Introduction into the sample cohorts RV144 and RV305 in order to investigate the role of effector functions and Fc-antibody characteristics

Major improvements in treatment options for the disease caused by human deficiency virus (HIV) like HAART were achieved in the last three decades. Unfortunately, access to drugs are limited in regions were the HIV epidemic is still ongoing like south Africa and parts of Asia. Therefore, one important goal in HIV research is still unreached: a preventive HIV vaccine. (WHO 2016; Day & Kublin 2013)

Out of the Phase II and III trials conducted in the last decade, only one recent HIV trial showed promising results: RV144, a vaccine trial conducted in Thailand showed a medium protection of 32.1% 3.5 years after final vaccination and is therefore the first HIV vaccine providing some level of efficacy. (Rerks-Ngarm et al. 2009) RV144 is a phase III HIV-1 vaccine trial, conducted in a community-based population in Thailand. The vaccine itself is composed of a prime and boost, with the canarypox vector prime containing gp120 Clade AE envelope which is the endemic circulating strain in Thailand (ALVAC-HIV, Sanofi Pasteur). The protein boost contains envelope protein from both Clade AE and B to induce breath (AIDSVAX B/E, Global Solutions for Infectious Diseases). (Kim et al. 2014) The trial was setup with two injections of the prime, followed by two administrations of both the prime and the boost. The injections were administered over a time course of 24 weeks (Fig 1). (Karasavvas et al. 2012)

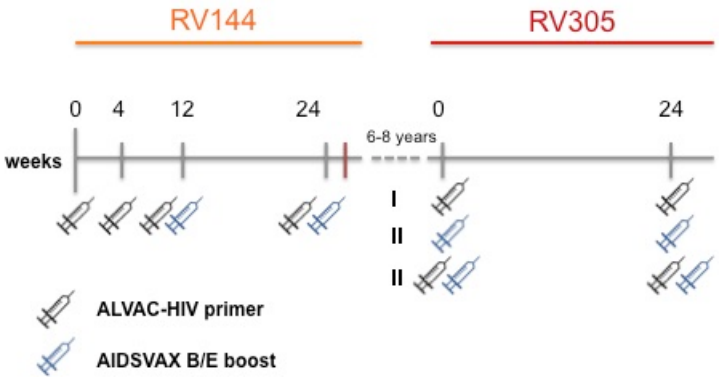

Figure 1: RV144 and RV305 vaccination schedule.

The vaccine trial showed an efficacy of 31.2% after 3 years and interestingly, after 1 year, initially 60% of the vaccinees were protected. This gave rise to the unique possibility of finding correlates of protection by comparing case control samples. These follow-up analyses showed that protection was partially achieved by non-neutralizing antibodies (nNAbs) and therefore it was stated that extra-neutralizing antibodies play an important role in HIV vaccines and their influence on vaccine efficacy and effector pathways should be investigated (Cohen & Dolin 2013; Gottardo et al. 2013)

The RV144 follow-up analyses revealed that antibody-dependent cytotoxicity (ADCC); an NK cell based response that is mediated by Fc-receptor activation on the effector cell by antibodies in the patient serum was associated with protection. Moreover, high levels of IgG1 and IgG3 against the V1V2 region of the gp120 HIV envelope protein were associated with a reduced risk of infection. On the other hand, high levels of IgA against gp120 were correlated with an increased risk of HIV acquisition which indicates that IgA antibodies could interfere with the protective effect of the vaccine. (Haynes et al. 2012) Primary analyses only included ADCP effector function by NK cells, and the other Fc-mediated functions by immune cells like monocytes, neutrophils or the complement system. These initial findings raised interest in the

question which antibody characteristics besides isotype selection track with protection and how effector functionality is regulated. Furthermore, could this prime and boost approach be used for other subtypes of HIV and how can the response be prolonged and enhanced.

These questions can be addressed by the systems serology approach developed in the lab of Galit Alter in Boston, Cambridge. This approach was recently published by Chung et al. (Chung et al. 2015). The array of assays allows the evaluation of the relationship between humoral responses in different vaccine trials and leads to a characterization of the extra-neutralizing IgG immune profile. The measurements include antibody-dependent cellular phagocytosis (ADCP), meaning phagocytosis of antibody-bound beads by macrophages, complement activation (ADCD), neutrophil phagocytosis (ADNP) and NK cell activation and degranulation. These functions are all mediated by one special part of the antibody, the conserved Fc- domain. (Cheeseman et al. 2016) Moreover, the array includes biophysical measurements like Fc-receptor binding affinity, antibody isotype selection and glycosylation of the Fc region which has been shown to highly tune antibody effector functions (Mahan et al. 2016).

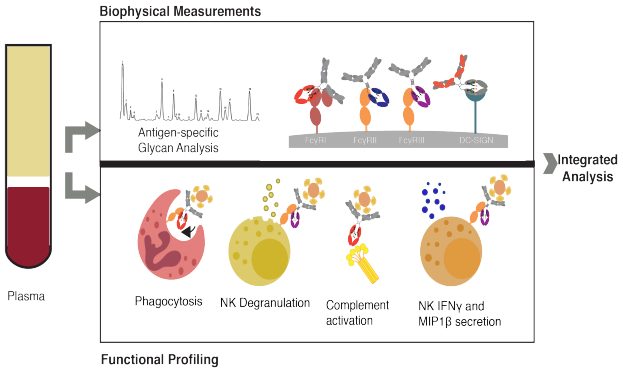

***Figure 2**: Systems serology s starting from patient plasma leading to an integrated analysis including different antibody features and functions. Image courtesy of Madeleine Jennewein (Alter Lab)*

Part of my PhD thesis involves two different sample sets: patient plasma from RV144 and RV305 vaccinees. RV305 is the follow-up study on the well-known RV144 trial in which participants that received the full vaccination cycle are again vaccinated to investigate if repeated administration could prolong and increase the protective effect. For RV144, we received 300 samples two weeks post vaccination, so the peak immunogenicity time point and all participants were injected with the same specimen. In contrast, for RV305, we will investigate samples from 318 patients at different time points after vaccination to see how the response changes over time after vaccination. Moreover, vacinees were injected with either only the prime, only the boost or with prime and boost after receiving the full RV144 vaccination cycle. This will hopefully give insight into which characteristics are mostly driven by prime or boost administration.

For RV144, I will focus on the specific question if patients that obtain high levels of IgG against V1V2 and low levels of IgA, so individuals that should be protected according to the correlates of protection show different antibody characteristics and functional responses and how can these responses be tuned by glycosylation. With such a big sample set of 300 patients, we can define different groups based on the correlates of protection and investigate functional response levels and antibody characterizations between the different groups by applying the systems serology approach.
For RV305, the same assays will be performed, but since we have different time points after the last vaccination, we will be able to directly compare between RV144 patients and RV305 vaccinees over time that received another round of prime/boost injections in different combinations.

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