CRISPR/CAS9 genetic modification of plasmodium falciparum and transgenic parasites in malaria vaccine research

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Stellingen behorende bij het proefschrift

"CRISPR/Cas9 genetic modification of *Plasmodium falciparum* and transgenic parasites in malaria vaccine research"

1. Transgenic *Plasmodium falciparum* reporter lines, expressing fluorescent and luminescent reporter proteins are not only being used to study the basic biology of malaria parasites but are now also being used as valuable tools to rapidly evaluate novel vaccines and vaccination strategies (this thesis).

2. Optimizing CRISPR/Cas9 technologies to edit the *P. falciparum* genome without the retention of drug-resistance genes in the parasite genome, greatly simplifies the production of parasites with multiple genetic modifications and thereby enhances their development as novel vaccines (this thesis).

3. The circumsporozoite protein (CSP) of the human malaria parasites, *P. falciparum* and *P. vivax*, can replace and complement the function of rodent malaria parasite CSP. However, the inability to create viable sporozoites after replacement of *P. falciparum* CSP with *P. vivax* CSP is unexpected and shows that full sporozoite maturation in *P. falciparum* is likely dependent on species-specific features of CSP (this thesis).

4. Although the processes of gamete formation, fertilization and zygote development are highly similar between rodent and human malaria parasites, critical differences exists in the function of several orthologous fertilization proteins of human and rodent parasites. This has implications of modelling and evaluating transmission blocking vaccines, *in vivo*, using rodent models of malaria (this thesis).

5. Despite the efficiency and flexibility of CRISPR/Cas9 methods to genetically modify multiple genomes, 'off-target' DNA breaks can often be introduced by single guide RNA/Cas9 expression, as has been demonstrated in mouse embryonic cells. Consequently, such unintended genomic damage caused by CRISPR-Cas9 editing may have pathogenic consequences in therapies that utilize this method to genetically repair deleterious mutations in humans (Kosicki, M. *et. al.* Nature Biotechnology, 2018).

6. While most research on human microbes is dedicated to their elimination, infectious organisms are now increasingly being seen as potential therapeutic agents, which have a large potential to play a role in the treatment of different diseases (Lukasiewicz, K. *et. al*. Journal of Immunology Research, 2018).

7. Mice often respond to experimental interventions in ways that differ strikingly from humans; therefore the use of mice in biomedical research needs to take into account the evolved differences in response to infection and disease as well as the physiological similarities of these hosts (Perlman, R.L. Evolution, Medicine and Public Health, 2016).

8. In debates about the nature of the disease caused by vector borne infections that can cross the placental barrier, such as Zika, it is important to take into account the possible long-term effects that may arise in the children years after infection of the mother (Zika Virus and Birth Defects — Reviewing the Evidence for Causality; NEJM Special Report 2016).

9. Unlike other mammals, hominids during their evolution lost their ability to synthesize the carbohydrate α-Gal, resulting in the ability to generate high antibody titters against α-Gal. This process may have conferred an advantage against a number of vector borne disease; however, despite this ability 17% of all infections of humans worldwide are vector borne diseases (Cruz, Valdes and de la Fuente (2016) Expert review of Vaccines).

10. "If curiosity and wonder are childlike, then so be it. When I think of science and scientists I think of kids who never lost their curiosity and wonder and then woke up one day as adults with the very same sense of search for what is and what is not true in the world." (Neil de Grasse Tyson in interview with Arianna Huffington 2017; Thrive Global). *Curiosity and the ability to wonder about simple things is the central basis of science.*

11. It does not take much strength to do things, but it requires a great deal of strength to decide what to do (Elbert Hubbard 1856-1915). *Spending time end energy for making decisions on what to do is important for reaching your goals.*

12. When you find your path, you must not be afraid. You need to have sufficient courage to make mistakes. Disappointment, defeat, and despair are the tools that God uses to show us the way (Paulo Coelho, Brida, 1990). *Mistakes, defeat and despair are the best teachers, they give an opportunity to learn and to start over better prepared.*

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