The project objectives included:

1. Application of information theory to the analysis of ultra-deep HIV-1 sequencing data obtained from HIV-infected subjects receiving antiretroviral therapy.
2. Application of phylogenetics and molecular evolution theory to characterize the HIV quasispecies, molecular epidemiology and the mechanisms of virological failure

To achieve these objectives, we first applied quantitative deep HIV-1 sequencing in a subject who developed virological failure to deep salvage therapy with raltegravir. HIV RNA was extracted and amplified in triplicate 3 weeks before initiation of salvage antiretroviral therapy (baseline) and at virological failure (VF), 24 weeks after treatment initiation. We found that most Q148R and N155H mutants detected at the time of virological failure originated from pre-existing minority Q148R and N155H variants through independent evolutionary clusters. Double 148R+N155H mutants were also detected in 1.7% of viruses at virological failure in association with E138K and/or G163R. Our findings illustrate the ability of HIV-1 to escape from suboptimal antiretroviral drug pressure through selection of pre-existing drug-resistant mutants, underscoring the importance of using fully active antiretroviral regimens to treat all HIV-1-infected subjects.

In a second project, we focused our interest in exploring the potential of deep HIV-1 sequencing for adding clinically relevant information relative to viral population sequencing in heavily pre-treated HIV-1-infected subjects. In a proof-of-concept study, deep sequencing was compared to population sequencing in HIV-1-infected individuals with previous triple-class virological failure who also developed virologic failure to deep salvage therapy including, at least, darunavir, tipranavir, etravirine or raltegravir. Viral susceptibility was inferred before salvage therapy initiation and at virological failure using deep and population sequencing genotypes interpreted with the HIVdb, Rega and ANRS algorithms. Deep sequencing data did not consistently modify the susceptibility predictions achieved with population sequencing for darunavir, tipranavir or raltegravir. We concluded that in this subset of heavily pre-treated individuals, deep sequencing improved the assessment of genotypic resistance to etravirine, but did not consistently provide additional information on darunavir, tipranavir or raltegravir susceptibility. These data may inform the design of future studies addressing the clinical value of minority drug-resistant variants in treatment-experienced subjects.

In a third project, we applied deep sequencing to HIV tropism testing. Accurate tropism testing is crucial for HIV management, but major controversies persist regarding which tools and settings confer better diagnostic performance in subjects with detectable and undetectable viremia. QDS of plasma viruses provides the highest diagnostic accuracy relative to ESTA. PS shows better accuracy at a 20% FPR and in proviral DNA. Tropism testing can be performed either in stored pre-treatment plasma samples or in contemporary proviral DNA in subjects with undetectable viremia not receiving CCR5 antagonists. Tropism testing in proviral DNA, however, requires clinical validation.

Overall, our work contributes to advancing the development of more sensitive and accurate diagnostic tools for HIV resistance and tropism, advancing in the field of personalized medicine for HIV-infected individuals. Our work shows that next-generation sequencing technologies are accurate, have the potential to be a cost-effective alternative to assess viral tropism and resistance and provide essential information to advance our understanding of HIV pathogenesis.