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SECTION I
General introduction
Chapter 1

Highly Active Antiretroviral Therapy in Children

The Disease, the Drug and the Patient: Current Issues and Potential Solutions

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Submitted for publication

Summary

Despite the enormous progresses observed in paediatric Highly Active Antiretroviral Therapy (HAART) in the last decades, a high percentage of children continue to experience treatment failure due to development of drug resistance, inadequate dosing and poor adherence. This review is aimed at exploring the current status of antiretroviral therapy in children with focus on the interaction between disease, drug and patient behaviour, all of which are strongly correlated and determinants of treatment outcome. With respect to the disease, an overview of viral characteristics and of the available antiretroviral drug classes currently combined to avoid development of resistance is provided. Taking into account pharmacokinetic and pharmacodynamic properties, we show the advantages and limitations of existing methodologies for dosing recommendation. Finally, the role of the patient is scrutinised: a detailed definition of adherence to therapy is provided, together with the main strategies used to enhance treatment compliance in children. The importance of adherence is also highlighted in terms of its implication for the development of resistance, which has been shown to differ for each class of antiretroviral drugs.

After having identified some of the challenges which need to be overcome to decrease viral failure in children, we propose the use of a model-based approach for exploring forgiveness of non-adherence, which may allow simplification of current dosing regimens taking into consideration inadequate compliance and its implication for efficacy and drug resistance. In conjunction with clinical trial simulations, we demonstrate that it is possible to evaluate relevant clinical scenarios and predict treatment outcome of simplified dosing regimens of antiretroviral drugs in hypothetical populations of HIV-infected children.
1.1. BACKGROUND

Paediatric HIV infection is a world-wide public health challenge disproportionately affecting children in the poorest parts of the world, where access to therapy is still quite limited. Major advances occurred during the past 15 years, such as effective prophylaxis and treatment in HIV-infected women, and administration of highly active antiretroviral therapy (HAART) to those babies who are infected (1). Early antiretroviral treatment has dramatically modified the course of HIV infection in children, reducing mortality by fivefold or more and resulting in high survival rates in adulthood (2;3). However, one of the greatest challenges for children living with HIV is maintaining effective antiretroviral treatment for life. A European study which looked at more than a thousand children on antiretroviral treatment found that 12 percent of children experienced treatment failure of three classes of drugs after 5 years, over two times the rate of adults (4).

Some of the reasons that lead to earlier treatment failure in children include a lack of choice of antiretroviral drugs for children, difficulties with adherence and inadequate dosing, together with the risk of running out of drug options sooner in case of drug resistance, and the need for psychosocial support - particularly during adolescence. From a therapeutic perspective, a comprehensive overview is required of the factors which influence treatment outcome and may lead to clinical failure, primarily involving the disease, the drug and the patient’s behaviour towards therapy. An extensive evaluation of such factors will be performed in the next paragraphs. Based on such an evaluation, specific goals and effective strategies in clinical practice can be identified, which may contribute to overcome existing issues and in turn to reduce the number of HIV-infected children experiencing viral failure.

The disease: why is combination therapy needed?

At present, a combination of at least three antiretroviral drugs from at least two drug classes is recommended for initial therapy in adults and in children. The antiretroviral classes currently approved in children are NRTIs (nucleoside reverse transcriptase inhibitors), NNRTIs (non-nucleoside reverse transcriptase inhibitors) and PIs (protease inhibitors). The mechanism of action of NRTIs and NNRTIs is based on the inhibition of reverse transcriptase, a viral DNA polymerase enzyme that retroviruses need to reproduce, while PIs block the HIV protease, an enzyme used by the virus to cleave nascent proteins for final assembly of new virions (5).

There are several causes related to the mechanism of infection and replicative capacity of the virus which elucidate the need for drug combination in HIV treatment: (i) the virus is able to replicate at a very fast rate (T1/2 approximately 1 day) once a cell has been infected; therefore one drug may be able to only decrease the rate of this process but not to interrupt it; (ii) given that viral replication depends on different enzymes which are the targets of the antiretroviral drugs, the possibility to reach two targets at the same time increases the chance of stopping HIV and protecting new cells from infection; (iii) the virus can infect different types of cells in different parts of the body; each drug differs in how well the virus can be attacked in these different cells; (iv) combination of anti-HIV drugs may overcome or delay the development of drug resistance (6,7). Drug resistance may emerge because of the replication program of HIV, which is rapid and error prone (mutation rate ca. $3 \times 10^{-5}$ mutations/base/replication cycle), resulting in large and genetically diverse populations in vivo (8). When HIV is allowed to replicate in the presence of antiretroviral drug concentrations, which are insufficient to exert complete suppression, antiretroviral drug-resistance mutations will almost invariably emerge (9;10). Depending on the site of viral mutations and their impact on viral fitness, different anti-HIV classes show higher or lower barriers to resistance. A combination of different antiretroviral classes is therefore needed to assure complete viral suppression and prevent the development of drug resistance.

In the past years direct nucleic acid sequencing has become a common mechanism to obtain resistance information; commercial genotyping services, as well as systems for laboratory use are available; routine testing with independent panels of resistant viruses is useful to maintaining proficiency in detection of mutations (11;12). Appropriate use of resistance testing provides valuable information useful in constructing regimens for treatment-experienced individuals with viremia during therapy.

To date, two therapeutic options are suggested as first-line choice in children: NNRTI- and PI-based regimens (13). Regarding NNRTI based regimens, only efavirenz (EFV) and nevirapine (NVP) are currently approved for paediatric patients. In children older than 3 years and able to swallow capsules, EFV is preferred as first-line therapy because of its once daily administration, absence of interaction with food and lower incidence of adverse events compared to nevirapine. Regarding PI-based regimens, most recent guidelines agree in recommending lopinavir/ritonavir (LPV/r) as a first line therapy in naïve paediatric patients. This drug is available as liquid formulation and appears to be safe and effective in children with regards to virological suppression and the increase in CD4 count.
Highly active antiretroviral therapy (HAART) in children

Table 1 Antiretroviral drugs currently approved in children (BID: twice daily, QD: once daily)

<table>
<thead>
<tr>
<th>ARV class</th>
<th>Drug</th>
<th>Half-life</th>
<th>IC50</th>
<th>Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>NRTI</td>
<td>Zidovudine</td>
<td>Serum 0.9-1.4 hrs Intracellular 3-4 hrs(15)</td>
<td>0.003-0.013 mcg/mL (16)</td>
<td>180-240mg/m^2-BID (17)</td>
</tr>
<tr>
<td>NRTI</td>
<td>Lamivudine</td>
<td>Serum 2-6 hrs Intracellular 10-15 hrs(18)</td>
<td>2 nM-15µM(19)</td>
<td>4mg/kg per dose-BID (17)</td>
</tr>
<tr>
<td>NRTI</td>
<td>Abacavir</td>
<td>Serum 1.5 hrs Intracellular 12-26 hrs(20)</td>
<td>0.26-4.0µM (21)</td>
<td>8-10 mg/kg per dose-BID(17)</td>
</tr>
<tr>
<td>NRTI</td>
<td>Didanosine</td>
<td>Serum 0.97-1.6 hrs (22)</td>
<td>0.49 µM(23)</td>
<td>&lt;3 months 50mg/m^2-BID -&gt;3 months 120 mg/m^2-BID(17).</td>
</tr>
<tr>
<td>NRTI</td>
<td>Stavudine</td>
<td>0.9-1.5 hrs (24)</td>
<td>0.009-4µM(25)</td>
<td>1 mg/kg per dose-BID (17)</td>
</tr>
<tr>
<td>NRTI</td>
<td>Emtricitabine</td>
<td>8-10 hrs (26)</td>
<td>0.0013-0.64µM(26)</td>
<td>&lt;3 months 3mg/kg-QD &gt;3 months 6mg/kg-QD (17)</td>
</tr>
<tr>
<td>NRTI</td>
<td>Tenofovir</td>
<td>17 hrs (27)</td>
<td>0.04 – 8.5 µM(28)</td>
<td>300 mg-QD (&lt; 12 years)(17)</td>
</tr>
<tr>
<td>NNRTI</td>
<td>Efavirenz</td>
<td>40-55 hrs after multiple doses (29)</td>
<td>0.51 mg/mL (30)</td>
<td>15-18.75mg/kg solid form or 19.5 mg/kg syrup-QD (17)</td>
</tr>
<tr>
<td>NNRTI</td>
<td>Nevirapine</td>
<td>25-30 hrs (31)</td>
<td>10mg/mL (31)</td>
<td>150-200mg/m2 per dose-BID (17)</td>
</tr>
<tr>
<td>Boosted PI</td>
<td>Lopinavir/ ritonavir</td>
<td>5-6 hrs (32)</td>
<td>0.04-0.18µg/mL(32)</td>
<td>&lt; 15 kg 12/3 mg/kg-BID &gt; 15 kg 10/2.5 mg/kg -BID (17)</td>
</tr>
<tr>
<td>PI</td>
<td>Nelfinavir</td>
<td>3.5-5 hrs (33)</td>
<td>0.06 µM(34)</td>
<td>&lt;10 kg -75 mg/kg/dose-BID 10 kg to 19.9 kg -60 mg/kg/dose-BID (17)</td>
</tr>
<tr>
<td>PI</td>
<td>Atazanavir</td>
<td>6-7 hrs (35)</td>
<td>2 - 5 nM(36)</td>
<td>&lt;20 kg 150mg + 100mg ritonavir &gt;20 kg +40 kg 200mg + 100mg ritonavir &gt;40 kg 300mg + 100mg ritonavir (17)</td>
</tr>
</tbody>
</table>

Two drugs from the NRTI class are needed to form the backbone of HAART, with six NRTIs (zidovudine, didanosine, lamivudine, stavudine, abacavir and emtricitabine) approved for HIV-infected children younger than 13 years of age. Combinations of lamivudine plus abacavir or zidovudine or didanosine are considered the preferred dual NRTIs backbone regimens for initial therapy in children. The selection of the initial regimen of antiretroviral drugs is generally based on several factors, such as comorbid conditions, potential adverse drug effects, potential drug interactions with other medications, results of genotypic drug resistance testing and convenience (e.g. pill burden, dosing frequency). In table 1 an overview of the antiretroviral drugs currently approved in children and their characteristics is provided, whilst table 2 summarises the antiretroviral combinations preferred as initial treatment in HIV-infected children.

An important point to consider which is still quite debatable is the optimal time to start HAART in children. According to the current WHO guidelines, all infants with confirmed HIV infection should be started on HAART, irrespective of the clinical or immunological stage, while for all children 12 months or older clinical and immunological thresholds should be used to identify those who need to start HAART (14).

Table 2 Antiretroviral regimens recommended for initial therapy for HIV infection in children (17)

<table>
<thead>
<tr>
<th>Preferred Regimens</th>
</tr>
</thead>
<tbody>
<tr>
<td>Children &gt;14 days and &lt;3 years</td>
</tr>
<tr>
<td>Two NRTIs plus lopinavir/ritonavir</td>
</tr>
<tr>
<td>Children ≥3 years</td>
</tr>
<tr>
<td>Two NRTIs plus efavirenz</td>
</tr>
<tr>
<td>Children age ≥6 years</td>
</tr>
<tr>
<td>Two NRTIs plus atazanavir plus low-dose ritonavir</td>
</tr>
<tr>
<td>Two NRTIs plus efavirenz</td>
</tr>
<tr>
<td>Two NRTIs plus lopinavir/ritonavir</td>
</tr>
</tbody>
</table>

2-NRTI Backbone Options for Use in Combination with Additional Drugs

<table>
<thead>
<tr>
<th>Preferred</th>
</tr>
</thead>
<tbody>
<tr>
<td>abacavir plus (lamivudine or emtricitabine)</td>
</tr>
<tr>
<td>(children ≥3 months)</td>
</tr>
<tr>
<td>tenofovir plus (lamivudine or emtricitabine)</td>
</tr>
<tr>
<td>(adolescents ≥12 years)</td>
</tr>
<tr>
<td>zidovudine plus (lamivudine or emtricitabine)</td>
</tr>
</tbody>
</table>

The drug: what is the right dose in HIV-infected children?

Up to now, empirical scaling from adults to children continues to be the mainstream method for dose selection in paediatrics, with adjustment for body weight as the most common approach (37). Although adjustment of drug pharmacokinetic parameters according to body weight or
body surface area (BSA) can occasionally explain the observed exposure differences between adult and paediatric patients, the direction and extent of these differences across age groups, in general, are not predictable. For example, some drugs are eliminated more rapidly or more slowly in younger paediatric patients, compared with adults or older paediatric patients (38). Bioavailability may also differ between children and adults due to differences in transit time or pH. There are extensive physiological changes with pharmacological impact that occur as a child matures from infancy to adulthood, and this process does not occur with precisely predicted timing or magnitude on an individual scale (39;40). Weight-based methods for determining paediatric doses may not account accurately for all variables related to the different stages of maturation and are unlikely to predict consistently the correct dose for each paediatric age group. All these aspects are in strong opposition to the concept of ‘one size fits all’ dosing for children (41). Rational considerations on the optimal dose for each child should be taken into consideration. Together with body weight, other confounders such as drug-drug interactions and demographic covariates, i.e. age, gender, body composition, functionality of liver and kidneys and maturation of enzymatic systems throughout the life span from neonates to adults (42) may affect the pharmacokinetics of a drug and consequently its exposure. When selecting the paediatric dose, these potential confounders must be taken into account in order to achieve an adequate exposure and to avoid the risk of toxicity or poor efficacy. A dosing regimen with more than the necessary doses, besides causing toxicity, might also increase the possibility of poor adherence, which is seriously related with occurrence of resistance. Suboptimal concentrations of antiretroviral drugs might as well be very dangerous because they may exert viral selection pressure and thus promote development of drug resistance.

Limited pharmacokinetic data remains one of the major issues in dosing recommendation for HIV-infected children. Although a similar disease progression in children and adults allows for dosing in children based on efficacy data in adults, performing pharmacokinetic trials to assess optimal dosing in children is critical to avoid inadequate exposure. Clinical studies to determine the optimal dosing in children of different ages are also critical to avoid under exposure or toxicity in children belonging to a certain age or weight group. Despite the indisputable need to perform clinical trials in HIV-infected children, one major limitation of such studies must be highlighted. In patients affected by chronic diseases who are obliged to take their medication for their whole life, adherence to therapy during the clinical trial may not be a realistic surrogate of patient adherence in real life, due to the limited duration of the study. Awareness is needed with regard to the implications that this may have on treatment outcome. Moreover participation to clinical trials may enhance adherence to treatment in chronic diseases (43), thus the pharmacokinetic profile of the drug might be altered in real life by different patterns of variable adherence.

The patient: adherence to HIV antiretroviral therapy

There are several reasons why antiretroviral treatment fails, of which poor adherence is a leading one (2;44-46). It has been shown that the role covered by the patient in achieving response to treatment is comparable to the one of the drug. A review of 17 studies on paediatric HIV treatment adherence found adherence in HIV-infected children ranging from 49 percent to 100 percent (47). Three-quarters of the studies showed adherence rates of 75 percent. Most of the studies in lower and middle-income countries revealed adherence rates above 75 percent, whereas adherence in higher-income countries was generally below 75 percent.

It is useful to provide an exhaustive definition of the term ‘adherence’ beyond the simple ‘patient’s tendency to follow medical advice’. Two substituent terms must be defined to have a comprehensive understanding of patient adherence: (i) compliance and (ii) persistence (48). The former is defined as ‘the degree of correspondence between the patient’s actual dosing history and the prescribed dosing regimens’. The latter is defined as ‘the time elapsed between the first dose taken and the time of treatment discontinuation’. The term compliance includes also the degree of correspondence between the patient’s actual dosing time and the prescribed dosing time. We handle this component as ‘quality’ of compliance. The different implications on treatment outcome of variable compliance (the patient sporadically misses some doses or takes the drug at different times) and variable persistence (‘drug holiday’) are shown in figure 1. It is important to mention that adherence is a critical issue in every chronic treatment, not only in HIV.

Numerous studies have investigated the effect of poor adherence in many therapeutic areas such as, hypertension (49), glaucoma (50;51) and osteoporosis (52).
Several factors pose specific challenges in children compared to adults (53). First of all young children’s adherence depends partly or entirely on a caregiver, who, especially in limited resources countries, may be sick or may need to work when the drug has to be administered (54;55). The identification of someone responsible for the child is difficult, especially when both parents died or are impaired. The expectation that older children should be able to take the medicine independently is often unrealistic (56). Moreover family members often have discrepant perceptions of a child’s level of responsibility for medication, especially in families with older children. Another reason which may affect compliance in antiretroviral therapy is the heavy pill burden that sometimes needs to be administered to perinatally infected children in need of savage therapy because of drug resistance and treatment experience (57). These complicated regimens pose greater issues in terms of adherence and therefore may lead to resistance which will create the need for even more complicated regimens. Furthermore, many of the current HIV medicines have an unpleasant taste, especially in syrups and powder form. This can make it difficult for children to take their antiretroviral drugs daily (58).

In the past years several strategies have been adopted to improve adherence in HIV-infected children, mainly based on the education of the caregivers or on peer support, self-monitoring and telephone follow-up (59;60). A brief period of hospitalization may help demonstrating the role of non-adherence on antiretroviral therapy and help identifying possible solutions. Material support such as pillboxes, drug identification charts, daily schedules, diaries and educational materials are provided to explain the schedules, risks and benefits of ART (61). Age-specific developmental-level protocols and teaching materials (e.g., cartoons, stories and drawings) have been developed to educate children about their treatment, their HIV status, and the importance of adherence and medical follow-up.

The possibility to reduce the dosing frequency of antiretroviral drugs is another important strategy to enhance adherence to treatment. It has been demonstrated that decreasing the pill burden and dosing frequency is associated with increased adherence (62-64). Several studies have already been performed to assess the feasibility of reduced dosing frequency of some antiretroviral drugs from three times a day to twice daily or from twice to once daily (65-67). However deep knowledge of the pharmacokinetic and pharmacodynamic properties of a drug is required to understand whether the dosing frequency could be reduced: it has been demonstrated that missing a dose when following once daily dosing regimens may be more dangerous than missing one dose on a twice daily regimen (68).

**Adherence-resistance relationship**

Failure to take the prescribed dose of antiretroviral drugs leads to ongoing viral replication in the presence of drug and the selection of drug-resistant HIV. It has been shown that poor adherence may increase the risk of drug resistance.
However, the relationship between adherence and development of resistance is not that simple as it may seem and it differs for each class of antiretroviral drugs. Bangsberg et al. have used a cohesive model (figure 2) to summarise this complex relationship for each class of antiretroviral drugs currently used as first line therapy (69).

![Figure 2: Schematic representation of the model developed by Bangsberg to summarise the relationship between adherence and resistance for each class of antiretroviral drugs. (Modified with permission from Bangsberg et al. (69)).](image)

According to this model, low levels of adherence are more likely to promote development of resistance to NNRTIs due to their low genetic barriers to drug resistance. On the other hand, higher selection pressure is required for single PIs given the high genetic barriers of this class to resistance; therefore a level of adherence close to perfect is more dangerous than low levels (70). Sporadic missed doses are unlikely to produce high risk combination of actively replicating virus and sub-therapeutic levels in NNRTIs, due to their long half-lives; conversely sub-therapeutic levels may easily be reached after long periods of treatment interruption and therefore the risk of resistance strongly increases. Unboosted PIs have a very short half-life and inferior antiviral efficacy compared to NNRTIs. Their rapid disappearance from circulation during non-adherent periods leads to lower frequency of new mutations compared to NNRTIs, therefore the majority of new PI resistance mutations occur in those with higher adherence rates. Boosted PIs instead have a high degree of antiviral efficacy and longer half-lives than unboosted PIs but shorter than NNRTIs. Their potency discourages the development of resistance in case of missed doses and their intermediate half-lives are impediments to the development of resistance mutations in patients who interrupt the treatment for long periods. The relationship between adherence and resistance is thus more complex than "non-adherence increases the risk of drug resistance". A good understanding of this relationship is a critical step in drug development and may lead to lower risk of viral failure in HAART.

**Assessment of New Highly Active Antiretroviral Therapy**

Based on the aforementioned data and considerations, optimal dosing regimen and adherence to prescribed treatment appear to be the main challenges in paediatric antiretroviral therapy and thus constitute the targets for future investigations of researchers and clinicians. The possibility to develop novel drugs with different mechanisms of action able to prevent the development of drug resistance and improve treatment outcome is an alternative solution which is beyond the scope of this study. Undoubtedly dosing frequency reduction may be very advantageous for the patients; however the impact of poor adherence on optimised dosing regimens must be taken into account. The possibility to evaluate which pharmacokinetic and/or pharmacodynamic properties of an antiretroviral drug make it less susceptible to suboptimal adherence and predict treatment outcome might be a powerful tool for dosing regimen optimisation. These considerations rely on an important concept in antiretroviral therapy, which still lacks an established and quantitative measure: forgiveness of non-adherence. Forgiveness of non-adherence is the ability of a drug or regimen to achieve and maintain viral suppression even in case of poor adherence (71). A variety of pharmacological, viral and host properties determine the level of forgiveness of any specific regimen. It is generally used as comparative descriptor of different classes of antiretroviral drugs, based upon the "anchor drug" of the regimen. In 2000 Paterson et al showed that extraordinarily high rates of adherence were necessary to achieve viral suppression in a group of HIV-infected patients receiving unboosted indinavir based regimen (45). These findings lead to the "95% rule", which means that patients must take at least 95% of the prescribed antiretroviral doses in order to control viral replication.

More recent studies have demonstrated that more moderate levels of adherence are needed to achieve and maintain viral suppression in patients treated with NNRTIs and boosted PIs based regimens. These findings gave birth to the evidence that some antiretroviral classes are more forgiving than others. This would be the starting point for future studies and investigations which may provide important information usable in clinical practice. Forgiveness is not defined in a quantitative manner and still lacks a specific scale and detailed thresholds which may influence therapeutic choices and improve the final outcome of the treatment. A quantitative and systematic definition of forgiveness will enable the exploration of new dosing regimens, which will provide advantages for the patients without risks of inadequate efficacy and in turn of drug resistance.
1.2. A MODEL-BASED APPROACH FOR THE EXPLORATION OF NOVEL DOSING REGIMENS

A systematic definition of forgiveness of non-adherence for different drugs and the exploration of alternative dosing regimens requires a quantitative methodology which allows evaluating contemporaneously the effect of disease, drug and patient behaviour.

A model-based approach in which pharmacokinetic-pharmacodynamic models for selected antiretroviral drugs are integrated with a model for viral dynamics may provide a powerful tool to predict efficacy of antiretroviral combination treatments. In addition, the inclusion of a model for patient adherence provides the basis for quantifying the relation between adherence, exposure and drug response as well as the impact of adherence on treatment outcome (72). The evaluation of adherence, however, may not be feasible in clinical practice due to ethical reasons and design issues. Thus an in silico approach is required to assess how different patterns of adherence may affect treatment outcome. Quantitative methods can provide insight into how forgiving antiretroviral drugs are and provide the scientific basis for alternative dosing regimens. Similar model-based methods have been already applied in different therapeutic areas, such as statin or antihypertensive therapies (73-75).

The possibility to integrate statistical models that describe disease mechanism, drug behaviour and patient adherence to treatment in clinical trial simulation will be crucial in the exploration of simplified dosing regimens of antiretroviral drugs in in silico populations of HIV-infected children based on hypothetical and real-life scenarios (76).

Disease models for viral dynamics
A statistical model that can accurately describe the disease in terms of viral replication and infection is the starting point to predict treatment response of combination antiretroviral therapy and to gain insight into possible mechanisms of treatment resistance (77).

HIV dynamics has been widely studied in the past twenty years and several models of different levels of complexity have been developed (8;78-80). The main advantage of this approach was the possibility to understand, quantify and parameterise viral processes such as replication, infection and death over time (81). For example modelling of viral dynamics has shown that HIV-1 is cleared from chronically infected patients at a rapid rate, with a half-life estimated to 6 hours. Furthermore quantitative estimates of viral parameters suggest that HIV-1 is a rapidly replicating virus and one that could respond to therapy. Finally, modelling has shown that the HIV virus can quickly become resistant to any single drug, particularly to those that require only one mutation to generate resistance. This phenomenon can be anticipated by the fact that every single possible mutation of the viral genome can be expected to occur hundreds or thousands times each day.

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Figure 3 depicts the basic model commonly used for viral dynamics. This model is widely used also to study the dynamics of hepatitis C virus, hepatitis B virus and cytomegalovirus infections in vivo.

The model considers a set of cells susceptible to infection, that is, target cells, T, which, through interactions with virus, V, become infected. Infected cells, I, are each assumed to produce new virus particles at a constant average rate p and to die at rate δ. The average lifespan of a productively infected cell is 1/δ and so if an infected cell produces a total of N virions during its lifetime, the average rate of virus replication per cell, p = N/δ. Newly produced virus particles, V, can either infect new cells or be cleared from the body at rate c per virion. This model is defined by a system of three differential equations (equation 1-3). These equations are applied to obtain minimal estimates for the parameters c and δ. From these estimates it is possible to calculate upper bounds for the half-life of virions in plasma and the half-life of productively infected cells.

\[
\begin{align*}
\frac{dT}{dt} & = \lambda - dT - kVT \\
\frac{dI}{dt} & = kVT - \delta I \\
\frac{dV}{dt} & = pI - cV
\end{align*}
\]

It has been observed that viral load decay in plasma takes place with an initial rapid exponential decline of nearly 2 logs of magnitude and continues subsequently with a slower exponential decline that leads to virus falling below the detection limit. The slope of this decline depends on the efficacy of therapy (82-84). The basic model was implemented to interpret this two-phase decay in viral load with the inclusion of more compartments representing a longer-lived popula-
tion of productively infected cells, activation of latently infected cells and release into the blood of virions trapped in tissue reservoirs (85;86).

Equations 1-3 represent the processes of viral infection and replication in absence of an antiretroviral drug. When the effect of an antiretroviral drug or regimen is analysed, the basic model is implemented as defined in equation 4-6, where $\gamma$ represents the antiviral efficacy (87).

$$
dT/dt = \lambda - dT - (1-\gamma)kVT \quad (4)
$$
$$
dI/dt = (1-\gamma)kVT - \delta I \quad (5)
$$
$$
dV/dt = pl - cV \quad (6)
$$

Given that reverse transcriptase inhibitors block the ability of HIV to infect a cell and protease inhibitors cause the production of non-infectious particles, the previous equations can be implemented taking into account the different mechanism of action of each class as shown in equations 7-10, where the viral compartment is decomposed in infectious and non-infectious virions ($V_i$ and $V_{NI}$) and $\epsilon_{RT}$ and $\epsilon_{PI}$ are the efficacies of RT and PI respectively (8).

$$
dT/dt = \lambda - dT - (1 - \epsilon_{RT})kVIT \quad (7)
$$
$$
dI/dt = (1 - \epsilon_{RT})kVIT - \delta I \quad (8)
$$
$$
dVI/dt = (1 - \epsilon_{PI})pl - cVI \quad (9)
$$
$$
dVNI/dt = \epsilon_{PI}pl - cVNI \quad (10)
$$

Such models have been widely validated and subsequently used to predict the time course of clinical endpoints and to design novel strategies in HIV treatment (88;89) given that they allow to link drug efficacy to long-term changes in HIV-1 viral load (87;90;91).

**Pharmacokinetic-pharmacodynamic modelling in children**

Characterisation of the pharmacokinetic-pharmacodynamic relationships is required to assess the correlation between plasma concentrations of antiretroviral drugs with changes in clinical endpoint. In order to define such relationships detailed information on pharmacokinetics and potentially also on pharmacodynamics of antiretroviral drugs in children need to be collected.

Given that only a limited number of observations can be obtained in paediatric subjects due to ethical and technical challenges, the population approach using nonlinear mixed effect modelling to obtain pharmacokinetics and pharmacodynamics parameters is the preferred approach (92;93).

The population approach is based on simultaneous analysis of all data of the entire population, while still taking into account that different observations come from different patients. Additionally, the population approach allows not only for the analysis of dense data, but also for sparse (limited number of observations per individual) and unbalanced data (unequal distribution of observations in various parts of the concentration–time profile in the individuals) or a combination of both. Finally, both the inter-individual and intra-individual variability are separately estimated in the dataset using this approach (94). The term “mixed” in nonlinear mixed effects modelling represents a mixture of fixed and random effects. For the fixed effects, a structural model describing the pharmacokinetics or pharmacodynamics is chosen (e.g. a two-compartment model for pharmacokinetics or an Emax model for pharmacodynamics). The random effects quantify the variability that is not explained by the fixed effects and include inter-subject and intra-subject random variability, which are both simultaneously and separately estimated (95). It is often assumed that the variability between subjects follows a normal distribution with a mean of zero and variance $\sigma^2$. Equation 11 is used to describe the relationship between individual and population parameter estimates:

$$
\theta_i = \theta_{\text{mean}} \cdot e^{\xi_i} \quad (11)
$$

Where $\theta_i$ represents the parameter of the $i^{th}$ subject, $\theta_{\text{mean}}$ the population mean, and $\xi_i$ the variability between subjects. The residual error is generally described using a proportional error (error is dependent on the concentration, which means a higher absolute error at higher concentrations (Eq. 12)) or an additive error (constant for all observations (Eq. 13)) or a combination of both. This means for the $j^{th}$ observed concentration of the $i^{th}$ individual the relation (Y$_{ij}$):

$$
Y_{ij} = c_{\text{pred}} e^{\eta_i} \cdot (1+\epsilon_i) \quad (12)
$$
$$
Y_{ij} = c_{\text{pred}} e^{\eta_i} + \epsilon_i \quad (13)
$$

Where $c_{\text{pred}}$ is predicted concentration and $\epsilon_i$ is a random variable with a mean of zero and a variance of $\sigma^2$.

The structural model uses fixed effects parameters such as clearance and volume of distribution for pharmacokinetics or $E_{\text{max}}$ and $EC_{50}$ for pharmacodynamics. The population values for these parameters are called typical values (TV). After selecting the structural model, the statistical submodel, which accounts for the inter-individual as well as the residual variability, is chosen and tested. Information on the inter- and intra-individual or residual variability is of clinical value, because it describes differences in clinical response between and within patients and may therefore provide guidance for rational dose adjustments. In the final step the covariate sub-model is determined, which expresses relationships between covariates and parameters of the structural model (e.g. influence of body weight on volume of distribution or clearance) (96).

**Covariate analysis**

Differently from adults, developmental changes in children (i.e. metabolising enzyme capacity, renal function, liver flow, body composition) profoundly affect the responses to medications. It
is important that such changes are considered in the context of all other sources of intra- and inter-individual variability resulting from genetic-, environmental- and disease-related factors and drug interactions (97). As shown in figure 4, pharmacokinetic-pharmacodynamic modelling permits the exploration of the influence of different demographic covariates to explain the variability in drug response. As previously mentioned, developmental changes may influence drug exposure and/or drug response. Thus the identification of the demographic covariates which are related to pharmacokinetic or pharmacodynamic parameters is crucial, specifically in children where such covariates may be strongly correlated.

The influence of developmental changes in childhood can be explored primarily by using size and/or age as covariates. Size (body weight) can be incorporated into the model using two different approaches. The first approach, the “allometric size approach”, includes size a priori by using a body weight-based exponential equation with a fixed exponent of 0.75 for clearance and 1 for volume of distribution (98;99). Once size is incorporated in the model using this fixed method, the influence of age is investigated, being the difference between the actual value of the pharmacokinetic parameter and the 0.75 allometric equation.

In the second approach, the “systematic covariate analysis”, body weight is regarded as covariate as any other, which means that the descriptive properties on the pharmacokinetic parameters are evaluated in a systematic covariate analysis as described below (42;100;101). In a systematic analysis, when studying the influence of covariates, scatter plots are used to screen for appropriate covariates to include in the covariate sub-model. Additionally, these plots are used to explore the nature of the influence of the covariate (linear, exponential, allometric, sub-populations, etc.). Likely candidate covariates are then added to the model (forward inclusion).

The identification of the correct covariates which are correlated to pharmacokinetic and/or pharmacodynamic parameters in children is particularly challenging due to the correlation between the covariates and to the limited number of subjects available for the analysis. In case of small populations, incorrect covariates might be selected due to lack of balance of the covariate distribution in the population analysed during model building; the selection of the erroneous covariates could have serious consequences in the prediction of pharmacokinetic or pharmacodynamic parameters in a different population.

Despite some limitations, pharmacokinetic-pharmacodynamic modelling in paediatrics constitutes a powerful and innovative approach to characterise pharmacokinetic-pharmacodynamic relationships and to optimise dosing regimens for children of different ages, body weights and genetic backgrounds. It has been widely applied to antiretroviral therapy to relate plasma concentration to efficacy and to identify the optimal dose of antiretroviral drugs in children (103;104).

**Modelling patient adherence**

In order to explore novel regimens of antiretroviral drugs or optimise existing ones, a third statistical element needs to be implemented, which describes the patients and their behaviour towards the treatment.

As explained in the previous part of this review, dosing patterns may differ between patients in terms of the actual dose (compliance), the timing of doses (quality) and the duration of treatment (persistence) (105-107). The consequences of variable adherence on treatment outcomes are determined by the magnitude of erratic dosing about the prescribed dosing times, the number and frequency of sequentially missed doses or “drug holidays” (when the patient stops taking the medication(s) for a period of time) and the pharmacological properties of the drug (108). Based on clinical data of adherence to treatment a very large inter-individual variability has been identified in dosing timing relative to the prescribed interdose interval. Indices of dose-taking compliance (the quantity of the dose) are usually less variable (109).
Clinical trial simulation

Given the characteristics of the HIV-infected population, a model-based approach is a potent instrument able to define and characterise the processes driving the disease, the pharmacokinetic and pharmacodynamic properties of the drug and the behaviour of the patient towards the prescribed treatment. Two elements of a model-based approach need to be distinguished: modelling and simulation. The former enables translation of the relevant features of a system into mathematical language (i.e. model parameters), whilst the latter allows the assessment of a system’s performance under hypothetical and real-life scenarios (i.e. “what-if” scenarios), yielding information about the implication of different experimental designs and quantitative predictions about treatment outcome, dosing requirements and covariate effects. In clinical trial simulation (CTS) multiple factors can be evaluated concurrently and relevant scenarios can be defined and investigated. The great advantage of the use of CTS in paediatric drug development and clinical practice is the possibility of exploring relevant scenarios before enrolling children into a clinical protocol. Simulations allow evaluation of a range of parameter values, including an assessment of critical scenarios, such as overdosing, that cannot be generated in real-life studies.

CTS has been widely used in the past in paediatric drug development and clinical practice. Läer et al. used CTS to develop an age-specific dosing regimen for sotalol in children, a CTS evaluation by Yim et al. was used to get US Food and Drug Administration approval to change the dosing regimen for etanercept in juvenile rheumatoid arthritis and CTS was applied to select rufinamide doses giving an exposure shown to be safe and efficacious in large paediatric populations. In CTS three important components are characterised: a disease/placebo model, a drug model, and the implementation model (trial design and decision criteria). Together with a model which describes the biological mechanisms underlying the disease and a drug-action model which comprises pharmacokinetic and pharmacodynamic factors, a trial model that simulates other important aspects of the trial, such as drop-out, compliance and protocol deviations, is required. Thus far, despite the widespread use of CTS in paediatrics, very few examples exist in which relevant design factors have been evaluated prospectively as part of the planning of a paediatric trial. In particular patient-related components, such as adherence and drop-out have not been encompassed in previous paediatric CTS.

Given the need to replace compliance data when not available or to test the impact of various non-compliance scenarios, several simulation models of treatment execution have been proposed in the past years. The simplest one assumes that the prescribed number of pills was taken correctly, but at different times than the prescribed ones. In those models, time intervals between two doses are drawn from normal distributions. Other models propose to simulate number of doses taken at each dose time according to a multinomial distribution allowing for 0, 1, 2 or more doses taken at each dosing time. Since this number may depend on the number of doses previously taken, an earlier attempt suggested using a Markov model, which has great flexibility and allows description of almost all different compliance profiles. The use of covariates in this model allows controlling, for example, the date at which the patient will have a “drug holiday”.

In conjunction with variable compliance, patient drop-out constitutes another fundamental element in clinical trials. Two types of drop-out exist: non-informative and informative drop-outs. Non-informative drop-outs simply mean that some patients may randomly stop to be reported in the trial, this independently from the treatment they received, and this independently of efficacy or toxic effects. On the contrary, disease progress can be correlated to the marker that is being followed. In this case, the drop-out is informative to the disease progress, and modelling the disease progress separately from the drop-out process may be inefficient and may produce bias estimates. For example, in a trial of HIV treatment, disease progress may lead a patient to drop-out to seek other treatment options.

Trial execution models, such as compliance and drop-out models, interact with the drug-disease models as depicted in figure 5.

Figure 5 Schematic representation of interactions between drug-disease models and two aspects of execution model: the compliance model and the dropout model. Drug-disease models interactions are shown with continuous lines, while execution models are presented with dotted line arrows. Notice that compliance influences pharmacokinetic and pharmacodynamic models, which in turn, by feedback mechanisms, may influence compliance. (source: http://www.euroformhealthcare.info/drug-development/protocol-deviations-and-execution-models.html).
1.3. CONCLUSIVE REMARKS

The previous paragraphs highlighted three main issues in antiretroviral paediatric therapy: the selection of the dose, the optimisation of the dosing regimen and the problem of adherence to treatment. The use of an integrated model-based approach in which pharmacokinetic-pharmacodynamic relationships, viral dynamics, patient behaviour and trial execution factors are incorporated provides the basis for comprehensive clinical trial simulation scenarios. CTS represents a critical step in the evaluation and planning of experimental protocols. It also offers the opportunity to explore conditions which may not be feasible or ethically acceptable in children. The possibility to evaluate in silico populations of HIV-infected children without exposing the patients to experimental settings will strongly simplify the identification of the best dose or dosing regimen for a selected group of HIV-infected children and the investigation of forgiveness of non-adherence of current or future treatments.
References


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