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/mm^3. 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 antiretroviral 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 significant 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.

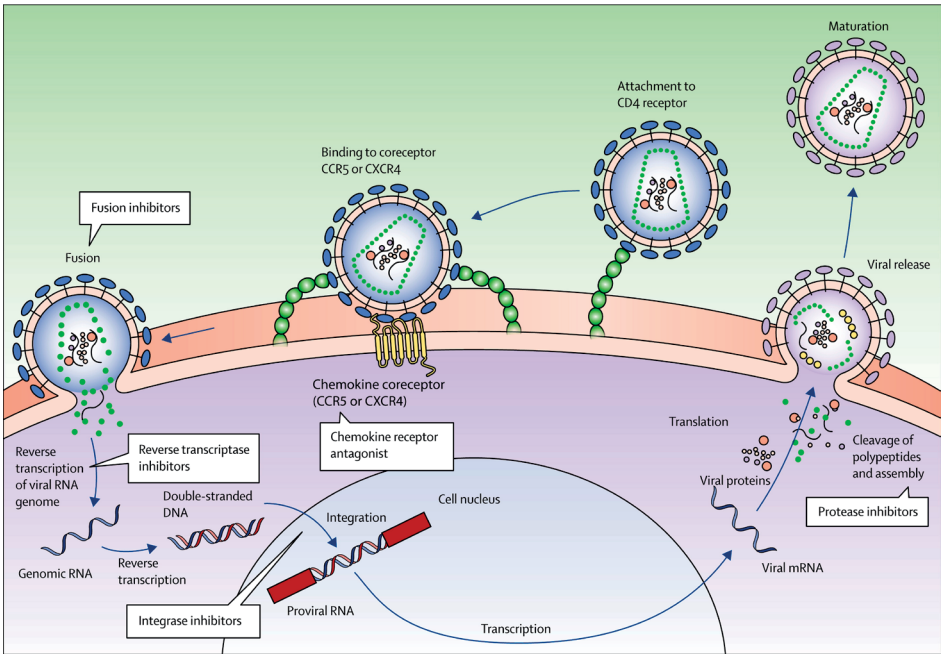

Figure 1. HIV-1 replication cycle. The white boxes indicate antiretroviral drug targets and accessory antiretroviral drug classes. Reproduced with permission from Walker et al.[21]

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 INSTI.[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]

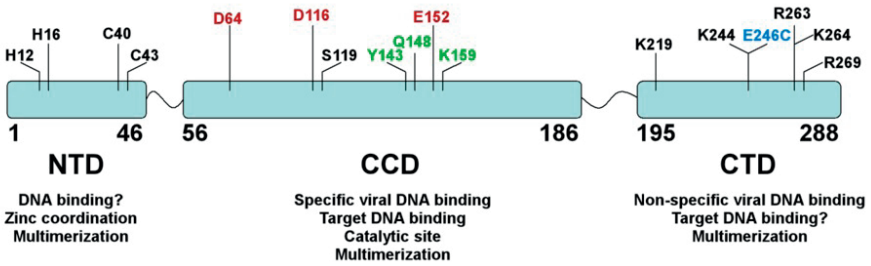

Figure 2. Schematic presentation of the structure of HIV-1 integrase. NTD=N-terminal domain; CCD=catalytic core domain; CTD=C-terminal domain. Reproduced with permission from Kessl et al.[62]

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]

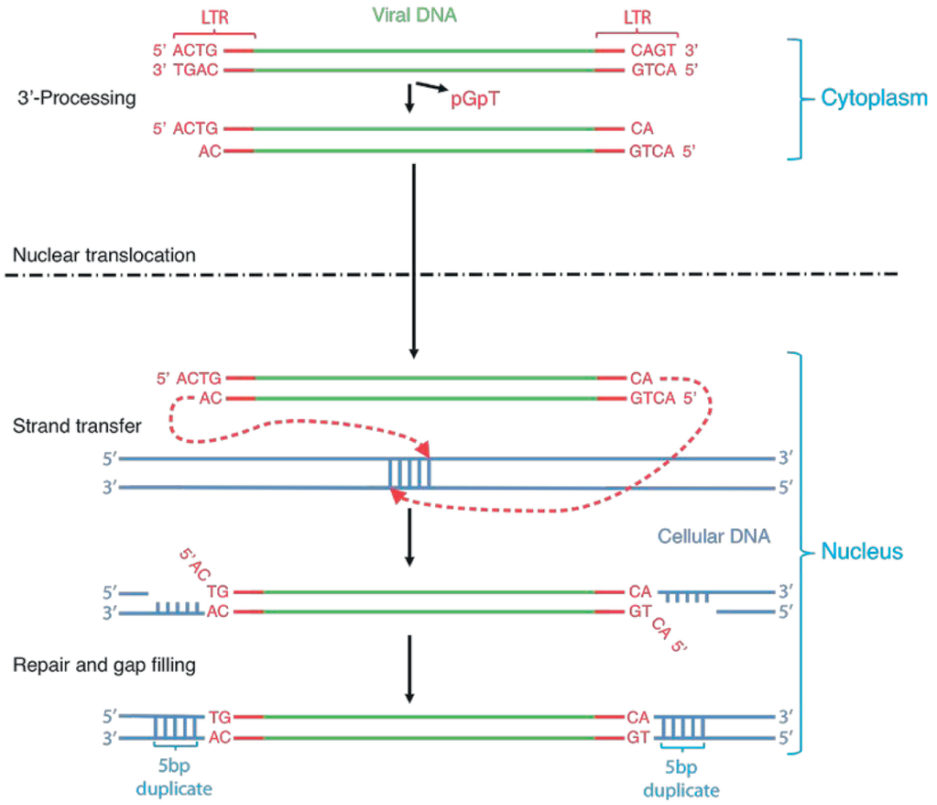

Figure 3. Schematic overview of integration of viral DNA into the host DNA. Reproduced with permission from Métifiot et al.[65]

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 T66K EVG: 10-40 fold DTG: 2-3 fold by T66K BIC: no effect of T66A/I
E92Q/G	RAL: >5 fold by E92Q EVG: >30 fold by E92Q, 10 fold by E92G DTG: 1.5 fold by E92Q BIC: no effect of E92Q
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 Y143C and R, 5-10 fold by Y143K/S/G/A EVG: no effect by Y143C and R DTG: no effect by Y143C and R **+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	Design	N	Main conclusion	Drug-related AEs RAL*	No of VF during RAL/No and type of INSTI RAMs[$]
Gatell et al, 2010[74]	II	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.	179	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	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.	703	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)

Rockstroh et al, 2013 (STARTMRK)[77,78]	III	Non-inferiority Double-blind Inclusion: cART-naive No resistance to TDF, FTC, and EFV Randomization: RAL or EFV, both combined with TDF/FTC.	563	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	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.	775	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/mm^3 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

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)

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)

Arribas et al, 2017 (STRATEGY-PI)[100–102]	III	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.	433	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

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

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.

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 1.43Log_{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 1.06Log_{10} in HIV-RNA, versus 0.10Log_{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 Inclusion: cART-naive patients No baseline RAMs Randomization: DTG QD or DRV/r, either + TDF/FTC or ABC/3TC, for 96 weeks.	484	Week 48 HIV-RNA < 50 c/mL: DTG: 90% DRV/r: 83% DTG superior to DRV/r. DTG was superior to DRV/r after 96 weeks in virological suppression rates. Faster virological suppression in DTG than in DRV/r. Similar increases of CD4 T-lymphocytes in both groups.	Similar rates of AEs. 2 drug-related SAEs in DTG: myocarditis and suicidality. Decrease in creatinine-clearance. Lower LDL in DTG than in DRV/r.	15/0

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), $=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 Inclusion: cART-naive No major RAMs 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.	286	Week 96 HIV-RNA<50 c/mL: Intramuscular CAB 4-weekly: 87% Intramuscular CAB 8-weekly: 94% Oral CAB: 84% Intramuscular CAB every 4 or 8 weeks combined with RPV as effective as CAB/ABC/3TC.	Two discontinuations for injection-site pain in the intramuscular group. SAEs comparable between groups and not drug-related.	3/2 (both in the 8-week group) R269R/G (not affecting CAB susceptibility) Q148R

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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