsai highlighted the importance of carefully defining the genome-wide activity of genome editors, because unlike gene therapy approaches with lentiviral vectors there are no defined genetic sequences that can be used to easily track editing-induced clonal expansion. For example, sensitive detection of off-target effects is important for translating CRISPR-Cas9 nucleases into human therapeutics. Even low-frequency off-target mutations may be relevant if they induce a cellular growth advantage. Defining off-target site locations is important, even if we cannot currently easily interpret their functional consequence.

Methods for defining off-targets as comprehensively as possible enable monitoring for clonal expansion of cells harbouring specific unintended edits. Dr. Tsai indicated that we now have many sensitive and unbiased genomic methods to understand nuclease specificity. Amongst these methods, GUIDE-seq and CIRCLE-seq[1] emerge as two complementary sensitive and unbiased methods for defining engineered nuclease activity. GUIDE-seq is a cell-based method for defining the genome-wide activity of genome editors based on the principle of efficiently integrating a short DNA tag into the sites of nuclease-induced double-stranded DNA breaks followed by tag-specific amplification and high-throughput sequencing. CIRCLE-seq is an *in vitro* method for selective sequencing of nuclease-cleaved genomic DNA fragments. It is important to use methods that can also identify and differentiate between off-target mutations and account for effects of cell-type-specific single-nucleotide polymorphisms (SNPs). Sensitive and unbiased empirical methods can guide choice of genome editing target, nuclease, and formulation. Experts debated whether the use of only one method is sufficient to detect off-target effects and what would be the combination of methods that can be used.

The next presentation on CRISPR in drug discovery and development, achievements, opportunities and challenges was presented by **Volker Lauschke** from the Karolinska Institute in Stockholm, Sweden. In his presentation, Volker Lauschke provided an overview of the possible use of CRISPR in drug discovery and development. Examples of such use are: drug screening in engineered cell lines, elucidating the mode of action of the medicine under development, identification of genes involved in drug sensitivity or resistance, identification of functional protein domains to guide drug development, as well as engineering of *in vitro* disease models. In addition, CRISPR offers the possibility for a more rapid and more specific generation of animal models, allowing for effective knock-outs in genetically inaccessible models, simultaneous targeting of multiple genes and the possibility of biallelic modification. This will potentially reduce the current restrictions of the choice of genetic disease models for the non-clinical evaluation of new medicines. Examples of CRISPR generated disease models are: Duchenne muscular dystrophy in rats and monkeys, congenital adrenal hypoplasia in monkeys and Parkinson's disease in pigs. Lastly, CRISPR is also used in the development of medicines. Relevant examples are the development of allogeneic CAR-T cell therapy where CRISPR is used to knock-out endogenous TCRs, or where TALEN is used to knock-out CD52 and TCRs to prevent graft versus host disease.

Richard Gabriel from the National Center for Tumor Diseases (NCT), Heidelberg, Germany, presented the currently available methods to analyse off-target and on-target activity of genome editing technologies, i.e. Zinc Finger nucleases, TALENS, CRISPR/CAS and Meganucleases. A lot of work is currently ongoing to develop comprehensive assays to quantify the creation of intended and unintended genomic changes both at on- and off-target sites. For the analysis of off-target modifications, apart from cytotoxicity assays or double-strand break (DSB) staining, there are experimental assays to detect designer nuclease-induced DSB (e.g. IDLV capture, Guide-Seq, HTGTS, ChIP-Seq) and *in silico* prediction tools of off-target sites, e.g. Prognos, E-Crisp, CCTop and ZiFiT. The prediction tools are very useful to design nucleases, but less useful to validate their specificities: there is still a need for experimental data to improve and validate these prediction tools. Detection methods after *in vitro* digestion, such as Digenome-Seq, CIRCLE-seq, suffer from false positive results. Cell-based detection methods such as whole genome sequencing (WGS), exome sequencing, ChIP-Seq and break labelling/next generation sequencing, allow for the analysis in 'real life' situations. Important disadvantages of these technologies have been highlighted;

[1] circularization for *in vitro* reporting of cleavage effects by sequencing (CIRCLE-seq)

- *WGS*: most comprehensive method to identify new mutations but only high frequency mutations can be detected and the cost for sufficient sequencing depth hampers WGS from being applied for sensitive off-target analysis
- *Exome Sequencing*: sufficient sensitivity for off-target detection but limited to a small part of the genome with high false-negative rate, i.e. many off-targets located in introns may be missed
- *ChIP-Seq*: many false positives; secondary analysis needed. Little overlap between ChIP-seq vs real off target sites
- *Break labelling/next generation sequencing (NGS)*: only detect DSBs present at the time of analysis, misses all breaks repaired earlier or occurring later

Further information was provided on the IDLV capture, Guide-Seq and HTGTS methods, as well as a comparison of these 3 methods. It was noted that no general conclusion can be drawn about the specificity of engineered nucleases: each nuclease / situation has to be evaluated individually to assess the impact on the cleavage of target sequence, enzyme structure, cell type and other variables. To analyse non-homologous end joining on-target modifications, mutation detection assays, target site sequencing and gene knockout analysis can be used. For homologous recombination on-target modifications, RFLP detection assay, target site sequencing and report gene integration assays are available. It was concluded that assessing unintended ON- and OFF-target modifications is crucial to minimise the risks associated with therapeutic genome editing.

Pietro Genovese from the San Raffaele Hospital, Milan, (IT) elaborated on clinical translation of haematopoietic stem cell genome editing for the treatment of inherited disease including experience with the use of genome editing to modify haematopoietic stem / progenitor cells (HSPC). He stated that genome editing has the potential for *in situ* gene correction and have some potential advantages over conventional gene therapy (gene replacement): it restores gene function and expression control, it may abrogate risk of genome-wide insertional mutagenesis and limits the genotoxic risk to off-target activity. The result of their research on targeted gene editing of HSPC was shown, including their experiments to correct SCID-X1 causing mutations using genome editing technologies. These included *in vivo* studies in a SCID-X1 mouse model. Their finding show that edited HSPC can rescue disease phenotype; there are safety concerns linked to low input of corrected cells and therefore partial myeloablative conditioning is required to allow engraftment of the edited HSPC and protect from replicative stress (and transformation) of correct thymic lymphoid progenitors. According to the presenter, these proof-of-concept studies established the rationale for clinical translation.

The last presentation of this session by **Bernd Gänsbacher**, member of the Committee for Advanced Therapies (CAT) gave an high level overview of the possible clinical applications of CRISPR-Cas, either to repair/restore (fix or modulate “broken” gene(s) to restore or preserve a healthy phenotype) or redirect/rewire (to program cells to carry out activities different from the normal state). At the time of the expert meeting, there were no clinical trials (in EU or US) with CRISPR-Cas based products, but main developments are close to entering clinical development: several non-clinical programmes are ongoing, many by academic sponsors and small developers (SMEs). There are ongoing clinical trials evaluating Zinc Finger Nucleases to target monogenic and infectious diseases. He concluded his talk highlighting the importance of early dialogue with the Regulatory Authorities to address the current challenges with this novel and promising technology. This will facilitate the progress into clinical trials and the future marketing authorisation and access to patients at EU and international level.

The second session provided industry stakeholder case studies using genome editing technologies, including CRISPR-Cas9, TALENs and Zinc finger nucleases.

Editas Medicines presented a genomic drug development to treat an inherited retinal dystrophy.

Translational challenges include potential for transformative therapies of genetically-defined and also genetically-treatable diseases. Further scientific questions include editing efficiency, cellular context, delivery & strategy, the biological and genetic context and specificity. Editas Medicine highlighted the need to balance the potential therapeutic benefit with risks and that innovative regulatory science is key to realize these medicines.

Editas Medicine demonstrated in non-clinical models that their editing strategy results in molecular correction of the gene deficit linked to Leber's congenital amaurosis, which is leading to paediatric blindness caused by the degeneration of photoreceptors, that the editing is efficient in a structurally complex eye when administered in the identical delivery vehicle and route of administration, and that the final product composition efficiently edits the appropriate human tissue.

CRISPR Therapeutics presented *ex vivo* genome editing with CRISPR-Cas9 to treat serious diseases. Persistent expression of foetal haemoglobin (HbF) beyond the neonatal period is a rare, naturally-occurring condition which substantially ameliorates the pathology of Sickle Cell Disease (SCD) and β-thalassemia (β-thal). The lead clinical product of CRISPR Therapeutics, CTX001, uses the CRISPR/Cas9 genome editing technology *ex vivo* with patient CD34+ haematopoietic stem and progenitor cells (HSPCs) to efficiently re-generate specific genetic variants associated with elevated HbF. Genetically modified Haematopoietic stem/progenitor cells (HSPCs), when reinfused into the patient, are to produce red blood cells containing high levels of the protective, naturally occurring HbF, to reduce or eliminate symptoms of SCD and β-thal.

CRISPR Therapeutics has established and optimized an innovative CRISPR/Cas9 approach to achieve editing efficiencies in human primary HSPCs at clinical scale of greater than 80%. This high rate of editing was associated with robust HbF expression following erythroid differentiation of edited cells from healthy donors and from patient samples, well into the range that may be clinically relevant.

They showed that HSPCs maintain their 'stem cell' characteristics, based on in vivo mouse models where haematopoietic differentiation or differentiation ex vivo is maintained and is not different from unedited cells used a control.

Safety is supported by no identified off-target editing after extensive deep next-generation sequencing at thousands of potential homologous sites or putative sites identified through a homology-independent method (Guide-Seq), as well as absence of safety signals in extensive tumourigenicity and biodistribution GLP toxicology studies.

Intellia Therapeutics presented several case studies of CRISPR-Cas9 technology for therapeutic applications to illustrate the spectrum of evidence generation paradigms and their relevance to various genetic disease targets and treatment strategies. Intellia's initial clinical application of CRISPR/Cas9 technology *in vivo* was described for the treatment of transthyretin (TTR) amyloidosis using a lipid nanoparticle (LNP)-based delivery approach. Data were shared demonstrating durable genome editing in mice out to 12 months post-treatment following a single LNP administration, associated with profound and sustained TTR knockdown. In this case, where the pharmacologic treatment is complete gene knockout, a single therapeutic may enable treatment of all patients regardless of their specific mutations.

For ultra-rare mutations, where clinical trials are not feasible, other means of evidence generation were outlined. An integrated evaluation of *in vitro* and *in vivo* animal models, and extrapolation of clinical data in other disease variants, was proposed as one possible alternative.

Intellia encouraged the community to maintain open-mindedness in the use of different analytic tools, and to take a pragmatic approach to the use of preclinical models. In addition, the audience was asked to consider advanced clinical trial designs including umbrella approaches and extrapolation, conditional marketing approval, incorporation of real-world evidence, and the development of guidelines to specifically address genome editing therapies. Going forward, the need for continued constructive dialogue on the therapeutic application of genome editing was considered paramount.

Sangamo Therapeutics focussed on non-clinical studies to support the clinical translation of zinc finger nuclease (ZFN)-based genome editing. Sangamo's therapeutic products are based on engineered zinc finger DNA-binding protein (ZFP) and adeno-associated virus (AAV) gene delivery platforms. ZFPs can be linked to the *Fok*I endonuclease domain to create ZFNs that enable genome editing by utilizing the cells own natural DNA repair pathways.

In non-clinical studies, they demonstrated that genome edited CD34+ HSPC can be manufactured in a GMP-compliant setting using a clinical-grade electroporation device to deliver the ZFN mRNAs *ex vivo*. Unbiased specificity studies demonstrated an exquisite amount of specificity, with high levels of on-target modification (~80%). Erythroid colony genotyping in enhancer targeted cells, showed bi-allelic modification of the BCL11A erythroid enhancer in >50% of HSPCs, resulting in >4-fold higher levels of gamma globin mRNA and protein compared to controls. Injection of the product into immune-deficient mice resulted in robust long-term (20 week) engraftment. Targeted gene modification was maintained through multi-lineage differentiation in the bone marrow and peripheral blood. Sangamo Therapeutics consider that these results support further clinical development of the product as a potential therapy for beta-thalassemia and sickle cell disease.

Cellectis presented on the development of transcription activator-like effector nucleases (TALEN)-edited allogeneic chimeric antigen receptor (CAR)-redirected T-cell therapies. Cellectis has developed a platform for generating CAR T-cells from healthy donors using TALEN (UCART product). In order to address potential concerns related to genome-editing-based therapeutics, Cellectis has taken a risk-based approach. Product related considerations include the cell type utilized (terminally differentiated), the nature of the genome modification (gene inactivation), target indication for the final product, the route of administration (*ex vivo*) and dose of the product. Process related considerations comprise delivery methods, nuclease and nuclease activity related. Due to potential safety risks, on-target and off-target effects are a principal regulatory concern. The major question for on-target cleavage is whether the genome-edited product is safe by design. For UCART products this has involved the demonstration of reduced Graft versus Host Disease (GvHD) risk following T-cell receptor (TCR) inactivation. In addition, when multiple sites are targeted there is the potential of generating translocation events. The presence of such events can be monitored by standard cytogenetic techniques (karyotyping and FISH analysis). In addition, absence of any proliferative advantage in cells with translocation can be demonstrated though *in vitro* long-term culture assays. Potential off-target effects can be addressed through multiple methods including *in silico* modelling as well as unbiased whole genome approaches. Finally, there are several challenges for clinical development of genome-editing related products in the European Union including GMO regulations, in particular the absence of a common application process and for genome-editing material, the definition of quality requirements and implementation of a Master File/formal filing process.

The last case study was presented by **Bluebird Bio** on scientific and regulatory considerations for the development of megaTAL genome editing products for the treatment of cancer and severe genetic diseases. Bluebird stated that genome editing offers the promise of site-specific modification to the genome and is an opportunity to improve safety over randomly integrating viral vector mediated strategies (assuming comparable efficacy). Risks highlighted as for any genome editing technology include undesired double-strand breaks (DSB) generation at sites beyond the intended target and genomic rearrangements driven by the induced DSBs. Bluebirds megaTALs are a hybrid endonuclease platform comprised of a fusion between TAL DNA binding array and meganuclease cleavage domain. They consist of small reagents (~2.5kb) but a long target recognition interface, 3' break overhang with catalytic specificity checkpoint and contain a monomeric architecture that lends itself to vectorization and multiplexing. An iterative specificity refinement model enables to be responsive to off-target discovery and verification data as protein engineering allows retention of target site throughout pre-clinical development. High target activity >80% routinely obtained and specificity demonstrated by reagents lead to undetectable off target cleavage genome wide possible. Considerations for Genome Editing Safety Risks resulting from the intended edit include intrinsic to the intended genetic and/or phenotypic product characteristics and risks that may arise from executing genome editing. Bluebird showed potential assays for non-clinical safety studies like molecular assays for assessing genomic integrity. The presentation finished with lessons learnt from Lentiviral vector developments and resulting considerations for genome editing strategies.

Roundtable discussion and conclusion

The experts acknowledged that regulatory dialogue has been sought via meeting with the Innovation Task Force (ITF) and Scientific advice (SA) and more product developments using this technology are in the pipeline.

It has been agreed that one of the major relevant important unknowns is the clinical relevance of **off target toxicity**. The group concluded that current scientific knowledge is far from fully understanding and interpretation of off-target findings, especially in genomic regions whose functions are unknown.

Potential off-target toxicity has to be evaluated and interpreted on a case by case basis and will depend on the treatment strategy, e.g. the target cells/organ and whether an in vivo or ex vivo application is envisaged and will therefore be product/indication specific. On- or off-target activity have been shown to be cell/tissue specific, i.e. depending on the cell differentiation stage, the chromatin structure or concentration and duration of the nuclease exposure, and needs to be investigated and considered accordingly. Stem-cell studies have been used to address the cell-cycle contribution to genome editing efficiency. Investigations using different tissue types are necessary to further evaluate tissue-specific risks in genome editing. The delivery system also needs to be considered, as it may affect the on/off- target effects due to changing exposure. Regulators enquired whether it was possible to identify and predict organs of "off-target" toxicity based on non-clinical data. It was considered premature to answer this question. The identification of organs affected by off-target toxicity would allow more focussed patient surveillance and implementation of targeted risk minimisation methods in clinical trials. Further experience needs to be gained in the field before regulators can provide specific recommendations and guidance.

The development of **immunogenicity**, e.g. due to long exposure to the nuclease, has been identified as an additional potential risk to be considered for these treatments. Research is ongoing aiming to reduce both off-target toxicity and immunogenicity. This includes engineering target sites that recognise larger binding sites, reducing the time of exposure and using different nucleases.

With regards to **methods and models** for the detection and quantification of intended and unintended genomic changes, experts indicated that all the available assays have limitations and need to be further improved, e.g. increasing sensitivity. Current *in silico* methods do not provide a reliable risk assessment on their own. Experimental data targeting identified sites in an *in vitro* model can provide useful information. A combination of complementary methods should be used. Verification e.g. by next generation sequencing in the possible target cells, to allow a comprehensive assessment of the potential risk for off-targeting in view of a clinical application, is expected.

The value of different animal species has been discussed and its use compared to *in vitro* human studies. The expert agreed that complex phenomena cannot be investigated *in vitro* only. However **animal models** also have their limitations as e.g. a homologous model may be necessary due to species specific gRNA. In this context the use of humanised organoids may be of value for non-clinical safety and efficacy investigations, e.g. predictive studies. It was noted that organoids can reflect certain disease aspects, but will not provide the full picture and should therefore be accompanied and supported by or support the evidence of other methods. Developers as well as Regulators will therefore need to collect, interpret and compare evidence from animal and human data.

The discussion concluded that currently available methods and models are at research stage and will need to be further developed and validated before being used as a therapeutic tool for the manufacturing of medicinal products.

In **summary** the Genome Editing technologies presented and discussed during this workshop are characterised by their potential of site-specific targeting and therefore may potentially provide safer gene modification than other currently available gene therapies. Experience is, however, still limited and the clinical relevance of non-clinical critical findings, like off-target effects, need to be further investigated allowing scientific, data-based conclusions that inform the benefit-risk assessment of a treatment.