HIV
Treatment
Strategies

The Role of Integrase
Strand Transfer Inhibitors

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HIV behandelstrategieën – de rol van integraseremmers

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General introduction and outline of the thesis

INTRODUCTION IN TREATMENT OF HIV-1

The rationale for immediate treatment initiation in HIV-1 infection

By initiating combination antiretroviral therapy (cART) early after the HIV diagnosis, almost all individuals achieve a suppressed plasma HIV viral load. This prevents the progression to an immunodeficient state due to loss and dysfunction of CD4 T-lymphocytes and, eventually, the Acquired Immune Deficiency Syndrome (AIDS).[1-5] The START and the TEMPRANO studies provided the important insight that immediate initiation of cART after the HIV diagnosis, irrespective of CD4 T-lymphocyte counts, results in a better outcome than deferring cART initiation until CD4 T-lymphocytes drop below 350 cells/mm³. This benefit is not only the consequence of a reduction in AIDS-related morbidity and mortality, but also of a reduction in non-AIDS-related morbidity and mortality.[6-8] These landmark studies led to the universal recommendations to initiate cART immediately after an HIV-diagnosis, regardless of CD4 T-lymphocytes count, which is in contrast with the previous recommendation to start cART below certain thresholds of CD4 T-lymphocytes.[9] Apart from the health benefit for the HIV infected patient, uninfected individuals benefit from treatment of HIV as well. People with a suppressed plasma HIV viral load as the consequence of cART have a negligible chance of transmitting HIV by sexual or vertical (mother to child, in utero, or peripartum or postpartum) transmission.[10-12] Thus, cART prevents development of AIDS and death, diminishes loss of immunity, and protects HIV uninfected individuals. The earlier cART is initiated during the course of an HIV-infection, the more advantageous it is expected to be.

The HIV replication cycle and antiretroviral resistance

HIV is an RNA lentivirus, belonging to the retrovirus subfamily. These viruses are characterized by the need of reverse transcription of viral RNA to synthesize viral DNA, which is part of their replication cycle. The replication cycle of HIV starts with binding of viral glycoprotein 120 (Gp120) to the surface of the CD4 T-lymphocyte. Thereafter, transformation of Gp120 enables binding of the virus to a co-receptor on the CD4 T-lymphocyte: the CCR5-receptor or the CXCR4-receptor. This enables viral Gp41 to fuse with the cell membrane, which is followed by the release of viral RNA and viral enzymes into the cytoplasm. After the formation of double-stranded HIV-DNA catalyzed by reverse transcriptase (RT), viral DNA, viral proteins, and host factors enter the nucleus. This so-called pre-integration complex (PIC) is integrated into the host DNA by the viral enzyme integrase. Transcription of this integrated HIV-DNA results in the formation of unspliced RNA or messenger RNA (mRNA), which then enters the cellular cytoplasm. The mRNA encodes for viral structural proteins and enzymes. Together with unspliced viral RNA, these proteins form a new free viral particle after successful assembly and detachment from the cell surface. Viral protease helps maturing the viral particle in order to be able to infect another cell.[13] Non-mutated viral strains, which are called wildtype viral strains, are considered to have the highest competence

to complete their replication cycle. The competence to complete the replication cycle is also called viral fitness. Viral fitness often becomes reduced when the virus mutates, compared to wildtype viral strains ('wildtype').[14] Development of mutations in the genome, causing resistance, is an important survival-mechanism of HIV to evade antiretroviral drugs. When a viral strain is susceptible to a specific antiretroviral agent, suppression of plasma HIV-RNA is the result from interruption of the HIV replication cycle by that antiviral agent. Resistance means that mutations lead to suboptimal interruption of the HIV replication cycle by an anti-retroviral drug, compared to wildtype virus at similar concentrations of cART. A distinction can be made between phenotypic and genotypic resistance. Genotypic resistance refers to the presence of resistance associated mutations (RAMs), whereas phenotypic resistance refers to the drug susceptibility of the virus by determining the concentration of an antiretroviral agent that inhibits viral replication. There are two mechanisms responsible for development of RAMs causing treatment failure: i) the rapid viral replication with the error prone reverse transcriptase-step causes random mutations which can be RAMs resulting in clinical sig-nificant resistance against cART even before treatment initiation and ii) RAMs that develop during cART, especially in settings with inadequate drug levels. The underlying mechanism of treatment failure is of importance for the selection of an adequate antiretroviral regimen. If the resistance to antiretroviral drugs is transmitted, the only possibility to achieve viral suppression is to initiate a cART-regimen consisting of antiretroviral agents with full antiviral activity. If the virus obtained mutations leading to antiretroviral resistance, the combination of an increased dose of the antiretroviral drug and the other antiretroviral drugs may result in viral resuppression.[15]

Antiretroviral drugs and treatment targets
Worldwide, five HIV treatment guidelines are commonly used.[16–20] In general, six classes of antiretroviral drugs are available: CCR5 antagonists, fusion inhibitors (FI), nucleoside reverse transcriptase inhibitors (NRTI), non-nucleoside reverse transcriptase inhibitors (NNRTI), integrase strand transfer inhibitors (INSTI), and protease inhibitors (PI). Figure 1 shows the HIV-1 replication cycle, including the different drug targets and antiretroviral drug classes. The different classes of antiretroviral drugs, including the individual drugs, are listed in Table 1. Only those that are in use in the Netherlands are provided. Table 2 provides an overview of the different recommended first-line antiretroviral treatment-regimens in five commonly used HIV treatment guidelines.
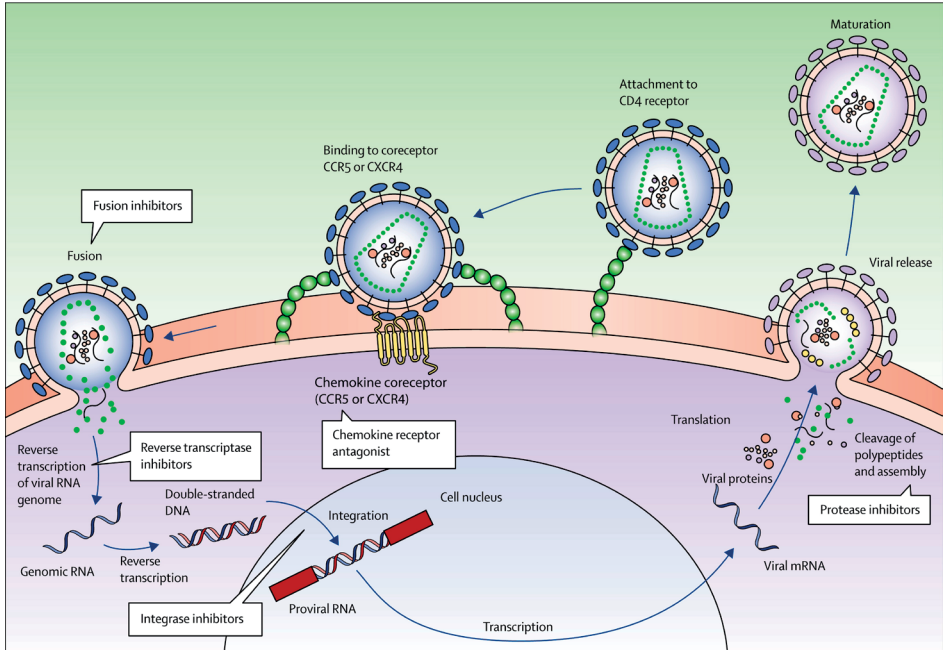
Figure 1. HIV-1 replication cycle. The white boxes indicate antiretroviral drug targets and accessory antiretroviral drug classes. Reproduced with permission from Walker et al.[21]

Drug class (abbreviation)	Name	Abbreviation
CCR5 antagonist	Maraviroc	MVC
Fusion inhibitor (FI)	Enfuvirtide	T20
Nucleoside reverse transcriptase inhibitor (NRTI)	Abacavir	ABC
	Didanosine	ddI
	Emtricitabine	FTC
	Lamivudine	3TC
	Tenofovir disoproxil fumarate	TDF
	Tenofovir alafenamide fumarate	TAF
	Zidovudine	ZDV
Non-nucleoside reverse transcriptase inhibitor (NNRTI)	Efavirenz	EFV
	Etravirine	ETV
	Nevirapine	NVP
	Rilpivirine	RPV
Integrase strand transfer inhibitor (INSTI)	Bictegravir	BIC
	Dolutegravir	DTG
	Elvitegravir	EVG
	Raltegravir	RAL
	Cabotegravir	CAB
Protease inhibitor (PI)	Atazanavir	ATV
	Darunavir	DRV
	Fosamprenavir	FPV
	Indinavir	IDV
	Lopinavir	LPV
	Nelfinavir	NFV
	Saquinavir	SQV
	Tipranavir	TPV
Pharmacoenhancer	Cobicistat	/c or COBI
	Ritonavir$^\$$	/r or RTV

Table 1. Available antiretroviral drugs for treatment of HIV. $^\$$ Ritonavir is a protease inihibitor, which was initially used as antiretroviral drug, but currently only is used as pharmacoenhancer.

In general, all guidelines recommend to initiate cART consisting of two NRTIs combined with a third agent, either an NNRTI, a PI boosted with a pharmacoenhancer, or an INSTI. Boosters inhibit the activity of cytochrome P450 (CYP450) enzymes which metabolize PI and EVG. This increases the plasma levels of PIs and EVG and reduces the necessary doses needed to achieve adequate plasma levels for virological suppression. The PI RTV differs from COBI because, unlike COBI, it is a booster with antiretroviral activity, especially when given in a therapeutic dosage. RTV also has an increased risk for gastro-intestinal side effects, dyslipidemia, and it inhibits multiple CYP450 coenzymes, whereas COBI is a more spe-

cific CYP450 inhibitor.[22] In contrast to RTV, COBI is an inhibitor of renal tubular creatinine excretion, by inhibition of tubular creatinine-transporter MATE-1, causing increased serum creatinine without truly affecting renal or glomerular function.[23]

Differences exist between HIV treatment guidelines. Whereas the American guideline DHHS recommends to initiate INSTI-containing cART-regimens as first line, the European guideline EACS recommends other non-INSTI regimens as well. Both the American and European guidelines make no distinction between ABC/3TC, TAF/FTC, or TDF/FTC as NRTI-backbone. However, when peak HIV-RNA is above 100.000 copies/mL (c/mL) and ATV/r or c, EFV, and RPV is considered as third agent, an ABC-containing backbone is not the preferred option in both guidelines. In resource-poor countries, the WHO-guideline is mostly used, which recommends an NNRTI-containing regimen, or DTG combined with NRTI backbones as alternative regimens. The limited availability of TAF and INSTI in resource-limited countries explains the differences between the WHO-guideline and the American and European Guidelines[16–20]

Guideline	Drug classes	Drugs
Department of Health and Human Services (DHHS)[16]	2 NRTIs + INSTI	ABC/3TC/DTG TAF/FTC/BIC TAF/FTC/DTG or TDF/FTC/DTG TAF/FTC/RAL or TDF/FTC/RAL
World Health Organisation (WHO)[17]	2 NRTIs + NNRTI (preferred)	TDF/FTC/EFV or TDF/3TC/EFV
	2 NRTIs + NNRTI (alternative)	TDF/FTC/NVP or TDF/3TC/NVP ZDV/3TC/EFV or ZDV/3TC/NVP
	2 NRTIs + INSTI (alternative)	TDF/FTC/DTG or TDF/3TC/DTG
European AIDS Clinical Society (EACS)[18]	2 NRTIs + INSTI	ABC/3TC/DTG TAF/FTC/BIC TAF/FTC/DTG or TDF/FTC/DTG TAF/FTC/EVG/c or TDF/FTC/EVG/c TAF/FTC/RAL or TDF/FTC/RAL
	2 NRTIs + NNRTI	TAF/FTC/RPV or TDF/FTC/RPV
	2 NRTIs + PI/r or PI/c	TAF/FTC/DRV/c or /r or TDF/FTC/DRV/c or /r
British HIV Association[19]	2 NRTIs + INSTI	TAF/FTC/DTG or TDF/FTC/DTG TAF/FTC/EVG/c or TDF/FTC/EVG/c TAF/FTC/RAL or TDF/FTC/RAL
	2 NRTIs + NNRTI	TAF/FTC/RPV or TDF/FTC/RPV
	2 NRTIs + PI/r	TAF/FTC/DRV/r or TDF/FTC/DRV/r TAF/FTC/ATV/r or TDF/FTC/ATV/r
International Antiviral Society-USA (IAS-USA)[20]	2 NRTIs + INSTI	ABC/3TC/DTG TAF/FTC/BIC TAF/FTC/DTG

Table 2. Overview of recommended first-line antiretroviral treatment-regimens in different HIV treatment guidelines. For the abbreviations, see Table 1.

Of course, the choice of which cART-regimen is initiated is a multifactorial decision of an HIV-treating physician with an individual patient, in which co-infections, comorbidity, sex (in relation to potential future pregnancies), use of concomitant medication, costs, and behavioral aspects should be taken into account. However, INSTI have become preferential agents for cART in resource-rich countries. An important advantage of initiating INSTI-containing cART is the very low risk of transmitted INSTI resistance mutations in the integrase gene, affecting INSTI efficacy, both on the viral population level and in viral subpopulations.[24] This contrasts with the prevalence of transmitted NRTI and NNRTI resistance.[25,26] Therefore, INSTI-containing cART could be initiated soon after HIV-diagnosis, unless there are risk factors for transmitted INSTI-resistance.[16,18,20] Additionally, the low risk of drug-drug-interactions and adverse events (AE), and the high genetic barrier against the development

of resistance of the second generation INSTIs (all discussed in detail later) further favor the recommendation to initiate INSTI-containing cART.[27–29] Since 2016, the consensus of HIV-treatment guidelines for resource-rich countries is to initiate INSTI-based regimens in cART-naive HIV-infected individuals, and this was followed by a worldwide uptake of INSTI based first-line treatments, in resource-rich countries.[16–20]

HIV treatment strategies and treatment simplification – a historical perspective

From the mid-80s until the early 2000s, studies investigating the virological efficacy of a new single NRTI or NNRTI therapy were conducted in cART naive patients, to evaluate the potency of these drugs.[30–32] Unfortunately, within a period of weeks, resistance associated mutations emerged with NRTI and NNRTI monotherapies.[30,32,33] Subsequent studies on dual and triple cART showed more durable virological responses without RAMs in most patients, although VF still occurred.[34–39] Preservation of virological suppression with cART improved when cART consisted of two NRTIs combined with an NNRTI, PI, or more recently IN-STI.[40–42]

Definitions

The main rationale behind the treatment of HIV infected individuals with cART consisting of three antiretroviral drugs targeting at least two targets in the HIV replication cycle (triple cART) is to suppress viral replication as efficacious as possible, to induce a sustained plasma viral suppression, and to minimize the risk of virological failure (VF) by the development of resistance. Different definitions of VF have been used. In phase 3 studies, virological suppression has often been defined as a plasma HIV-RNA below c/mL, although guidelines consider virological suppression as an undetectable plasma HIV-RNA, which means that the HIV-RNA has to be below the limit of detection of the local laboratory thresholds. These thresholds vary between 20 and 50 c/mL. Detectable plasma HIV-RNA may lead to development of RAMs, leading to a reduced susceptibility of the virus to that agent, which is permanent, even after resuppression of the virus with other agents. The consequence of RAMs is, that also in the future, treatment with the particular agent is not possible. Sometimes, a temporary increase of plasma HIV-RNA during use of antiretroviral therapy occurs, a blip or viral rebound. A blip is usually defined as a temporary increase of the plasma viral load from undetectable to 50 to 200 c/mL. A viral rebound means a substantial amount of detectable plasma HIV-RNA, and the virus might spontaneously get resuppressed again. Although the effects of viral rebounds below 20.000 c/mL on the risk of development of an AIDS-defining illness are shown to be small, low-level viremia is a risk factor for future VF and viremia is associated with elevated pro-inflammatory markers.[43–45]

Simplification of HIV treatment has always been an important subject of research. Simplification can consist of the reduction in the number of pills and/or the number of antiretroviral

agents. However, reasons to simplify therapy to reduce AE, pill burden, or costs, should not or only minimally increase the risk of VF. A decade after the introduction of triple cART in 1996, the concept of simplification of cART was investigated with PI monotherapy, because PIs have a higher genetic barrier against resistance than NRTIs and NNRTIs.[46–48] The results of the first of these PI-monotherapy studies were promising, but larger studies eventually showed that PI monotherapy was not the golden bullet. A landmark-study on PI monotherapy is the PIVOT-study.[49] In this open-label non-inferiority study, 587 patients were randomized to continuation of cART or to a switch to PI monotherapy, either DRV/r or LPV/r. The patients were suppressed (plasma HIV-RNA below 50 c/mL) on triple cART with NNRTI or PI plus 2 NRTI, and they had a CD4 T-lymphocyte count higher than 100 cells/mm^3. Furthermore, they had no PI RAMs or previous VF, which means that their virus should have had optimal susceptibility to PIs, and that patients were likely to be adherent. These characteristics are considered essential for simplified antiretroviral regimens to work. VF during the study was defined as three consecutive HIV-RNA measurements higher than 50 c/mL, of which the second one was one week after the first measurement, and the third measurement was four weeks after the first one. The primary endpoint of the study was loss of future cART treatment options after three years of follow-up. This was defined as acquired resistance to the PI. After a median follow-up of 44 months, 2/291 patients in the cART-group and 6/296 patients in the PI monotherapy group lost future treatment options, which proved non-inferiority of the PI-monotherapy strategy. In 8/291 patients in the cART-group, but in 95/296 patients in the PI monotherapy group, plasma HIV-RNA became detectable again at least once. This viral rebound in the PI-group often occurred in the first year, and in all patients with available follow-up, spontaneous resuppression on monotherapy or resuppression after the addition of two NRTIs (which means re-initiation of cART) occurred. In conclusion, this study showed that in the setting of a clinical trial, PI monotherapy as a simplification strategy in patients who are suppressed on cART rarely leads to the loss of future treatment options, because even if a viral rebound occurs, viral resuppression occurs in almost all patients. However, the effect of viral rebounds on the inflammation markers and the risk on future VF were not discussed. Furthermore, the very frequent VL monitoring, which was part of the study protocol, makes it difficult to extrapolate the study results to real-life, as patients typically have their VL monitored twice a year when they are stable on cART. A number of other randomized clinical trials was performed to compare the virological efficacy of boosted PI maintenance monotherapy with PI-containing triple cART, and in 2015 a meta-analysis was published. This study analyzed 13 randomized clinical trials, investigating PI maintenance monotherapy versus triple cART in patients with suppressed HIV-RNA prior to PI monotherapy initiation and included a total of 2303 patients. Patients switched to monotherapy with DRV/r (4 studies, N=784), LPV/r (7 studies, N=829), ATV/r (1 study, N=103), or were allowed to switch to one of the three mentioned PIs (PIVOT study, N=587). The authors considered a switch from monotherapy to triple therapy as treatment failure, and the absolute difference in percentages

of treatment failure between PI monotherapy and cART was -8.3% (73.9 versus 82.0%, 95% confidence interval (CI) -4.8% to -11.9%), and was statistically significant (p<0.0001). In a switch-included-analysis, in which a confirmed elevation of HIV-RNA higher than 50 c/mL was defined as treatment failure, but intensification of PI monotherapy to cART was not, no significant difference in virological suppression was observed. Furthermore, the risk of development of any NRTI or PI RAMs was low (1.3% in PI monotherapy and 0.6% in cART) and comparable between groups. However, the results of the PIVOT trial were not included in this resistance analysis, as they were not yet available.[50] The results of this meta-analysis were therefore not convincingly in favor of PI monotherapy nor against the use of PI monotherapy. Disadvantages which hampered the global introduction of PI monotherapy were concerns regarding insufficient virological suppression, the fact that boosted PIs monotherapy still meant two or more large pills, the occurrence of AEs, and the potential for drug-drug-interactions. However, the concept of monotherapy remained appealing, and with the introduction of INSTIs this strategy was reconsidered. INSTIs have better tolerability and less drug-drug-interactions than PIs. In 2013, the second-generation INSTI DTG became available. With the availability of DTG with its high genetic barrier to resistance, new possibilities regarding treatment-simplification maintenance therapy became available. To provide insights in the favorable properties of INSTIs, and differences between drugs within this antiretroviral class, the INSTIs are described in more detail in the next chapter.

VIRAL INTEGRASE AND INTEGRASE STRAND TRANSFER INHIBITORS

Viral integrase

A pivotal step in the replication cycle of the retrovirus HIV is the integration of HIV-DNA in the host DNA of infected cells. After integration, transcription initiation of viral genes results in mRNA and production of viral proteins and eventually new viral particles that can infect other cells. The viral enzyme integrase enables integration of the proviral DNA into the host DNA. Integrase is a 32kDa amino-acid, encoded by the viral *pol* gene. It is produced as part of the *gag-pol* polypeptide precursor by viral protease.[51] Integrase acts as multimer, usually as dimer, which consists of two integrase-proteins, or as tetramer, which consists of four integrase-proteins. It has three functional domains:[52–58] i) the N-terminal domain, which consists of amino acids 1-46. The exact function of the N-terminal domain in the integration-process has currently not been fully elucidated. However, the domain carries a HHCC motif (because of the His and Cys residues it contains), and the HHCC motif is necessary for binding of the N-terminal domain to zinc, which is subsequently needed for optimal 3'-processing and the strand transfer step. The N-terminal domain is also involved in formation of integrase-multimers (multimerization). ii) The central core domain, which consists of amino acids 56-212. The central core domain of integrase is considered the most important

part of the enzyme. The central core domain encompasses a D,D-35-E motif (responsible for the arrangement of the D64, D116, and E152 acidic amino acid residues)[59], which is pivotal for the binding of integrase to the host DNA and the catalytic activity of the integrase process. The central core domain contains two divalent cations (often magnesium (Mg^{2+}) or manganese (Mn^{2+})), which are needed as cofactor for 3'-processing and to destabilize target DNA for integration.[60] Furthermore, the core domain is also involved in multimerization, and it promotes the disintegration process (see below). iii) The C-terminal domain, which consists of amino acids 195-288. The C-terminal domain also is involved in multimerization, it binds to random localizations in the DNA, and is mainly responsible for the stability of the viral-host-DNA-complex.[61] Figure 2 shows a schematic overview of the structure of the HIV-1 integrase.[62]
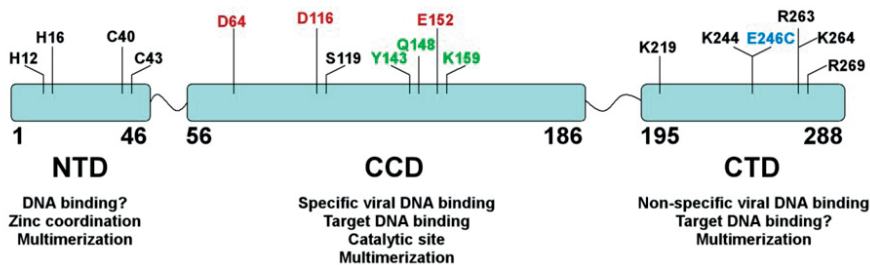
Figure 2. Schematic presentation of the structure of HIV-1 integrase. NTD=N-terminal domain; CCD=catalytic core domain; CTD=C-terminal domain. Reproduced with permission from Kessl et al.[62]

Integration of viral DNA into host DNA

Integration of viral DNA into host DNA is a two-step mechanism. First, integrase binds to both ends of the viral DNA, the long terminal repeats (LTR), forming the PIC. After binding to the LTR, integrase catalyzes a process of cleavage of two nucleotides from each 3'-end of the viral DNA. This is known as 3'-processing. After this step, the DNA is integrated into the DNA of the host cell by a strand transfer step, which is characterized by 'cuts' in the host DNA, creating 5'-protrusions, followed by binding of the viral 3'-ends and the host 5'-ends. It is likely that the position at which the viral DNA integrates into the host DNA is not random, but that it preferentially integrates into active transcription sites, in order to promote viral gene expression after integration.[63] The integration is completed by removal of the unpaired nucleotides at the 5'-ends of the viral DNA and repair of single gaps between viral and host DNA. The whole process of integration-strand-transfer is accompanied *in vitro* with the step of disintegration, which seems not to occur *in vivo*. This step is characterized by a reversed strand transfer step, resulting in the release of viral DNA from the host DNA and repair of the host DNA strand.[51, 57, 64] The exact role of the disintegration-step has to be further elucidated *in vivo*. The whole process of integration of HIV DNA into host DNA is presented schematically in Figure 3.[65]
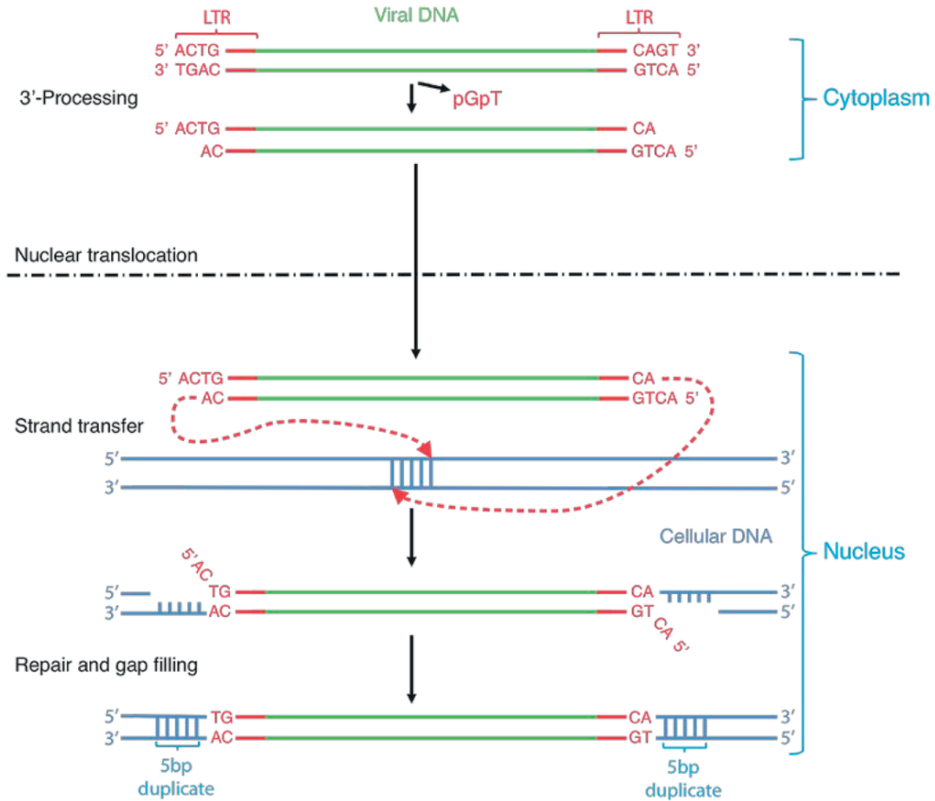
Figure 3. Schematic overview of integration of viral DNA into the host DNA. Reproduced with permission from Métifiot et al.[65]

Integrase strand transfer inhibitors

The antiretroviral effect of INSTIs is the result of inhibition of the strand-transfer step. INSTIs do not influence the binding between integrase and viral DNA and 3'-processing, but they bind to the PIC and subsequently inhibit the strand transfer reaction. The unique property of INSTIs compared to other antiretroviral agents is that both viral integrase and viral pre-integrated DNA are needed as a complex for the INSTIs to bind to, as INSTIs cannot bind to one of those factors individually.[66] The binding of INSTIs results both in cleavage of Mg^{2+}, which inactivates integrase's catalytic function, and to displacement of the 3'-hydroxyl end of the viral DNA-strands.[60,67] In conclusion, the inhibition of the strand transfer step by INSTIs is the result of i) their binding to the viral integrase, with ii) concomitant binding to the 3'LTR region of viral DNA, resulting in iii) inhibition of PIC-host DNA complex formation. All INSTIs have two components of major importance: a metal-binding part which cleaves the Mg^{2+}-ions of integrase, and a hydrophobic part which interacts with the viral DNA and the integrase. Especially the hydrophobic components are important for the affinity and specificity of the INSTI-viral DNA-integrase-complex.[60]

Integrase strand transfer inhibitor resistance

Although increasing, the prevalence of transmitted INSTI-resistance mutations is low.[25,26,68–71] With a few exceptions, INSTI-resistance is typically caused by RAMs occurring during viremia in the setting of inadequate plasma INSTI-concentrations, caused by suboptimal adherence to antiretroviral drugs, or insufficient drug penetration. An overview of INSTI-RAMs, including their effect on INSTI-susceptibility according to the Stanford University Drug Resistance Database is shown in Table 3.[72]

Mutation associated with INSTI-resistance	Reduction of INSTI-susceptibility
Major primary mutations	
T66A/I/K	RAL: 10-20 fold by T66<u>K</u> EVG: 10-40 fold DTG: 2-3 fold by T66<u>K</u> BIC: no effect of T66<u>A/I</u>
E92Q/G	RAL: >5 fold by E92<u>Q</u> EVG: >30 fold by E92<u>Q</u>, 10 fold by E92<u>G</u> DTG: 1.5 fold by E92<u>Q</u> BIC: no effect of E92<u>Q</u>
E138K/A/T	Usually occurs in combination with Q148 mutations. Alone: no reduction of INSTI-susceptibility **+Q148:** RAL: >100 fold EVG: >100 fold DTG: 10 fold
G140S/A/C	Usually occur in combination with Q148H/R/K. Alone: no reduction of RAL and DTG-susceptibility, 3-5 fold reduction of EVG-susceptibility. **+Q148:** RAL: >100 fold EVG: >100 fold DTG: 10 fold
Y143C/R/H/K/S/G/A	Alone: RAL: 5-20 fold by Y143<u>C</u> and <u>R</u>, 5-10 fold by Y143<u>K/S/G/A</u> EVG: no effect by Y143<u>C</u> and <u>R</u> DTG: no effect by Y143<u>C</u> and <u>R</u> **+T97A (and other accessory mutations):** RAL: >100 fold EVG: 10-20 fold DTG: no effect
S147G	RAL: minimal effect EVG: 5 fold DTG: minimal effect

Q148H/K/R/N

Q148H alone:
RAL: 5-10 fold
EVG: 5-10 fold
DTG: minimal effect
BIC: minimal effect

Q148R/K alone:
RAL: 30-100 fold
EVG: 30-100 fold
DTG: minimal effect
BIC: minimal effect

Q148H/K/R+G140S/A:
RAL: >100 fold
EVG: > 100 fold
DTG (or + E138K/T/A): 10 fold
BIC (or + E138K/T/A): 10 fold

Q148N: low-level EVG-resistance

N155H/S/T/D

RAL: >10 fold
EVG: > 30 fold
DTG: reduced susceptibility in combination with other INSTI-RAMs

Rare primary mutations

G118R

Varying from no effect to 10 fold reduction to each of the INSTIs

F121Y

RAL: 5 fold
EVG: >10 fold
DTG: no effect

P145S

High-level EVG-resistance

Q146P

EVG: 2-5 fold

R263K

EVG: 6 fold
DTG: 2 fold
BIC: 2 fold

Major accessory mutations

H51Y

Alone:
RAL: no effect
EVG: 2-3 fold
DTG: no effect
BIC: no effect

+R263K:
DTG: 7 fold

L74M/I/F

Alone: minimal if any effect

+ any of the Primary resistance mutations: reduced susceptibility to all INSTIs

T97A	Alone:
	RAL: no effect
	EVG: 3 fold
	DTG: no effect
	+Y143CR:
	Synergistical reduction of RAL and EVG susceptibility
Q95K	Alone: minimal if any effect
V151I/L/A	V151I: minimal if any effect
	V151L:
	RAL: 10-15 fold
	EVG: 20-30 fold
	DTG: 2-3 fold
	V151A:
	EVG: 3 fold
S153Y/F	EVG, DTG, and BIC: 2-3 fold
E157Q	RAL: minimal effect
	EVG: minimal effect
	DTG: minimal effect
G163R/K	Usually occurs in combination with other INSTI-RAMs, usually N155H
S230R	EVG: > 2 fold
	DTG: 2 fold
	BIC: 2 fold

Table 3. Overview of integrase strand transfer inhibitor resistance associated mutations. Adapted from the Stanford University HIV Drug Resistance Database.[72]

An important mechanism to take into account, when cART is switched, is cross-resistance between different agents. Cross-resistance means that RAMs, which develop during use of a specific antiretroviral agent, also confer resistance to another antiretroviral agent of the same ART class. This is a common phenomenon for RAL and EVG for example. The consequence of development of cross-resistance is, that with the 'loss' of one treatment option, another treatment option is lost as well (e.g. loss of EVG in the case of development of a Q148K during RAL-use).

Characteristics of clinical available integrase strand transfer inhibitors

At this moment, the INSTIs RAL, EVG, DTG, and BIC are widely available in clinic. Another INSTI, cabotegravir (CAB) has shown promising results in clinical phase 2 and 3 studies. All have shown favorable clinical and virological properties. The individual INSTIs will be discussed below.

Raltegravir

Raltegravir (MK-0518, RAL) was the first clinical approved INSTI after its authorization in 2007.[73] RAL has been investigated in phase 2 and phase 3 studies with cART-naive or INSTI-

naive individuals, and it was combined with TDF plus FTC or 3TC, or with an optimized background regimen (OBR) in case of resistance against multiple antiretroviral classes. Its efficacy was high, AEs were comparable or lower, and development of RAMs occurred less frequent compared to regimens with two NRTIs with NNRTIs or PIs. For a summary of the results of the phase 2 and phase 3 studies on RAL-containing cART, see Table 4.

RAL's 95% inhibitory concentration effect (IC_{95}) of HIV-replication is at 16 ng/mL. The time to peak plasma-concentration after oral administration is 0.5 to 1.3 hours. This peak is followed by a biphasic decline in plasma-concentration, with a half-life ($t_{1/2}$) of 1 hour and 7–12 hours in the first and second phase respectively. With RAL 400mg twice daily, the steady state (where drug concentrations over time are constant due to nearly equal absorption and elimination) is reached after two days. In individuals receiving 400 mg twice daily, a mean viral load reduction after 10 days of 1.66 $\log_{10}$ c/ml was observed regardless of HIV-subtypes.[73,80,82,83] RAL is primarily hepatically metabolized via uridine diphosphate glucuronosyltransferase (UGT) 1A1, and excreted in feces and urine.[84] There is no interaction with cytochrome P450 enzymes including CYP3A4, hormonal contraceptives, the membrane transporter P-glycoprotein, and UGT enzymes. This dramatically reduces the drug-drug-interaction risk.[85] Only drugs that contain divalent cations (e.g. Mg^{2+} containing antacids) should be taken with caution due to their binding to INSTIs in the gastro-intestinal tract reducing absorption.[86] In clinical trials, RAL was well tolerated; its most frequently reported AEs were gastrointestinal and neuropsychiatric, or increases in creatinine kinase (CK) and liver enzymes. Discontinuation rates of RAL for AEs were infrequent compared to placebo or EFV.[74–81] Of note, RAL is not available in a single tablet regimen (STR), so cART-regimens containing RAL cause a relatively high pill burden.[73]

Study	Phase	N	Design	Main conclusion	Drug-related AEs RAL*	No of VF during RAL/No and type of INSTI RAMs§
Gatell et al, 2010[74]	II	179	Dose-ranging Triple-blind Inclusion: cART-experienced (INSTI-naive) Resistance to NRTI and NNRTI and PI. Randomization Addition of RAL 200 mg/400 mg/600 mg BID or placebo to OBR.	Week 48 HIV-RNA: <400 c/mL: 68% <50 c/mL: 55% In RAL-users. Viral suppression at week 96 of RAL 200/400/600 BID + OBR better than placebo + OBR in patients with few remaining treatment options	AEs similar in both groups (58%), most were of gastrointestinal origin in RAL. 2 SAEs: pancreatitis+metabolic acidosis with renal insufficiency. Lab AEs comparable (22% vs 18%). 1 lab SAE: thrombopenia. 1 lab discontinuation: not reported.	38/35 Q148H/K/R + 138 and/or 140 or N155H + 74/92/97/143/163 (N=33)
Eron et al, 2013 (BENCHMRK)[75,76]	III	703	Double-blind Placebo or RAL, followed by open-label RAL after 156 weeks. Inclusion: cART-experienced (INSTI-naive) Resistance to NRTI and NNRTI and PI Randomization: Addition of RAL 400 mg BID or placebo to OBR.	Week 48 HIV-RNA < 50 c/mL: 62.1% in RAL 32.9% in placebo. Viral suppression at week 240 in RAL: <400 c/mL: 45% <50 c/ml: 42% Changes in HIV-RNA and CD4 T-lymphocytes greater in the RAL-group after 156 weeks.	AEs similar in both groups (61% and 62%), most were of gastrointestinal origin in RAL. SAEs and discontinuations were comparable. Lab AEs were comparable or lower in RAL than in placebo, except for CK elevations (more in RAL).	166/148 Y143/Q148/N155 (N=89) Other (N=59)

Study	Phase	N	Design	Efficacy	Adverse events	Ratio	Resistance mutations
Rockstroh et al, 2013 (STARTMRK)[77,78]	III	563	Non-inferiority Double-blind Inclusion: cART-naive No resistance to TDF, FTC, and EFV Randomization: RAL or EFV, both combined with TDF/FTC.	Week 48 HIV-RNA < 50 c/mL: RAL: 86.1% EFV: 81.8% RAL non-inferior to EFV. HIV-RNA at week 240 < 50 c/mL: RAL: 71.0% EFV: 61.3% RAL superior to EFV. Increases in CD4 T-lymphocytes greater in the RAL-group.	Less AEs in the RAL-group: 52.0% versus 80.1% in the EFV-group, with significantly less neuropsychiatric AEs. Comparable SAEs. Less discontinuations for AEs, 1 discontinuation for an SAE. No discontinuations for lab AEs. Most RAL-related AEs were of gastro-intestinal or neuropsychiatric origin.	55/5	Q148H+G140S (N=1) Q148R+G140S (N=1) Y143Y+L74L/M+E92Q +T97A (N=1) Y143R (N=1) Other (N=1)
Eron et al, 2011 (QDMRK)[79]	III	775	Double-blind Non-inferiority Inclusion: cART-naive No resistance to TDF and FTC. Randomization: Treatment with RAL 400 mg QD or BID, both combined with TDF/FTC.	Week 48 HIV-RNA < 50 c/mL: RAL QD: 83% RAL BID: 89% RAL QD not non-inferior to RAL BID. Longer time to virological response in RAL QD after 48 weeks.	AEs similar in both groups: 26.2 vs 24.2% 3 SAEs, 5 discontinuations for AE, 2 discontinuations for SAEs. 14 lab AEs, no SAEs and no discontinuations for lab AEs.	88/11	T97A+Y143R (N=1) I203M+N155N/H (N=1) E92E/Q+G163G/R+N155N/H (N=1) F121C+L74I (N=1) G163G/R+Y143R/C (N=1) N155H (N=1) N155H+V151V/I (N=1) L74L/M+T97M+Y143R (N=1) E92E/Q(N=1) E92Q+L74M+T97A (N=1) E92Q+T97A+Y143Y/R/H/C (N=1)

| Gotuzzo et al, 2012[80,81] | II | Double-blind

Inclusion:
cART-naive
Susceptibility to TDF, 3TC, and EFV.

Randomization:
Treatment with RAL or EFV, both combined with TDF/3TC. | 198 | At week 48, virological suppression rates in RAL-users were similar to those in EFV-users.

Viral suppression similar in both groups (68.8 vs 63.2%) after 5 years. | Less drug-related AEs in the RAL-group (55.0%) than in the EFV-group (76.3%). Mainly due to neuropsychiatric AEs.

Most RAL-related AEs were of gastrointestinal and neuropsychiatric origin.

Comparable rates of discontinuations for AEs and comparable rates of lab AEs.

1 discontinuation for lab AE: a CK elevation. | 10/3 | N155H+V151I+L74M+L74M/L (N=1)
N155H (N=1)
S230R+Y143C (N=1) |

Table 4. Overview of clinical studies on the efficacy and safety of raltegravir. *=main considerations regarding adverse events (AE) related to use of raltegravir (RAL), $=number of patients with virological failure (VF) during RAL / the number and type of developed resistance associated mutations (RAMs) causing resistance against integrase strand transfer inhibitors (INSTIs) during use of RAL, cART=combination antiretroviral therapy, NRTI=nucleoside reverse transcriptase inhibitor, NNRTI=non-nucleoside reverse transcriptase inhibitor, PI=protease inhibitor, BID=twice daily, OBR=optimized background regimen (i.e. the cART-regimen with the highest virological efficacy), CK=creatine kinase, SAE=serious adverse event, TDF=tenofovir disoproxil fumarate, FTC=emtricitabine, EFV=efavirenz, QD=once daily, 3TC=lamivudine, AE=adverse event.

Elvitegravir

Elvitegravir (GS-9137, EVG) was the second first-generation INSTI after FDA authorization in 2012. EVG/c has been investigated in phase 2 and phase 3 studies with cART-naive or INSTI-naive individuals. EVG/c's efficacy was high, AEs were comparable or lower, and development of RAMs occurred less frequent compared to two NRTIs with NNRTIs or PI. For a summary of the results of the phase 2 and phase 3 studies on EVG/c-containing cART, see Table 5.

EVG/c was initially available with TDF/FTC as STR. In 2015, EVG/c/TAF/FTC became available as alternative STR, and this combination has the alleged advantage of less risk of renal deterioration. EVG/c's IC_{95} is 45 ng/mL, and the time to peak plasma-concentration is 4.0 to 4.5 hours. $T_{1/2}$ is approximately 9 hours. In individuals receiving 50 mg of EVG/c QD, the mean viral load reduction was $1.91\log_{10}$ c/mL after 10 days, and EVG/c is equally active against all HIV subtypes.[103–105] Besides CYP3A4, EVG/c is hepatically metabolized by UGT1A1 and UGT1A3, which leads to excretion of the majority of metabolites in faeces, and another small part in urine.[103] The CYP3A4-inhibiting properties of COBI result in a risk on drug-drug-interactions between EVG/c-containing cART and a number of drug-classes, e.g. anticonvulsants, anticoagulants, direct acting antivirals, and statins.[14] As with all INSTIs, drugs containing bivalent cations should be taken with caution. On the other hand, the pill-burden of the EVG/c-containing STRs is low.

Study	Phase	Design	N	Main conclusion	Drug-related AEs EVG*	No of VF during EVG/No and type of INSTI RAMs[§]
Zolopa et al, 2010[87]	II	Dose-ranging Partially blinded (to the EVG dose) Inclusion: cART-experienced (INSTI-naive) ≥1 PI-RAM Randomization (1:1:1:1): PI or 20, 50, or 125 mg EVG + OBR for 48 weeks	278	At week 48: Higher $\log_{10}$ decrease in EVG 125 than in EVG 50 mg, EVG 125 superior to PI. EVG 20 mg discontinued for higher VF rates, addition of PI allowed in the other EVG-arms (added in 10%). EVG 50 mg non-inferior, 125 mg superior to PI after 24 weeks. Comparable increases in CD4 T-lymphocytes in all groups at weeks 24 and 48.	Similar rates of AEs and study drug discontinuations. 2 SAEs: syncope and hypersensitivity reaction (in a patient with a history of multiple drug allergies). No deaths. Numerical details not reported.	Not reported
Cohen et al, 2011[88]	II	Double-blind Inclusion: cART-naive CD4 T-lymphocytes > 50 cells/mm³ Randomization: 2:1 to EVG/c or EFV + TDF/FTC for 48 weeks	71	Week 48 HIV-RNA < 50 c/mL: EVG: 90% EFV: 83% Faster HIV-RNA decline and higher viral suppression rates at week 48 in the EVG/c group than in the EFV-group.	More neuropsychiatric AEs in EFV (26%) than in EVG (10%). No discontinuation for AEs and no deaths. Small decrease in eGFR in EVG, stabilized in the first 24 weeks. No discontinuations for lab AEs.	0/0

Study	Phase	Design	N	Outcomes	Adverse events	Resistance
Wohl et al, 2014[89-91]	III	Double-blind Non-inferiority Inclusion: cART-naive Viral susceptibility to EFV, FTC, and TDF. Randomization (1:1): EVG/c or EFV, both + TDF/FTC.	700	Week 48 HIV-RNA < 50 c/mL: EVG: 88% EFV: 84% Comparable virological suppression at week 144 between both groups (80.2% for EVG versus 75.3% for EFV).	Similar rates of discontinuation for AEs in both groups (6.0-7.4%), but the relation with study drugs was not specified. One discontinuation in EVG was for increased serum creatinine. Lower rates of neuropsychiatric AEs in EVG/c. Smaller increases in LDL and HDL than in EFV, comparable triglycerides and total cholesterol increase.	21/9 Primarily E92Q, not further specified.
Clumeck et al, 2014[92-94]	III	Double-blind Non-inferiority Inclusion: cART-naive Randomization (1:1): EVG/c or ATV/r + TDF/FTC for 192 weeks.	708	Week 48 HIV-RNA < 50 c/mL: EVG: 89.5% ATV: 86.8% EVG non-inferior to ATV. At week 144, EVG/c was non-inferior to ATV/r in virological suppression rates (77.6% versus 74.5%). Comparable CD4 T-lymphocyte increases.	AE rates comparable, less SAEs in EVG/c than in ATV/r (relation with EVG not reported). Increase in serum creatinine in EVG/c.	24 (week 96)/8 week (144) E92Q (2) N155H (2) Q148R (2) T66I (1) T97A (1)

Study	Phase	Design	N	Efficacy	Safety	Resistance
Elion et al, 2013[95,96]	III	Double-blind Non-inferiority. Inclusion: cART-experienced (INSTI-naive). Resistance to at least 2 ART classes *or* 6 months cART-experience with or without RAM. Randomization 1:1 to EVG or RAL BID, combined with a PI and another ART agent (NRTI, ETV, MVC, or T20) for 96 weeks.	702	Week 48 HIV-RNA < 50 c/mL: EVG: 59% RAL 58%. EVG non-inferior to RAL. Comparable rates of virological suppression in the RAL (45%) group and the EVG (48%) group after 96 weeks.	Similar low rates of AEs and SAEs in both groups (<5% all). Low rates of drug discontinuations in both groups, including nausea in 3 patients with EVG. No drug-related deaths. Similar rates of lab abnormalities. More ALT and AST elevations in the RAL group.	EVG 87/23 RAL 93/26. Only registered until week 48: T66I/A (7) E92G (6) T97A (6) S147G (3) Q148H/R (7) N155H (12) Y143R/C/H (1)
Pozniak et al, 2017 (STRATEGY-NNRTI)[97–99]	III	Open-label Non-inferiority. Inclusion: cART-experienced (INSTI-naive). HIV-RNA < 50 c/mL for at least 6 months on TDF/FTC + NNRTI. Randomization (2:1): Switch to EVG/c +TDF/FTC or continue NNRTI +TDF/FTC, for 96 weeks.	439	Week 48 HIV-RNA < 50 c/mL: EVG: 93% NNRTI: 88% EVG non-inferior to NNRTI. A switch to EVG/c is non-inferior to continuation of NNRTI + TDF/FTC after 96 weeks.	Comparable rates of AEs in both groups. Improvement of neuropsychiatric and gastrointestinal AEs in the group who switched from EFV to EVG/c. Increase in serum creatinine in the EVG/c group causing 3 discontinuations	19/0 (at week 48)

Study	Phase	N	Design	Results	AE	
Arribas et al, 2017 (STRATEGY-PI)[100–102]	III	433	Open-label, Non-inferiority Inclusion: cART-experienced (INSTI-naive) HIV-RNA < 50 c/mL for at least 6 months on TDF/FTC + PI. Randomization (2:1): Switch to EVG/c +TDF/FTC or continue PI +TDF/FTC, for 96 weeks.	Week 48 HIV-RNA < 50 c/mL: EVG: 93.8% PI: 87.1% EVG superior to PI (mainly driven by more discontinuations for non-virological reasons in the PI-group). A switch to EVG/c is superior to continuation of PI + TDF/FTC after 96 weeks. This is the result of less VF combined with less discontinuations for non-virological reasons.	Comparable rates of AEs in both groups. Improvements of gastrointestinal AEs in the group who switched from PI to EVG. Increase in serum creatinine and decrease in TG with EVG/c	3/0 (at week 96)

Table 5. Overview of clinical studies on the efficacy and safety of elvitegravir. *=main considerations regarding adverse events (AE) related to use of elvitegravir (EVG), $=number of patients with virological failure (VF) during EVG / the number and type of developed resistance associated mutations (RAMs) causing resistance against integrase strand transfer inhibitors (INSTIs) during use of EVG, cART=combination antiretroviral therapy, PI=protease inhibitor, OBR=optimized background regimen (i.e. the cART-regimen with the highest virological efficacy), SAE=serious adverse event, EFV=efavirenz, TDF=tenofovir disoproxil fumarate, FTC=emtricitabine, eGFR=estimated glomerular filtration rate, LDL=Low-density lipoprotein cholesterol, HDL=high-density lipoprotein cholesterol, ATV=atazanavir, BID=twice daily, NRTI=non-nucleoside reverse transcriptase inhibitor, ETV=etravirine, MVC=maraviroc, T20=enfuvirtide, AST=aspartate aminotransferase, ALT=alanine aminotransferase, NNRTI=non-nucleoside reverse transcriptase inhibitor.

Dolutegravir

Dolutegravir (S/GSK1349572, DTG) came available as second-generation INSTI in 2013. The distinction between first- and second-generation INSTIs is based on the risk of development of INSTI-RAMs, which is lower in second generation INSTIs. This lower risk on RAMs is caused by changes in the structure of the INSTI, compared to the first generation: the zinc-binding component and peripheral structural elements differ from RAL's and EVG's.[106] HIV develops RAMs compromising RAL and EVG/c efficacy relatively easily when used in INSTI-naive patients, and significant cross-resistance between these agents occurs. DTG as one of the second-generation INSTIs is less susceptible to the development of RAMs: INSTI-RAMs develop very infrequently in INSTI-naive patients, and DTG often remains active against RAL- or EVG/c-resistant viral strains.[29,107,108] The phase 2 and 3 studies showed that DTG-containing cART had non-inferior and sometimes superior virological efficacy compared to NNRTI-, PI-, and RAL- and EVG-containing cART in INSTI-naive and INSTI-experienced patients with a low risk on development of RAMs. Furthermore, it has antiretroviral activity against RAL- and EVG- resistant viral strains, it has a favorable AE profile, and has a low risk on drug-drug-interactions. For an overview of the phase 2 and 3 studies, see Table 6.

The time to peak plasma-concentration of DTG is 0.5 to 1.25 hours, and $t_{1/2}$ is 13 to 15 hours. The IC_{90} is 64 ng/mL. A steady-state is reached after five days.[122] In patients receiving the recommended 50mg dose once daily, plasma HIV-RNA decreased 2.46 $\log_{10}$ c/mL in 10 days.[109] Like the other INSTIs, DTG has good antiviral activity against HIV B- and non-B subtypes.[123–125] DTG is metabolized by UGT1A1 and CYP3A4, and excreted in feces (~50%) and urine.[126] Like RAL, there is a low risk on drug-drug-interactions, but cation-containing medication should be taken with caution. DTG's inhibition of the renal organic cation transporter 2 (OCT2) and MATE1 is of importance for drug-drug-interaction risks. These transporters aid in drug clearance (e.g. metformin) and tubular creatinine transport. This results in serum creatinine increases, consequently decreasing the creatinine based estimation of the estimated glomerular filtration rate (eGFR). However, this does not reflect an actual decline in glomerular renal function.[125,126] Despite the favorable data regarding AEs in the phase 2 and 3 studies, there are rising concerns about the higher incidence of neuropsychiatric AEs in post marketing studies.[125,127,128]

Study	Phase	Design	N	Main conclusion	Drug-related AEs DTG*	No of VF during DTG/No and type of INSTI RAMs[§]
Min et al, 2011[109]	II	Double-blind Dose-ranging Inclusion: cART-naive and cART-experienced, but INSTI-naive patients CD4 T-lymphocytes ≥ 100 Randomization: Placebo, 2, 10, or 50 mg DTG monotherapy for 10 days.	35	DTG (all doses) showed virological efficacy compared with placebo. Highest proportion of virological suppression in 50 mg group, with fastest HIV-RNA decline.	No discontinuations, SAEs, or deaths. One patient with lipase increase, resolved at the end of follow-up.	NA / 1 (2 mg DTG) L74I/L/M
Stellbrink et al, 2013[110,111] (SPRING-1)	II	Dose-blinded Dose-ranging Inclusion: cART-naive patients CD4 T-lymphocytes ≥ 200 Randomization: 10, 25, or 50 mg DTG, or EFV, either + TDF/FTC or ABC/3TC for 96 weeks.	205	Week 48 HIV-RNA < 50 c/mL: DTG: 87% EFV: 82% More non-responders in DTG 10 or 25 mg than in 50 mg, but all DTG doses had higher rates of viral suppression than EFV-users (79, 78, and 88% versus 72%). Greater increase in CD4 T-lymphocytes with DTG than with EFV.	Lower rates of AEs in users of DTG and EFV. More headache and nausea in DTG. Less discontinuations due to AEs in DTG users than in EFV users. 1 SAE leading to drug discontinuation: myocardial infarction. An increase in serum creatinine in users of DTG.	13/0

Study	Phase	Design	N	Results	Safety	
Raffi et al, [112,113] (SPRING-2)	III	Double-blind Non-inferiority Inclusion: cART-naive patients Randomization (1:1): DTG QD or RAL BID, either with TDF/FTC or ABC/3TC for 96 weeks.	822	Week 48 HIV-RNA < 50 c/mL: DTG: 88% RAL: 85% DTG QD non-inferior to RAL BID DTG was non-inferior to RAL BID after 96 weeks. Similar increases of CD4 T-lymphocytes.	Similar rates of AE between groups. No deaths or SAEs related to study drugs. Serum creatinine increase with DTG	22/0
Walmsley et al, 2015[26,114] (SINGLE)	III	Double-blind Non-inferiority Inclusion: cART-naive patients Randomization (1:1): DTG + ABC/3TC or EFV + TDF/FTC for 144 weeks.	833	Week 48 HIV-RNA < 50 c/mL: DTG: 88% EFV: 81% DTG superior to EFV. DTG + ABC/3TC was superior to EFV + TDF/FTC in maintaining viral suppression after 144 weeks. Greater increase in CD4 T-lymphocytes in DTG than in EFV.	More insomnia in DTG-users than in EFV-users. Less SAEs in DTG than EFV.	39/0

	Phase	Design / Inclusion	N	Efficacy	Safety	Resistance
Cahn et al, 2013[115] (SAILING)	III	Double-blind Non-inferiority Inclusion: cART-experienced (INSTI-naive) patients with 2 consecutive HIV-RNA ≥ 400 c/mL RAMs to 2 or more ART classes with 1 or 2 fully active drugs for OBR. Randomization: DTG QD or RAL BID with OBR.	715	Week 48 HIV-RNA < 50 c/mL: DTG QD: 71% RAL BID: 64% DTG + OBR superior to RAL BID + OBR in cART-experienced patients. Earlier and more frequent VF in RAL BID. Comparable increases in CD4 T-lymphocytes in both groups.	Comparable rates of AEs. No deaths during DTG. Comparable low rates of drug-related SAEs in both groups (1%) Increase in serum creatinine in both groups.	21/4 (of note: 1 RAM was present at baseline and did not develop during DTG) Q148H + G140S (baseline) R263R or R263K (2) V151V/I
Eron et al, 2013[116] (VIKING)	II	Single-arm Pilot study 2 sequential cohorts Inclusion: INSTI-experienced patients RAL treatment failure (HIV-RNA ≥ 1000 c/mL) RAL resistance at screening Resistance to at least 1 PI and 1 NNRTI and 1 NRTI. Treatment with RAL-containing cART + 50 mg DTG QD (cohort 1) or BID (cohort 2) for 10 days + 11 days DTG QD or BID with OBR (which not necessarily consists of RAL) with ≥ 1 fully active agent, extended at most for 24 weeks.	51	78% in cohort 1 and 96% in cohort 2 showed a decrease in HIV-RNA of ≥ 0.7 c/mL, 41% in cohort 1 and 54% in cohort 2 had HIV-RNA < 400 c/mL at day 11. Cohort 2 had a significantly larger reduction in HIV-RNA. Comparable increases in CD4 T-lymphocytes between groups.	No drug-related SAEs. Increases of serum creatinine in both cohorts.	17/7 (of note: all patients had INSTI-RAMs at baseline) L74I/M + E138E/K L74L/I/M + T97A + G140S+Q148H N155N/H (2) T97T/ A+E138K+N155H E92E/Q + T97T/A E138E/K+N155H.

	Phase	Design / Inclusion	N	Results	Adverse events	RAMs
Castagna et al, 2014[117] (VIKING-3)	III	Single-arm Open-label Inclusion: INSTI-experienced patients RAL or EVG treatment failure (HIV-RNA ≥ 500 c/mL) RAMs to RAL or EVG/c and to ≥ 2 other classes, with at least 1 fully active agent. Substitution of RAL or EVG/c by DTG 50 mg BID for 7 days, followed by optimization of OBR, for at least 24 weeks.	183	After 8 days DTG BID caused a decrease of $1.43 \log_{10}$ in HIV-RNA. At week 24, 69% had HIV-RNA < 50 c/mL.	Most common AEs: diarrhea, nausea, and headache. 1 SAE: syncope. 1 SAE probably DTG-related: generalized rash, nausea and vomiting. Increase in serum creatinine.	Not reported.
Akil et al, 2015[118,119] (VIKING-4)	III	Double-blind Inclusion: INSTI-experienced patients RAL or EVG treatment failure (HIV-RNA ≥ 1000) RAMs to RAL or EVG/c and to ≥ 2 other classes, with at least 1 fully active agent. Randomization: Addition of placebo or DTG 50 mg BID to the cART regimen for 8 days, followed by an open-label phase with all participants receiving OBR + DTG BID until they have no longer clinical benefit.	30	Week 48: HIV-RNA < 50 c/mL: 40% HIV-RNA < 400 c/mL: 53% After 8 days DTG BID showed a decrease of $1.06 \log_{10}$ in HIV-RNA, versus $0.10 \log_{10}$ in the placebo group. After 24 weeks, 47% and 57% had HIV-RNA <50 and <400 c/mL.	No drug discontinuations due to AEs. Decrease in creatinine-clearance.	7/5 (more than 1 RAM per patient) L74I/M T97A (3) E138E/K E138K S147G N155N/H

Molina et al, 2015[120,121] (FLAMINGO)	III	Open-label Non-inferiority	484	Week 48 HIV-RNA < 50 c/mL: DTG: 90% DRV/r: 83% DTG superior to DRV/r.	Similar rates of AEs.	15/0
					2 drug-related SAEs in DTG: myocarditis and suicidality.	
		Inclusion: cART-naive patients No baseline RAMs		DTG was superior to DRV/r after 96 weeks in virological suppression rates.	Decrease in creatinine-clearance.	
		Randomization: DTG QD or DRV/r, either + TDF/FTC or ABC/3TC, for 96 weeks.		Faster virological suppression in DTG than in DRV/r.	Lower LDL in DTG than in DRV/r.	
				Similar increases of CD4 T-lymphocytes in both groups.		

Table 6. Overview of clinical studies on the efficacy and safety of dolutegravir. *=main considerations regarding adverse events (AE) related to use of dolutegravir (DTG), s=number of patients with virological failure (VF) during DTG / the number and type of developed resistance associated mutations (RAMs) causing resistance against integrase strand transfer inhibitors (INSTIs) during use of DTG, cART=combination antiretroviral therapy, SAE=serious adverse event, EFV=efavirenz, TDF=tenofovir disoproxil fumarate, FTC=emtricitabine, ABC=abacavir, 3TC=lamivudine, QD=once daily, RAL=raltegravir, BID=twice daily, OBR=optimized background regimen (i.e. the cART-regimen with the highest virological efficacy), PI=protease inhibitor, NNRTI=non-nucleoside reverse transcriptase inhibitor, NRTI=non-nucleoside reverse transcriptase inhibitor, DRV/r=ritonavir boosted darunavir, LDL=Low-density lipoprotein cholesterol.

Bictegravir

Bictegravir (GS-9883, BIC) is the fourth available INSTI belonging to the second-generation INSTIs. It was introduced in 2017. Like DTG, the phase 2 and 3 studies on BIC showed good antiretroviral activity in cART-naive and cART–experienced individuals. However, its efficacy in INSTI-experienced patients remains to be demonstrated. Besides, the AE profile is favorable, and the risk on drug-drug-interactions is low. Table 7 shows an overview of the clinical trials on BIC.

The time to peak plasma-concentration after oral administration of BIC is 1 to 3 hours, with a $t_{1/2}$ of 16 to 22 hours, and a steady-state after 10 days. BIC has an IC_{95} of 162 ng/mL. It has antiviral activity against all HIV-subtypes. In individuals receiving 50 mg QD, HIV-RNA decreased mean $1.37Log_{10}$ after 11 days. BIC is metabolized by CYP3A4 and UGT1A1, and excreted in feces and urine. The risk on drug-drug-interactions is limited to inhibitors of both CYP3A4 and UGT1A1 (e.g. atazanavir) or inducers of CYP3A4 (e.g. rifampicin).[129,135,136] Medications containing bivalent cations should be taken with caution. An important advantage of BIC above DTG and EVG/c is its absent effect on tubular creatinine clearance.

Study	Phase	Design	N	Main conclusion	Drug-related AEs BIC*	No of VF during BIC/ No and type of INSTI RAMs[$]
Gallant et al, 2017[129]	I	Double-blind Dose-ranging Sequential cohorts Inclusion: cART-naive or cART-experienced but INSTI-naive. No INSTI-RAMs. Randomization: N=10: 1:1 cohort 1 (BIC 25 mg) or cohort 2 (BIC 100 mg), consequently N=10: 1:1 cohort 3 (BIC 5 mg) or cohort 4 (BIC 50 mg), all for 10 days with 7 days follow-up.	20	At day 11, mean reduction in $\log_{10}$ HIV-RNA ranged from 1.45 to 2.43for increasing BIC doses. HIV-RNA < 50 c/mL in 3 patients at the end of the study.	No discontinuations.	NA/0
Sax et al, 2017[130]	II	Double-blind Inclusion: cART-naive No RAMs against TDF or FTC. Randomization (2:1): BIC or DTG, either + TAF/FTC for 48 weeks.	98	Week 48 HIV-RNA < 50 c/mL: BIC: 96.9% DTG: 93.9%	No drug-related SAEs or deaths. One discontinuation for AE: urticaria.	2/0

Gallant et al, 2017[131]	III	Double-blind Non-inferiority Inclusion: cART-naive No RAMs against TDF, FTC, 3TC and ABC. Randomization (1:1): BIC/FTC/TAF or DTG/ABC/3TC for 144 weeks.	631	Week 48 HIV-RNA < 50 c/mL: BIC/FTC/TAF: 92.4% DTG/ABC/3TC: 93.0% BIC/FTC/TAF non-inferior to DTG/ABC/3TC.	Less nausea in BIC users than in DTG users. Less drug-related AEs in BIC users.	1/0
Sax et al, 2017[132]	III	Double-blind Non-inferiority Inclusion: cART-naive patients. Randomization (1:1): BIC/TAF/FTC or DTG/TAF/FTC for 144 weeks.	657	Week 48 HIV-RNA < 50 c/mL: BIC: 89% DTG: 93% BIC non-inferior to DTG.	Similar AE rates between groups. Less drug-related AEs in BIC than in DTG users.	3/0
Molina et al, 2018[133]	III	Double-blind Non-inferiority. Inclusion: Suppressed (HIV-RNA < 50 c/mL) on DTG/ABC/3TC No RAMs to BIC, TAF, or FTC. Randomization (1:1): Continuation of DTG/ABC/3TC or switch to BIC/TAF/FTC for at least 48 weeks.	563	Week 48 HIV-RNA < 50 c/mL: BIC: 93.6% DTG: 95.0% BIC/TAF/FTC non-inferior to continuation of DTG/ABC/3TC after 48 weeks.	Less drug-related AEs in the BIC-group. No drug-related discontinuations or deaths.	3/0

| Daar et al, 2017[134] | III | Open-label Non-inferiority cART-experienced Suppressed on ATV/r or DRV/r + either TDF/FTC or ABC/3TC. Randomization (1:1) Continuation of PI-containing cART or switch to BIC/TAF/FTC for at least 48 weeks. | 577 | Week 48 HIV-RNA < 50 c/mL: BIC: 92.1% PI: 88.9% BIC/TAF/FTC non-inferior to continuation of PI-containing cART in patients suppressed on PI-containing cART after 48 weeks. | Similar rates of AEs in both groups. | 5/0 |

Table 7. Overview of clinical studies on the efficacy and safety of bictegravir. *=main considerations regarding adverse events (AE) related to use of bictegravir (BIC), $=number of patients with virological failure (VF) during BIC / the number and type of developed resistance associated mutations (RAMs) causing resistance against integrase strand transfer inhibitors (INSTIs) during use of BIC, cART=combination antiretroviral therapy, TDF=tenofovir disoproxil fumarate, FTC=emtricitabine, DTG=dolutegravir, TAF=tenofovir alafenamide fumarate, SAE=serious adverse event, 3TC=lamivudine, ABC=abacavir, ATV/r=ritonavir boosted atazanavir, DRV/r=ritonavir boosted darunavir, PI=protease inhibitor.

Cabotegravir

Cabotegravir (GSK1265744, CAB) is an INSTI which has not yet been approved for treatment of HIV. The unique property of CAB is that it can be administered orally or as injectable. The effectivity of CAB was evaluated in the LATTE-I and LATTE-II studies, see Table 8. For the randomized ATLAS and ATLAS-2M phase 3 studies on CAB, an oral CAB dosage of 30 mg was chosen.

The time to peak plasma-concentration after parenteral administration of CAB 800 mg is 6 days, and $t_{1/2}$ is 40 days. CAB has an IC_{90} of 166 ng/mL. A steady-state is reached after 3 months, when injections are given every 4 weeks.[139,140] The time for oral CAB 30 mg to peak in plasma is 2 hours, and steady-state is reached after 14 days.[141,142] CAB has antiretroviral activity against all HIV-subtypes, and a mean HIV-RNA reduction of $2.3Log_{10}$ is observed after 11 days of monotherapy at a dose of 30 mg QD.[143,143] Metabolization of CAB is primarily by UGT1A1 with excretion in feces and urine.[141] The risk on drug-drug-interactions is low, but when given orally, CAB should be administered 2 hours before or 6 hours after medications containing bivalent cations. The genetic barrier of CAB against development of INSTI-RAMs is higher than RAL and EVG/c, but not as high as DTG's and BIC's.[145]

In conclusion, the class of INSTIs consists of agents with equal or superior antiretroviral activity compared to NNRTIs and PIs. The second-generation INSTI have a high barrier against development of RAMs, a favorable AE-profile, and a low drug-drug-interactions risk. These properties make second-generation INSTIs potential candidates for simplification strategies, which will be studied in this thesis.

Study	Phase	Design	N	Main conclusion	Drug-related AEs CAB*	No of VF during CAB/No and type of INSTI RAMs[§]
Margolis et al, 2015[137] (LATTE-I)	II	Double-blind Dose-ranging Sequential cohorts Inclusion: cART-naive No major RAMs Randomization (1:1:1:1): Induction with oral CAB 10, 30, or 60 mg QD or EFV 600 mg QD, combined with an investigator-selected backbone during 24 weeks, followed by a maintenance phase with replacement of the NRTI-backbone by RPV in patients using CAB,for 72 weeks.	243	Week 48 HIV-RNA <50 c/mL: CAB 10 mg: 80% CAB 30 mg: 80% CAB 60 mg: 87% EFV 600 mg: 74% Viral suppression rates in all CAB+RPV groups higher than in EVF+NRTI-backbone. Selection of CAB 30 mg for further assessment, based on efficacy, safety, tolerability, and viral resistance.	Less drug-related AEs during CAB than during EFV-use. Less drug-related discontinuations during CAB than during EFV, mostly because of liver toxicity. Most common drug-related AE during CAB: nausea.	6/1 (an individual using 10 mg CAB, during maintenance) Q148R

Margolis et al, 2017[138] (LATTE-2)	II	Double-blind Dose- and interval-ranging Sequential cohorts	286	Week 96 HIV-RNA<50 c/mL: Intramuscular CAB 4-weekly: 87%	Two discontinuations for injection-site pain in the intramuscular group.	3/2 (both in the 8-week group)
				Intramuscular CAB 8-weekly: 94%	SAEs comparable between groups and not drug-related.	R269R/G (not affecting CAB susceptibility)
		Inclusion: cART-naïve No major RAMs		Oral CAB: 84%		Q148R
				Intramuscular CAB every 4 or 8 weeks combined with RPV as effective as CAB/ABC/3TC.		
		Randomization (2:2:1): Induction in all individuals with oral CAB 30 mg + ABC/3TC for 20 weeks, followed by addition of RPV 16 weeks after randomization. After induction, maintenance with long-acting intramuscular CAB 400 mg + RPV 600 mg every 4 weeks or intramuscular CAB 600 mg + RPV 900 mg or continuation of oral CAB 30 mg + ABC/3TC for 96 weeks.				

Table 8. Overview of clinical studies on the efficacy and safety of cabotegravir. *=main considerations regarding adverse events (AE) related to use of cabotegravir (CAB), �=number of patients with virological failure (VF) during CAB / the number and type of developed resistance associated mutations (RAMs) causing resistance against integrase strand transfer inhibitors (INSTIs) during use of CAB, cART=combination antiretroviral therapy, QD=once daily, EFV=efavirenz, NRTI=nucleoside reverse transcriptase inhibitor, RPV=rilpivirine, ABC=abacavir, 3TC=lamivudine, SAE=serious adverse event.

RISKS OF INTEGRASE STRAND TRANSFER INHIBITOR CONTAINING COMBINATION ANTIRETROVIRAL THERAPY

Although treatment with antiretroviral therapy is life-saving for HIV-infected individuals, it is not without potential toxicity and risks. Initiation of cART in therapy-naive patients who are severely immunocompromised, may lead to an excessive immune response. This phenomenon is called the immune reconstitution inflammatory syndrome (IRIS) and can be associated with significant morbidity and in certain subgroups of patients also with increased mortality. Beside the risk for IRIS, which is limited to patients with a low CD4 T-lymphocyte count at initiation of cART, short and long-term side effects of cART can occur in all HIV-infected patients. Both IRIS and direct cART-toxicities should be considered when initiating or switching cART. Below, these aspects will be discussed.

Immune reconstitution inflammatory syndrome

IRIS in HIV is a group of heterogeneous clinical symptoms, all caused by an excessive immune response against antigens of opportunistic infections (OI). Two key mechanisms play a role in development of IRIS: i) a severe CD4 T-lymphocyte deficiency might lead to the presence of OIs, as well as an inadequate immune response against them, and ii) immune dysfunction. Studies suggest that a deficient CD4 T-lymphocyte function also leads to an impaired innate immunity (including antigen presenting cells like monocytes, neutrophils, and macrophages). This results in antigen accumulation, and cART-induced immune recovery causes priming of innate immune cells and an excessive inflammatory response against antigens of OI. Second, although differences in antigen-specific T-lymphocyte responses have not been consistently detected between those who develop IRIS and those who do not, an imbalance of immune regulation by Th1- and Th2-lymphocytes may in part be responsible. Furthermore, patients who develop IRIS have higher levels of pro-inflammatory cytokines (IL-2, interferon-γ) and lower levels of anti-inflammatory cytokines (IL-10, IL-4).[146–148] Two types of IRIS can be distinguished: paradoxical IRIS and unmasking IRIS. In paradoxical IRIS, an OI is diagnosed before cART-initiation. After cART-initiation, the clinical course of the OI first improves as a consequence of directed OI therapy. However, afterwards, a deterioration in the clinical course occurs, with recurrence of signs and symptoms of the initial OI. This deterioration is considered secondary to restoration of the immune response, not failing OI treatment. In unmasking IRIS, an OI is not diagnosed before cART-initiation, often because there are no typical signs and symptoms of the OI as consequence of severe immune deficiency. After initiation of cART, a deterioration of the clinical course occurs, due to an OI which is only then diagnosed.[148] Mortality rates up to 30% in central nerve system IRIS have been reported. Mortality and morbidity depend on the causative OI, and consequently the localization of IRIS and the severity of the disease.[148,149] A conditio sine qua non for IRIS is a low CD4 T-lymphocyte count at the initiation of cART. Furthermore, treatment-associated

risk-factors are a steep decline in HIV-RNA and a fast increase of CD4 T-lymphocytes.[150–152] The use of INSTI-containing cART has been associated with a faster HIV-RNA decline and CD4 T-lymphocyte increase than PI- or NNRTI-containing cART.[77,93,121] The low risk for drug-drug-interactions, their efficacy, and good safety profile make INSTIs good candidates for the treatment of severely immunocompromised AIDS patients with complex comorbidities. However this should be reconsidered when the IRIS-risk is increased in AIDS patients initiating INSTI-containing cART. Currently, adequate studies on the relationship between initiation of INSTI-containing cART and the risk for IRIS are lacking.

Toxicity of INSTI-containing cART

More often than VF, cART-related toxicities are the main reason to discontinue cART. Side-effects also decrease adherence, one of the cornerstones of successful treatment of HIV.[153,154] Toxicities can be related to drug classes, or to specific individual agents within these classes. Simplification of cART, using INSTIs, may help to overcome this important problem. However, when simplification to an antiretroviral regimen without (N)NRTIs is not possible, other strategies to overcome cART-toxicity may be helpful. Within the class of NRTIs, ABC is associated with a potentially lethal hypersensitivity reaction in individuals who have the HLA-B5701 allele (approximately 5% of the Caucasian population), but this risk has been eliminated by testing for HLA-B5701-positivity. Additionally, several large cohort studies found an association of ABC use and cardiovascular events particularly in patients at increased cardiovascular disease risk.[155–157] TDF, another commonly used NRTI, sometimes causes renal toxicity. This is reflected by a progressive eGFR-decline or by proximal tubular dysfunction (PTD).[158–160] TDF can also decrease bone mineral density and may result in an increased fracture risk, in particular in an ageing HIV-population.[161,162] Since August 2016, TDF-related toxicity can be avoided by replacing it by TAF. TAF and TDF are different prodrugs of tenofovir. TAF is more stable in plasma than TDF, and it is hydrolyzed to tenofovir by cathepsin A in the CD4 T-lymphocytes, which leads to higher intracellular tenofovir-concentrations, and 90% lower plasma tenofovir-concentrations. This 90% lower overall exposure to tenofovir leads to less renal and bone exposure to tenofovir.[163] Comparable to HLA-B5701 screening for ABC hypersensitivity, screenings for the development of TDF-associated toxicity would be useful, as TDF is still commonly used in cART. Besides, TDF is used for pre-exposure prophylaxis (PrEP) for HIV-negative individuals as well, to prevent HIV-transmission. In the cellular DNA-metabolism, toxic by-products are produced. A group of toxic compounds of the cellular DNA-metabolism is the group of non-canonical nucleoside triphosphates (NTPs), and incorporation in the DNA leads to an increased risk on mutagenesis. NTPs could be removed by the 'housekeeping-enzyme' inosine triphosphatase (ITPase).[164] Purines are the building blocks of DNA and RNA, and tenofovir and ABC are analogues of purines. Tenofovir is an adenine nucleotide analogue, and ABC is a guanine nucleotide analogue. Therefore, tenofovir and ABC might be potential substrates for ITPase.

Recently, a study showed a potential relationship between ITPase activity and toxicity caused by analogues of the purine metabolism. A decreased ITPase-activity showed to be protective against occurrence of TDF-associated AEs, while it was associated with an increase in ABC-related AEs. The exact underlying mechanism for this finding has not been elucidated yet, but probably it can be explained by the fact that tenofovir is an adenine nucleotide analogue, wherease ABC is a guanine nucleotide analogue, which have different chemical structures. However, the AEs in this study were not specified, so the relationship between ITPase-activity and TDF-associated nephrotoxicity and bone-toxicity remains to be studied, as well as the potential of recovery of TDF-associated nephrotoxicity.[165]

Also other ART classes all have their specific toxicities. Within the class of NNRTIs, particularly EFV is associated with more neuropsychiatric events compared to other NNRTIs. Furthermore, the NNRTIs are associated with occurrence of rash and hepatitis. [166–169] PIs have gastrointestinal complaints and increases of serum lipids as their common side effects, and because they need to be combined with a strong CYP3A4 inhibitor, drug-drug-interactions are frequent.[170,171] As discussed above, INSTIs are well tolerated, but there are some concerns about potential neuropsychiatric side effects of DTG. Also, CK increase is occasionally reported, and nausea is reported by some patients, in particular during the first weeks.[127,172]

An historical overview of toxicity developments of cART is studied by the Dutch ATHENA (AIDS Therapy Evaluation in The Netherlands) cohort study. Apart from showing the changes in first line cART preferences, the incidence of switching cART due to toxicity almost halved from 26% in 1996-2000 to 14% in 2006-2010. Especially patients with stavudine-, didanosine-, and AZT-based regimens had a high toxicity risk. Patients during 1996-2000 usually switched due to gastrointestinal (36.2%), hepatological (15.2%), and hematological (10.1%) AE. In 2001-2005 gastrointestinal (24.9%), neuropsychiatric (14.9%), and hematological (12.9%) were the most reported AEs, and between 2006 and 2010, these were neuropsychiatric (30.7%), dermatological (16.6%), and gastrointestinal (15.3%) AEs.[173] Although these data did not include patients on INSTI-containing cART, the toxicity driven cART-discontinuations were obviously high. Reduction of cART-toxicity therefore remains an important research area, also in the era of INSTI-containing cART.

OUTLINE OF THIS THESIS

The studies that will be described in **Chapter 2 to 8**, and which resulted in this thesis, aim to evaluate the effectiveness and safety of new HIV treatment strategies with INSTIs, especially DTG, in HIV-1 infected individuals. Furthermore, several aspects of cART-toxicity with dual NRTI backbones in combination with an INSTI, PI, or NNRTI are evaluated. The ultimate

goal is a more individualized and patient-centered therapy by balancing virological and immunological efficacy with reduction of the risk of adverse events. This thesis focuses on three issues.

Part 1 focuses on the effects of a switch from triple cART to DTG as maintenance monotherapy. Given DTG's high genetic barrier against resistance, the low potential for drug-drug-interactions, and the favorable side-effects-profile, DTG could be a good candidate for monotherapy. The virological efficacy of DTG maintenance monotherapy compared to triple cART is investigated in **Chapter 2**. **Chapter 3** describes resistance dynamics in the patients who experienced VF during DTG maintenance monotherapy, and **Chapter 4** studies clinical and virological factors which are associated with VF during DTG maintenance monotherapy. In **Chapter 5,** the effects of a switch from cART to DTG maintenance monotherapy on metabolic markers are evaluated.

In part 2, the safety of initiating INSTI-containing cART in HIV late presenters with compromised immunity on the risk of IRIS is discussed. In **Chapter 6**, the hypothesis is tested that use of INSTI-containing cART as first line therapy in HIV late presenters is associated with an increased risk of development of IRIS.

Part 3 concentrates on aspects regarding safety of the NRTI backbone in INSTI, PI, or NNRTI containing cART and possibilities to further individualize HIV treatment. The association between ITPase activity and occurrence and recovery of renal toxicity in TDF-users is investigated in **Chapter 7.** In **Chapter 8**, the renal effects of a switch to TAF or ABC in patients with a TDF-associated eGFR-decline or PTD are studied.

Finally, **Chapter 9** provides a summary with a discussion of the study results and their implications for future directions.

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Part 1

**Effects of a switch from cART to dolutegravir
maintenance monotherapy**

Chapter 2

Dolutegravir as maintenance monotherapy for HIV (DOMONO): a phase 2, randomised non-inferiority trial.

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ABSTRACT

Introduction

The high genetic barrier to resistance of dolutegravir (DTG) might allow for its use as maintenance monotherapy in patients with HIV. We investigated whether DTG monotherapy was non-inferior to combination antiretroviral therapy (cART) for maintaining virological suppression in HIV-1 patients successfully treated with cART.

Methods

We did this open-label, phase 2, randomised non-inferiority trial at two medical centres in the Netherlands. Eligible patients (aged ≥ 18 years) were on cART, had been virologically suppressed (plasma HIV-RNA <50 c/mL) for at least 6 months, and had CD4-nadirs of 200 cells/mm³ or higher, HIV-RNA zeniths of less than 100.000 c/mL, and no history of virological failure (VF). Patients were randomly assigned (1:1) via a web-based block randomization method (variable block sizes of 4 and 6) to switch to dolutegravir monotherapy (50 mg once a day) either immediately or after a delay of 24 weeks of continued cART. Randomisation was stratified by HIV-RNA zenith (<50.000 c/mL or 50.000-99.999 c/mL). Investigators and patients were not masked to group allocation. The primary endpoint was the proportion of patients with plasma HIV-RNA viral loads of less than 200 c/mL at week 24, with a non-inferiority margin of 12%. We did analyses in the on-treatment and intention-to-treat populations. This trial is registered with ClinicalTrials.gov, NCT02401828.

Results

Between March 10, 2015, and Feb 4, 2016, we randomly assigned 51 patients to the immediate switch group and 53 to the delayed switch group. One patient who received immediate monotherapy discontinued dolutegravir at week 12 because of disturbed sleep. At week 24, dolutegravir monotherapy was non-inferior to cART with plasma HIV-RNA loads of 200 c/mL or higher observed in 2% (1/50) during immediate dolutegravir monotherapy and in 0/53 patients in the delayed dolutegravir monotherapy group (difference 2%, exact 95% CI -5%,+12%). Of patients assigned to the delayed switch group, 47 of 53 patients (89%) switched to dolutegravir monotherapy at week 24, and two of them (4%) subsequently discontinued dolutegravir monotherapy because of headache (N=1) and disturbed sleep (N=1). Eight (8%) of the 95 patients who remained on dolutegravir monotherapy had VF; all had therapeutic DTG plasma-concentrations. In three of the eight patients, mutations associated with resistance were detected in the integrase gene. According to a predefined stopping rule, detection of these mutations led to premature study discontinuation.

Conclusions

Dolutegravir monotherapy was non-inferior to cART at 24 weeks. However VF continued to occur thereafter and led to dolutegravir resistance in three patients. Dolutegravir should not be used as maintenance monotherapy.

INTRODUCTION

Combination antiretroviral therapy (cART) regimens containing the second-generation integrase inhibitor dolutegravir (DTG) showed equal or superior virological suppression rates compared with raltegravir, efavirenz, or darunavir containing cART in treatment of HIV-1 infected adult patients.[1–3] This high virological efficacy, the favorable safety profile, and the high genetic resistance barrier of DTG has led to the recommendation of DTG-containing cART as first-line strategy in HIV treatment guidelines.[4,5] Although existing cART regimens are effective, maintenance therapy with one or two drugs might have advantages, including reduced side-effects, pill burden, and costs. Various factors make DTG a suitable candidate for maintenance monotherapy: the development of resistance is rare in integrase inhibitor-naive patients; the risk of drug-drug-interactions is low, the drug has a good tolerability, a once-daily dosing schedule, a small pill size, and a neutral effect on serum lipids.[1,6,7] Previous studies have not shown monotherapy with protease inhibitors to be virologically non-inferior to cART, although virological failure (VF) during protease inhibitor monotherapy has not been associated with an increased incidence of resistance to protease inhibitors.[8,9] However, in one study of virologically suppressed patients fulfilling strict criteria regarding HIV-RNA zenith (<100.000 c/mL) and CD4-nadir (>200 cells/mm^3) protease inhibitor maintenance monotherapy was non-inferior to cART.[10] We found DTG maintenance monotherapy to be promising in a retrospective observational study of five patients, although no control group was used.[11] Therefore, we conducted the randomized DOMONO trial to evaluate whether a switch to DTG monotherapy would be non-inferior to continuation of cART in maintaining virological suppression in HIV-1 infected patients.

METHODS

Study design and participants

We conducted this open-label, phase 2, randomized, non-inferiority trial in two university medical centres in the Netherlands: the Erasmus MC and the University Medical Center Groningen (UMCG). Eligible patients were HIV-1 infected adults, on cART and virologically suppressed (HIV-RNA <50 c/mL) for at least 6 months at the time of screening, with an HIV-RNA zenith of less than 100.000 c/mL and a CD4-nadir of 200 cells/mm^3 or higher. A previous HIV-RNA zenith of 100.000 c/mL or more was allowed if measured during an untreated acute HIV-infection. We excluded patients with a chronic hepatitis B virus (HBV) infection or without anti-HBs antibodies and not willing to undergo HBV vaccination. We also excluded subjects with previous VF on any cART or with any documented HIV-1 resistance with at least low-level resistance according to the Stanford HIV drug resistance database.[12] Patients had to have a self-reported adherence of at least 95%. For a complete list of the inclusion

criteria and exclusion criteria, see Table 1 of the Supplementary Data. The study was approved by the Dutch competent authority and the Institutional Review Board of the Erasmus MC Rotterdam (NL51858.078.15). The study was done in accordance with Good Clinical Practice and the Helsinki Declaration. All participating subjects provided verbal and written informed consent in the language they could read (Dutch or English) before study procedures.

Randomisation and masking

We randomly assigned (1:1) eligible patients, via a web-based block randomization method (variable block sizes of 4 and 6) to switch to DTG monotherapy either immediately or after a delay of 24 weeks of continued cART (control). Randomization was stratified by HIV-RNA zenith (<50.000 c/mL or 50.000-99.999 c/mL). Because patients in the control group also switched to DTG monotherapy after 24 weeks, no randomized control group on cART was available after that timepoint. Therefore, we also collected data from a concurrent control group, which included eligible HIV-1 patients on cART who did not want to switch therapy. These patients remained in standard care for HIV-1, underwent no study procedures, and provided verbal consent for use of their clinical data for research purposes. Investigators and patients were not masked to group allocation.

Study procedures

We prescreened patients by reviewing their files for the inclusion and exclusion criteria. Eligible patients first received information about the study from their physician, and those who were interested in the study were referred to an investigator for a formal screening visit. We did clinical and laboratory assessments, including HIV-RNA, renal, urinary, and hepatic variables at weeks 0, 12, 24, 36, and 48. Additionally, HIV-RNA was also measured at weeks 4, 8, and 18 with the COBAS® ampliprep/COBAS®Taqman® HIV-1 v2 test (Roche diagnostics, Almere, The Netherlands). We defined VF as two consecutive HIV-RNA measurements of 200 c/mL or higher. We contacted all patients with an HIV-RNA of 200 c/mL or higher and retested them immediately to confirm the result. Patients with confirmed VF were taken off DTG monotherapy and restarted cART. We did Sanger sequence analysis of the integrase gene with in-house primers from EDTA-containing plasma that had been collected at the time of VF and before cART initiation, and we measured DTG plasma-concentrationsin these plasma samples. We contacted patients with a viral load above 20 but below 200 copies per mL and instructed them to take DTG with food to increase absorption, we then measured their plasma DTG levels concentrations in stored plasma to check for therapy compliance. After 48 weeks on DTG monotherapy, patients with viral loads less than 50 c/mL could choose to continue DTG monotherapy with plasma HIV-RNA measurements every 12 weeks, or to reinitiate cART. We informed patients that continuation of DTG monotherapy would be off label use and documented their consent again in the patient file. To protect the safety of the study participants, predefined stopping were the detection of resistance associated mutations

(RAMs) in the integrase gene in more than two patients during the study and failure of DTG monotherapy in more than 20 patients at any time during the study.

Outcomes

The primary endpoint of the study was the proportion of patients with plasma HIV-RNA of less than 200 c/mL at 24 weeks in the on-treatment (OT) population. The OT population consisted of all patients initiating DTG monotherapy except for those who discontinued DTG because of an adverse event while virologically suppressed at the time of DTG discontinuation. The intention to treat population (ITT) consisted of all patients who started DTG monotherapy. A temporary increase of the plasma HIV-RNA from less than 50 c/mL to 50-200 c/mL is not infrequent during cART. Furthermore, given the relatively small sample size of a phase 2 study, we expected only one to three patients with VF in each group. As such, the use of a cutoff of 50 c/mL could have led to inappropriate statistical conclusions about non-inferiority; therefore, we used 200 c/mL as cutoff for the primary analysis. Predefined secondary endpoints reported herein were the proportion of patients in the OT-population with plasma HIV-RNA of less than 50 c/mL at week 24, the proportion of patients in the entire population on DTG monotherapy after 48 weeks with plasma HIV-RNA less than 200 c/mL, the proportion of patients in the ITT population with plasma HIV-RNA of less than 200 c/mL at week 24, and the number and type of RAMs in the integrase gene of patients with confirmed HIV-RNA of 200 c/mL or higher at any time-point during DTG monotherapy. Other predefined secondary endpoints included bone, renal, and inflammatory markers and will be reported elsewhere. We registered adverse events according to the Common Terminology Criteria for Adverse Events version 4.0. Because of the study design, it would not have been fair to compare the groups for all adverse events that were not considered drug related (eg. Bronchitis, headache, diarrhea) because patients in the immediate switch group were seen or contacted seven times during the first 24 weeks, whereas patients in the delayed switch group were seen or contacted only twice. Therefore, adverse events would have been more frequent in the immediate switch group.

Statistical analyses

The sample size calculation was based on a non-inferiority design comparing DTG monotherapy with cART. Assuming virological suppression (HIV-RNA < 200 c/mL) in 95% of patients in both groups (Pa=Pb=0.95), a non-inferiority margin of 12%, 80% power and a one-sided confidence interval of 97.5% (α=0.025), a sample size of 104 patients would be needed. For the primary endpoint, we calculated 95% exact confidence intervals for differences in proportions.[13] In a post-hoc analysis, we used Fisher's Exact test to compare virological suppression rates between the entire population on DTG monotherapy at study discontinuation and the concurrent control population. We did all analyses with the statistical software package R (version 3.3.1). This trial is registered with ClinicalTrials.gov (NCT02401828).

RESULTS

Between March 10, 2015 and February 4, 2016, we randomly assigned 104 patients to receive immediate (N=51) or delayed (N=53) DTG monotherapy (Figure 1). Baseline characteristics were similar between the groups (Table 1).

For our concurrent control group, we recruited 152 consecutive patients who had chosen not to participate in the DOMONO study. Patients in this group had to have been available for follow-up for at least one year and have a viral load measurement available between 44 and 56 weeks after they were initially considered eligible for the DOMONO study. The entire OT-population receiving DTG monotherapy consisted of 95 patients (N=50 in the immediate switch group and N=45 in the delayed switch group, Figure 1). The OT-population assessed for the week 24 primary endpoint consisted of 50 patients in the immediate switch group and 53 patients in the delayed switch group (Figure 1).

	Immediate DTG monotherapy (N=51)	Delayed DTG monotherapy (N=53)
Male sex, N(%)	47 (92)	48 (91)
Age, median (Q1,Q3)	46 (37,56)	45 (40,51)
Transmission route, MSM, N(%)	41 (80)	41 (77)
Ethnicity, Caucasian, N(%)	44 (86)	42 (79)
cART regimen before switch, N(%)		
NNRTI + 2 NRTI	41 (80)	43 (81)
PI + 2 NRTI	2 (4)	1 (2)
INI + 2 NRTI	7 (14)	9 (17)
Other	1 (2)	0 (0)
Receiving an STR, N(%)	32 (63)	41 (77)
On TDF before switch, N(%)	44 (86)	45 (85)
Median (Q1,Q3) time on cART, months	35 (24,61)	43 (25,68)
Median (Q1,Q3) time suppressed on cART, months	31 (20,54)	39 (21,60)
Median (Q1,Q3) HIV-RNA zenith, copies per mL	29.300 (14.800-76.900)	44.877 (16.100-63.100)
Median (Q1,Q3) CD4 T-lymphocyte nadir, cells/mm^3	320 (250-490)	380 (285-515)

Table 1. Baseline characteristics of the immediate DTG monotherapy and the delayed DTG monotherapy group. MSM=Men having Sex with Men, NNRTI=Non-Nucleoside Reverse Transcriptase Inhibitor, NRTI=Nucleoside Reverse Transcriptase Inhibitor, PI=Protease Inhibitor, INI=Integrase Inhibitor, STR=Single Tablet Regimen, TDF=Tenofovir Disoproxil Fumarate, cART= combination AntiRetroviral Therapy.
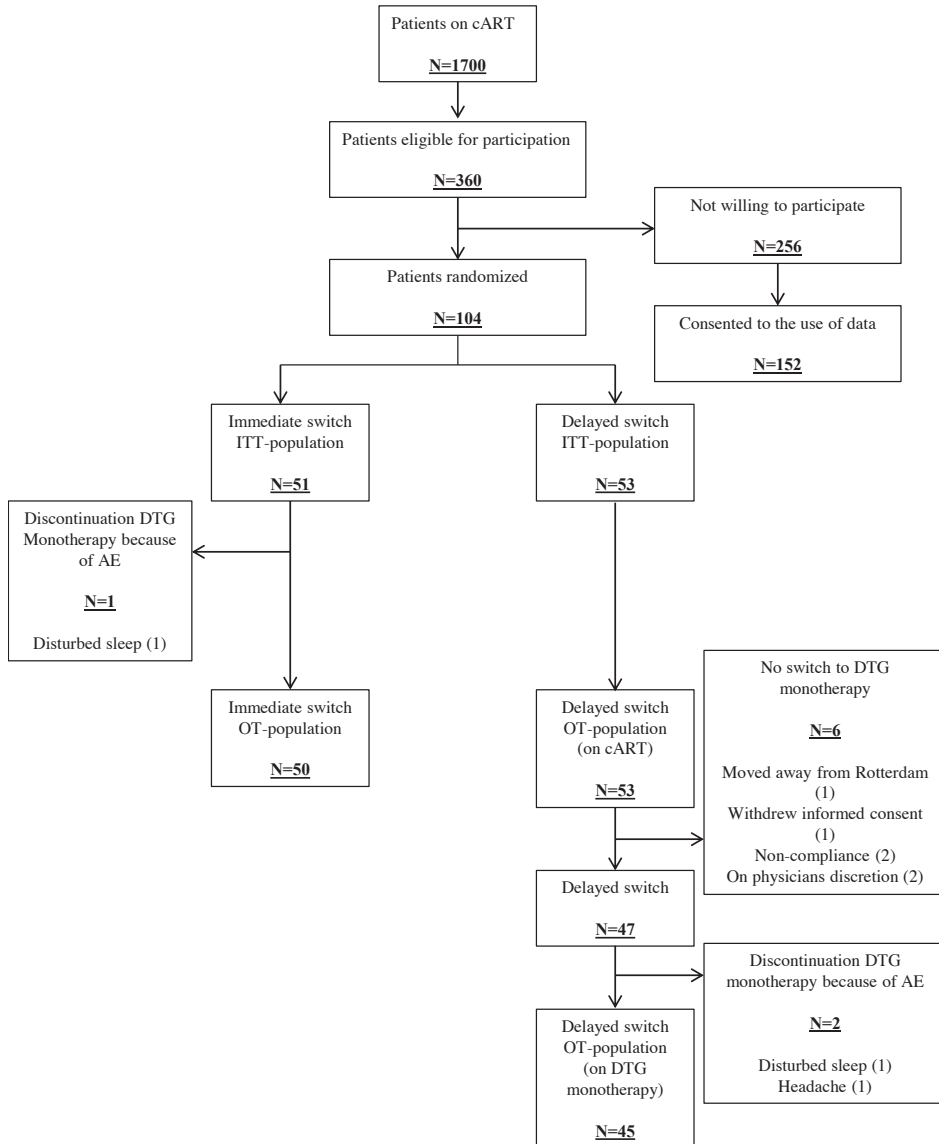
Figure 1. Patient disposition in the study. cART=combination AntiRetroviral Therapy, ITT=intention to treat, OT=on treatment, DTG=dolutegravir, AE=adverse event.

At 24 weeks, the proportions of patients with plasma HIV-RNA of 200 c/mL or higher were 2% (1/50) in the immediate DTG monotherapy group and 0% in the delayed DTG monotherapy group (difference 2%, exact 95% confidence interval -5% to +12%; Figure 2). The plasma HIV-RNA of the single patient in the immediate DTG monotherapy group who had VF was 71.600 c/mL, detected at week 4 of DTG monotherapy. The patient had a self-reported adher-

Virological suppression at week 24 OT

D = 2%, Exact 95% CI [+12%,-5%]
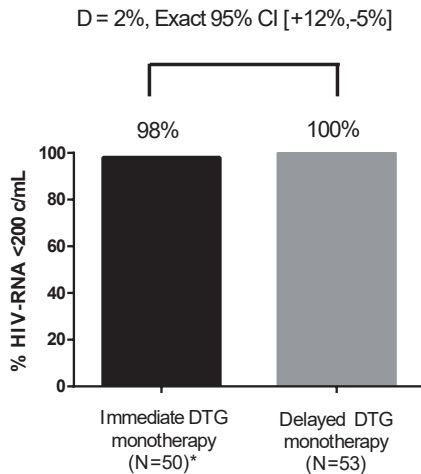
Figure 2. Percentages of virological suppression at week 24: on treatment analysis. * 1/51 patients discontinued DTG monotherapy at week 12 (HIV-RNA < 50 c/mL) because of disturbed sleep.

ence of 100%, an adequate DTG plasma-concentration (1.29 mg/mL, measured 14 hours after intake), and the integrase sequence showed no DTG-RAMs. However, we realize that even a 100% correct pill count and adequate plasma-concentrations at preplanned blood draws cannot exclude temporary incompliance with certainty. The patient reinitiated cART with single-tablet tenofovir disoproxil fumarate, emtricitabine, and rilpivirine, and had a plasma HIV-RNA of less than 50 c/mL within 12 weeks after cART reinitiation (Table 2). After 24 weeks of treatment, 8% (4/50) of the patients in the immediate DTG monotherapy group and 0% of the 53 patients in the delayed switch group had a plasma HIV-RNA of 50 c/mL or higher (difference 8%, exact 95% confidence interval [-1%, +20%]). In the ITT-analysis at week 24, 4% (2/51) of patients in the immediate switch group and no patients in the delayed switch group had a plasma HIV-RNA of 200 c/mL or higher (difference 4%, exact 95% confidence interval [-4%, 15%]).

Of the 95 patients on DTG monotherapy in the OT-population, 78 (82%) had reached the week 48 endpoint when we decided to discontinue the study early in agreement with the pre-defined stopping criteria. At that time, eight patients on DTG monotherapy had VF; N=6 in the immediate switch group and N=2 in the delayed switch group (Table 2). Two patients had VF before week 24 and the other six had VF after week 24 (Table 2). In all patients with VF, DTG plasma-concentrations were therapeutic and self-reported adherence was greater than 95% (Table 2). In patient 7, a period of suboptimal DTG plasma-concentrations might have occurred because of gastro-enteritis. Integrase sequencing was successful in six patients who had VF, three of whom had RAMs in the integrase gene, including a R263K in the patient

	Duration of DTG monotherapy at the time of failure (weeks)	HIV-RNA zenith (copies/mL)	CD4-T-lymphocyte nadir (cells/mm^3)	cART before DTG monotherapy	Time suppressed* on cART before DTG monotherapy (years months)	HIV-RNA at failure on DTG monotherapy (copies/mL)	DTG-plasma concentration at failure (mg/mL)§	Adherence (self-reported)	IN sequence at failure
Failure 1	4	18.500	290	TDF/FTC/RPV	2Y5M	71.600	1·29 (+14h)	>95%	No RAM's
Failure 2	12	7.420	220	TDF/FTC/EFV	8Y7M	678	2·00 (+19h)	>95%	Not successful
Failure 3	30	17.500	280	TDF/FTC/RPV	3Y11M	3.510	2·59 (+16h)	>95%	No RAM's
Failure 4	30	99.270	330	TDF/FTC/RPV	1Y10M	1.570	2·96 (+22h)	>95%	**S230R**
Failure 5	36	56.300	210	TDF/FTC/DTG	4Y0M	1.440	1·00 (+24h)	>95%	Not successful
Failure 6	48	67.000	230	TDF/FTC/RPV	5Y4M	4.990	1·44 (+24h)	>95%	No RAM's
Failure 7	60	34.600	240	TDF/FTC/NVP	7Y0M	3.470	0·70 (+13h)	>95%**	**R263K**
Failure 8	72	20.100	380	TDF/FTC/NVP	1Y2M	4.180	2·15 (+9h)	>95%	**N155H**

Table 2. Overview of characteristics of the patients with virological failure. TDF=Tenofovir Disoproxil Fumarate, FTC=Emtricitabine, RPV=Rilpivirine, EFV=Efavirenz, DTG=Dolutegravir, NVP=Nevirapine, RAM=Resistance Associated Mutation, IN=integrase. *Suppressed is defined as HIV-RNA <50 copies/ml. §The hours mentioned after the DTG-plasma concentration was measured after the last DTG intake. **Probably suboptimal gastrointestinal uptake of DTG during 10 days due to gastroenteritis.

with VF at week 60 and the N155H in the patient with VF at week 72. In the patient with VF at week 30, the S230R mutation was detected (Table 2). Integrase sequencing of stored plasma collected before initiation of cART showed that these mutations were not present at that time. After the decision was made to stop the study prematurely, all participants were contacted by the study team and were instructed to reinitiate their previous cART regimen. Another informed option was addition of two nucleoside reverse transcriptase inhibitors to DTG, if the subject's plasma HIV-RNA was still undetectable.

We followed up 83 (the five patients who had VF before week 48 plus 78 other patients) of the 95 patients who received DTG monotherapy for at least 48 weeks. When the study was discontinued, 77 (93%) of these patients had an HIV-RNA of less than 200 c/mL, and the last HIV-RNA to be measured was less than 50 c/mL in 76 patients (92%). In only one of the eight patients who had VF, the VF was preceded by two consecutive HIV viral loads of greater than 50 c/mL. Therefore, in the seven other patients with a confirmed viral load of 200 c/mL or higher, VF occurred suddenly with no preceding low-level viral replication.

Of the 61 patients who completed the 48 week 48 follow-up and were virologically suppressed at that time, 59 chose to continue DTG monotherapy, whereas two preferred to switch back to cART. One patient had VF at week 48 and also reinitiated cART. Sixteen patients were in the 44-48 week follow-up window when the study was discontinued, and therefore, were not given the option to continue DTG monotherapy. We measured HIV-RNA measurements every 12 weeks thereafter, and the median follow-up in the 59 patients who continued DTG monotherapy was 64 weeks. In two of them, VF occurred after 48 weeks; the remaining 57 patients had HIV-RNA of less than 50 c/mL.

DTG monotherapy was inferior to cART in a post-hoc analysis comparing the overall virological suppression rate (<200 c/mL) in patients in the concurrent control group (149/152, 98%) with that in the 95 patients on DTG monotherapy in the OT-population at week 48 (87/95, 92%) (difference 6%, Exact 95% CI 0.5, 14.5, p=0·02, Figure 3 and Figure 3 of the Supplementary Data).

Virological suppression:
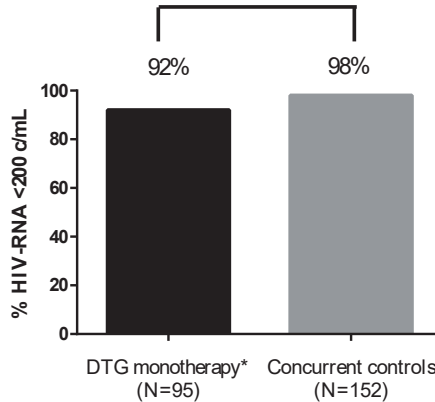**Entire study population on DTG monotherapy versus concurrent controls**

Figure 3. Percentages of virological suppression in entire study population: on treatment analysis. *8/53 patients in the delayed DTG monotherapy group did not switch to DTG monotherapy and were long enough to follow-up for inclusion in the OT-analysis, which brings the total number of patients on DTG to 95.

DISCUSSION

DTG monotherapy was non-inferior to cART in maintaining virological suppression for 24 weeks, with VF recorded in only one patient in the group of patients who switched immediately to DTG monotherapy and in no patient in the delayed switch group. No RAMs in the integrase gene were detected in the virus of the one patient with VF at week 24, and viral replication in this patient was re-suppressed soon after reinitiation of cART. Despite these promising results, VF was observed in seven additional patients after week 24, which led to virological suppression rate in 92% of patients at the time of study discontinuation. This result was statistically inferior to the 98% suppression rate observed in the concurrent control group. Because all eight patients with VF achieved re-suppression of the plasma viral load soon after reinitiation of cART, this observation alone would not contraindicate DTG monotherapy. However, the results of integrase sequencing at the time of VF clearly showed that DTG monotherapy cannot replace cART, even in patients with a CD4-nadir above 200 cells/mm^3 and an HIV-RNA zenith of less than 100.000 c/mL. In two of the six patients in whom integrase sequencing was successful, well defined resistance associated mutations were detected at position 263 and 155 of the integrase gene. Additionally, in one patient, a change at position 230 was observed, which is an accessory mutation that has been previously described in combination with other RAMs in the integrase gene of patients with VF on raltegravir or elvitegravir.[14] The three mutations could not be detected in viral RNA from

stored plasma collected before initiation of cART. The presence of acquired RAMs in the integrase gene in three of the 95 patients was inconsistent with the results from three phase 3 studies on DTG-containing cART.[2,3,15] No mutations associated with decreased susceptibility to integrase inhibitors was observed in any of the 1067 treatment-naive patients who started DTG-containing cART in the phase 3 FLAMINGO, SINGLE, and SPRING-2 studies.[2,3,15] Given the development of RAMs in the integrase gene of more than two patients on DTG monotherapy in our study, with the potential for cross-resistance to other available and future integrase inhibitors, one of the stopping rules was met and the study was terminated. Development of RAMs in the integrase gene has been extremely rare in patients on DTG previously untreated with integrase inhibitors and with a history of VF on other antiretroviral drugs; in the SAILING study, such mutations were observed in only two of 354 patients during the first 48 weeks of follow-up.[1] More recently, five small observational studies found that VF led to the development of a new RAM in the integrase gene in five of 118 patients treated with DTG monotherapy.[11,16–19] However, these studies were done in patients undergoing routine clinical care with non-standardized monitoring and without formal approval from any ethics committee.

We documented good self-reported adherence and therapeutic DTG plasma-concentrations in all patients who had VF on DTG monotherapy, and no other patient-related causes (such as intercurrent diseases or use of concomitant medications) could be identified as a possible causes of VF. However, we realize that self-reported adherence is not always reliable. The development of VF, with or without RAMs, is possibly the result of ongoing (low-level) viral replication. Therefore, DTG monotherapy seems to be insufficiently potent, and its genetic barrier to resistance insufficient, to decrease viral replication in all tissues to levels low enough to avoid development of resistance. Notably, in two of the three patients who developed resistance, we observed VF after plasma HIV-RNA levels had been below 20 c/mL at eight consecutive visits, and was therefore not preceded by documented viral replication. We can only speculate about the possible mechanisms of virological escape in the three patients in whom known RAMs in the integrase gene were not detected and therapeutic DTG plasma-concentrations were measured. Possibly, resistance to integrase inhibitors can also develop outside the integrase gene as recently described in *in vitro* experiments.[20] A similar outcome has been described in the context of resistance against protease inhibitors, wherein mutations at the protease cleavage side were involved in protease inhibitor resistance.[21]

Several hypotheses might explain the occurrence of VF in our cohort. First, the decision to start cART in patients was more often based on a decreasing CD4 T-lymphocyte count than on HIV-RNA or other factors. Therefore, HIV-RNA measurements from a time close to cART initiation were not available in four of the eight patients who had VF, meaning that the HIV-RNA zeniths in these patients just before cART intiation might have been greater than

100.000 c/mL. The multivariate analysis in the PROTEA-trial showed the relevance of this criterion, darunavir monotherapy was inferior to darunavir-containing cART in patients with an HIV-RNA of greater than 100.000 c/mL before initiation of cART.[22] However, in all three patients with RAMs in the DOMONO study, the HIV RNA plasma viral load on the day of cART initiation had been measured and was less than 100.000 c/mL in all patients, and was as low as 20.000 c/mL in one patient.

Besides the HIV-RNAzenith, an estimation of the size of the viral reservoir might be a more reliable predictor of VF during maintenance therapy with fewer drugs.[23] In the MONOI-trial, a higher baseline total HIV-DNA copy number was associated with virological rebound on darunavir monotherapy.[24] If this finding can be confirmed in future studies of maintenance therapy with fewer drugs, quantification of HIV-DNA as marker of the viral reservoir has the clear advantage as marker of the reservoir that it can be measured before simplification of cART.

Whether other mechanisms, such as differences in drug-concentrations between plasma and sanctuary sites (eg lymphoid tissue), are involved in failure of DTG maintenance mono-therapy remains unknown.[25,26] We examined other more obvious factors that could have been associated with VF during DTG monotherapy, such as time on type of cART, CD4-nadir, and height of the peak viral load before initiation of cART. Given the strict inclusion and exclusion criteria of the study, and the few events, it is not surprising that the eight patients with VF were similar to the 87 other patients with regard to these factors (p>0.05 for all). Another explanation for the development of mutations associated with resistance to DTG could be that patients had archived replication-competent viruses with pre-existing RAMs, and that this virus was reactivated during monotherapy. Finally, although plasma-concentrations of DTG were therapeutic in all patients, intermittent non-adherence could not be entirely excluded. However, even if patients had been intermittently non-adherent, a switch to DTG monotherapy in those who had been virologically suppressed on cART for years led to an unacceptable number of patients with VF and RAMs in the integrase.

Our study has several limitations. First, the study was small and was only intended to be a proof-of-concept study before designing a larger study with a smaller non-inferiority margin and a primary endpoint of 50 c/mL. Second, the 24 week delayed-switch design has been used in most randomized studies of treatment switches and has the advantage that study results are available 48 weeks after the last patient is randomized. However, several patients in this study had VF between week 24 and 48. Therefore, future switch studies should use week 48, rather than week 24, as the primary endpoint. Finally, given the very strict inclusion and exclusion criteria, the external validity is limited to a small proportion of HIV patients in care.

In conclusion, although DTG monotherapy was non-inferior to cART after 24 weeks, it led to VF in a relatively high number of patients during longer-term follow-up. Moreover, three patients with VF developed resistance to integrase inhibitors. DTG should therefore not be used as maintenance monotherapy.

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SUPPLEMENTARY DATA CHAPTER 2

Inclusion criteria	Exclusion criteria
Documented HIV-1 positive by ELISA or Western Blot or Plasma HIV-RNA >1000 copies/mL	Previous virological failure on any cART
18 years or older	Patients without anti-HBs antibodies who are not willing to undergo hepatitis B vaccination
HIV-RNA <50 copies/mL for >24 weeks	Subjects positive for hepatitis B at screening (HBsAg+)
Historical baseline HIV-RNA plasma load <100.000 copies/mL. A HIV-RNA plasma load >100.000 copies/mL is allowed, if measured during an acute HIV infection. Acute means within 6 months after a negative HIV-1 test or during the documentation of an incomplete HIV-1 Western Blot antibody-test.	No record of the historical baseline plasma viral load available
CD4-T-lyphocyte nadir ≥200 cells per μL	Subjects with concomitant CDC-C opportunistic infections within 90 days of screening
Not on strong UGT1A1 or CYP3A4 inducing agents as stated in DTG SPC	Subjects with history of allergy to INI
General medication is not interfering with trial procedures (on investigators' discretion)	Subjects with creatinine clearance <50 ml/min according to CKD-EPI
Females should have no plans of becoming pregnant during the next 18 months after baseline visit	Subjects with hepatic impairment of at least Child-Pugh B
	Exposure to experimental drug or experimental HIV-1 vaccine within 90 days of start DTG
	Screening ALT >5x ULN or ALT >3x ULN and bilirubin >2x ULN
	Patient planning or hoping to conceive a child or become pregnant during the study
	Patients who cannot take DTG 2 hours before or 6 hours after antacids, calciumcarbonate, or iron supplements

Table S1. Overview of inclusion criteria and exclusion criteria.

Reasons for not switching to DTG monotherapy	Total (N=6)
Moved away from treating hospital, N=1	Moved away from treating hospital, so was not able to comply with all scheduled study procedures.
Withdrew informed consent, N=1	Was satisfied with cART-regimen, so did not want to switch from cART to DTG monotherapy
Other, N=2	No show at scheduled visit for switch from cART to DTG monotherapy and following visits
Physician's decision, N=2	Diagnosis and surgery for prostate carcinoma during the cART period.
	In need of use of Mg^{2+}, so drug-drug interactions with DTG expected.

Table S2. Reasons for not switching to DTG monotherapy

Survival curve: percentages of virological suppression
DTG monotherapy versus Concurrent Controls
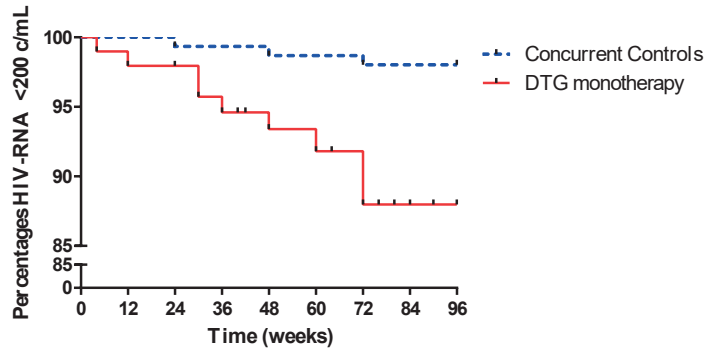
	W0	W4	W12	W24	W30	W36	W40	W42	W48	W60	W64	W72	W76	W80	W84	W90	W96
DTG monotherapy																	
No at risk	98	98	96	93	88	86	80	79	78	59	33	24	18	14	11	4	1
Censored	0	1	2	5	0	5	1	1	18	25	9	5	4	3	7	3	1
VF	0	1	1	0	2	1	0	0	1	1	0	1	0	0	0	0	0
Concurrent Controls																	
No at risk	152	152	152	152	151	151	151	151	151	150	150	150	150	150	150	150	150
Censored	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
VF	0	0	0	1	0	0	0	0	1	0	0	1	0	0	0	0	0

Figure S3. Kaplan Meier curve of percentages virological suppression (HIV-RNA < 200 copies/mL) in the entire study population on DTG monotherapy versus Concurrent Controls. DTG=dolutegravir, VF=virological failure.

Chapter 3

HIV-1 resistance dynamics in patients with virological failure to dolutegravir maintenance monotherapy.

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ABSTRACT

Introduction

A high genetic barrier to resistance to the integrase strand transfer inhibitor (INSTI) dolute-gravir has been reported *in vitro* and *in vivo*. We describe the dynamics of INSTI resistance associated mutations (INSTI-RAMs) and mutations in the 3'-polypurine tract (3'-PPT) in relation to virological failure (VF) observed in the randomized dolutegravir as maintenance monotherapy study (DOMONO, NCT02401828).

Methods

From ten patients with VF, plasma samples were collected before the start of cART and during VF, and were used to generate Sanger sequences of integrase, the 5' terminal bases of the 3' long terminal repeat (LTR), and the 3'-PPT.

Results

Median HIV-RNA at VF was 3,490 (interquartile range 1.440-4.990) c/mL. INSTI-RAMs were detected in 4 patients (S230R, R263K, N155H, and E92Q+N155H), no INSTI-RAMs were detected in 4 patients, and sequencing of the integrase gene was unsuccessful in 2 patients. The time to VF ranged from 4 to 72 weeks. In 1 patient, mutations developed in the highly conserved 3'-PPT. No changes in the terminal bases of the 3'-LTR were observed.

Conclusions

The genetic barrier to resistance is too low to justify dolutegravir maintenance monotherapy, because single INSTI-RAMs are sufficient to cause VF. The large variation in time to VF suggests that stochastic reactivation of a pre-existing provirus containing a single INSTI-RAM is the mechanism for failure. Changes in the 3'-PPT point to a new dolutegravir resistance mechanism *in vivo*.

INTRODUCTION

The second-generation integrase strand transfer inhibitor (INSTI) dolutegravir (DTG) seems to have a high genetic barrier to resistance *in vitro*, with significant loss of replication capacity in viruses with INSTI resistance associated mutations (RAMs) compared with wildtype viruses.[1,2] The high genetic barrier to DTG resistance when administered as DTG-containing cART is already demonstrated *in vivo*: no RAMs associated with decreased DTG susceptibility were observed in any of the 1067 treatment-naive patients who initiated DTG-containing cART in pivotal phase 3 studies.[3–5] In treatment-experienced but INSTI-naive patients taking a DTG-containing cART-regimen, virological failure (VF) with development of INSTI-RAMs is rare.[6,7] Furthermore, mutations selected by the first generation INSTI raltegravir (RAL) and elvitegravir (EVG) might have limited impact on DTG susceptibility, enabling successful treatment with DTG-containing cART in a large proportion of patients who previously developed VF during use of RAL- or EVG-containing cART, although dosing DTG twice daily is necessary for certain INSTI-RAMs.[7–11]

Given the high genetic barrier to DTG resistance and other favorable properties of DTG, such as few drug-drug-interactions, few adverse events, and a low pill-burden, we hypothesized that DTG could be used as monotherapy to maintain viral suppression in HIV-1 infected adults with long-term virological suppression during cART. More recently, DTG maintenance monotherapy has been investigated in small, retrospective, observational studies and case series, which showed high rates of virological suppression without emergence of INSTI-RAMs in INSTI-naive patients. These studies included a total of 118 patients, in five of whom VF led to development of INSTI-RAMs, but all five were INSTI-experienced or had treatment-compliance issues. Also, these studies were done in routine clinical care and without standardized monitoring and ethics committee approval.[12–16] As these results lacked a control arm, we conducted the randomized, controlled Dolutegravir as Maintenance Monotherapy for HIV-1 study (DOMONO, NCT02401828) and showed that DTG maintenance monotherapy in HIV-1 infected patients led to a higher rate of VF (8/95, 8%) compared with patients who continued cART (3/152, 2%).[17]

Since INSTI-RAMs in the integrase gene are rarely detected in patients with VF to DTG-containing cART,[5,6] novel DTG resistance pathways outside of the integrase gene may exist. Experiments with purified integrase enzyme and long terminal repeat (LTR) duplexes showed that mutations in the four terminal bases of the LTR could confer INSTI resistance.[18] Furthermore, *in vitro* DTG resistance selection experiments showed that mutations in the 3'-polypurine tract (3'PPT) confer high-level resistance to RAL, EVG, and DTG.[19] Both resistance pathways have not been reported yet in HIV-infected individuals. Here, we describe the dynamics of resistance-associated mutations in the integrase gene and the 3'-PPT in HIV-1 infected individuals with VF during DTG maintenance monotherapy.

METHODS

Study population

Patients with VF during DTG maintenance monotherapy were included from two studies performed in the outpatient clinic of the Erasmus University Medical Center (Rotterdam, The Netherlands) and the University Medical Center Groningen (Groningen, the Netherlands). The first group of patients consisted of participants in the DOMONO main study. The most important inclusion criteria were a CD4 T-lymphocyte nadir *above* 200 cells/mm^3, an HIV-RNA zenith below 100.000 c/mL (except when measured during the acute phase of infection), and no previous VF and/or any documented RAMs, according to The Stanford HIV Drug Resistance Database.[20] The second group of patients consisted of participants in the DOMONO pilot study. This pilot study had the same inclusion and exclusion criteria as the main study, with the exception that patients with a CD4 T-lymphocyte nadir *below* 200 cells/mm^3 were included.

Study procedures

HIV-1 RNA in plasma was quantified with the COBAS® ampliprep/COBAS®Taqman® HIV-1 v2 test (Roche diagnostics, Almere, The Netherlands). VF was defined as a confirmed plasma HIV-RNA level above 200 c/mL. Adherence was defined as the percentage self-reported adherence. DTG plasma-concentrations at the moment of VF were determined and interpreted according to the Therapeutic Drug Monitoring protocol of Radboud University Medical Center (Nijmegen, the Netherlands).[21] In all patients, Sanger sequences covering the integrase gene, and the 3'-PPT and the four 5' terminal bases of the 3'-LTR in the nef gene were determined using stored plasma samples with detectable HIV-RNA before patients started on cART (baseline samples) and in plasma samples at time of VF during treatment with DTG monotherapy. For nef amplification, RNA was isolated with the High Pure Viral RNA kit (Roche, #11858882001), according to the manufacturer's instructions. Of 60 μL of eluted RNA, 12.5 μL was used for complementary DNA synthesis, using 50 pmol oligo dT primer (20T+TRAAG, Eurogentec) and superscript IV (ThermoFisher) with the following polymerase chain reaction (PCR) conditions: 5 minutes at 30°C, 2 hours at 50°C, and 10 minutes 80°C. After RNase H inactivation (by incubation for 20 minutes at 37°C and 15 minutes at 70°C), 5 μL of complementary DNA was used for PCR analysis. Primers were as follows: HXB2-8343for//T (or C): AGAGTTAGGCAGGGATAT(C)TCACC and HXB2-9632rev: GCACTCAAGGCAAGCTTTATTGAGGCT. PCR conditions were as follows: 2 minutes at 94°C and 40 cycles of 10 seconds 94°C, 30 seconds at 60°C, 1 minute at 68°C, and 10 minutes at 68°C (extension). PCR samples were separated on 1% agarose, and bands were cut and extracted with QIAEX II gel extraction kit (Qiagen) before sequencing. For integrase sequencing, nucleic acids were extracted using MPLC (Roche) after ultracentrifugation of 1.5 mL ethylenediaminetetraacid-plasma at 26.000xg for 1 hour at 4°C. Subsequently, the HIV-1

integrase gene was amplified with the OneStep RT-PCR kit (Qiagen, Venlo, The Netherlands) and 20 pmol of the primers described in supplementary Table 1, according to manufacturer's protocol, using the following thermal profile: 30 minutes at 50°C, 15 minutes at 95°C, and 25 cycles of 1 minute at 95°C, 1 minute at 55°C, 2 minutes at 72°C, and 10 minutes at 72°C. Nnested PCR was then performed with the HotStar HiFidelity Polymerase kit according to the manufacturer's protocol and the following thermal profile: 5 minutes at 95°C and 30 cycles of 15 seconds at 94°C, 1 minute at 50°C, 2 minutes at 72°C and 10 minutes at 72°C. One microliter of the amplicon was sequenced using the BigDye Terminator v3.1 cycle sequencing kit (Applied Biosystems, Nieuwerkerk a/d IJssel, The Netherlands) and 5 pmol of sequencing primers described in supplementary Table 1. The sequenced PCR products were purified using Performa DTR V3 purification plate (Edgebio, Sopachem, Ochten, The Netherlands) and analyzed on an ABI 3130XL sequencer (Applied Biosystems). The sequence data were analyzed using a Sequence Navigator software sequencer (Applied Biosystems) and SeqMan v10.1.2 (DNASTAR, Madison,WI). Resulting HIV-1 integrase sequences were further analyzed using the Stanford database to check for known drug RAMs, and baseline sequences were compared to rebound sequences using BioEdit, v7.2.0.[20]

Ethical considerations

The DOMONO main study and its pilot study were approved by the Dutch Medical Ethics Committee and were performed in accordance with good clinical practice and the Helsinki Declaration. All participating subjects provided verbal and written informed consent before study procedures.

RESULTS

Patient population and baseline characteristics

Eight of 95 participants of the DOMONO main study had VF. Of the four participants of the DOMONO pilot study, two had VF. In total, 10 patients experienced VF during DTG maintenance monotherapy. Baseline characteristics at entry of DTG maintenance monotherapy study are listed in Table 1. All participants were male with a median age of 46 years and were infected with HIV-1 subtype B. The median HIV-RNA zenith was 29.750 c/mL and the median CD4 T-lymphocyte nadir was 235 cells/mm^3. In three patients (patient 5, 9, 10) cART was initiated within a year after the HIV-diagnosis. Nine of the participants with VF were INSTI-naive, whereas one patient (patient 3) was previously treated successfully with RAL-containing cART and DTG-containing cART. The median duration of plasma HIV-RNA suppression (defined as a plasma HIV-RNA lower than 50 c/mL) during cART (ie. before initiation of DTG maintenance monotherapy) was 61 months.

	Patients (N=10)
Male sex, N (%)	10 (100)
Age, years, median (Q1,Q3)	46 (39,52)
Mode of transmission, N (%)	
MSM	7 (70)
HSX	2 (20)
Other	1 (10)
Ethnicity, N (%)	
Caucasian	7 (70)
Caribbean/Surinam	3 (30)
Time on cART, median (Q1, Q3), months	71 (47, 104)
Time suppressed on cART, median (Q1, Q3), months	61 (41, 101)
INSTI-naive, N (%)	9 (90)
HIV-1 subtype B, N (%)	10 (100)
HIV-RNA zenith, median (Q1, Q3), c/ml	29.750 (18.250, 66.625)
CD4 T-lymphocyte nadir, median (Q1, Q3), cells/mm^3	235 (183, 300)

Table 1. Baseline characteristics of patients with virological failure on dolutegravir maintenance monotherapy. DTG=dolutegravir, MSM=men having sex with men, HSX=heterosexual, cART=combination antiretroviral therapy, INSTI=integrase strand transfer inhibitor.

Characteristics of virological failure during DTG maintenance monotherapy

Clinical and virological characteristics at the moment of VF are shown in Table 2 and 3. Median HIV-RNA (IQR) at VF was 3.490 (1.440-4.990) c/mL. Interestingly, the time to VF after the start of DTG maintenance monotherapy varied considerably, with patient 4 already experiencing VF at week 4 and patient 7 experiencing VF at week 72. The median time to VF was 33 weeks. Self-reported adherence was more than 95%, and DTG plasma-concentrations during DTG maintenance monotherapy were therapeutic (i.e. above 0.50 mg/L) in all patients at time of VF. Two patients received co-medication (patient 3: oxazepam, buprenorphine/naloxone, fluticasone, quetiapine, levomepromazine, and prednisolone; patient 5: simvastatin, acetylsalicylic acid, tolbutamid, perindopril, and metoprolol), but no drug-drug-interactions were detected. No INSTI-RAMs were detected in the samples obtained from patients before they started therapy. At the moment of VF detection, INSTI-RAMs were detected in patients 2, 5, 7, and 9, whereas no known INSTI-RAMs were detected in patients 1, 4, 8, and 10; integrase gene sequencing was unsuccessful in patients 3 and 6 owing to low plasma HIV-RNA levels of 1.440 and 678 c/mL respectively. Single INSTI-RAMs were detected in three patients: S230R (in patient 2), R263K (in patient 5), and N155H (in patient 7). In patient 9, the combination of E92Q and N155H was detected. Figure 1 shows the courses of plasma HIV-RNA levels and the INSTI-RAMs detected during treatment with DTG maintenance monotherapy.

Patient	Study	Relevant medical history	HIV-RNA zenith (c/ml)	CD4-T-lymphocyte nadir (cells/mm³)	cART before start DTG*	INSTI naive*	Interval between HIV-diagnosis and start cART (months)	Time suppressed on cART before DTG* (months)	HIV-RNA at VF (c/mL)	DTG-plasma level at VF in mg/L$	Adherence (according to clinician)
4	Main	-	18.500	290	TDF/FTC/RPV	Yes	27	29	71.600	1.29 (+14h)	>95%
6	Main	-	7.420	220	TDF/FTC/EFV	Yes	28	103	678	2.00 (+19h)	>95%
10	Pilot	-	66.500	100	TDF/FTC/RPV	Yes	1	59	798	5.31 (+19h)	>95%
1	Main	-	17.500	280	TDF/FTC/RPV	Yes	66	47	3.510	2.59 (+16h)	>95%
2	Main	-	99.270	330	TDF/FTC/RPV	Yes	51	22	1.570	2.96 (+22h)	>95%
3	Main	Psychiatric problems, alcohol-abuse, ablatio retinae with operation.	56.300	210	TDF/FTC/DTG	No (RAL, DTG)	105	48	1.440	1.00 (+24h)	>95%
9	Pilot	-	24.900	70	TDF/FTC/EFV	Yes	0	100	54.200	0.86 (+16)	>95%
8	Main	-	67.000	230	TDF/FTC/RPV	Yes	47	64	4.990	1.44 (+24h)	>95%
5	Main	POAD, coronary artery disease, DM2	34.600	240	TDF/FTC/NVP	Yes	7	84	3.470	0.70 (+13h)	>95%
7	Main	-	20.100	380	TDF/FTC/NVP	Yes	58	14	4.180	2.15 (+9h)	>95%

Table 2. Overview of clinical characteristics of patients failing dolutegravir maintenance monotherapy. VF=virological failure, INSTI=integrase strand transfer inhibitor, DTG=dolutegravir, TDF=tenofovir disoproxil fumarate, FTC=emtricitabine, RPV=rilpivirine, EFV=efavirenz, RAL=raltegravir, NVP=nevirapine, cART=combination antiretroviral therapy, POAD=peripheral occlusive arterial disease, DM2=Diabetes Mellitus type 2. DTG*=DTG maintenance monotherapy. $ Timing of sample in hours after last DTG intake.

Patient	Study	Time to VF*	INSTI-RAMs detected at VF	INSTI-RAMs detected at baseline	Additional changes in integrase at VF compared to baseline
4	Main	4	No	No	VI32I, LS45L, T112A
6	Main	12	below limit of detection	No	-
10	Pilot	24	No	No	IV72V, LI101I
1	Main	30	No	No	A10D, D11E, N17S, L45Q, I50M, T111K, A112T, T124S, I135V, KR211K, I220L
2	Main	30	S230R	No	EV13E, LV45L
3	Main	36	below limit of detection	No	-
9	Pilot	42	E92Q, N155H	No	T112I
8	Main	48	No	No	G24GNDS, R111RK, A124AT, A229D, S283G
5	Main	60	R263K	No	ED10E, ED11E, L45I, F121FV, RK231K, LF234L
7	Main	72	N155H	No	D41G, T111A, S119R, N155H, M208I

Table 3. Virological characteristics and mutations in the integrase gene of HIV from patients with VF on dolutegravir maintenance monotherapy. VF=Virological Failure, DTG=dolutegravir, INSTI-RAMs=Integrase Strand Transfer Inhibitor – Resistance Associated Mutations. *In weeks after start DTG maintenance monotherapy.
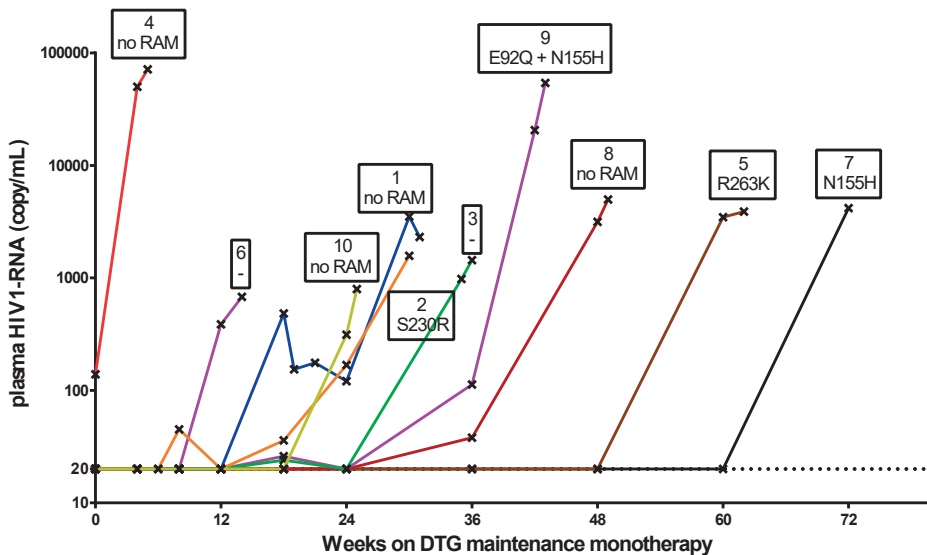

Figure 1. Overview of the course of the plasma HIV-RNA and INSTI-RAMs in all patients with VF. The dotted line represents the limit of quantification of plasma HIV-RNA (20 copy/mL). -=sequencing of integrase gene unsuccessful due to low plasma HIV-RNA level.

To investigate the role of an alternative resistance mechanism, we sequenced the nef gene, including the 3'-PPT and four 5' terminal bases of the 3'-LTR, because mutations in these sites can confer resistance to INSTIs.[18,19] No mutations were observed in the four 5' terminal bases of the 3'-LTR at baseline or at VF in any of the patients. In 9 of 10 patients, we did not detect changes in the 3'-PPT located in the nef gene. However, in patient 10, who did not develop INSTI-RAMs in the integrase gene, the wildtype G-stretch of the 3'-PPT was observed at baseline (i.e. GGGGGG), but at VF two changes were detected, i.e. GGG**A**G**C** (Figure 2).
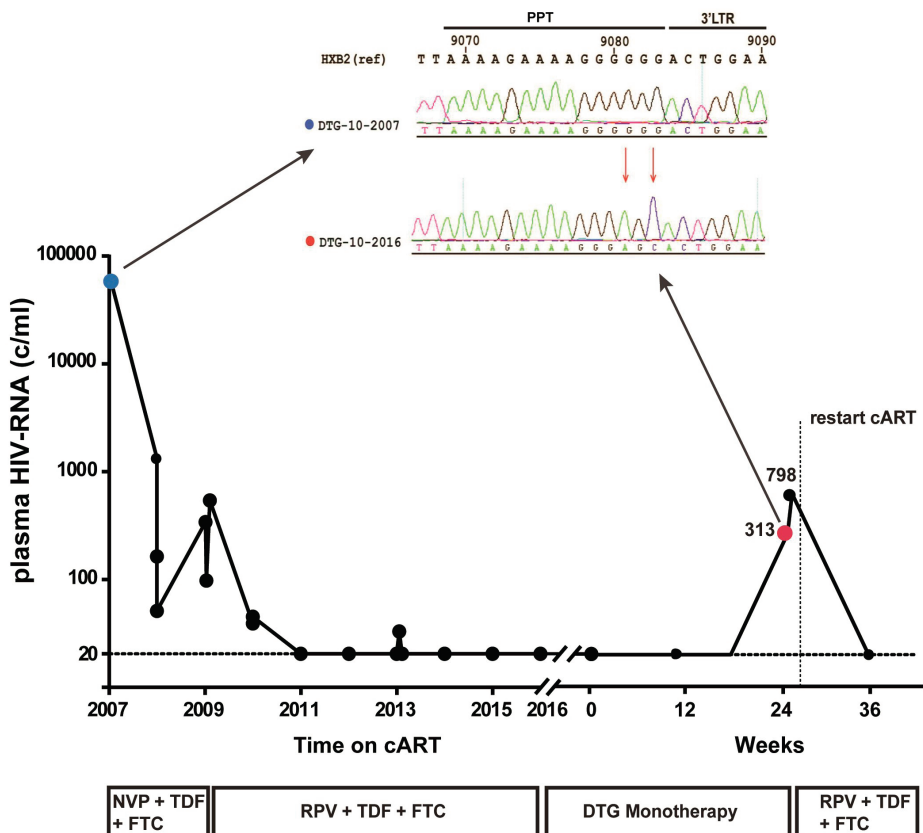
Figure 2. Changes in the G-stretch of the 3'-PPT in patient 10 at VF and course of plasma HIV-RNA. NVP=nevirapine, TDF=tenofovir disoproxil fumarate, FTC=emtricitabine, RPV=rilpivirine, DTG=dolutegravir. DTG-10-2007 is the sequence generated from HIV-RNA in plasma prior to cART initiation. DTG-10-2016 is the sequence generated from HIV-RNA in plasma at VF during DTG maintenance monotherapy. HXB2 is the sequence from the HXB2 reference strain.

Virological response after restart of cART

At the moment of confirmed VF, DTG maintenance monotherapy was stopped, and all patients restarted their previous cART regimen. The patients responded well to cART reinitiation, including the patient who restarted with dolutegravir, emtricitabine, and tenofovir. In all patients, plasma HIV-RNA levels declined to lower than 20 c/mL and remained lower than 20 c/mL for a mean of 39.7 weeks of follow-up.

DISCUSSION

In the DOMONO study, we recently showed that a higher proportion of patients treated with DTG maintenance monotherapy experienced VF, compared with patients who continued to receive cART.[17] In addition, 50% (2/4) of participants in the subsequent DOMONO pilot study, in which patients had a CD4 T-lymphocyte nadir of lower than 200 cells/mm^3, had VF. The same observation was previously made during protease inhibitor maintenance monotherapy.[22] Moreover, of the ten patients with VF in the main study and pilot study combined, we observed the emergence of INSTI-RAMs in four INSTI-naive patients: N155H (in patient 7), E92Q + N155H (in patient 9), R263K (in patient 5), and S230R (in patient 2). These results are in contrast with findings of observational cohorts, in which VF during DTG maintenance monotherapy often occurred in INSTI-experienced patients, in patients with previous VF during INSTI-containing therapy, and/or in patients with suboptimal adherence.[12–16] Only Blanco et al are reporting VF with emergence of INSTI-RAMs in INSTI-naive patients.[23] Emergence of the R263K mutation has previously been observed in the SAILING study in treatment-experienced, INSTI-naive patients who experienced VF on DTG-containing cART[6,24], and in the VIKING study, the N155H mutation emerged in patients with HIV resistant to RAL and/or EVG, who were treated with DTG-containing cART.[11,25] Furthermore, emergence of the N155H and E92Q+N155H was noted in the retrospective, observational Redomo-study in patients with VF on DTG maintenance monotherapy.[23] To our best knowledge, the S230R mutation has been described previously in one patient experiencing VF during DTG-containing cART.[26] Interestingly, development of the S230R mutation was observed in 1 of 5 HIV-1 infected humanized mice treated with DTG monotherapy. However, in that study, the S230R mutation was detected in combination with E138K, G140S, Q148H and N155H.[27] *In vitro*, the N155H, N155H + E92Q and the R263K mutation caused 1-2-, 2.5-, and 2-4-fold changes in the IC$_{50}$ of DTG and conferred only low-level resistance to DTG.[9,28,29] Pham et al. characterized the S230R substitution and showed that it resulted in a 4-fold change in IC$_{50}$ of DTG in tissue culture resulting in low level resistance to DTG.[30] When combining these results with our observations *in vivo*, it appears that single INSTI-RAMs that result in a relatively small fold increase in the IC$_{50}$ of DTG are sufficient to cause VF in patients receiving DTG maintenance monotherapy. In our study, plasma HIV-RNA levels were relatively low in eight

of the ten patients who experienced VF (lower than 1000 c/mL) compared with their pre-cART HIV-RNA zenith. This suggests that DTG still exhibited partial antiretroviral activity. It should be noted that our patients received DTG 50 mg once daily and that treatment with higher doses of DTG may be sufficient to overcome this partial resistance and fully suppress replication of these viruses.[11]

In four patients with VF, no known INSTI-RAMs were detected (patient 1, 4, 8, and 10). For these patients, we compared the pre-cART integrase sequence with the integrase sequence during VF and found amino acid changes at the following positions: E10, E11, S17, S24, V32, L45, M50, I72, L101, K111, T112, T124, I135, K211, I220, D229, and S283. Mutations at these positions occur at frequencies of more than 1% in INSTI-naive patients.[31] These mutations have not been shown to increase the IC_{50} of DTG *in vitro*,[31-33] although we cannot exclude that certain combinations of these mutations result in a decreased susceptibility to DTG. VF could also be explained by therapy non-adherence, but this seems unlikely because only therapy-adherent patients with no history of VF were included in our study, and DTG plasma-concentrations were therapeutic at the time of VF.

To find an explanation for VF, we searched for mutations outside of the integrase gene that may confer INSTI resistance in all ten patients. Therefore, we sequenced the 5' terminal bases of the U3 region of the LTR (i.e. ACTG), which are the substrate for integrase in the integration process.[18] Dicker et al showed with purified integrase enzymes and LTR duplexes that certain mutations in the four terminal bases of the LTR led to decreased binding of INSTI to integrase, while the strand-transfer activity of integrase remained relatively intact.[18] These mutations have not been reported in HIV culture experiments or *in vivo*. In this study, we did not detect changes in the ACTG motif of the 3'-LTR in patients with VF.

Simultaneously, we sequenced the 3'-PPT region adjacent to the 3'-LTR, because mutations in this region have been linked to INSTI resistance.[19] Malet et al recently showed in *in vitro* HIV culture experiments involving high doses of DTG that mutations located in the nef gene led to INSTI resistance.[19] The AàC mutation located 6 nucleotides upstream of the 3'-PPT motif in combination with four changes in the G-stretch of the 3'-PPT motif (GGGGGG à **GCAGTdel**), conferred high level resistance to DTG, RAL, and EVG. In our study, we detected mutations in the G-stretch of the 3'-PPT in the virus from patient 10 that were not present at baseline (GGGGGG à GGGA**GC**). Interestingly, a high DTG plasma-concentration was detected during VF in this patient, and the mutations in 3'-PPT described by Malet et al were also selected using high DTG concentrations *in vitro*. In addition, no known INSTI-RAMs in the integrase gene were detected in this patient.

The 3'-PPT is more than 99.9% conserved among HIV-1 sequences in the Los Alamos database, as it serves as hybridization site for the RNA primer for plus-strand DNA synthesis during reverse transcription. Removal of these RNA primers is pivotal because it defines the end of the linear proviral DNA for integration.[34] The GGGGGG à GGGAGC mutations in the 3'-PPT observed in patient 10 result in a glycine to alanine change in the Nef protein, and the mutations in the 3'-PPT described by Malet et al result in a truncated Nef protein. It seems unlikely, however, that the Nef protein plays a role in INSTI resistance, since there is hardly data that the Nef protein plays any role in the proviral integration process and because a previous study reported full susceptibility to RAL of Nef-deficient HIV.[35] The terminal 200–250 base pairs of each proviral DNA end are the primary protein binding sites of the HIV intasome.[36,37] Since the 3'-PPT in the unintegrated linear proviral DNA intermediate is located approximately 9000 base pairs from the 5'-LTR and approximately 800 base pairs from the end of the 3'-LTR, it is unlikely that the 3'-PPT interacts with the HIV intasome.

Notwithstanding the findings above, the 3'-PPT plays a major role in defining the proviral DNA end of the 5'-LTR. Previous studies on the reverse transcription process showed that mutations in the G-stretch of the 3'-PPT can lead to incomplete removal of the 3'-PPT RNA primer by RNAse H or to an alternative starting point for plus-strand DNA synthesis.[34,38-40] Both scenarios would lead to an altered end of the unintegrated linear proviral DNA intermediate at the 5'-LTR site. In addition, production of viral particles from unintegrated proviral DNA has been proposed. In patient 10, we observed the emergence of different mutations in the 3'-PPT, compared with the virus described by Malet et al. This indicates that other mutations in 3'-PPT may also cause INSTI resistance. However, experiments with site-directed mutants are needed to prove that the GGGGGG à GGGAGC mutations in 3'-PPT indeed cause INSTI resistance. In addition, further studies are needed to unravel the mechanism of INSTI resistance caused by mutations in 3'-PPT.

Still, for three patients we could not find a cause for virological failure. Of note is patient 4 who had adequate plasma DTG levels and already failed four weeks after start of DTG maintenance monotherapy with a plasma HIV-RNA level of 71.600 c/mL. Ultra-deep sequencing of plasma HIV-1 RNA or proviral DNA in peripheral blood mononuclear cells during VF might show minority variants that harbor RAMs in integrase or 3'-PPT, which could partially explain VF. Unfortunately, samples needed for these analyses were not available. VF in these patients might also be explained by yet another novel resistance pathway, which would require whole-virus genome sequencing at baseline and during VF, and phenotyping of the emerged mutations with site-directed mutants. In addition, VF in these patients could be explained by replication of HIV in sanctuary sites in which DTG does not penetrate with subsequent spillover of HIV to peripheral blood.

The origin of the viruses detected during VF in this study is currently unknown. These viruses might originate from ongoing low level replication in sanctuary sites during cART that are not detectable in plasma. However, Joos et al showed that after patients with long-term virological suppression (plasma HIV-1 RNA lower than 50 c/mL) stopping cART, the rebound viruses detected in plasma originate from reactivation of latently infected cells instead of viral lineages that continuously replicate at low levels.[41] Reactivation of HIV from latently infected cells is a stochastic process which occurs on average every 5–8 days, and depends on the size of the replication competent viral reservoir. This may also explain why two of the four patients who had a CD4 T-lymphocyte nadir lower than 200 cells/mm^3 and were included in the DOMONO pilot study, went on to have VF. Indeed, a low CD4 T-lymphocyte nadir is correlated with a larger viral reservoir.[42,43] Owing to the error-prone nature of HIV replication, minority populations with drug resistant mutants harboring single resistance mutations are most likely generated before therapy is initiated and persist in the proviral archive.[44,45] We hypothesize that the viruses detected during VF in this study originate from stochastic reactivation of a single cell harboring a provirus with a preexisting INSTI-RAMs. Reactivation of wild type virus from latently infected cells will not result in further rounds of replication, because these viruses are inhibited by DTG. However, reactivation of a virus carrying a single INSTI-RAM is not fully inhibited by DTG and further rounds of replication will lead to detectable HIV-RNA levels in plasma. This hypothesis is supported by the large variation observed in the timing to failure due to stochastic nature of reactivation of proviruses carrying a single INSTI-RAM.

In conclusion, the genetic barrier to DTG resistance is too low to justify DTG maintenance monotherapy, even in treatment-adherent patients with a relatively low HIV-RNA zenith and high CD4 T-lymphocyte nadir. The exact mechanism of VF in patients with long-term virological suppression who switch to DTG maintenance monotherapy is currently unclear, but we propose stochastic reactivation of a single cell harboring a provirus with a pre-existing INSTI-RAM as mechanism for VF. Mutations in the G-stretch of the 3-PPT region might confer an alternative DTG resistance pathway.

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SUPPLEMENTARY DATA CHAPTER 3

Primer	Sequence (5'-3')	Position according to HXB2*
Primers outer PCR		
5'INoutF1	ggAATCATTCAAgCACAACCAgA	4059-4081
3'INoutR2	TGTATGCAGACCCCAATATGTT	5262-5241
Primers nested PCR		
5'INinF1	TATCTggCATgggTACCAgCAC	4143-4164
3'INinR1	TAgTgggATgTgTACTTCTgAAC	5217-5195
Sequence primers		
KVL076	GCACAYAAAGGRATTGGAGGAAATGAAC	4161-4188
KVL082	GGVATTCCCTACAATCCCCAAAG	4647-4669
INT3	TTCGGGTTTATTACAG	4897-4912
INTSeq5	CTGGCTACATGAACTGCTAC	4470-4452
INT4	CTTGTATTACTACTGC	4986-4971
3'INinR1	TAgTgggATgTgTACTTCTgAAC	5217-5195

Table S1. Primers for integrase sequencing. * Genbank accession no. K034550.

Chapter 4

Predictors of virological failure in HIV-1 infected patients switching to dolutegravir maintenance monotherapy.

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ABSTRACT

Introduction

The Dolutegravir Maintenance Monotherapy for HIV-1 (DOMONO; NCT02401828) study showed that maintenance monotherapy with dolutegravir (DTG) is associated with virological failure (VF) and leads to DTG resistance, and as a result should not be used. However, data on clinical and virological factors associated with VF during DTG monotherapy are lacking. We identified factors associated with VF during DTG monotherapy.

Methods

A **r**andomized trial was carried out in which patients on combination antiretroviral therapy (cART) with an HIV-1 RNA zenith <100.000 c/mL, a CD4 T-lymphocyte nadir ≥200 cells/mm^3, who had never experienced VF, switched to DTG monotherapy. Clinical and virological factors were compared between patients with and without VF, using univariable analyses.

Results

Eight of the 95 patients developed VF during DTG monotherapy. A total of 78 participants had reached week 48 when the study was discontinued. The median (IQR) CD4 T-lymphocyte nadir was lower in patients with VF than in patients without VF (260 (223-320) cells/mm^3 versus 380 (290-520) cells/mm^3, respectively, p=0.011). Patients with VF had a longer time between HIV-diagnosis and cART initiation than those without VF (49 (27-64) versus 15 (1-38) months, respectively, p=0.015). The median total peripheral blood mononuclear cells (PBMC) HIV-DNA copy number (min, max) PBMCs was higher in patients with VF than in those without VF (417 (85-4151) versus 147 (16-4132) copies/10^6 PBMCs, respectively, p=0.022).

Conclusions

A lower CD4 T-lymphocyte nadir, a longer time between HIV diagnosis and cART initiation, and a higher HIV-DNA copy number at the time of DTG monotherapy initiation were associated with VF. While there clearly is no future role for DTG monotherapy, ongoing and future studies on the efficacy of maintenance dual therapy (e.g. DTG with lamivudine) may have to take these variables into account in their study design and analysis.

INTRODUCTION

Dolutegravir (DTG) based combination antiretroviral therapy (cART) is one of the preferred treatment options in current guidelines for HIV-1 treatment. Given the high genetic barrier to resistance of DTG maintenance monotherapy based on previously determined *in vitro* data,[1–5] we studied its efficacy in the Dolutegravir Monotherapy for HIV (DOMONO, NCT02401828) study.[6] In the DOMONO study, 95 virologically suppressed patients on cART, selected on strict criteria regarding CD4 T-lymphocyte nadir and HIV-RNA zenith, started DTG monotherapy. The study was discontinued prematurely, because virological failure (VF) was observed in eight patients, of whom three had integrase inhibitor resistance associated mutations.[6–8] Previous studies on protease inhibitor (PI) maintenance monotherapy identified time on cART, drug adherence during monotherapy, the presence of very low-level viremia (plasma HIV-RNA less than 50 c/mL) at baseline, and CD4 T-lymphocyte nadir as predictors for failure.[9,10] Additionally, the peripheral blood mononuclear cell (PBMC) HIV-DNA copy number was associated with the risk of VF in the MONOI study and PROTEA study.[9,11] Predictors of VF during integrase inhibitor monotherapy have not been described. Here we determined which clinical and virological factors are associated with VF during DTG monotherapy.

METHODS

The DOMONO study was a randomized clinical non-inferiority trial. Participants provided written informed consent, and the study was approved by the ethics committee (METC Erasmus MC, MEC2015-043) and performed in accordance with the Helsinki Declaration. Details can be found elsewhere, but in brief, 95 patients, who were virologically supressed on cART, had never failed any antiretroviral regimen, had a CD4 T-lymphocyte nadir of at least 200 cells/mm^3 and an HIV-RNA zenith lower than 100.000 c/mL, consented to switch from cART to DTG monotherapy.[6] The primary outcome of this study was virological suppression at week 24 during DTG monotherapy, and we defined VF as a confirmed plasma HIV-RNA of more than 200 c/mL. Clinical and virological factors were compared between patients with and without VF using unpaired T-tests, Mann Whitney U Tests, and Fisher's exact tests, when applicable. As a consequence of the relatively low number of patients with VF at the time when the study was discontinued, a multivariable analysis could not be performed. Factors included were as follows: age, sex, the pre-cART HIV-RNA zenith and CD4 T-lymphocyte nadir, the CD4 T-lymphocyte count at the start of DTG monotherapy, and the time between HIV diagnosis and cART initiation. Other evaluated factors were the type of cART-regimen before switch to DTG monotherapy (non-nucleoside reverse transcriptase inhibitor versus PI- versus integrase strand transfer inhibitor-containing cART), the time on cART, whether

the patient had a detectable viral load at the start of DTG monotherapy (defined as a plasma HIV viral load of more than 20 c/mL or a detectable HIV-RNA but lower than 20 c/mL), DTG plasma-concentration, and the total HIV-DNA copy number in PBMCs at the start of DTG monotherapy. Total HIV-DNA quantification was performed by droplet digital PCR (ddPCR), as described elsewhere, and could be done on 77 patients (eight patients with VF and 69 without VF) from whom PBMC had been successfully harvested.[12–14]

RESULTS

Seventy-eight of the 95 participants had reached the week 48 endpoint when the study was discontinued prematurely in accordance with one of the predefined stopping rules. At the time of study discontinuation, VF had been observed in eight patients. Median (IQR) follow up duration was 59 (48-71) weeks and for 17 patients, including five with VF, the follow-up was shorter than 48 weeks. The characteristics of the patients with and without VF are described in Table 1.

	no VF during DTG monotherapy (N=87)	VF during DTG monotherapy (N=8)	P-value (test)
Age, years, mean (SD)	47 (11.0)	47 (11.2)	0.891 (UTT)
Male sex, N (%)	80 (92)	8 (100)	1.00 (FET)
HIV RNA zenith, copies/mL	37000 (12950,65625)	27350 (17750,64325)	0.973 (MWU)
Viremia at start DTG*, N (%)	10 (11.5)	2 (25.0)	0.266 (FET)
HIV DNA, copies/10^6 PBMCs	147 (69,338)	417 (181,837)	0.022 (MWU)
Log10 HIV DNA/10^6 PBMCs, mean (SD)	2.16 (0.53)	2.57 (0.40)	0.037 (UTT)
CD4 T-lymphocyte nadir, cells/mm^3	380 (290,520)	260 (223,320)	0.011 (MWU)
CD4 T-lymphocyte count at start DTG, cells/mm^3	650 (540,825)	830 (573,1030)	0.153 (MWU)
CD4:CD8-ratio at start DTG	1.05 (0.74,1.50)	1.41 (0.74,2.00)	0.507 (MWU)
C-reactive protein at start DTG, mg/L	1.20 (0.40,2.70)	1.45 (0.73,3.08)	0.673 (MWU)
DTG plasma-concentration, mg/L	1.65 (1.23,3.75)	1.70 (1.05,2.40)	0.308 (MWU)
DTG plasma-concentration, % deviation from population average	12.9 (-43.2,55.2)	10.9 (-27.6,45.5)	0.879 (MWU)
cART before DTG, N(%)			
NNRTI	69 (79.3)	7 (87.5)	0.783 (CST)
PI	4 (4.6)	0 (0)	
INI	14 (16.1)	1 (12.5)	
Time between HIV-diagnosis and start cART, months	15 (1,38)	49 (27,64)	0.015 (MWU)
Time suppressed on cART, months	31 (20,54)	57 (28,94)	0.104 (MWU)

Table 1. Baseline characteristics of patients with and without VF during DTG maintenance monotherapy, including p-values resulting from univariate analysis. Data documented as median (Q1,Q3), unless stated otherwise, DTG=dolutegravir, VF=virological failure, SD=standard deviation, UTT=unpaired T-test, FET=Fisher's Exact test, MWU=Mann Whitney U test, * residual viremia is defined as HIV-RNA detectable but < 20 c/mL or > 20 c/mL, PBMC=peripheral blood mononuclear cells, cART=combination antiretroviral therapy, NNRTI=non-nucleoside reverse transcriptase inhibitor, PI=protease inhibitor, INI=integrase inhibitor, CST=Chi Square test.

Per study protocol, the median HIV-RNA zenith was low and the median CD4 T-lymphocyte nadir was relatively high with a minimum of 200 cells/mm³. The median (IQR) CD4 T-lymphocyte nadir was significantly lower in the patients with VF at 260 (223-320) cells/mm^3 than in those without VF (380 (290-520) cells/mm^3, p=0.011). The median time between HIV-diagnosis and cART initiation was longer in patients with VF: 49 (27-64) months versus 15 (1-38) months for patients without VF on monotherapy (p=0.015). At the start of DTG monotherapy, no significant differences were observed between patients with and without VF regarding the number of patients with a detectable palsma HIV-RNA, the CD4 T-lymphocyte count, CD4:CD8-ratio or the C-reactive protein (CRP) concentration. In contrast, the median (min-max) total HIV-DNA copy number in PBMCs at the time of DTG monotherapy initiation differed significantly between both groups: 147 (16-4132) versus 417 (85-4151) copies

per 10^6 PBMCs respectively (p=0.022). DTG plasma levels were adequate (i.e. > 0.1 mg/L) in all patients with VF and no difference in median (min-max) DTG plasma-concentrations was observed between the patients with VF and 20 randomly selected patients without VF: 1.70 (0.70-2.90) mg/L versus 1.65 (0.70-4.50) mg/L. See Figure 1 for boxplots of CD4 T-lymphocyte nadir, time between HIV diagnosis and start cART, total HIV-DNA copy number in PBMCs, and DTG plasma-concentrations in patients without and with VF.
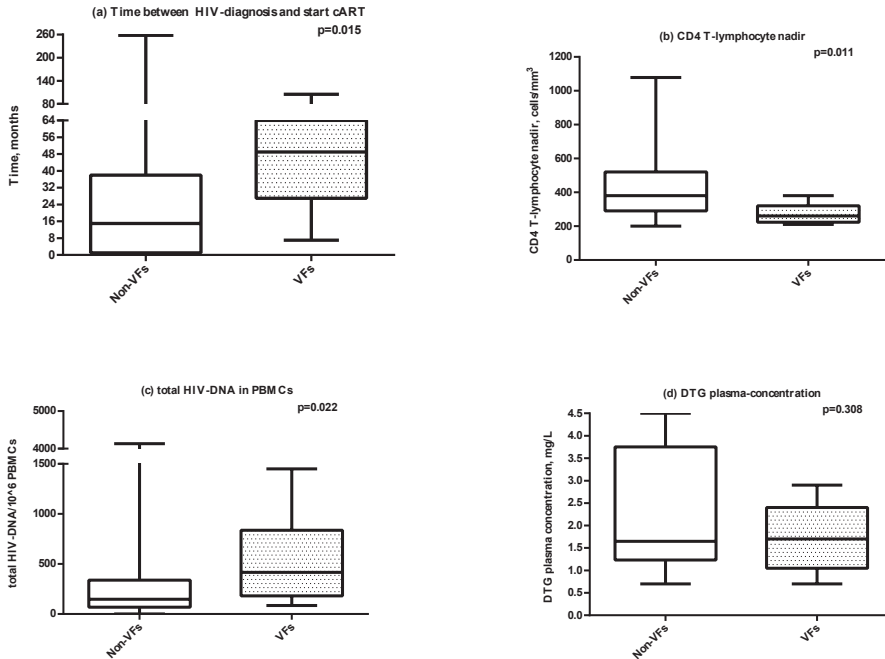

Figure 1. Distributions of time between HIV-diagnosis and start cART (a), CD4 T-lymphocyte nadir (b), total HIV-DNA in PBMCs (c), and DTG plasma-concentration (d) in patients without and with VF during DTG maintenance monotherapy. cART=combination antiRetroviral therapy, PBMC=peripheral blood mononuclear cell, DTG=dolutegravir.

DISCUSSION

In the DOMONO study, we clearly showed that DTG maintenance monotherapy is associated with VF and the development of DTG resistance, and it should not be used as maintenance monotherapy. In the current study we evaluated potential predictors of VF during integrase inhibitor monotherapy. We showed that a higher level of cell-associated total HIV-DNA copy number at start of monotherapy, a lower CD4 T-lymphocyte nadir, and a longer time between HIV-diagnosis and start cART were significantly associated with VF. A lower CD4 T-lympho-

cyte nadir and a higher level of cell-associated HIV-DNA copy number have previously also been described as risk factors for virological failure during PI monotherapy.[9–11] In contrast, none of the following ten factors were associated with VF during DTG monotherapy: gender, age, CD4 T-lymphocyte count, CRP, CD4:CD8-ratio, the type of the cART-regimen, whether the patient had detectable plasma HIV-RNA (all at the time of DTG monotherapy initiation), DTG plasma-concentrations during monotherapy, the duration of viral suppression on cART, and the HIV-RNA zenith before DTG monotherapy initiation. Also, no differences were observed regarding plasma viral load detectability in the 12 months preceding DTG mono-therapy initiation: three of 87 patients without VF versus zero of eight patients with VF had HIV-RNA more than 20 c/mL in the 12 months preceding the switch to DTG monotherapy.

Various PI monotherapy studies identified suboptimal adherence as a risk factor for VF.[9,15,16] We were unable to analyze adherence as a predictor of VF, because the inclusion and exclu-sion criteria of the study led to the selection of a very therapy-adherent study population: no history of VF on any previous cART regimen, a self-reported adherence during DTG mono-therapy of more than 95%, and therapeutic DTG plasma-concentrations in all patients with and without VF. DTG plasma-concentrations were adequate in both groups and there was no significant difference in DTG plasma-concentrations between patients with and without VF, which consistent with previous studies which did not identify lower PI plasma-concentrations as a risk factor for VF in patients receiving PI monotherapy.[16,17] It must be noted that drug level measurement was only performed at single time-points, so the possibility of temporary non-adherence between study visits cannot be ruled out.

The limiting factor of this study is the relatively small number of patients who experienced VF in comparison to the previously mentioned PI studies. Even if we had considered an iso-lated and unconfirmed viral load of more than 50 c/mL as VF (as observed in 14 patients), the number of VF would have been too small to enable a multivariate analysis to be performed. Therefore, we were not able to assess whether CD4 T-lymphocyte nadir, time between HIV-diagnosis and start cART, and cell-associated HIV-DNA copy number are independent risk factors for VF during DTG maintenance monotherapy. Actually, CD4 T-lymphocyte nadir, time between HIV-diagnosis and start cART, and cell-associated HIV-DNA levels could very well be correlated. Indeed, Boulassel et al previously showed the inverse relationship between CD4 T-lymphocyte nadir and cell-associated HIV-DNA levels[18], and longer time between HIV-diagnosis and start cART is associated with higher cell-associated HIV-DNA levels.[19] This implies that the size of the viral reservoir is probably the most important determinant of VF, as the cell-associated total HIV-DNA level is a measure for the size of the viral reservoir in virologically suppressed patients. Reactivation of HIV from latently infected cells is a stochastic process, which occurs on average every five to eight days, and depends on the size of the replication competent viral reservoir.[20] Our observation that a higher HIV-DNA level

is associated with VF is in agreement with stochastic reactivation of pre-existing provirus harboring a single integrase inhibitor mutation. It would have been useful to provide data on the size of the reservoir at the time of VF. Unfortunately, we did not collect PBMCs at the time of VF and therefore, we are not able to provide these data.

In conclusion, a longer time between HIV diagnosis and cART initiation, a lower CD4 T-lymphocyte nadir, and a higher total HIV-DNA copy number increased the risk of VF during DTG monotherapy. While there clearly is no future role for DTG monotherapy, ongoing and future studies on the efficacy of maintenance dual therapy (e.g. DTG with lamivudine) should take these variables into account in their study design and analysis.

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Part 4

Perspectives

Chapter 9

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 signifi-cant 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 de-scribed.[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/mm³. 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 neuro-psychiatric 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.

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Chapter 10

Nederlandse samenvatting

NEDERLANDSE SAMENVATTING

Het humaan immuundeficiëntie virus type 1 (HIV) infecteert afweercellen, voornamelijk CD4 T-cellen, in het menselijk lichaam. Zonder behandeling leidt dit tot een steeds ergere aantasting van de afweer. Het gevolg hiervan is dat mensen het acquired immune deficiency syndrome (beter bekend als AIDS) ontwikkelen. AIDS is het eindstadium van een HIV-infectie waarbij mensen zo weinig afweercellen hebben, dat ze ernstige infecties krijgen. Dit zijn specifieke infecties die alleen mensen met een slechte afweer kunnen krijgen, en mensen met een gezond afweersysteem niet. Uiteindelijk overlijden mensen aan deze infecties. Levenslang dagelijkse behandeling met medicijnen tegen HIV, ofwel antiretrovirale therapie, zorgt ervoor dat het virus onderdrukt wordt. Dit leidt tot herstel van de afweer, waardoor AIDS en overlijden aan de bijbehorende specifieke infecties voorkomen worden. De levensverwachting van een HIV-geïnfecteerd persoon is dan nagenoeg hetzelfde als die van een ongeïnfecteerd persoon. Verder is de kans op overdracht van HIV via seksueel contact en van moeder op kind heel erg klein bij mensen met een virus dat in het bloed door antiretrovirale therapie onderdrukt is. Hierdoor zijn mensen die het virus niet hebben, beschermd tegen het krijgen van een HIV-infectie. Naast virologische onderdrukking leidt antiretrovirale therapie ook tot vermindering van een ontstekingsreactie in het lichaam, veroorzaakt door HIV. Dit soort reacties kunnen onder andere vaatschade geven, en daarmee leiden tot bijvoorbeeld hart- en vaatziekten. Ook verkleint behandeling van HIV het risico op diverse kankersoorten.

HIV is een retrovirus. Dat wil zeggen dat het virus het eiwit reverse transcriptase bevat. Reverse transcriptase kan het genetisch materiaal van het virus, het ribonucleïnezuur (RNA), omzetten in desoxyribonucleïnezuur (DNA). Het eiwit integrase kan vervolgens viraal DNA in het humaan DNA in cellen inbouwen. HIV misbruikt vervolgens deze cel om nieuwe virusdeeltjes te maken, die in het bloed terechtkomen. Deze nieuwe virusdeeltjes kunnen vervolgens weer nieuwe cellen besmetten. Op deze manier vermenigvuldigt HIV zich in het lichaam. Dit staat bekend als de replicatiecyclus van HIV. Verschillende stappen in deze replicatiecyclus kunnen we tegenwoordig remmen met antiretrovirale therapie.

Vijf vrijwel overlappende HIV-behandelrichtlijnen zijn wereldwijd het belangrijkst. De gemeenschappelijke aanbeveling van de richtlijnen is om alle mensen met HIV zo snel mogelijk te behandelen met een combinatie van drie antiretrovirale medicijnen. Dit wordt ook wel triple antiretrovirale therapie genoemd. De reden hiervoor is dat de genetische informatie van HIV snel kan veranderen doordat er mutaties optreden. Door mutaties wordt het virus ongevoelig (ook wel resistent genoemd) voor één of meerdere medicijnen tegen HIV. Hierdoor kan het virus weer ongeremd in het lichaam gaan vermenigvuldigen. Door HIV met een combinatie van verschillende antiretrovirale medicijnen te behandelen, is de kans op resistentie erg klein. Daardoor is de kans dat het virus in het bloed onderdrukt blijft, groot. De huidige triple

antiretrovirale therapie remt op twee verschillende manieren de replicatiecyclus. Momenteel wordt geadviseerd om twee remmers van het eiwit reverse transcriptase te combineren met een derde HIV-remmer die een ander onderdeel van de replicatiecyclus remt. Deze derde HIV-remmer kan een ook een reverse transcriptase remmer met een ander aangrijpingspunt zijn, een remmer van het virale eiwit protease, dat het virus nodig heeft om tot een functioneel virusdeeltje te worden na het vrijkomen uit de cel, of een remmer van het virale eiwit integrase. Voor Westerse landen adviseren de richtlijnen nu een behandeling met een integraseremmer in combinatie met twee reverse transcriptase remmers. Omdat integraseremmers nieuw zijn, en weinig beschikbaar in armere landen, wordt in deze landen nog veelal een combinatie van twee reverse transcriptase remmers en een reverse transcriptase remmer met een ander aangrijpingspunt gebruikt.

Bij het starten van triple antiretrovirale therapie komt meer kijken dan alleen een zo goed mogelijke onderdrukking van het virus. Er dient ook rekening gehouden te worden met de beschikbaarheid van medicijnen in bepaalde delen van de wereld, de kosten van de medicatie, de bijwerkingen en het innamegemak. Een integraseremmer geeft hele goede onderdrukking van het virus, vaak beter dan andere HIV-remmers. Daarom gebruikt men bij voorkeur triple antiretrovirale therapie die een integraseremmer bevat. Daarnaast geeft triple antiretrovirale therapie met een integraseremmer weinig bijwerkingen en is het makkelijk in te nemen.

Hoewel de behandeling van HIV volgens de behandelrichtlijnen met triple antiretrovirale therapie leidt tot goede onderdrukking van het virus, is het onbekend of drie medicijnen voor alle HIV-geinfecteerde mensen noodzakelijk zijn. Daarnaast zijn de risico's van het gebruik van integraseremmers bij mensen met AIDS of andere aandoeningen naast hun HIV-infectie nog niet zo goed bekend. De vraag is dus in hoeverre triple antiretrovirale therapie op deze patiëntencategorieën van toepassing is, en of er mogelijkheid of noodzaak is om bij individuele patiënten daarvan van af te wijken. Hierdoor zou hun behandeling nog beter, veiliger en minder belastend kunnen worden. Het doel van dit proefschrift is om met nieuwe kennis bij te dragen aan een optimale en veilige onderdrukking van HIV met nieuwe en bestaande antiretrovirale behandelingen, waarbij integraseremmers centraal staan.

Men probeert al heel lang om HIV met minder dan drie antiretrovirale medicijnen te behandelen. Dit heet versimpeling van therapie. Succesvolle versimpeling kan vele voordelen hebben, waaronder minder bijwerkingen, minder kosten en meer gebruiksgemak. Dit lukte niet met alleen reverse transcriptase remmers of protease remmers, doordat het virus in het bloed onvoldoende onderdrukt bleef. Daarnaast werd het virus te vaak resistent tegen deze middelen, en kwam het terug in het bloed. Als het virus terugkomt in het bloed doordat antiretrovirale therapie niet werkt, bijvoorbeeld door resistentie, wordt gesproken van virologisch falen.

Sinds een aantal jaar zijn de integraseremmers beschikbaar voor de behandeling van HIV. Er zijn twee groepen integraseremmers: de eerste en de tweede generatie. De tweede generatie integraseremmers zijn zeer krachtig in het onderdrukken van het virus, en resistentie tegen deze klasse ontstaat nauwelijks. Eén van de tweede generatie integraseremmers is dolutegravir. Tot nu toe lijkt de ontwikkeling van resistentie tegen dolutegravir nauwelijks mogelijk bij HIV-patiënten die dolutegravir als eerste integraseremmer gebruiken, dus zonder dat er eerder een eerste generatie integraseremmer gebruikt is. De gunstige eigenschappen van dolutegravir zouden het mogelijk kunnen maken om HIV-behandeling met triple antiretrovirale therapie te versimpelen tot alleen dolutegravir, dus zonder andere antiretrovirale medicijnen erbij. Dit noemen we monotherapie. In deel 1 van dit proefschrift onderzoeken we daarom of alleen dolutegravir voldoende is om het virus te onderdrukken. Behandeling met deze therapie vergelijken we met de gebruikelijke behandeling met triple antiretrovirale therapie.

In **hoofdstuk 2** vergelijken we in het DOlutegravir MONOtherapie voor HIV-1 onderzoek (DOMONO) de effectiviteit en veiligheid van dolutegravir monotherapie met die van triple antiretrovirale therapie bij 95 HIV-patiënten met een onderdrukt virus door triple antiretrovirale therapie. De helft van deze mensen stopte de triple antiretrovirale therapie en kreeg dolutegravir monotherapie, en de andere helft continueerde de triple antiretrovirale therapie. Gedurende het eerste half jaar was de mate van onderdrukking van het virus vergelijkbaar in beide groepen, maar daarna ontwikkelden maar liefst acht patiënten met dolutegravir monotherapie virologisch falen ten opzichte van nul patiënten met virologisch falen die triple antiretrovirale therapie gebruikten. Drie van deze acht mensen hadden een gemuteerd virus. Vanwege deze ontwikkeling hebben we de studie gestaakt. Wij vinden dat bij mensen met HIV, bij wie het virus onderdrukt is met triple antiretrovirale therapie, versimpeling door dolutegravir monotherapie niet geschikt is.

In **hoofdstuk 3** gaan we dieper in op deze gemuteerde virussen. In een verwant onderzoek hadden nog twee mensen virologisch falen tijdens dolutegravir monotherapie. Hun virussen, samen met de virussen van de mensen met virologisch falen uit het DOMONO-onderzoek, hebben we onderzocht. In totaal hebben we tien virussen onderzocht. Bij resistentie onderzoek bekijken we normaal gesproken een klein stukje virus dat codeert voor het integrase eiwit (het integrase gen). Hierin zoeken we naar mutaties. Het virus is echter groter en mutaties buiten dit stukje kunnen mogelijk ook van invloed zijn op de gevoeligheid van het virus voor dolutegravir. Daarom hebben we nu ook gekeken naar het virus buiten het integrase gen. Bij vier van de patiënten met virologisch falen ontwikkelde het virus tijdens monotherapie mutaties in het integrase gen die zorgen voor resistentie tegen integraseremmers. Uniek was dat bij één andere patiënt de mutaties op een zeldzame plek buiten het integrase gen werden gevonden. Dit is een nieuw resistentiemechanisme, dat nog nooit bij een virus in een HIV-patiënt aangetoond was. Dit impliceert dat bij virologisch falen de mutaties, die zorgen voor

resistentie tegen de behandeling, ook in andere delen van het virus kunnen optreden. De oorzaak van het virologisch falen met monotherapie berust mogelijk op het feit dat HIV nooit helemaal weg is uit het lichaam, ondanks antiretrovirale therapie. Het verstopt zich namelijk in zogenaamde reservoirs waar het gedurende jaren aanwezig kan zijn in vrijwel inactieve staat. In het reservoir kunnen ook virusdeeltjes zitten die al een mutatie bevatten. Wanneer zo'n virusdeeltje uit het reservoir actief wordt, lijkt monotherapie onvoldoende om de vermenigvuldiging te stoppen. Het is nu nog niet te voorspellen of en wanneer dit gebeurt, waardoor het onzeker is of de huidige en toekomstige behandelstrategieën met monotherapie tot goede virologische onderdrukking zonder ontwikkeling van resistentie kunnen gaan leiden.

In **hoofdstuk 4** zoeken we naar voorspellers van het virologisch falen in het DOMONO-onderzoek. Een belangrijke factor die we vonden, was de totale hoeveelheid genetisch materiaal van het virus in het lichaam: het HIV-DNA. Het HIV-DNA vormt een maat voor de grootte van het HIV-reservoir. Het HIV-reservoir is van belang, omdat een groter of actiever reservoir de kans op virologisch falen hoogstwaarschijnlijk vergroot. Virologisch falen in het DOMONO-onderzoek bleek gerelateerd aan een groter reservoir, een slechtere afweer, en een langere periode zonder behandeling met medicijnen na het stellen van de HIV-diagnose. Deze factoren houden verband met elkaar: des te langer er gewacht wordt met behandeling van HIV, des te groter het HIV-reservoir wordt, en des te meer de afweer wordt aangetast. Deze bevindingen onderschrijven dat de grootte van het reservoir en de staat van de afweer belangrijke voorspellers zijn van virologisch falen op monotherapie.

In **hoofdstuk 5** beschrijven we de effecten van een versimpeling van triple antiretrovirale therapie naar dolutegravir monotherapie op de metabole processen in het lichaam. Bijna alle deelnemers aan het DOMONO-onderzoek gebruikten voor hun versimpeling naar dolutegravir monotherapie het middel tenofovir disoproxil fumaraat (TDF) in hun triple antiretrovirale medicatie. TDF kan schadelijk zijn voor de nieren en voor de botsterkte. Aan de andere kant is een gunstig metabool effect van TDF dat het zorgt voor een lager cholesterol. Aangezien veel patiënten TDF staakten, verwachtten we dat de nieren en botsterkte zouden verbeteren, maar ook dat het cholesterol zou stijgen. Bij de deelnemers aan het DOMONO-onderzoek vonden we een verbetering van de nierfunctie, maar de waarden van de botsterkte, het cholesterol, de afweer en ontsteking in het lichaam bleven stabiel. De relatief gezonde studiepopulatie en de onderzoeksduur van slechts één jaar hebben mogelijk bijgedragen aan deze bevindingen.

Het gebruik van integraseremmer bevattende triple antiretrovirale therapie geeft een snelle daling van de hoeveelheid virusdeeltjes in het lichaam, en het leidt tot snel herstel van de afweer, sneller dan andere klassen medicatie. Deze effecten zijn helaas risicofactoren voor het optreden van een heftige afweerreactie. Deze afweerreactie staat bekend als het immuun reconstitutie inflammatoir syndroom (IRIS). IRIS kan optreden bij mensen die AIDS hebben

en bij wie de afweer plotseling snel herstelt. Van IRIS kunnen mensen erg ziek worden en zelfs overlijden. Vaak zijn ziekenhuisopnames of extra medicijnen noodzakelijk. De veiligheid van integraseremmer bevattende triple antiretrovirale therapie bij AIDS-patiënten wordt onderzocht in deel 2 van dit proefschrift.

In **hoofdstuk 6** wordt de relatie onderzocht tussen het starten van integraseremmer bevattende triple antiretrovirale therapie bij mensen met AIDS en het optreden van IRIS. In een groep van 672 AIDS-patiënten die startten met triple antiretrovirale therapie, hebben we onderzocht of zij IRIS ontwikkelden. Mensen die een integraseremmer startten, hadden inderdaad een verhoogd IRIS-risico ten opzichte van mensen die geen integraseremmer startten. Opvallend was dat dit vooral patiënten betrof die de eerste generatie integraseremmer raltegravir gebruikten, terwijl we dit bij dolutegravir en de eerste generatie integraseremmer elvitegravir niet vonden. We vermoeden dat dit resultaat wordt verklaard door andere verschillen tussen patiënten die deze medicatie gebruikten.

Voor 2016, toen integraseremmer bevattende triple antiretrovirale therapie nog niet door de behandelrichtlijnen aanbevolen werd, en dolutegravir nog maar net op de markt was, werd raltegravir alleen voorgeschreven aan HIV-patiënten die erg ziek waren. Deze patiënten hadden bijvoorbeeld lymfeklierkanker, tuberculose of hersenvliesontsteking door cryptococcen (cryptococcen meningitis) bij hun HIV. Bij deze aandoeningen moeten veel andere medicijnen voorgeschreven worden. Het is dan van belang om het risico op medicatie-interacties zo laag mogelijk te houden, en dat gaat het beste met een integraseremmer. In die tijd was dit doorgaans raltegravir. Mensen die het hoogste risico liepen op een IRIS kregen daarom ook het vaakst de integraseremmer raltegravir, en minder vaak een behandeling met een antiretroviraal medicijn uit een andere klasse. Hierdoor is een vertroebeling van de resultaten opgetreden. Daarnaast wordt de ontwikkeling van IRIS bij mensen die raltegravir kregen vermoedelijk verder versterkt door onbekende factoren. Of de eigenschappen van integraseremmers leiden tot meer of andere immuunreacties in vergelijking met andere HIV-remmers is nog onduidelijk. Vooralsnog lijken de nadelen niet op te wegen tegen de voordelen van integraseremmers bij AIDS-patiënten.

Triple antiretrovirale therapie moet in een substantieel aantal patiënten vroeg of laat wel eens onderbroken worden vanwege bijwerkingen. Integraseremmers worden altijd samen met reverse transcriptase remmers gebruikt. De reverse transcriptase remmers worden daarom veel gebruikt en kunnen ook bijwerkingen veroorzaken. In de klasse van reverse transcriptase remmers zijn drie belangrijke medicijnen beschikbaar: abacavir, TDF en tenofovir alafenamide fumaraat (TAF). Deze middelen zijn allemaal in gelijke mate effectief, maar verschillen qua bijwerkingen. Het gebruik van abacavir kan leiden tot een ernstige allergische reactie, en is geassocieerd met hart- en vaatziekten bij bepaalde risicogroepen. Nierschade is een

belangrijke bijwerking van langdurig TDF-gebruik. TAF en TDF lijken erg op elkaar, allebei de middelen bevatten namelijk tenofovir. TAF en TDF zijn in gelijke mate effectief tegen het virus. Een belangrijke bijwerking van langdurig gebruik van middelen met tenofovir is nierschade. Omdat de dosering van TAF veel lager is dan die van TDF, hoeft minder TAF gebruikt te worden en treden de bijwerkingen van tenofovir minder op. TDF wordt niet alleen als behandeling van HIV gebruikt. Het voorkomt ook overdracht van HIV bij mannen die seks hebben met mannen, en kan gebruikt worden bij hepatitis B-infecties. De komende jaren zal TDF in grote delen van de wereld een veelgebruikt middel blijven, ondanks dat er soms betere alternatieven beschikbaar zijn. Om deze reden zullen de bijwerkingen van TDF een belangrijk probleem blijven. In het derde deel van dit proefschrift onderzoeken we of we de nierschade van TDF beperkt kunnen houden.

Hoofdstuk 7 beschrijft onderzoek naar een bloedbepaling die mogelijk uit zou kunnen wijzen of mensen een verhoogd risico hebben om tijdens TDF-gebruik nierschade te ontwikkelen. Het inosine 5'-trifosfatase (ITPase) is een enzym dat onder andere betrokken is bij de afbraak van TDF in de cel. De meeste mensen hebben een normaal werkend ITPase, maar sommigen hebben een verminderde werking van het ITPase. Het stukje genetische informatie dat codeert voor ITPase heet het *ITPA* gen. Het is niet duidelijk in welke mate mutaties in het *ITPA* gen en de ITPase activiteit gerelateerd zijn aan schadelijkheid van TDF. HIV-geïnfecteerden met een normale ITPase-activiteit vertonen een iets grotere achteruitgang in nierfunctie tijdens TDF gebruik, maar ook een meer uitgesproken herstel na het staken ervan, ten opzichte van mensen met een verminderde ITPase activiteit. Er werd geen duidelijk verband gevonden tussen mutaties in het *ITPA* gen en het optreden en herstellen van nierschade door TDF-gebruik. Alhoewel het precieze werkingsmechanisme onbekend is, en er meer onderzoek moet volgen, zou ITPase mogelijk in de toekomst gebruikt kunnen worden om te voorspellen bij welke patiënten nierschade door TDF optreedt.

In **hoofdstuk 8** onderzoeken we of het vervangen van TDF door TAF- of abacavir-bevattende triple antiretrovirale therapie bij mensen met nierschade door TDF goede en vergelijkbare alternatieve behandelopties zijn. Bij mensen die naar TAF- of naar abacavir-bevattende triple antiretrovirale therapie switchen, stabiliseerde de nierfunctie in gelijke mate. TAF- en abacavir-bevattende triple antiretrovirale therapie lijken beide dus goede alternatieven voor TDF-bevattende triple antiretrovirale therapie, wanneer nierschade door TDF is opgetreden.

De inzichten die in dit proefschrift verworven zijn, worden samengevat en bediscussieerd in **hoofdstuk 9**. De belangrijkste conclusies van dit proefschrift zijn:

i) dolutegravir is niet geschikt om als monotherapie het virus in het bloed onderdrukt te houden. Resistentie van het virus tegen integraseremmers treedt veelvuldig op bij mo-

notherapie en kan veroorzaakt worden door mutaties buiten het integrase gen. Het HIV-reservoir, evenals de staat van de afweer zijn bruikbaar als voorspeller van effectiviteit van HIV-behandeling tijdens versimpelingsstrategieën.

ii) integraseremmer bevattende triple antiretrovirale therapie is veilig bij AIDS-patiënten zonder een duidelijke associatie met het ontwikkelen van IRIS.

iii) ITPase enzym activiteit kan helpen bij het voorspellen en voorkomen van nierschade door TDF. Als er nierschade optreedt, dan zijn abacavir en TAF bevattende triple antiretrovirale therapie gelijkwaardig om te gebruiken om de toename van nierfunctieverlies te voorkomen.

Het doel van dit proefschrift is om met nieuwe kennis bij te dragen aan een optimale en veilige onderdrukking van HIV met nieuwe en bestaande antiretrovirale behandelingen met een focus op integraseremmers. Deze conclusies helpen om een betere HIV-behandeling op maat te leveren aan individuele personen die leven met een HIV-infectie.

Chapter 11

Publications

PhD portfolio

Curriculum Vitae

Dankwoord

PUBLICATIONS

This thesis

1. I.E.A. Wijting, C. Rokx , C.A.B. Boucher, J.J.A. van Kampen, S.D. Pas, T.E.M.S. de Vries-Sluijs, C.A.M. Schurink, H.I. Bax, M. Derksen, E.R. Andrinopoulou, M.E. van der Ende, E.C.M. van Gorp, J.L. Nouwen, A. Verbon, W.F.W. Bierman, B.J.A. Rijnders. Dolutegravir as maintenance monotherapy for HIV (DOMONO): a phase 2, randomised, non-inferiority trial. *Lancet HIV,* 2017;**4**:e547-e554.

2. I.E.A. Wijting, C. Lungu, B.J.A. Rijnders, M.E. van der Ende, H.T. Pham, T. Mesplède, S.D. Pas, J.J.C. Voermans, R. Schuurman, D.A.M.C. van de Vijver, P.H.M. Boers, R.A. Gruters, C.A.B. Boucher, J.J.A. van Kampen. HIV-1 resistance dynamics in patients with virologic failure to dolutegravir maintenance monotherapy. *J Infect Dis* 2018;**218**:688-697.

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Other

4. H.T. Pham, L. Labrie, I.E.A. Wijting, S. Hassounah, K.Y. Lok, I. Portna, M.E. Goring, Y. Han, C. Lungu, M.E. van der Ende, B.G. Brenner, C.A.B. Boucher, B.J.A. Rijnders, J.J.A. van Kampen, T. Mesplède, M.A. Wainberg.

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PHD PORTFOLIO

Name PhD student: Ingeborg E. A. Wijting
Erasmus MC Department: Internal Medicine and Infectious Diseases
PhD period: 01-09-2015 – 01-09-2018
Promotor: Prof. Dr. A. Verbon

	Year	Workload (ECTS)
General courses		
'Basiscursus Regelgeving en Organisatie voor Klinisch onderzoekers' (BROK-course)	03-2016	1,5
Course 'Biostatistical methods 1: basic principles Part A' (CC02A)	03-2016	2,0
Biomedical English Writing and Communication	09-2016	3,0
Research Integrity	01-2017	0,2
Seminars and workshops		
HIV masterclass	2015-2016	1,0
Oral and poster presentations		
Oral presentations:		
NVHB wintervergadering*: 'De DOMONO-studie – Dolutegravir antiretrovirale monotherapie voor HIV-1.'*	01-2016	1,0
2nd European HIV Clinical Forum: Integrase Inhibitors: *'Switching from combination antiretroviral therapy to DOlutegravir MONOtherapy in virologically suppressed HIV-1 infected adults: a randomized multicenter, non-inferiority clinical trial'*	10-2016	1,0
Netherlands Conference on HIV Pathogenesis, Prevention and Treatment 2016: *'Switching from combination antiretroviral therapy to DOlutegravir MONOtherapy in virologically suppressed HIV-1 infected adults: A randomized multicenter, non-inferiority clinical trial'* and: *'Integrase Inhibitor use is an independent risk factor for immune reconstitution inflammatory syndrome (IRIS) in HIV-1 late presenters in the Dutch ATHENA cohort'*	11-2016	2,0
Wetenschapsdagen Internal Medicine Erasmus MC, Antwerp, Belgium: *'Integrase Inhibitor use is an independent risk factor for immune reconstitution inflammatory syndrome (IRIS) in HIV-1 late presenters in the Dutch ATHENA cohort'*	01-2017	1,0
NVHB wintervergadering: *'Switchen van combinatie antiretrovirale therapie naar DOlutegravir MONOtherapie bij virologisch onderdrukte HIV-1 geïnfecteerde volwassenen: een gerandomiseerde multicenter non-inferiority klinische studie (DOMONO).*	03-2017	1,0
European AIDS conference, Milan, Italy: *Use of integrase inhibitors is an independent risk factor for immune reconstitution inflammatory syndrome (IRIS) in HIV-1 late presenters: an ATHENA cohort study.'*	10-2017	1,0

NVHB wintervergadering: *'Het gebruik van integraseremmer bevattende cART is een onafhankelijke risicofactor voor Immuun Reconstitutie Inflammatoir Syndroom (IRIS) bij HIV-1 late presenters: een ATHENA-cohort studie.*	01-2018	1,0

Poster presentations:

HIV Glasgow 2016: *'Switching from cART to dolutegravir monotherapy in virologically suppressed HIV-1 infected patients: A randomized clinical trial (DOMONO)*	10-2016	1,0
Conference on Retroviruses and Opportunistic Infections 2017: *'Integrase Inhibitors are an Independent Risk Factor for IRIS: an ATHENA-Cohort Study'* and: *'Dolutegravir as Maintenance Monotherapy for HIV-1: a Randomized Clinical Trial'*	02-2017	2,0
European AIDS conference, Milan, Italy: *'Dynamics of viral rebound and development of resistance associated mutations in the DOlutegravir maintenance MONOtherapy (DOMONO) study.'*	10-2017	1,0
Conference on Retroviruses and Opportunistic Infections 2018: *'Factors predicting virological failure during dolutegravir maintenance monotherapy.'* and: *'Bone, renal and inflammation markers in the dolutegravir monotherapy (DOMONO) study.'*	03-2018	2,0
22nd International AIDS Conference 2018: '*Incidence, timing, and outcome of IRIS in relation to specific opportunistic infections in the ATHENA cohort.'*	07-2018	1,0

National and international conferences

'Netherlands Conference on HIV Pathogenesis, Prevention and Treatment 2015' – Amsterdam.	11-2015	1,0
'Science days Internal Medicine, Erasmus MC 2016' - Antwerp	01-2016	1,0
'Going Beyond Undetectable 2016' – Vienna.	03-2016	1,0
'Healthy Living with HIV 2016' – Barcelona.	09-2016	1,0
'HIV Glasgow 2016' –Glasgow.	10-2016	1,0
'2nd European HIV Clinical Forum: Integrase Inhibitors' – Glasgow.	10-2016	1,0
'Netherlands Conference on HIV Pathogenesis, Prevention and Treatment 2016' – Amsterdam.	11-2016	1,0

'Science days Internal Medicine, Erasmus MC 2017' – Antwerp.	01-2017	1,0
'Conference on Retroviruses and Opportunistic Infections 2017' – Seattle.	02-2017	1,0
'European AIDS conference' – Milan.	10-2017	1,0
'Netherlands Conference on HIV Pathogenesis, Prevention and Treatment 2017' – Amsterdam.	11-2017	1,0
'Conference on Retroviruses and Opportunistic Infections 2018' – Boston.	03-2018	1,0
'EACS Young Investigators Conference 2018' – Brussels.	04-2018	1,0
'Global HIV Clinical Forum: Integrase Inhibitors 2018' – Amsterdam	07-2018	1,0
'22nd International AIDS conference' –Amsterdam.	07-2018	1,0

Scholarships and Travel Grants:

HIV Glasgow 2016 – Junior Investigator Scholarship	10-2016
Conference on Retroviruses and Opportunistic Infections 2017 – Young Investigator Scholarship	02-2017
European AIDS conference, Milan, Italy - Junior Investigator Scholarship	10-2017
Conference on Retroviruses and Opportunistic Infections 2018 – New Investigator Scholarship	03-2018
22nd International AIDS Conference – Scholarship	07-2018

Awards:

Netherlands Conference on HIV Pathogenesis, Prevention and Treatment 2016 – Joep Lange and Jacqueline van Tongeren Award	11-2016

Other

Subinvestigator TANGO study	04-2018 – 09-2018	2,0

Supervising Masters theses

A. Postma – masterthesis about 'Type of cART regimen and the risk for immune reconstitution and inflammatory syndrome in HIV-1 infected persons. Is integrase inhibitor use an independent risk factor?	03-2016 – 08-2016	2,0
G. Bouchtoubi – masterthesis about 'Het gebruik van een integraseremmer als een onafhankelijke risicofactor voor het immuun reconstitutie inflammatoir syndroom (IRIS) bij HIV-1 late presenters in het Nederlands ATHENA-cohort'	09-2016 – 01-2017	2,0

V. Bloemen – masterthesis about 'Use of integrase inhibitor-containing cART as an independent risk factor for the development of the immune reconstitution inflammatory syndrome (IRIS).'	03-2017 – 08-2017	2,0
J. Broers – masterthesis about 'Switching to Tenofovir Alafenamide Fumarate or Abacavir in patients with Tenofovir Disoproxil Fumarate associated eGFR decline. A randomized clinical trial and a retrospective observational study.'	03-2017 – 08-2017	2,0
M. Langeveld – masterthesis about 'The BACTAF-study: switching to tenofovir alafenamide fumarate or abacavir in patients with tenofovir disoproxil fumarate associated eGFR decline.'	11-2017 – 04-2018	2,0

Other

Supervising 'Kennismaking met de Beroepspraktijk'(KBP) for medical students	2015-2016	0,2

Total workload (ECTS)	**49,9**

CURRICULUM VITAE

Ingeborg Wijting was born on October 28th 1988 in Oldenzaal, the Netherlands, and in 1990 she moved to Gorinchem. In 2006, she graduated from the Gymnasium Camphusianum in Gorinchem. Subsequently, she started her medical training at the Erasmus University Medical Center in the same year. In 2010, she obtained her doctorate degree after performing graduation research focusing on drug therapy after primary coronary intervention. In 2013, she obtained her Medical Doctor degree, and she started working as a resident at the department of internal medicine at the Amphia hospital in Breda, under the supervision of dr. C. van Guldener and dr. J. W. J. van Esser. In 2015, she intiated her PhD-research under the supervision of dr. B. J. A. Rijnders, dr. C. Rokx, and Prof. dr. A. Verbon. In 2018, she continued working as a resident at the Amphia hospital in Breda. In 2019, she will initiate her medical specialisation in internal medicine at the Amphia hospital in Breda under the supervision of dr. J. W. J. van Esser, as part of her medical specialisation at the Erasmus Medical Center in Rotterdam, supervised by dr. A. A. M. Zandbergen. Ingeborg is living in Gorinchem together with Sander and they have a son, Tobias (2017).

DANKWOORD

Daar zijn ze dan, die o zo belangrijke laatste pagina's van dit proefschrift. Op een positie in het proefschrift waar ze makkelijk en snel te vinden zijn, zodat de inhoud ervan niet aan de verdiende aandacht ontsnapt. Tijdens mijn promotieonderzoek en de totstandkoming van dit proefschrift kende ik dalen en pieken waar ik nooit eerder mee geconfronteerd was. Degenen zonder wie dit proefschrift nooit het succes geworden zou zijn wat het nu is, en degenen die me in voor- en tegenspoed bijstonden, wil ik graag bedanken.

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Mijn co-promotor dr. Bart Rijnders, beste Bart, wat ben ik ontzettend trots en wat voel ik me nog altijd vereerd dat ik promotieonderzoek heb mogen doen onder jouw supervisie. De keren dat ik blij verrast werd door je vindingrijkheid en je creativiteit zijn ontelbaar, en ik heb ontzettend veel geleerd van de steengoede internist-infectioloog en wetenschapper die je bent. Bedankt voor deze fantastische ervaring en het vertrouwen dat ik altijd van je gekregen heb. Ik had nooit kunnen vermoeden dat onze eerste kennismaking in de Bazar in Rotterdam tot zo veel goeds zou leiden.

Mijn co-promotor dr. Casper Rokx, lieve Casper, CrisprCas, ik weet niet waar ik moet beginnen. Je bent het niet erg van me gewend, maar in dit geval heb ik toch moeite om mijn dank aan jou te verwoorden. Van je hulp bij het opzetten van studies tot het kritisch becommentariëren van stukken, zonder jou was dit proefschrift er nooit gekomen. Niet onbelangrijk is ook mijn introductie door jou in de Rotterdamse borrelscene, een essentieel stukje opvoeding voor iedere promovendus, in ons geval een succesformule met een lach en een traan. Alle goeds gewenst samen met Lotte en jullie kindjes, en ik ben trots op de internist-infectioloog en wetenschapper die je ook bent! Toedeledokie, IngeMountain.

Mijn promotor Prof. dr. Annelies Verbon, beste Annelies, ik bewonder je niet alleen als wetenschapper, maar ook als clinicus en organisator. Naast je immer verhelderende visie op wetenschap, waarbij door jouw scherpe blik stukken altijd veel beter werden, zag jij met jouw creativiteit altijd mogelijkheden voor nieuwe inspirerende projecten en stukken. Daarnaast ben je ook op organisatorisch en klinisch gebied een groot voorbeeld voor me. Heel veel dank voor alle kansen die ik van je gekregen heb en alles wat ik van je heb mogen leren.

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Dank aan alle internist-infectiologen, Prof. dr. A. Verbon, dr. B.J.A. Rijnders, dr. C.A.M. Schurink, dr. T.E.M.S. de Vries-Sluijs, dr. H.I. Bax, dr. J.L. Nouwen, Prof. dr. E.C.M. van Gorp, dr. M.E. van der Ende en N.C. Peltenburg, beste Annelies, Bart, Karin, Dorine, Hannelore, Jan, Eric, Ineke en Chantal, dank voor jullie bijdrage aan de studies en de manuscripten. Ook wil ik jullie hartelijk danken voor de leerzame jaren waarin ik als internist in spé zoveel heb mogen leren over HIV en infectieziekten in bredere zin. Mijn grote dank gaat ook uit naar Jan, Nadine, Laura en Marion, die me met enige regelmaat op de poli infectieziekten hielpen met de zorg, mijn kennis bijspijkerden en met wie ik ook gewoon eens een fijn babbeltje kon maken. René, dank je voor alle hulp bij de studies en ál het prikken! Kader, geen mailtje was jou en je collega's teveel, zonder jou was de agenda een zooi geworden. Sandra, zelfs voor die ene DEXA-scan had je altijd een gaatje, ook als er zelfs geen gaatje meer was eigenlijk! Ik waardeer je inspanningen enorm!

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Boston. Charles Boucher, beste Charles, je enthousiasme en je geniale blik op de virologie enthousiasmeerden me keer op keer. Ook dank voor alle inzichten in de virologie die je me verschaft hebt. Jeroen van Kampen, beste Jeroen, nadat ik in het begin een enkele keer per ongeluk op vrijdagmiddag laat nog PBMC's liet komen, startte het eigenlijk pas echt, de succesvolle samenwerking tussen virologie en kliniek voor de DOMONO-studie. Veel dank voor de fijne samenwerking, de brainstormsessies en de successen die we geboekt hebben! Leonie de Groot en collega's van de SHM, door jullie is het IRIS-onderzoek een gigantisch succes geworden, dank daarvoor.

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Arts-assistenten Interne geneeskunde van het Amphia ziekenhuis, dank jullie voor het warme welkom, die borrels staan op mijn prioriteitenlijst, écht! Lieve oud-Amphia-collega's, het waren hele mooie tijden waar ik jullie heel dankbaar voor ben! De koffie-momentjes in het Erasmus MC met jullie hielden me op de been. Beste internisten en MDL-artsen van het Amphia ziekenhuis, veel dank voor al jullie geduld en alle leermomenten. Joost, jouw speech aan mijn adres over Winston Churchill was één van de kleine zetjes in de goede richting. Hopelijk volgen er nog vele. Coen, ik kreeg als piepjong doktertje van jou de kans, oneindig

veel dank daarvoor. Gerba, toen het echt nodig was, bood je een luisterend oor en zei je het juiste, dank daarvoor.

AIOS Infectieziekten en Medische microbiologie van het Erasmus MC, op een koude maandagochtend in december 2015 stond ik opeens met mijn boodschappentassen vol ordners op Na09. Ik vond een plekje en ik mocht altijd deel uitmaken van de gezellige werksfeer, alle sociale activiteiten en de koffierondjes. Daarnaast kon ik altijd wel iemand om promotie-gerelateerd advies vragen. Veel dank dat ik deel uit mocht maken van de groep. Ook heel veel dank voor jullie oneindige geduld ten aanzien van mijn matige lunchgewoonten, het blijft een zwakke plek, maar ik doe mijn best!

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Merel, lieve Merel, weet je nog hoe het allemaal begon, zo'n 10 jaar geleden? Wat hebben we door de jaren heen veel meegemaakt en een fijn luisterend oor aan elkaar gehad! Jij bent inmiddels hard op weg om een supergoede huisarts te worden, ik gepromoveerd en over een paar jaar internist. Zullen we nog heel lang elkaars luisterend oor blijven, een kop thee of biertje drinken en Ticket to Ride spelen met de mannen?

Pimmie, één appje (of soms twee) was genoeg om je naar beneden te lokken om onder het genot van een bakkie koffie te discussiëren over essentiële promotiezaken, zoals wat de beste voetbalclub van Nederland is, waar de mooiste grasmat ligt, en of beschouwend of snijdend nou de mooiste tak van sport is. Veel dank daarvoor! Hou je de moed erin? Dat deed ik voor 2017 ook al 18 jaar, dus dan hou jij het deze laatste driekwart jaar voor je boekje ook wel vol hè?

Lieve Ruth, Lot en Notorious, wie had dit toch gedacht? 18 jaar geleden stonden we als D'tjes op het hockeyveld, nu zitten we onder het genot van een glas wijn en een lekker hapje eten levens- en carrière-ervaringen uit te wisselen. Dank voor alles wat ik met jullie mee heb mogen maken, jullie vriendschap is me ontzettend dierbaar! Ik ben trots op jullie! Ik kijk uit naar de vele goede avondjes die we nog voor de boeg hebben!

Lieve Juud, Sharon, Mars en Joyce, meiden, wat ben ik toch blij met jullie! Jullie hielden me altijd met beide benen op de grond en onze altijd hilarische dates leidden tot de broodnodige ontspanning bij mij! Laten we vanavond op de borrel eens een uitzondering op de regel maken, en voor één keer helemaal niet gezellig, luidruchtig en vervelend doen! Ik neem een potje kaneel mee!

Judith, lieve Juud, zo'n fijn maatje zo dichtbij, wat ben ik blij met onze vriendschap! Vriendschap met een kop thee die soms uitmondt in een wijntje of biertje (in de stad, nog ééntje dan), een luisterend oor, spelletjes met de mannen en vooral al die ongelooflijk gezellige en grappige momenten (inclusief flauwe woordgrappen) die we samen mee hebben gemaakt en gaan maken! Daarnaast ben je één van de hele weinige personen van wie ik in al mijn eigenwijsheid écht nog wel eens wat aanneem! Heel veel dank voor alles, zonder jouw ontnuchterende Zeeuwse visie op de essentiële zaken in het leven liep ik nu denk ik nog steeds als een naïef kippetje zonder kop over het voetbalveld rond! Veel liefde en geluk gewenst, ook samen met jouw fantastische smikkelbeer-kanjers Jan en Siem. O enne... mijn naam is....^-_-^!

Lotte, lieve Lotje, ik kan eigenlijk niet goed verwoorden hoeveel onze vriendschap me waard is. Een half woord of zelfs maar een blik is al genoeg, hoewel ik ook altijd met heel veel plezier (en een kop thee en een koekje) jouw epistels lees en er één terug produceer. Dank je voor alle kopjes thee, koekjes, lunchjes, high teas, shopsessies, uitjes, etentjes, sushi-dates – en sinds vorig jaar pogingen tot het ongestoord ondernemen van eerdergenoemde activiteiten – en ervaringen die we over de kuikentjes uitwisselden. Jij begrijpt als geen ander hoe het is om alle ballen hoog te moeten houden, en je was een hele belangrijke steun voor me. Oneindig veel dank dat je aan mijn zijde staat op deze belangrijke dag, en natuurlijk voor je prachtige ontwerp van de cover van dit proefschrift. Ik wens je het allerbeste en –mooiste samen met jouw lieve Bas en Guusje.

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kan er dan beter aan mijn zijde staan op deze bijzondere dag dan jij? Veel succes met de laatste loodjes voor je proefschrift, en veel geluk en liefde gewenst samen met Daphne!

Djurre en Phi, ik ben blij met de familie die we vormen! Ik genoot afgelopen jaren van de tripjes naar Vinkeveen en de dierentuin, gourmet-sessies met de beste dresscode ever, de manier waarop we kerstcadeautjes verdelen en niet te vergeten een maag vol met Vietnamese loempia's. Ik heb vanaf nu weer meer tijd in mijn agenda, zullen we snel lekker uit eten gaan met elkaar en de kindjes? Mila, mijn allerliefste grote knappe prinses, tante is hartstikke trots op jou! Addy, tante Puntje, we zijn completer met jou erbij. Zal ik nu toch m'n GVB maar gaan halen?

Lieve Alexander en Aniek, dank jullie voor jullie eeuwige interesse en support, jullie hulp bij het schrijven van de Nederlandse samenvatting, alle gezelligheid, de fijne etentjes en niet te vergeten de diensten van jullie oppasbureau! Aniek, 36 maanden promotieonderzoek betekent 71.459 koppen thee en 18 maanden koude voeten, toch? Ro, van jou leerde ik denk ik de eerste les over serendipiteit: een zeker spel genaamd Stinksok. Lien, jij begrijpt als geen ander wat de sleutel tot succes is: tram 4 + Capadocia. Ik ben trots op jullie!

Lieve pap en mam, vanaf het allereerste moment dat ik zei dokter te willen worden, hebben jullie je vertrouwen in mij uitgesproken. Ook in de afgelopen jaren, zelfs als ik zelf de moed dreigde te verliezen, stond voor jullie als een paal boven water dat het allemaal zou gaan lukken en dat ik mijn doelen zou bereiken. Pap, ik begrijp nu pas, dat mij opvoeden als Feyenoord-fan me in de breedste zin des woords gevormd heeft tot doorzetter pur sang! Dank voor jullie oneindige vertrouwen in mij en alle andere dingen die jullie voor mij en voor ons betekend hebben, waaronder ook alle oppas-momenten, als er weer gewerkt moest worden door ons. Tobias had zich geen leukere en lievere opa en oma kunnen wensen, wij ons geen betere (schoon)ouders, ik hou van jullie.

Lieve lieve Tobias, mannetje, wat ben ik trots op jou en wat hou ik ongelooflijk veel van je. Jij hebt pas echt als geen ander mijn ogen geopend voor wat ik wil, en jij vormt voor mij de allergrootste motivatie om dingen te doen, ze goed te doen, en ze af te maken. Zeker de laatste maanden waren niet altijd leuk, als ik weer eens geen tijd had om met je te spelen of knuffelen, maar daar gaan we verandering in brengen, hè vriendje? Heel veel kusjes!

Sander, allerliefste Sander, jij kent me zo door en door dat ik eigenlijk niet op zoek hoef naar woorden om je te bedanken voor alles wat je voor me betekent. Deze promotie was niet alleen voor mij, maar ook voor ons af en toe een behoorlijke uitdaging, die ik zonder jou nooit tot een goed einde had kunnen brengen. En jij werd denk ik het allermeest de dupe van het

gebrek aan tijd in mijn agenda. Zullen we vanaf nu maar weer gaan doen waar we echt goed in zijn: Genieten? Ik hou oneindig veel van je, en dank je voor alles! Kus!