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Curriculum Vitae

Sanne Terryn was born on the 20th of March 1987 in Anderlecht. After her high school studies with majors in science and mathematics at the Koninklijk Atheneum Ukkel, she commenced the study of bio-engineering at the University of Brussels (VUB). In 2010, she obtained the degree of master in bio-engineering sciences (MSc) with distinction.

After obtaining her degree, Sanne remained highly fascinated by science and decided to pursue a pre-doctoral research project on Buruli ulcer for one year. After this project, she started her doctoral research project on the rabies virus at the Viral Diseases Laboratory of the Scientific Institute of Public Health.

In her doctoral work, she studied the potential benefits of camelid heavy chain antibodies for the prophylaxis and treatment of rabies using mouse models. Her work was financed by a scholarship from the Scientific Institute of Public Health.

Sanne Terryn is first author of two international scientific publications and was invited as a speaker to present her work on national and international congresses.

UNIVERSITEIT GENT

UITNODIGING

Openbare verdediging van het doctoraal proefschrift van

Sanne Terryn

24 november 2016

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Vakgroep Virologie, Parasitologie en Immunologie

FACULTEIT DIERGENEESKUNDE
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U wordt vriendelijk uitgenodigd voor de openbare verdediging van het doctoraal proefschrift van

Sanne TERRYN

Titel van het proefschrift:

Development and evaluation of antiviral immunoglobulin single variable domains for prophylaxis of rabies in mice

De verdediging zal plaatsvinden op
donderdag 24 november 2016 om 17 uur
in Auditorium Hoogbouw van de
Faculteit Diergeneeskunde
Universiteit Gent
Salisburylaan 133, Merelbeke

Na de verdediging volgt een receptie waarop u vriendelijk wordt uitgenodigd

Indien u de receptie zult bijwonen, gelieve dit telefonisch (02/373.33.52) of per mail (sanne.terryn@wiv-isp.be) te melden vóór 16 november 2016

Samenvatting van het proefschrift

Rabies virus causes an invariable fatal infectious disease in a large range of animals and humans. Yearly, rabies causes 59000 fatalities, mainly in developing countries. When people are exposed to the virus, disease can be prevented by treatment with a combination of vaccination and rabies immunoglobulins (RIG). RIG are purified from plasma of vaccinated persons or horses. Because RIG are scarce and expensive, they are often unavailable to those most in need. The WHO has therefore launched a call for the development of alternatives.

In this thesis, it was investigated whether immunoglobulin single variable domains or VHH might serve as an alternative for RIG in rabies post-exposure prophylaxis. VHH are the antigen-binding fragments of heavy chain antibodies found in *Camelidae*. These small antibody fragments are easily produced in microbial expression systems and can easily be formatted in multimeric constructs.

The general aim of this thesis was to develop and validate several types of anti-rabies VHH constructs for rabies prophylaxis in mice. It was investigated whether VHH can be used for the neutralization of the rabies virus and prevention of infection and disease in mice. We attempted to determine the advantages and limitations of VHH in this model and investigated a number of modifications that could be applied to improve the therapeutic efficacy of anti-rabies VHH.

In chapter 3, the intranasal virus inoculation model is discussed. This model is used since 2010 by our laboratory, but is not the standard infection route for the rabies virus. This chapter gives a retrospective study of this model and compares it to other inoculation methods. It shows that the intranasal inoculation model is a highly reproducible technique with a high mortality rate which makes it very suitable to study experimental treatments with potentially low differences in the median or overall survival.

In vitro tests showed that VHH were highly potent to neutralize the rabies virus in cell culture and virus-neutralization could be increased by formatting VHH in multimeric constructs containing two different clones. After this initial *in vitro* screening, the intranasal infection model was used to test the *in vivo* efficacy of anti-rabies VHH (Chapter 4).

Co-administration experiments with a pre-incubated mix of VHH and virus, showed that VHH were able to completely neutralize the virus in susceptible body compartments of mice. When VHH were administered before virus challenge in mice, complete protection could be observed. However, when VHH were administered after virus challenge, the level of protection depended on the route and timing of administration. The protective efficacy of VHH was limited due to the relative short circulatory half-life of the VHH. This led to the development of a VHH construct with an extended circulatory half-life, by addition of a third VHH directed against human albumin. This significantly increased the circulatory half-life in mice from 1.6h to 30.5h. Upon systemic administration, the half-life extended VHH could rescue up to 71% of the animals, compared to 0% survival rate for the non-half-life extended VHH. This result stresses the importance of circulatory half-life on the efficacy of VHH.

A major hurdle in the treatment of infection of the central nervous system is the presence of the blood-brain barrier, which limits the entry of therapeutics from the blood to the brain. In an attempt to circumvent this problem, VHH were linked to neurotropic peptides or proteins (Chapter 5) in order to give them an address tag to better reach the target organ. Although this tagging led to better binding to or entry into neuronal cells in cell culture, these constructs failed to improve protection in the mouse infection model.

Half-life extended VHH were also tested in combination with anti-rabies vaccination (chapter 6). This resembles the treatment protocol for rabies in humans, in which vaccine is combined with RIG. In this experimental setting, it was shown that the combination therapy of VHH and vaccine was more effective than treatment with either VHH or vaccine alone.

The main conclusions of this thesis are formulated in chapter 7. It was shown that VHH, especially in a bivalent format, can potently neutralize rabies virus and can prevent disease in mice. In the post exposure setting, the protective effect of VHH was improved by prolonging the half-life and combining VHH treatment with vaccination. Our results are promising for the use of VHH as alternatives for immunoglobulins. Steps to further validate and develop anti-rabies VHH for application in humans are discussed in the perspectives of the thesis.