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AREVIR-GENAFOR-Meeting

April 19-20, 2007, Bonn

Abstracts

Edited by

Mark Oette, Joachim Selbig, Daniel Hoffmann,
Gabriele Poggensee, Osamah Hamouda, Thomas Lengauer,
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FOREWORD

This supplement of the European Journal of Medical Research presents the abstracts of the 12th AREVIR-GENAFOR-Symposium. The symposium was held as a joined meeting of the AREVIR-GENAFOR platform, the cooperation project “Monitoring of resistant HIV in recently and chronically infected patients in Germany” and the EURESIST-network from 19th to 20th April 2007 at the CAESAR-Foundation in Bonn, Germany.

HIV resistance testing has become standard of care in the treatment of HIV infection. However, there is an urgent need of further research on this topic, as understanding of resistance mechanisms is incomplete, technology of testing is still developing, and adequate interpretation of genotypes is under debate. The contributors of the symposium are experts in the field of HIV drug resistance.

AREVIR is a cooperation of virologists, bioinformatics experts, and clinicians, who developed a large database including an interpretation tool of HIV drug resistance results. GENAFOR is the associated foundation for the funding of scientific projects in the field. The cooperation project “Monitoring of resistant HIV in recently and chronically infected patients in Germany” is a research group on the surveillance of HIV drug resistance, financed by the federal Ministry of Health and Social Services. Major partners within this project are the Robert Koch Institute, the Paul Ehrlich Institute, the National Reference Center for Retrovirology of the University of Erlangen and study centers at the Universities of Köln and Düsseldorf. For the first time, the meeting took place under participation of researchers of the European EURESIST project. This project integrated several large European databases on HIV resistance testing results and clinical outcome and uses machine learning techniques and data mining for better application of resistance testing.

The symposium covered different aspects of resistance testing. This included new interpretation tools like HIV-GRADE or THEO, results of national and international cooperations on HIV resistance, HBV resistance, and epidemiological projects. Furthermore, talks on the molecular basics of drug resistance, ultra-sensitive testing, evolution of resistance over time, resistance associated with new antiretroviral compounds, and current databases were presented. Technical problems and patient-orientated aspects completed the program.

Since the year 2000, the AREVIR-GENAFOR meeting gathers researchers and physicians to discuss actual results of biomedical, clinical, virological, and bioinformatical research on HIV drug resistance and the more recent emerging topic of HBV drug resistance. The symposium continues to be held every spring, with the next date at 10th to 11th of April 2008. We hope that this supplement will help to draw attention to the medical and economical aspects of HIV and HBV drug resistance.

Rolf Kaiser and Mark Oette



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HIV-GRADE CLINICAL VALIDATION –
IDENTIFICATION OF PROBLEMS
IN THE PREDICTION OF DRUG RESISTANCE BY
APPLICATION OF A MULTIPLE LINEAR
REGRESSION MODEL

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Objectives: Clinical validation of interpretation systems is the strongest argument for their use in therapeutical decisions. A retrospective analysis of 332 therapy experienced patients after therapy failure from 5 centres was performed. Datasets comprised suitable information on therapy regimen and viral load at the time of resistance testing and after 3 months. The value of the correlation of therapy response with the predicted regimen activity allows a direct measurement of the significance of various interpretation systems.

Methods: A multiple linear regression model was defined to compare the predictive power of several rules-based interpretation systems. The number of active drugs according to the applied algorithms and the viral load at the time of resistance testing (VLbC) were correlated by a multiple linear regression model to observed viral load shifts. Using this model a correlation factor for each given drug could be calculated. With the HIV-GRADE Internet-Tool the results of 4 different free available drug resistance algorithms were compared (HIV-GRADE version 12/2006, ANRS version 07/2006, Stanford HIVdb version 4.2.6 and REGA version 7.0).

Results: Based on the multiple linear regression model coefficients of determination (R^2) were calculated on the complete database as following: HIV-GRADE (0.36), Stanford HIVdb (0.36), REGA (0.35) and ANRS (0.30). All of the analysed algorithms showed a bad correlation for their clinical interpretation of resistance to Abacavir (ABC) and the observed viral load change in this cohort. To prove this observation, the data of all patients not having received ABC within the observation period was analysed. As expected R^2 -values were increased in this subset ($n=164$): HIV-GRADE (0.45), Stanford HIVdb (0.44), REGA (0.42) and ANRS (0.41).

Furthermore, variations of the predictive power between several algorithms can be explained using the described model. Amprenavir (APV) for example, showed the following p-values for the correlation factor: GRADE (<0.01), Stanford HIVdb (<0.01), REGA (<0.05) and ANRS (0.16) compared to AZT where all of the algorithms showed p-values <0.01 . The inclusion of the viral load prior to change in treatment (VLbC) in the model improves its correspondence to

the data. (HIV-GRADE without VLbC R^2 : 0.22 / with VLbC: 0.36)

Conclusions: Although analysis of retrospective data can not substitute a prospective study, multiple linear regression models can be very helpful in analysing the predictive power of drug resistance algorithms. Such models also allow determining problems in the interpretation of resistance against specific drugs as shown here for ABC and APV. Even if low coefficients of determination can be raised by including parameters like viral load before treatment change, further optimisation of the regression model is still needed. The influence of factors such as compliance, complete treatment history as an indicator for archived resistance and centre specific differences are not taken into account at the moment.

References:

HIV-GRADE <http://www.hiv-grade.de>

ANRS <http://www.hivfrenchresistance.org/>

Stanford HIVdb <http://hivdb.stanford.edu/>

REGA http://www.kuleuven.be/regag/cev/links/regag_algorithm/index.htm

GENO2PHENO: DETERMINATION
OF CLINICALLY RELEVANT CUT-OFFS

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Introduction: There is wide agreement that genotypic and phenotypic definitions of HIV drug resistance should rely on correlation with virologic and clinical outcome. Establishing these "clinical cut-offs" has proved to be a significant challenge because (i) monotherapy is obsolete, (ii) treatment response to a drug combination is usually attributed to more than one drug, and (iii) other factors apart from drug resistance may confound the analysis (e.g. drug adherence).

Methods: For determination of clinically relevant cut-offs we combined the quantitative phenotype prediction tool geno2pheno [1] with the multi-center Arevir database. Geno2pheno is an HIV drug resistance interpretation system based on machine learning techniques, whereas the Arevir database contains clinical and virological data [2]. In a first approach the database was screened for Lopinavir (LPV) add-on- and functional monotherapies (therapies where LPV was the only drug estimated to be active) to deduce virologic

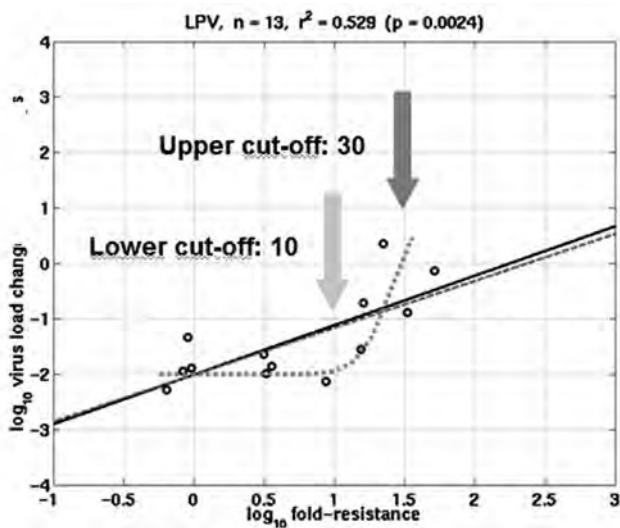


Fig. 1. Correlation of fold resistance and viral load change in LPV/r functional monotherapies an determination of lower and upper cut-offs.

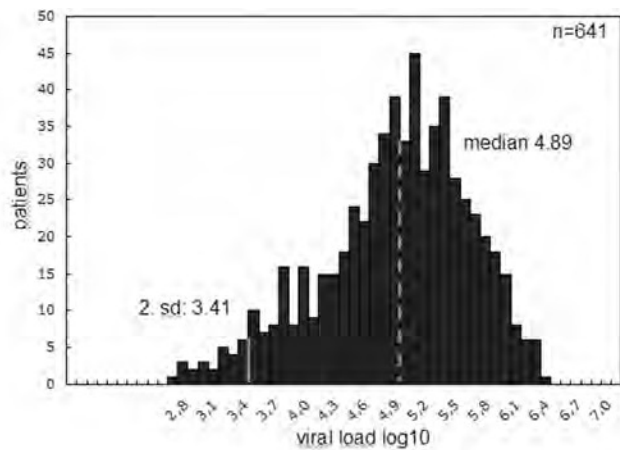


Fig. 2. Distribution of viral load in therapy-naive patients.

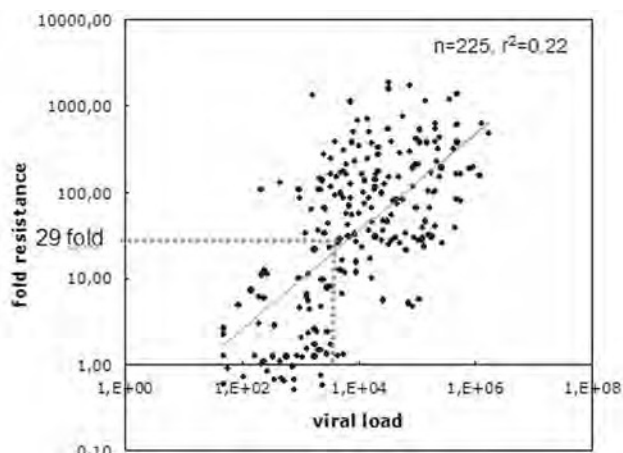


Fig. 3. Correlation of AZT fold resistance and viral load in AZT-containing, failing regimens.

response to LPV. Resistance factors (RFs) for LPV were predicted from HIV-genotypes before therapy switch and correlated with the observed viral load (VL) change four to six weeks post initiation of the LPV containing therapy (Fig. 1). In a second approach the upper cut-offs for all other drugs were determined by estimating the residual drug activity in failing regimens. A remaining activity was assumed when the viral load did not exceed 3.41 log₁₀ cop/mL (second standard deviation from the mean of the distribution of viral load in therapy-naïve patients; Fig. 2). Consequently, the upper cut-off was assessed by correlating fold resistance and viral load, e.g. for AZT in AZT-containing, failing regimens (Fig. 3). The lower cut-offs remained biological cut-offs, based on the distribution of RFs in therapy-naïve patients (2. standard deviation from the mean).

Results and discussion: The maximum VL reduction after therapy switch was observed for genotypes with LPV-specific resistance factors (RFs) <10 fold (lower cut-off). Patients with no VL change harboured viruses with genotypes of predicted RFs >30 fold (upper cut-off).

The determination of clinically relevant cut-offs for the geno2pheno system enables a differentiated prediction of an “intermediate” state of resistance. The graphical output (geno2pheno interpretation button) allows the continuous discrimination between “intermediate almost susceptible” and “intermediate almost resistant”, which is most helpful in salvage situations, where interpretation is most challenging, particularly for patients with limited therapy options. For clinical validation of the geno2pheno cut-offs see abstract by A. Altmann, geno2pheno-THEO: Predicting clinical outcome of combination ART.

References:

1. Niko Beerenwinkel, Martin Däumer, Mark Oette, et al. Geno2pheno: Estimating phenotypic drug resistance from HIV-1 genotypes. *Nucleic Acids Res.* 31:3850-3855, 2003.
2. Roomp K, Beerenwinkel N, Sing T, et al. Arevir: a secure platform for designing personalized antiretroviral therapies against HIV. *Proceedings 3rd Int. Workshop on Data Integration in the Life Sciences (DILS)*, Springer: 2006.

DERIVING CLINICALLY RELEVANT PHENOTYPIC CUT-OFF VALUES FOR THE VIRCO TYPE HIV-1 RESISTANCE ANALYSIS

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Background: Quantitative HIV-1 resistance information, either measured via a conventional phenotypic drug susceptibility assay, or predicted from the viral genotype, can provide a refined assessment of the activity of antiretroviral drugs against an individual pati-

ent's virus. For optimal utility, cut-off values, which serve as landmarks along the resistance/response continuum, should be defined based on the response in treated patients.

Methods: Based on clinical isolates with both drug susceptibility phenotypes (Antivirogram) and viral genotypes, linear regression models have been developed to predict fold change in IC_{50} (FC) for all available antiretrovirals (with the exception of FTC, nevirapine and efavirenz).

Using treatment response data from clinical trials and cohorts, separate linear regression models were developed to predict 8-week change in viral load for regimens containing the individual drugs as a function of

- Log baseline viral load,
- FC as predicted by virtualPhenotype-LM from the baseline viral genotype
- Activity of the background regimen summarized as a continuous Phenotypic Susceptibility Score (cPSS)
- Use of T20 for the first time
- For ATV/r, concomitant tenofovir use.

Results: Two clinical cut-offs were defined, corresponding to predicted FC values associated with a 20% or 80% loss of response (as predicted for subjects infected with wild type strains) to individual drugs.

Conclusions:

- Linear regression models have been developed to predict drug susceptibility from the viral genotype utilizing very large datasets of HIV-1 clinical isolates for which both individual drug susceptibility phenotype and the viral genotype are known. For each drug the models identify which mutations and interacting mutation combinations affect drug susceptibility, and by how much. The resulting virtualPhenotype-LM provides an accurate prediction of the measured phenotype (Antivirogram or PhenoSense).
- Both the dynamic range of resistance observed in HIV-1 clinical isolates and the clinical cut-offs defined based on response in treated patients are drug specific.
- VircoTYPE resistance analysis applies a systematic and consistent approach to defining clinical cut-offs for phenotypic resistance data predicted from the viral genotype.
- Two clinical cut-offs have been defined for each NRTI (except FTC) and boosted PI by this methodology.
- This information can facilitate selection of active treatment regimens, especially for treatment-experienced patients.

GENO2PHENO[CO-RECEPTOR]

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Coreceptor antagonists are a promising new class of drugs supporting the treatment of HIV-1. The virus

can gain entry into host cells by using the cellular CD4-receptor in combination with a coreceptor. The most important of these coreceptors are the chemokine receptors CCR5 and CXCR4. Three types of viruses exist: viruses only capable of using the CCR5 coreceptor (R5), X4 viruses being able to use only the CXCR4 coreceptor, and dual-tropic (R5X4) viruses able to enter cells using both coreceptors. R5 viruses are generally present over the entire course of infection, while variants capable of using CXCR4 emerge in approximately half of the infected patients over the course of disease. The emergence of these viral strains is associated with accelerated disease progression. Therefore, monitoring coreceptor usage is of great importance. In addition, the first coreceptor antagonist being available, Maraviroc from Pfizer, is a CCR5-antagonist. It should only be administered to people having exclusively R5 viruses. This is due to the fact that X4 viruses are naturally resistant against them and there are major concerns that the selective pressure towards X4 viruses can increase the risk of disease progression.

At present, two phenotypic assays for monitoring coreceptor usage are commercially available: Trofile from Monogram Biosciences and Phenoscript from VIRalliance. However, phenotyping has several limitations. It is very time consuming, costly and has a limit in its sensitivity for detection of minority quasispecies in the presence of mixed viral populations (Skrabal et al., 2007).

Prediction-methods based on genotypic data can be a fast and cheap alternative. They are based on sequence information of the third hypervariable loop (V3) of HIV envelope glycoprotein gp120 which is considered to be the major determinant for coreceptor specificity.

Several methods have been proposed for prediction of coreceptor tropism. The classical 11/25 rule considers only sequence positions 11 and 25 in the V3 loop for classification. Despite its moderate sensitivity in identifying x4 viruses and its lack of a continuous prediction scale, it has nevertheless enjoyed ongoing popularity as a genotypic monitoring method. More sophisticated methods use Neural Networks, Position Specific Scoring Matrices, or Support Vector Machines (SVM).

Geno2pheno[coreceptor] (Sing et al., 2004) is a freely available web service for predicting viral coreceptor usage based on Support Vector Machines. These SVMs have shown the best results in a comparison of six statistical learning methods on a dataset of 1110 matched genotype-phenotype pairs from different subtypes. They performed significantly better than all other methods except for PSSMs which were almost equally reliable. The web server uses prediction models trained on this dataset. Unlike other web services, it allows the user to paste in (or upload) unaligned sequences rather than sequences which have to be aligned to a specific reference manually. It is also not necessary to cut out the V3 loop from a larger sequence, geno2pheno[coreceptor] finds the V3 loop automatically. It is possible to choose between six significance levels. The result of a prediction is an output page containing some general information (fasta-header, prediction date), the aligned V3-region, and the predicted pheno-

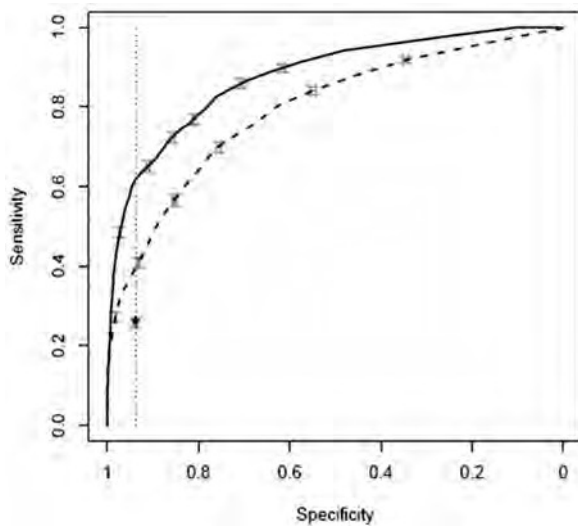


Fig. 1. ROC curves for prediction of coreceptor usage on population-based data. The dashed line shows predictions based on sequence-information alone whereas the star marks the 11/25-rule. The inclusion of markers leads to considerable improvement in predictive performance (solid line).

type. The predicted phenotype describes if a virus can use the CXCR4-coreceptor or not. The reported p-value describes the confidence of a prediction.

Geno2pheno_[coreceptor] has been widely used in the past; more than 15000 predictions have been done since June 2004. To enhance the understanding of coreceptor usage and to move forward the use of prediction-based coreceptor methods in routine clinical practice, new methods have been developed and are currently implemented into the web service.

Significant improvements could be achieved on clinical data from therapy-naïve patients (Sing et al., submitted). Unlike clonal data, clinical samples are usually obtained using bulk or population-based approaches resulting in sequence ambiguities. Therefore, the performance of prediction methods substantially decreases in comparison to clonal data. To address this problem, a strategy for dealing with mixtures of sequences was developed. In addition, it could be shown that the incorporation of clinical markers such as CD4-percentages or host CCR5₃₂-heterozygosity significantly improves predictions. In comparison to the 11/25 rule, a 2.4-fold improvement in detecting X4-viruses was achieved.

Established prediction methods for coreceptor usage are based on sequence information alone. To improve these methods methodologically, the first structure-based approach has been described in a recent study (Sander et al., 2007). A better understanding of the underlying determining factors regarding sequence and structural aspects should be gained from this method.

The prediction method is based on a crystal structure of the V3-loop which acts as a template for viral variants. For each variant, the backbone conformation is kept rigid and the side-chains are modeled. Pairwise distance distributions between functional atoms are computed in order to capture the spatial arrangement of physico-chemical properties. These distributions are used as a vectorial input to a SVM which predicts the coreceptor usage. By using statistical importance measures, structural features relevant for coreceptor usage can be mapped onto the structure allowing for visual and quantitative interpretation.

The method has been evaluated on a dataset of 432 V3-sequences. The predictions of the structure-based approach were significantly better than the 11/25 rule as well as predictions from a Support Vector Machines using sequence-information. Further improvements could be gained by combining the structural-descriptors with the sequence-representation.

Due to the length of the loop in the crystal structure, the method can so far only handle V3 sequences of length 35. Future developments will lead to improved modelling of V3 variants containing insertions or deletions and will be concerned with relaxation of the backbone rigidity

References:

- Sing T, Beerenwinkel N, Lengauer T, Learning mixtures of localized rules by maximizing the area under the ROC curve, Proc. ROCAI-2004: 1st International Workshop on ROC Analysis in Artificial Intelligence, Valencia, Spain, August 22, 2004; pp. 89-96.
- Skrabal K, Low AJ, Dong W, et al., Determining human immunodeficiency virus coreceptor use in a clinical setting: degree of correlation between two phenotypic assays and a bioinformatic model., J Clin Microbiol. 2007
- Sander O, Sing T, Sommer T, et al., Structural Descriptors of gp120 V3 Loop for the Prediction of HIV-1 coreceptor Usage, PLoS Comput Biol., 2007
- Sing T, Sander O, Beerenwinkel N, et al., Predictive Models of Human Immunodeficiency Virus type 1 (HIV-1) coreceptor Usage Based on Genetic, and Clinical Covariates, submitted

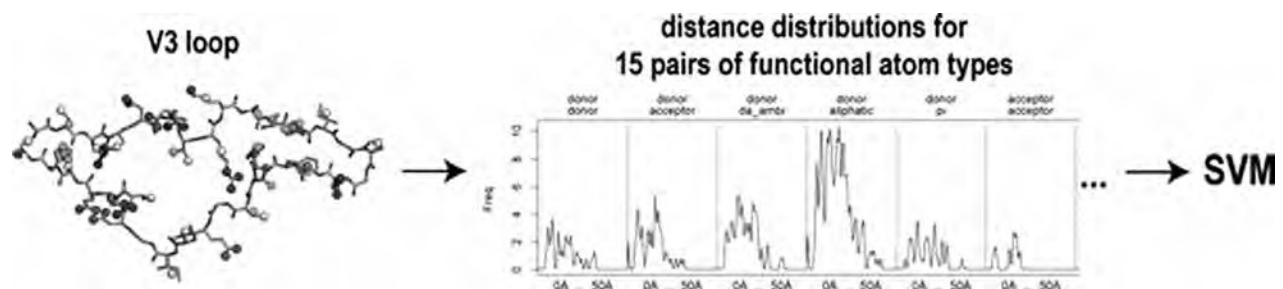


Fig. 2. Schematic Overview of the Structural Descriptor Computation and coreceptor Usage Prediction.

TOWARDS MOLECULAR MODEL BASED PREDICTION OF HIV DRUG RESISTANCE

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Background: Occurrence of phenotypic HIV drug resistance is highly correlated with specific mutations in the drug target proteins, and it is plausible to assume that the molecular basis of resistance is a change of protein-drug interaction due to these mutations. Under this assumption and in view of the advances in molecular modelling, the increase of knowledge of drug-target structures, and the growth of computing power, it could become possible to predict resistance of HIV of given genotype against a given drug from computational modelling of the involved molecules and their interactions. Such capabilities would support diagnostics and therapy decisions.

Methods: We have developed an automated computational protocol for the modelling of HIV protease (PR) mutants, their complexes with drugs, and the estimation of drug-protein affinities. The protocol (1) takes a given amino acid sequence of a PR mutant and models its mutations onto experimentally determined 3D-structures of PR in the Protein Databank (<http://www.pdb.org>), (2) prepares a 3D-structure of the PR mutant in complex with a given drug, (3) computes with force field methods the differences in free energy of binding between, on one hand, drug and PR mutant, and on the other hand, drug and PR wild type. For resistance mutants of PR it is expected that the estimate yields a decrease of drug-PR binding affinity for the mutant with respect to the wild type. Since the protocol implies generation of 3D-structures, the physico-chemical basis of the changed drug-PR interactions can be studied in detail.

Results: We have compared measured phenotypical resistance factors (RF) with the computed affinity changes due to resistance mutations for the drug Lopinavir. The comparison shows that strong increases in RF correlate well with particularly low drug-PR affinities, as expected. However, the overall correlation of affinity estimates with measured RFs is by far not good enough to be useful for resistance prediction. We have investigated the reasons for this lack of accuracy for single drug resistance mutations in greater detail. E.g. we have studied the mutant L76V associated with resistance to Lopinavir and Amprenavir and resensitisation with respect to Saquinavir. The modelled changes in molecular electrostatics were in qualitative agreement with the measured changes for all three drugs, but whereas the model correctly predicted a lower affinity of the drug to the L76V mutant in the cases of Lopinavir and Amprenavir, the model did not correctly predict the expected higher affinity to Saquinavir.

Closer inspection of the modelled 3D-structures showed that the latter may be due to larger scale structural rearrangements caused by the mutation, which were not adequately reproduced in the modelling procedure. **Conclusions:** The current status of molecular model based prediction of HIV drug resistance can be described as being useful for qualitative investigations. The methods have not yet matured to a point that would allow for accurate predictions. Nevertheless, with the steady improvements of modelling methods and increasing computing power the aim of quantitative prediction from molecular models seems to be in reach within the next decade.

DETECTION OF MINOR VARIANTS IN THERAPY NAIVE PATIENTS

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Background: In therapy naive patients resistance is associated with a poor treatment outcome of the first-line regimen. The observed rate of transmitted resistance in therapy naive HIV-1 patients varies between 10-20% as reported in different studies. The high replication and mutation rate of HIV leads to the coexistence of different strains in one patient. The selective pressure of drugs results in predominance of one strain while other variants form minorities. With the method of Population Based Sequencing only quasispecies of more than 20% can be measured. The major variant can be replaced by minorities when drug pressure is changing. For NNRTIs a single resistance mutation, such as K103N, is sufficient for therapy failure. Primary virological failure in first PI regimens is rare and only seldom described with resistance associated mutations. This suggests a superiority of protease inhibitor based firstline regimens compared to NNRTI regimens, when resistance transmission is suspected. The following investigations should give more information on resistance transmission problems.

Patients and methods: 163 therapy naive HIV-1 patients from multicenter study RESINA underwent genotypic resistance test. Using realtime-PCR on a Light Cycler 2.0 with one labelled probe and two different primerpairs described by Lecossier et al. [1] the amount of mutant variant at aminoacid position 103 of the RT of each patient sample was detected, with a detection limit of 0.1 %.

In a second analysis one patient from the RESINA cohort failing his initial therapy with LPV/r was analysed at baseline, initial therapy and at therapy failure.

Population sequencing of PCR-products was performed with ViroSeq HIV Genotyping system. A primerspecific PCR for detection of minorities at position 82 for these three samples was performed with the Light Cycler 2.0. The realtime-assay was a modified method of Hance et al. [2] with 0.2% minority detection limit.

Results: 128 of these patients harboured a HIV-1 subtype B and the other 35 a non-B subtype. With population based sequencing NRTI associated mutations could be detected in 11 of 163 patients (6.7%). One of these patients harboured 3 NRTI mutations, each of the other 10 patients 1 mutation. In 8 patients a revertant could be seen at position 215. In two patients (1.2%) 1 NNRTI mutation was detected, but in none of the patients was K103N found by this method. The number of NRTI- and NNRTI mutations in these 163 patients detected by Population Based Sequencing was representative for the RESINA cohort of 831 patients (NRTI 5.4%, NNRTI 3%). Using the realtime PCR assay minority variants of at least 0.1% of the total viral population could be safely proved. In the population of 163 patients 34 samples (21%) showed a resistant minority K103N. Comparing the different subgroups of subtype B and non-B, a difference of 16% vs. 40% transmitted resistant minority was found. Because of the low number of non-B patients these results have to be confirmed by further analysis.

In the second analysis one patient from the RESINA cohort failed his firstline treatment with AZT, 3TC and LPV/r. The baseline virus population showed a wildtype without V82A in the population based sequence in the protease (PR) and in the reverse transcriptase (RT) a not resistant associated revertant at aa position 215. The mutational profile at therapy failure was: in the PR V82A and in the RT M41L, T215Y/C and M184V. These patterns suggest a newly acquired LPV resistance mutation at position V82A. Due to the revertant T215A an educated guess of a transmission of resistant variants was made. The minority realtime-assay discovered a resistant minority of 0.2% V82A. In the second sample in 7% mutation were detected shortly after starting therapy and in 100% after failure of treatment

Conclusions: The resistant minority at position 103, which was detected in 21 % of the patients, may lead to an early virologic failure because the resistant minority can replace the majority. In HIV-1 non-B patients the prevalence of a K103N minority seems to be higher than in HIV-1 subtype B patients. The transmission of resistance at position 103 is higher than estimated by the results of sequencing.

The case report of one patient failing his first line regimen with AZT, 3TC and LPV/r showed that transmitted resistant minorities can become dominant under the selective pressure of drugs and cause therapy failure. A minority mutation at PR position 82 could be measured at baseline and the transmitted revertant at RT position 215 can give a hint at transmitted resistance of other drug classes.

For drugs with a low genetic barrier or in cases with a suspicion of transmitted resistance, it could be important to detect minorities in therapy naïve patients.

References:

1. Lecossier et al., J Acquir Immune Defic Syndr 2005; 38:37-42
2. Hance et al., J Virol 2001;75:6410-7

PRIMARY HBV RESISTANCE

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Despite safe and efficient vaccines the human hepatitis B virus (HBV) remains a major medical problem worldwide. In Germany, according to the Robert-Koch-Institute (www.rki.de), an estimated percentage of 0.8% of all residents, which is a total number of 640.000 patients, are chronically infected with HBV. Provided that this percentage is true for the total European Community, which had an estimated number of 459 million residents in 2005 (<http://europa.eu>), more than 3.67 million patients are chronically infected with HBV in Europe. Besides the medical problem socio-economic costs per patient and year are calculated with up to 25.000 € and therefore are also a severe economic problem for the European Community.

The HBV infection, once it has become chronic, is a major cause of subsequent liver disease that initiates with fibrosis, leads to cirrhosis and finally causes hepatocellular carcinoma that can often only be treated by liver transplantation or lead to a fatal outcome if they remain untreated. Recent studies have shown that the progression of chronic HBV infection and thus the progression of liver disease can be efficiently retarded by vigorous antiviral therapy. These antiviral therapies are based on interferons (with a broad range of mild to severe side effects) and an increasing number of nucleoside and nucleotide analogues [1]. The latter groups of compounds were initially used for HIV therapy in which they inhibit the viral RNA dependent DNA polymerase (reverse transcriptase), an enzyme that is also encoded by the HBV genome. The mechanism of action of nucleos(t)ide analogues against HBV is similar as they also inhibit the viral reverse transcriptase which results in chain termination during the viral genome replication and thus, consequently, to the reduction of the viremia. Despite the increasing number of anti-HBV drugs that are in clinical studies or at least in development, only 4 compounds are approved for HBV therapy yet, namely lamivudine, adefovir, entecavir, and, for the HIV coinfecting patients, tenofovir. As observed with HIV therapy, the usage of antiviral agents is accompanied (or followed) by the development of antiviral resistance that in most cases can be referred to mutations in the viral target enzymes, i.e. in case of HBV and HIV, the viral reverse transcriptase(s). Taking this into account it was rather surprising that with the introduction of adefovir several cases of nonresponse to the drug were observed that were associated not only with mutations or newly detected variants of the virus (recently reviewed by [2]). In fact, despite proven compliance, in some cases the

drug was unable to reduce the viremia although no resistance mutations were observed. These observations led to the conclusion that in some patients (in case of adefovir and tenofovir) (a) either the prodrugs are not efficiently processed into the active metabolites, (b) the drugs are not (efficiently) delivered to the infected cell by a defective or altered transport mechanism, or (c) that the drugs are not efficiently phosphorylated [2]. These assumptions are supported by a number of previous observations on the metabolism of the nucleos(t)ide analogues: First, especially for adefovir and tenofovir which are administered as prodrugs, defective intracellular esterolytic cleavage may lead to treatment failures [3]. Second, as it is known e.g. for the ATP binding cassette transporters, there are significant inter-individual differences in form of polymorphisms in the respective genes/enzymes that result in altered drug response, which in turn may induce treatment failure or severe side effects. Third, studies on the metabolism of adefovir (9-(-2)-phosphonyl-methoxyethyl)adenine), an adenosine derivative probably absorbed by the ATP binding cassette, revealed that the compound is actively transported into the cytoplasm by a 50 kDa protein also against a concentration gradient before it is phosphorylated to its diphosphorylated derivative PMEApp by the 5-phosphoribosyl-1-pyrophosphate (PRPP) synthase and/or adenylate kinase 2; the diphosphorylated can be incorporated into the viral genome. Thereby adefovir can reach intracellular concentrations of up to 10 pmol per one million cells. Polymorphisms in any of the enzymes involved in these up-take cascades may also lead to impaired antiviral activity of the analogues. Nevertheless, from these earlier studies and also from newer studies on the metabolism of adefovir and also tenofovir, it remains unclear to what extent other enzymes are involved in the processing of these drugs, as no related data from the respective clinical cases with non-response of assumed metabolic origin were hitherto published.

However, although a respectable portion of non-response to antiviral therapy may be explained by these potential mechanisms of metabolic resistance to antiviral compounds, there are still a (limited) number of viral factors to be investigated with respect to their potential to mediate antiviral resistance. Some of these factors, namely mutations in the viral reverse transcriptase, have already been described and were extensively investigated in vitro (summarized by [4]). However, for some of these mutations it remains yet unclear whether they in fact induce resistance, whereas the role of other mutations (that have clear clinical correlates) are still disbelieved to play a role in antiviral resistance [5]. In all studies, only the small domain of the viral polymerase that is responsible for reverse transcription is taken into account for analyses, whereas the role and structural influence of the remaining domains on this domain remain to be investigated.

References

1. Hadziyannis SJ. New developments in the treatment of chronic hepatitis B. *Expert Opin Biol Ther.* 2006; 6:913-21.
2. Tillmann HL. Antiviral therapy and resistance with hepa-

titis B virus infection. *World J Gastroenterol.* 2007; 13:125-40.

3. Ray AS, Vela JE, Olson L, et al. Effective metabolism and long intracellular half life of the anti-hepatitis B agent adefovir in hepatic cells. *Biochem Pharmacol.* 2004; 68:1825-31.
4. Zoulim F. In vitro models for studying hepatitis B virus drug resistance. *Semin Liver Dis.* 2006; 26:171-80.
5. Wong SN & Lok AS. Tenofovir disoproxil fumarate: role in hepatitis B treatment. *Comment on Hepatology.* 2006; 44:318-25; *Hepatology.* 2006; 44:309-13

FULLY AUTOMATED ANALYSIS OF HEPATITIS B SEQUENCES FOR INTERPRETATION OF DRUG RESISTANCE AND HBs-ANTIGEN ESCAPE MUTATIONS USING THE HIV-GRADE HBV INTERNET TOOL

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Objectives: Inhibitors of reverse transcriptase are a well established treatment option for patients with chronic hepatitis B infection. The widespread use of these antiviral drugs results in an increase of drug resistance if long-term treatment is necessary. Although public-available tools for analysis of HIV-sequences are frequently used to assess drug resistance, such tools are not commonly used for Hepatitis B. A new easily accessible tool for Hepatitis B resistance pattern analysis was developed on the basis of the well established HIV-GRADE internet tool.

Methods: The hepatitis B drug resistance tool is based on the HIV-GRADE tool, which itself is derived from the HIValg module of the Stanford HIVdb tool. As these tools are specialised on HIV drug-resistance, substantial changes in the internal structure of the software were introduced to allow the analysis of HBV resistance patterns including adjustments of the reference sequences for alignment and genotyping. As there is no standard reference sequence for hepatitis B like HxB2 for HIV, sequences are aligned to their specific genotype consensus sequence after a pre-alignment to appoint the genotype. Thus detected mutations are automatically corrected for genotype, excluding the display of amino-acid variations which are not associated with drug resistance. In addition to this drug resistance interpretation the tool allows the reliable analysis of HBsAg-escape mutations.

The underlying algorithm is based on a comprehensive analysis of available literature and includes our own observations of clinically resistant hepatitis B strains.

As the tool uses the Algorithm Specification Interface (ASI) developed by Shafer et al. for the Stanford HIVdb software, the algorithm can be coded and stored in XML format. The application of the ASI also allows the users to create their own algorithm and utilize it with this tool.

50 previously analysed sequences at the Max von Pettenkofer Institute showed no difference when re-analysed with the newly designed tool.

Conclusions: With this freely available software analysis tool fast and reliable Hepatitis B drug resistance and HBsAg-escape mutation profiles can be easily generated.

The tool is available at the internet address <http://www.hiv-grade.de>

THE MEDEORA-HIV DATABASE

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Background: Sufficient capturing and management of information is the most important step before data can be analyzed and conclusions can be drawn. We introduce the database MEDEORA-HIV which puts a strong emphasis on the collection and the processing of routinely gathered clinical data of HIV infected patients. Furthermore the database was developed to support physicians in their daily routine with a wide

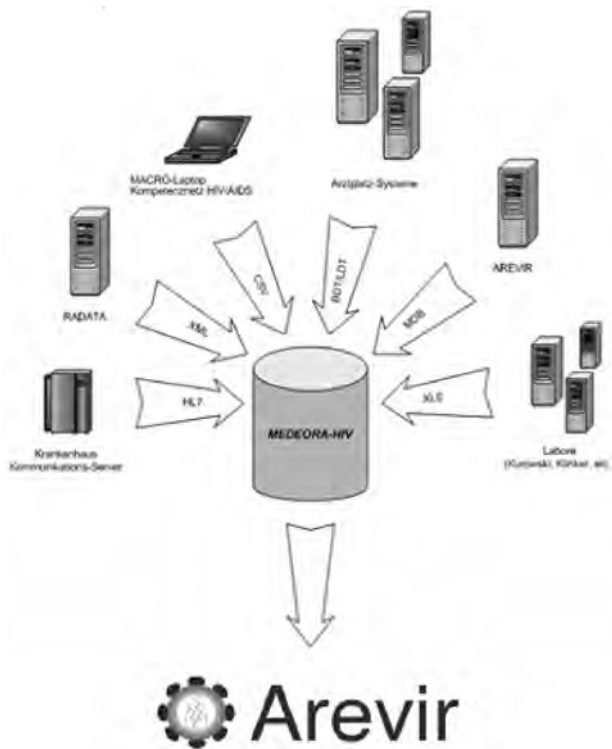


Fig. 1. MEDEORA interfaces and data transfer to AREVIR.

variety of interfaces and reporting tools (e.g. discharge letter or diagrams of the patient's clinical course).

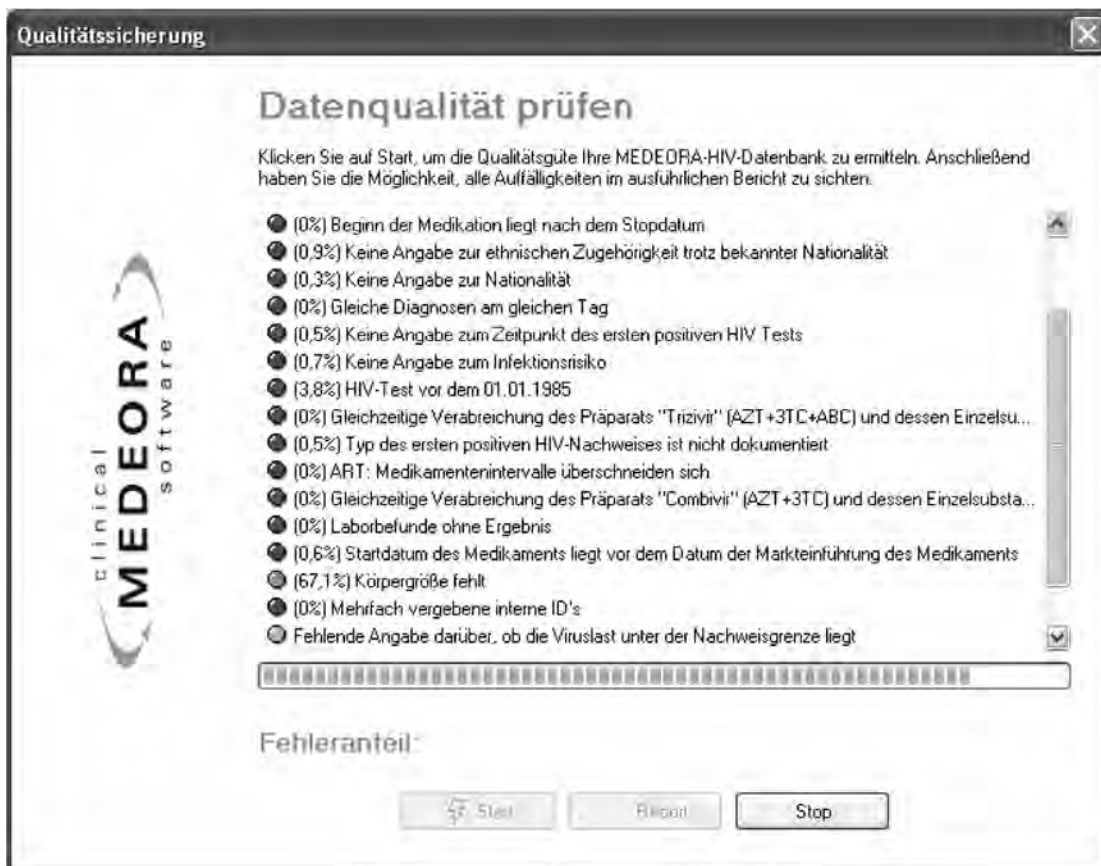


Fig. 2. The interactive QA-module.

To guarantee high data quality, compulsory boxes, edit checks, reference ranges and data validation via a quality assurance (QA) module were implemented.

Meanwhile more than 120 users have registered for MEDEORA-HIV and more than 20.000 German patients (>10.000 of them under treatment) are documented in a standard data format.

Methods: A wide variety of interfaces allows the mobilisation of data gathered during the treatment of patients in clinical routine. BDT and LDT interfaces allow communication with information systems of private practitioners or laboratory information systems (LIS) and a HL7 interface is able to collect data from most hospital information systems (HIS, see Fig. 1).

To assure a high data quality we introduced an interactive QA module (Fig. 2). This module validates the data by using a wizard that cross checks the dataset searching for logical errors. More than 60 validation queries are in use looking for e.g.: double diagnosis, overlapping medication periods or laboratory measurements without a results. A detailed report allows the correction of possible errors.

Results: Using the structure and functionality described above we implemented a bi-directional interface for data transfer that allowed data exchange with the AREVIR project. Clinical data of 953 patients with 1274 genotypes were exported to AREVIR (09/2006) and matching virological data were imported to the local MEDEORA-databases in several outpatient centers.

This example proved the benefit of MEDEORA-HIV for daily use in clinical routine and supports our approach to store data in a standard format and combine data from different sources. An approach that may help to find new hints and clues for further research projects.

GERMAN HIV COHORT STUDY

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The process of implementation of the German HIV cohort study, funded by the BMBF, was started in 2004. The prospective cohort study enrolls HIV-infected patients in 68 centres throughout Germany. In this network different disciplines such as clinical medicine, dermatology, neurology, paediatrics and obstetrics are brought together. Currently, there are data from more than 14.000 patients.

Per follow-up at 6-month intervals, information on approximately 560 different variables is collected. 11.000 first follow-ups exist, 7.500 patients have two follow ups, and 4.500 even have 3 follow-ups. High quality retrospective data exists for 4.000 patients. The

database also contains information on asserved blood and serum samples and biological material such as lymph nodes. This information is immediately available for further analysis together with extensive medical data.

Essential for the successful operation is effective communication and cooperation among a variety of different institutions and centres. In September 2005, the 2nd funding period, the coordinating centre for clinical Trials (KKS), an Institution of the Medical Faculty of the University of Cologne, took over the data management for the cohort. Physicians, scientists, KKS staff, patient representatives and colleagues from the competence net central meet on a regular basis and contribute substantially to further improvement of the cohort.

The data collected from each patient has the following components: The basic module comprises socio-demographic information, onset of disease, ART and laboratory parameters. The hepatitis module documents vaccination status and potential hepatitis infection, specific lab values and medication. The children's module collects data from HIV positive children. It currently contains data from 120 children. The exposed children's module contains information on children that have been exposed to HIV during pregnancy. This module is connected to the pregnancy module. In this module data related to the pregnancy of HIV positive women is soon going to be collected. The neurology module is a separate module documenting drug abuse and neurological diseases, symptoms and deficits.

Quantity is nothing without quality.

Standard operating procedures (SOP) form the basis for all processes associated with management of the cohort. They exist, for example for on site monitoring, development and management of data and project management. These are constantly kept up to date in cooperation with the SOP group and the quality management department of the KKS. The use of InferMed's validated study documentation software MACRO guarantees data keeping on a high level according to GCP. For example, data entry is only allowed for authorised users: All manipulations of the data such as entry and modification are documented by an audit trail. The helpdesk is the primary contact point for all problems. It coordinates and directs all requests to the respective staff in charge. Such a procedure contributes to effective support and guarantees standardized solutions. Various checks for plausibility and inconsistencies are integrated into the database. For example, numerical data are associated with ranges, and a warning will be generated if these ranges are exceeded. Central monitoring optimises the data through queries removing imprecise data. The HIV on-site monitoring is a unique feature of registers in German competence networks. It is carried out by 3 people in accordance with the monitoring manual. It has various functions: patients' concerns are being kept, documenting staff is adequately trained, and data entry is standardised. Apart from organisational questions and source data verification it is checked whether written informed consent exists. In addition, the correct asservation of blood and serum samples is controlled.

A crucial aspect is the identification and treatment of duplicates: The HIV/AIDS competence network is going to use TMF's data security concept B for the elimination of duplicates. It draws together data from different centres and is able to effectively avoid duplicates through a PID generator, a pseudonymisation tool and an electronic data custodian. The PID generator was implemented in last summer and the pseudonymisation tool in December 2006. As the competence network has made electronic custodianship in a TMF project more precise, we expect the concept to be implemented soon. The competence net HIV is thus in a leading position of bringing a suitable concept for data protection to work. This concept will be useful for all German competence networks. Until then, identification of duplicates happens as follows: Patients can be identified as duplicate when they inform a second physician that they are already participating in the competence network. If the patient wishes, his 2 PIDs can be unified on a central server for analytical purposes.

The weekly on-line reports also contribute to quality management.

Each centre can consult a list of their own patients whose CD4 cell count has been overdue for more than 8 months. The list reflects follow-ups that are still missing. The respective patients need to be documented.

The cohort has received attention on an international level. Tests have demonstrated that data quality and data collection meet international standards. Interested in Co-operations are Cohere (Collaboration of Observational HIV Epidemiological Research Europe), MITOC (Mitochondrial Toxicity in Children and NRTI Exposure during pregnancy) and the AIDS Research centre Elite-controller project.

Conclusion: Quality has top-priority, which is ensured by a wide variety of measures such as SOPs, monitoring, queries and the usage of study documentation software. Online reports create transparency and provide feedback for the participating centres. 14.000 patients, with 560 variables covered in different modules such as children, hepatitis, neurology and pregnancy, as well as a direct link to blood and serum sample base are available.

All this is substantially contributing to both a detailed description of the current state of HIV therapy in Germany as well as to a continuous improvement in therapy by generating hypotheses for clinical trials. The cohort makes a valuable contribution to vertical and horizontal knowledge transfer nationally as well as internationally.

AREVIR – A DATABASE TO SUPPORT THE ANALYSIS OF RESISTANCE MUTATIONS OF HUMAN IMMUNODEFICIENCY VIRUS

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Background: Despite the large amount of information about drug resistance associated mutations in the HIV genome, the interpretation of mutations is challenging and moreover, single mutations might not reflect the overall complexity of HIV resistance. To investigate with bioinformatics methods correlations between HIV genotypes from patients under antiretroviral therapy and the clinical outcome of the patients with bioinformatics methods, the Arevir database was created (Arevir = analysis of retroviral drug resistance). Special emphases of the implementation were the protection of the privacy of patients, physical protection of the data and data integrity, as well as quality control of the imported data.

Methods: Arevir consists of a MySQL 5.x database (<http://www.mysql.com>) and a web user interface for data access. The database comprises 50 tables organized in 5 groups holding: patient demographic data, therapy data, isolate related data, HIV genotypes and administrative data. To protect the privacy of the patients all data are completely anonymized. Physical protection is achieved by a high-security server room and network access restricted solely to SSH (<http://www.openssh.com>). A standalone, offline application 'Rosie' for data input was written to facilitate data acquisition and management at HIV treatment centers.

Results: To guarantee anonymity, each patient record is accessed by a meaningless serial number. Instead of storing patient names a pseudonym (hash code) based on SHA-1 [1] is generated from the patient's name and birth date. This irreversible pseudonym is not visible to the Arevir users. It is used to avoid duplicate patient records under different serial numbers. To improve the recognition of duplicate copies when receiving follow up data, a pre-processing step was introduced in the pseudonym generator. This step minimized the impact of spelling errors in patient names and increased the number of extracted standard datum records (STDR) as shown in Table 1. A STDR consists of a selection of clinical data and a HIV genotype, in a time slot around the start of a new or modified antiretroviral therapy.

Table 1. record counts and number of extracted STDs from the Arevir database in 2005 and 2007.

	June 2005	January 2007
patients	4.500	2.500
therapies	12.000	6.000
CD4 values	52.000	53.000
HIV RNA values	41.000	26.000
HIV genotypes	3.000	2.200
STDs	350	750

A very high network security is achieved by enabling only one port on the Arevir server (for SSH connections). Furthermore, login is only possible with a private key file and from computers whose IP address is registered at the Arevir server. The administrative tables in the database provide additional content protection and are used by the web interface application to guarantee that users can only see data from patients they are responsible for.

To improve the quality and integrity of the data, data cleansing was established as a continuous process. Several checks are performed either at input time or by analysis scripts to take batch imports into consideration. One example of the importance of quality analysis is shown in Figure 1. In this case report the success or failure of a new therapy after virological failure is measured by the viral load closest to week 12 after the TCE (therapy change event).

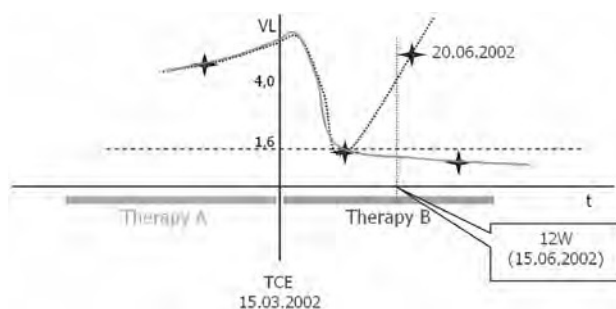


Fig. 1. Assumed (dotted line) and real (solid line) viral load progression interpretation depending on a mistyped date.

The viral load value at 20.06.2002 was exposed as typing error in the date, by a quality check warning, due to more than one viral load encountered in a time slot of 20 days. Without this check, Therapy B would be erroneously classified as a failure.

Conclusions: The new pseudonym algorithm increased the number of available standard datum records and reduced the number of duplicates. The Arevir database is now capable to incorporate data from other patient databases with lower risk of duplicates. Furthermore, the quality checks improved the overall reliability of Arevir data.

References

1. Eastlake & Jones, RFC3174 2001 (<http://tools.ietf.org/html/rfc3174>)

EuResist: INTEGRATING AND EXCHANGING EUROPEAN HIV DATA

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Background: The EuResist project aims at developing an integrated system for the clinical management of antiretroviral drug resistance. The system will provide clinicians with a prediction of the HIV patient's response to antiretroviral treatment and thus help select the best drugs and drug combinations for any given HIV genetic variant. To this end, a large European integrated data set has been created by merging three of the largest existing resistance databases: ARCA (Italy), AREVIR (Germany) and Karolinska (Sweden). Several distinct prediction engines are being used in the study and preliminary results are encouraging.

Methods: The EuResist project brings together clinical virology units with experts in machine learning and data mining. The project consortium aims at integrating clinical and virological data from a large group of patients to provide the information for training multiple machine learning approaches. In order to be reliable, some of the most promising prediction methods available require a critical mass of high-quality data, which has not been available from a single source. To this end, three large and expanding databases, containing historical and prospective data from different European countries, have been integrated. Since several variables have a potential impact on the response to treatment, gathering data from multiple locations ensures that different and potentially influential scenarios (e.g., drug prescription attitudes, HIV subtypes) are represented in the integrated database.

Each learning strategy results in an engine that generates a prediction of a short term viral load for a patient, given at least one particular drug combination and a genotype. These engines will be combined in a final engine, which is freely accessible on the web, to assist infectious disease specialists in building the optimal treatment regimen.

Integrating data: The EuResist Integrated Database (IDB) is generated by integrating three pre-existing independent data sources: ARCA (Italy), AREVIR (Germany) and Karolinska (Sweden). It includes patient demographics, CD4 and viral load values, genotypes, and treatment histories. The data is fully anonymised so it is impossible to trace back to a patient from the EuResist IDB content. Physical data consolidation was selected as the integration strategy, since it ensures high availability, high performance, and a high level of data quality – the three essential requirements for successfully training prediction engines. Data Clean-

sing is an integral part of the data integration and feeding process. The quality of the data is crucial for the training and validation of the prediction engines. Although quality checks exist in each of the integrated data sources, a set of domain specific rules has been defined for the EuResist IDB, thus leveraging and enhancing the overall data quality.

Table 1. The number of data items integrated in the latest version of the EuResist IDB, released on April, 2007.

	ARCA	Arevir	Karo-linska	EuResist
Patients	9074	2444	4295	15813
Therapies	29977	6154	13881	50012
Therapy Compounds	88338	18233	42868	149439
CD4 Isolates	114521	28678	89173	232372
Viral Load Isolates	93640	25972	61074	180686
Raw Sequences	14942	2180	1266	18388
Protease Sequences	13372	2180	1135	16687
RT Sequences	13313	2179	918	16410

Exchanging data: Health Level Seven (HL7) is a standard, domain specific, common protocol for the exchange of healthcare information. HL7 is the *lingua franca* of the healthcare industry and is widely used for exchanging healthcare data. The latest version, HL7 v3, consists of two main elements: a messaging standard and an information model referred to as the RIM – Reference Information Model.

The EuResist IDB defines an HIV-specific data mart, which can be derived from the more generic HL7 RIM. By mapping the EuResist IDB schema to HL7 v3, a standard interface for exchanging HIV data is achieved. Although there are no HIV specific messages in HL7 v3, the standard provides a generic way of representing any medical information. A mapping between the EuResist proprietary HIV data mart to a standard HL7 CDA (Clinical Document Architecture) is being defined as part of the EuResist project.

The CDA document is a defined and complete information object that can be sent inside an HL7 message and can exist independently. Our goal is to define a *CDA Template for HIV treatment*. This template holds demographical and clinical data for a patient, as well as viral genomic data through links to the HL7 Clinical Genomics models, and would provide a much-

needed standard way for HIV scientists/caregivers to exchange information (Fig.1).

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EVOLUTION OF HIV GENO- AND PHENOTYPES DURING ANTI-RETROVIRAL THERAPY

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The HIV-1 envelope protein (Env) facilitates entry of the virus into target cells. This process is initiated by the interaction of HIV-1 Env with the CD4 receptor molecule on the surface of the target cell [1]. CD4 binding induces a conformational change in Env and allows the binding of a coreceptor of the chemokine receptor family. The coreceptor usage is virus strain dependent: R5-tropic viruses, which do not induce syncytia, use CCR5 and X4-tropic viruses, which induce syncytia, use CXCR4. X4R5 or dual-tropic strains can use CXCR4 as well as CCR5 for entry. R5-tropic strains are found early after infection and replicate efficiently in memory T cells, which express high levels of CCR5 and are particularly abundant in the gut-associated lymphoid tissue, the site of primary HIV repli-

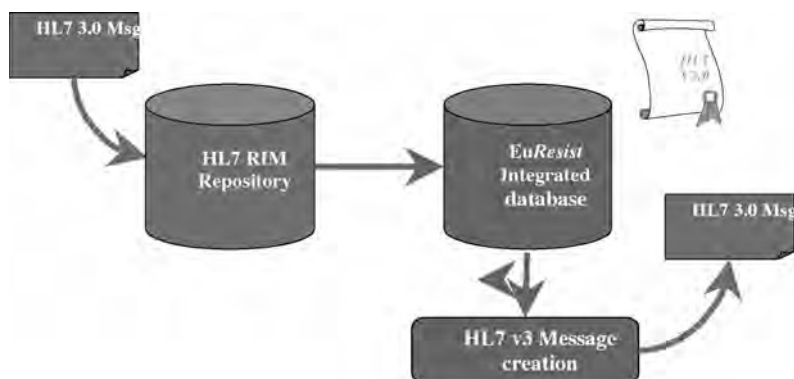


Fig. 1. The EuResist HIV specific data mart is aligned with the generic HL7 v3 standard.

cation. However, in half of the infected individuals, HIV strains emerge that use CXCR4, which coincides with a rapid depletion of CD4-positive T-lymphocytes. Therefore, we are conducting a longitudinal analysis of the coreceptor usage of HIV isolated from infected individuals [2]. These data should help to elucidate the still unanswered question of whether the appearance of X4-tropic HIV is the result or the cause of the immunodeficiency. In addition the study should evaluate the influence of anti-retroviral therapy (ART) on coreceptor switch with the focus on the reversibility of the switch by ART.

The primary determinant of coreceptor usage is the third variable (V3) region of the HIV envelope glycoprotein. Coreceptor usage can be predicted from the sequence of the V3 region by using bioinformatics tool as geno2pheno [3]. We are currently evaluating the coreceptor usage by sequence analysis of the V3 region of the HIV Env obtained from HIV isolated from infected individuals. The correlation of the experimental data with patient's therapeutic regime will hopefully enable us to make prediction about coreceptor switch and the onset of AIDS and might allow us to improve ART to further delay the emergence of highly pathogenic HIV variants.

References

1. Siegert, S., Schnierle, P., and Schnierle, B.S. (2006). Novel anti-viral therapy: drugs that block HIV entry at different target sites. *Mini. Rev. Med. Chem.* 6, 557-562.
2. Holtkamp, N., Otteken, A., Findhammer, S., et al. (2000). Unexpected coreceptor usage for primary human immunodeficiency viruses type 1 isolates from viremic patients under highly active antiretroviral therapy. *J. Inf. Dis.* 181, 513-521.
3. Beerenwinkel N., Daumer M., Oette M., et al. (2003). Geno2pheno: Estimating phenotypic drug resistance from HIV-1 genotypes. *Nucleic Acids Res.* 31, 3850-3855.

EPIDEMIOLOGY AND TREATMENT IMPLICATIONS OF PRIMARY DRUG RESISTANCE IN CHRONICALLY HIV-INFECTED PATIENTS IN GERMANY, 2001-2006

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Background: Primary HIV drug resistance is defined by the presence of resistance-associated mutations in previously untreated HIV-infected patients. It may lead to suboptimal virological outcome of highly active antiretroviral therapy (HAART), as shown by a number of prospective trials. In these investigations,

treatment choice was not based on prior resistance testing. We studied the epidemiology of primary resistance in Nordrhein-Westfalen, Germany. Furthermore, the aim of this study was to evaluate the efficacy of first-line HAART guided by resistance testing and to identify predictors of virological failure.

Methods: In an ongoing prospective multicenter study in Germany, genotypic resistance testing was performed before initiation of first-line HAART in chronically HIV-infected patients. Mutations were classified according to published criteria on reporting about primary resistance (Shafer RW, *AIDS* 2007; 21: 215-23). The components of therapy were chosen using the geno2pheno® interpretation tool of resistance testing. Treatment was monitored for 48 weeks.

Results: Altogether, 37 centers specialized in the treatment of HIV-infected patients in Nordrhein-Westfalen, collaborated this study. 1097 patients were recruited from January 2001 to December 2006. Of these, 78.6% were males, the mean age was 39 years (standard deviation (SD): 10.2), 52.7% were homosexuals, the mean duration of HIV diagnosis was 1.6 years (SD: 3.4), 31.2% were at the stage of AIDS, mean CD4-cell count was 244/μl (SD: 212), mean viral load was 189,442 copies/ml (SD: 461,460). Resistance-associated mutations were found in 102 cases (9.3%; 95% CI, 7.6-11.0). Information on detected mutations is provided in Table 1 (page 14):

Primary resistance was found significantly more often in males ($p = 0.01$), in patients with high viral load ($p = 0.01$), in patients with HIV subtype B ($p = 0.02$), and in homosexuals ($p = 0.04$), respectively. The following graph shows the development of epidemiology over time (Fig.1).

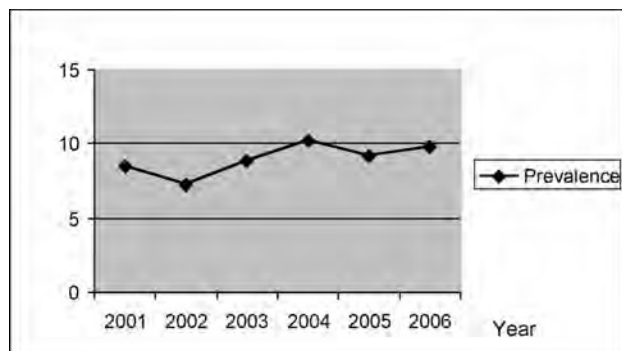


Fig. 1. Trends of Primary Resistance.

Treatment data are available for 455 individuals. In this group, the prevalence of primary drug resistance was 8.8% (95%-CI, 6.2-11.4). In intent to treat-analysis, a viral load (VL) below 50 copies (c)/mL at 48 weeks was seen in 72.5% of patients with primary drug resistance and in 75.9% of patients with wild type virus ($p = 0.7$). In on treatment-analysis, VL <50 c/mL was found in 82.9% of patients with primary resistance and in 86.8% of patients with wild type virus ($p = 0.6$). Treatment was significantly less effective in patients presenting revertant variants (T215C/D/E/L/S/V; VL <50 c/mL in 52.6 %),

Table 1.

	n	%	95%- confidence interval	Mutations
NRTI-associated mutations	63	5.7	4.4-7.1	M41L, K65R, D67N/G/del, T69D/ins, K70R, L74V, V75A/M/T/S, F77L, Y115F, F116Y, Q151M, M184V/I, L210W, T215Y/F, K219Q/E/R
NNRTI-associated mutations	34	3.1	2.1-4.1	L100I, K101E, K103N/S, V106A/M, Y181C/I, Y188L/K/C, G190A/S/E/Q, P225H, M230L, P236L
PI-associated mutations	24	2.2	1.3-3.1	L24I, D30N, V32I, M46I, I47A/V, G48V, I50V/L, F53L, I54V/L/M/A/T/S, G73C/S/T/A, V82A/F/TS/M, I84V/A/C, N88D/S, L90M
Revertants	38	3.5	2.4-4.5	T215C/D/E/S/I/V
2-class-resistance	11	1.0	0.4-1.6	Combinations of the above mentioned mutations
3-class-resistance	3	0.3	-0.03-0.6	

NRTI: Nucleoside reverse transkriptase-inhibitors

NNRTI: Non-NRTI

PI: Protease inhibitors

NRTI-mutations contain resistance associated mutations and revertants

compared to 76.6% in cases without revertants ($p = 0.03$). In patients at the clinical stage of AIDS, a VL <50 c/mL was seen in 70.1%, and 78.8% in cases at stages CDC A or B ($p = 0.04$). These differences were confirmed by multivariate analysis, with a relative risk of having a detectable viral load of 3.44 (95%-CI; 1.3-9.0) in individuals harbouring revertants and 1.62 (95%-CI; 1.1-2.6) in individuals with AIDS. The parameters age, gender, nationality, ethnic origin, duration of HIV-diagnosis, route of HIV-transmission, baseline CD4 cell-count and viral load, primary drug resistance in general, drug class-specific resistance, multi-drug-resistance, HIV subtype, and number of treatment modifications, were not associated with virological failure.

Conclusions: We found a prevalence of primary drug resistance of about 10% in chronically HIV-infected patients. The majority of mutations were found in the NRTI substance group. We identified risk factors for the presence of primary resistance. Application of resistance testing resulted in similar efficacy of HAART in cases showing resistant virus as compared to cases with wild-type virus. Thus, first-line HAART should not be administered without prior genotypic resistance testing. The strongest independent predictor of virological failure was the presence of revertant variants. Consequently, treatment options for patients with an increased risk of virological failure need to be improved.

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CORRELATIONS BETWEEN TRANSMITTED HIV DRUG RESISTANCE MUTATIONS AND HLA OF THERAPY-NAIVE HIV-PATIENTS

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Background: About 10% of HIV infected therapy-naïve patients possess mutations in the pol-region of their virus, which are associated with resistances to certain drugs. In general, mutations are afflicted with disadvantages for the replication cycle of the virus, in an environment without drug pressure. Nevertheless, in the course of infection a mutated virus was transmitted to patients.

Pressure from the immune system is also continuously present on the virus, therefore the HLA-A and -B type of previously selected therapy-naïve patients were determined to see whether correlations between the HLA-type and the observed drug resistances exist. HLA molecules display processed peptides of virus particles on cell surfaces. In case of an escape by the virus this process cannot be guaranteed and cytotoxic T-cells are not able to respond.

Patients and Methods: The HLA-A and -B type of 103 therapy-naïve patients from the RESINA cohort with

Table 1. Correlation of resistance mutations and HLA types.

Mutation	HLA-A / -B	Frequency in genotype		Lit. Value	P value	
		Subgroup	Whole group			
K103R	B*44	41.7 %	9.3 %	5.8 %	0.0018	
V118I	B*07 + B*40	17.6 %	5.3 %	0.8 %	0.0344	
L210F	B*44	40 %	9.3 %	5.8 %	0.0074	
V75I	A*11	50 %	8 %	6.2 %	0.0323	
L33F	A*01 + B*35	50%	5.3 %	1.5 % Caucasians	0.5 % Africans	0.0125
M46I/L	A*03	40 %	14 %	13.4 %	0.0344	
M46I/L	B*35	40 %	14.7 %	9.7 %	0.0406	
M46I/L	A*03 + B*35	60%	12 %	1.3 %	0.0109	

documented drug resistance mutations in protease or/and reverse transcriptase were determined by sequencing (n = 75). The frequencies of HLA-types were correlated with mutations in protease and reverse transcriptase. The significance of correlation was determined by using Fisher's exact probability test.

Results: 75/103 ART naive patients who harbour HIV drug resistance mutations were typed for both HLA-A & -B. For 8 resistance-associated positions significant correlations between drug resistance mutations and HLA-type were found which have not yet been reported:

K103R & HLA-B*44; V118I & HLA-B*07 + HLA-B*40; L210F & HLA-B*44; V75I & HLA-A*11; L33F & HLA-A*01 + HLA-B*35; M46I/L & HLA-A*03; M46I/L & HLA-B*35; M46I/L & HLA-A*03 + HLA-B*35 (Table 1).

The already reported correlation D121x & HLA-B*35 [1] was also found in our cohort, which proves that this study cohort is representative for HIV positive patients and the previous selection of patients did not cause a shift (bias) in the normal distribution of variation. Our results support the finding that 2/3 (67%) of the CTL epitopes on HIV are HLA-B restricted [2]. Four of the 6 possible escape variants are HLA-B and only 2 are HLA-A restricted. According to a study with heavily antiretroviral-treated patients, L210W in combination with HLA-B*44 is less frequently recognized than the wild-type [3]. Our study revealed L210F in combination with HLA-B*44 as a possible escape mutation (Phe (F) is homologous to Trp (W)).

Conclusion: The results of this study contribute to the understanding of the relationship between HIV infection and immune response. The knowledge about HLA restricted HIV drug resistance mutations might be helpful in designing new therapy strategies. The results of this study indicate that the prevalence of transmitted drug resistance could be effectively higher than determined by several nationwide survey programmes.

References:

1. Moore CB, John M, James IR, et al. (2002). Evidence of HIV-1 adaptation to HLA-restricted immune responses at a population level. *Science*;296:1439-43

2. Kiepiela P, Leslie AJ, Honeyborne I, et al. (2004). Dominant influence of HLA-B in mediating the potential co-evolution of HIV and HLA. *Nature*;432:769-75
3. Mason RD, Bowmer MI, Howley CM, et al. (2004). Antiretroviral drug resistance mutations sustain or enhance CTL recognition of common HIV-1 Pol epitopes. *J Immunol*;172:7212-9

HIV DRUG RESISTANCE IN CLINICAL PRACTICE

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In the last decade, efficacy of highly active antiretroviral therapy (HAART) of HIV infection has increased significantly (Bartlett JA, *AIDS* 2006; 20: 2051-64). However, the duration of effective treatment is limited due to a significant number of patients with premature cessation of therapy (Mocroft A, *AIDS Res Hum Retroviruses* 2005; 21: 743-52). Besides toxicity, a major reason for the termination of therapy is virological failure, often associated with the development of resistance-associated mutations. Another way of acquiring resistance is continuous treatment without fully suppressed viral replication. Thus, suboptimal HAART may lead to a continuous increase of mutations. Consecutively, the prevalence of resistant virus strains is rising over time (Sabin C, *BMJ* 2005; 330: 695). From a clinicians point of view, the most important question in this context is the clinical relevance of drug resistance. Several studies have shown that the presence of mutated viruses may not only reduce treatment options, but also the clinical prognosis of patients (Hirsch MS, *Clin Infect Dis* 2003; 37: 113-28). An unfavourable outcome has been shown especially in the situation of multi-class-resistance (Zaccarelli M, *AIDS* 2005; 19: 1081-9). In summary, drug resistance has become one of the major obstacles of current antiretroviral treatment.

A large number of studies on resistance testing for salvage therapy have been published. Most of these demonstrated the value of genotyping in comparison to standard of care, which was a therapy switch according to the physician's choice at that time (Panidou ET, AIDS 2004; 18: 2153-61). However, the follow-up duration of investigations was too low to give an impression of the long-term use of resistance testing. Nowadays, a switch of HAART due to virological failure without such testing is regarded as substandard. Therefore, information about the impact of resistance testing is derived from current studies on the use of new drugs in salvage therapy. The phase-II- and III-trials on compounds like Enfuvirtide, Tipranavir, Maraviroc, Darunavir, and integrase inhibitors have taught us that the number of active drugs in the new HAART counts. This means that resistance testing has the important task to identify a high number of substances without resistance to become the choice in subsequent therapy. This combination should contain at least two, if possible three, compounds without resistance. This strategy has been called genotypic or phenotypic sensitivity score (GSS or PSS). Due to very promising results of salvage therapy in the mentioned studies, the purpose of treatment should nowadays be a viral load below the limit of detection in almost all cases. Most international societies have included this strategy for salvage therapy in their guidelines. Correspondingly, an investigation has shown that access to resistance testing may improve survival in patients with multiple failures of HAART episodes (Wegner SA, Clin Infect Dis 2004; 38: 723-30).

Due to the specific selective pressure of each antiretroviral drug and whole combinations, several studies have investigated treatment strategies with a focus on remaining options after virological failure. In a meta-analysis of first-line therapies, the aspect of "resistance cost" of first-line therapy was evaluated (Bartlett JA. JAIDS 2006; 41: 323-31). Despite similar virological outcome, the NNRTI-containing study arms resulted in less treatment options at the time point of virological failure as compared to PI-containing therapies.

In conclusion, the development of drug resistance during failing HAART may not only reduce treatment options, but is also associated with reduced life expectancy. Sensible application of resistance testing may improve the efficacy of the following treatment episodes significantly. Thus, resistance testing together with rational interpretation of results are important elements of contemporal salvage therapy. Current guidelines recommend resistance testing before treatment initiation and before switch or cessation of combination therapy due to virological failure. The aim of salvage therapy is the full suppression of viral replication.

TMC114 ASSOCIATED HIV gag MUTATIONS IN THERAPY-NAIVE AND THERAPY-EXPERIENCED PATIENTS

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Background: Gag cleavage site (CS) mutations occur commonly under selective pressure of protease inhibitors (PI) in vivo and in vitro. Several CS mutations correlated with the presence of protease (PR) mutations and were linked to resistance profiles. CS mutations can confer PI resistance by an increased cleavage rate of precursor proteins and compensate for reduced enzymatic activity of drug resistant PR mutants. Most recently de Meyer et al. (2006) found certain gag mutations (G123E, H124Y, M200I, V390D, R409K, E428K, A431D, I437D, Y441H, S473P) after in vitro selection, solely responsible for 2-5-fold increase of resistance to TMC114. However, little is known about frequencies of these mutations in therapy-naïve and therapy-experienced patients and their impact on the genetic barrier of TMC114 containing therapies.

Patients and Methods: We analysed HIV subtype B isolates from therapy-naïve (n = 414) and therapy-experienced (n = 242) patients in the C-terminal gag region. The obtained sequences were assembled and edited by using the Seqman software and translated thereafter into protein sequences according to the gag and pol reading frame. Each sequence was aligned to the HxB2 reference sequence and amino acid substitutions were documented. Statistical significance was determined using Fisher's exact test (p < 0.05*, p < 0.01**).

Results: We found eleven therapy-associated CS mutations accumulating significantly in TE viruses (E428D*, A431V**, K436R*, I437V**, L449F**/V**/H*, S451T*, R452S*, P453L**, P453A**) and few natural polymorphisms with similar frequencies in both groups (I437L, L449P, S451N). In therapy-experienced HIV with three or more major protease mutations at least one additional therapy-associated CS mutation was found in 87%. In contrast neither therapy-naïve nor therapy-experienced viruses harboured gag mutations E428K, A431D and I437D. However, few viruses had the S473P mutation. Y441H also included in the gag mutation list selected in vitro under TMC114 exposure was found in the majority of HIV isolates (n = 572) and should therefore be classified as natural polymorphism in p1. Interestingly, I437V, which is a frequent therapy-associated CS mutation, could be an intermediate between WT and I437D. So far, no analysed HIV from patients with detectable

viral loads with a TMC114 containing antiretroviral therapy (n=4) showed one of these unique gag mutations. Moreover, in one patient with primary protease and primary therapy-associated cleavage-site mutations (A431V, I437V, P453L) sustained reduction of viral replication below the detection could be achieved with TMC114.

Conclusions: Therapy-associated CS mutations are present on therapy-naïve and therapy-experienced HIV. Especially viruses with three or more major PR mutations had additional CS mutations. Gag mutations (E428K, A431D and I437D) selected in vitro under TMC114 exposure could not be found in either groups so far. It is not clear due to host-virus interactions whether these gag mutations can occur in vivo. Moreover, the meaning of therapy-associated gag mutations for failure of TMC114 containing antiretroviral therapies has to be evaluated in further studies. Detection and interpretation of gag-mutations should be integrated into HIV resistance analyses for further optimised therapy strategies.

References

1. De Meyer S, Azijn H, Franssen E, et al. The pathway leading to TMC114 resistance is different for TMC114 compared with other protease inhibitors. *Antiviral Therapy* 2006; 11:S24

ANALYSIS OF T-20 DRUG RESISTANCE USING THE HIV-GRADE INTERNET TOOL

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T-20 is a 36 amino acid (AA) synthetic peptide, homologous to parts of helix region 2 (HR2) of gp41. Its binding to HR1 of HIV-1 disturbs the formation of a hairpin structure and blocks thereby virus-cell fusion. So far, T-20 was mainly used in salvage therapy, where the emergence of T-20 resistant viruses was predictable.

The HIV-GRADE internet tool is the first freely available HIV drug resistance interpretation tool that allows the analysis of gp41 sequences. To test the performance of the internet tool 80 blood samples from 24 heavily pre-treated patients with insufficient virus suppression were collected before, during and after T-20 therapy. Viral RNA was extracted from plasma. After amplification by nested PCR, a 511 bp region of gp41 including HR1 and HR2 was sequenced. The generated sequences were analysed with the HIV-GRADE internet tool.

All but one of the patients had mutations at conserved AA positions. Mutations within HR1 appeared in

decreasing frequency at positions 43, 42, 38, 36, and 45 in 10, 6, 6, 4, and 2 patients respectively. In HR2, mutations peaked at positions 126, 132, and 138. In the region between HR1 and HR2, mutations accumulated at the C-terminus (positions 107 to 115). Fifteen of 18 patients with mutations in HR1 had additional mutations in HR2. A pairwise association between mutations was found at positions 38/138, 43/126, and 45/138.

In most patients mutations at positions 36, 38, 42, 43, and 45 in HR1 can sufficiently explain treatment failure. Mutations in HR2 seem to occur mainly as secondary mutations to improve viral fitness.

Charpentier et al. showed that treatment duration was directly correlated to the length of time until the mutations in the HR1 disappeared. An explanation for this observation could be the development of compensatory HR2 mutations. Further studies are needed to prove whether T-20 reusability can be predicted by the analysis of HR2 mutations.

The HIV-GRADE tool is available at the internet address <http://www.hiv-grade.de>

References:

- C. Charpentier et al., Kinetics of Disappearance of Enfuvirtide-Resistance HIV-1 Mutations After Drug Discontinuation ..., 14 th CROI, Los Angeles, 2007; Poster M-134

TECHNICAL PROBLEMS AND INTERPRETATION TOOLS FOR CORECEPTOR USAGE

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Background: HIV-1 uses CD4 as a receptor for cell entry and as a coreceptor, either CCR5 or CXCR4. In early stages of disease, HIV strains using CCR5 are found, these viruses are named “R5-Viruses”. In later stages, virus strains which use CXCR4 (“X4-Virus”) can occur either exclusively or alternatively to CCR5. The latter are therefore named “Dualtropic”. In addition, different strains with different coreceptor usage can persist in one patient (“Mixed Tropic”). In a patient infected with HIV the occurrence of X4-viruses is associated with higher mortality. Moreover, these strains are resistant to CCR5-blockers, a new class of anti-retrovirals effective against R5-Viruses. Maraviroc is the first drug of the new class to come into the expanded access program. Hence, diagnosis of X4-viruses is necessary to avoid costly and ineffective treatments and for focusing on effective therapies. For the-

rapeutical aspects the test to identify the coreceptor usage need not differentiate between virus strains that use only X4, Dualtropic or Mixed Tropic variants, because these situations lead to replication by using CXCR4. Therefore, the tests and interpretation programs basically need to analyse whether CXCR4 usage is possible or not.

Methods: There are several commercially available phenotypic assays to analyse the coreceptor usage. The best known test is the Trofile-assay from Monogram in San Francisco. Studies with the coreceptor blockers from Pfizer (Maraviroc), Schering (Vicriviroc) and GSK (Aplaviroc) have been analysed with the Trofile assay. But there are also assays available from Virco in Mechelen, EuroFins (TRT) in Paris and InPheno (DeCIPhR) in Basel. Additionally there are non-commercial assays in the Paul Ehrlich Institute, Langen, in the Institute of Virology, Bonn and in the University of Amsterdam. It is now a challenge to predict the coreceptor usage by sequence analysis. Fouchier and others described the V3-region of the Env-gp120 as important for the coreceptor usage, especially the aminoacid positions 11 and 25. Donaldson et al. also showed that the V3-region was predictive, but this group used the attributes net charge and difference from a consensus sequence. Freely available internet tools analyse the V3-sequence with bioinformatics techniques:

- PSSM (Mark Jensen et al), <http://ubik.microbiol.washington.edu/computing/pssm/>;
- WetCat (SVM, Pillai et al.) <http://genomiac2.ucsd.edu:8080/wetcat/v3.html>;
- geno2pheno[coreceptor] (SVM, T. Sing et al.) <http://www.genafor.org> (Fig. 1)

All of these systems use sequence information, while a newer version of geno2pheno[coreceptor] uses structure based information and a combination of both leads to an improved sensitivity of X4-Viurs detection from 79% for the sequence based interpretation system to 84.7% for the structure based version and 86.4% using the combination of both.

Results: The Trofile assay is the most widely used assay. A study with the Eurofins TRT assay showed a 86% concordance with the Trofile assay (Skrabal et al J. Clin. Microbiol, 2007). Analyses in therapy naïve patients with the Trofile assay showed that 81 – 88% (Brumme ZL, et al. J Infect Dis. 2005;192:466-474. Moyle GJ, et al. J Infect Dis. 2005;191:866-872. Demarest J, et al. ICAAC 2004. Abstract H-1136.) of the patients carry only R5-strains. In therapy experienced patients these numbers vary from 48 – 62%. (Whitcomb JM, et al. CROI 2003. Abstract 557. Paxinos EE, et al. ICAAC 2002. Abstract 2040. Wilkin T, et al. CROI 2006. Abstract 655.) Two studies show analyses with a genotypic approach. One describes 71.2% therapy naïves with R5 only and 58.8% R5 only in therapy experienced patients C.Soulie et al. 2006 EU AIDS-Congress) using the PSSM-Tool and geno2pheno. The other group (Lehmann et al 2006) found that 56% of the heavily pre-treated patients had a stable CCR5 coreceptor usage and 27% had a stable CXCR4-usage over the observed time period, while 5 out of 45 patients showed a coreceptor switch. In these analyses geno2pheno[coreceptor] was used. A coreceptor switch was also observed in the Motivate study: Within six weeks of screening of therapy with the coreceptor blocker, 8% of the patients showed a

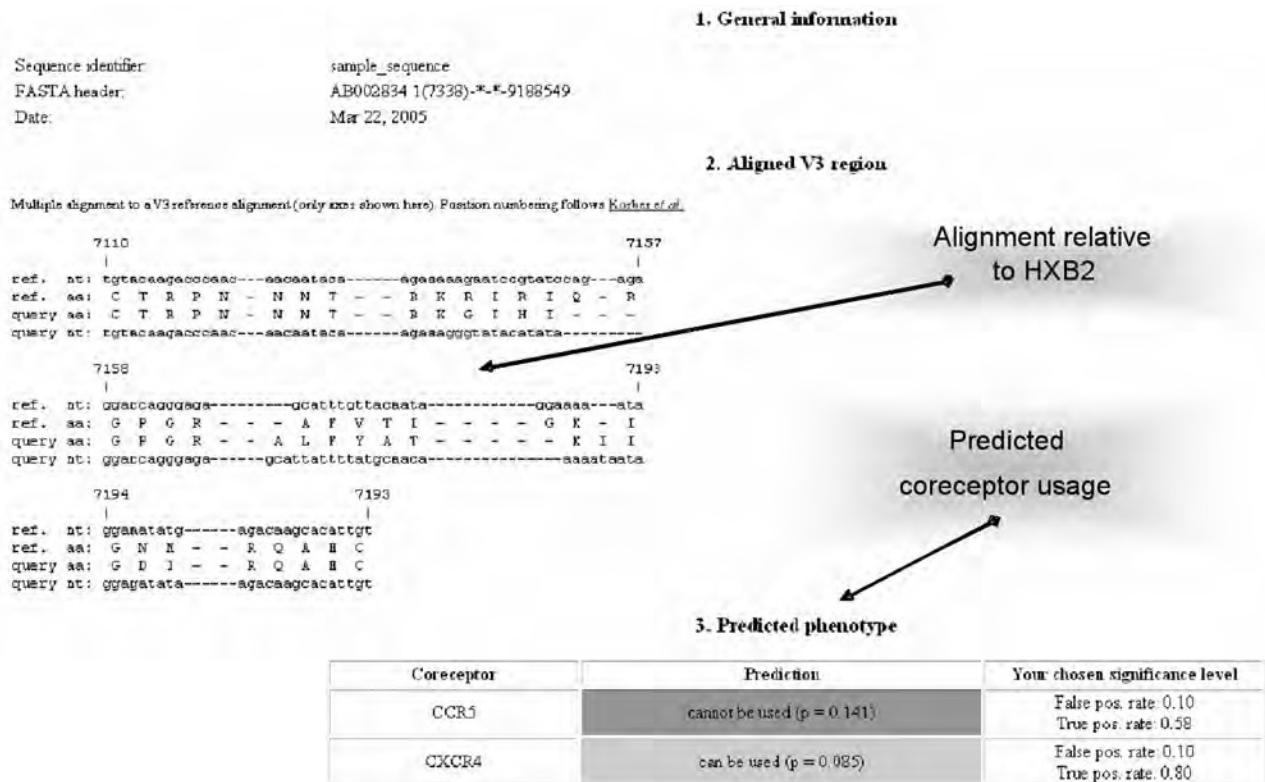


Fig. 1. Output from geno2pheno[coreceptor] interpretation system for HIV coreceptor usage based on the sequence of HIV-1 env-V3.

switch and about the same number of patients with virologic failure showed a switch under treatment with Maraviroc. Despite these cases of switches, the treatment with Maraviroc was superior to the control arm. Most likely this switch is due to the outgrowth of X4 minorities. The risk of switch correlates inversely with the CD4 cell count (Brumme et al JID, 2005, 192(3):466 – 474). Below 200 cells there is a significant higher risk of switch to X4. Resistance to Maraviroc can also occur as cell culture experiments show (Westby et al., Journal of Virology, Mar. 2007).

Conclusions: There are two reasons to determine the coreceptor usage of HIV. In the first place, the occurrence of X4-Viurs strains is correlated with a higher risk of disease progression. In the second place, a new class of antiretroviral drugs blocks the CCR5-receptor, so that R5-Virus strains can be inhibited. Phenotypic and genotypic assays are available today and are used to detect X4-Viruses in patients. If no X4-Viruses are detected, CCR5-coreceptor blockers like Maraviroc can be used. The risk of early virological failure caused by the use of coreceptor blockers appears to be small as shown in studies.

References:

1. Fouchier RA, Groenink M, Kootstra NA, et al. (1992). Phenotype-associated sequence variation in the third variable domain of the human immunodeficiency virus type 1 gp120 molecule. *J Virol.* 66(5):3183-7.
2. Donaldson YK, Bell JE, Holmes EC, et al. (1994). In vivo distribution and cytopathology of variants of human immunodeficiency virus type 1 showing restricted sequence variability in the V3 loop. *J Virol.* 68(9):5991-6005.
3. Pillai S, Good B, Richman D, Corbeil J (2003). A new perspective on V3 phenotype prediction (2003) *AIDS Res Hum Retroviruses* 19(2):145-9.
4. Jensen MA, Li FS, van 't Wout AB, et al. (2003) Improved coreceptor usage prediction and genotypic monitoring of R5-to-X4 transition by motif analysis of human immunodeficiency virus type 1 env V3 loop sequences. *J Virol.* 77(24):13376-88.
5. Sing, T.;Sander, O.;Beerenwinkel, N. et al. Learning mixtures of localized rules by maximizing the area under the ROC curve. Proceedings of the First International Workshop on ROC Analysis in Artificial Intelligence. 2004. pp. 96–98.
6. Skrabal K, Low AJ, Dong W, et al. (2007). Determining human immunodeficiency virus coreceptor use in a clinical setting: degree of correlation between two phenotypic assays and a bioinformatic model. *J Clin Microbiol*;45(2): 279-84. Epub 2006 Nov 22.
7. Lehmann C, Daumer M, Boussaad I, et al. (2006). Stable coreceptor usage of HIV in patients with ongoing treatment failure on HAART. *J Clin Virol.* 2006 Dec;37(4): 300-4. Epub Sep 26.
8. Brumme ZL, Goodrich J, Mayer HB, et al. (2005) Molecular and clinical epidemiology of CXCR4-using HIV-1 in a large population of antiretroviral-naive individuals. *J Infect Dis.* 192(3):466-74.

MK-0518

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Raltegravir (MK-0518) is a novel HIV-1 Integrase Inhibitor which has no cross resistance to currently approved antiretroviral drugs and is a new mechanism of action as a Strand Transfer Inhibitor (STI) [1]. BENCHMARK-1 (Protocol 018 enrolled in Europe, Peru, Asia and the Pacific: n = 350) and 2 (Protocol 019 enrolled in North, Central and South America: n = 349) are ongoing multi-centre, triple-blind randomized studies to evaluate the safety and efficacy of oral Raltegravir 400 mg twice daily vs placebo (2:1 randomisation), each plus optimized background therapy (OBT), in HIV-infected patients failing ART with HIV resistant to three classes of oral ART. Efficacy endpoints included percentage of patients with HIV RNA <400 and <50 copies/ml and change from baseline CD4 cell counts. Baseline characteristics were similar across treatment arms. Pre-planned 16-week analysis demonstrates superior efficacy of Raltegravir over placebo. 61 % -62 % of the patients in Raltegravir + OBT arms vs 33- 36 % in Placebo + OBT arms (BENCHMARK-1-2) reach viral loads below the level of detection (HIV-RNA < 50 copies/ml; Non-completer = failure) at week 16. Raltegravir resistance was partially analysed based on genotyping 41 Raltegravir failures (32 with integrase changes; 9 with no consistent changes from baseline). Virological failure was observed in 76 (16 %) patients on Raltegravir vs 121 (51 %) on placebo. Raltegravir failure was generally associated with one of two genetic pathways: N155H or Q148K/R/H. Additional mutations were observed with both pathways: N155H + E92Q, V151I, T97A, G163R, L74M or Q148K/R/H + G140S/A, E138K. Raltegravir was generally well tolerated with an adverse experience profile similar to that of placebo. In these Phase III studies in patients failing ART with triple-class resistant HIV oral Raltegravir 400 mg twice daily plus OBT demonstrated potent and superior antiretroviral effect compared to placebo plus OBT at week 16 [2, 3].

References

1. Markowitz M, Morales-Ramirez JO, Nguyen BY, et al. Antiretroviral activity, pharmacokinetics, and tolerability of MK-0518, a novel inhibitor of HIV-1 integrase, dosed as monotherapy for 10 days in treatment-naive HIV-1-infected individuals. *J Acquir Immune Defic Syndr* 2006;43: 509-15
2. D Cooper, J Gatell, J Rockstroh, et al., for the BENCHMARK-1 Study Group; Results of Benchmark-1, a Phase III Study evaluating the efficacy and safety of MK-0518, a novel HIV-1 Integrase Inhibitor in patients with triple-class resistant virus: Abstract 105aLB session 33, 14th CROI 25-28 February 2007, Los Angeles USA
3. R Steigbigel, P Kumar, J Eron, et al., for the BENCHMARK-2 Study Group; Results of Benchmark-2, a Phase III Study evaluating the efficacy and safety of MK-0518, a novel HIV-1 Integrase Inhibitor in patients with triple-class resistant virus: Abstract 105bLB session 33, 14th CROI 25-28 February 2007, Los Angeles USA

VARIABILITY OF HIV-1 GROUP M INTEGRASE IN TREATMENT-NAIVE AND EXPERIENCED (BUT INI-NAIVE) PATIENTS

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Background: HIV-1 integrase inhibitors (INIs) comprise a new class of antiretroviral compounds. Hitherto, more than 20 mutations within the integrase were reported as to be associated with an elevated fold change to several INIs. Mutations linked to high level resistance in cell culture studies are T66I, F121Y, A128T, E138K, G140S, Q146K, Q148K, V151I, S153Y, N155S, and V249I. Mutations observed during INI treatment and possibly associated with resistance and/or fitness are I72V, L74I/M, E92Q, T97A, T125A/V, E138K, G140S/A, Q148K/R/H, V151I, M154I, N155H/S, G163R, V165I, V201I and S230N [1, 2]. As known for the protease and the reverse transcriptase there are subtype-specific polymorphisms having an impact on the effectiveness of antiretroviral drugs. In general there are low amounts of natural resistance-associated mutations to INIs in therapy-naïve HIV-1 infected patients [3]. Since there is an increasing frequency of drug-treated patients in Germany infected with non-B subtypes, we analyzed the variability of the HIV-1 integrase gene of subtype B and non-B subtypes in treatment-naïve (TN) and experienced (TE) but INI-naïve patients, to identify polymorphisms possibly associated with INI resistance.

Material and Methods: The complete integrase genes of 148 INI-naïve patients were amplified by RT-PCR and sequenced on an ABI-3100 Avant. Of these 148 patients 43 were harbouring a subtype B (23 TE and 20 TN), whereas 105 patients (65 TE and 40 TN) were carrying non-B subtypes. The sequences were screened for mutations in comparison to the reference strain HXB2.

Results: We analyzed 148 samples from INIs-naïve patients including subtype B (n = 45) and non-B subtypes (n = 105). Irrespective of subtype and drug-treatment the residues within the catalytic triad (D64-D116-E152) and the HHCC zinc-binding site (H12-H16-C40-C43) were fully conserved. Moreover, none of the ten mutations associated with high-level resistance to INIs were detectable (T66I, F121Y, A128T, E138K, G140S, Q146K, Q148K/R/H, S153Y, N155HS, V249I), neither in naïve nor in treated patients. The mutation I72V, which increases the level of resistance together with F121Y, T125K and V151I, occurred >45% in both B and non-B subtypes, whereas the mu-

tations T125A and V201I were more frequent in non-B-subtypes (88.6% / 94.3%) in comparison to subtype B (25.6% / 51.2%). Furthermore, there were no significant differences between treated and untreated patients. In contrast, the mutation L74I was exclusively detected in non-B subtypes (15.2%), irrespective of treatment status. M154I, K156N and V165I occurred at 3% frequency not influenced by subtype or drug-therapy. The polymorphism S230N showed a higher prevalence in subtype B sequences (14%) than in those of non-B subtypes (2%). V151I, which is known to confer high level INI-resistance was more frequent in the non-B subtypes (CRF13_cpx) of drug-treated patients (non-B: TE (2.9%), TN (0.9%); B: TN (2.3%)). L74M and T97A, also conferring high level INI-resistance, were detected in subtype G, AG and F1/F2 with a frequency of <3% including untreated patients.

Conclusion: The prevalence of INI-associated resistance mutations in INI-naïve patients is higher in non-B subtypes than in subtype B. T97A and L74M were detected in subtypes G, AG and F1/F2 independent of drug-experience, indicating a natural subtype-dependent INI-resistance. In contrast, V151I may occur more frequently in treated patients than in drug naïve.

References:

1. Malet I, Fabeni L, de Mendoza C, et al. Specific mutations associated with in-vitro resistance to HIV-1 integrase inhibitors are present in untreated and NRTI/NNRTI/PI-treated HIVinfected patients. 5th European HIV Drug Resistance Workshop. March 28-30, 2007. Cascais, Portugal. Abstract 52.
2. Cooper D, Gatell J, Rockstroh J, et al, for the BENCHMRK-1 Study Group. Results of BENCHMRK-1, a phase III study evaluating the efficacy and safety of MK-0518, a novel HIV-1 integrase inhibitor, in patients with triple-class resistant virus. In: Program and abstracts of the 14th Conference on Retroviruses and Opportunistic Infections; February 25-28, 2007; Los Angeles, Calif. Oral 105aLB.
3. Cuevas M, Perez-Alvarez L, Sierra M, et al. Low frequency of natural resistance associated mutations to integrase inhibitors in HIV-1 infected patients. 5th European HIV Drug Resistance Workshop. March 28-30, 2007. Cascais, Portugal. Abstract 51.

SELF-HELP - QUO VADIS?

CONCEPT FOR A SELF-HELP-MODEL-PROJECT REGARDING RESISTANCE TO ANTIRETROVIRAL DRUGS

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The call for research that is connected with medical care in reference to patient orientated therapies cannot be overheard any longer. According to A. Pestalozzi's sentence: "Who does not want to help himself can not be helped" a new setup of the self-help after 25/5 years of HIV /Hepatitis is eagerly demanded from the point of view of patients and all relevant people.

Due to the fact that new, patient orientated literature about resistance (HIV/Hepatitis) is not available either on the internet or in print - the question is: what can be done about this matter?

Antiretroviral (ARV) therapy has remarkably reduced HIV-related mortality and increased survival rates. Because of these facts the area "resistance" is gaining importance.

The model-project consists of the following four pillars:

- Expertise
- Transparency
- Communication
- Semantic Web

Expertise: Expertise here is mainly the expertise of the person concerned, as a model example the "Deutsche Leberhilfe e. V." [1], organised according to the expertise network model set-up of the "Kompetenznetz Hepatitis" [2].

Well-informed patients [3] generate better compliance, reduced expenses and an increase in turnover. This means that "we" depend on the results of the scientific research of the pharmaceutical industry. A main source for the expertise will be the Arevir-GenaFor-Forum [4].

The Arevir-GenaFor-Forum is a combination of 35 institutions, laboratories, hospitals and medical practices with an emphasis on HIV/Hepatitis, with the aim of scientific exchange.

Transparency: Transparency in reference to the following two fields of activity: The 1st would be generating a legal structure within which the structure, way

of self-help work, becomes comprehensible. This includes scientific measures and quality measurement [5] regarding efficacy and efficiency.

This also means complete transparency regarding financial issues. This would significantly improve the bad reputation of the pharmaceutical industry regarding sponsoring and financial control. The first signs of a trend towards this are clearly visible already. It is up to "us" to create a new, more positive image. Up to now, there has not been any good information that was free of charge [6] "Good money for good literature".

The state health insurance companies are in demand as well: despite the legal regulations (SGBV §20 Abs. 4) the realisation of their help is still rudimentary. *Communication:* By success- and target orientated work within the individual disciplines the information that reaches the end-user is fairly poor. The patient is dependent on a drug combination according to the resistance monitoring (if successful and well evaluated). Because the suggested therapy is based on computer generated data and on - often poorly completed - questionnaires, the tolerance and prognosis of the specific therapy regime is rather unpredictable for the individual patient.

Patient orientated interdisciplinary communication simply does not take place at the moment, it has to be redefined if not recreated.

A good way of realising this would be the implementation of a Semantic Web

[5]. "The semantic web is the idea of having data on the web, defined and linked in a way, not just for display purposes but for being used in various applications".

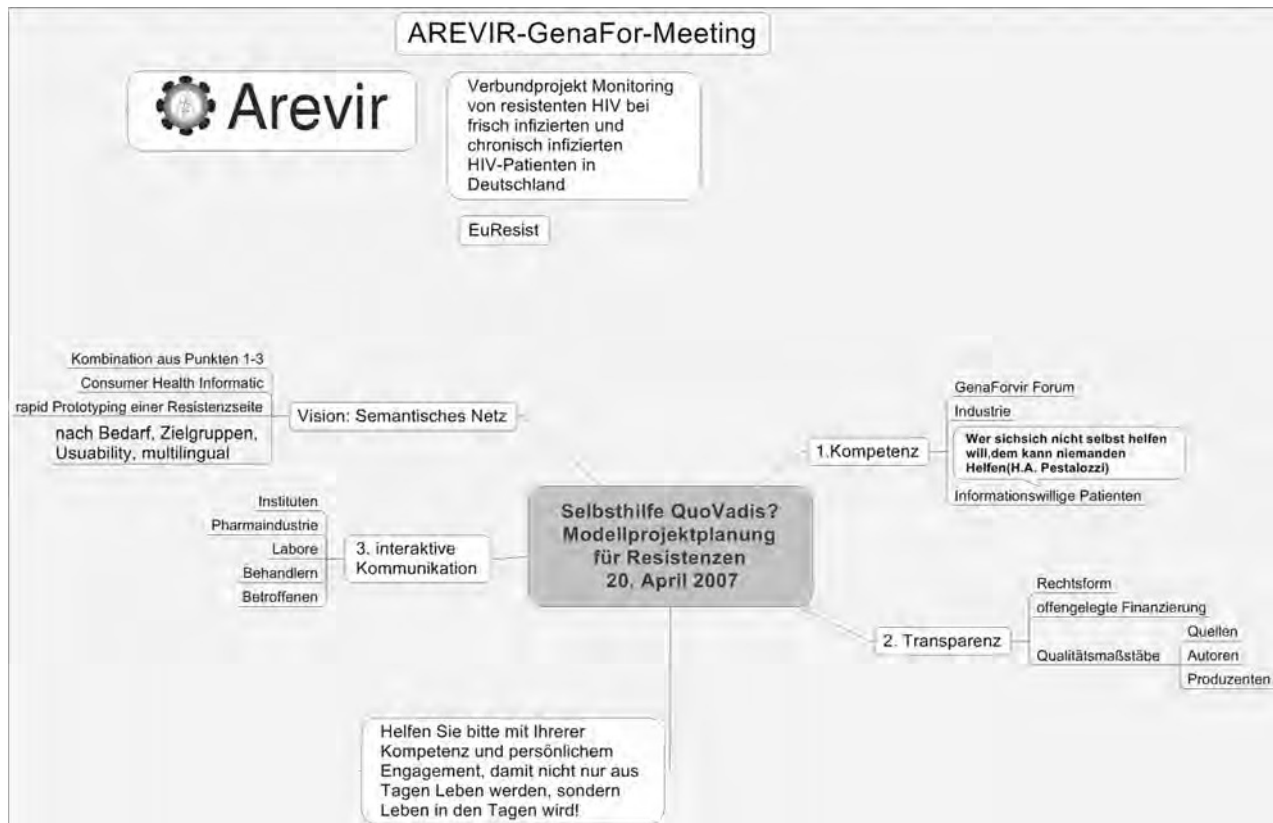


Fig. 1. Diagram illustrating the process.

Description [5]: The web as it exists today has played a significant role in fostering consumerism in healthcare. The current web provides an abundance of information, but “providing” information for the patient is certainly not enough. The ultimate goal is to enhance “knowledge”. The opportunities of the Semantic web therefore go much beyond scheduling appointments with a doctor. The Semantic Web enhances the possibility to support “knowledge translation” for consumers, the translation of information into knowledge, or the “uptake of health research in a manner that improves health and healthcare of people through improved understandings, processes, services products or systems” (Canadian Institute for Health Research, definition of “Knowledge Translation”). Doctors who are confronted with “web-informed” patients complain that patients often find irrelevant information on the web - information the patient (and the clinician) have to sift through and evaluate, and which is often not applicable to the individual situation.“

Conclusion: In order to generate proper patient orientated care the suggestions of our Health Care system can just be realised by a combination of legal regulations, financial incentives and financial help, as well as corresponding activities of scientific societies and political organisations in cooperation with the self help-groups. The aim of the project is:

1. Rapid prototyping of Internet presence regarding resistance, it has to be patient orientated under the premises of usability and contains multilingual options [7].
2. A change of the personal life circumstances and an impact on social and political circumstances. (Fig. 1).

References

1. Deutsche Leberhilfe e.V. Köln: www.leberhilfe.org
2. Kompetenznetz Hepatitis:www.kompetenznetz-hepatitis.de
3. Manual Patienteninformation, äzq Schriftenreihe Band 25, 2006
4. www.genafor.org
5. Lewis D. Eysenbach G. et al. Consumer Health Informatics. Springer 2005;34-60
6. 2- Münchner Aidswerkstatt. Michael Kochen. Wissenschaftliche Veröffentlichungen und Industrieinfluß. Blackwell Verlag.2007,24/25
7. Eysenbach G,Köhler C. Health-Related searches on the Internet. JAMA2004; 291:2946

CLINICAL IMPACT OF THE HIV-1 PROTEASE MUTATION L76V

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Introduction: L76V is a rarely observed mutation in clinical isolates of HIV-1 infected patients. It is selected

under Lopinavir-, Amprenavir- and possibly Darunavir containing regimens. While L76V appears to decrease susceptibility to Lopinavir and Amprenavir, it increases susceptibility to Saquinavir and Atazanavir [1]. An analysis of Virco’s genotypic database indicated that the prevalence of L76V increased from 0.17% in 1998 to 1.5% in 2005. For this reason, different interpretation systems of resistance (HIV Grade, Virco Type, geno2pheno) take into account the resensitizing effect of L76V. Our objective was to elucidate the clinical implication of the L76V mutation in the response to Protease inhibitor-containing regimen in patients with strongly limited therapy options.

Methods: Virological, Immunological and genotypical data of 30 therapy-experienced, HIV-1 multiclass-resistant and L76V-positive patients were obtained retrospectively. All patients were pretreated with a mean duration of 83 months, mean CD4 cell count was 222 ± 179 cells/mL and median viral load (VL) was 20,163 copies/mL (range: 59-890,000). The most frequent Protease-mutations before the start of a new therapy were: L10F/I/V (96%), M46I/L (83%), I54V/L (63%) and V82A/F 54%. 24 patients were three-class resistant and 6 showed NRTI- and PI-resistance. 11 patients started a new regimen containing boosted Atazanavir and/or Saquinavir (Group A). 10 patients switched to Atazanavir or Saquinavir plus Lopinavir or Amprenavir to maintain selection pressure on L76V (Group B); and 9 patients received Lopinavir or Amprenavir regimens (Group C). 26 patients received an optimized backbone therapy, mostly NRTIs. 4 patients received double-PIs. VL and CD4-counts were determined at baseline and at week 12-96. Long term success of therapy was defined as VL-reduction <50 copies/mL for the whole time period of 12-96 weeks during evaluation. Resistance predictions were performed by using Stanford HIVDB and HIV Grade. Effects of resistance and resensitization were verified in phenotypic analysis of 10 patients as previously described by Walter et al. 1999 [2].

Results: Long term therapy success was observed in 4/11 of group A (three included new drug classes) and 5/10 of group B, where selection pressure on L76V was constantly maintained (1 included new drug classes). Additionally, two patients of group B showed an initial reduction of VL below 50 copies/mL followed by a virological failure after 24 weeks of treatment. There was no success of therapy observed in group C. Mean CD4-cell counts slightly increased in group A and B. 3 patients of each group and with failing treatment received a second resistance-test. While L76V was then undetectable in group A, it persisted in group B and group C.

While both resistance interpretation systems HIV Grade and Stanford predicted an almost correct phenotype against Lopinavir and Amprenavir, the predicted resistance level for Atazanavir and Saquinavir was less concordant with the phenotype prediction. The actual phenotype showed almost complete sensitivity for Atazanavir and Saquinavir in the majority of all analysed samples. Failure of therapy in group A and B was noticeable associated with the co-existence of protease gene mutation L90M. The effect of resensi-

tization was additionally verified in 2 patients before and after establishment of L76V.

Discussion: L76V was detected under LPV/r-, APV/r- or rarely under Inidinavir-containing regimens. Ritonavir boosted Atazanavir and/or Saquinavir-containing regimens were more successful than regimens without these drugs. Thus, Atazanavir and/or Saquinavir are encouraging options in deep salvage-situations with viruses carrying the L76V mutation. Like in group B, it seems to be an advantage for long-term success to maintain selection-pressure on L76V by combining Atazanavir or Saquinavir with L76V-selecting drugs like Amprenavir or Lopinavir. Virological failure seems to be noticeable associated with protease gene mutation L90M. Its clinical impact has to be shown in the future.

References:

1. Mueller et al., 2004; Abstr. 38. XIII Int. Drug Resistance Workshop
2. Walter et al.; Rapid, phenotypic HIV-1 drug sensitivity assay for protease and reverse transcriptase inhibitors. J Clin Virol 1999;13:71-80

GENO2PHENO-THEO: PREDICTING RESPONSE TO COMBINATION ART ON A LARGE INDEPENDENT DATASET

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Background: Genotypic assays are standard methods for guiding treatment selection for patients infected with HIV-1. To date, several tools exist that support the interpretation of complex genotypic patterns with regard to drug resistance and clinical outcome of therapy. The majority of the available tools use a set of expert-crafted rules for every drug. Application of these rules to an HIV sequence rates the virus to be susceptible or resistant against the compound in question. The remaining tools are data-driven rather than based on expert rules. For example, geno2pheno_[resistance] and VirtualPhenotype™ apply methods from statistical learning to predict phenotypic resistance from genotype. However, all those tools have a shortcoming, since they provide ratings for single drugs while ratings for treatments comprising multiple compounds are needed.

In recent work we investigated prediction of treatment response from the applied drug combination and the genetic constellation of the virus population at baseline [1]. As an additional predictor we investigated three different models for estimating the evolutionary potential of the virus for escaping from drug pressure.

The first predictor estimates the activity of the drug cocktail against the virus population. Evolution is mo-

deled by generating *in silico* mutants and searching the sequence space for mutants of least activity [2]. The second method is a quantitative and probabilistic description of the genetic barrier to drug resistance. It estimates the probability of the virus not escaping from drug pressure by developing further mutations [3]. The third approach is termed genetic progression score (GPS). The GPS of a genotype is defined as the expected waiting time for the mutational pattern to occur [4].

Based on 6,337 treatment-sequence-pairs extracted from the Stanford HIV Drug Resistance Database and a large US clinic-based patient population, we compared pairs of sets of predictive sequence features and statistical learning methods using cross-validation. The combination of genetic barrier as sequence evolutionary feature and Logistic Model Trees as statistical learning method outperformed all other combinations. Thus it was implemented in geno2pheno-THEO (THErapy Optimizer).

In order to validate the approach, it is applied to predict response to combination ART on a large independent clinical dataset. Results are compared to three different expert-based approaches: Stanford HIV-DB, ANRS V2006.07, REGA V6.4.1.

Methods: The approach implemented in geno2pheno-THEO reduces the viral genotype to 49 mutation indicator variables, each representing one resistance-associated position listed in [5]. The same encoding is applied for the regimen; giving raise to 17 drug indicators. This baseline encoding is enhanced by the genetic barrier to drug resistance.

In this analysis response to combination ART is dichotomized to success and failure. Any available genotype obtained during an ongoing treatment is considered as evidence of a failing regimen, because in general, sequencing can only be performed if the viral load exceeds ~1,000 copies/ml. Successful regimens are defined by inspecting therapies that follow a genotype measurement. The respective treatment is considered a success if the viral load decreases below 400 copies/ml at least once during the course of the follow-up therapy. As a further constraint the genotype must not be obtained earlier than 90 days before the start of this treatment.

According to this definition, we extracted a dataset from a large European database (the EuResist integrated database) comprising data from Germany (Arevir), Italy (ARCA), and Sweden (Karolinska). From a total of 44,220 therapies, 16,243 sequences, and 13,811 patients, 5,224 treatment-sequence-pairs (904 successes, 4,320 failures) were extracted. On this dataset the approach implemented in geno2pheno-THEO is used to directly compute treatment scores for every treatment-sequence-pair. For comparison, drug activity scores for every compound in the regimen using three expert-based algorithms are computed. Since these methods provide only scores for single drugs, genotypic susceptibility scores (GSS) are used as treatment scores. The GSS is defined as the sum of single drugs scores. Classification results are evaluated using the area under the receiver operating characteristic (ROC) curve (AUC).

Results: The ROC curves of the expert-based methods do not exhibit significant differences (Fig. 1). This is

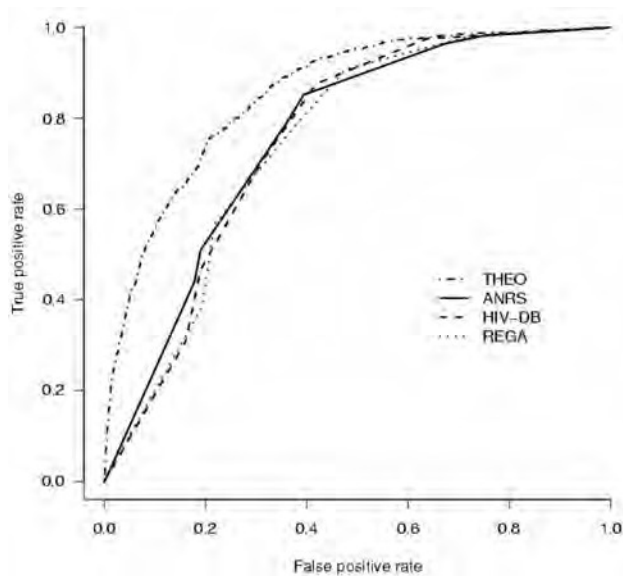


Fig. 1. ROC curves comparing the classifier performance of THEO and three expert-based methods.

also reflected by the corresponding AUC values: 0.759, 0.752, and 0.748 for ANRS, HIV-DB, and REGA, respectively. The ROC curve produced by geno2pheno-THEO is located above all other curves with a higher AUC of 0.855.

Table 1 shows one specific part of the ROC curves that is focused on therapy success. Using the expert-based methods, a GSS of 2.5 or more indicates a success. The cut-off for geno2pheno-THEO is adapted in order to allow for equal false positive rate. Under this condition THEO detects 20-25%-points more successes than the expert-based methods. Table 2 shows another part of the ROC curves which focuses on detecting therapy failure. Here a GSS of 1.0 or less is used to identify a failing therapy. The cut-off for geno2pheno was adapted to match the false negative rate of the other approaches. The benefit of our approach is reduced to 7-9%-points when predicting therapy failure.

Conclusion: On this independent data we find that the approach implemented in geno2pheno-THEO outperforms all three expert-based approaches studied here. By contrast, no significant differences between the expert-based methods are observed. Compared to expert-based methods geno2pheno-THEO is better in predicting therapy failure. Moreover, this benefit is even increased when focusing on the detection of therapy success. The elevated performance can be explained by the objective of geno2pheno-THEO, since it is designed to predict response to ART directly without intermediate interpretations for single drugs. Moreover, a large clinical database, storing viral sequences and applied combination therapy forms the basis for training the statistical model. Thus, the learned model applied in geno2pheno-THEO generalizes decisions made by clinicians when confronted with a viral variant.

Given a viral sequence geno2pheno-THEO uses the best performing approach to compute the treat-

Table 1. Predicting therapy success. The table displays for all expert-based methods under study (rows) the percentage of correctly classified successes (column: true positive rate) and percentage of failures classified as successes (column: false positive rate) if a GSS of 2.5 or more is required to predict a success. For comparison the true positive rate for THEO is given (column: THEO) if the cut-off is selected such that it gives the same false positive rate (vertical lines in the ROC plot).

	True positive rate in %	THEO	False positive rate in %
ANRS	50.8	71.5	19.0
HIV-DB	46.6	71.5	19.0
REGA	56.6	76.1	21.7

Table 2. Predicting therapy failure. The table displays for all expert-based methods under study (rows) the percentage of correctly classified failures (column: true negative rate) and percentage of successes classified as failures (column: false negative rate) if a GSS of 1.0 or less is required to predict a failure. For comparison the true negative rate for THEO is given (column: THEO) if the cut-off is selected such that it gives the same false negative rate (horizontal lines in the ROC plot).

	True negative rate in %	THEO	False negative rate in %
ANRS	60.6	68.6	14.8
HIV-DB	58.1	65.5	12.4
REGA	52.3	61.2	9.0

ment score for a fixed set of drug combinations. The combination therapies are ranked using the computed scores. Geno2pheno-THEO is freely available for research purposes at <http://www.geno2pheno.org>.

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References:

1. Altmann A, Beerenwinkel N, Sing T, Savenkov I, Däumer M, Kaiser R, Rhee SY, Fessel WJ, Shafer RW, Lengauer T. Improved prediction of response to antiretroviral combination therapy using the genetic barrier to drug resistance. *Antiv Ther* 2007; 12: 169-178
2. Beerenwinkel N, Lengauer T, Daumer M, Kaiser R, Walter H, Korn K, Hoffmann D, Selbig J. Methods for optimizing antiviral combination therapies. *Bioinformatics* 2003; 19 Suppl 1:i16-25.
3. Beerenwinkel N, Daumer M, Sing T, Rahnenfuhrer J, Lengauer T, Selbig J, Hoffmann D, Kaiser R. Estimating HIV evolutionary pathways and the genetic barrier to drug resistance. *J Infect Dis* 2005; 191:1953-1960.
4. Rahnenfuhrer J, Beerenwinkel N, Schulz WA, Hartmann C, von Deimling A, Wullich B, Lengauer T. Estimating cancer survival and clinical outcome based on genetic tumor progression scores. *Bioinformatics* 2005; 21:2438-2446.
5. Johnson VA, Brun-Vezinet F, Clotet B, Conway B, Kuritzkes DR, Pillay D, Schapiro JM, Telenti A, Richman DD. Update of the drug resistance mutations in HIV-1: Fall 2005. *Top HIV Med* 2005 Oct-Nov;13:125-31.

EURESIST: EUROPEAN DATA – EUROPEAN INTERPRETATION SYSTEMS

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Background: Although much progress has been done on the interpretation of drug resistance data, both genotypic and phenotypic information often remain difficult to translate into clinical decisions on individual patients, particularly when heavily pretreated. In recent years, several machine learning techniques have been explored to model prediction of response to treatment. While these methods show a promising development, definition of the optimal training attributes is complex and there is a crucial need for rich and massive, high-quality and updated clinical and virological data.

Methods: The EuResist project gathers clinical virology units and experts in machine learning and data mining. The consortium aims at integrating clinical and virologic data from a large cohort of patients to provide the information for training multiple machine learning approaches. Each learning strategy results in an engine that generates a prediction of short term viral load for a patient given at least a particular drug combination and a genotype. A much larger set of attributes to be provided to the prediction engine is optional. As the data provided is multifaceted, a significant component of the learning is the feature: selection and extraction phase. This process identifies optional data attributes that contribute to the accuracy of prediction and evaluates different ways of representing the features. Different engines are based on different subsets of the attributes and the attributes may be represented in the engines differently. These engines are then to be combined in a final engine freely accessible on the web to assist the infectious diseases specialist in building the best treatment regimen.

Data description: The EuResist database is generated by integrating three pre-existing independent data sources: Arca, Arevir and Karolinska. It includes patient demographics, CD4 and viral load values, genotypes and treatment histories. The latest release includes data from 16,000 patients and a total of 50,000 therapies. Each of the therapies is labelled. Success is defined as achievement of HIV RNA <500 copies/ml or a decrease of two log in the viral load. Here we focus on short term therapy. If a therapy is not successful it is labelled as a failure, except for cases when the viral load at start and end of therapy is not available. These cases are the unlabeled therapies. Figure 1 shows the success/failure and unlabeled therapies per database source. There are around 15,000 labelled therapies of

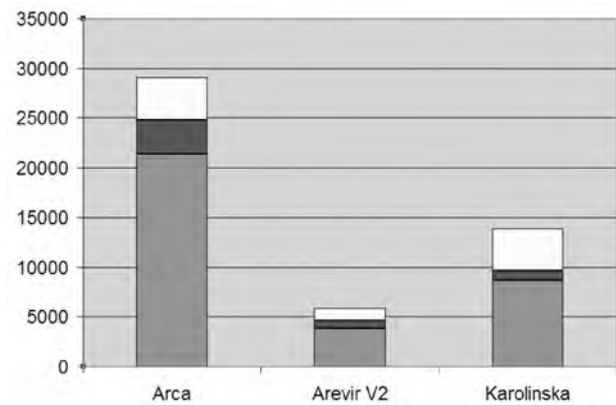


Fig. 1. Number of available therapies per database provided. Bottom (blue) part stands for the number of unlabeled therapies, middle (violet) part stands for failure therapies and upper (yellow) part stands for successful therapies.

which 10,000 are successful. Genotype at start of therapy is available only for around 15% of the labelled therapies and those are the therapies used for training and testing of the engines. The engine's target is either to predict the label of a new therapy or the viral load after 8 weeks of a therapy.

Results: The contribution of the following attributes to the accuracy of the prediction has been studied:

- Previous therapies: (a) one option is to put a flag for any drug that has been used previously. (b) Another alternative is to put a flag only for drugs that have been previously cumulatively used for at least three months.
- Days since first therapy: (c) one option is to simply count the days since first therapy. (d) Another option is to group the information into few categories as follows - put all the very first therapies into one category, all those that started 1-10 days since the first therapy into a different category, those that started 11-100 days since the first therapy into a different category and so on.
- RISKID: (e) indicates the most likely transmission route of the infection. Possible values are - IVDA (Intravenous Drug Abuse), homosexual, heterosexual, blood / blood products, vertical transmission, bisexual, other, unknown.
- Genotype history: (f) represent all past sequences as a separate attribute.
- In vitro score: (g) phenotypic data is available and can be used for training on each drug separately. The combined in vitro score for a drug combination is used as a newly derived feature.

In addition to those optional attributes the prediction based on the minimal attributes – genotype and drugs is tested. Results are obtained from five-fold cross validation, see Table 1. We employed filtering feature selection method where each of the features receives a score for its ability to predict the outcome. We used linear regressor (LR) and naïve-Bayes for learning.

The results summarized in Table 1 show that while HIV genotype is a recognized key factor, the inclusion

Table 1. Precision of prediction by LR and Naïve Bayes of labelled therapies and correlation between predicted viral loads obtained by LR and correct viral load given different attribute sets.

	Linear Regressor		Naïve Bayes
	Correlation	Precision	Precision
Random Model	0	0.66	0.66
Previous therapies (a)	0.32	0.7	0.66
Previous therapies 3 month (b)	0.34	0.71	0.66
Days since 1st therapy (c)	0.13	0.66	
Days since 1st therapy- categorial (d)	0.16	0.66	0.67
Risk ID (e)	0.01	0.66	0.68
Genotype history (f)	0.26	0.69	0.66
In-vitro score (g)	0.18	0.67	
Minimal	0.43	0.73	
All	0.46	0.75	

of additional patient data and sequence-derived features can improve the accuracy of the prediction. Three attributes that seem to be most important are the previous genotypes, the treatment history and the in-vitro scores.

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