Summarizing discussion

SUMMARIZING DISCUSSION

Since the introduction of combination antiretroviral therapy (cART) to treat HIV-infection, an immense number of lives have been saved. However, this lifesaving treatment comes at a cost in a substantial number of individuals due to adverse events (AE), high pill-burdens, drug-drug-interactions, or lack of efficacy with the subsequent emergence of resistant viruses. It remains difficult to define the optimal cART regimen due to large interclass ART differences and, more importantly, to align them per individual clinical situation. Therefore, the central aim of this thesis is to evaluate the efficacy and safety of existing and new cART strategies, with a focus on integrase strand transfer inhibitors (INSTI), which could help to tailor antiretroviral regimens to the need of individual patients. The discussion of this thesis is divided in three parts. The first part describes the efficacy of a maintenance monotherapy simplification strategy with the second-generation INSTI dolutegravir (DTG), the second part is on the safety of INSTI initiation in the most vulnerable HIV population, and the third part further focuses on cART related toxicities and potential solutions. In this last part of the thesis, the clinical relevance of these findings is summarized (also in Dutch).

THE ROLE OF DOLUTEGRAVIR IN SIMPLIFIED ANTIRETROVIRAL REGIMENS

Simplification of triple cART might have advantages, including reduction of AEs, pill burden, and drug-drug-interactions, and cost-effectiveness. DTG has a high virological efficacy and a high genetic barrier to resistance. Even in patients with previous treatment failure, DTG often remains active.[1–5] The good virological properties, combined with a low risk on AEs and DDI, made DTG an ideal candidate to be studied as maintenance monotherapy simplification strategy in HIV patients.

Dolutegravir maintenance monotherapy

In the randomized clinical non-inferiority DOlutegravir maintenance MONOtherapy for HIV-1 infected adults (DOMONO) study, presented in **Chapter 2**, the aim was to prove that simplification to DTG maintenance monotherapy was non-inferior to cART. This strategy turned out to be of no clinical use, despite formal non-inferiority at the week 24 primary endpoint, since in total eight patients had virological failure (VF) on monotherapy and none in the control group continuing cART. Three of the eight patients also acquired mutations associated with resistance (RAM) in the integrase gene, which is in sharp contrast to the absence of any mutated virus in patients on DTG in the published phase 3 trials. The study was therefore prematurely terminated, with the conclusion that DTG should not be used as maintenance monotherapy.[6] Five observational studies of DTG monotherapy in 118 patients

in total were conducted. Of note, they were all conducted prior to DOMONO and without report of ethical committee approval in the manuscripts. Interestingly, no VF with acquisition of resistance associated mutations (RAM) was found in patients who were never treated with INSTIs before and who did not have a history of VF.[7–11] Other randomized studies confirmed the DOMONO results, however. The DOLAM-study in 91 patients included a simplification DTG maintenance monotherapy arm and compared this to cART and DTG dual therapy with lamivudine (3TC). Two patients on DTG monotherapy had VF, both with clinically significant emerging RAMs (S147G/Q148R/N155H and E138K/G140S/N155H), compared to one patient on DTG/3TC without RAM in the integrase gene.[12,13] Also, the MONCAY-study had seven patients with VF, including two with RAMs in the integrase gene, compromising clinical management (S147G/N155H and R263K) among a total of 78 patients on DTG monotherapy. A plasma HIV-RNA load between 0 and 20 copies/mL (c/mL) and low CD4 T-lymphocyte counts seemed to predict VF in these patients.[14] On the other hand, a randomized trial on 68 patients, who initiated cART during a primary HIV-infection showed non-inferiority of DTG maintenance monotherapy to cART, although the follow up period was limited. In this trial, one patient experienced VF, but no RAM was identified in the rebounding virus.[15] The results of these four randomized trials clearly show that simplification to DTG maintenance monotherapy is not a useful clinical strategy with the current armamentarium of effective triple drug based cART. Currently, INSTI monotherapy is not recommended by international guidelines.[16–18]

In **Chapter 3**, viral dynamics of patients with VF on DTG maintenance monotherapy are described.[19] Besides, DOMONO, we also conducted a DOMONO pilot study. In the latter study, we included patients with the same inclusion criteria as in DOMONO, except for a CD4 T-lymphocyte nadir below 200 cells/mm^3. In our DOMONO studies combined, ten patients had VF, including four with acquired INSTI-RAMs during DTG monotherapy compared to none on triple drug cART. This shows that the genetic barrier to resistance of DTG monotherapy is too low for maintenance of viral suppression. The large time variation to VF after start of DTG monotherapy (from 4 to 72 weeks) suggests that stochastic reactivation of pre-treatment existing proviruses, containing a single INSTI-RAM, may be the responsible mechanism for VF. Interestingly, we found that one patient with VF without RAMs in integrase had changes in the 3'polypurinetract (3'PPT). This points to a new HIV antiretroviral resistance mechanism *in vivo*, which, until now, has only been described *in vitro*.[20] This mutation is thought to result in alterations of the four terminal bases of the long-terminal repeat leading to a decreased binding capacity of INSTIs to the integrase, while leaving its strand-transfer-activity intact.[21] This resembles a resistance mechanism also described for protease inhibitors (PIs).[22] In four patients with VF during DTG monotherapy, the viral integrase and the 3'PPT were unaffected. However, it should be kept in mind that VF during INSTI-containing cART without the detection of RAMs in the integrasegene is observed in a substantial number of

patients in phase 3 studies with different INSTIs. Sequencing areas outside integrase should therefore be considered in these cases. Preferably this should also be done in the genome of non-B HIV subtypes, as the majority of data regarding mutations outside the integrase causing antiretroviral resistance is obtained from HIV subtype B.

We only can speculate about an ideal antiretroviral agent maintaining plasma virological suppression when applied as maintenance monotherapy. A number of virus- and patient-related factors are known to be associated with plasma viral rebound. One of them is the viral reservoir, which could be defined as all the cells in the body that are infected with HIV. A theory is that in some parts of this reservoir, ongoing viral replication due to low antiretroviral drug levels might lead to selection of viruses with the emergence of RAMs.[23–26] A larger reservoir size might increase this chance. In **Chapter 4** we therefore investigated characteristics, including reservoir size measured as total HIV-DNA, associated with VF during DTG maintenance monotherapy. We found that (similar to PI monotherapy) VF was associated with a lower CD4 T-lymphocyte nadir, a longer time between HIV diagnosis and cART initiation, and a higher HIV-DNA at the time of DTG monotherapy initiation.[27–31] Thus, the efficacy of future simplification strategies might be based on the time of infection at start of first line cART but this assumption needs further investigation. A low CD4 T-lymphocyte nadir, a longer time between HIV diagnosis and cART initiation, and a higher HIV-DNA at the time of DTG monotherapy initiation however seem intertwined since the longer the time between HIV-diagnosis and initiation of cART, the lower the CD4 T-lymphocyte nadir, and the higher total HIV-DNA is likely to be.[32,33] Nevertheless, the results imply that the viral reservoir size, measured here as total HIV-DNA, plays an important role in plasma viral outcomes in patients on monotherapy. Future studies on simplification strategies should evaluate the relevance of the relation between total HIV-DNA as marker for the reservoir, virological suppression, and non-virological markers.[34–37]

Next, we investigated metabolic effects of a switch from cART to DTG maintenance monotherapy in **Chapter 5**. We found that DTG monotherapy led to creatinine based estimated glomerular filtration rate (eGFR) decreases but amelioration of proteinuria. No major clinically relevant effects on bone density, lipids, or inflammation were observed. The vast majority of participants in the DOMONO study was using tenofovir disoproxil fumarate (TDF)-containing cART, and use of TDF is associated with a number of metabolic effects: i) an accelerated eGFR decline, ii) renal proximal tubular dysfunction (PTD), iii) a decrease in bone mineral density (BMD), and iv) lowering of plasma lipids.[38–43] It is therefore surprising that we did not observe relevant lipid changes or improvements in BMD. The eGFR decrease after TDF-discontinuation is unsurprising, given DTG's inhibitory activity of renal tubular creatinine clearance. This leads to eGFR underestimation since this creatinine increase does not affect true glomerular function. True glomerular function was not measured in DOMONO,

but the measurements reflecting tubular function all ameliorated. The trabecular bone score (TBS) has not often been studied in HIV patients. It can provide additional information about the bone microarchitecture. Several studies suggest that a lower TBS increases the risk for osteoporotic fractures independently of the BMD and therefore leads to a better prediction of the fracture risk.[44–46] Our data did not show a clinically relevant change in TBS in HIV patients after discontinuing TDF. It has to be studied whether TBS is a better predictor of the fracture risk than BMD, especially in patients at risk for osteoporosis. The stable lipid parameters might be a consequence of including predominantly healthy middle-aged males, which is also illustrated by the low Framingham Risk Score (FRS) indicating a low ten-year cardiovascular risk in our patient cohort. Besides, stable virological suppression during cART-use, not necessarily TDF-containing cART, has been associated with stable low levels of inflammation, as well as with adequate but not normalized T-cell immunity compared to HIV-negative individuals. Both these factors are associated with less mortality.[47,48] However, the results of these explorative analysis on metabolic markers still has to be interpreted with caution due to intrinsic limitations in our study design such as relatively few elderly, female, or non-Caucasion patients included, and the lack of a control-arm. Furthermore, prolonged simplified regimens, even in the absence of frank viremia, might still result in unfavorable metabolic changes due to a persistent suboptimal state of virological suppression exemplified by frequent viral blips for example. Although DTG monotherapy is virologically inferior to cART and should therefore not be considered as a useful clinical simplification strategy, the findings in **Chapter 4** and **Chapter 5** remain relevant for the studies on DTG-containing treatment strategies such as dual therapy with DTG/rilpivirine (RPV) or DTG/3TC.

To conclude, with the studies mentioned in **Chapters 2** to **5**, unique data are available about the consequences of a switch to DTG monotherapy, which may have consequences for the use of current second-generation INSTI-containing dual therapy simplification strategies. In fact, several clinical trials already show favorable results of DTG/3TC, DTG/RPV, and cabotegravir (CAB)/RPV in virologically suppressed, but also cART-naive patients, so data on virological and non-virological consequences of simplification of cART using a second-generation INSTI remain very important.[49–54]

Implications and future directions

Although DTG failed as maintenance monotherapy, it can still be a useful drug for simplification strategies. Dual drug strategies including an INSTI have shown promising initial results and might well become the first simplification strategies that are also effective in the long term. This treatment strategy is the first successful switch from triple drug containing cART to more simplified antiretroviral strategies since the failed dual therapy strategies in the 90s with NRTIs. The success of dual therapy strategies with INSTI and NRTI or NNRTI might origin from the ongoing inhibition of the reverse transcriptase step in the HIV replication

cycle, as also observed for PI dual therapy.[55–57] However, unfavorable immunological or reservoir characteristics might still interfere with maintenance of virological suppression by a DTG-containing dual antiretroviral regimen, illustrated by the GEMINI study where a subgroup analysis showed more treatment failure in patients with low CD4 T-lymphocyte count.[49] Although VF was rare, these factors should be taken into account in future research on, and implementation of, INSTI-based simplification strategies. Expanding DTG dual therapy strategies with boosted PI would be interesting and can also provide essential information about the necessity to inhibit the reverse transcriptase step to maintain adequate viral suppression rates. Furthermore, whether once daily DTG 50 mg in dual therapy strategies is sufficient in patients with resistance against ART classes, and the influence of dual therapies on reservoir evolution and inflammation, remain areas of research. It would be interesting to determine the most important reservoir sites for rebounding virus in individual patients and to improve methods to guarantee adequate intracellular antiretroviral drug-concentrations to stop replication. This may lead to less viral replication, less development of resistance, and improved virological suppression. However, these research and treatment goals seem to be particularly of interest for resource-rich settings. At this moment, DTG-containing dual therapy does not seem to be a good treatment option in the context of the 90-90-90 treatment goals in resource-poor countries with highly prevalent (transmitted) drug-resistance to (N)NRTI, less clinical and safety monitoring options, less drug-adherence, and a very high prevalence of *Mycobacterium tuberculosis* co-infections with a high risk of drug-drug-interactions and lower effective INSTI concentrations due to rifampicin based TB co-treatment.

With the expected inclusion of INSTI-containing dual therapy in guidelines as recommended HIV-treatment strategy, a unique era is approaching. After decades of studies that came to the conclusion that triple drug based cART should include treatment with multiple drug classes, resulting in varying virological efficacy, sometimes high pill burdens, drug-drug-interactions, and AE risks, the second generation INSTIs are the first class that can be used in clinical effective simplification strategies, without the risk of drug-drug-interactions that hindered PI simplification strategies. In the future, INSTI-containing dual therapy could very well become the cornerstone of HIV induction and maintenance treatment, while new monotherapy strategies and cure strategies are getting developed. One of these new strategies are new ways to administer cART, e.g. by injection. With prolonged adequate plasma drug-concentrations as a result of injection of antiviral drugs the antiretroviral effect in the cells likely will be guaranteed. Besides, injection of antiretrovirals will cause a further reduction in pill burden. Another interesting future option would be if we would be able to determine, using whole genome sequencing, the proportions of INSTI-resistant viruses and their localization in the reservoir, which would help to make an a priori estimation of the virological success of INSTI-containing cART. With periodical repeated assessment of localization, activity, and sequence analyses of the whole viral population in an individual patient, instead of the current

practice of plasma HIV-RNA monitoring, an increasing risk on VF may be better predicted and emergence of RAMs may be prevented prior to the occurrence of plasma viral rebound.

SAFETY OF INTEGRASE STRAND TRANSFER INHIBITORS

The second part of the discussion of this thesis focuses on the safety of initiation of INSTI-containing cART in vulnerable patients with the acquired immunodeficiency syndrome (AIDS). The fast HIV-RNA decline and immunological recovery that is associated with INSTI use, could promote the development of an immune reconstitution inflammatory syndrome (IRIS), especially in those with severely immunocompromised states or opportunistic infections (OI).[58–60] In **Chapter 6** we test the hypothesis that INSTI-containing cART, initiated during AIDS, increases the IRIS risk. In our cohort of 672 AIDS patients, we found that raltegravir (RAL), but not DTG or elvitegravir (EVG) initiation was associated with more IRIS development compared to non-INSTI regimens. Furthermore, patients initiating INSTI had more steroid exposure, but hospitalization rates and mortality were comparable to those who initiated non-INSTI regimens. Our findings are in line with another cohort study from a resource-rich setting among 2287 hospitalized AIDS patients, although this study was hampered by a limited follow up period, and no distinction between INSTI were made.[61] In another study of 417 patients, a similar pattern in those exposed to INSTI was observed. Notably, the use of DTG and EVG, but not RAL, increased IRIS risk which is in contrast to our study and might partially be explained by differences in IRIS definitions.[62] The findings from these cohort studies were, however, not reproduced in three randomized trials in which INSTI-containing cART was started in AIDS patients. Although IRIS risk was not the primary endpoint in these trials, nor were these studies designed to assess this risk, no higher incidence of IRIS after initiation of INSTI was reported in these studies. In the multifactorial REALITY-trial from sub-Sahara Africa, AIDS patients either received usual cART or intensified cART with RAL, next to other interventions including additional food or enhanced prophylaxis for OI. Despite a faster HIV-RNA decline in the RAL group, the all-cause and IRIS related mortality were not increased with RAL exposure.[63] In the INSPIRING-study, HIV/*Mycobacterium tuberculosis* co-infected patients were simultaneously treated with rifampicin-containing antimycobacterial therapy and DTG twice daily 50 mg or efavirenz (EFV) in combination with two NRTIs, and IRIS-rates were similar in both groups.[64] Also in the OPTIMAL-trial, initiation of INSTIs was not associated with an increased IRIS-risk.[65] Currently, the ADVANCE-trial, including 1110 HIV patients, is ongoing in a resource limited setting where a high proportion of AIDS patients can be expected and in which patients are randomized to DTG based regimens or TDF/emtricitabine (FTC)/EFV. This study started enrollment in 2017, and week 48 data are expected the first quarter of 2019.[66,67] Taken together, these trials do not provide evidence for

an increased IRIS risk with INSTI, but definite conclusions can nonetheless not be drawn due to the multifactorial designs and non-unified IRIS classifications.

The difference in IRIS risk between observational studies and randomized clinical trials is striking. It should be kept in mind however, that IRIS definitions varied between the studies and no uniform way to diagnose or study this heterogeneous disease is available.[68–70] Importantly, IRIS risk seems to differ between observational studies depending on type of INSTI used which hints on the difficulty of diagnosing IRIS, especially in observational and retrospective studies. Also, for specific OIs, additional IRIS-definitions were used by the investigators, which further increases the variability of IRIS definitions. Furthermore, confounding by indication in observational studies is likely a main confounder, which can divided in different considerations. Until recently, INSTI-containing cART was predominantly used in special situations. Particularly patients at a high risk of drug-drug-interactions initiated INSTI-containing cART, which is also the population that consists mainly of patients with mycobacterial infections, cancers, or other OIs.[71] From 2016 on, increasing proportions of patients initiated DTG-containing cART, as this was recommended by guidelines. This means that over time, the indication for INSTI-containing cART changed, as well as the type of INSTI initiated. This might reduce the IRIS-risk over time, and it also increases the IRIS-risk of RAL relatively to DTG. However, the awareness for IRIS as a consequence of initiation of INSTI-containing cART may have increased, which may have led to an increase in IRIS-diagnoses by clinicians. On the other hand, the fear for IRIS may have increased, which may have resulted in avoidance of INSTIs in certain patients. These three factors might interact, and the net influence on the observed IRIS risk in observational studies, including ours, cannot be specified. Observational studies cannot fully correct for these types of biases in multivariable models and as such, our data should be interpreted with caution.

Until future data show otherwise, the risk of IRIS should not restrict INSTI use in AIDS patients. In the future, it can be expected that the incidence of IRIS will decline in resource-rich countries with the developments in healthcare leading to a decrease in HIV late presenters. However, in resource-poor settings, the AIDS incidence remains high, as well as the burden of OIs. For these settings, awareness for any potential increased IRIS risk with INSTI use should remain high, especially in light of the limited availability of care facilities to treat this complication of antiretroviral treatment. Given the fact that IRIS is not an HIV-specific problem (it also occurs in transplant recipients who discontinue immunosuppressive medication), knowledge about the immunological pathophysiology might be gained from these areas and could be extrapolated to HIV infected patients. The determination of the specific pro-inflammatory mediators could contribute to the answer of the question whether the IRIS risk is increased during INSTI use, as the conclusions of cohort studies and clinical trials differ. Measuring IRIS-specific markers in HIV late presenters using either INSTI- or non-INSTI

containing cART, and relating them to clinical IRIS development, might help to distinguish between IRIS and other mechanisms responsible for clinical deterioration in a patient, and it would also help in solving the issue of heterogeneity of the IRIS syndrome.

After marketing of DTG, there is still an ongoing debate on neuropsychiatric AEs. The DOMONO study also provided insights regarding the association between neuropsychiatric AE and INSTIs, In the DOMONO study, 2% discontinued DTG for drug-related neuropsychiatric AEs, which is higher than observed in phase 3 registration trials. Other studies showed mixed signals: a meta-analysis of all phase 3 studies did not show an increased risk, but large cohort studies did also show an increased risk on neuropsychiatric AE. A representative prospective cohort-study including 1315 patients who discontinued INSTI-containing cART, showed an increased risk for patients using DTG to discontinue treatment because of neuropsychiatric AEs.[72,73] These studies demonstrate important differences between AEs in trials and in real-life, and the risk on neuropsychiatric AEs can therefore not be ignored. Further research is warranted on the occurrence and deterioration of well-monitored neuropsychiatric AEs in patients initiating INSTI-containing cART. Moreover, the underlying pathophysiological mechanism for INSTI-related neuropsychiatric AEs is unknown. In the future, it would be useful to determine neuropsychiatric substrates of INSTIs, for example neurotransmitter-concentrations in plasma or cerebrospinal fluid, or markers of immune activation which are known to be associated with neuropsychiatric symptoms. When these biomarkers could be determined, a screening test for the risk of neuropsychiatric AEs may be developed.

OPTIMIZING THE NRTI-BACKBONE

Despite all advances in safety, use of cART is still associated with toxicity in a considerable number of patients. For now, the use of one or two (N)NRTIs remains the cornerstones of cART, as was also illustrated by the results of our DTG monotherapy study. HIV-treatment guidelines therefore recommend treatment with an INSTI and either an ABC- or a tenofovir (TDF or tenofovir alafenamide fumarate (TAF)) containing NRTI-backbone. These backbones are unfortunately associated with specific toxicities. TDF is used worldwide as WHO recommended cART. Since TDF is also generically available, recommended as pre-exposure prophylaxis, and active against hepatitis B, its frequent use may still result in a significant number of patients with, predominantly renal, toxicity.[74,75] The optimal way to handle TDF-associated renal toxicity is unknown, but the availability of TAF broadens potential NRTI switch options. Part 3 of this thesis centers on safety aspects of TDF-containing cART and possibilities to further individualize treatment of an HIV-infected individual.

Inosine 5'-triphosphatase as predictor for TDF-associated nephrotoxicity

A way to prevent TDF-associated nephrotoxicity is to identify patient-related risk factors. In **Chapter 7**, the influence of *ITPA* genotype and Inosine 5'-triphosphatase (ITPase) activity on TDF-associated nephrotoxicity is determined. In this study, albeit the number of cases was limited, patients with TDF associated nephrotoxicity more frequently had a wildtype (wt)/wt *ITPA* genotype with normal ITPase activity. ITPase activity or *ITPA* genotype therefore might function as a screening- and prognostic tool for TDF-associated nephrotoxicity. In order to become a useful screening tool, several important issues need to be addressed. First, knowledge about ITPase activity related to HIV-treatment is sparse. ITPase is an enzyme involved in the purine metabolism, which is responsible for the formation of DNA, and involved in intracellular energy-metabolism. Apart from HIV, purine analogues are also frequently used in the treatment of malignancies, inflammatory bowel diseases, organ transplant recipients, and their cellular metabolism depends partially on ITPase activity.[76–78] HIV-infected individuals have a lower ITPase activity in their lymphocytes and erythrocytes, and the exact mechanism and effect of ITPase on the metabolism of various NRTIs is variable.[76,79] Also, ITPase activity seems to differ between different human tissues, and it is not known whether erythrocyte ITPase activity, which was measured in our study, is a good surrogate of activity in renal tubular cells.[80] Second, the definition of TDF-associated nephrotoxicity has to be more strict for successful screening. Also in our study, the interpretation of the results depends on the definition of TDF-associated nephrotoxicity, as we used a logical but non-validated definition leading to potential misclassification of patients with renal dysfunction that were actually not caused by TDF.[40] Third, the external validation of ITPase activity in predicting renal dysfunction and other TDF related toxicities (e.g. decreased BMD) in various populations is essential, including an optimal cut-off. Also cost-effectiveness analyses should be done to assess whether testing is beneficial. The remarkable extent of recovery after stopping TDF in patients with normal ITPase activity cannot fully be explained by the removal of TDF, given a natural irreversible eGFR-decline of 1 mL/min as a result of aging and a long duration of TDF-use in many patients. Another explanation might be that, besides being involved in intracellular oxidative stress, a normal functioning ITPase also catalyzes the hydrolyzation of 6-N-hydroxylaminopurine (HAP), which prevents incorporation of HAP in the DNA, which prevents the cellular DNA from HAP's mutagenic effects, and therefore catalyzes faster DNA-recovery.[81] A translation of this knowledge to the relation between *ITPA* genotype, ITPase activity, and use of TAF should also be made. It is important to gain more insight in factors influencing ITPase-activity, and the epidemiology of *ITPA* genotype and ITPase activity. The latter provides information about the proportion of HIV-infected individuals who do not develop NRTI-related AEs while having a wt/wt *ITPA* genotype and normal ITPase activity. This may contribute to the usefulness of ITPase as screeningstool for tenofovir-toxicity. However, even in patients already using TDF-containing cART, determination of ITPase activity could contribute to more individualized care. In patients using TDF,

serum and urine markers of renal function should be measured at least once a year to detect TDF-associated nephrotoxicity as early as possible. Patients with normal ITPase activity, in whom TDF is considered as part of antiretroviral therapy, should be informed about the increased risk on TDF-associated nephrotoxicity. Patients with reduced ITPase activity, who use TDF, should be monitored more frequently, for example twice a year, and when the first signals of nephrotoxicity occur, TDF should immediately be discontinued, as recovery of TDF-associated nephrotoxicity is less likely in those patients. In conclusion, ITPase activity might be useful in cases of TDF treatment or where TDF initiation is considered, but its exact place should be elucidated.

Recovery of TDF-associated nephrotoxicity

HIV-treatment guidelines advise to discontinue TDF in case of renal tubulopathy or evidence of decreased glomerular function, and to initiate ABC- or TAF-containing cART-regimens instead.[17,18] ABC and TAF in NRTI backbones of cART have comparable virological efficacy. However, direct comparative renal recovery analyses of ABC versus TAF in patients with TDF-associated nephrotoxicity are unavailable.[82] In **Chapter 8**, this knowledge gap has been studied and renal recovery rates after discontinuing TDF for renal toxicity are reported. This interim analysis showed that regardless of switching to ABC or TAF containing cART, the eGFR decline stabilizes. Furthermore, proteinuria decreased, virological suppression rates remained high, and the beneficial lipid effect of TDF waned similarly in both groups. Longer follow up should further strengthen these results.[83–88] These findings, especially in combination with ITPase findings, can contribute to further individualization of HIV-treatment.

Future perspectives on tenofovir-containing antiretroviral therapy

In the future, patients will likely be more in the lead of their own healthcare process, which could be an improvement for their dedication to their own health. Providing patients with exact knowledge about the advantages and risks of TDF, and other ART, and gaining more knowledge on factors involved in toxicity could help them and their health care professionals to make the best choices for their HIV-treatment. Patients at the lowest risk of VF and AE could be identified, and this knowledge could be valuable to tailor cART regimens for individual patients.

CONCLUDING REMARKS

The effectiveness and safety of new HIV treatment strategies including INSTIs and NRTI backbones in HIV infected individuals will likely remain important in the upcoming decades since the prospect of a cure is still unclear. This thesis helps to individualize patient care by showing what and how several first-line drugs, which will remain important in the upcoming

decade due to worldwide roll-out should be used, and which strategies should be avoided. The main conclusions are that:

i) DTG should not be used as maintenance monotherapy for viral efficacy, since INSTI-resistance acquired on DTG monotherapy is frequent and may be caused by mutations outside the integrase gene. The viral reservoir size and activity are likely to be useful as predictor for VF during simplification strategies.
ii) INSTI containing cART is safe in AIDS patients and not associated with an increased IRIS-related mortality risk.
iii) *ITPA* genotype and ITPase activity may be future biomarkers for toxicity during TDF use, and ABC or TAF can both be used for optimal renal recovery in cases of TDF-related nephrotoxicity.

Future research should remain focused on safe and efficacious HIV-treatment strategies especially with newer simplification regimens, development and effect of existing and new antiretroviral resistance mechanisms, and improve ways to predict virological success and toxicity on ART. These research goals contribute to more individualized care, which will further optimize HIV-treatment, until curing HIV is possible.

REFERENCES

1. Raffi F, Jaeger H, Quiros-Roldan E et al. Once-daily dolutegravir versus twice-daily raltegravir in antiretroviral-naive adults with HIV-1 infection (SPRING-2 study): 96 week results from a randomised, double-blind, non-inferiority trial. *Lancet HIV* 2013;**13**:927–935.
2. Molina JM, Clotet B, van Lunzen J et al. Once-daily dolutegravir versus darunavir plus ritonavir for treatment –naive adults with HIV-1 infection (FLAMINGO): 96 week results from a randomised, open-label, phase 3b study. *Lancet HIV* 2015;**2**:e127–e136.
3. Walmsley S, Baumgarten A, Berenguer J et al. Brief Report: Dolutegravir Plus Abacavir/Lamivudine for the Treatment of HIV-1 Infection in Antiretroviral Therapy-Naive Patients: Week 96 and Week 144 Results From the SINGLE Randomized Clinical Trial. *J Acquir Immune Defic Syndr* 2015;**70**:515–519.
4. Cahn P, Pozniak AL, Mingrone H et al. Dolutegravir versus raltegravir in antiretroviral-experienced, integrase-inhibitor-naive adults with HIV: week 48 results from the randomised, double-blind, non-inferiority SAILING study. *Lancet* 2013;**382**:700–708.
5. Castagna A, Maggiolo F, Penco G et al. Dolutegravir in antiretroviral-experienced patients with raltegravir- and/or elvitegravir-resistant HIV-1: 24-week results of the phase III VIKING-3 study. *J Infect Dis* 2014;**210**:354–362.
6. Wijting IEA, Rokx C, Boucher CAB et al. Dolutegravir as maintenance monotherapy for HIV (DOMONO): a phase 2, randomised, non-inferiority trial. *Lancet HIV* 2017;**4**:e547–e554.
7. Rokx C, Schurink CA, Boucher CAB, Rijnders BJA. Dolutegravir as maintenance monotherapy: first experiences in HIV-patients. *J Antimicrob Chemotherapy* 2016;**71**:1632–1636.
8. Gubavu C, Prazuck T, Niang M et al. Dolutegravir-based monotherapy or dual therapy maintains a high proportion of viral suppression even in highly experienced HIV-1-infected patients. *J Antimicrob Chemother* 2016;**71**:1046–1050.
9. Rojas J, Blanco JL, Marcos MA et al. Dolutegravir monotherapy in HIV-infected patients with sustained viral suppression. *J Antimicrob Chemother* 2016;**71**:1975–1981.
10. Katlama C, Soulié C, Caby F et al. Dolutegravir as monotherapy in HIV-1 infected individuals with suppressed HIV viremia. *J Antimicrob Chemother* 2016;**71**:2646–2650.
11. Oldenbuettel C, Wolf E, Ritter A et al. Dolutegravir monotherapy as treatment de-escalation in HIV-infected adults with virological control: DoluMono cohort results. *Antivir Ther* 2017;**22**:169–172.
12. Blanco JL, Rojas J, Paredes R et al. Dolutegravir-based maintenance monotherapy versus dual therapy with lamivudine: a planned 24 week analysis of the DOLAM randomized clinical trial. *J Antimicrob Chemother* 2018. DOI:10.1093/jac/dky093.
13. Stanford University Drug Resistance Database. Online available at https://hivdb.stanford.edu/.
14. Hocqueloux L, Allavena C, Prazuck T et al. Dolutegravir monotherapy versus dolutegravir/abacavir/lamivudine fomr HIV-1-infected virologically suppressed patients: results from the randomized non-inferiority MONCAY trial. Abstract TUAB0103, International AIDS Conference, July 218, Amsterdam, The Netherlands.
15. Braun DL, Turk T, Hampel B et al. Simplification to dolutegravir monotherapy is non-inferior compared to continuation of combination antiretroviral therapy in patients who initiated combination antiretroviral therapy during primary HIV infection: a randomized, controlled, non-inferiority trial. Abstract TUAB0102, International AIDS Conference, July 2018, Amsterdam, The Netherlands.
16. Blanco JL, Marcelin AG, Katlama C, Martinez E. Dolutegravir resistance mutations: lessons from monotherapy studies. *Curr Opin Infect Dis* 2018;**31**:237–245.
17. European AIDS Clinical Society Guidelines. European Guidelines for treatment of HIV-positive adults in Europe. Available at http://www.eacsociety.org/guidelines/eacs-guidelines/eacs-guidelines.html.

18. Panel on Antiretroviral Guidelines for Adults and Adolescents. Guidelines for the Use of Antiretroviral Agents in Adults and Adolescents Living with HIV. Department of Health and Human Services. Available at https://aidsinfo.nih.gov/contentfiles/lvguidelines/adultandadolescentgl.pdf.
19. Wijting IEA, Lungu C, Rijnders BJA et al. HIV-1 resistance dynamics in patients failing dolutegravir maintenance monotherapy. *J Infect Dis* 2018. DOI:10.1093/infdis/jiy176.
20. Malet I, Subra F, Charpentier C et al. Mutations located outside the integrase gene can confer resistance to HIV-1 integrase strand transfer inhibitors. *MBio* 2017. DOI:10.1128/mBio.00922-17.
21. Dicker IB, Samanta HK, Li Z et al. Changes to the HIV long terminal repeat and to HIV integrase differentially impact HIV integrase assembly, activity, and the binding of strand transfer inhibitors. *J Biol Chem* 2007;**282**:31186–31196.
22. Fun A, Wensing AM, Verheyen J, Nijhuis M. Human immunodeficiency virus gag and protease: partners in resistance. *Retrovirology* 2012;**9**:63.
23. Folks T, Powell DM, Lightfoote MM et al. Induction of HTLV-III/LAV from a nonvirus-producing T-cell line: implications for latency. *Science* 1986;**213**:600–602.
24. Lorenzo-Redondo R, Fryer HR, Bedford T et al. Persistent HIV-1 replication maintains the tissue reservoir during therapy. *Nature* 2016;**520**:51–56.
25. Finzi D, Hermankova M, Pierson T et al. Identification of a reservoir for HIV-1 in patients on highly active antiretroviral therapy. *Science* 1997;**278**:1295–1300.
26. Fletcher CV, Thorkelson A, Winchester L et al. Comparative lymphoid tissue pharmacokinetics (PK) of integrase inhibitors (INSTI). Abstract 27, Conference on Retroviruses and Opportunistic Infections 2018, Boston, MA, USA.
27. Lambert-Niclot S, Flandre P, Valantin MA et al. Factors associated with virological failure in HIV-1-infected patients receiving darunavir/ritonavir monotherapy. *J Infect Dis* 2011;**204**:1211–1216.
28. Rutsaert S, De Spiegelaere W, De Clercq L et al. HIV DNA as a Predictive Marker for Virologic Failure of Darunavir/r Monotherapy: A Substudy of the PROTEA Trial to Define a Cut-off for Success. Abstract PS6/2. European aids Clinical Society Conference, October 2017, Milan, Italy.
29. Torres-Cornejo A, Benmarzouk-Hidalgo OJ, Gutiérrez-Valencia A et al. Cellular HIV reservoir replenishment is not affected by blip or intermittent viremia episodes during darunavir/ritonavir monotherapy. *AIDS* 2014;**28**:201–208.
30. Lopez-Cortes LF, Ruiz-Valderas R, Sánchez-Rivas E et al. Lopinavir Plasma Concentration and Virological Outcome with Lopinavir-Ritonavir Monotherapy in HIV-1-Infected Patients. *Antimicrob Agents Chemother* 2013;**57**:3746–3751
31. Campo RE, Da Silva BA, Cotte L et al. Predictors of loss of virologic response in subjects who simplified to lopinavir/ritonavir monotherapy from lopinavir/ritonavir plus zidovudine/lamivudine. *AIDS Res Hum Retroviruses* 2009;**25**:269–275.
32. Boulassel MR, Chomont N, Pai NP, Gilmore N, Sékaly RP, Routy JP. CD4 T cell nadir independently predicts the magnitude of the HIV reservoir after prolonged suppressive antiretroviral therapy. *J Clin Virol* 2012;**53**:29–32.
33. Avettand-Fènoel V, Hocqueloux L, Ghosn J *et al*. Total HIV-1 DNA, a Marker of Viral Reservoir Dynamics with Clinical Implications. *Clin Microbiol Rev* 2016;**29**:859–880.
34. Avettand-Fènoël V, Hocqueloux L, Ghosn J et al. Total HIV-1 DNA, a marker of viral reservoir dynamics with clinical implications. *Clin Microbiol Rev* 2016;**29**:859–880.
35. Gandhi RT, McMahon DK, Bosch RJ et al. Levels of HIV-1 persistence on antiretroviral therapy are not associated with markers of inflammation or activation. *PLoS Pathog* 2017;**13**:e1006285.
36. Nordell AD, McKenna M, Borges AH *et al*. Severity of cardiovascular disease outcomes among patients with HIV is related to markers of inflammation and coagulation. *J Am Heart Assoc* 2014;**3**:e000844.

37. Borges AH, O'Connor JL, Philips AN *et al*. Factors associated with plasma IL-6 levels during HIV infection. *J Infect Dis* 2016;**212**:585–595.
38. Hall AM, Hendry BM, Nitsch D, Connolly JO. Tenofovir-associated kidney toxicity in HIV-infected patients: a review of the evidence. *Am J Kidney Dis.* 2011;**57**:773–780.
39. Kinai E, Hanabusa H. Progressive renal tubular dysfunction associated with long-term use of tenofovir DF. *AIDS Res Hum Retroviruses* 2009;**25**:387–394.
40. Rokx C, Alshangi H, Verbon A, Zietse R, Hoorn EJ, Rijnders BJ. Renal toxicity of Concomitant Exposure to Tenofovir and Inhibitors of Tenofovir's Renal Efflux Transporters in Patients Infected With HIV Type 1. *J Infect Dis* 2016;**213**:561–568.
41. Grant PM, Kitch D, McComsey GA *et al*. Long-term Bone Mineral Density Changes in Antiretroviral-Treated HIV-Infected Individuals. *J Infect Dis* 2016;**214**:607–611.
42. Santos JR, Saumoy M, Curran A *et al*. The lipid-lowering effect of tenofovir/emtricitabine: a randomized, crossover, double-blind, placebo-controlled trial. *Clin Infect Dis,* 2015;**61**:403–408.
43. Arribas JR, Thompson M, Sax PE *et al*. Brief Report: Randomized, Double-Blind Comparison of Tenofovir Alafenamide (TAF) vs Tenofovir Disoproxil Fumarate (TDF), Each Coformulated With Elvitegravir, Cobicistat, and Emtricitabine (E/C/F) for Initial HIV-1 Treatment: Week 144 Results. *J Acquir Immune Defic Syndr,* 2017;**75**:211–218.
44. Güerri-Fernández R, Molina-Morant D, Villar-García J et al. Bone density, microarchitecture and tissue quality after long-term treatment with tenofovir/emtricitabine or abacavir/lamivudine. *J Acquir Immune Defic Syndr.* 2017; DOI: 10.1097/QAI.0000000000001396.
45. Calmy A, Chevalley T, Delhumeau C et al. Long-term HIV infection and antiretroviral therapy are associated with bone microstructure alterations in premenopausal women. *Osteoporos Int,* 2013;**24**:1843-1852.
46. McCloskey EV, Odén A, Harvey NC et al. A Meta-Analysis of Trabecular Bone Score in Fracture Risk Prediction and Its relationship to FRAX. *J Bone Miner Res*, 2016; **31**: 940-948.
47. Serrano-Villar S, Sainz T, Lee SA *et al*. HIV-infected individuals with low CD4/CD8 ratio despite effective antiretroviral therapy exhibit altered T cell subsets, heightened CD8+ T cell activation, and increased risk of non-AIDS morbidity and mortality. *PLoS Pathog,* 2014;**10**:DOI 10.1371/journal.ppat.1004078.
48. Van den Dries L, Claassen MAA, Groothuismink ZMA, van Gorp E, Boonstra A. Immune activation in prolonged cART-suppressed HIV patients is comparable to that of healthy controls. *Virology* 2017;**509**:133–139.
49. Cahn P, Sierra Madero J, Arribas J et al. Non-inferior efficacy of dolutegravir (DTG) plus lamivudine (3TC) versus DTG plus tenofovir/emtricitabine (TDF/FTC) fixed-dose combination in antiretroviral treatment-naive adults with HIV-1 infection – 48-week results from the GEMINI studies. Abstract TUAB0106LB, International AIDS Conference, July 218, Amsterdam, The Netherlands.
50. Switch study to evaluate dolutegravir plus lamivudine in virologically suppressed human immunodeficiency virus type 1 positive adults (TANGO). https://www.clinicaltrials.gov, NCT03446573.
51. Llibre JM, Hung CC, Brinson C et al. Efficacy, safety, and tolerability of dolutegravir-rilpivirine for the maintenance of virological suppression in adults with HIV-1: phase 3, randomised, non-inferiority SWORD-1 and SWORD-2 studies. *Lancet* 2018;**391**:839–849.
52. Aboud M, Orkin C, Podzamczer D et al. Durable suppression 2 years after switch to DTG + RPV 2-drug regimen: SWORD-1 and SWORD-2 studies. Abstract THPEB047, International AIDS Conference, July 218, Amsterdam, The Netherlands.
53. Margolis DA, Brinson CC, Smith GHR et al. Cabotegravir plus rilpivirine, once a day, after induction with cabotegravir plus nucleoside reverse transcriptase inhibitors in antiretroviral-naïve adults

with HIV-1 infection (LATTE): a randomised, phase 2b, dose-ranging trial. *Lancet Infect Dis* 2015;**15**:1145–1155.

54. Margolis DA, Gonzalez-Garcia J, Stellbrink HJ et al. Long-acting intramuscular cabotegravir and rilpivirine in adults with HIV-1 infection (LATTE-2): 96-week results of a randomised, open-label, phase 2b, non-inferiority trial. *Lancet* 2017;**390**:1499–1510.
55. Arribas JR, Girard PM, Landman R et al. Dual treatment with lopinavir-ritonavir plus lamivudine versus triple treatment with lopinavir-ritonavir plus lamivudine or emtricitabine and a second nucleos(t)ide reverse transcriptase inhibitor for maintenance of HIV-1 viral suppression (OLE): a randomised, open-label, non-inferiority trial. *Lancet Infect Dis* 2015;**15**:785–792.
56. Perez-Molina JA, Rubio R, Rivero A et al. Dual treatment with atazanavir-ritonavir plus lamivudine versus triple treatment with atazanavir-ritonavir plus two nucleos(t)ides in virologically stable patients with HIV-1 (SALT): 48 week results from a randomised, open-label, non-inferiority trial. *Lancet Infect Dis* 2015;**15**:775–784.
57. Cahn P, Andrade-Villanueva J, Arribas JR et al. Dual theray with lopinavir and ritonavir plus lamivudine versus triple therapy with lopinavir and ritonavir plus two nucleoside reverse transcriptase inhibitors in antiretroviral-therapy-naive adults with HIV-1 infection: week 48 results of the randomised, open label, non-inferiority GARDEL trial. *Lancet Infect Dis* 2014;**14**:572–580.
58. Grant PM, Komarow L, Andersen J et al. Risk factor analyses for immune reconstitution inflammatory syndrome in a randomized study of early vs. deferred ART during an opportunistic infection. *PLoS One.* 2010; DOI 10.1371/journal.pone.0011416.
59. Valin N, Pacanowski J, Denoeud L et al. Risk factors for 'unmasking immune reconstitution inflammatory syndrome' presentation of tuberculosis following combination antiretroviral therapy initiation in HIV-infected patients. *AIDS.* 2010; **24**: 1519–1525.
60. Manabe YC, Campbell JD, Sydnor E, Moore RD. Immune reconstitution inflammatory syndrome: risk factors and treatment implications. *J Acquir Immune Defic Syndr* 2007; **46**: 456–462.
61. Dutertre M, Cuzin L, Demonchy E et al. Initiation of antiretroviral therapy containing integrase inhibitors increases the risk of IRIS requiring hospitalization. *J Acquir Immune Defic Syndr* 2017;**76**:e23–e26.
62. Psichogiou M, Basoulis D, Tsikala-Vafea M et al. Integrase strand transfer inhibitors and the emergence of immune reconstitution inflammatory syndrome (IRIS). *Curr HIV Res* 2017;**15**:405–410.
63. Gibb D, Szubert AJ, Chidziva E et al. Impact of raltegravir intensification of first-line ART on IRIS in the REALITY trial. Abstract 23, Conference on Retroviruses and Opportunistic Infections 2018, Boston, MA, USA.
64. Dooley K, Kaplan R, Mwelase T et al. Safety and efficacy of dolutegravir-based ART in TB/HIV co-infected adults at week 48. Abstract TUAB0206, International AIDS Conference, July 218, Amsterdam, The Netherlands.
65. Lelievre JD, Assoumou L, Aznar E et al. Are integrase inhibitors a risk factor for IRIS in the ANRS 146 OPTIMAL trial? Abstract 495, Conference on Retroviruses and Opportunistic Infections 2018, Boston, MA, USA.
66. Venter WDF, Clayden P, Serenata C, OPTIMIZE Consortium. The ADVANCE study: a groundbreaking trial to evaluate a candidate universal antiretroviral regimen. *Curr Opin HIV AIDS* 2017;**12**:351–354.
67. Study record details of ADVANCE-trial in ClinicalTrials.gov. Available at https://clinicaltrials.gov/ct2/show/record/NCT03122262. Accessed at 06/08/2018.
68. Meintjes G, Boulle A. Immune reconstitution inflammatory syndrome in a large multicenter cohort study: case definition and comparability. *Expert Rev Anti Infect Ther* 2012;**10**:737–741.

69. French MA, Price P, Stone SE. Immune restoration disease after antiretroviral therapy. *AIDS* 2004;**18**:1615–1627.
70. Robertson J, Meier M, Wall J et al. Immune reconstitution syndrome in HIV: validating a case definition and identifying clinical predictors in persons initiating antiretroviral therapy. *Clin Infect Dis* 2006;**42**:1639–1646.
71. Panel on Opportunistic Infections in HIV-infected Adults and Adolescents. Guidelines for the prevention and treatment of opportunistic infections in HIV-infected adults and adolescents: recommendations from the Centers for Disease Control and Prevention, the National Institutes of Health, and the HIV Medicine Association of the Infectious Diseases Society of America. Available at https://aidsinfo.nih.gov/contentfiles/lvguidelines/adult_oi.pdf.
72. van Wyk J, Oyee J, Barthel S et al. Variables associated with neuropsychiatric symptoms in PLWH receiving dolutegravir based therapy in phase III clinical trials. Abstract TUPDB0102, International AIDS Conference, July 218, Amsterdam, The Netherlands.
73. Cuzin L, Pugliese P, Katlama C et al. Integrase inhibitors and neuropsychiatric adverse events in a large prospective cohort. Abstract TUPDB0107, International AIDS Conference, July 218, Amsterdam, The Netherlands.
74. Panel on Antiretroviral Guidelines for Adults and Adolescents. Guidelines for the Use of Antiretroviral Agents in Adults and Adolescents Living with HIV. Department of Health and Human Services. Available at http://www.aidsinfo.nih.gov/ContentFiles/ AdultandAdolescentGL.pdf.
75. Center for Disease Control and Prevention: US Public Health Service: Preexposure prophylaxis for the prevention of HIV infection in the United States–2017 Update: a clinical practice guideline. https://www.cdc.gov/hiv/pdf/risk/prep/cdc-hiv-prep-guidelines-2017.pdf. Published March 2018.
76. Peltenburg NC, Bierau J, Bakker JA et al. Erythrocyte inosine triphosphatase activity: a potential biomarker for adverse events during combination antiretroviral therapy for HIV. *PLoS One* 2018;**13**:e0191069.
77. Moon W, Loftus EV Jr. Review article: recent advances in pharmacogenetics and pharmacokinetics for safe and effective thiopurine therapy in inflammatory bowel disease. *Aliment Pharmacol Ther* 2016;**43**:863–883.
78. Burgis NE. A disease spectrum for ITPA variation: advances in biochemical and clinical research. *J Biomed Sci* 2016;**23**:73.
79. Peltenburg NC, Bierau J, Bakker JA et al. Inosine triphosphate pyrophosphohydrolase expression: decreased in leukocytes of HIV-infected patients using combination antiretroviral therapy. *J Acquir Immune Defic Syndr* 2016;**73**:390–395.
80. Holmes SL, Turner BM, Hirschhorn K. Human inosine triphosphatase: catalytic properties and population studies. *Clin Chim Acta* 1979;**97**:143–153.
81. Galperin MY, Moroz OV, Wilson KS, Murzin AG. House cleaning, a part of good housekeeping. *Mol Microbiol* 2006;**59**:5–19.
82. Winston A, Post FA, DeJesus E et al. Tenofovir alafenamide plus emtricitabine versus abacavir plus lamivudine for treatment of virologically suppressed HIV-1-infected adults: a randomised, double-blind, active-controlled, non-inferiority phase 3 trial. *Lancet HIV* 2018;**5**:e162–e171.
83. Jose S, Hamzah L, Campbell LJ et al. Incomplete reversibility of estimated glomerular filtration rate decline following tenofovir disoproxil fumarate exposure. *J Infect Dis* 2014;**210**:363–373.
84. Bonjoch A, Echeverria P, Perez-Alvarez N et al. Prospective study to assess progression of renal markers after interruption of tenofovir due to nephrotoxicity. *Biomed Res Int* 2016;**2016**:4380845.

85. Bonjoch A, Echeverria P, Perez-Alvarez N et al. High rate of reversibility of renal damage in a cohort of HIV-infected patients receiving tenofovir-containing antiretroviral therapy. *Antiviral Res* 2012;**96**:65–69.
86. Cha A, Besignano-Long AR, Rothberger N, Shah B. Reversibility of renal dysfunction after discontinuation of tenofovir. *J Am Pharm Assoc* 2016;**56**:280–283.
87. Young J, Wang Q, Fux CA et al. The rate of recovery in renal function when patients with HIV infection discontinue treatment with tenofovir. *HIV Med* 2014;**15**:505–510.
88. Yoshino M, Yagura H, Kushida H et al. Assessing recovery of renal function after tenofovir disoproxil discontinuation. *J Infect Chemother* 2012;**18**:169–174.