

# Molecular Virology



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## Immunologic HIV control during continuous, highly effective therapy promotes tropism-dependent loss of infected cells

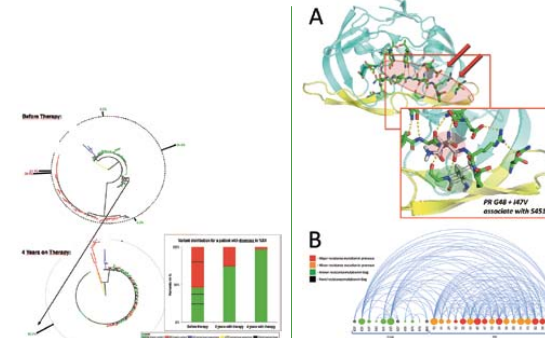
In the past decades successful therapy has completely changed the global landscape of HIV infection: New drugs with excellent side-effect profiles are today available for long therapy and a perspective of life-long virus suppression; now the role of regained or retained immune functions is back in the focus of interest. Can the improved immune capacity reduce virus spread and disease? Yet, despite all therapeutic developments the unavoidable flip-side of decade-long therapy continues to be the increased risk of drug-resistant HIV and treatment failure. While most of the responsible mutations in the respective therapy target genes have been characterized, cases with persisting "low level viremia" or virologic failure without overt mutations remain puzzling. Therefore, a better understanding of alternative resistance mechanisms, e.g. via mutations in enzyme substrates may prove clinically important. As practical, clinical contributions of our research-driven molecular work, and as elements of capacity building towards improved clinical care my research group got engaged in projects with rural settings of our globe. Our efforts intend to address aspects of the ambitious WHO 90-90-90 aim towards ending the global HIV epidemic: Identify by year 2020 globally 90% of all HIV infected people, provide treatment to 90% of them with virus suppression in 90%. The current focus of ongoing work in my research group is thus on three key questions:

### 1) How can immune function complement effective HIV therapy?

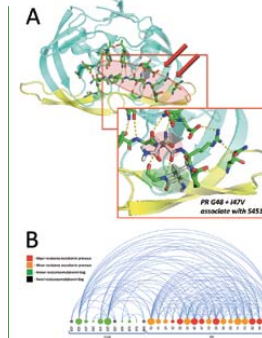
We have recently discovered that recovering immune functions can crucially change the fate of HIV-1 in a given patient. Such competence might assist therapeutic strategies to arm the natural defense over time in concert with HIV drug therapy. Published work revealed that HIV tends to change its host tropism during the course of infection: Early, close to 90% of patients harbor mostly HIV that uses the chemokine receptor CCR5 for cell entry, late in the disease up to 50% of viruses are CXCR4-tropic. CXCR4-use therefore seemed to indicate the emergence of more aggressive virus variants, characteristic for late-stage disease. We set out to probe this hypothesis by a longitudinal follow-up of patients in the Swiss HIV cohort study by deep-sequencing approaches. To our surprise, and against expectation the study demonstrated the opposite trend: In patients with long term suppression of virus replication, CCR5-tropic HIV variants re-appeared or remained, while CXCR4 tropic virus in most cases drastically declined (Fig. 1), obviously as result of therapeutic pressure. This new finding correlates well with other key properties of CXCR4-viruses: lower envelope glycosylation, stronger induction of inflammation and cell activation. It is thus likely that immune-stimulating features enable preferable control and elimination of CXCR4-tropic HIV variants and -infected cells under therapy.

### 2) Does HIV resistance evolve under long-term therapy?

Although combination therapy has become superbly successful for the global fight against HIV, escape from therapy continues to be a serious issue for all inhibitor classes, including the highly potent protease inhibitors (PI). Mechanistically most PI-resistances correlate with genetic changes within the protease gene, i.e. drug-specific mutations or those that confer broad viral resistance to most drugs of the class. However, clinical situations are observed, where no such obvious link can be established. Our investigations identified genetic changes in the gag gene for structural HIV proteins, which serve as protease substrate. Fig. 2a details H-bridges between enzyme and substrate that are crucial for proper interaction and Gag-



**Fig. 1:** Next generation sequencing-based phylogenetic tree before and during treatment for a representative patient. The population of CXCR4-tropic variants declines massively. Green dots represent CCR5-tropic HIV-variants in the virus population, red dots CXCR4-tropic ones. Blue: position of V3 consensus sequences of subtypes A, B, and C. In yellow: consensus HIV-1 subtype B LTR sequence used for rooting. The most prominent outgrowing variant under therapy is marked with a black arrow. Black bars indicate the frequency (in percent) of a given variant in the entire HIV sequence pool. The bar graph summarizes the relative frequencies of the 5 most prominent viruses over time.



**Fig. 2A:** HIV-1 Protease complexed with the decapeptide at the p1/p6 Gag substrate junction (top). Specific polar contacts of Gag S451 with PR G48 mediate the immediate neighboring of mutated position 147V with Gag S451N under therapy (bottom), which causes a simultaneous loss of a polar contact between PR D30 and PR Q58 (next to PR flap region). Altered residues are in purple. Polar contacts between substrate and enzyme are shown as yellow dots; arrows indicate position of lost polar contacts. **Fig. 2B:** Arch-diagram depicting the interactions between residues in Gag and Protease (PR). Blue lines as well as sizes of dots indicate the number of connections of a given amino acid (numbers indicate position) with other residues. The most active positions correlate with known or suspected drug resistances.

processing. We analyzed data in the resistance database of the Swiss HIV Cohort Study for identifying more of the possible links. As summarized in the arch diagram in Fig. 2b several links between protease and Gag were unraveled that associate with therapy. Currently their role is being verified by phenotyping in cell-based assays in the presence of inhibitors. This may help identify new clinically relevant mutations to be considered in HIV resistance testing

### 3) Modern diagnostic tools and patient care in rural Africa.

With a translational, practical effort my research group is involved in the establishment and implementation of quantitative viral load-testing and the genotypic determination of HIV drug resistance in a rural, resource-limited setting. In collaboration with N. Labhardt and the Swiss NGO SolidarMed we study therapy efficacy, viral resistance-emergence as well as the successful retention in antiretroviral care over time in the North of Lesotho. Up to here the project was already able to demonstrate that nurse-led hospitals can achieve, similar to Swiss settings, about 90% treatment success. Pleasantly surprising, also patients' therapy adherence is similarly high (Fig. 3). However, in very sharp contrast to the positive adult situation, virus control and retention in care for children and adolescents were shown to pose massive challenges. Therefore, along with our most recent introduction of sensitive virus-load measurement and sequence-based resistance testing in Butha-Buthe/Seboche we are currently concentrating our work on new concepts to accompany and improve clinical work towards long-term virus suppression for these key populations and the overall health towards prolonging the lives of the most-affected in a country that, with an overall prevalence of 20% HIV-1-positivity, is among those hardest hit by HIV/AIDS.

## Connection to Clinical Practice

**Niklaus Labhardt**  
STPHI, Basel

**Molecular tools for HIV diagnostics in order to reach the WHO goals of "90-90-90" in rural Africa**  
(See point 3 in the abstract to the left)

## Selected Publications

- Bader J, Däumer M, Schöni-Affolter F, Böni J, Gorgievski-Hrisoho M, Martinetti G, Thielens A, Klimkait T, and the Swiss HIV Cohort Study. Therapeutic Immune Recovery and Reduction of CXCR4- Tropic HIV-1. Clinical Infectious Diseases 10.1093/cid/ciw737, Advance Access published Nov. 12, 2016
- Bader J, Schöni-Affolter F, Böni J, Gorgievski-Hrisoho M, Martinetti G, Battegay M, Klimkait T & the Swiss HIV Cohort Study. (2016) Correlating HIV tropism with immunological response under combination antiretroviral therapy. HIV Med. 2016 Mar 17; doi: 10.1111/hiv.12365
- Scherer AU, von Wyl V, Yang WL, Kouyos R, Böni J, Yerly S, Klimkait T, Aubert V, Cavasini M, Battegay M, Furrer H, Calmy A, Vernazza P, Bernasconi E, Günthard HF. Swiss HIV Cohort Study. Emergence of acquired HIV-1 drug resistance has almost been stopped in Switzerland – a 15 year prospective cohort analysis. Clin Infect Dis. 2016; 15:62(10):1310–7
- Wagner S, Kurz M, Klimkait T. the Swiss HIV Cohort Study. Algorithm evolution for drug resistance prediction: comparison of systems for HIV-1 genotyping. Antivir. Ther. 2015; 20(6):661–5
- Edwards S, Stucki H, Bader J, Vidal V, Kaiser R, Battegay M, Klimkait T. the Swiss HIV Cohort Study. HIV-1 Tropism – A Diagnostic system based on Sequence-Relatedness. J Clin Microbiol. 2015; 53(2):597–610

