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Dynamics of Drug-resistant minor viral populations and phenotypic drug susceptibility in diverse HIV-1 subtypes

AKADEMISK AVHANDLING

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ABSTRACT

Antiretroviral therapy (ART) has significantly reduced mortality in human immunodeficiency virus type 1 (HIV-1) infection, both in high- and low/middle-income countries (LMIC). However, the development of drug resistance following exposure to subtherapeutic concentrations of antiretrovirals (ARV) and transmitted drug resistance mutations (DRM) adversely affects the outcome of ART.

In my thesis, drug resistance patterns of diverse HIV-1 subtypes were described, at both genotypic and phenotypic level. A high throughput sequencing (HTS) method was utilized to amplify and sequence the *gag-pol* of different HIV-1 subtypes for identification and quantification of DRM present in < 20% of the viral population. A novel bioinformatics pipeline was developed, MiDRM*pol*, which integrates genomic variations and mapping of minor populations with DRM. Phenotypic drug sensitivity assays were performed to study *in vitro* potency of newer ARV. Also, polymerase independent increase of virulence and replication competence of HIV-1, which may influence the outcome of ART, was studied.

Paper I describes the pipeline, MiDRM*pol*, which can be used without any prior knowledge and does not require on-site bioinformatics support. The raw data from HTS in the fastq format are uploaded to get an easily readable format. One of the unique features, when compared to other available pipelines, is the FastUniq tool, which removes the duplicate reads generated by PCR, thus reducing the pseudo-bias of few variants. Another feature is the subtype-specific adaptation during the analysis. In **Paper II** and **Paper III**, I studied the potency of three newer ARV. 4'-Ethylnyl-2-fluoro-2'-deoxyadenosine (EFdA), a novel translocation defective reverse transcriptase inhibitor (RTI), was found to inhibit both wild-type and RTI resistant viruses efficiently in a subtype-independent manner.

Similarly, second-generation integrase strand inhibitors (INSTI) cabotegravir (CAB) and bictegravir (BIC) were shown to have equal or higher potency against non-B subtypes as compared to HIV-1B. This confirms the suitability of these drugs for use in countries dominated by non-B subtypes. In **Paper IV**, I observed that a PYxE insertion in the *gag* plays a role in increased virulence and replication capacity in HIV-1C viruses and seems to be associated with suboptimal CD4⁺ T-cell gain following ART initiation. Even though there was no effect of PYxE or PTAPP on the susceptibility to 20 ARVs, the enhanced replication capacity might increase the time to reach viral suppression during ART and thereby increased the risk for the virus to develop DRM.

In conclusion, identification and quantification of DRM at frequencies <20% is a major hurdle in current ART monitoring, and our MiDRM*pol* facilitates the analysis of such HTS data. However, we found that also polymerase independent mutations which increase the replication capacity/virulence of HIV-1 may influence the outcome of ART. The new ARVs EFdA, CAB, and BIC suppress the viral load *in vitro* in a subtype independent manner. This is important since if not all subtypes are suppressed efficiently the risk of a global increase of acquired DRM would be further increased.