Eur J Med Res (2007) 12: 473-482

Determination of HIV-1 Coreceptor Tropism in Clinical Practise

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Abstract
Several studies showed that the upcoming drug class of CCR5 coreceptor antagonists have potent virological and immunological activity in treatment experienced patients. In patients failing a CCR5 antagonists-based regimen, the emergence of CXCR4-tropic viral variants has been demonstrated. Clonal analysis of viral isolates from a limited number of patients revealed that these CXCR4-tropic strains did not develop by mutation of a CCR5-tropic virus during therapy, but emerged from a minor population of CXCR4-tropic variants already present in the patients at baseline. Obviously, screening for CXCR4-tropic strains with a functional assay and subsequent exclusion of positive individuals from clinical studies could not completely avoid the selection of CXCR4-tropic strains during failure. But emergence of CXCR4-tropic viruses on therapy may require a critical threshold of CXCR4 viral load at baseline, which may not be the case in patients with a very low proportion of CXCR4-using variants. Therefore, this review addresses to what extent currently available methods are suitable to detect CXCR4-tropic strains in clinical settings. Available functional assays are based on recombinant viruses. These assays are generally restricted to a few laboratories and cannot be easily included in daily clinical settings. Whereas minority detection limits of sequence analyses are generally high with 15 to 30%, functional assays achieve lower detection limits for minorities of 5%. Sequence analyses require an additional interpretation step, and the accuracy of interpretation from clinical samples by current predictions systems has to be improved. In consequence, new methods are arising: genotyping may be improved by hybridisation assays, which quantify CXCR4-tropic viruses by their homology down to 1% minorities, and functional non-infectious cell fusion assays may overcome security restrictions and make phenotypic methods suitable for routine clinical laboratory practise. The highly sensitive detection of CXCR4-tropic viruses may provide the opportunity to clarify the conditions of clinical relevance for CXCR4-tropic minorities.

Key words: HIV, coreceptor, tropism, CCR5, CXCR4, genotype, phenotype, recombinant virus assay, minority

Abbreviations: R5 virus: CCR5-tropic virus; X4 virus: CXCR4-tropic virus; HIV : human immunodeficiency virus

Introduction

Viral entry into the host cell is a highly regulated step of the viral life cycle [1]. HIV entry is mediated through the CD4 receptor, which is required for efficient attachment of the particle to the cell membrane, and a coreceptor, which can be either CCR5 or CXCR4 and binds to the third of five loop regions (V3) of the viral envelope protein gp120 [2, 3]. This binding initiates the membrane fusion process via the second envelope protein gp41 ending with the freed nucleocapsid in the cytoplasma and the initiation of viral uncoating and reverse transcription [1]. Inhibition of viral entry is a highly efficient way to block viral replication and the viral spread in the body. Good long term experiences have been achieved with the fusion inhibitor enfuvirtide and recent clinical studies showed the high potency and efficacy of CCR5 coreceptor antagonists [4-7].

In vivo, CCR5-tropic strains or R5 viruses are described to be the most frequent viruses [2, 8], and CXCR4-tropic viruses or X4 viruses have been attributed to faster disease progression [9-11]. However, although these viruses tend to appear more frequently in later stages of disease, it is yet unclear, if they are cause or symptom of progression, or both [12]. It is obvious, that X4 viruses have to have disadvantages in comparison to R5 viruses, otherwise they would be the most prevalent subspecies of the virus. The character of this disadvantage is still under discussion [12]. Recent results showed that a substantial percentage of HIV-1 infected individuals harbour X4 viruses, which seems to be correlated to both, the disease progression and previous antiretroviral treatment [13, 14]. This suggests, that X4 viruses may play a role during all disease stages, although their relevance may be minor in earlier stages. In a recent clinical study patients harbouring dual tropic viruses, which can use both CCR5 and CXCR4 to enter the cells, were treated with CCR5 coreceptor antagonists. Despite the lack of virological response, an increase of CD4 T cells in comparison to the placebo group and no evidence of disease progression could be observed [15]. This suggests that the selection of X4 viruses by CCR5 coreceptor antagonists is not associated with disease progression. Thus, X4 viruses might be more pathogenic in case they evolved naturally rather than due to selection pressure by CCR5 coreceptor antagonists. Since no sufficient virological therapy response could be observed, patients were screened for

the presence of X4 viruses and in case of positive results they were excluded from all clinical studies.

Therefore, there is a need to determine X4 viruses in HIV infected individuals and to assess their clinical relevance in terms of sustained therapy response to CCR5 coreceptor antagonists. This review aims to show, which methods are currently available to detect X4 viruses in patients' plasma samples, and discusses their appropriateness for daily clinical laboratory practise.

Resistance and Tropism Switch – Two Mechanism to Escape

HIV tends to escape all selective pressure by mutation and subsequent selection of escape variants, which fit better to the selective pressure. This process takes more time if the virus needs to accumulate several mutations to escape efficiently [16]. After this time, maximum viral load in the plasma can be achieved and the variant appearing to be the plasma majority is the best choice for effective replication in a given environment. In case of failure under treatment with CCR5 coreceptor antagonists two different escape mechanisms were identified, both allowing the unlimited replication of the selected viral variants: drug resistance development and coreceptor tropism switch [17, 18].

Drug resistant variants are characterised by the abilities to bind to the CCR5 coreceptor and to enter the cells despite the presence of the antagonist [18]. Cross-resistance to other compounds of the drug class was not observed. Resistant viruses harboured several mutations (high genetic barrier) and developed very rarely *in vivo* and in vitro [18]. Therefore, this escape mechanism may have minor clinical relevance. The more frequently observed escape mechanism was the coreceptor tropism switch [17]. Although this was limited to a few patients only, it appears to be the major viral escape mechanism in case of virological failure, potentially affecting the clinical use of the upcoming drug class.

Relevance of X4 Viral Minorities in the Plasma

Since only individuals with no detectable X4 viruses were included in the clinical studies, either preexisting X4 viral minorities were overlooked or the shift of tropism was caused by new mutations developed during the treatment. In the cases of therapy failure, phylogenetic analyses showed that the X4 viruses were more likely to be selected from the viral reservoirs than directly mutated from the R5 viruses present at baseline [17]. In addition, the genetic barrier for a coreceptor tropism switch appears to be only slightly lower than for CCR5 resistance development. Although there are a few examples for viruses switching with only one mutation, most strains develop slower and accumulate two to five mutations to switch their coreceptor tropism [19]. The evolution is probably dependent on many factors including the pressure by the immune system and the viral backbone [20]. A retrospective examination of the baseline samples, taken only a few weeks after the screening samples, revealed the presence of X4 viruses in approximately 10% of all negatively screened individuals [21]. There is ongoing discussion about the immunological implications of these findings in case of virological failure. Furthermore, the data indicates that there are relevant minorities of X4 viruses which can contribute to therapy failure in CCR5 coreceptor antagonists containing regimen, and these had not been sufficiently detectable during the screening.

Minorities in the plasma clearly contribute to therapeutic failure in the context of drug resistance development and in the context of treatment interruptions [22-24]. In fact, the need to combine several antiretrovirals is based on the idea that single resistant minorities are always present [16]. Considering the way of viral spread in the infected body and the high viral mutation rate, it is obvious that there is a large number of distinct variants present in the plasma [25]. The composition of these quasispecies is as unique as the infected individual harbouring it and results from sum and relative potencies of the selective pressures present in each individual. In particular if the genetic barrier to resistance is low like for 3TC or the NNRTI, drug resistant mutants, which develop by a point mutation, are generated constantly [26]. Failure in these cases – like the failure observed under CCR5 coreceptor antagonists - is less a consequence of a mutation event under therapy but more likely of selection of an already existing variant and has been described for 3TC and the NNRTI, which both led to short term failure if used in a monotherapy setting [27, 28]. However, the selection of pre-existing resistant variants can be prevented by the combination of several antiretrovirals. If not, all NNRTI- or 3TC-containing regimen would always have to fail. There are mathematical models to support this idea, which take into account the following aspects: the genetic barrier to become a certain mutant, the viral mutation rate, the viral recombination rate, the total viral replication rate, the individual replication rate of a variant, the number of infectable cells, the half life of infected cells and of viruses, and several other factors [16, 29-32]. This data suggests that the probability of an escape mutant to become a major variant is decreased, the lower the total and the relative amount of replication is at the time, when the selective pressure starts providing an advantage for this particular variant. In other words, even if there is an X4 viral minority, there may be a certain level of absolute and relative replication for this individual variant, that could function as a threshold for being selected successfully when treated with CCR5 coreceptor antagonists.

This may explain, how currently available genotypic methods for drug resistance testing - detecting no more than 15 % to 30 % of drug resistant plasma minorities - provide predictive results in clinical settings. Although, there are exceptions: the knowledge about NNRTI pretreatment seems to be more predictive than actually detectable NNRTI resistance associated mutations, as it has been described for treatment with efavirenz after failure under nevirapine [33]. Nevertheless, all this implies that there may be a threshold of clinical relevance for X4 viral minorities, which requires further investigation. The two open questions

are, what amount of an X4 viral minority is relevant and are they detectable?

Methodological Requirements for the Detection of CXCR4 Viruses

Most R5 viruses and X4 viruses can clearly be discriminated, because the tropism is determined mainly by the V3 loop of the viral envelope [34, 35]. *In vivo* the viruses can appear in mixtures of both variants, so that both coreceptors can be used at the same time by different viruses (Fig. 1A). Additionally, there are viruses with a real intermediate coreceptor tropism, which are able to use both coreceptors, but with different efficacy. These viruses are also named R5X4 viruses (Fig. 1B) [34, 35]. According to the current understanding, it only seems to be relevant to detect any viruses able to use CXCR4, irrespective whether they are pure, mixed, or intermediate virus populations. The clinical relevance of R5X4 viruses for CCR5 coreceptor antagonists has been demonstrated recently [15]. However, in order to establish an appropriate minority detection and the possible introduction of a clinical relevance threshold, the discrimination between the mixtures or R5X4 viruses is crucial. One has to keep in mind, that viruses with an intermediate tropism may appear with lower activity according to their binding affinity to the CXCR4 coreceptor under inhibition by CCR5 coreceptor antagonists. This phenomenon is hard to distinguish from mixtures of viruses with distinct tropisms.

In contrast to other human retroviruses, HIV-1 is replicating efficiently and causes a viral load from 10.000 to 1.000.000 copies of viral RNA per ml plasma. Replication itself is located predominantly in the lymph nodes, from where infected cells and free virus are spreading over the whole body via the lymphatic fluid and the blood. Therefore, plasma viruses represent the most recently replicated virus populations and are easily accessible in the blood [36, 37]. In addition, the half-life of plasma virus is discussed to be within the range from minutes to hours [31, 32]. In contrast, infected cells in the peripheral blood are not necessarily actively replicating the virus and may not have been infected recently. Therefore, some major discrepancies may occur between plasma viruses and RNA or proviral DNA derived from peripheral blood cells [38, 39].

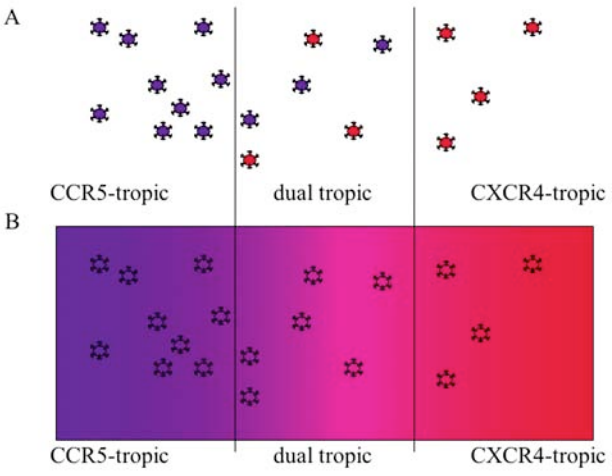

Fig. 1. HIV can use two coreceptors CCR5 and CXCR4. Most of the strains are restricted to CCR5, some are to CXCR4. Some clinical isolates are able to use both coreceptors (dual tropic) at the same time, either (A) due to mixtures of CCR5- and CXCR4-tropic strains or (B) due to real intermediate viruses. The latter are thought to be intermediates, slowly evolving from CCR5 to CXCR4 tropism or vice versa.

Finally, in terms of daily practise, methods should provide results within one or two weeks, they should be cost-effective, which means within the range of common drug resistance testing for the pol gene. They should be able to address drug resistance issues at least for CCR5 coreceptor antagonists and maybe extending also to other envelope-associated inhibitors. The laboratory procedures should be accessible to standard diagnostic laboratory conditions in terms of workload and biosafety.

If only one of the requirements mentioned above is not fulfilled by a method, drug response prediction with this method must be performed outside routine drug resistance testing and may be more time-consuming and expensive.

Phenotypic Methods for the Determination of Viral Coreceptor Tropism

Generally, there are two ways to determine the coreceptor tropism: a functional and a knowledge based way. Both methods are widely used and have certain advantages and disadvantages. Functional or phenotypic methods are based on the biological entry process. First systems have been developed already in the 90ies, isolated viruses from the peripheral blood leukocytes and tested the virus in the presence of blocking cytokines or in cell lines, which were known to show different kind of cytopathogenic effect according to the viral tropism [40-48]. Since none of the coreceptors had been identified yet, the viruses were named by this phenotype: syncytia inducing or SI viruses for the X4 viruses and non-syncytia inducing or NSI viruses for R5 viruses [49]. Meanwhile, recombinant virus assays have substituted these early assays, which overcome the selection of ancient and less resistant viruses during the viral isolation phase [50-55]. Recombinant viruses are generated by combining a part of the patients' genome – in our case the whole or a part of the viral envelope gene – with a matched deletion mutant of a laboratory HIV strain (most commonly used is NL4-3). Recombination occurs either on the level of DNA *in vitro* by a ligation step [54, 55] or intracellularly by the transfected cells via overlapping DNA fragments [52, 53] or simply all viral proteins are expressed in the same cell by co-transfection and used for particle formation [50, 51]. Besides from recombinant virus assays, there are cell cell fusion assays based on envelope expression only. Using a biological system and simulating the viral entry is the method, which is the closest to the *in vivo* situation. In combination with smart ways to enhance the detection of membrane penetration by the use of suitable indicators, these methods may provide the opportunity to detect viral tropism with appropriate efficacy and sensitivity.

However, looking at the data summarised in Table

Table 1. Systems for the detection of coreceptor tropism. Phenotypic assays share the principle to detect membrane fusion events, provide functional results and improve current knowledge. But they require higher security standards and more time for the results, and they may lack sufficient minority detection rates. This limits their accessibility. Genotypic assays characterise the viral sequence according to previous knowledge. They have no security restrictions, are fast, but conventional sequence analyses fail to detect sufficient amount of minorities. Concerning the detection of dual tropic viruses the systems do not differ, however, only a few functional assays are able to discriminate between intermediate tropic strains and mixtures of CCR5-tropic and CXCR4-tropic viruses. Parallel detection of drug resistance is generally possible, as long as the PCR product size allows it. Since fusion is mediated by gp41 upstream from the V3 loop, fusion inhibitor resistance is not generally detectable, but some assays provide the possibility for an extension.

		Detection of virus								Detection of drug resistance to	
Name	Principle of assay	Viral origin	CCR5-tropic	CXCR4-tropic	intermediate tropic	mixed	Minority detection rate	Time (after laboratory entry)	Security level	coreceptor antagonists	fusion inhibitors
Phenotyping											
Isolation of Virus and infection of selected cells	Infection	Provirus, plasma	yes	yes	yes	yes	n.a.	2-5 weeks	3	+	+
Cell cell fusion assay	Cell membrane fusion	Plasma	yes	yes	yes	yes	1%	~ 2 weeks	2	+	(+)
Trofile™ Monogram Biosciences	Infection of pseudotyped viruses	Plasma	yes	yes	yes	yes	5-10%	16 days	2b	+	(+)
Phenoscript™ HIV-1 Entry Inhibitor	Infection of recombinant viruses	Plasma	yes	yes	yes	yes	5-10%	~ 2 weeks	3	+	
PhenXR™ inPheno	Infection of recombinant viruses	Plasma	yes	yes	yes	yes	1%	~ 2 weeks	3	+	
Virco® Type HIV-1 Virco	Infection of recombinant viruses	Plasma	yes	yes	yes	yes	< 10%	12 days	3	+	
Genotyping											
Sequence analyses	Sequence comparison	Plasma	yes	yes	yes	yes	15-30%	7 days	1	+	(+)
XTrackC™ inPheno	DNA-Hybridisation, capillary separation	Plasma	yes	yes	yes	yes	1%	4 days	1		

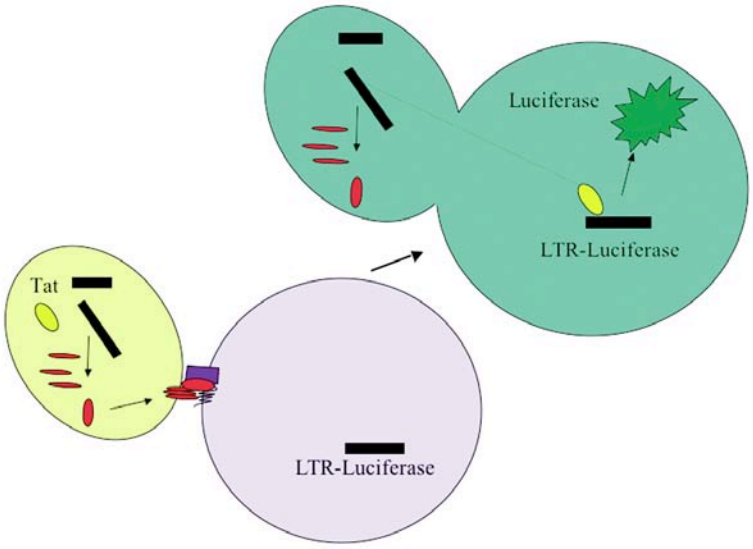

Fig. 2. Cell cell fusion assay. The assay avoids the generation of real virus and fuses envelope-expressing and HIV-infectable cells directly. The major advantage is that the assay does not require dealing with infectious agents. Gp160 (red) is expressed by the transfected cell (yellow) and transported to the cell membrane, where it mediates cell cell fusion with an HIV infectable cell (blue) according to coreceptor expression and viral tropism. The fusion liberates the coexpressed promotorprotein Tat to induce the expression of an indicator gene, which is under the control of the Tat-Promotor gene LTR.

1, functional assays also have some disadvantages. The minority detection rate for most of the assays is five to ten percent only. As mentioned before, this may not be sufficient as mentioned before [21]. Only one of the commercially available assays exhibits a minority detection rate of 1%, which may fulfil to the requirements [54]. The assay can ensure the higher sensitivity by the enrichment of the fully replication competent X4 viruses in cell culture over four replication cycles. However, the assay is restricted – like all the others based on recombinant viruses – to laboratories with a high security level. Only the procedure based on cell cell fusion can be done in security 2 laboratories, which may be more frequently available. This system uses - similar to recombinant virus assays - DNA vectors. In contrast to recombinant virus assays, in cell cell fusion assays only the patient derived envelope genes are expressed on the surface of the transfected cells and no infectious virus is generated. Transfected cells are enabled to fuse with HIV-infectable cells with distinct coreceptor expression status. Fusion events can be detected easily if the transfected cells promote an indicator in the infectable cells (Fig. 2). However, the validation of the method as a diagnostic tool is currently ongoing, although the system is already in use for research purposes for years.

Most phenotypic assays cannot easily discriminate between viral mixtures of R5 viruses and X4 viruses and viruses with intermediate tropism. In both cases activity from both coreceptors will be reported. However, the *in vitro* use of CCR5 and CXCR4 coreceptor antagonists provide the opportunity for this discrimination.

Genotypic Methods for the Determination of Viral Coreceptor Tropism

The second general concept for the determination of the viral coreceptor usage is based on sequencing of the viral envelope gene, with a strong focus on the V3 loop of gp120. The method is – like the recombinant virus assays – based on RT-PCR, which is directly followed by the sequence analysis. The last step is to interpret the identified mutations according to knowledge based interpretation systems [34, 56-63]. Discrimination between mixtures of viruses with different tropism and R5X4 viruses is a direct function of sequence analysis. Overall turnover time from blood sampling to the result is between seven to ten days in daily practise. In addition, sequencing is a method used for diagnostic purposes, in particular in the context of drug resistance testing for HIV-1. This clearly indicates, that the infrastructure for a genotypic test is already present. Furthermore, systems predicting the coreceptor usage of HIV-1 from V3 loop sequence data have been developed a long time ago. The most famous algorithm is the rule 11/25, which determines the viral coreceptor tropism from these two amino acids of the V3 loop only [56]. The rule convinces mainly by its simplicity, but recent publications indicate a poor performance [13, 58]. According to a recent comparison of four coreceptor prediction systems, the sensitvity of the two best prediction systems, WebPSSM and geno2pheno, was approximately 50% at the 90% specificity level, if predictions were done for clinical samples [65]. Other systems were even worse with sensitivities below 30%. There is an urgent need to improve these prediction systems, otherwise sequencing based methods will not reach the required level of accuracy. Interestingly, the prediction rates are much higher, if clonal sequences were used [65]. This may be explained by another constraint of the method of conventional sequencing: Only minorities of at least 15 to 30% can be detected. In the clinical studies a phenotypic coreceptor determination assay was used which detects minorities of five to ten percent [21]. Nevertheless, unexpected failure occurred most probably due to the selection of undetected X4 viruses [17]. It is likely, that the use of genotypic methods with higher detection limits for minorities will result in higher rates of unpredicted failure. The combination of detection rates of both sequencing and interpretation processes may have resulted in the decrease of prediction sensitivity from 80% for clonal analyses to 50% for plasma derived sequences.

On the other side, there has been substantial progress in identifying predictive markers to improve

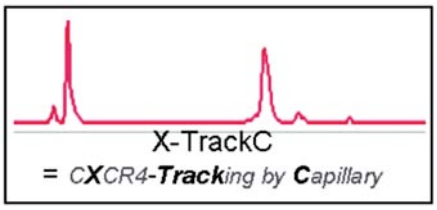

Fig. 3. X-TrackC is the first commercially available genotypic assay, that detects viral minorities of X4 viruses of 1% in four days. The system is based on hybridisation of the patients' derived PCR product of the viral envelope gene and a labelled reference DNA, which represents R5 viruses or X4 viruses. Tracked in a capillary the labelled DNA moves differently and gives distinct signals according to the amount of hybridisation. In addition, PCR products of intermediate tropic strains may hybridise with both reference sequences resulting in intermediate signals. It is obvious that the choice of the reference DNA is the key for the quality of the system, and ongoing clinical and functional validation is required and assured. (modified figure from inPheno).

the prediction rate of the geno2pheno system [63, 68]. First of all, the inclusion of sequence data up- and downstream of the V3 loop, which has been shown to possibly have an impact on the coreceptor tropism, should improve and stabilise the predictions [65-67]. The same is true for the inclusion of structural data in the prediction tools, which may include a complete new perspective on the data [68]. The so far most striking improvement of the coreceptor prediction was achieved by the inclusion of the patients' CD4 cell count [63]. This data suggests that either the CD4 cell count is an independent predictor for the coreceptor tropism, or more interestingly, that the sequence harbours distinguishable information about the coreceptor tropism, which becomes accessible for interpretation if the immune status of the patient is known.

Last but not least, the most recently developed genotypic system provides the feature to be no longer dependent on subsequent interpretation [54]. After the RT-PCR steps the amplicon is hybridised to a fluorescence-labelled reference DNA, which represents either a consensual R5 virus sequence or an X4 virus sequence, respectively. Quantitative detection according to the different labelling enables the system to detect minorities as well as dual tropic strains with a sensitivity of 1% (Fig. 3). Mixtures appear as two different peaks, whereas R5X4 viruses appear as a bulk peak localised between the reference peaks for pure viruses. Since the system is new, it needs to be further validated and the provider recommends real phenotyping in cases of mixtures and intermediate variants. In particular, it is necessary to validate the appropriateness of the labelled probes in terms of the detection of the whole variety of HIV-1, e.g. non-B subtypes. Nonetheless, the assay produces results within four days with a high sensitivity for X4 viral minorities, which is very promising. Finally, one major limitation of this method is the restriction to a single laboratory of the commercial provider.

Conclusions

CCR5 coreceptor antagonists are compounds of a new drug class with high safety and efficacy. In the cases of virological failure, the selection of drug resistant or X4 viruses was observed. Whereas drug resistance development appeared to be extremely rare, several cases of X4 virus selection have been reported. Therefore, the determination of X4 viruses before initiation of CCR5 coreceptor antagonists containing regimen turned out to be essential for sustained virological therapy response. Hence, there is a need for determination methods that fulfil the requirements of routine laboratory testing and provide a high sensitivity and specificity in a combination with cost-effectiveness, acceptable workload and biosafety.

Generally, there are two ways to detect HIV-1 coreceptor tropism: based on the genome or based on the entry process. Since genotypic methods generally are based on fast and cheap conventional sequencing, they need to refer to current knowledge about which mutations and polymorphisms are contributing to the function. This interpretation is provided by several systems, but needs to be improved for clinical samples. Promising increases of the predictivity of combined sequence and immunological data have been recently published [63]. However, it is unclear, if the achievable improvements of the interpretation systems are able to overcome the relatively low detection limit for minorities below the 15% to 30% level plasma presence. New approaches based on DNA-hybridisation may overcome this disadvantage [54]. Concerning this new method, one should keep in mind: the major challenge is, how to define the reference DNA.

Functional assays are based on the entry process and are definitely required, either directly as diagnostic tools or indirectly for ongoing support of genotypic assays. At the moment, X4 viral minorities may be easier detectable by functional assays. Furthermore, CCR5 and CXCR4 coreceptor antagonists can be added in vitro to improve the sensitivity and specificity of the assays [54]. Suitable assays are based on recombinant viruses and work with fully replication competent or pseudotyped viruses. At the moment, these assays are restricted to specialised laboratories due to the high security level while dealing with recombinant viruses. Although phenotypic assays yield results within an acceptable time frame of two weeks for most assays, the assays require highly specialised know-how and are relatively expensive. Most of the functional assays might not detect all relevant X4 viral minorities with a plasma presence below 5%. Cell cell fusion assays could overcome most of these disadvantages, and therefore be an alternative to current strategies, but have to be further validated.

Minority detection and discrimination between viruses with intermediate tropism and mixtures of R5 and X4 viruses are specific needs for assays aiming X4 virus detection. Whereas the latter phenomenon can be handled with little effort in all methods, minority detection may be a major challenge. X4 virus selection has been associated to virological failure in patients, who had been screened negative for presence of X4 viruses [17]. However, since nearly no currently available method is able to detect minorities below the level of 5% plasma presence, at the moment insufficient data is available to prove the relevance of X4 viral mi-

norities or to define the threshold of which minority will cause virological failure.

In summary, there is clear evidence for the clinical relevance of X4 viral minorities, both from the experience with drug resistant minorities and from clinical data about therapy failure under treatment with CCR5 coreceptor antagonists. Since overall failure rates under CCR5 coreceptor antagonists are low and since the detection of X4 viruses before treatment initiation is correlating with the subsequent failure, no general limitation of CCR5 coreceptor antagonists can be concluded from the data. Nonetheless, the major reason for therapeutic failure is the selection of X4 viruses. In conclusion, there is a need to develop methods with higher sensitivity for X4 viral minorities to further investigate their clinical relevance.

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Received: August 16, 2007 / Accepted: August 18, 2007

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Curriculum Vitae

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Curriculum Vitae

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