CRISPR Summit

CRISPR Delivery Challenges and Applications within Pharma

Expert Interviews

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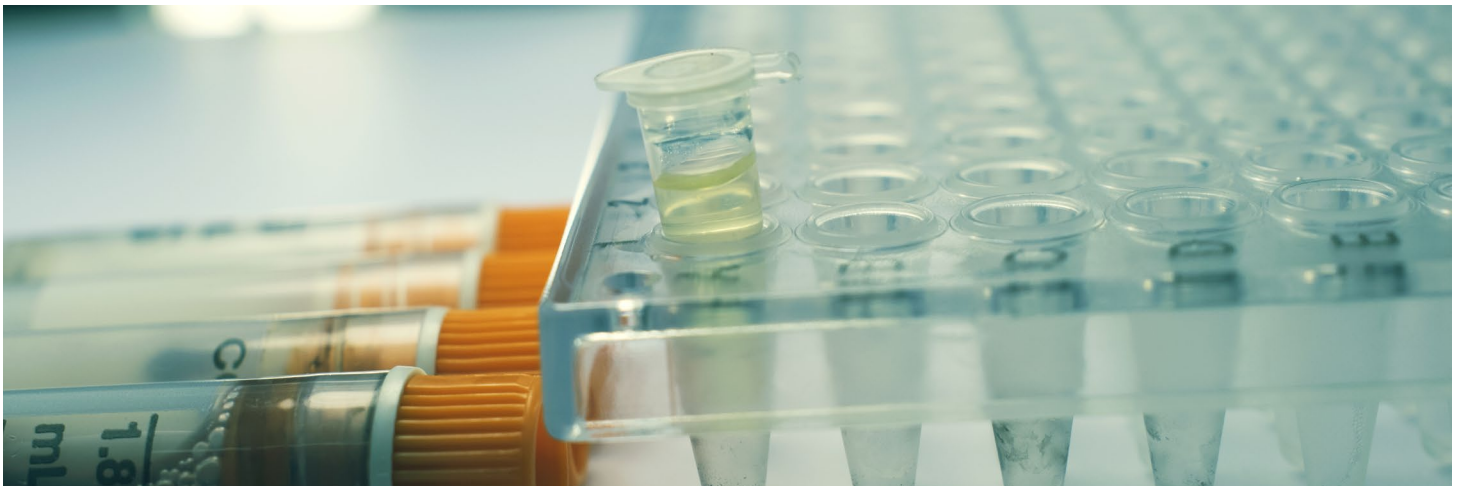

CRISPR is now one of the most affordable forms of gene editing causing a boom in R&D over the last few years. However, the technique does have its limitations which include maintaining end to end control on genetic manipulation, avoiding off targets effects and pathway recovery post double strand break.

Ahead of the 2017 CRISPR summit Javier Terriente, CSO, ZeClinics and Danilo Maddalo Lab Head ONC Pharmacology, Novartis Institutes for BioMedical Research discuss the latest regarding CRISPR delivery challenges and applications within Pharma.

The Line up

Javier Terriente
CSO
ZeClinics

"I've been working with CRISPR both in the generic, transgenic or disease models in therapies, also in different cell lines. In ZeClinics we do both types of works but mostly focus on therapies. We've been working in generating knockouts but also we have been doing some knock ins or targeted mutagenesis with differing success rates.

Danilo Maddalo
Lab Head ONC
Pharmacology
Novartis Institutes for
BioMedical Research

"I mainly work in vivo.: So in the generation of pre-clinical models that can be used for research and for drug discovery and target identification. Basically somatic engineering of mouse cell lines to generate a specific type of tumour that could be either a specific oncogenic signature that is already known or some sort of target identification for finding out whether that signature is indeed oncogenic."

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Danilo Maddalo, Lab Head ONC Pharmacology, Novartis Institutes for BioMedical Research

What would you say are the main delivery challenges with this technique and what impacts do these have on market progress?

"Well, the problem of delivery is two-fold. First you are delivering a bacterial protein, so this means that of course it's not something really physiological for any organism you're going to work with, from fish to mouse. So you are going to expect a certain degree of rejections from these organisms because of the expression of a foreign protein itself.

"The second problem is definitely the size. So that's what's limiting a lot of the delivery in general. Whatever means of delivery you are using, the Cas9 is pretty big. There have been efforts in identifying similar endonucleases doing the same job that are a little bit smaller. This has been partially successful but it's still a big issue. Of course there is a limitation with certain tissues that cannot really be reached so far by the Cas9 with any type of delivery, so this limits the application."

What challenges are experienced with in vivo or in vitro application of CRISPR?

"Well, again, one of the problems is to maintain a certain level of expression of the protein. If the protein is given externally to a cell line or to an organism, this is because sometimes it could be toxic itself , of course the cells expressed in the Cas9 would change the behaviour compared to their original behaviour and that would give you a confounding readout when you're looking for pro-apoptotic or pro-survival agents etc.

"The other improvement again is to find efficient ways of delivering the Cas9 to a specific cell type. This is something that has not been achieved yet, up to my knowledge, in [any] of the applications, academic or industry. This is definitely something that's going to be a major point for the future."

What has been a highlight in your career in terms of the application of CRISPR?

"From my point of view the use of CRISPR was impactful in the generation of animal models. The model that I generated was an animal model of chromosomal rearrangement, so this model was really never [seen] before. There were a few models that were mimicking this situation and to generate them this would have taken a couple of years and several hundreds of dollars to generate. You went from a situation like that to a

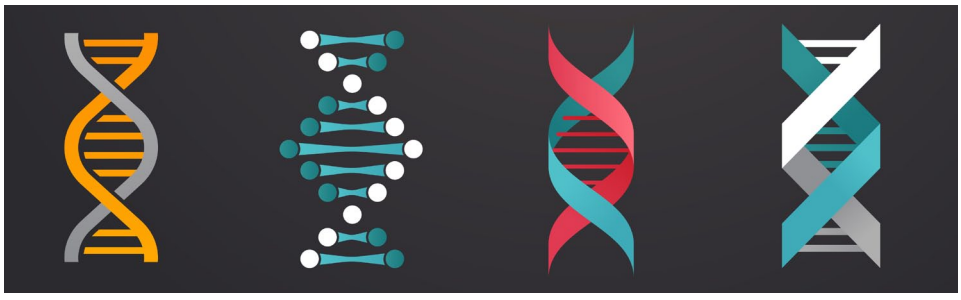
model that takes around six months to be generated and probably less then $10,000 in terms of price, so the impact has been massive."

How quickly do you think the CRISPR technique will progress in the next three years?

"We are kind of reaching a plateau in terms of the applications, all the things that you can do at the cellular level. Of course the big question, or the big challenge I have in general for CRISPR would be to see some sort of application in patients.

"In humans there are many model limitations which [include] safety and ability to predict off targets.[These would probably] need an impulse from another discipline, for example, nanotechnology or any other type of applied technology to biological sciences that could help out the technique to develop.

"Most likely – and I speak as a CRISPR scientist – we will end up having something that is completely different in terms of biological entity compared to what we are using now. Probably the Cas9 approach that we are using now will be completely obsolete in three years because it [may]be replaced by a smaller endonuclease,



a more precise one, etc. The concept will be still the same. The concept of the CRISPR/Cas9 itself was already at least ten years old [when it came out] because people tried with zinc finger and TALENs to cut the genome and edit it. So of course this is something that is going to progress. [Although], I don't know how quickly it's going to be in the next three years."

What is holding back the progression of CRISPR and how are we going to see it affect general research in genetic manipulation application within humans?

"There are many challenges and I would like to see a little bit more thorough verification of the method. Of course it's something that excites everyone because it's the very first time in the history that we can really do what we want with the genome – that's never happened before. Like before I was working with plasmids – this is amazing. You take a human being or an animal and you can modify the genome – this is completely new.

"So, even the scientists I guess, they are not even mature enough to go through this at the moment. We are still in this hangover, the CRISPR hangover. We got drunk on it and now we're having this hangover and it would be wise to sober up a little bit before going into the serious stuff, because human [assays] can give you bad surprises even few years later and you don't want to be responsible for that just because you wanted to try [it] out.

"Having said that, the potential [of CRISPR] is there and cannot be ignored. So at some point there is also a certain type of responsibility to go for it.

"If you look at it historically TALENS or zinc finger nucleases were discovered a longer time ago and there are very little applications in humans. The enthusiasm that you have in the research field gets a little bit milder when you go for humans and it's the responsibility of everyone to make people aware that yes - that could be a possibility, but it's definitely not something immediate."

What would you say are the

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Javier Terriente, CSO, ZeClinics

main delivery challenges with this technique and what impacts do these have on market progress?

"If we consider only the work we do with CRISPR, which is basically to generate either disease models or [target] mutants or so on, the main challenge is the knocking efficacy. This has proven very hard to work in therapies at least. I think it's a bit better in rodents but in [unclear] it has been very difficult to integrate in a precise location a certain piece of DNA.

"If you want to consider also the use of CRISPR as a gene therapy method, the main challenge is the delivery method.

"There are different challenges to be approached. In general scientists will want to do gene therapy integrating a specific protein in a precise location, for example, either to repair or to enhance activation of some gene."

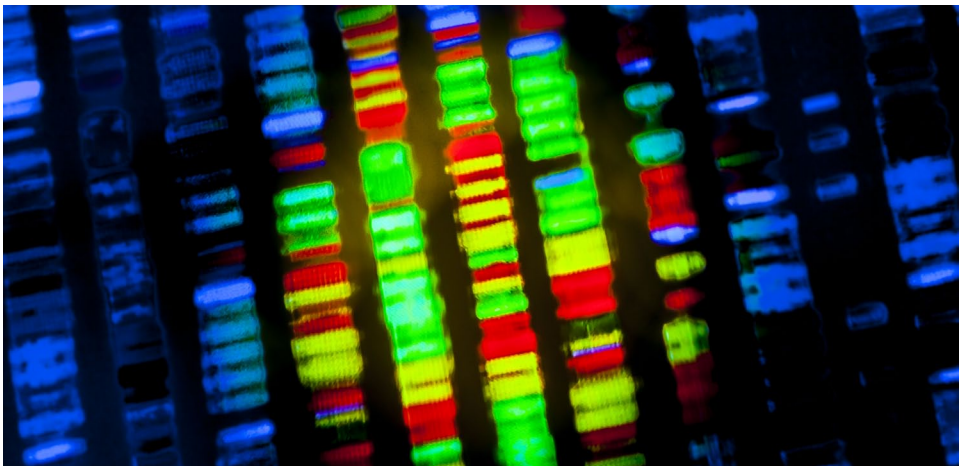
How far would you say is the industry advanced in terms of tackling these issues?

"As far as I know, there have been several publications from academics, tackling both issues that I just mentioned but my understanding is that they are far from being solved.

"I think a lot of people are working on something [and] at the end we aim to solve this also. So, it's more a matter of time than a matter of impossibility."

What challenges are experienced with in vivo or in vitro application of CRISPR?

"Knock-in, is one challenge. Another challenge is the screening. When you work with mice, you want some phenotypical screening of animals but that's a general issue when you work with mutants. That is a very time-consuming thing [and] I think at some point we have to [make] better ways to streamline the process. Probably if the knock-in worked better, we will be able to use some type of fluorophore proteins more often that can tell us better if we have a mutant heterozygous or homozygous. At the end everything comes together - we will have tacks that allow us to check for heterozygosity or homozygosity in an easier way than just standard sequencing, for example."



What has been a highlight in your career in terms of the application of CRISPR, this can be first-hand or anything that you've witnessed?

"So, we managed to get the patent licence from the MIT and that has allowed us to start commercialising services as a company, making new models for academics and other industry players. So, I think that's an important highlight, at least for us but in general I think it's good that people can do that.

"Then the second highlight would be more in a scientific point of view. We have managed to generate a lot of different mutants and targeted mutagenesis mutants. We have, in fact, seen that in making big knock-ins like in tabulating large species of DNA which is, I guess, our next goal. But for the moment we have more or less mastered the use of CRISPR for many different applications. So, I think that's an important highlight and, again, it changes in general in the CRISPR technology for genetic manipulation for basic research and also for therapeutical applications."

What would you say is holding back the progression of CRISPR and how are we going to see that effect on general research in genetic manipulation?

"I think [there] will be a point where anybody almost from the garages in their houses will be able to manipulate species, either plants or animals. Because it is actually as easy as that, which means that we will have to have a tight control on who does what.

"On the one point [this] is very exciting for scientists but I see this as, obviously, a bit worrying. From a biosecurity point of view, we will have to be careful [on] how to release information and from an environmental point of view too.

"[CRISPR] has to [progress] together with a lot of ethical regulations and it won't be able to progress that far if other fields in the genomic field don't advance as fast as the genetic manipulation."

How quickly do you think CRISPR will progress in the next three years?

"Very quickly. There are more and more people working in the field and the more people working in the field, the more brains working [to solve]problems. So I think it's going to be a very quick race of different applications and [concepts] that we cannot even think of now. The [significant challenges] are more the legal and ethical limitations that are around [rather]than scientific limitations on the possibilities of CRISPR.

"At some point [there has to be] some type of solution between the different patent holders that can be applicable to all the people that have a patent or want to use CRISPR with a commercial purpose. Then we will have a good ethical review of how to use CRISPR in humans because it might change the way our societies behave in the future."

17th-18th May 2017
London, UK

Unlocking The True Capabilities of CRISPRThe only summit commited to unlocking CRISPR's potential, trouble shooting the challenges within CRISPR research and exploring new ideas to improve the CRISPR/Cas9 system

4 Reasons to Attend:

- Uncover different research techniques using CRISPR
- Understand how the right application for your modification will decrease unwanted immunogenic responses
- Discover how CRISPR screening has been used to improve the level understanding in research
- Gain insight into how pharma companies have overcome precision challenges in pre clinical mouse models and cell line generation

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