The 'Triple Study': viral dynamics and immune reconstitution in HIV-1 infection during potent antiretroviral therapy
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Citation for published version (APA):
CHAPTER X

DISCUSSION AND CONCLUSION

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Viral dynamics and immune reconstitution during potent antiretroviral therapy

Potent antiretroviral combination therapy, consisting of a protease inhibitor (PI) with two nucleoside analogue reverse transcriptase inhibitors (NRTIs), was first used in clinical trials in 1995 and in the year thereafter introduced into the clinical practice. At that time, a number of uncertainties existed concerning the effects to be expected: 1) the possibility to obtain a strong and durable suppression of viral replication was uncertain; resistance might develop as it did with mono- and double therapies and the effects of potential sanctuary sites were not clear. 2) It was not certain that the virological and immunological responses seen in blood were representative for the lymphoid tissue (LT), which harbors most virus and CD4+ T-cells. 3) Even if a strong suppression of viral replication could be sustained, the extent and durability of immune reconstitution that could be obtained was uncertain, especially in subjects with advanced disease. 4) Experience with the drugs was very limited and long-term side effects were unknown. Furthermore, it would be difficult for patients to remain adherent to the strict intake requirements, the complex dosage schedules and the food requirements.

SUPPRESSION OF VIRAL REPLICATION

With triple therapy, we observed a rapid, biphasic decline of HIV-RNA to levels below the cutoff level of the assay used (Chapters II and IV), comparable to the effects seen with several other PI-containing triple regimens. The suppression of the virus was maintained for at least two years in the subjects remaining on therapy (Chapter VII). Using a so-called ultrasensitive assay modification, with a lower cutoff level of 100 copies/mL, HIV-RNA levels had also declined to below this new cutoff level (Chapter VIII). To date, only a very limited number of reports on trials or patient cohorts with a follow-up of at least two years has been published. Some data on two to three years of therapy are available from regional subgroups of international trials, or from smaller, specifically selected groups of subjects. Furthermore, a few cross-sectional evaluations of selected subjects who received potent therapy for a prolonged time, occasionally for more than three years, have been published.

Viral replication can thus be suppressed for a period of at least two to three years and probably considerably longer. Evidence has been found, however, of continuing viral evolution despite the use of potent therapy. Different groups have reported ongoing mutations in the env gene, thus not related to drug resistance, in a number of subjects with HIV-RNA levels below the cutoff level for a prolonged time. This suggests that viral replication is not fully suppressed in at least part of the subjects, despite unquantifiable plasma HIV-RNA levels in ultrasensitive assays. Over time, this might lead to the selection of drug-resistant virus.

Furthermore, viral mRNA expression in infected peripheral blood mononuclear cells (PBMCs) has been detected in subjects who have
unquantifiable plasma HIV-RNA levels for prolonged times.\textsuperscript{118,23,25} The presence of mRNA does not necessarily reflect ongoing viral replication, as the antiretroviral therapy does not block transcription of DNA into RNA, but at least it shows the presence of long-lived infected cells. Using a highly sensitive HIV-RNA assay, measuring down to 5 copies/mL, low levels of HIV-RNA have been detected in plasma in one study in randomly selected patients.\textsuperscript{22} Another study has suggested the presence of circular viral DNA as proof of ongoing viral replication while using potent therapy.\textsuperscript{26}

There are more indications that suppression of viral replication can be improved upon. Correlations of plasma concentrations of PIs or non-nucleoside RTIs (NNRTIs) with the decline rate of viral RNA have been found, suggesting that therapy efficacy often is not 100\%.\textsuperscript{27,28} Using a five-drug regimen, HIV-RNA levels decline more rapidly below the cutoff level of an ultrasensitive assay than in our subjects.\textsuperscript{29} Furthermore, subjects using this five-drug regimen generally have plasma HIV-RNA levels sustained below 10 copies/mL (Chapter VIII).\textsuperscript{8}

\section*{POTENTIAL SANCTUARIES}

Initial studies on the amount of HIV in lymphoid tissue (LT) showed no or limited effects with NRTI mono- or double therapy.\textsuperscript{10-12} With the more potent PI-NRTI triple combination, we observed a strong decline in HIV-RNA, confirming that the strong decline observed in blood does reflect a suppression of viral replication in LT (\textit{Chapters II and III}).\textsuperscript{11,33}

Some care must be taken though to extrapolate HIV-RNA levels in plasma to LT as the dynamics of free virus in the blood might not be the same as intracellular viral RNA or FDC-associated virus in LT.\textsuperscript{34,35} Treatment interruptions can lead to a rapid spread of virus in the LT, with a subsequent slower decline of HIV-RNA levels in LT than in plasma, following restart of treatment.\textsuperscript{36} Furthermore, it has been suggested that treatment with non-nucleoside RT inhibitor (NNRTI)-containing regimens results in a lower suppression of HIV-RNA in LT than treatment with PI combinations, even if equally low plasma HIV-RNA levels are obtained.\textsuperscript{37} Other studies, however, showed that a strong decline of HIV RNA in LT could be achieved with NNRTI-containing regimens.\textsuperscript{38,39}

A more detailed analysis of the LT of our subjects with \textit{in situ} hybridization showed that the largest pool of HIV in the body, consisting of viral particles stored on the surface follicular dendritic cells (FDCs), rapidly declined (\textit{Chapter III}).\textsuperscript{33} as has been confirmed by others.\textsuperscript{40-42} Thereby it is shown that the largest pool of virus in the body is affected by potent antiretroviral therapy. FDC-associated viral proteins like p24 have been shown to persist longer than virions while using antiretroviral therapy.\textsuperscript{40,42,41} It is not clear whether this antigen persistence reflects a much slower turnover of FDC-associated viral proteins compared to viral RNA, a quantitative difference as a virion contains only two RNA copies
and many p24 molecules, ongoing protein production in persistently infected cells, or ongoing viral replication while using therapy.

Other potential sanctuaries were not studied in the work described in this thesis, but a discussion of recent findings is important to understand the possibilities and limitations of potent antiretroviral therapy.

The reservoir of latently infected cells is considered an important obstacle for attempts of viral eradication. In 1997, several groups showed that in subjects on therapy, with unquantifiable plasma HIV-RNA levels for a prolonged time, replication-competent virus could be recovered from their blood. Latently infected resting CD4+ memory T cells could produce infectious virus upon stimulation in vitro, suggesting that in vivo immune activation would result in virus production if the medication would be interrupted. Estimates of the half-life of these cells in subjects using potent therapy ranged from 6 to 44 months, suggesting that eradication of this pool might take between ten and sixty years. Furthermore, in subjects with occasional quantifiable plasma HIV-RNA, as evidence of ongoing viral replication, the decay of these cells is considerably slower or does not occur at all.

Theoretically, activation of these latently activated cells through medical interventions, under the protection of potent antiretroviral therapy might lead to a more rapid decline in this pool. Cytokines like interleukin-2 (IL-2) and stimulation of the T-cell receptor with monoclonal antibodies to CD3 (OKT3) can induce viral replication through cell activation. Evaluation of patients receiving potent antiretroviral therapy with IL-2 suggested that a stronger decline in the pool of latently infected cells can be achieved. A pilot study with OKT3 plus IL-2 showed T-cell activation and virus replication, but the effect on the size of the pool of latently infected cells was not clear and the treatment had considerable side effects.

The central nervous system (CNS) is known to be less accessible for many drugs because of the blood-brain barrier. Usually the cerebro-spinal fluid (CSF) is studied as a reflection of the amount of virus and drug penetration in the CNS, although it is not certain how well the CSF represents the CNS. Most NRTIs and NNRTIs, except ddi, penetrate well into the CSF. PIs are generally heavily protein-bound in plasma and penetrate much less easily into the CSF, possibly with the exception of indinavir. Several studies with PI + NRTI triple regimens observed strong declines of HIV-RNA in the CSF. A study with a double PI regimen showed a considerably lower effect on HIV-RNA levels in the CSF when no NRTIs were given concomitantly, suggesting that the NRTIs might be mostly responsible for the observed antiviral effects.

Antiretrovirals have been predicted to penetrate well into semen, although little actual data are available. PIs with extensive plasma protein-binding appear to penetrate poorly into semen, however, while another PI and an NNRTI could be detected in reasonable...
concentrations. Studies on the anti viral effects of present-day combinations show a good suppression of HIV-RNA levels in semen. Proviral DNA could still be detected in seminal cells despite unquantifiable HIV-RNA levels, however, indicating that infectivity might not be (completely) suppressed.

In conclusion, with our triple regimen, viral replication was strongly suppressed in LT and blood, although ongoing replication might occur at low levels and stronger combinations might have additive effects. Published data beyond two years of therapy are very scarce. Studies with comparable regimens suggest that other anatomical reservoirs such as the brain and testes/prostate can be reached by most, but not all antiretrovirals. Long-term studies on antiviral efficacy and the possibility of local resistance development in these reservoirs are needed. Latently infected cells decline very slowly, if at all, with the present combinations and it is unclear to date whether this reservoir can be eliminated completely by activation of the cells.

MODELS OF VIRAL DYNAMICS

Mathematical models can be used to gain insight into the complex relationship of HIV with the immune system. The models published by Wei et al. and Ho et al., published in early 1995, considerably changed our view on HIV. Furthermore, debate on the biological assumptions underlying these models can show where our knowledge and understanding is lacking and provide challenges for further research. Examples are studies to more precisely measure the clearance rate of the virus, which resulted in much shorter half-life estimates. Also, T-cell turnover has been measured more directly (reviewed in).

Models might be used to more rapidly evaluate the potency of different drug regimes. The model described in Chapter IV showed that during the first three weeks, triple therapy resulted in lower HIV-RNA levels and a higher decline rate of productively infected cells than PI monotherapy. It has been shown that the lowest RNA level that can be achieved with antiretroviral therapy is indicative for the success of therapy. The relationship of decay rates of virus and infected cells with long term success has not been fully investigated thus far. One study found a relationship between viral RNA decay rates during quadruple therapy and virological failure during a subsequent two-drug maintenance.

Newer models have been described, incorporating LT HIV-RNA copy numbers and CD4 T-cell counts, differences in drug efficacy and virus-induced CD4 T-cell activation.
IMMUNE RECONSTITUTION

Upon start of potent antiretroviral therapy, CD4+ T-cell counts in the blood rapidly increase, mainly because of an increase in memory T-cells (CD45RO+ with or without CD45RA-CD62Lneg; Chapter V). In Chapter V, we suggest that this increase is caused by a rapid redistribution of the memory CD4+ T cells from the LT into the circulation, simultaneous with CD8+ memory T cells and CD19+ B cells. The increase rate of CD4+ T cells with therapy is inversely correlated with the baseline CD4+ T-cell counts, suggesting that cell-trapping in LT increases with disease progression and that cell counts in the blood might overestimate the extent of immune deficiency. The latter is confirmed in Chapter VI, where it is shown that the CD4+ T-cell depletion in our subjects relative to uninfected controls, is less in LT as compared to the blood. A rapid decrease in inflammatory cytokines in LT with antiretroviral therapy could explain the release of cells from the LT. Other studies have confirmed the occurrence of initial redistribution as an explanation for the rapid increase in peripheral CD4+ T-cell counts.

Following the increase during the first few weeks, a further increase in memory CD4+ T-cell counts is limited. Naive CD4+ T cells (CD45RA+CD62L+) increase at a lower rate, but their increase is more sustained (Chapters V and VII). The increase rate of the naive cells in the peripheral blood is comparable to that in the lymphoid tissue (Chapter VI).

An important requirement for the achievement of a considerable immune improvement is a successful suppression of the viral replication. Even though, discrepancies in CD4+ T-cell- and HIV-RNA responses can occur. We therefore evaluated long-term immune reconstitution in subjects with well-suppressed HIV-RNA levels to study other determining factors. The long-term increase in total CD4+ T-cell counts is to a large extent determined by the increase in naive CD4+ T cells. The most important predictive factor for the increase in total CD4+ T-cell counts was the baseline naive CD4+ T-cell count (Chapter VII). Other studies have found a stronger increase in naive CD4+ T cell counts in subjects with higher baseline naive CD4+ T-cell counts, partially confirming our findings.

In half of the subjects analyzed, total CD4+ T-cell counts did not further increase or even declined after 72 weeks of therapy, because of a decrease in memory CD4+ T cells, although naive cells continued to increase (Chapter VII). To date, only a limited number of studies on long-term immune reconstitution of at least 2 years in virologically well suppressed subjects have been published. One study has reported no further change in CD4+ T-cell counts between the second and third year of follow-up. Furthermore, most studies did not study naive and memory T-cell subsets nor examined the influence of baseline naive CD4+ T-cell counts.
The importance of baseline naive CD4+ T-cell counts for long-term CD4+ T-cell reconstitution and the correlation between naive CD4+ and CD8+ T-cell increase rates, suggest that thymic output might be involved in the immune reconstitution (Chapter VII). Furthermore, the capacity of peripheral circulating precursor cells to generate mature T cells in an in vitro thymic organ culture system was found to improve strongly during therapy and the increase in this capacity correlated with the increase in naive T-cell numbers. Abundant thymic tissue has been detected in a considerable proportion of HIV-1 infected adults and the amount of tissue correlated with the number of circulating naive T cells. Furthermore, increases in thymic size with therapy correlated with the increase in naive CD4+ T-cell counts. The role of the thymus in immune reconstitution is further supported by the observation of an increase in recent thymic emigrants with antiretroviral therapy. This increase does not always correspond to the increase in naive CD4+ T-cell counts, however, and thymectomy does not prevent an increase in naive CD4+ T cells. It can therefore be concluded that the role of the thymus in immune reconstitution is complex and has not yet been completely elucidated.

T-cell activation, measured by the percentage of CD8+ T cells expressing markers like CD38 or HLA-DR, declines rapidly, although not completely, with potent antiretroviral therapy (Chapter VII). The hypergammaglobulinemia, as reflection of general B-cell activation, also declines, as do antibodies against p24 and gp120. The decline in HIV-specific antibodies correlated with the change in memory CD8+ T-cell counts and with the percentage of activated (CD38+) memory CD8+ T cells. This suggests a common underlying mechanism: possibly the decline in CD8+ T-cell subsets mostly reflects a decline in HIV-specific cells and both HIV-specific antibodies and -cells respond to the decline in the amount of viral antigen (Chapter IX).

The immune system does not only recover in numbers but also in function. Both aspecific and antigen-specific cell responses improve with therapy (Chapters V and VII). In contrast, the recovery of HIV-specific CD4+ and CD8+ T-cell responses is very limited. HIV-specific immune responses might possibly be preserved if therapy is started soon after infection, preferably before seroconversion.

The clinical relevance of the recovery of the immune system is shown by the clear reduction in HIV-disease progression and death, as observed in clinical studies. On a larger scale, the therapy has resulted in a reduction in the number of new AIDS diagnoses and HIV-related hospital admissions and deaths in the whole Western world. Immune reconstitution can result in clearance of pathogens and HIV-related conditions that were difficult to treat before. Also, prophylaxis for opportunistic infections can safely be discontinued.

In conclusion, potent antiretroviral therapy leads to significant improvement of the immune system, in laboratory parameters and clinically. The patterns of recovery and the relationships between different
parameters have given more insight into HIV-related immunopathogenesis. However, the clinical relevance of the different patterns of immune reconstitution needs to be elucidated. Furthermore, whether immune reconstitution occurs to the same extent with non-PI containing regimens and what the impact is of stronger suppression of viral replication on the immune system needs to be further investigated.\textsuperscript{11,26} Truly long-term data are not yet available and it is not clear to date whether the immune reconstitution can result in complete normalization of all parameters, especially of immunity against HIV itself.

NEW DEVELOPMENTS IN ANTIRETROVIRAL THERAPY

With the introduction of potent antiretroviral therapy, the aim of treatment has shifted from delaying to preventing disease progression through suppression of viral replication as strongly as possible for as long as possible and thereby preventing or restoring damage to the immune system.

As viral eradication does not seem feasible with current antiretroviral drug-combinations within the foreseeable future, life-long use needs to be anticipated. Toxicity, both short- and long-term, the strict dosage regimes and the accompanying food restrictions can severely compromise adequate usage of the drugs, with the risk of viral resistance development. To minimize these problems a number of therapy-strategies have been or are being attempted.

Certain PIs are combined to favorably alter their pharmacokinetic profiles, thereby increasing their potency and the dosing interval, and reducing the number of pills to be taken and the accompanying food restrictions.\textsuperscript{113-136} The number of side-effects associated with PIs, including lipodystrophy and other metabolic abnormalities seen frequently with the use of PIs, next to the more convenient dosage regimes, has stimulated the use of NNRTIs.\textsuperscript{117-140} Antiviral and immunological effects are more thoroughly investigated for PIs, however, and only recently study results of direct comparisons have become available, showing equal antiviral potency of NNRTIs.\textsuperscript{141,142} Whether immune reconstitution occurs to the same extent as with PIs needs to be further investigated.\textsuperscript{111}

Another treatment strategy that has been investigated is induction-maintenance. After a period with a common three- or four-drug-combination induction-phase, patients are switched to a maintenance regimen of one or two drugs. Thus far, this strategy has not been successful in maintaining suppression of HIV-RNA levels.\textsuperscript{62,143,144}

Several treatment strategies involve the immune system. IL-2 has been shown to increase CD4\textsuperscript{+} T-cell counts, but following administration HIV-RNA levels were temporarily increased.\textsuperscript{145} Stimulation of the immune reconstitution while controlling viral replication has been attempted by combining IL-2 concomitant with potent antiretroviral therapy. To date
only a number of small studies have been published, showing a stronger increase in CD4+ T-cell counts, but a clinical benefit has not yet been shown.\textsuperscript{146,147}

Immunological control of the virus has been attempted with 'therapeutic' vaccination of HIV-infected subjects while taking antiretroviral therapy. Studies with different vaccines are being undertaken, although to date it has not been proven that a significant clinical benefit or immunological control of HIV replication can be achieved through vaccination.\textsuperscript{148-150} A few case reports exist on a sort of 'auto-immunization' in subjects who started antiretroviral treatment soon after infection. They repeatedly interrupted therapy, which was initially followed by rises in viral replication. This appeared to have acted like a booster for their immune system and allowed them to finally discontinue therapy without a rise in HIV-RNA levels.\textsuperscript{151,152} In contrast, therapy interruptions in chronically infected patients inevitably lead to a rapid rebound in HIV-RNA.\textsuperscript{153} Structured treatment interruptions are now being investigated in chronically infected patients to study whether HIV-specific immunity can be elicited this way.\textsuperscript{154}

New drugs are being developed, either of the same classes as the existing ones or aiming at other viral targets. For new drugs of the same class, issues like tolerance, dosage regime and (cross)-resistance patterns are taken into account. Among the new drugs aiming at other viral targets are fusion inhibitors, which have already shown to be efficacious in humans,\textsuperscript{155} and integrase inhibitors.\textsuperscript{156} The antimalarials chloroquine and hydrochloroquine have antiretroviral activity, possibly through interference with gp120 glycosylation, although inhibition of integration has also been observed.\textsuperscript{157-158} Limited clinical data on the antiretroviral effects of either drug has been published to date, but they might provide more affordable constituents of combination therapy in developing countries.\textsuperscript{159} (For an overview of known or potential anti-HIV compounds see \textsuperscript{160}.)

As the antiviral drugs are used only since a few years, not only long-term efficacy is unknown, but also long-term side effects are not clear. Lipodystrophy was first reported one-and-a-half years after the widespread introduction into clinical practice and the pathophysiology of the syndrome is still not known.\textsuperscript{161,162} Furthermore, the long-term health risks of lipodystrophy and the metabolic abnormalities associated with antiretroviral therapy are not clear.

In conclusion, present-day antiretroviral therapy has clear clinical benefit, resulting from the strong suppression of the viral replication that allows for immune reconstitution and -preservation. Much has been learned about the pathogenesis of HIV from studying the effects of potent antiretroviral therapy, but many questions still remain. Future developments will include research on the possibility of viral eradication or permanent immunological control of the virus. On the other hand,
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regimens better suited for life-long therapy are investigated and new drugs are developed for those who harbor resistant virus or cannot tolerate the current drugs.

However, in the fight against HIV-infection and AIDS, prevention of transmission through proper information and the development of a vaccine remains of crucial importance. Not only since the treatment options are far from ideal, but especially since worldwide for the overwhelming majority of HIV-infected people long-lasting antiretroviral therapy and proper health care are not affordable.

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Chapter X. Discussion and conclusion


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