CRISPR in HIV: dangers of CCR5 deletion

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Dr. He Jiankui, an associate professor in the Department of Biology of the Southern University of Science and Technology (SUSTech) in Shenzhen, China, reported during the Second International Summit on Human Genome Editing, located in Hong Kong between 27 and 29 November 2018, the results of his *in vivo* experiment with Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) technique [1]. The experiment consisted of a molecular knock-out surgery named CRISPR to obtain the deletion of 32 amino acids located in the gene which codifies the C-C chemokine receptor type 5 (CCR5). The experiment aimed to obtain the genetic variant Delta 32 of the CCR5 which have been demonstrated to gather protection against Human Immunodeficiency virus (HIV) infection [2, 3]. The experiment was performed on embryos of one day of age obtained from *in vitro* fecundation of male and female gametocytes. In particular, the male gametocyte was obtained from an HIV positive subject. It is well known that the Delta32 variant of CCR5 has a protective effect against the entrance of HIV infection into the cells by blocking one of the two co-receptors (the other one is the C-X-C chemokine receptor type 4 (CXCR4)) which HIV needs to bind concomitantly to CD4 to enter into cells [4, 5]. It is worth mentioning that viruses, which are able to bind the CXCR4 co-receptor, are not influenced by this deletion.

The aim of Dr. Jiankui, as he reported before the conference by social media (*i.e.* YouTube), was to "protect" the twins born using the *in vitro* fecundation, from HIV infection. Several serious drawbacks have to be taken into account when considering this experiment. First, in regard to the regulatory laws, Dr. Jiankui did not request any authorization from the bioethical commission of his institution before performing his experiments [6-8]. Due to the fact that at the time of the experiment's performance Dr. Jiankui was on sabbatical leave, it is not clear where the experiments were performed. Moreover, it is consequently not sure if the laboratories where the experiments were carried out was properly certified.

A great harmfulness of this kind of human experiments arose from this absence of ethical clearance. In fact, all the interventional studies, ranging from experimental use of drugs and vaccines to genetic therapy, require an informed consent which explains the subject's will. In this particular scenario, the clear absence of an informed consent represents a serious violation of human rights [6]. When considering the

implication of such an intervention on a neo-formed embryo several things have to be taken into account. First, the modification inserted in the germinal line will persist into the genome of the subject [9]. Second, the subject will transmit this modification to future generations. In the end, this technique is imperfect and its potentially detrimental effect of off-target deletions must be taken into account.

It is well known that several methods have been demonstrated to be effective against HIV transmission. First, the first objective of 90-90-90 target of the World Health Organization to be reached in 2030 has the aim to expand the number of people who know of their HIV serostatus [10]. The second step is to deeply extend the antiretroviral treatment. This preventive strategy is known as Therapy as Prevention (TasP) and is effective at the individual and population level [11]. TasP relies on the assumption of undetectable equals untransmittable (U=U). In other words, people who are under antiretroviral treatment with an undetectable virus in their blood are not capable of HIV transmission [12]. Moreover, in high risk groups for HIV transmission, burdened by an inconsistent condom use, pre exposure prophylaxis has been demonstrated to reduce the risk of HIV acquisition [13]. Taking these scientific evidences all together, the use of CRISPR technique to reduce the risk of HIV acquisition seems to be without benefit after balancing the potential risks for patients.

The gene editing with CRISPR technology or other kinds of techniques seem to be promising in several fields ranging from agriculture [14], to some infectious diseases such as malaria [15]. One of the most promising application of CRISPR technology is in congenital inherited diseases where a single gene is involved in the development of the disease [16]. Nevertheless, it should be considered only when no other alternatives are suitable.

In the end, the deletion of CCR5 is protective against the acquisition of HIV but its role in other infectious diseases is still matter of debate. In particular the frequency of Delta32 deletion in Caucasian subjects has led to speculate that it could produce a protection against *Yersinia pestis* infection. In particular, a reduced uptake of *Y. pestis* by macrophages with CCR5 deletion has been observed *in vitro*. Nevertheless, *in vivo* this mutation does not affect the survival of infected mice [17]. Moreover, an increase in frequency and

severity of West Nile virus infection have been observed [18]. Whereas, the role of CCR5 deletion in predisposing to influenza infection remains more controversial. According to some reports, it seems that Caucasians with a Delta32 deletion of CCR5 have an increased risk of severe influenza [19]. Nevertheless, these findings were not replicated in subsequent analyses [20, 21]. Thus, the CCR5 receptor maintains specific biological functions due to an increased risk of some infections. Moreover, this technology introduces a permanent deletion into the genome of the subject which is different when compared to the temporary pharmacological block of the coreceptor caused by antiretroviral compounds, such as maraviroc, or the hematopoietic stem cells transplantation with Delta32 deletion of CCR5 which lead the "Berlin patient" to HIV eradication [22].

Nowadays, the risk of HIV acquisition could potentially be reduced by a Delta32 CCR5 deletion, nevertheless the potentially detrimental effect for future generations warrants a fight against this kind of approach. The absence of the CCR5 co-receptor could represent a serious threat for new generations challenged by new infections. In fact, the introduction of a genetic alteration in future generations, which is not evolutionary driven but arbitrary chosen is an hazard. By thinking at the process of natural selection this intervention could expose the affected individuals to potential unpredictable risks in the case of an emergent pathogen with the ability to hit people with this specific deletion. Looking at the ethical concerns raised by the modification of germinal line, it is unlikely that in the next future these could be overcome. In particular it is possible that in the future this technique will be able to reduce the off-target error and reduce the risk of off-target deletion and/or mutations [23]. Nevertheless, the risks of such a technique to prevent a transmittable disease such as HIV, with codified and scientifically demonstrated preventive strategies, could overcome potential benefits. Moreover, the Delta32 deletion of CCR5 does not protect from all the strains of HIV. In particular, the host would be potentially at risk to be infected with a CXCR4-tropic virus after the gene editing. Furthermore, the modification of embryo cells poses serious ethical concerns that require an extensive discussion by the scientific community.

Taking all these concerns together, the experiment conducted by Dr. Jiankui and his team should be considered unethical, and without any logical reasons after considering all the current available strategies to prevent HIV acquisition without posing a serious threat for current and future generations. His indictment in December 2019 may be the end of this awful story.

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