

# The Importance of Bio-Computational Tools for Predicting HIV Drug Resistance

Antonio Carvajal-Rodríguez\*

Departamento de Bioquímica, Genética e Inmunología. Universidad de Vigo, 36310 Vigo, Spain

Received: November 17, 2006; Accepted: December 4, 2006; Revised: December 11, 2006

**Abstract:** The battle against retrovirus HIV-1 has reached a critical point. Antiretroviral resistance appears under highly active anti-retroviral therapy and the use of new drug combinations capable to overcome the emerged resistance is necessary. After detecting drug resistance two main approaches are possible. The phenotypic assays study *in vitro* the replication ability of virus variants in the presence or absence of drugs. This approach is expensive and time consuming. The genotypic assays try to obtain information from viral sequences coding for the drug targets in order to detect mutations with low susceptibility to drugs. Although this approach is faster and cheaper, a clear interpretation of the results is not always possible. In this work, I comment and analyze some new patents that point towards more efficient resistance detection and integral data management and prediction systems performing an efficient personalized combined therapy. In the future, computational tools will be essential as exploratory and interpretation systems in order to obtain a better support of clinical decisions concerning both the prediction and the evolution of drug resistance. Importantly, the revised patents conform to this trend.

**Keywords:** HIV-1, antiretroviral therapy, protease inhibitors, drug resistance test.

## INTRODUCTION

The main types of antiretroviral drugs against HIV-1 target different phases in the retrovirus live cycle. The reverse transcriptase inhibitors (RTIs), both nucleotide and non-nucleotide, inhibit reverse transcription of RNA to DNA. The protease inhibitors (PIs) lead to the production of immature non-infectious viral particles. Finally, the entry inhibitors block the penetration of HIV virions into their target cells.

The HIV protease has become a prime target in drug design since the discovery that the chemical inhibition or the mutational inactivation of the enzyme generates non-infectious viral particles. Furthermore, the protease gene has a relatively small coding region, and the enzyme an stringent substrate specificity. These properties provided an initial expectation of narrow spectra of mutations that could occur without decreasing the virus fitness below a tolerable threshold. However, HIV protease has proved to be very plastic through the development of resistant variants under the selection pressure of PIs [1]. In fact the prevalence of drug resistant mutations increased in the last few years [2-4].

Because antiretroviral resistance appears even under highly active anti-retroviral therapy (HAART), in which different combinations of anti-retroviral agents are used to combat HIV-1 [5, 6], resistance seems difficult to overcome, even in patients on effective combination therapy. This is mainly due to the lifelong persistence and the latent infection of the virus [7]. Furthermore, cross resistance (resistance against an unused drug) suggests that HIV-1 would be able to adapt to any combination of the available drugs targeting the same enzyme [8].

After treatment failure it is necessary the use of new drug combinations capable to overcome the emerged resistance. In order to design these new drug combinations two main approaches are used. The first approach consists on a phenotypic test in which the replication ability of virus variants is studied *in vitro*, via cell culture assays, in the presence or absence of drug. This approach is expensive and time consuming. The second approach is the use of genotypic assays in which viral sequences coding for the targets of a given drug are analyzed in order to detect mutations with low susceptibility to drugs. Although, this approach is faster and cheaper, a clear interpretation of results is not always possible due to the existence of many different mutations and mutation patterns that confer resistance. For instance, both the interaction between mutations (epistasis) and cross-resistance make difficult the interpretation of genotypic tests. Thus, the elucidation of results requires knowledge of the mutations selected due to the effect of different antiretroviral drugs and of the potential for cross-resistance to other drugs conferred by certain mutations. Resensitization, the occurrence of a new mutation which annihilates a previous resistance effect [9], further complicate the scenario. So, the correlation of specific genotypes with resistant phenotypes after genotypic assays needs new computational methods that allow effective therapy design against drug resistant HIV variants [9-11].

In summary, the battle against HIV requires three related foreheads. First, the design of new protease inhibitors active on multi-drug resistant viruses [6,12-14]. Second, the development of new experimental approaches allowing for a fast detection of old or new resistance mutations. And third, the design of new computational strategies to interpret the large amount of genotypic data and to choose among the huge amount (up to 10,000) of possible combination therapies [9].

\*Address correspondence to this author at the Departamento de Bioquímica, Genética e Inmunología. Universidad de Vigo, 36310 Vigo, Spain; Tel: 34 986813828; Fax: 34 986812556; Email: acraaj@uvigo.es

Although the use of computational tools is already paramount in the design of antiretroviral drugs [14-18], the growing impact of genotypic interpretation systems is revealed by the fast increase in the number of software tools and projects intended to predict viral drug susceptibility. Some examples are the Arevir database project, the Geno2Pheno web server [19] and the DR\_SEQAN program [20]. There is also software to describe viral evolution (mtreemix) [21] and for therapy optimization (Theo) via selection of optimal drug combinations [22]. Therefore, in the near future computational tools will be important not only for designing new drugs but also as exploratory and interpretation systems to better support clinical decisions concerning both prediction and evolution of drug resistance [11].

In the present work, after briefly reviewing some recent developments regarding experimental detection of HIV-1 drug resistance, I will be mainly concerned with new interpretation computer based systems. Finally, I will take a quick look at recent developments on quantum mechanics and nanotechnology applications to molecular modeling, diagnose and therapy monitoring.

### DETECTING RESISTANCE MUTATIONS

Although HIV-1 developed resistance to all protease inhibitor drugs by mutating its protease, there is no effective rule to design resistance-proof to HIV protease inhibitors. In addition, some basic questions regarding retroviral protease activation and regulation still remain unanswered [1]. The understanding of the structural changes that undergoes the wild type protease to become resistant will be of major interest for the development of new ideas and concepts to design resistance invulnerable inhibitors. This knowledge will also imply the improvement of the techniques for resistance detection. Although this review is not concerned with the design of improved drugs based upon existing ones, I will make an exception in this regard with a recently

developed new strategy for designing PIs [23] due to its importance and possible implications in future drug design and resistance detection. This invention is based on the use of two isosteres (molecules of similar size and the same valence) on the inhibitors to more effectively bind the protease. Because a single conformational change in the protease can confer resistance to many, traditional, one isostere, PIs [24, 25] it is likely that placing two isosteres in each inhibitor will difficult mutations driving to the needed conformational changes for resistance. These increase in the genetic barrier (number of mutations needed) for the apparition of resistance to PIs could help to prevent cross-resistance.

Returning to the main subject of this paper, the fast and precise identification of resistant variants will avoid therapy failing and will facilitate therapy redesign after failure. Some of the most recent patents regarding phenotypic and genotypic resistance detection are given in Table 1 in the same order of their apparition in this text. New fast PCR-mediated genotypic assays for detecting specific resistant genotypes are emerging from patients on HAART [26,27]. There are also new combined phenotype-genotype assays, which allow detection of new mutations and mutational profiles linking them with known phenotypes that cause alterations in sensitivity to anti-HIV drugs.

Patents [28,29] in Table 1, are examples of this kind of assays for RTIs and patent [30] for PIs.

A new flexible and customized phenotypic assay using plasmid recombinant cells, with reporter gene sequence, allows testing the susceptibility of HIV to drug treatment [31]. The core subject of this invention relates with the ability of producing recombinant cells susceptible to productive infection of virtually all HIV strains, allowing for the detection of a wide spectrum of resistance mutations. Such recombinant cells, that can be easily monitored and measured, could be of great interest for designing patient customized anti HIV drug cocktail treatments, thus allowing

**Table 1. Recent Patents Involving Phenotypic and/or Genotypic Resistance Tests, Arranged by Order of Apparition in the Text**

Ref	Inventor	Patent Number	Year	Title
[26]	Stevenson	US20046797464	2004	Detection of drug-resistant human immunodeficiency virus
[27]	Yang	US20056946254	2005	Amplification of HIV -1 gag sequences for detection of sequences associated with drug-resistance mutations
[28]	Azjin	US20050239053A1	2005	New mutational profiles in HIV-1 reverse transcriptase correlated with phenotypic drug resistance
[29]	Parkin	WO2006050237	2006	Methods and compositions for determining resistance or susceptibility of HIV-1 to stavudine
[30]	Parkin	US20056869759	2005	Means and methods for monitoring protease inhibitor antiretroviral therapy and guiding therapeutic decisions in the treatment of HIV/AIDS
[31]	Dong	US20040106136A1	2004	Method for testing drug susceptibility of HIV
[32]	Capon	US20056942969	2005	Compositions and methods for determining anti-viral drug susceptibility and resistance and anti-viral drug screening
[33]	Boucher	EP1605064A1	2005	HIV Variant showing a novel resistance mechanism against protease inhibitors, assays for detecting the variant, and methods for identifying drugs effective against the resistant virus

for an important improvement on phenotypic assays. This also concerns with the screening of new compounds. Another invention also linked with monitoring drug resistance, candidate drugs effectiveness and screening of new compounds is that of reference [32].

As new mutational variants appear continuously, another major goal is the detection of such new variants and the identification of their resistance profile for subsequent diagnostic assays and drug development. This has been recently accomplished for a new PIs resistant strain [33] and further relates with the design of new diagnostic assays to determine the presence in a sample of that HIV variant.

## RESULTS INTERPRETATION SYSTEMS

After resistance appears a proper interpretation of the mutation patterns and their relationships is essential to choose the correct drug combination to overcome it.

### Phenotype Prediction from Genotypes

The use of different algorithms implemented in computer programs will allow the analysis of complex genotypes related with drug susceptibility. Among these algorithms we can distinguish rules-based computer algorithms based on updated published resistance data and virological response to each considered drug in the presence of defined sets of mutations [10]. However, other methodologies refer to quantitative prediction (virtual phenotypes) based on a linear

regression correlating stored genotypic information with phenotypic profiles measured experimentally [34].

I will mention some new recent computer systems and tools for genotype interpretation and data integration (Table 2). A new method use targeted populations to detect new mutational profiles of reverse transcriptase or protease and correlate them with resistance of HIV strains [35]. Another approach is to quantify the individual contribution of a mutation or combination of mutations to the drug resistance phenotype exhibited by HIV and then adjusting models that compute the combined effect of these mutations [36]. Other invention [37] refers to computer-implemented methods for determining HIV resistance due to single or different combined interacting mutations. This method also allows, by means of genotype interpretation algorithms, determining whether likelihood exists for reduced PI susceptibility of a HIV in a subject.

There are also other more sophisticated machine learning approaches, such as artificial neural networks, support vector machines (SVM) and decision tree classification [9]. A new method is based on a neural network for predicting phenotypic resistance from genotype information based upon previous training of the network on HIV genotype-phenotype database [38].

When studying drug resistance, a direct measure of susceptibility is dependent on the "cut-off" value of the fold

**Table 2. Recent Patents Involving Computational Methods for Predicting Resistance, Arranged by Order of Apparition in the Text**

Ref	Inventor	Patent Number	Year	Title
[35]	Wang	MX3003476A	2004	New mutational profiles in HIV-1 reverse transcriptase correlated with phenotypic drug resistance
[36]	Van Marck	WO04111907	2004	Quantitative prediction method
[37]	Chappey	US20050214749A1	2005	Method for determining reduced susceptibility of HIV to protease inhibitor treatment
[38]	Larder	US20067058616B1	2006	Method and system for predicting resistance of a disease to a therapeutic agent using neural network
[41]	Bachelor	WO05086061A2	2005	Estimation of clinical cut offs
[42]	Acosta	US20050080570A1	2005	Predicting probabilities of achieving a desired minimum through level for an anti-infective agent
[43]	Bonhoeffer	US20050214752A1	2005	Compositions and methods for determining epistatic relationships between HIV mutations that affect replication capacity
[48]	Elcock	US2006136139	2006	Rapid computational identification of targets
[49]	Ramnarayan	US2006141480	2006	Use of computationally derived protein structures of genetic polymorphisms in pharmacogenomics and clinical applications
[50]	Fuerst	US20060036619A1	2006	Method for accessing and analyzing medically related information from multiple sources collected into one or more databases for deriving illness probability
[51]	Osborne	US2006212414	2006	Artificial intelligent systems for genetic analysis
[53]	Fernandez	US20060178841A1	2006	Integrated biosensor and simulation system for diagnosis and therapy
[54]	Sandeep	EP1406199	2004	Method and apparatus for monitoring therapy effectiveness
[55]	Sandeep	US7047136	2004	Technique for quantifying biological markers using quantum resonance interferometry.

increase in, for example, the half maximal inhibitory concentration ( $IC_{50}$ ) at which a pathogen is considered resistant. There has been a recent debate about the relevance of some cut-off values [39,40]. In consequence, new methods, as in patents [41,42] in Table 2, assessing the impact of pre-existing variations in drug susceptibility, on treatment response and/or calculating the probability of achieving desired concentrations for a desired dosing regimen are of full interest for managing drug treatments for patients.

Another remarkable and original approach bases its idea on population genetics theory. It is possible to measure resistance or susceptibility and to identify new resistant mutant combinations that could be interesting targets for antiviral therapy. The idea is to determine the location of mutations in the viral genome that affect replication, and the epistatic relationships between them [43]. Note that this last invention also concerns with the importance of taking an evolutionary perspective when designing drug therapy. This approach is not as usual as should be, although some recent considerations have been made on it [44-47].

Other prediction systems are physics-based methods, which combine inhibitor-binding affinities and genotype mutations with phenotypic drug resistance factors. This approach could allow, for example, for the rapid computational identification of drug targets to recognize the protein receptors likely to bind a drug. This can provide accurate predictions of the drug's ability to bind to each homologue of the receptor [48,49].

### **Complete Systems for Data Integration, Management and Therapy Monitoring**

A new proposed complete system for data integration, management and therapy monitoring, uses a combination of a centralized computer controlling a network of data entry and reporting access points for accessing medically related information [50]. The system can perform analysis to derive illness probability, predicting risk of susceptibility to one or more drugs, and providing support for the necessary decisions along the course of treatment. This system will further include data-mining models and tools that assist in the selection of a specific sequence of drugs to take. Another example of this kind of integrated schemes is a new computerized artificial intelligence method for acquiring and processing DNA hybridization patterns [51]. This method integrates primary and secondary genomic information with any other relevant information by means of neural network algorithms and comparing the processed patterns with databases for clinical or research applications.

### **NANOTECHNOLOGY AND QUANTUM MECHANICS APPLICATIONS**

Nanotechnology is the ability to work at the atomic, molecular and supra-molecular levels at the nanometer scale where the laws of quantum mechanics prevail [52]. The revolution of nanotechnology will offer new solutions for the transformation and manipulation of biosystems. This, undoubtedly, will include systems as viruses and their interaction with drugs, allowing in the future improved drug discovery and rapid and cheap near-patient genotype analysis.

As a final part of this work, we will briefly mention two new methods that consider the use of nanotechnology in relation with the problem we are facing. The first invention [53] refers to a based integral biosensor-simulation system to detect a wide range of conditions in a biological target and allows modeling the data coming from the sensor to provide therapy, diagnosis, or other automated feedback. For instance, a virus vector sensor can be used for detection of genome mutations conferring drug resistance. The second invention use DNA biomicroarray for analyzing hybridized patterns employing quantum resonance interferometry, which allows for monitoring therapy effectiveness using viral load measurements. This will provide much more precise and reliable estimates of the viral load and, in particular, it will be capable of reducing the nadir of detection significantly [54,55].

### **CURRENT & FUTURE DEVELOPMENTS**

The importance of resistance detection during clinical setting is clear. The development of computational methods for predicting resistance and designing more effective anti-HIV therapies is already a fact [9,11]. However, the results interpretation systems currently in use are mainly based on the individual evaluation of each drug [9]. Improved interpretation systems taking into account the combined effect of mutations and genetic background, i.e. epistasis, jointly with drug combinations are lacking. I have presented here some new recent developments based on the prediction of the outcome of drug combinations. The improvement of interpretation systems is important, as there is still a long way to go on resistance prediction. As an example, when designing algorithms, only codons with reported association to PI resistance are usually analyzed, but it may be necessary to include all codons of the protease for a more precise prediction of resistance.

The approach for resistance testing called virtual phenotype, consists on a large database of samples with paired genotypic and phenotypic data to predict a "virtual phenotype" from a given problem genotype. The use of virtual phenotypes has been demonstrated to be at least as effective as the real phenotype when used to select an optimized treatment for patients who have failed one or more antiretroviral regimens [34,56].

We have also looked at new physics-based methods that link genotype information with inhibitor-binding affinities to predict resistance. Currently, quantum chemical calculation of protein-drug interactions is feasible for providing a qualitative molecular understanding of drug resistance, thus allowing an improvement in structure drug design [57,58].

The above settings, jointly with new integral data management and prediction systems, are not so far in future. This kind of methods should produce the changing from negative prediction (identifying and predicting drug resistance) towards positive prediction (the performing of efficient personalized combined therapy) [9]. However, a lot of research is still pending. Consider, for example, the phenotypic prediction from genotypes by studying correlation of specific point mutations, patterns of mutations or conformational changes produced by such mutations in the specific genes. One could ask, are there any DNA

patterns, outside of the target genes, related with drug susceptibility? Indeed in a target gene or specific region, is the distribution of k-words of nucleotide sequences uniform among drug susceptible and non-susceptible populations?. Can such distribution have an effect in conformational changes and, consequently, in inhibitor affinity? And so on.

Therefore, further integration of genomic, phenotypic, and clinical data, and new computational and artificial intelligent models, together with the upcoming nanotechnological revolution, will support hopefully the design of effective combined and personalized therapies in the future.

## ACKNOWLEDGEMENTS

I am grateful to S.T Ramilo, H.Quesada, A. Caballero and J. Pasantes for helpful discussion and English corrections in this manuscript.

## REFERENCES

- [1] Prejdova J, Soucek M, Konvalinka J. Determining and overcoming resistance to HIV protease inhibitors. *Curr Drug Targets Infect Disord* 2004; 4:137-52.
- [2] Johnson VA, Brun-Vezinet F, Clotet B, Conway B, D'Aquila RT, Demeter LM, Kuritzkes DR, Pillay D, Schapiro JM, Teienti A, Richman DD. Update of the drug resistance mutations in HIV-1: 2004. *Top HIV Med* 2004; 12:119-24.
- [3] Johnson VA, Brun-Vezinet F, Clotet B, Conway B, Kuritzkes DR, Pillay D, Schapiro J, Teienti A, Richman D. Update of the Drug Resistance Mutations in HIV-1: 2005. *Top HIV Med* 2005; 13: 51-57.
- [4] Johnson VA, Brun-Vezinet F, Clotet B, Kuritzkes DR, Pillay D, Schapiro JM, Richman DD. Update of the drug resistance mutations in HIV-1: Fall 2006. *Top HIV Med* 2006; 14:125-30.
- [5] Richman DD, Morton SC, Wrin T, Hellmann N, Berry S, Shapiro MF, Bozzette SA. The prevalence of antiretroviral drug resistance in the United States. *Aids* 2004; 18:1393-401.
- [6] Seigny G, Stranix B, Tian B, Dubois A, Sauve G, Petropoulos C, Lie Y, Hellmann N, Conway B, Yelle J. Antiviral activity and cross-resistance profile of P-1946, a novel human immunodeficiency virus type 1 protease inhibitor. *Antiviral Res* 2006; 70:17-20.
- [7] Finzi D, Blankson J, Siliciano JD, Margolick JB, Chadwick K, Pierson T, Smith K, Lisiewicz J, Lori F, Flexner C, Quinn TC, Chaisson RE, Rosenberg E, Walker B, Gange S, Gallant J, Siliciano RF. Latent infection of CD4(+) T cells provides a mechanism for lifelong persistence of HIV-1, even in patients on effective combination therapy. *Nat Med* 1999; 5:512-517.
- [8] Morand-Joubert L, Charpentier C, Poizat G, Chene G, Dam E, Raguin G, Taburet AM, Girard PM, Hance AJ, Clavel F. Low genetic barrier to large increases in HIV-1 cross-resistance to protease inhibitors during salvage therapy. *Antivir Ther* 2006; 11:143-54.
- [9] Cordes F, Kaiser R, Selbig J. Bioinformatics approach to predicting HIV drug resistance. *Expert Rev Mol Diagn* 2006; 6:207-15.
- [10] Zazzi M, Romano L, Venturi G, Shafer RW, Reid C, Dal Bello F, Parolin C, Palu G, Valensin PE. Comparative evaluation of three computerized algorithms for prediction of antiretroviral susceptibility from HIV type 1 genotype. *J Antimicrob Chemother* 2004; 53:356-60.
- [11] Beerenwinkel N, Sing T, Lengauer T, Rahnenfuhrer J, Roomp K, Savenkov I, Fischer R, Hoffmann D, Selbig J, Korn K, Walter H, Berg T, Braun P, Fatkenheuer G, Oette M, Rockstroh J, Kupfer B, Kaiser R, Daumer M. Computational methods for the design of effective therapies against drug resistant HIV strains. *Bioinformatics* 2005; 21:3943-50.
- [12] Surleraux DL, de Kock HA, Verschuereen WG, Pille GM, Maes LJ, Peeters A, Vendeville S, De Meyer S, Azijn H, Pauwels R, de Bethune MP, King NM, Prabu-Jeyabalan M, Schiffer CA, Wigerinck PB. Design of HIV-1 protease inhibitors active on multidrug-resistant virus. *J Med Chem* 2005; 48:1965-73.
- [13] Surleraux DL, Tahri A, Verschuereen WG, Pille GM, de Kock HA, Jonckers TH, Peeters A, De Meyer S, Azijn H, Pauwels R, de Bethune MP, King NM, Prabu-Jeyabalan M, Schiffer CA, Wigerinck PB. Discovery and selection of TMC114, a next generation HIV-1 protease inhibitor. *J Med Chem* 2005; 48:1813-22.
- [14] Yin PD, Das D, Mitsuya H. Overcoming HIV drug resistance through rational drug design based on molecular, biochemical, and structural profiles of HIV resistance. *Cell Mol Life Sci* 2006; 63:1706-24.
- [15] Wlodawer A, Vondrasek J. Inhibitors of HIV-1 protease: a major success of structure-assisted drug design. *Annu Rev Biophys Biomol Struct* 1998; 27:249-84.
- [16] Jorgensen WL. The many roles of computation in drug discovery. *Science* 2004; 303:1813-18.
- [17] Ghosh AK, Sridhar PR, Leshchenko S, Hussain AK, Li J, Kovalevsky AY, Walters DE, Wedekind JE, Grum-Tokars V, Das D, Koh Y, Maeda K, Gatanaga H, Weber IT, Mitsuya H. Structure-based design of novel HIV-1 protease inhibitors to combat drug resistance. *J Med Chem* 2006; 49:5252-61.
- [18] Ambesi-Impombato A, di Bernardo D. Computational Biology and Drug Discovery: from single-target to network drugs. *Curr Bioinform* 2006; 1:3-13.
- [19] Beerenwinkel N, Daumer M, Oette M, Korn K, Hoffmann D, Kaiser R, Lengauer T, Selbig J, Walter H. Geno2pheno: Estimating phenotypic drug resistance from HIV-1 genotypes. *Nucleic Acids Res* 2003; 31:3850-55.
- [20] Garriga C, Menendez-Arias L. DR\_SEQAN: a PC/Windows-based software to evaluate drug resistance using human immunodeficiency virus type 1 genotypes. *BMC Infect Dis* 2006; 6:44.
- [21] Beerenwinkel N, Rahnenfuhrer J, Daumer M, Hoffmann D, Kaiser R, Selbig J, Lengauer T. Learning multiple evolutionary pathways from cross-sectional data. *J Comput Biol* 2005; 12:584-98.
- [22] 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.
- \*[23] Tang, J.J.N., Ghosh, A.K.: US20056969731 (2005).
- [24] Yoshimura K, Kato R, Kavlick MF, Nguyen A, Maroun V, Maeda K, Hussain KA, Ghosh AK, Gulnik SV, Erickson JW, Mitsuya H.: A potent human immunodeficiency virus type 1 protease inhibitor, UIC-94003 (TMC-126), and selection of a novel (A28S) mutation in the protease active site. *J Virol* 2002; 76:1349-58.
- [25] Mahalingam B, Louis JM, Hung J, Harrison RW, Weber IT. Structural implications of drug-resistant mutants of HIV-1 protease: high-resolution crystal structures of the mutant protease/substrate analogue complexes. *Proteins* 2001; 43:455-64.
- [26] Stevenson, M., Sharkey, M.: US20046797464 (2004).
- [27] Yang, Y.Y., Brentano, S.T., Babola, O., Tran, N., Vernet, G.: US20056946254 (2005).
- [28] Azjin, H., Bethune, M.-P.D., Vingerhoets, J.H.J.: US20050239053A1 (2005).
- [29] Parkin, N.T.: WO06050237 (2006).
- [30] Parkin, N.T., Ziermann, R.A.: US20056869759 (2005).
- \*[31] Dong, J.-Y.: US20046884576 (2004).
- [32] Capon, D.J., Petropoulos, C.J. US20056942969: (2005).
- [33] Boucher, C.A.B., Schipper, P.J, van Maarseveen, N.M., Nijhuis, M.J.G.: EP1605064A1 (2005).
- [34] Perez-Elias MJ, Garcia-Arota I, Munoz V, Santos I, Sanz J, Abreira V, Arribas JR, Gonzalez J, Moreno A, Dronda F, Antela A, Pumares M, Marti-Belda P, Casado JL, Geijos P, Moreno S. Phenotype or virtual phenotype for choosing antiretroviral therapy after failure: a prospective, randomized study. *Antivir Ther* 2003; 8:577-84.
- [35] Wang, D.: MX3003476A (2004).
- [36] Van Marck, H.G.E., Bulcke, T.G.V.D., Hans, V.V.: WO04111907 (2004).
- [37] Chappay, C., Petropoulos, C.J., Parkin, N.T.: US20050214749A1 (2005).
- \*[38] Larder, B., Wang, D.: US2006058616B1 (2006).
- [39] Lafeuillade A, Poggi C, Hittinger G, Chadapaud S. Phenotypic and genotypic resistance to nucleoside reverse transcriptase inhibitors in HIV-1 clinical isolates. *HIV Med* 2001; 2:231-35.

- [40] Larder B, Harrigan P. Establishment of biologically relevant cut-offs for HIV drug resistance testing. *AIDS* 2000; 14 (Suppl. 4).
- [41] Bachelier, L.T.: WO05086061 (2005).
- [42] Acosta, E.P.: US2005080570 (2005).
- \*[43] Bonhoeffer, S.: US20050214752A1 (2005).
- [44] Nijhuis M, Boucher CA, Schipper P, Leitner T, Schuurman R, Albert J. Stochastic processes strongly influence HIV-1 evolution during suboptimal protease-inhibitor therapy. *Proc Natl Acad Sci USA* 1998; 95:14441-46.
- [45] Boeri E, Gianotti N, Canducci F, *et al.* Evolutionary characteristics of HIV type 1 variants resistant to protease inhibitors in the absence of drug-selective pressure. *AIDS Res Hum Retroviruses* 2003; 19:1151-53.
- [46] Menendez-Arias L, Martinez MA, Quinones-Mateu ME, Martinez-Picado J. Fitness variations and their impact on the evolution of antiretroviral drug resistance. *Curr Drug Targets Infect Disord* 2003; 3:355-71.
- [47] Troyer RM, Collins KR, Abraha A, Fraundorf E, Moore DM, Krizan RW, Toossi Z, Colebunders RL, Jensen MA, Mullins JI, Vanham G, Arts EJ. Changes in human immunodeficiency virus type 1 fitness and genetic diversity during disease progression. *J Virol* 2005; 79:9006-18.
- \*[48] Elcock, A.H.: US2006136139 (2006).
- \*[49] Ramnarayan, K., Maggio, E.T.: US2006141480 (2006).
- [50] Fuerst, O., Fuerst, T.: US2006036619 (2006).
- \*[51] Osborne, G.F., Chin, S.S.M., McDonald, P., Schneider, S.: US2006212414 (2006).
- [52] Roco MC. Nanotechnology: convergence with modern biology and medicine. *Curr Opin Biotechnol* 2003; 14:337-46.
- \*[53] Fernandez, D.S.: US20060178841A1 (2006).
- [54] Sandeep, G.: EP1406199 (2004).
- [55] Sandeep, G.: US20047047136 (2004).
- [56] Mazzotta F, Lo Caputo S, Torti C, *et al.* Real versus virtual phenotype to guide treatment in heavily pretreated patients: 48-week follow-up of the Genotipo-Fenotipo di Resistenza (GenPheRex) trial. *J Acquir Immune Defic Syndr* 2003; 32:268-80.
- [57] He X, Mei Y, Xiang Y, Zhang DW, Zhang JZ. Quantum computational analysis for drug resistance of HIV-1 reverse transcriptase to nevirapine through point mutations. *Proteins* 2005; 61:423-32.
- [58] Mei Y, He X, Xiang Y, Zhang DW, Zhang JZ. Quantum study of mutational effect in binding of efavirenz to HIV-1 RT. *Proteins* 2005; 59:489-95.