THERAPEUTIC MAINTENANCE OF THE M184V MUTATION IN THE REVERSE TRANSCRIPTASE GENE OF HUMAN IMMUNODEFICIENCY VIRUS TYPE-1 BY NUCLEOSIDE ANALOGUE INHIBITORS: IMPLICATIONS FOR CLINICAL MANAGEMENT

By

Marco Petrella

A thesis submitted to the Faculty of Graduate and Postdoctoral Studies, McGill University, in partial fulfillment of the requirement for the degree of Doctor of Philosophy

Department of Medicine

Division of Experimental Medicine

McGill University, Montreal, Canada

Submitted in March of 2005

Your file Votre référence
ISBN: 978-0-494-21689-7
Our file Notre référence
ISBN: 978-0-494-21689-7

ABSTRACT

The research for this doctoral dissertation was undertaken to: (1) establish a rationale that is supported by published laboratory and clinical data for prospective clinical investigation of the therapeutic maintenance of the M184V mutation in human immunodeficiency virus type-1 (HIV-1) reverse transcriptase (RT) in antiretroviral therapy-experienced patients with virological failure, and, (2) to study the relative effectiveness of various nucleoside analogue reverse transcriptase inhibitors (NRTIs) that are structurally-unrelated to 3TC in selecting and/or maintaining M184V in tissue culture and whether continuous exposure to therapeutic levels of 3TC is the only means of attaining this objective. Accordingly, we have evaluated the ability of zalcitabine (ddC), didanosine (ddI), abacavir (ABC) and the novel nucleoside analogue SPD754, in addition to 3TC, to maintain the presence of M184V in tissue culture and have shown that each of SPD754, ABC and 3TC were able to preserve M184V in mixed dual infections consisting of wild-type viruses and clinical isolates in which the M184V mutation was present either alone or in a genetic background consisting of several other resistance-conferring mutations in the RT gene. Moreover, M184V could also be maintained in these cultures when a subtherapeutic concentration of 3TC (i.e., 0.05 μM) was used. In contrast, neither ddI nor ddC were able to maintain M184V to the same extent as the other drugs tested after up to ten weeks of tissue culture in mixtures of wild-type viruses and isolates containing only M184V, especially when the latter represented a minority species in the viral population. The M184V substitution in HIV-1 RT develops rapidly following initiation of

therapy with 3TC and confers high-level phenotypic resistance to this drug and a related analogue, FTC, both *in vitro* and *in vivo*. Interestingly, the proximity of the M184V mutation in relation to the polymerase active site is also responsible for several alterations of RT biochemical activity (i.e., decreased enzymatic processivity, reduced nucleotide-dependent primer unblocking, increased fidelity, hypersensitization to other NRTIs, impaired viral fitness, and delayed appearance of mutations in the HIV-1 *pol* gene that confer resistance to certain NRTIs, and possibly, to PIs) that, collectively, might be of therapeutic consequence. Indeed, the results of numerous clinical studies are suggestive of improved virological and/or immunological responses with continued usage of 3TC and maintenance of the M184V mutation. However, with the exception of the COLATE Trial, these trials were not specifically designed to study maintenance of the M184V mutation as a therapeutic intervention in prospective fashion. There is a need for both additional research and randomized controlled clinical trials in order to validate the M184V benefit hypothesis in treatment-experienced patients with HIV-1 infection, and, in particular, for those patients requiring salvage antiretroviral therapy in whom management options are limited.

RÉSUMÉ

Cette thèse a été effectuée afin de: (1) développer une rationnelle qui est bien appuyée par des données publiées de laboratoire et clinique pour l'étude prospective de la maintenance thérapeutique de la mutation M184V dans la transcriptase inverse (TI) du virus de l'immunodéficience humaine de type-1 (VIH-1) chez les patients expérimentés à la thérapie antirétrovirale et qui subissent un échec virologique, et, (2) d'étudier l'efficacité de différents inhibiteurs nucléosidiques de la TI (INTI) qui possèdent une structure non-reliée au 3TC a sélectionner et/ou maintenir la mutation M184V en culture cellulaire et d'affirmer si l'exposition à des niveaux thérapeutiques de 3TC serait la seule façon d'atteindre cet objectif. Nous avons donc évalué la capacité de la zalcitabine (ddC), didanosine (ddI), l'abacavir (ABC), un nouvel inhibiteur nucléosidique, le SPD754, et bien sur du 3TC a maintenir la présence de la mutation M184V en culture cellulaire et avons démontré que le SPD754, ABC et 3TC pouvait tous effectivement préserver cette mutation dans des infections mixtes qui contenait des virus de type sauvage ainsi que des isolats dans laquelle la mutation M184V était présente uniquement ou apparaissait en présence d'autres mutations dans le gène de la TI. Nous avons aussi pu constater que la mutation M184V pouvait être maintenu dans ces cultures aussi bien par une exposition à des niveaux sous thérapeutique du 3TC (0.05μM). Par contre, ni le ddC ou le ddI ont pu manifester cette même capacité à préserver la mutation M184V et ceci pendant une période allant jusqu'a dix semaine dans les cultures mixtes infectées avec des virus de type sauvage et des isolats dans laquelle

M184V était la seule mutation et de plus quand cette mutation ne constituait qu'une minorité dans la population virale. La présence de la mutation M184V dans la TI du VIH-1 se manifeste rapidement suite au début d'un traitement avec le 3TC tout en étant responsable pour le développement d'une importante résistance phénotypique à ce médicament et à un analogue relié, le FTC, et ceci in vitro et in vivo. De plus, la proximité de la mutation M184V par apport au site catalytique de la TI serait aussi une raison pour plusieurs des altérations de la fonction biochimique de cet enzyme (diminution de la processivité de la TI, diminution de la « fitness » et de la réplication virale, augmentation de la fidélité de la TI, inhibition du déblocage de l'amorce ou de la pyrophosphorolyse, augmentation de la susceptibilité à la zidovudine et le tenofovir) et qui dans leur ensemble pourraient contribuer à un effet thérapeutique. Vraisemblablement, les résultats provenant de nombreuses études cliniques suggèrent la possibilité d'une amélioration des réponses virologiques et/ou immunologiques avec l'utilisation continu du 3TC et la maintenance de la mutation M184V. Cependant, ces mêmes études, avec l'exception de l'étude COLATE, n'étaient pas conçues *a priori* spécifiquement pour tester la préservation de la mutation M184V en tant qu'intervention thérapeutique et ceci de façon prospective. Par conséquence, il demeure un besoin important pour de plus amples recherches et d'études cliniques randomisées afin de bien valider le potentielle ce cette application chez les patients qui sont expérimentées à la thérapie antirétrovirale, et plus particulièrement, pour les patients qui nécessitent des thérapies de sauvetage et pour qui les options thérapeutiques peuvent êtres rapidement épuisées.

PREFACE

This Ph.D. thesis was written in accordance with the Guidelines for Thesis Preparation from the Faculty of Graduate and Postdoctoral Studies at McGill University. The structure and contents of the thesis conforms to the option which states:

"Candidates have the option of including, as part of the thesis, the text of one or more papers submitted, or to be submitted, for publication, or the clearly-duplicated text (not the reprints) of one or more published papers. These texts must conform to the "Guidelines for Thesis Preparation" with respect to font size, line spacing and margin sizes and must be bound together as an integral part of the thesis. (Reprints of published papers can be included in the appendices at the end of the thesis.) The thesis must be more than a collection of manuscripts. All components must be integrated into a cohesive unit with a logical progression from one chapter to the next. In order to ensure that the thesis has continuity, connecting texts that provide logical bridges preceding and following each manuscript are mandatory. The thesis must conform to all other requirements of the "Guidelines for Thesis Preparation" in addition to the manuscripts. The thesis must include the following: (1) a table of contents; (2) a brief abstract in both English and French; (3) an introduction which clearly states the rational and objectives of the research; (4) a comprehensive review of the literature (in addition to that covered in the introduction to each paper); (5) a final conclusion and summary; (6) a thorough bibliography; (7) Appendix containing an ethics certificate in the case of research involving human or animal subjects, microorganisms, living cells, other biohazards and/or radioactive material.

As manuscripts for publication are frequently very concise documents, where appropriate, additional material must be provided (e.g., in appendices) in sufficient detail to allow a clear and precise judgement to be made of the importance and originality of the research reported in the thesis.

In general, when co-authored papers are included in a thesis the candidate must have made a substantial contribution to all papers included in the thesis. In addition, the candidate is required to make an explicit statement in the thesis as to who contributed to such work and to what extent. This statement should appear in a single section entitled "Contributions of Authors" as a preface to the thesis. The supervisor must attest to the accuracy of this statement at the doctoral oral defence. Since the task of the examiners is made more difficult in these cases, it is in the candidate's interest to clearly specify the responsibilities of all the authors of the co-authored papers."

Original publications presented in chapters 2 and 3 have been published in refereed scientific journals. Chapter 1 includes a literature review and general introduction that clearly states the rationale and objectives of this research. A final conclusion and summary is presented in chapter 4. The candidate's contribution to original knowledge and references appear at the end of the thesis.

Two original papers described in chapter 2 and 3 are presented as follows:

Chapter 2: Petrella M., and M.A. Wainberg. 2002. Might the M184V substitution in HIV-1 RT confer clinical benefit? AIDS Rev. 4: 224-232.

Chapter 3: Petrella M., M. Oliveira, D. Moisi, M. Detorio, B.G. Brenner, and M.A. Wainberg. 2004. Differential maintenance of the M184V substitution in the reverse transcriptase of human immunodeficiency virus type-1 by various nucleoside antiretroviral agents in tissue culture. Antimicrob. Agents Chemother. 48: 4189-4194.

CONTRIBUTIONS OF AUTHORS

The candidate was responsible for all work described in this thesis, with the exception of parts of the work regarding virus isolation, preparation and stimulation of umbilical cord blood mononuclear cells (CBMCs), sequencing of HIV-1 *pol* genes, and cell culture of clinical isolates. Mervi Detorio and Maureen Oliveira (chapter 3) contributed to the virus isolation, collection, preparation, and stimulation of CBMCs. Maureen Oliveira also contributed to the tissue culture selection experiments with different clinical isolates. Daniela Moisi (chapter 3) contributed to the sequencing of the HIV-1 *pol* gene of the different clinical isolates and clonal variants used for this research. All work was performed in the laboratory of Dr. Mark A. Wainberg.

ACKNOWLEDGEMENTS

First and foremost, I would like to express my sincere appreciation and gratitude to Dr. Mark A. Wainberg, my research supervisor at McGill University, for having provided me with this unique opportunity to pursue my doctoral studies in his laboratory. Dr. Wainberg's mentorship and support were instrumental to the realization of my academic interests. Moreover, Dr. Wainberg's scientific leadership, commitment, and numerous contributions to HIV/AIDS research have been an important source of inspiration for many students like myself that also endeavor to make a contribution to this challenging and scientifically demanding field.

I would also like to thank Drs. Norbert Gilmore, Christos Tsoukas, Ralph Germinario and Chen Liang who as members of my thesis committee followed my progress and provided constructive criticism in regard to my research. I would also like to acknowledge Dr. Bluma Brenner for her scientific input and contribution to the work presented in chapter 3 of this thesis. In addition, a special note of appreciation is owed to Dr. Julio Montaner of the University of British Columbia for his advice, stimulating discussions, and contribution to our recently co-authored paper concerning both antiretroviral and cancer therapy that was also co-authored with Drs. Gerald Batist and Mark Wainberg. I would also like to convey my thanks to Drs. Dan Turner, Hugues Loemba, and Florence Doualla-Bell for their helpful comments. I am also extending thanks to my academic advisor, Dr. Konstantinos Pantopoulos, the late Dr. Gerald Price, Dominique

Besso, and Dr. Hugh Bennett for their guidance in the Division of Experimental Medicine at McGill University.

My thanks and appreciation is also extended to Maureen Oliveira, Mervi Detorio and Daniela Moisi for their expert technical assistance and to Bonnie Spira, Dr. Wainberg's laboratory manager, for her help and logistics support. I would also like to thank Estrella Moyal and Monica Marcela Romero for their clerical help with the preparation and submission of our manuscripts.

Finally, I am very grateful to my wife, Almerinda Pizzanelli, and my children, Victor and Amanda, for their patience and understanding during this time.

TABLE OF CONTENTS

Abstract	II
Résumé	IV
Preface	VI
Contributions of authors	VIII
Acknowledgements	IX
Table of contents	XI
List of figures and tables	XIV
List of abbreviations	XVI
Chapter 1: Literature review and general introduction	1
1.1. Literature review	2
1.1.1. Overview of antiretroviral therapy	2
1.1.2. Classification and availability of antiretroviral agents (ARV)	4
1.1.3.1. The emergence of drug resistance in HIV-1 infection	8
1.1.3.2.1. Competitive and non-competitive inhibitors of HIV-1 reverse transcriptase	10
1.1.3.2.2. Nucleoside analogue (NRTI) and nucleotide reverse transcriptase inhibitors (NtRTI)	10
1.1.3.2.3. Non-nucleoside analogue reverse transcriptase inhibitors (NNRTI)	13
1.1.3.3. HIV-1 protease inhibitors (PI)	15
1.1.3.4. Viral entry/fusion inhibitors (FI)	17
1.1.4.1. Monitoring resistance to antiretroviral therapy	19

1.1.5.1. Mechanisms of resistance conferred by NAMs 22

1.1.5.2. The M184V/I mutation 26

1.1.5.3. The V118I and E44D/A substitutions 28

1.1.5.4. Thymidine analogue mutations (TAMs) 29

1.1.5.5. Genetic pathways for development of TAMs 30

1.1.5.6. Effect of TAMs on excision 32

1.1.5.7 Multi-nucleoside resistance (MNR) 34

1.1.6.1. Viral fitness 35

1.1.7.1. Residual antiviral activity of 3TC against M184V-mutated HIV-1 37

1.1.8.1. Cross-resistance amongst NRTIs 38

1.2. General introduction 40

Chapter 2: Might the M184V substitution in HIV-1 RT confer clinical benefit? 44

Preface to chapter 2 44

2.1. Abstract 46

2.2. Introduction 47

2.3. Development of M184V in HIV-1 RT 49

2.4. M184V does not confer significant cross-resistance to other NRTIs 51

2.5. Reversal of resistance to AZT and synergistic antiviral activity with other drugs 53

2.6. Improved HIV-1 RT fidelity with M184V and delayed emergence of TAMs 55

2.7. Diminished HIV-1 RT processivity and impaired viral fitness with M184V 61

2.8. Effect of M184V on HIV disease outcomes and need for additional clinical trials 66

2.9. Conclusion 67

Chapter 3: Differential maintenance of the M184V substitution in the Reverse Transcriptase of Human Immunodeficiency Virus Type-1 by various nucleoside antiretroviral agents in tissue culture 70

Preface to chapter 3 70

3.1. Abstract 72

3.2. Introduction 73

3.3. Materials and methods 76

3.3.1. Tissue culture selection experiments 76

3.3.2. Genotypic analysis 77

3.3.3. Phenotypic resistance testing 78

3.4. Results 80

3.5. Discussion 88

3.6. Acknowledgments 93

Chapter 4: Final conclusion and summary 94

Contribution to original knowledge 106

References 111

LIST OF FIGURES AND TABLES

Chapter 1:

Figure 1: Schematic representation of the HIV-1 life cycle illustrating the viral targets for different classes of FDA-approved ARVs 6

Table 1: Antiretroviral drugs approved by the United States Food and Drug Administration (FDA) for the treatment of HIV/AIDS as of July 2004 7

Table 2: Mutations in HIV-1 reverse transcriptase commonly associated with resistance to NRTIs/NtRTIs 25

Chapter 2:

Table 1: Incidence of various resistance-conferring mutations in patients on initial triple combination therapy regimens 60

Chapter 3:

Table 1: IC_{50} of various NRTIs for clinical isolates and fold-resistance of clinical isolates to the various NRTIs 81

Table 2: Effects of different NRTIs on maintenance of the M184V mutation in tissue culture 83

Table 3: Effect of 9 weeks of antiretroviral drug pressure on maintenance of M184V in mixtures of HXB2D and clinical isolate 3350, an M184V-containing HIV-1 variant 85

Table 4: Selection and/or maintenance of the M184V mutation during 10 weeks of antiretroviral drug exposure in mixtures of HIV-1 isolates 87

LIST OF ABBREVIATIONS

3TC: lamivudine

3TC-TP: 3TC triphosphate

AAUCMB: area under the curve over time minus changes from baseline

ABC: abacavir

AIDS: acquired immunodeficiency syndrome

APV: amprenavir

ARV: antiretroviral agent or antiretroviral drug

ATP: adenosine triphosphate

CA: capsid protein

CBMC: umbilical cord blood mononuclear cell

CHIP: Copenhagen HIV Programme

d4T: stavudine

dCTP: deoxycytosine triphosphate

ddC: zalcitabine

ddI: didanosine

ddNTP: dideoxynucleotide triphosphate

DLV: delavirdine

DNA: deoxyribonucleic acid

dNTP: deoxynucleotide triphosphate

DRG: drug resistance genotyping

DSMB: data safety monitoring board

EFV: efavirenz

env: envelope gene

f-APV: fosamprenavir

FC: fold-change

FDA: United States Food and Drug Administration

FDC: fixed-dose combination

FI: fusion inhibitor

FTC: emtricitabine

GART: genotypic antiretroviral resistance testing

HAART: highly active antiretroviral therapy

HBV: hepatitis B virus

HIV-1: human immunodeficiency virus type-1

HR-1: heptad repeat domain 1

HR-2: heptad repeat domain 2

IC_{50}: fifty percent inhibitory concentration

IDV: indinavir

IN: integrase

LiPA: line probe assay

LPV: lopinavir

MNR: multi-nucleoside resistance

NAM: nucleoside analogue mutation or nucleoside/nucleotide-associated mutation

NEM: nucleoside excision mutation

NFV: nelfinavir

NNRTI: non-nucleoside reverse transcriptase inhibitor

NRTI: nucleoside analogue reverse transcriptase inhibitor

NtRTI: nucleotide reverse transcriptase inhibitor

NVP: nevirapine

PBMC: peripheral blood mononuclear cell

PCR: polymerase chain reaction

PHI: primary HIV infection

PI: protease inhibitor

PMEA: adefovir

PMPA: tenofovir dipivoxil fumarate (TDF)

pol: polymerase gene

PPi: pyrophosphate

PR: protease

RC: replication capacity

RCT: randomized controlled clinical trial

RNA: ribonucleic acid

RNase H: ribonuclease H

RT: reverse transcriptase

RTV: ritonavir

SIV: simian immunodeficiency virus

SOC: standard of care

SQV: saquinavir

STI: structured treatment interruption

T-20: enfuvirtide

TAM: thymidine analogue mutation or thymidine-associated mutation

TI: treatment interruption

VL: viral load

WT: wild-type

YMDD: tyrosine-methionine-aspartate-aspartate

ZDV: zidovudine (AZT)

ZDVMP: zidovudine monophosphate

CHAPTER 1

Literature Review and General Introduction

1.1. Literature review

1.1.1. Overview of antiretroviral therapy

The introduction of efficacious combination antiviral chemotherapy for human immunodeficiency virus type-1 (HIV-1) infection and acquired immunodeficiency syndrome (AIDS), commonly referred to as highly active antiretroviral therapy (HAART), during the past decade is widely recognized as a major realization in the clinical management of this presently incurable infectious disease with global prevalence (UNAIDS/WHO, 2004). The initial demonstration of HAART was achieved in therapy-naïve and zidovudine (ZDV)-experienced patients with HIV-1 infection through intensification of incompletely suppressive regimens consisting of two licensed nucleoside analogue reverse transcriptase inhibitors (NRTI) with a third drug from a novel and distinct class of antiretroviral agents (ARV) known as HIV-1 protease inhibitors (PI) (Collier et al., 1996b; Gulick et al., 1997; Mathez et al., 1997). This strategy was based on the principle of divergent combination therapy in which drugs with complementary mechanisms of action are combined to achieve increased therapeutic activity (Lange, 1995) and was already well-established for the treatment of diseases such as mycobacterium tuberculosis infection (Eliopoulos and Moellering, 1982; Snider et al., 1985; Frieden et al, 2003; Di Perri and Bonora, 2004) and cancer (Frei, 1985; Fan et al., 1998; Hortobagyi, 1997; Bunn, 2001; Yung and Linch, 2003). In comparison to the limited treatment modalities that were available during the pre-HAART era (i.e., ZDV monotherapy and dual NRTI regimens), the use of this approach which involved three drugs from

different classes of ARVs allowed for the first time effective suppression of plasma HIV-1 RNA levels to below the limit of quantitation of the currently available viral load (VL) assays, and consequently, resulted in superior gains in HIV/AIDS-related health outcomes (Collier et al., 1996a; Gulick et al., 1997; Cameron et al., 1998). The primary goals of modern combination antiretroviral therapy are multiple and include: maximal and durable suppression of viral replication as monitored through reduction of plasma HIV-1 RNA, restoration of immune function and prevention and/or delay of both disease progression and the emergence of drug resistance (Fischl, 1999; Lederman, 2001; Dybul et al., 2002; Louie and Markowitz, 2002; Yeni et al., 2004).

Today, the availability of a wide array of ARVs in conjunction with improved knowledge of HIV-1 pathogenesis has been instrumental for both the continued development and attainment of the goals of antiretroviral therapy for an increasing number of patients with HIV/AIDS. Clinicians are now able to construct potent regimens that can be individualized to some extent with three or more drugs for initiation, maintenance and salvage antiretroviral therapy (Yeni et al., 2004). Numerous clinical studies have also demonstrated significantly reduced HIV/AIDS morbidity and mortality associated with increased use of HAART in North America and Europe (Hogg et al., 1998; Pallela et al, 1998; Vittinghoff et al., 1999; Mocroft et al., 2000), and in industrialized countries, HAART has been adopted as the standard of care (SOC) for the treatment of HIV-infection and AIDS. The rational implementation of combination antiretroviral therapy on a

larger scale in resource-poor countries commensurate with wider access to anti-HIV drugs is also viewed as an urgent and important goal (Petrella et al., 2001; Doualla-Bell et al., 2004; Kuritzkes, 2004a; Wainberg, 2004a).

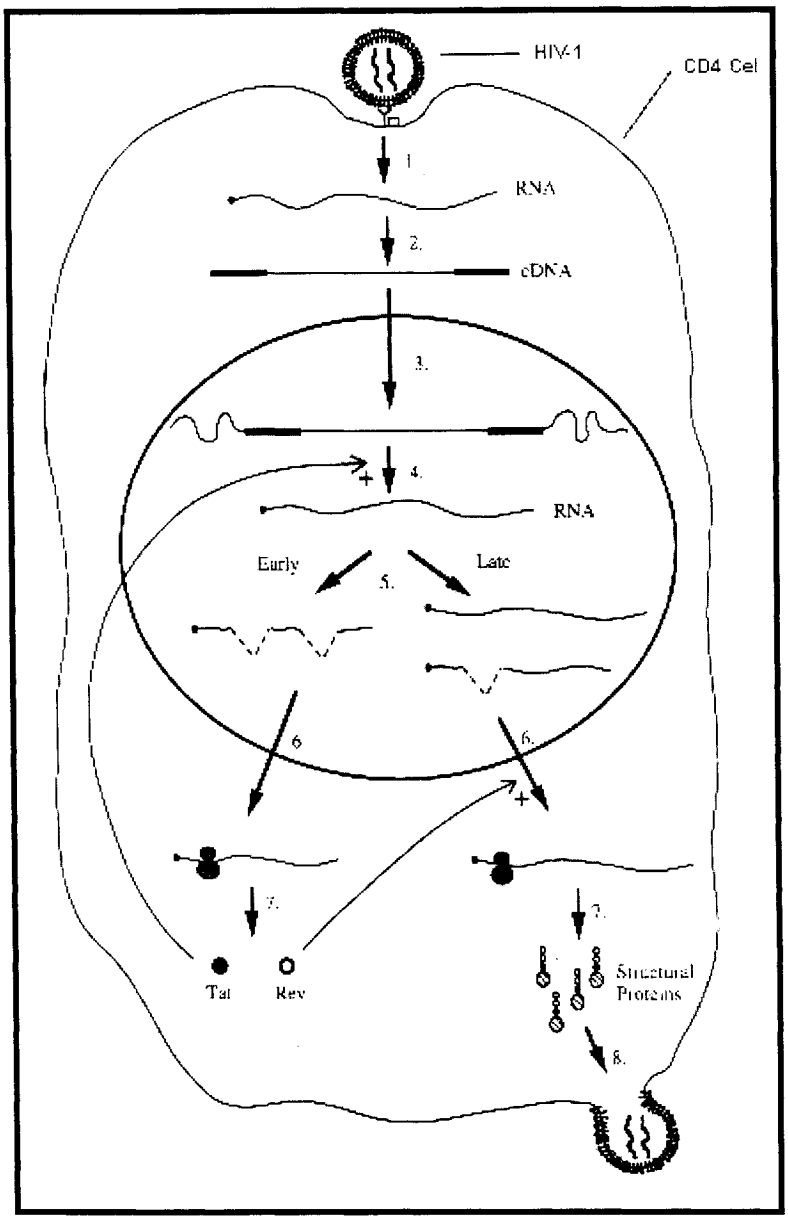
1.1.2. Classification and availability of antiretroviral agents (ARV)

The ARVs that are currently available for treatment of HIV/AIDS are listed in Table 1 and belong to four different classes that include: nucleoside analogue (NRTI) and nucleotide (NtRTI) reverse transcriptase (RT) inhibitors, non-nucleoside reverse transcriptase inhibitors (NNRTI), protease inhibitors (PI) and viral entry or fusion inhibitors (FI). In addition, several fixed-dose combination products (FDC) in which two or three HIV-1 RT inhibitors (i.e., NRTI, NtRTI or NNRTI) are co-formulated have recently become available. FDCs have been of considerable value in helping to lessen the high pill burden that is typically associated with many antiretroviral therapy regimens (Chesney et al., 1999; Tapper et al., 2004; Valenti, 2004).

As shown in Figure 1, the pharmacological activity of approved ARVs is dependent on viral target inhibition and in *in vitro* assays, these compounds have been shown to be potent and selective antagonists of HIV-1 enzymes or receptors that are essential for the retroviral life cycle (Johnston and Hoth, 1993; Gottfredsson and Bohjanen, 1997; Moore and Stevenson, 2000; Wlodawer, 2002). Additionally, although many combinations of ARVs can display additive or synergistic effects *in vitro* (Merrill et al., 1996; Tremblay et al., 2003),

antagonism between drugs belonging to the same or different class is also possible. For example, incompatibility between the NRTIs ZDV and d4T has been observed and is thought to result from competition for metabolic pathways that are required for the activation of these compounds (Ho and Hitchcock, 1989; Hoggard et al., 1997; Havlir et al., 2000). Management with ARVs may be also associated with clinically important toxicities that can seriously diminish the tolerability, and ultimately, effectiveness of therapy (Brinkman et al., 1999; Carr and Cooper, 2000; Jain et al., 2001; Anderson, 2002; Côté et al., 2002). Since some treatment-limiting adverse reactions also have been shown to occur with higher frequency within certain classes of ARVs (i.e., class-specific toxicities), careful selection and/or sequencing of the drugs that are included in combination regimens for treatment of HIV-1 infection is necessary in order to prevent pharmacokinetic drug interactions (Burger et al., 1997; de Maat et al., 2003) and potentially dangerous overlapping toxicities.

Figure 1: Schematic representation of the HIV-1 life cycle illustrating the viral targets for different classes of FDA-approved ARVs



1. **Viral Binding and Entry (Fusion Inhibitors)**

2. **Reverse Transcription (NRTIs/NtRTIs and NNRTIs)**

3. Nuclear Import and Integration

4. Transcription

5. Splicing

6. Nuclear Export

7. Translation

8. **Protein Processing and Viral Assembly (PIs)**

Adapted with permission from: Gottfredsson M., and P.R. Bohjanen. 1997. Front. Biosci. 2: 619-634.

Table 1: Antiretroviral drugs approved by the United States Food and Drug Administration (FDA) for the treatment of HIV/AIDS as of July 2004

Antiretroviral Class	Drug Name	FDA Approval Date
NRTI/ NtRTI	Zidovudine (AZT)	March 1987
	Didanosine (ddI)	October 1991
	Zalcitabine (ddC)	June 1992
	Stavudine (d4T)	June 1994
	Lamivudine (3TC)	November 1995
	Abacavir	December 1998
	Tenofovir DF (PMPA)	October 2001
	Emtricitabine (FTC)	July 2003
NNRTI	Nevirapine	June 1996
	Delavirdine	April 1997
	Efavirenz	September 1998
PI	Saquinavir	December 1995
	Ritonavir	March 1996
	Indinavir	March 1996
	Nelfinavir	March 1997
	Amprenavir	April 1999
	Lopinavir/ritonavir	September 2000
	Atazanavir	June 2003
	Fosamprenavir	October 2003
FI	Enfuvirtide (T-20)	March 2003

1.1.3.1. The emergence of drug resistance in HIV-1 infection

Antiretroviral drug resistance is an important limitation in treatment of HIV/AIDS that can significantly diminish the effectiveness and duration of benefit associated with HAART (Lorenzi et al., 1999; Winters et al., 2000; Quirós-Roldán, 2001; Rousseau et al., 2001; Johnson et al., 2004). Resistance mutations to all approved and investigational ARVs can emerge spontaneously because of the error-prone replication of HIV-1 and, in addition, are selected and maintained both *in vitro* and *in vivo* by drug pressure (Roberts et al., 1988; Preston and Dougherty, 1996; Rezende et al., 1998; Menendez-Arias, 2002). Several factors including RT processivity, viral fitness, viral pool size, and availability of target cells for infection, in addition to the low fidelity of DNA synthesis by HIV-1 RT are also important determinants of HIV-1 mutagenesis (Coffin, 1995; Drosopoulos et al., 1998; Colgrove and Japour, 1999; Overbaugh and Bangham, 2001). Alterations in any one or combination of these factors might influence the development of drug resistance. Drug-resistant HIV-1 variants may also be acquired through maternal-fetal (i.e., vertical), sexual and other forms of transmission (Tyndall, 2003; Gallant, 2004; Hamers and Downs, 2004; Scarlatti, 2004).

Although, resistance-conferring mutations may precede the initiation of therapy, the development of HIV-1 drug resistance is primarily a consequence of persistent viral replication and the accumulation of resistance mutations during administration of incompletely suppressive regimens (de Jong et al., 1996;

Mayers, 1997; Balotta et al., 2000; Clavel and Hance, 2004). Genotypic analysis has shown that continued exposure to failing combination therapy regimens is associated with complex and often overlapping patterns of drug resistance mutations that predict increasing levels of resistance and, for that matter, cross-resistance, to antiretroviral therapy. In general, multiple drug mutations need to be selected in order to produce clinical resistance to most ARVs, and in particular, the PIs. Exceptions to this include lamivudine (3TC) and a structurally-related compound, emtricitabine (FTC), and the NNRTIs which all have low genetic barriers for resistance since only a single drug resistance mutation in RT can lead to substantial loss of antiviral activity (Tisdale et al., 1993; Quan et al., 1996; Gao et al., 2000; Deeks, 2001; Quirós-Roldán et al., 2002).

In the following sections, genotypic resistance to NRTIs/NtRTIs, NNRTIs, PIs and viral entry inhibitors will be reviewed with reference to important aspects of the pharmacology and mechanism of action of these drugs. Moreover, the emergence and role of the M184V mutation in HIV-1 RT and its relevance to other NRTI/NtRTI-associated resistance mutations (NAMs) are fundamental topics with respect to the scope and objectives of this doctoral dissertation, and therefore, will be discussed more completely hereinafter.

1.1.3.2.1. Competitive and non-competitive inhibitors of HIV-1 reverse transcriptase

Together with protease (PR) and integrase (IN), RT is one of three enymes that is encoded by the HIV-1 polymerase (*pol*) gene and is responsible for the transcription of double-stranded proviral DNA from viral genomic RNA (Jacobo-Molina and Arnold, 1991; Katz and Skalka, 1994; Tarrago-Litvak et al., 1994). RT also contains ribonuclease H (RNase H) enzymatic activity that degrades genomic RNA generating purine-rich fragments that serve as primers during retroviral DNA synthesis (Schatz et al., 1990; Davies et al., 1991; Tarrago-Litvak et al., 1994; Nikolenko et al., 2005). Two categories of drugs have been developed to block RT; these are the nucleoside analogue (NRTI) and nucleotide (NtRTI) RT inhibitors that function as competitive antagonists, and secondly, the non-nucleoside RT inhibitors (NNRTI) that act as non-competitive antagonists of RT enzyme activity (Yeni et al., 2004).

1.1.3.2.2. Nucleoside analogue (NRTI) and nucleotide reverse transcriptase inhibitors (NtRTI)

NRTIs/NtRTIs are pharmacologically inactive in their administered form and are phosphorylated to their virologically competent triphosphate moieties by host cellular kinases (De Clercq, 1992; Gray et al., 1995; Peter and Gambertoglio, 1998; Schneider et al., 2000; Hoggard et al., 2001; Stein and Moore, 2001). These analogues resemble naturally occurring deoxynucleotide triphosphates (dNTPs) and can effectively compete with these substrates for binding to HIV-1 RT and

incorporation into viral DNA (Furman et al., 1986; Hart et al., 1992). However, NRTIs/NtRTIs either lack or contain other functional groups that replace the 2'- and/or 3'-hydroxyl groups on the nucleoside pentose ring that is necessary for DNA polymerization; hence, the mechanism of action underlying the antiviral activity of these compounds is based on termination of viral DNA strand elongation (Mitsuya et al., 1987; Furman and Barry, 1988; Goody et al., 1991; Furman et al., 2000; Wlodawer, 2002).

Including zidovudine (AZT, ZDV), the first antiretroviral drug developed for the treatment of patients with HIV-1 infection (Mitsuya and Broder, 1986; Fischl et al., 1987; Loveday, 2001), seven NRTIs i.e., didanosine (ddI), zalcitabine (ddC), stavudine (d4T), lamivudine (3TC), abacavir (ABC) and emticitabine (FTC) have been approved to date by the United States Food and Drug Administration (FDA) and by regulatory agencies elsewhere for use in antiretroviral combination therapy regimens (Table 1) (Yeni et al., 2004). In addition, tenofovir dipivoxil fumarate (TDF, PMPA), a potent NtRTI, has been approved for the treatment of HIV-1 (Fung et al., 2002; Schooley et al., 2002; Louie et al., 2003) while a related NtRTI, adefovir dipivoxil, is available for treatment of hepatitis B (HBV) infections (Hadziyannis et al., 2003; Karayiannis, 2003; Marcellin et al., 2003); 3TC is also approved for the latter indication (Dienstag et al., 1999; Jonas et al., 2002). Although they share some structural similarity with NRTIs, NtRTIs contain a phosphonate group which reduces the requirement of these drugs for phosphorylation. This property is believed to

impart certain advantages to TDF such as heightened antiviral potency and longer intracellular half-life compared to most existing NRTIs (Squires, 2001).

Resistance-conferring mutations to NRTIs/NtRTIs can be selected both in tissue culture protocols by exposure to increasing drug levels, and similarly, in patients following treatment with NRTI- or NtRTI-containing regimens. These mutations encode amino acid substitutions in HIV-1 RT at codons that are responsible for diminished antiviral susceptibility to these inhibitors and, in general, are predictive of worse virological responses to antiretroviral therapy (Lorenzi et al., 1999; Winters et al., 2000; Johnson et al., 2004). Drug resistance to different NRTI/NtRTIs appears to be mediated by two principal mechanisms. Firstly, some resistance mutations in RT are associated with increased drug discrimination, a situation where the binding and/or incorporation of antiviral drugs by the mutated RT is significantly attenuated (Götte and Wainberg, 2000; Soriano and de Mendoza, 2002). The M184V mutation in RT, which confers high-level phenotypic resistance to 3TC (i.e., 500- to 1,000-fold increase in IC_{50}) and FTC is considered to be a classical discriminatory mutation (Wainberg et al., 1995; Keulen et al., 1997) Alternatively, another group of RT mutations known as thymidine analogue mutations (TAMs) promote resistance to ZDV and d4T as a result of increased levels of nucleotide primer uncoupling. With this mechanism, ZDV- or d4T-monophosphate is excised from the terminus of blocked primer templates by RT containing TAMs, thus restoring DNA polymerization and

permitting HIV-1 replication to continue in the presence of these antiviral drugs (Götte and Wainberg, 2000; Miller and Larder, 2001).

1.1.3.2.3. Non-nucleoside analogue reverse transcriptase inhibitors (NNRTI)

NNRTIs include a heterogeneous group of compounds that act as non-competitive inhibitors of HIV-1 RT. The basis for their antiviral activity is not completely understood but is known to involve the binding of these compounds to a hydrophobic pocket that is located approximately 10 Å from the RT substrate binding site in the p66 subunit (Wlodawer, 2002). The NNRTI binding pocket also contains a few amino acid residues from the smaller and catalytically incompetent p51subunit of RT (Wu et al., 1991; Ding et al., 1995). It is thought that the inhibition of HIV-1 replication by NNRTIs may be mediated through allosteric effects which lock the RT active site into a configuration that is incompatible with DNA polymerization. NNRTIs reduce the catalytic rate of polymerization without affecting nucleotide binding or nucleotide-induced conformational change (Spence et al., 1995). These drugs are particularly active at template positions at which the RT enzyme naturally pauses and, moreover, do not appear to influence the competition between dideoxynucleotide triphosphates (ddNTPs) and the naturally occurring dNTPs for insertion into the growing proviral DNA chain (Sardana et al., 1992; Dueweke et al., 1993; Gu et al., 1995; Deeks, 2001). There are presently three NNRTIs, i.e., nevirapine (NVP), delavirdine (DLV) and efavirenz (EFV), that have been approved for use in antiretroviral combination therapy regimens (Yeni et al., 2004) (Table 1).

Diminished sensitivity to NNRTIs appears quickly both in tissue culture selection protocols and in patients (Wu et al., 1991; Ding et al., 1995; Deeks, 2001). NNRTIs share a common binding site, and mutations that encode NNRTI resistance are located within the binding pocket that makes drug contact (Richman et al., 1991; Wu et al., 1991; Chong et al., 1994; Vandamme et al., 1994; Ding et al., 1995; Esnouf et al., 1995; Gu et al., 1995; Spence et al., 1995). This explains the finding that extensive cross-resistance is observed among all currently approved NNRTIs (Byrnes et al., 1993; Fletcher et al., 1995; Deeks, 2001). A substitution at codon 181 (i.e., Y181C) is a common mutation that encodes cross-resistance among many NNRTIs (Richman et al., 1991; Balzarini et al., 1993; Byrnes et al., 1993; Deeks, 2001). Replacement of Y181 by a serine or histidine also conferred HIV-1 resistance to NNRTIs (Sardana et al., 1992). A mutation at amino acid 236 (i.e., P236L), conferring resistance to a particular class of NNRTIs that include DLV, can also diminish resistance to NVP and other NNRTIs, particularly if a Y181C mutation is also present in the same virus (Dueweke et al., 1993). Y188C and Y188H are other important mutations that can also confer resistance to NNRTIs. Another drug resistance mutation namely, K103N, is also commonly observed and is responsible for reduced susceptibility to all approved NNRTIs (Richman et al., 1991; Balzarini et al., 1993; Byrnes et al., 1993; Deeks, 2001). Substitution of K103N results in alteration of interactions between NNRTIs and RT. The K103N mutation shows synergy with Y181C in regard to resistance to NNRTIs, unlike antagonistic interactions involving Y181C and P236L (Nunberg et al., 1991).

1.1.3.3. HIV-1 protease inhibitors (PI)

The HIV-1 specific aspartyl protease (PR) is responsible for the processing of gag and gag-pol polyprotein precursors into the functional proteins (i.e., p17, p24, p7, p6, p2, p1, RT, IN and PR) that are required to generate infectious virions (Kohl et al., 1988; Navia et al., 1989; Navia and McKeever, 1990; Debouck, 1992; Bhat et al., 1994). HIV-1 protease inhibitors (PI) used for antiretroviral therapy are generally small (i.e., molecular weight less than 1,000) synthetic molecules that structurally resemble the viral substrates normally cleaved by PR (Roberts et al., 1990; Lam et al., 1994; Wlodawer and Vondrasek, 1998; Wlodawer, 2002). These compounds are competitive antagonists of PR and bind to the enzyme's catalytic site with a high degree of affinity and selectivity. Treatment of HIV-1 infected cells with PIs in tissue culture leads to the formation of immature viral progeny that cannot establish further rounds of infection. The following PIs are currently approved for treatment of HIV-1 infection: saquinavir (SQV), ritonavir (RTV), indinavir (IDV), nelfinavir (NFV), amprenavir (APV), lopinavir (LPV), atazanavir (ATZ) and fosamprenavir (f-APV) (Yeni et al., 2004) (Table 1).

Drug-resistant viruses have been observed in the case of all PIs developed to date. In addition, some strains of HIV have displayed cross-resistance to a variety of PIs after either clinical use or *in vitro* drug exposure (Condra, 1998; Deeks, 1999; Murphy, 1999; Johnson et al., 2004). In general, the patterns of mutations observed with PIs are more complex and extensive than those observed

with RT antagonists (Johnson et al., 2004). This involves greater variability, as well, in temporal patterns of appearance of different mutations and the manner in which different combinations of mutations can give rise to phenotypic resistance. These data suggest that the viral PR enzyme can adapt more easily than RT to pressures exerted by ARVs. At least 40 mutations in PR have been identified as responsible for resistance to PIs (Condra, 1998; Deeks, 1999; Murphy, 1999; Johnson et al., 2004). Certain of the mutations within the HIV-1 PR are more important than others and can confer resistance, virtually on their own, to at least certain PIs (Condra, 1998; Deeks, 1999; Murphy, 1999; Miller, 2001). One mutation, in particular, D30N, is probably unique to NFV, a potent HIV protease inhibitor (Markowitz et al., 1998; Patick et al., 1998; Sugiura et al., 2002). However, a variety of other mutations may confer cross-resistance among multiple drugs within the PI family. In addition, wide arrays of secondary mutations have been observed, that, when combined with primary mutations, can cause increased levels of resistance to occur (Servais et al., 2001b; Clemente et al., 2003; Turner et al., 2004). Although the presence of secondary mutations on their own may not lead to increased levels of drug resistance, some of the amino acid changes encoded by secondary mutations can mediate a compensatory effect to improve the replication capacity of viruses that contain primary PR mutations while also conferring cross-resistance to other PIs (Nijhuis et al., 1999; Gatanaga et al., 2002; Menzo et al., 2003; Weber et al., 2003). In addition, it should be noted that resistance to PIs can also result from mutations within the substrates of the PR enzyme, i.e., the gag and gag-pol precursor proteins of HIV. A variety of

studies have now shown that mutations at cleavage sites within these substrates can be responsible for drug resistance, both in tissue culture as well as in treated patients (Zhang et al., 1997; Bally et al., 2000; Miller, 2001; Whitehurst et al., 2003). The full clinical significance of these cleavage site mutations in regard to PI resistance, however, remains to be elucidated.

1.1.3.4. Viral entry/fusion inhibitors (FI)

Enfuvirtide (T-20) is the first entry in a novel class of antiretroviral agents known as HIV-1 entry inhibitors and has recently been approved for the treatment of HIV-1 infection (Cervia and Smith, 2003). This compound is a synthetic peptide consisting of 36 amino acids that are homologous to the residues located at positions 127 to 162 of the C-terminus of the heptad repeat 2 (HR-2) domain in the gp41 transmembrane glycoprotein of the viral envelope. T-20 binds competitively to the HR-1 domain within gp41, thus preventing interaction with HR-2 and formation of the hairpin-like structure that is required for fusion of the viral and host cell membranes (Tomaras and Greenberg, 2001; Cervia and Smith, 2003).

In the TORO-1 and TORO-2 studies, the addition of T-20 to optimized background therapy consisting of 3 to 5 active antiretroviral drugs, that were selected using genotypic drug resistance testing, was shown to result in significant reduction of plasma HIV-1 RNA and CD4 cell count increases compared to optimized background therapy alone in heavily treatment-experienced patients

with HIV-1 infection that was resistant to NRTIs, NNRTIs and PIs (Lalezari et al., 2003c; Lazzarin et al., 2003). The results from additional open-label and controlled clinical trials with this drug have similarly demonstrated improved treatment outcomes for up to 48 weeks in HIV-1 patients that were experiencing virological failure on previous regimens (Lalezari et al., 2003a; Lalezari et al., 2003b).

In phase 1 clinical testing, resistance to T-20 developed rapidly and was evidenced by rebounding plasma HIV-1 RNA after 14 days of monotherapy in four patients receiving an intermediate dose (i.e., 30 mg twice daily) of T-20 (Wei et al., 2002). Genotypic analysis of cloned virus from these patients showed that resistance to T-20 was produced by substitutions in the highly conserved GIV motif which comprises a three amino acid sequence between residues 36 to 38 within the HR-1 domain that is essential for fusion of viral and cellular membranes to occur. Mutants that contained a single amino acid substitution in GIV (i.e., G36D, I37V and V38A/M) were frequently detected (Wei et al., 2002). G36D, and in particular, V38A both elicited significant fold-increases in the IC_{50} for T-20 compared to HIV-1 strains with wild-type envelope sequences. In addition, dual mutants that contained G36D together with substitutions at other amino acid residues within HR-1 (i.e., Q32H/R and Q39R) were also observed and were shown to confer reduced susceptibility to T-20 to an extent similar to that produced with G36D by itself (Wei et al., 2002). Interestingly, variability in the HR-1 domain at positions that are associated with resistance to T-20 has been

demonstrated in both subtype B (i.e., residues 37, 39 and 42) and in non-B (i.e., residue 42) HIV-1 strains isolated from T-20-naïve patients (Roman et al., 2003). However, the major GIV mutants commonly associated with T-20 resistant isolates were not observed in the absence of drug treatment suggesting that primary genotypic resistance to this drug is uncommon (Roman et al., 2003). Further study is needed to better understand the long-term implications of these uncommon resistance mutations in HIV-1 patients undergoing therapy with fusion inhibitors.

1.1.4.1. Monitoring resistance to antiretroviral therapy

Two types of technology for measuring HIV-1 drug resistance, referred to as genotypic and phenotypic tests, are available to clinicians to help guide decisions in antiretroviral therapy. These tests permit characterization of specific changes in the genomic nucleotide sequence of viral isolates in comparison to a HIV-1 reference strain to monitor the development of resistance to antiretroviral therapy (Arens, 2001; Smith, 2001). With genotyping, mutations in the HIV-1 *pol* or envelope (*env*) genes are commonly detected by automated techniques based on the Sanger method for dideoxy-terminator nucleotide sequencing (Arens, 2001) or, alternatively, with hybridization tests such as the line probe assay (LiPA) that detect point mutations at codons known to be important for resistance to specific drugs (Stuyver et al., 1997; Arens, 2001; Servais et al., 2001a). The effective utilization of these genotypic tests requires expert clinical interpretation of mutational patterns; this task may be facilitated by computerized algorithms

specifically designed for HIV-1 genotypic analysis (Hanna and D'Aquila, 2001; Schmidt et al., 2002; Youree and D'Aquila, 2002). In contrast to genotyping, phenotypic tests represent a more direct method for measurement of HIV-1 drug resistance based on changes in the concentration of a particular drug that is required to inhibit viral replication by 50 percent (i.e., IC_{50}) when studying clinical isolates of HIV-1 (Dunne et al., 2001; Demeter and Haubrich, 2001; Lerma and Heneine, 2001). Phenotypic resistance can be determined with traditional tissue culture techniques in which p24 core antigen capsid (CA) protein production by HIV-1 infected cells is measured, or more recently, using automated recombinant vector-based assays that work in conjunction with a luciferase colorimetric readout to detect HIV-1 activity during single-cycle viral replication in the presence of the test ARVs (Petropoulos et al., 2000; Parkin et al., 2004). The phenotypic resistance displayed by an HIV-1 isolate to a specific ARV is customarily expressed as the fold-change (FC) or ratio of IC_{50} values for the isolate and wild-type (WT) HIV-1, respectively. Thus, higher FC values obtained after several months of treatment reflect a loss in antiretroviral susceptibility. Genotypic analysis has confirmed that the number of mutations associated with drug resistance also increases concomitantly with increases in IC_{50} values.

Mutations that encode single or multiple amino acid substitutions in RT, PR or the HR-1 domain of gp41 in the HIV-1 envelope have been shown to be directly responsible for diminished susceptibility to the inhibitors of these viral

targets and, may therefore, be viewed as important molecular markers that are predictive of drug resistance (Johnson et al., 2004). The prognostic value of genotypic resistance testing in improving virological outcomes to antiretroviral therapy for HIV-1 infection has been documented in several prospective and retrospective clinical studies, including comparisons against standard of care (Durant et al., 1999; Baxter et al., 2000; Meynard et al., 2002; Torre and Tambini, 2002). In addition, health economics analyses from the CPCRA 046 (Weinstein et al., 2001) and VIRADAPT (Chaix et al., 2000) studies have confirmed the benefit conferred by genotypic resistance testing when used for guiding therapy choice decisions in patients who experienced virological failure on an initial antiretroviral regimen. In CPCRA 046, patients receiving standard antiretroviral therapy regimens were randomly assigned to one of two study groups in which therapeutic decisions were determined by clinical judgment alone or, alternatively, using genotypic antiretroviral resistance testing (GART) as an adjunct to clinical judgment (Baxter et al., 2000). With GART, 34% of patients were reported to achieve a successful virological response compared to 22% of patients in which therapy choice decisions were based entirely on physician clinical judgment (Baxter et al., 2000; Torre and Tambini, 2002). Similar results have also been reported from the VIRADAPT study (Durant et al., 1999), in which 32% of patients assigned to the drug resistance genotyping (DRG) group responded satisfactorily to antiretroviral therapy compared to a response rate of 14% in patients without DRG (Durant et al., 1999; Weinstein et al., 2001).

The benefit conferred by HIV-1 genotyping in treatment-experienced patients has also been further corroborated by the Havana trial (Tural et al., 2002). In this study, a significantly greater proportion of patients in whom genotyping was used to guide therapy choice decisions achieved undetectable plasma viremia (i.e., HIV-1 RNA <400 copies/ml) after 24 weeks of therapy as compared to patients managed in accordance with the standard of care alone (Tural et al., 2002). Additionally, the use of expert advice to assist with treatment decisions was also shown to be associated with improved virological response, especially in patients that had experienced a second virological failure (Tural et al., 2002). Thus, the results from CPCRA 046, VIRADAPT and Havana, as well as those from other related studies conducted in settings that may more closely reflect current clinical practice (Badri et al., 2003), support the use of genotypic drug resistance assays and expert advice as important interventions to help improve the sustained effectiveness of antiretroviral therapy in patients with HIV-1 infection.

1.1.5.1. Mechanisms of resistance conferred by NAMs

Table 2 illustrates drug resistance mutations in HIV-1 RT that are commonly associated with reduced antiviral susceptibility to NRTIs/NtRTIs (Johnson et al., 2004). The resultant amino acid substitutions in mutated RT result in resistance through one of two mechanisms, i.e., increased discrimination against antiviral drugs or increased levels of excision of incorporated NRTI triphosphates or NtRTI diphosphates from elongating chains of viral DNA; a process also referred to as nucleotide-dependent primer unblocking (Meyer et al.,

1998; Götte and Wainberg, 2000). The first mechanism affects the affinity and/or incorporation of the active phosphorylated forms of NRTIs/NtRTIs elongating into proviral DNA (Gao et al., 2000; Götte and Wainberg, 2000; de Mendoza et al., 2002; Soriano and de Mendoza, 2002). A classic example of a discriminatory resistance mutation is the M184I/V substitution in RT that is associated with high-level phenotypic drug resistance to 3TC. Discriminatory mutations can confer cross-resistance by also lessening the affinity and/or incorporation into viral DNA by drugs other than those which may have selected them (Miller and Larder, 2001). Cross-resistance among NRTIs/NtRTIs has been extensively documented and probably occurs to a greater extent than initially thought (Kuritzkes, 2002). Cross-resistance is commonly due to the selection by different compounds of resistance-conferring mutations that result in diminished antiviral activity for several drugs, but may also be produced by specific mutational patterns that are selected by a single ARV (Miller and Larder, 2001). The extent of cross-resistance may increase with the selection of additional mutations following breakthrough viral replication in the presence of incompletely suppressive regimens (Lange, 2001; Loveday, 2001). Some have argued that this problem can be forestalled by reserving the use of those NRTIs/NtRTIs with the greatest potential for the development of cross-resistance for post first-line therapy (Lange, 2001).

The excision mechanism can be thought of in some ways as the opposite of the reverse transcription reaction. Enhanced levels of ATP-dependent primer

unblocking and/or pyrophosphorolysis are mediated by TAMs that are responsible for diminished susceptibility to ZDV and d4T (Götte and Wainberg, 2000; Lange, 2001; Loveday, 2001; Miller and Larder, 2001). More recently, the term nucleoside excision mutation (NEM) has also been used to characterize this group of mutations.

Table 2: Mutations in HIV-1 reverse transcriptase commonly associated with resistance to NRTIs/NtRTIs

Drug	**Common RT Resistance Mutations**
Zidovudine	M41L, E44A/D, D67N, K70R, V118I, L210W, T215Y/F, K219Q/E
Didanosine	K65R, L74V
Zalcitabine	K65R, T69D, L74V, M184V
Lamivudine	E44A/D, V118I, M184I/V
Emtricitabine	M184I/V
Abacavir	K65R, L74V, Y115F, M184V
Stavudine	M41L, E44A/D, D67N, K70R, V118I, L210W, T215Y/F, K219Q/E
Tenofovir DF	K65R
Thymidine Analogue Mutations (TAMs)	M41L, D67N, K70R, L210W, T215Y/F, K219Q/E
Multinucleoside Resistance	Q151M resistance complex: A62V; V75I; F77L; F116Y; Q151M
Multinucleoside Resistance	69XXX insertion: Usually observed with TAMs

1.1.5.2. The M184V/I mutation

The M184V substitution is selected rapidly in tissue culture following serial passage of wild-type HIV-1 in the presence of increasing concentrations of 3TC, and 3TC-resistant virus can also be isolated from patients who experience virological failure as early as eight weeks following initiation of a 3TC-containing regimen (Larder et al., 1995; Schuurman et al., 1995; Wainberg et al., 1995). In contrast, longer times (e.g., six months) are commonly required for the development of drug resistance to other NRTIs such as ZDV (Mayers, 1996; Mayers, 1997; Miller and Larder, 2001). Resistance to 3TC and FTC is conferred by a single mutation in the HIV-1 RT gene that encodes a methionine to valine amino acid substitution at position 184 (i.e., M184V) (Larder et al., 1995; Schuurman et al., 1995; Wainberg et al., 1995; Gao et al., 2000; Richman, 2001). The appearance of M184V is commonly preceded by another more transient mutation, M184I, which also results in high-level resistance to both 3TC and FTC (Keulen et al., 1997). The reason that M184I is often transient is that HIV-1 variants harboring M184I are less fit than M184V-mutated viruses and are therefore rapidly out-competed both *in vitro* and *in vivo* by the latter (Kavlick et al., 1995; Back et al., 1996; Frost et al., 2000; Devereux et al., 2001).

M184V is a discriminatory mutation that significantly reduces the affinity of HIV-1 RT for 3TC triphosphate (3TC-TP) compared to its naturally occurring counterpart nucleoside, i.e., deoxycytosine triphosphate (dCTP), as a preferred substrate for incorporation (Larder et al., 1995; Wainberg et al., 1995; Gao et al.,

2000; Götte and Wainberg, 2000; de Mendoza et al., 2002). Resistance is due to steric hindrance effects between the beta side-chains of the isoleucine or valine amino acid residues at position 184 in the mutated RT and the oxathiolane ring of 3TC-TP that would otherwise result in a catalytically incompetent complex (Larder et al., 1995; Schuurman et al., 1995; Wainberg et al., 1995; Gao et al., 2000; Lee et al., 2001; Miller and Larder, 2001).

As stated, M184V is also selected by FTC (Gao et al., 2000; Lee et al., 2001; Richman, 2001; Schinazi et al., 2001), which is structurally-related to 3TC, but additionally by structurally-unrelated NRTIs such as ABC (Harrigan et al., 2000; Miller et al., 2000; Walter et al., 2002) and less frequently by ddC or ddI (Gu et al., 1992; Gao et al., 1993; Winters et al., 1997). In fact, M184V is usually the first resistance mutation that emerges following *in vitro* or *in vivo* exposure to ABC but confers only low-level resistance (i.e., 2- to 4-fold increases in IC_{50}) to this drug. The development of meaningful drug resistance to ABC requires overcoming a higher genetic barrier that involves the accumulation of several TAMs and/or other NAMs in RT (e.g., K65R, L74Vand Y115F) before significant loss of antiviral activity (i.e., >10-fold increase in IC_{50}) is observed (Tisdale et al., 1997; Moyle, 2001; Ray et al., 2002). Similarly M184V usually only results in significant resistance to ddC when other mutations in RT are also present (Gu et al., 1992; Gao et al., 1993). Hence, M184V does not on its own predict broad cross-resistance to most other NRTIs, including ZDV, ddI, ddC and ABC (Miller V. et al., 1998; Miller and Larder, 2001) and the presence of M184V

does not usually limit sequencing options in therapy or compromise the effectiveness of ABC or ddI in subsequent treatment (Mouroux et al., 2001; Ait-Khaled et al., 2002a). For example, the presence of M184V which was detected in 60% of patients experiencing virological failure in first-line regimens containing 3TC, did not compromise virological outcomes after subsequent treatment with a new regimen that included d4T plus ddI (Winters et al., 2002; Winters et al., 2003), and the percentage of patients in which M184V was detected after such treatment diminished to 27% indicating that d4T/ddI were effective against M184V-containing viruses; in contrast, M184V remained present in 100% of patients who received an alternative regimen of 3TC/d4T (Winters et al., 2003). Similar results have also been reported in other studies (Eron et al., 2002).

1.1.5.3. The V118I and E44D/A substitutions

Two mutations in RT that frequently appear together are E44D/A with V118I; this combination has been shown to confer moderate-level resistance (i.e., 4- to 50-fold) (Hertogs et al., 2000) to 3TC in the absence of M184V (Delaugerre et al., 2001; Montes and Segondy, 2002; Romano et al., 2002). Unlike M184I/V, the E44D/A and V118I mutations are not selected by 3TC (Hertogs et al., 2000; Delaugerre et al., 2001; Montes and Segondy, 2002) and are generally seen only in treatment-experienced patients. The presence of these mutations may reach 30-33% in patients previously treated with NRTIs (Delaugerre et al., 2001; Montes and Segondy, 2002). In addition, the prevalence of E44D/A and V118I has been

shown to increase as a function of number of NRTIs used in prior therapy and can be as high as 42% in individuals treated with at least four antiretroviral regimens (Montes and Segondy, 2002). The selection of E44D/A and/or V118I may also be strongly correlated with prior exposure to ddI and the concomitant presence of specific clusters of TAMs including M41L and T215Y/F or D67N and L210W (Montes and Segondy, 2002). These data point to a possible relationship between these mutations and the prior use of ZDV- or d4T-containing regimens with more extensive cross-resistance among NRTIs as a consequence (Montes and Segondy, 2002; Romano et al., 2002).

1.1.5.4. Thymidine analogue mutations (TAMs)

The TAMs (Table 2) comprise a group of six drug resistance mutations (i.e., M41L, D67N, K70R, L210W, T215Y/F and K219Q/E) (Götte and Wainberg, 2000; Johnson et al., 2004) in RT that were initially described in association with resistance to ZDV (Götte and Wainberg, 2000; Lange, 2001; Loveday, 2001; Miller and Larder, 2001; de Mendoza et al., 2002; Soriano and Mendoza, 2002). Subsequent research has also implicated these mutations in diminished susceptibility to d4T (Götte and Wainberg, 2000; Lange, 2001; Loveday, 2001; Miller and Larder, 2001; Ross and Henry et al., 2001a; Ross and Scarsella et al., 2001b; Johnson et al., 2004). The presence of TAMs also results in modest moderate levels of resistance to other NRTIs/NtRTIs, including ddI, ddC, ABC, and TDF (Miller et al., 2002a; Miller et al., 2002b), depending on the mutational pattern that is present. However, L74V is the primary mutation that is

selected by ddI and has been shown to be responsible for the greatest loss of antiviral activity with this drug (Götte and Wainberg, 2000; Lange, 2001; Miller and Larder, 2001; Johnson et al., 2004). Other resistance-conferring mutations such as L74V, K65R and T69N are also important in regard to resistance to these compounds (Lange, 2001; Miller and Larder, 2001; Johnson et al., 2004). The latter, like M184V, are discriminatory mutations in RT and generally yield resistance only against the drugs that select them; in contrast, TAMs can mediate increased levels of resistance to multiple unrelated NRTIs so long as other mutations are also present (Götte and Wainberg, 2000; Miller and Larder, 2001; Soriano and de Mendoza, 2002).

1.1.5.5. Genetic pathways for development of TAMs

Two major genetic pathways exist in regard to the development of resistance to d4T and ZDV, as manifested through detection of TAMs (Marcelin et al., 2002; Flandre et al., 2003; Van Houtte et al., 2003). Each of the M41L and T215Y/F mutations are commonly present in both pathways (Marcelin et al., 2002) and are followed by the stepwise accumulation of other TAMs at positions 210 and 215 (i.e., 41L-210W-215Y/F pattern) or, alternatively, positions 67, 70 and 219 (i.e., 67N-70R-219Q/E pattern) (Marcelin et al., 2002; Flandre et al., 2003). The specific sequence of accumulation of TAMs may depend on whether HIV-infected patients had initially received ZDV monotherapy or dual NRTI combinations, i.e., ZDV monotherapy is more commonly associated with the initial appearance of the K70R mutation leading predominantly to the 67N-70R-

219Q/E pattern (Flandre et al., 2003). In contrast, patients who started treatment with either ZDV/ddI or ZDV/ddC usually developed T215Y/F as an initial mutation followed by 41L and 210W (Flandre et al., 2003). In addition, different subtypes of HIV-1 may follow distinct pathways in this regard; these preferences are referred to as dichotomous pathways in the development of drug resistance.

Genotypic analysis has shown that the 41L-210W-215Y pattern is more prevalent than 67N-70R-219Q/E (Van Houtte et al., 2003). In addition, the two other mutations associated with long-term ZDV/d4T usage, i.e., V118I and E44A/D, cluster together with the 41L-210W-215Y pathway but were only present individually in association with the 67N-70R-219Q/E pattern (Van Houtte et al., 2003). The 41L-210W-215Y pathway generally yields higher levels of resistance to ZDV/d4T and cross-resistance to other NRTIs in association with other mutations than does the 67N-70R-219Q/E pathway (Van Houtte et al., 2003).

Differences in the initial presentation and incidence of TAMs have been reported in therapy-naïve patients treated with regimens that included ZDV versus d4T. One study showed that the incidence of TAMs in patients on a ZDV-containing regimen (23.8%) was higher than in patients treated with d4T, despite the fact that the latter had lower CD4 cell counts and higher plasma HIV-1 RNA pre-treatment values (Bocket et al., 2003). The T215Y/F mutation was also detected in a higher proportion of patients who began therapy with a ZDV-

containing regimen (10.4%) in comparison to d4T (1.8%) (Bocket et al., 2003). Another study on treatment-naïve patients (i.e., ACTG 306 rolled-over into ACTG 370), showed that the frequency of individual TAMs at positions 41, 67, 70, 210, 215, 219 was similar in patients who had received either ZDV/3TC or d4T/3TC (Kuritzkes et al., 2002). However, M41L was detected earlier in patients who began with ZDV-based regimens. There was also a trend toward accumulation of two or more TAMs in the ACTG 370 patients who began with ZDV (29.4 %) compared to d4T (3.8 %) (Kuritzkes et al., 2002).

1.1.5.6. Effect of TAMs on excision

Enhanced excision of incorporated nucleotide triphosphates, which is due to either nucleotide-dependent primer unblocking and less commonly to pyrophosphorolysis, has been identified as the mechanisms that results in resistance to both ZDV and d4T (Götte and Wainberg, 2000; Loveday, 2001; Miller and Larder, 2001; de Mendoza et al., 2002; Soriano and de Mendoza, 2002). In the case of ZDV, rescue of viral DNA synthesis by either mechanism requires the excision of ZDV 5'-monophosphate (ZDVMP) from the 3' terminus of the polymerizing c-DNA strand. This reaction is facilitated by ATP or, alternatively, by pyrophosphate (PPi) (Naeger et al., 2002). Furthermore, levels of ATP binding, and uncoupling of nucleotide monophosphate-terminated primers have been reported to increase concomitantly with the accumulation of TAMs (Meyer et al., 1999; Meyer et al., 2000; Shulman et al., 2002).

An increased level of nucleotide primer unblocking or pyrophosphorolysis by HIV-1 RT has not been demonstrated in all situations, and therefore, does not represent the major mechanism responsible for attenuated antiviral susceptibility to non-thymidine analogues (Naeger et al., 2001b; Naeger et al., 2002). It is noteworthy that the efficiency of removal of such NRTIs as 3TC, ddC, ddI and ABC by RT containing TAMs at positions 67, 70 and 215 has been reported to be reduced in comparison to ZDV and d4T (Naeger et al., 2002). Furthermore, removal of TDF by ATP-dependent primer unblocking in the presence of specific TAMs (i.e., D67N, K70R and T215Y/F) has been shown to be 22- to 35-fold less efficient than that observed for either d4T or ZDV, respectively (Naeger et al., 2002). These findings suggest that not all TAMs may mediate decreased susceptibility to TDF to the same degree, a finding corroborated in virological studies performed with the latter drug (Miller et al., 2001). TDF can retain antiviral activity against HIV-1 that contains as many as three TAMs, as long as these do not include either M41L or L210W (Miller et al., 2001; Miller et al., 2002a; Miller et al., 2002b). These findings help to account for the fact that TDF can often be used successfully to treat individuals previously treated with a variety of NRTI-based regimens (Squires et al., 2002).

Resistance to TDF is principally associated with the K65R mutation. Although this mutation was first shown to be selected by TDF in tissue culture (Wainberg et al., 1999), it was initially rare in patients experiencing virological failure with this drug. More recent data show that it is now increasing in

incidence, probably as a consequence of increased usage of TDF. The development of K65R on its own appears to compromise virological responses with TDF, although this is exacerbated when additional mutations in RT (i.e., TAMs) are also present (Margot et al., 2002). K65R on its own decreases sensitivity to TDF by 3- to 4-fold (Wainberg et al., 1999; Margot et al., 2002); K65R is also associated with resistance to other NRTIs, most notably ddI, ddC and ABC (Johnson et al., 2004). An increased prevalence of K65R has also been reported in antiretroviral therapy-naïve HIV-1 patients who failed treatment with a regimen consisting of ABC/ddI/d4T (Roge et al., 2003). Hence, resistance to TDF and several other NRTIs can develop via genetic pathways that involve either the TAMs or K65R as hallmark resistance mutations (Winston et al., 2002), supporting the view that HIV-1 can select multiple pathways toward drug resistance (Hertogs et al., 2000; Miller and Larder, 2001; Winston et al., 2002; Flandre et al., 2003).

1.1.5.7 Multi-nucleoside resistance (MNR)

Mutational patterns associated with the development of broad cross-resistance to multiple NRTIs include the Q151 multi-nucleoside resistance (MNR) complex which can be encoded by five distinct mutations in RT: A62V, V75I, F77L, F116Y and Q151M; the latter is considered to be the signature mutation for this MNR cluster (Johnson et al., 2004) (Table 2). These mutations were initially observed in viral isolates from patients who received combination therapy with ZDV plus either ddC or ddI for over one year (Shirasaka et al., 1995;

Ueno et al., 1995) and who lacked other substitutions associated with resistance against ZDV, ddI or ddC. Q151M is the first of these five mutations to appear *in vivo* and produces the greatest attenuation of antiviral activity to NRTIs (Ueno et al., 1995).

In addition, a family of insertion mutations between codons 67 and 70 in RT can cause resistance to a variety of NRTIs including ZDV, 3TC, ddI, ddC and d4T (Johnson et al., 2004). These insertions confer multi-nucleoside resistance and usually develop against a background of resistance to ZDV (Larder et al., 1999; Boyer et al., 2002a). As with the Q151M MNR pattern, the development of these resistance-conferring insertions is associated with prior treatment with ZDV/ddI and ZDV/ddC combination regimens. The prevalence of the insertion mutations is, however, lower than that reported for the Q151M MNR pattern (Van Vaerenbergh et al., 2000).

1.1.6.1. Viral fitness

HIV-1 variants that are resistant to NRTIs may display diminished fitness in comparison to viruses that remain sensitive to these drugs (Harrigan et al., 1998; Dykes et al., 2001; Quiñones-Mateu et al., 2001; Brenner et al., 2002). In the absence of antiretroviral pressure, wild-type (WT) virus is the fittest and most prevalent quasispecies in the replicating population. The eventual outgrowth of resistant variants arises due to their replication advantage under conditions of selective drug pressure (Coffin, 1995). Estimates for the fitness of NRTI-resistant

mutants can vary depending on laboratory methodology and the viral strain utilized. Estimates of the replication impairment of viruses containing single mutations such as M184V or L74V ranged between 3% to 60% compared to WT in initial studies (Sharma and Crumpacker, 1999; Devereux et al., 2001), but, more recent studies based on recombinant methods that employed a luciferase colorimetric readout, showed that the extent of fitness impairment attributable to M184V ranged between 48% to 57% (Miller et al., 2003). The K65R mutation was also found to diminish viral replication capacity by this method (Miller et al., 2003). Specific combinations of NRTI-resistance mutations were shown to act additively in some studies to further impair fitness; e.g., both M184V and K65R together resulted in about half of the level of replication that would have been expected if either mutation was present alone (Miller et al., 2003).

Viral fitness is an important determinant of HIV-1 pathogenesis, and infectivity, and may also help to predict treatment outcome, since inefficient viral replication is associated with reduced plasma viremia, delayed mutagenesis, and improved immunological responses to antiretroviral therapy. This may sometimes manifest itself in the form of immunological and virological discordance, in which CD4 cell counts are stabilized or increase despite detectable plasma HIV-1 RNA. Virological-immunological discordance has also been attributed to the emergence of fitness-reducing resistance mutations such as D30N in the HIV-1 protease gene (Antinori et al., 2001; Deeks et al., 2001; Stoddart et al., 2001), and has recently been reported with M184V (Nicastri et al., 2003) in HIV-infected

patients who experienced virological failure on regimens that included drugs that had selected for this mutation.

1.1.7.1. Residual antiviral activity of 3TC against M184V-mutated HIV-1

The M184V substitution in RT may be a mitigating factor in regard to HIV-1 replication capacity, since clinical studies, have shown that this mutation is associated with lower levels of plasma RNA even after resistance to 3TC has developed. This was first observed in the NUCA3001 study in which viral loads consistently remained both below baseline (0.6 to 0.3 $\log_{10}$ viral load reduction) and lower than those resulting from ZDV monotherapy over 52-weeks (Kuritzkes et al., 1996). Similarly, the development of M184V in 3TC/ZDV-treated patients enrolled in the AVANTI 2 and 3 trials was associated with lower plasma viremia than would be expected with ZDV monotherapy, even after the detection of M184V (Maguire et al., 2000).

Clinical and laboratory data also demonstrate that 3TC possesses a degree of residual antiviral activity following the development of M184V, and this may be independent of the effect of M184V on replication capacity. Interruption of 3TC therapy in patients experiencing virological failure resulted in a rebound of plasma HIV-1 RNA that was about 0.5 $\log_{10}$ above previous levels (Campbell et al., 2003). In this limited analysis, genotyping revealed that the M184V mutation was still present at the time that plasma viremia rebounded, commensurate with the discontinuation of 3TC (Campbell et al., 2003).

Laboratory data has also shown that high concentrations of 3TC-TP can effectively inhibit reverse transcription in cell-free assays that were performed with purified M184V-RT (Quan et al., 2003). The degree of inhibition caused by 3TC-TP was found to increase with elongation of the RT products that were generated (Quan et al., 2003). These observations were confirmed in tissue culture experiments, in which the formation of full-length RT products in peripheral blood mononuclear cells (PBMCs) newly-infected with M184V-HIV-1 could be inhibited with 2 to 10 μM of 3TC (Quan et al., 2003). Thus, 3TC may possess a residual antiviral effect in the presence of M184V that is not easily revealed with current phenotypic assays.

1.1.8.1. Cross-resistance amongst NRTIs

Cross-resistance to NRTIs constitutes an ongoing challenge for the successful long-term management of patients with HIV-1 infection, due to loss of treatment options. The development and transmission of NRTI resistance-conferring mutations are also of serious concern, since these mutations can be selected by suboptimal therapy. The prevalence of drug resistance to NRTIs may be as high as 70% in patients on failing regimens (i.e., HIV-1 plasma RNA >500 c/ml). In contrast, resistance to PIs and NNRTIs was detected in only 42% and 31% of patients in this cohort, respectively (Richman et al., 2004). In another study, the horizontal transmission of viruses manifesting high-level resistance to NRTIs increased from 3% (1995 to 1998) to 7.6% (1999 to 2000) (Little et al., 2002). Strategies aimed at avoidance of the development and transmission of

NAMs are important, since the accumulation of additional mutations in RT is associated with increased levels of cross-resistance to NRTIs (Loveday, 2001; Miller and Larder, 2001).

A possible exception to the adverse role of NAMs on cross-resistance to NRTIs may be M184V, since delayed or reduced selection of TAMs and/or the Q151M MNR mutational pattern has been reported in patients with M184V-mutated viruses (Miller V. et al., 1998; Mouroux et al., 2001; Ait-Khaled et al., 2002a). However, other studies reported that M184V did not significantly restrict the development of resistance-conferring mutations to either PIs (Keulen et al., 1999) or NNRTIs (Jonckheere et al., 1998). Interestingly, the emergence of mutations following selection with EFV and APV (i.e., K103N, V106M in RT, I54M/L/V in PR) was significantly delayed in HIV-1 subtype B and C clinical isolates that contained M184V (Diallo et al., 2003a). Therefore, the protective effect of M184V may not be limited to delayed emergence of TAMs and other mutations responsible for diminished susceptibility to NRTIs. Numerous alterations of RT enzymatic function that include decreased RT processivity, reduced levels of nucleotide-dependent primer unblocking/pyrophosphorolysis, increased polymerase fidelity in biochemical analyses, diminished rates of initiation of reverse transcription (Diallo et al., 2003b), hypersensitization to other NRTIs such as ZDV and TDF, and impaired viral fitness/replication capacity may contribute to these effects (Miller V. et al., 2002; Petrella and Wainberg, 2002).

1.2. General introduction

As reviewed previously, significant progress has been made with respect to the optimization of combination antiretroviral therapy for patients with HIV/AIDS largely as a result of the development of several novel ARVs and the routine implementation of sophisticated laboratory tools for monitoring drug resistance in diverse clinical settings.

Nevertheless, in spite of these important accomplishments, the avoidance of drug resistance in HIV-1 infection has remained one of the more elusive goals of HAART to date, and moreover, may be most serious in patients with advanced infection such as those requiring salvage therapy. These patients generally have a higher plasma HIV-1 RNA levels and a broader range of quasispecies than newly-infected individuals as a result of the longer duration of HIV-1 infection and sequential exposure to multiple failing antiretroviral therapy regimens (Lorenzi et al., 1999; Rousseau et al., 2001; Bongiovanni et al., 2003). The progressive depletion of CD4 cells in patients with later stage HIV-1 infection can also diminishes the capacity for immunological control of viral replication, possibly leading to more rapid evolution of drug resistance. In addition, such patients are also more likely to harbor archival drug-resistant HIV-1 variants in latent cells and other viral reservoirs (Ghosn et al., 2004; Kuritzkes, 2004b; Siliciano and Siliciano, 2004; Zhang et al., 2004) that can lead to recurrence of drug resistance when antiretroviral drug pressure is re-introduced as is the case following repeated structured treatment interruptions (STI) (Kijak et al., 2002; Martinez-

Picado et al., 2002; Schweighardt et al., 2002; Fischer et al., 2003; Metzner et al., 2003).

The occurrence of HIV-1 drug resistance in conjunction with cross-resistance between ARVs belonging to the same class, both of which have been shown to increase over prolonged periods of treatment, can seriously limit management options especially for extensively pretreated patients. Salvage therapy for HIV-1 infection undoubtedly represents a clinical challenge and alternative treatment strategies might include; the recycling of drugs in different ways. These ideas should be pursued contemporaneously with the discovery and development of novel ARVs with more robust genetic barriers and a broader spectrum of antiviral activity against drug-resistant HIV-1 variants.

One such possible approach that merits further investigation exploits the inverse relationship that exists between drug resistance and viral fitness in the presence of certain mutations in the HIV-1 *pol* gene (Berkhout, 1999; Quiñones-Mateu and Arts, 2002; Bates et al., 2003) and more specifically, as concerns this research project, with the M184V substitution in RT. Although selection of M184V in HIV-1 RT has been shown in several studies to result in attenuated viral fitness (Miller et al., 2003; Deval et al., 2004; Wainberg, 2004b), this mutation by itself confers only minimal cross-resistance, and therefore, preserves most treatment options with NRTIs/NtRTIs except for FTC. In addition, M184V-containing viruses also have a reduced propensity for further mutagenesis *in vitro*

(Wainberg et al., 1996c; Whitney et al., 2002; Diallo et al., 2003a) which in turn may delay the development of resistance, and hence, extend the clinical effectiveness of other ARVs, and in particular, that of NRTIs (Miller V. et al., 2002; Petrella and Wainberg, 2002).

In summary, M184V-mediated alterations of RT biochemical function in conjunction with the impairment of viral replication capacity produced by this mutation may be of therapeutic consequence by improving virological and/or immunological responses to antiretroviral therapy in the aftermath of drug resistance. Of course, in the context of initial regimens, the role of antiretroviral therapy should always be to reduce viral load to as great an extent as possible and as durably as possible, by maximizing the effectiveness of all anti-HIV drugs.

The primary objectives of this research project were two-fold as follows:

(1) To establish the rationale for prospective investigation of the potential benefit associated with therapeutic maintenance of the M184V substitution in HIV-1 RT by antiretroviral therapy in patients with HIV-1 infection that experienced virological failure with a regimen that initially selected for this mutation;

(2) To study whether NRTIs that are structurally-unrelated to 3TC in addition to subtherapeutic concentrations of this drug can exert adequate

pharmacological pressure in tissue culture to maintain the M184V mutation in RT in several HIV-1 clinical isolates harboring different patterns of NAMs.

CHAPTER 2

Might the M184V Substitution in HIV-1 RT Confer Clinical Benefit?

Petrella M. and M.A. Wainberg

Preface to Chapter 2:

In chapter 1, the basis for highly active antiretroviral therapy (i.e., HAART) for HIV-1 infection and AIDS, the development of genotypic resistance to currently approved ARVs, and the prognostic value of laboratory tests to monitor resistance and guide therapy with these drugs have been reviewed. The emergence of the M184V mutation in the HIV-1 RT gene and its mitigating effect in regard to mechanisms responsible for resistance to NRTIs/NtRTIs is also discussed at length. Finally, the possibility that several of the M184V-mediated

alterations of RT biochemical activity, and in particular, the impairment of viral fitness/replication capacity that is mediated by this mutation may be of therapeutic significance in certain clinical settings is introduced.

2.1. Abstract

The M184V substitution in HIV-1 RT develops rapidly following initiation of therapy with 3TC and confers high-level phenotypic resistance to this drug both *in vitro* and *in vivo*. Interestingly, the presence of M184V is also associated with alteration of several mechanisms relating to RT function that include decreased RT processivity, reduced nucleotide-dependent primer unblocking, increased fidelity, hypersensitization to other NRTIs, impaired viral fitness, and delayed appearance of mutations in RT that are responsible for resistance to thymidine analogues (i.e., thymidine-associated mutations or TAMs). Collectively, these factors might explain the residual antiviral effect and clinical benefit observed with continued use of 3TC in combination therapy regimens following the emergence of M184V. Indeed, the results of numerous controlled as well as observational clinical studies are suggestive of improved therapeutic outcome associated with continued usage of 3TC and maintenance of the M184V mutation. However, several of these trials did not possess adequate statistical power to resolve whether or not continued use of 3TC provided actual benefit, nor were they specifically designed to test the M184V benefit hypothesis in prospective fashion. There is a need for randomized clinical trials of this type in order to validate the potential benefit of maintenance of M184V and whether continued use of 3TC is the only means of attaining this objective.

2.2. Introduction

The emergence of drug-resistant HIV-1 is both a consequence and limitation of antiretroviral therapy and has been shown to significantly diminish the effectiveness and duration of benefit associated with combination therapy regimens for the treatment of HIV/AIDS (Lorenzi et al., 1999; Winters et al., 2000; Quirós-Roldán et al., 2001; Rousseau et al., 2001; Yeni et al., 2002). Although resistance-conferring mutations in both the HIV-1 reverse transcriptase (RT) and protease (PR) genes may often precede the initiation of therapy, due to both spontaneous mutagenesis and the spread of resistant viruses by sexual and other means of transmission, it is generally believed that multiple drug mutations to any single or combination of antiretroviral agents (ARVs) are selected during continued viral replication in the presence of incompletely suppressive drug regimens (De Jong et al., 1996; Mayers, 1997; Balotta et al., 2000). For the protease inhibitors (PIs) (Molla et al., 1996; Condra, 1998; Deeks, 1999), and most nucleoside analogue reverse transcriptase inhibitors (NRTIs), the development of progressive high-level phenotypic drug resistance follows the accumulation of primary resistance-conferring mutations in the HIV-1 PR and RT genes, respectively (Frost et al., 2000; Götte and Wainberg, 2000; Loveday, 2001). However, in the case of the non-nucleoside reverse transcriptase inhibitors (NNRTIs), which have lower genetic barriers for the development of drug resistance, a single primary drug resistance mutation is generally sufficient to abrogate antiviral activity and produce extensive cross-resistance within this class of ARVs (Bacheler et al., 2001; Deeks, 2001). Similarly, a single resistance-

conferring mutation encoding a methionine to valine amino acid substitution at position 184 (i.e., M184V) in the RT enzyme also rapidly results in high-level resistance (i.e., 100 to 1000 fold increase in IC_{50}) to the nucleoside analogue lamivudine ([-]-2', 3'-dideoxy-3'-thiacytidine, 3TC) both *in vitro* and *in vivo* (Boucher et al., 1993; Gao et al., 1993; Tisdale et al., 1993; Quan et al., 1996; Hertogs et al., 2000). Unlike the situation with the NNRTIs, there is also considerable evidence at this time suggesting that lamivudine may, in fact, continue to contribute to the effectiveness of antiretroviral combination therapy regimens, even after the appearance of the M184V mutation and development of high-level phenotypic drug resistance to 3TC as confirmed by *in vitro* drug susceptibility assays (Wainberg et al., 1996b; Wainberg and Parniak, 1997; Bean, 2002; Miller V. et al., 2002).

In this review, recent laboratory findings on the effects of the M184V mutation on RT function and viral replication kinetics will be discussed in relation to clinical studies in which the presence of the M184V mutation has been associated with a positive treatment outcome. The clinical implications of HIV-1 drug resistance are significant and illustrate the need for continued research in this area. In order to confront HIV-1 drug resistance, the continued optimization of antiretroviral therapy constitutes an important goal that needs to be pursued in tandem with new drug discovery. Approaches based on the maintenance of the M184V substitution in HIV-1 RT through the use of sufficiently selective

antiretroviral regimens may represent a viable intervention that should be considered alongside other therapeutic options.

2.3. Development of M184V in HIV-1 RT

Lamivudine ([-]-2', 3'-dideoxy-3'-thiacytidine, 3TC) is a potent and highly selective nucleoside analogue inhibitor of wild-type HIV-1 RT (Schinazi et al., 1993; Pluda et al., 1995; Perry and Faulds, 1997; Anderson, 2001). As with other members of this class of antiretroviral drugs, 3TC is phosphorylated to its active triphosphosphate form (3TCTP) by host cellular kinases. 3TCTP lacks a 3'-hydroxyl group on the nucleoside pentose ring that is required for DNA polymerization and, hence, the antiviral activity of 3TC and other NRTIs is based on the ability of these compounds to prematurely terminate viral DNA strand elongation (Mitsuya et al., 1987; Furman and Barry, 1988; Goody et al., 1991; Furman et al., 2000; Anderson, 2002). Resistance to 3TC is rapidly selected in tissue culture following serial passage of wild-type HIV-1 in the presence of increasing concentrations of drug. In addition, 3TC-resistant HIV-1 can be isolated from patients who experience virological failure as early as eight weeks following initiation of a 3TC-containing regimen (Larder et al., 1995; Schuurman et al., 1995; Wainberg et al., 1995). Resistance to 3TC follows the development of a single primary mutation in the HIV-1 RT gene that encodes a methionine to valine amino acid substitution at position 184 (i.e., M184V) in both the p66 and p51 subunits of HIV-1 RT. The appearance of this mutation is usually preceded

by another more transient mutation, in which the methionine residue at position 184 is replaced with isoleucine (i.e., M184I) (Keulen et al., 1997).

Use of the limited dilution method to quantify the relative proportions of HIV-1 variants that are selected by 3TC *in vitro* has determined that the frequency of M184I (56%) is initially more than 4 times greater than that observed for M184V (12.5%) (Keulen et al., 1997). These findings indicate that HIV-1 RT has a mutational bias for the M184I substitution which explains the earlier appearance of this variant over M184V following initiation of treatment with 3TC (Keulen et al., 1997; Rezende et al., 1998). Both the M184I and M184V substitutions each only require a single nucleotide change or mutation in the HIV-1 genetic sequence. However, HIV-1 variants harboring M184I are less fit than their M184V counterparts and are therefore rapidly out-competed both *in vitro* and *in vivo* by the latter (Back et al., 1996; Keulen et al., 1997; Frost et al., 2000; Devereux et al., 2001).

The proximity of the methionine amino acid residue at position 184 in relation to the active site of HIV-1 RT is important for RT enzyme function (Kohlstaedt et al., 1992; Huang et al., 1998; Feng and Anderson, 1999). M184V is a discriminatory mutation (Soriano and De Mendoza, 2002) that significantly reduces the affinity of HIV-1 RT for some NRTIs in comparison with naturally occurring deoxynucleoside triphosphates (dNTPs) as preferential substrates for the mutated enzyme (Gao et al., 2000; De Mendoza et al., 2002). This altered

selectivity of M184V RT is responsible for high-level phenotypic drug resistance to 3TC *in vitro* and has been shown to increase the concentration of drug needed to inhibit viral replication by 50 percent (i.e., IC_{50}) for M184V HIV-1 from 100- to 1000-times over levels observed for wild-type virus (Kavlick et al., 1995; Larder et al., 1995; Schuurman et al., 1995; Wainberg et al., 1995; Wainberg et al., 1996a).

2.4. M184V does not confer significant cross-resistance to other NRTIs

The M184V mutation can be selected by structurally unrelated NRTIs such as abacavir (ABC) (Harrigan et al., 2000; Miller V. et al., 2000; Walter et al., 2002) and less frequently by didanosine (ddI) or zalcitabine (ddC) (Gu et al., 1992; Gao et al., 1993; Winters et al., 1997). M184V is, in fact, the first resistance mutation that emerges following *in vitro* or *in vivo* exposure to ABC and, contrary to the situation with 3TC, confers only low-level resistance (i.e., 2- to 4-fold increases in IC_{50}) to ABC. Indeed, the latter represents a high genetic barrier compound in regard to development of drug resistance, and requires the accumulation of several nucleoside analogue mutations (NAMs) in RT (e.g., M41L, K65R and Y115F in addition to M184V) before significant loss of antiviral activity (i.e., >10 fold increase in IC_{50}) is observed *in vitro* (Tisdale et al., 1997; Ray et al., 2002). Similar attenuation of antiviral drug susceptibility with M184V has been reported for both ddI and ddC only in the presence of additional mutations (Gao et al., 1993). These laboratory findings are of clinical relevance and predict that the emergence of the M184V mutation should not be

associated with broad cross-resistance to most NRTIs including zidovudine (ZDV), ddI, ddC and ABC; this has been confirmed by observational and controlled clinical trials (Miller V. et al., 1998; Miller and Larder, 2001).

In the CNA3003 study, for example, antiretroviral-naïve patients initially randomized to a dual NRTI regimen consisting of 3TC and ZDV were eligible to receive ABC with or without additional ARVs following the sixteen-week double-blind phase of this study. Despite the presence of the M184V mutation in more than 70 percent of patients, the intensification of therapy with ABC produced further suppression of viral replication and 65 percent of patients attained <400 copies/ml plasma HIV-1 RNA after 48 weeks of therapy. Thymidine analogue mutations (TAMs) were also observed infrequently in this group of patients (Ait-Khaled et al., 2002a). Low selection rates for TAMs or for the Q151M multi-dideoxynucleoside resistance mutation in the presence of M184V have been described in other studies in which patients were treated for up to 48 weeks with a stavudine (d4T)/3TC dual NRTI regimen (Mouroux et al., 2001). Taken together, the results from these and related protocols strongly suggest that the presence of the M184V mutation does not, by itself, limit treatment-sequencing options available with most other NRTIs or compromise the clinical effectiveness of either ABC- or ddI-containing regimens.

2.5. Reversal of resistance to AZT and synergistic antiviral activity with other drugs

The M184V substitution in HIV-1 RT may also have a role in the reversal of phenotypic susceptibility to ZDV in HIV-1 variants that have already acquired ZDV resistance mutations (Larder, 1995; Staszewski, 1995; Catucci et al., 1999; Loveday, 2001). For example, in the DELTA roll-over study, selection of the M184V mutation was associated with a transient resensitization to ZDV during a one-year follow-up period in 20 of 29 patients in whom baseline HIV-1 isolates were phenotypically resistant to ZDV (Masquelier et al., 1999). Restoration of antiviral susceptibility to ZDV observed during concomitant treatment with 3TC is thought to be mediated primarily by impaired rescue of dideoxy-terminated primers by HIV-1 RT containing the M184V resistance mutation (Götte et al., 2000). Two related mechanisms, notably enhanced pyrophosphorolysis and nucleotide-dependent primer unblocking, have been identified as the underlying cause of resistance to ZDV and d4T (Götte and Wainberg, 2000; Boyer et al., 2001; Isel at al., 2001; Soriano and De Mendoza, 2002). Rescue of viral DNA synthesis by either mechanism requires the excision of ZDV 5'-monophosphate (ZDVMP) from the 3' terminus of the polymerizing c-DNA strand and is facilitated by pyrophosphate (PPi), or alternatively, by ATP which is believed to be the principal PPi donor *in vivo*. Furthermore, ATP binding and consequently, uncoupling of ZDVMP-terminated primers, have been reported to increase concomitantly with the development of TAMs (Meyer at al., 1999; Meyer et al., 2000; Meyer et al., 2002).

In addition to reports of a potential benefit regarding reversal of ZDV resistance, the M184V mutation may also enable a synergistic interaction between 3TC and ZDV that temporarily boosts the *in vitro* antiviral activity of ZDV (Quan et al., 1998; Boyer et al., 2002b). Similarly, the IC_{50} values for two related nucleotide analogue inhibitors of HIV-1 RT, i.e., adefovir (PMEA) and tenofovir (PMPA or TDF), are approximately two-fold lower for M184V-containing HIV-1 in comparison to wild-type virus and appear to be unaffected by the presence of ZDV resistance-conferring mutations in RT (Miller et al., 1999; Wainberg et al., 1999; Miller et al., 2001; Naeger et al., 2001b). Reduced nucleotide-dependent primer unblocking and reduced levels of pyrophosphorolysis have been documented in HIV-1 RT that contains the M184V mutation (Götte and Wainberg, 2000; Götte et al., 2000), and this provides a possible mechanism to explain the resensitization that occurs when viruses that are initially resistant to ZDV regain susceptibility to this drug. Notably, the incorporation of ZDV triphosphate into a growing viral DNA chain may not be as easily reversed in the case of viruses and RT enzymes containing the M184V substitution. Hence, in this situation, DNA chain termination will still be expected to occur to some extent at least. Furthermore, since even wild-type RT possesses some degree of nucleotide primer unblocking activity, it also follows that drugs such as d4T, PMEA and PMPA might also display heightened antiviral activity against M184V-containing viruses for the same reason. It is only the later accumulation of other mutations in RT, such as V118I, that may play a negative compensatory

role in regard to M184V, which may reverse these effects (Hertogs et al., 2000; Delaugerre et al., 2001; Romano et al., 2002).

Indeed, the possibility that viruses containing M184V may remain minimally sensitive to 3TC for these same reasons should not be discounted. It is also correct that M184V discriminates against incorporation of 3TC triphosphate at levels between 50- to 200-fold, depending on how these measurements are performed; nonetheless, once a single molecule of 3TC-TP is incorporated into viral DNA, the likelihood of its excision is reduced compared to wild-type RT because of the M184V effect on pyrophosphorolysis/nucleotide primer unblocking. In this context, it has been shown that modest concentrations of 3TC-TP can exert chain termination effects against M184V-containing RT in biochemical assays (Quan et al., 1996; Quan et al., 1998). However, reduced pyrophosphorolysis/nucleotide primer unblocking by HIV-1 RT containing the M184V substitution has not been consistently demonstrated in all situations and, therefore, may not represent the sole mechanism responsible for heightened antiviral susceptibility to other compounds (Naeger et al., 2001a).

2.6. Improved HIV-1 RT fidelity with M184V and delayed emergence of TAMs

Resistance mutations to ARVs arise spontaneously as a result of the error-prone replication of HIV-1 and, in addition, are selected both *in vitro* and *in vivo* by pharmacological pressure (Roberts et al., 1988; Preston and Dougherty, 1996; Menéndez-Arias, 2002). The high rate of spontaneous mutation in HIV-1 has

been largely attributed to the absence of a 3'->5'exonuclease proof-reading mechanism. Sequence analyses of HIV-1 DNA have detected several types of mutations including base substitutions, additions and deletions (Roberts et al., 1988). The frequency of spontaneous mutation for HIV-1 varies considerably as a result of differences among viral strains studied *in vitro* (Rezende et al., 1998). Overall mutation rates for wild-type laboratory strains of HIV-1 have been reported to range from 97 X 10^{-4} to 200 X10^{-4} per nucleotide for HXB2 to as high as 800 X 10^{-4} per nucleotide for the HIV-1 NY5 strain (Roberts et al., 1988; Rezende et al., 1998).

In addition to the low fidelity of DNA synthesis by HIV-1 RT, other interdependent factors that affect rates of HIV mutagenesis include RT processivity, fitness, viral pool size, and availability of target cells for infection (Coffin, 1995; Drosopoulos et al., 1998; Colgrove and Japour, 1999; Overbaugh and Bangham, 2001). It follows that an alteration in any single one or combination of these factors might influence the development of HIV drug resistance. Of relevance is the positive effect of the M184V substitution on HIV-1 RT fidelity (Wainberg et al., 1996c; Wainberg, 1997; Feng and Anderson, 1999). Furthermore, the presence of the M184V substitution in both HIV-1 and in simian immunodeficiency virus (SIV), containing large genomic deletions, results in a relative inability to regain replication competency due to compensatory mutations and reversions compared to matched wild-type variants lacking M184V (Whitney et al., 2002). Clinical benefit due to M184V is not evident for all classes of ARVs,

and may be limited to the delayed emergence of TAMs (Jonckheere et al., 1998; Keulen et al., 1999). In the ALBI trial (Molina et al., 1999) for example, the T215Y mutation developed in a significantly higher proportion of patients randomized to treatment with ddI/d4T (62%) compared to those who received ZDV/3TC (10%) (Picard et al., 2001). The Q151M multi-nucleoside resistance mutation was also observed less frequently in patients treated with 3TC (Picard et al., 2001).

Similar results have also been obtained following a retrospective analysis of the effect of the M184V substitution in RT on the incidence of TAMs and fold differences in phenotypic resistance to ZDV and d4T among baseline HIV-1 clinical isolates from NRTI-experienced patients enrolled in the CNAB 3002 study. Patients previously treated with 3TC prior to initiating a new regimen with 3TC, ABC and ZDV were observed to have a significantly lower proportion of isolates that contained 3 or more TAMs (9%) in comparison to 3TC-naïve patients (36%). In addition, the frequency of viral isolates containing D67N, L210W and T215Y/F was also lower in 3TC-experienced patients. This reduction in proportion of TAMs was independent of levels of plasma HIV-1 RNA and duration of prior treatment with ARVs. Levels of phenotypic resistance to ZDV and d4T were also reduced in patients in whom M184V was selected as a result of previous exposure to 3TC, compared to cases in which this mutation was not present (Ait-Khaled et al., 2002b).

The development of ZDV resistance was also evaluated in patients experiencing virological failure with 3TC-containing regimens in the AVANTI 2 and 3 clinical studies. In these trials, antiretroviral therapy-naïve patients with HIV infection were randomly assigned to treatment with 3TC/ZDV or 3TC/ZDV/IDV for 52 weeks in AVANTI 2 or with 3TC/ZDV and nelfinavir (NFV) for 28 weeks in the case of AVANTI 3 (The Avanti Study Group, 2000; Gartland and The Avanti Study Group, 2001). Using combined data from both trials, genotypic analysis revealed ZDV resistance-conferring mutations in 27% of patients from the 3TC/ZDV arm of AVANTI 2, whereas these mutations were absent in patients from both arms of AVANTI 3, as well as in patients who received 3TC/ZDV/IDV in AVANTI 2 (Maguire et al., 2000). The M184V mutation, in these studies, was present in viral isolates from most patients who were treated with 3TC/ZDV. Overall, these results compare favorably to those from the CNA3003 study of ABC intensification therapy, in which selection rates for TAMs and Q151M were also reduced following appearance of M184V (Ait-Khaled et al., 2002a). In contrast to these findings, it has been demonstrated that the presence of either the M184I or M184V substitutions in RT did not significantly restrict the kinetics or extent of mutagenesis in the PR gene of HIV-1 compared to wild-type virus during tissue culture selection with protease inhibitors (Keulen et al., 1999). In other experiments, the development of drug resistance to the NNRTIs nevirapine and loviride was not delayed in M184V-containing HIV-1 compared with wild-type HIV-1 strain IIIB (Jonckheere et al., 1998). Hence, the potential protective effects of M184V against selection of

resistance-conferring mutations may be limited to delayed emergence of TAMs and further research on this topic is required. Of course, the multiple alterations in RT enzyme function associated with M184V may contribute to these effects.

In fact, a number of clinical trials with triple drug combination therapy regimens have been performed in which the first and most prevalent mutation to have arisen in the context of an initial regimen was M184V (Table 1). The finding that the occurrence of M184V is so extensive suggests that this substitution should be considered to be a marker of ongoing viral replication in the face of drug pressure as much as a determinant of resistance to 3TC. The various clinical trials in Table 1 represent situations in which the development of M184V was not necessarily accompanied by a sharp rebound in viral load, as long as the other two drugs in the regimen continued to maintain antiviral effect. This is also reflected by the observation that patients in each case had a significantly reduced likelihood of developing TAMs or mutations associated with protease inhibitor resistance. Thus, while the occurrence of M184V may sometimes be predictive of treatment failure, this is not always the case. Moreover, viruses containing M184V remain susceptible to all other approved antiviral drugs.

Table 1: Incidence of various resistance-conferring mutations in patients on initial triple combination therapy regimens

			% Patients with		
Study	**Treatment**	**No.**	**M184V**	**TAMs**	**Protease inhibitor resistance**
Start I & II	3TC/ZDV/IDV	34	59	0	6
AVANTI 2	3TC/ZDV/IDV	11	45	0	0
AVANTI 3	3TC/ZDV/NFV	7	43	0	14
CNA3005	3TC/ZDV/IDV	29	70	0	5
NZTA4002	3TC/ZDV/NFV	33	61	3	52
ACTG 347	3TC/ZDV/APV	7	57	0	14
ACTG 343	3TC/ZDV/IDV	17	82	0	0

* Sampling for genotypic analysis was performed on clinical isolates from patients whose viral load had rebounded to >400 copies HIV-1 RNA/ml.

2.7. Diminished HIV-1 RT processivity and impaired viral fitness with M184V

The processivity of the HIV-1 RT enzyme may be affected by the presence of several NAMs. These mutations which include L74V and M184V reduce the processivity of RT, while it is unclear what role the zidovudine resistance-conferring mutations (i.e., D67N, K70R and T215Y/F) may play in this regard (Boyer and Hughes, 1995; Caliendo et al., 1996; Oude Essink et al., 1997; Sharma and Crumpacker, 1999). Furthermore, certain combinations of M184V in the presence of TAMs, in particular the T215Y/F mutation, have been shown to interact additively or synergistically to inhibit RT processivity to a higher degree than produced by M184V alone (Arion et al., 1998). The acquisition of a compensatory mutation at position 219 in RT together with T215Y/F may result in higher RT processivity than is observed for wild-type virus (Back and Berkhout, 1997; Miller M.D. et al., 1998).

HIV-1 RT processivity may be a major determinant of viral replication capacity or fitness (Caliendo et al., 1996; Back and Berkhout, 1997; Sharma and Crumpacker, 1999; Naeger at al., 2001a). It has been shown that HIV-1 harboring drug resistance mutations to nucleoside analogues have a measurable replication disadvantage in comparison to wild-type virus. However, it has also been reported that the extent of the impairment of HIV-1 fitness, associated with RT mutations, is less than that produced by primary PR drug resistance mutations (Harrigan et al., 1998; Berkhout, 1999; Kosalaraska et al., 1999; Quiñones-Mateu et al., 2000; Dykes et al., 2001; Nijhuis et al., 2001; Quiñones-Mateu et al., 2001). Although

estimates for the fitness of M184V HIV-1 mutants vary considerably depending on laboratory methodology and the viral strain utilized, the replication efficiency of these viruses appears to be reduced by about 3 to 10 percent in comparison to wild-type HIV-1 (Sharma and Crumpacker, 1999; Devereux et al., 2001; Miller V. et al., 2002). In a recent study that examined the fitness of multi-class resistant HIV-1 acquired during primary HIV infection (PHI), it was observed that plasma HIV-1 RNA levels in two cases were initially suppressed but increased to levels comparable to those for PHI patients without these mutations following disappearance of the M184V mutation. Remarkably, a third PHI case infected with M184V virus maintained consistently low levels of plasma HIV-1 RNA for up to five years from the estimated time of seroconversion. Furthermore, virus from another individual could only be isolated for growth competition experiments following the loss of the M184V mutation (Brenner et al., 2002). Of relevance to these observations, the M184V mutation has also been reported to produce a slight impairment of SIV fitness, although this did not affect disease outcome in a macaque study of SIV infection (Van Rompay et al., 2002).

As stated previously, in either SIV or HIV-1 variants containing large deletions in the viral genome, the simultaneous presence of M184V in RT has been shown to severely restrict the ability of these initially attenuated viruses to regain viral replication competence as a result of compensatory mutagenesis (Whitney et al., 2002). HIV-1 variants containing M184V have also been reported to show slower escape from neutralizing antibodies as consequence of mutations

in the envelope (*env*) gene, than did wild-type virus (Wainberg et al., 1996b; Wainberg and Parniak, 1997; Inouye et al., 1998).

Diminished fitness may also be advantageous in helping to improve HIV-1-related disease outcome, as inefficient viral replication has been shown to be associated with reduced plasma viremia, delayed emergence of resistance mutations, and improved immunological responses to antiretroviral therapy. With respect to the latter, immunological and virological discordance, in which CD4 cell counts are stabilized or increase despite detectable plasma HIV-1 RNA, has been partially attributed to the emergence of fitness-reducing resistance mutations such as D30N in the HIV-1 PR gene in patients continuing treatment with failing PR inhibitor-based regimens (Picchio et al., 2000; Antinori et al., 2001; Liegler et al., 2001; Stoddart et al., 2001)

Similarly, impaired fitness associated with M184V may explain residual antiviral activity reported for 3TC following the development of high-level resistance to this drug. In the NUCA3001 study, 366 patients with baseline CD4 cell counts between 200 to 500 cells/mm^3 and less than 4 weeks of prior exposure to ZDV were randomized to receive treatment with 3TC monotherapy (300 mg every 12 h), ZDV monotherapy (200 mg every 8 h) or combination therapy with 3TC (150 or 300 mg every 12 h) with ZDV for up to 52 weeks (Eron et al., 1995; Eron, 1996). In this study, plasma HIV-1 RNA in the 3TC monotherapy arm attained a nadir of -1.2 $\log_{10}$ by week 4 after initiation of treatment before

rebound occurred concomitant with the appearance of M184V; however, viral load levels consistently remained below baseline (0.6 to 0.3 $\log_{10}$ viral load reduction), and were significantly lower than those resulting from treatment with ZDV alone, for the trial's 52-week duration (Kuritzkes, 1996). The development of the M184V mutation in 3TC/ZDV-treated patients enrolled in the AVANTI 2 and 3 trials was also associated with significant reduction of baseline plasma HIV-1 RNA that was, in fact, greater than would be expected with ZDV monotherapy (Maguire et al., 2000). Collectively, these results from the NUCA3001 and AVANTI trials provide further evidence for a residual antiviral effect with 3TC following the emergence of M184V.

Coincidently, lamivudine (3TC)-resistant hepatitis B virus (HBV) variants have been selected in patients following prolonged treatment with this drug. As with HIV-1, this resistance results from either isoleucine (I) or valine (V) substitutions in place of methionine (M) within the C domain of the highly-conserved tyrosine-methionine-aspartate-aspartate (i.e., YMDD) motif of the HBV DNA polymerase. In most patients with chronic HBV infection, serum HBV-DNA remains suppressed below baseline so long as treatment with 3TC is continued even after emergence of M184V (Fischer et al., 2001; Liaw, 2001; Rizzetto, 2002). HBV variants that contain M184V are thought to have decreased replication capacity compared to wild-type virus, which helps to explain the sustained antiviral activity of 3TC in this circumstance (Melegari et al., 1998; Fischer et al., 2001; Papatheodoridis et al., 2002).

The Trilège trial was designed to evaluate virological outcomes with induction antiretroviral therapy followed by maintenance therapy with a less potent regimen. A total of 378 antiretroviral-naïve patients with HIV-1 infection received treatment during the induction phase of the trial with 3TC/ZDV/IDV for a 12-week period. Of these patients, 279 attained the virologic endpoint for the induction phase which required a reduction of plasma HIV-1 RNA levels to <500 copies/ml and were randomly assigned to the maintenance phase to continue treatment with 3TC/ZDV/IDV or, alternatively, with 3TC/ZDV or ZDV/IDV (Pialoux et al., 1998). The effectiveness of either dual combination regimen to maintain plasma HIV-1 RNA below 500 c/ml or to produce further suppression to below 50 c/ml was diminished in comparison to that noted with 3TC/ZDV/IDV. Furthermore, despite reduced antiviral potency, maintenance therapy with 3TC/ZDV or ZDV/IDV in the Trilège trial did not compromise the virological benefit conferred by subsequent treatment with either the original induction regimen or other antiretroviral combinations (Flandre et al., 2002). Removal of 3TC from the triple-drug induction regimen was associated with rapid rebound of HIV-1 RNA that increased from -1.66 $\log_{10}$ at the time of virological failure to near pre-treatment levels (i.e., -0.31 $\log_{10}$) six weeks later. In contrast, plasma HIV-1 RNA in the 3TC/ZDV group did not rebound as sharply as was the case in patients treated with ZDV/IDV, and remained suppressed at a level of -1.38 $\log_{10}$ below baseline for the six-week period following removal of IDV from the induction regimen (Descamps et al., 2000).

Others have also reported differential kinetics of plasma HIV-1 RNA rebound in patients experiencing virological failure on triple antiretroviral therapy regimens. In these studies, it was noted that the slope of plasma HIV-1 RNA for virus escaping with the M184V 3TC-resistance mutation was lower and did not attain as high levels compared to those cases in which virological failure followed the emergence of HIV-1 containing NNRTI resistance-conferring mutations (e.g., Y181C or K103N) (Press et al., 2002).

2.8. Effect of M184V on HIV disease outcomes and need for additional clinical trials

Further clinical evidence regarding continued use of 3TC in the face of the M184V mutation is provided by the CAESAR trial. Briefly, patients with HIV-1 infection were randomized to receive either placebo, 3TC or, alternatively, a combination of 3TC and loviride, an NNRTI, added onto a ZDV-based regimen for up to 52 weeks. The results on 1,080 patients revealed that treatment with 3TC resulted in significantly less HIV disease progression and death compared to the placebo arm (CAESAR Coordinating Committee, 1997; Montaner et al., 1998). However, the clinical benefit conferred by 3TC in this study was of limited duration, most likely due to accumulation of other resistance-conferring mutations.

To date, clinical benefits resulting from selection of M184V in 3TC-containing regimens have been largely inferred from mechanistic studies of RT

function and the results of *post-hoc* and meta-analyses from numerous clinical trials. However, discordant findings that interrogate the utility of continuing treatment with 3TC after the development of high-level resistance have also been published. A notable example is ACTG 370 in which suppression of baseline plasma HIV-1 RNA levels to $\leq$ 200 copies/ml was reported to be superior after 24 weeks of therapy when 3TC was replaced by delavirdine (DLV) (73% response for DLV versus 58% response for 3TC maintenance) in NRTI-experienced patients also treated with IDV and ZDV (Kuritzkes et al., 2000). Although differences in virologic outcome between both treatment groups in this study were not statistically significant ($p = 0.29$), these results nevertheless reflect the need for other randomized clinical trials that will be sufficiently powered to validate the M184V benefit hypothesis. In this regard, the COLATE trial, a large multi-center European study initiated by the Copenhagen HIV Programme (CHIP), may help to address this important objective. In this study, 160 patients experiencing viral load rebound (plasma HIV-1 RNA $\geq$1000 copies/ml) on an initial 3TC-containing regimen will be randomized to one of two treatment groups in which 3TC is either continued or substituted by another drug in individualized second-line combination therapy regimens. This study also involves new restrictions to be placed in regard to use of other ARVs.

2.9. Conclusion

Several mechanisms including decreased RT processivity, reduced nucleotide-dependent primer unblocking, increased fidelity, hypersensitization to

other NRTIs, and impaired viral fitness have been invoked to explain the clinical benefits associated with continued 3TC therapy following emergence of the M184V substitution in RT. However, the importance of each of these factors in regard to therapeutic outcome may be difficult to ascertain, and, indeed, it is increasingly clear that M184V can have multiple simultaneous effects based, in large part, on its strategic location close to the active catalytic site of RT. Thus, multiple mechanisms may, in fact, be responsible, including reduced RT processivity and impairment of viral fitness. In designing future clinical trials to test the M184V benefit hypothesis, as is the case with COLATE, consideration of these mechanisms alongside the potential for augmentation of the antiviral activity of other drugs will be important factors to help guide the selection of antiretroviral drugs to be used in combination with 3TC.

3TC was one of the first ARVs to result in reductions in HIV/AIDS-related morbidity and mortality and remains a cornerstone of current antiretroviral therapy. Hopefully, continued research to further study the potential benefits of M184V will lead to optimized therapy with available drugs and provide insight into future optimization of combination regimens. It should be noted, as well, that continued 3TC usage may not be the only means of preserving M184V and that alternative ways of attaining this goal could be explored. These could include a variety of measures that would keep pressure on M184V including the use of ABC and/or low doses of 3TC. These and related concepts could likewise constitute the basis of future clinical trials although, to be sure, continued 3TC

usage is the only clinically proven means of preserving the M184V mutation at this time.

Finally, none of the points made in this paper in regard to potential benefits of M184V would justify, as some have suggested, that this substitution be deliberately selected by 3TC as part of a therapeutic strategy. Antiviral drugs should ideally be used for their intended purpose which is to arrest viral replication and reduce viral load. The arguments raised here pertain only to the wisdom of whether to maintain M184V once it has already been selected.

CHAPTER 3

Differential Maintenance of the M184V Substitution in the Reverse Transcriptase of Human Immunodeficiency Virus Type-1 by Various Nucleoside Antiretroviral Agents in Tissue Culture

Petrella M., M. Oliveira, D. Moisi, M. Detorio, B.G. Brenner and M.A. Wainberg

Preface to Chapter 3:

In chapter 2, we discussed the results of several clinical trials in which improvements in virological and/or immunological responses to 3TC-containing combination therapy regimens were positively correlated with the presence of the M184V substitution in HIV-1 RT. These outcomes have also been explained on the basis of the many modifications of RT biochemical activity and viral fitness

that result from the M184V substitution. Collectively, these findings create a rationale for further investigation of the therapeutic relevance associated with maintenance of the M184V mutation, a concept referred to as the M184V benefit hypothesis.

3.1. Abstract

The M184V substitution in human immunodeficiency virus type-1 reverse transcriptase (RT) is rapidly selected in tissue culture following serial passage of wild-type virus in the presence of increasing concentrations of lamivudine (3TC). M184V is also associated with several alterations of RT enzymatic function *in vitro* that may adversely affect viral fitness or replication capacity, which creates a potential rationale for its maintenance once it has been selected by antiviral chemotherapy. However, the relative effectiveness of nucleoside RT inhibitors that are structurally-unrelated to 3TC in selecting and/or maintaining M184V has not been investigated. In the present study, we have studied the abilities of a variety of drugs, i.e., zalcitabine (ddC), didanosine (ddI), abacavir (ABC), and the novel nucleoside SPD754, in addition to 3TC, to maintain the presence of M184V in tissue culture and have shown that SPD754, ABC and 3TC are able to preserve M184V in mixed dual infections consisting of wild-type viruses and clinical isolates which contained the M184V mutation. Moreover, M184V could also be maintained in these cultures when a subtherapeutic concentration of 3TC (i.e., (0.05 μM) was used. In contrast, neither ddI nor ddC was able to maintain M184V to the same extent as the other drugs after 10 weeks of tissue culture in mixtures of wild-type viruses and isolates containing M184V in different proportions.

3.2. Introduction

Lamivudine [3TC; (-)-2', 3'-dideoxy-3'-thiacytidine] is a potent nucleoside analogue inhibitor (nucleoside reverse transcriptase [RT] inhibitor [NRTI]) of human immunodeficiency virus type-1 (HIV-1) RT (Hart et al., 1992; Schuurman et al., 1995; Gray et al., 1995). 3TC is an integral component of many combination therapy regimens (Yeni et al., 2002), and intensification of zidovudine (ZDV) monotherapy or dual therapy with ZDV-zalcitabine (ddC) or ZDV-didanosine (ddI) with 3TC was shown to result in significant reductions in HIV- and AIDS-related morbidity and mortality (CAESAR Coordinating Committee, 1997; Montaner et al., 1998). Resistance to 3TC is rapidly selected in tissue culture following serial passage of wild-type (WT) HIV-1 in the presence of increasing concentrations of drug (Boucher et al., 1993; Gao et al., 1993; Tisdale et al., 1993). In addition, HIV-1 variants with high-level phenotypic resistance to 3TC can be isolated from patients who experience virological failure as early as 8 weeks following the initiation of therapeutic regimens that include 3TC (Larder et al., 1995; Schuurman et al., 1995; Wainberg et al., 1995).

A single mutation at position 184 (i.e., M184V) in the HIV-1 RT gene is responsible for high-level resistance (i.e., ≈500-fold) to 3TC (Quan et al., 1996; Frost et al., 2000; Götte and Wainberg, 2000), although a more transient substitution, M184I, usually develops first (Keulen et al., 1997; Rezende et al., 1998). However, HIV-1 variants harboring M184I are less fit than their M184V

counterparts and are therefore rapidly outcompeted by the latter both *in vitro* and *in vivo* (Back et al., 1996).

The M184V mutation can also be selected by structurally related compounds such as (-)-beta-2', 3'-dideoxy-5-fluoro-3'-thiacytidine (FTC; emtricitabine,) (Gao et al., 2000; Lee and Chu, 2001) as well as by several structurally-unrelated NRTIs such as abacavir (ABC) (Harrigan et al., 2000; Miller V. et al., 2000). Two other NRTIs that select less frequently for M184V are ddI and ddC, and both ddI and ABC retain clinical efficacy against viruses that contain the M184V substitution (Gu et al., 1992; Gao et al., 1993; Winters et al., 1997). This is due, in part at least, to the fact that M184V confers only very low levels of resistance to such drugs as ddI and ABC in tissue culture (i.e., two- to four-fold increases in the 50% inhibitory concentration (IC_{50}) (Tisdale et al., 1997; Moyle, 2001; Ray et al., 2002), in contrast to its effect against 3TC. Hence, the potential ddI-, ABC-, and ddC-resistance-conferring effect of M184V may be manifested only in the presence of additional mutations in the RT gene (Gao et al., 1993; Miller V. et al., 1998). Although M184V also confers high-level resistance to FTC (Schinazi et al., 1993; Tisdale et al., 1993; Richman, 2001), clinical experience with this compound is limited, and it is not clear whether FTC selects for this mutation as efficiently as 3TC does in patients receiving therapy.

The M184V substitution is also associated with altered RT function as a possible consequence of the location of M184V within a conserved YMDD motif

that constitutes part of the enzymatic active site (Kohlstaedt et al., 1992; Jacobo-Molina et al., 1993). These altered functions include decreased RT processivity, reduced nucleotide-dependent primer unblocking, increased polymerase fidelity in biochemical analyses, diminished rates of initiation of reverse transcription (Diallo et al., 2003b), hypersensitization to other NRTIs, impaired viral fitness, and delayed appearance of mutations in the RT gene that are responsible for resistance to thymidine analogue inhibitors such as ZDV and stavudine (d4T) (Miller V. et al., 2002; Petrella and Wainberg, 2002).

For these reasons, some clinicians believe that the M184V mutation should be maintained once it has been initially selected by antiretroviral therapy. However, it is not known whether continuous exposure to therapeutic levels of 3TC is the only means of achieving this goal or if other drugs might also be able to maintain the pressure required to prevent the disappearance of the M184V mutation once it has been selected. Other methods of maintaining M184V could potentially include the use of other NRTIs, combinations of drugs, or even low doses of 3TC. This study was performed to address this subject.

3.3. Materials and methods

3.3.1. Tissue culture selection experiments

Stocks of HIV-1 HXB2D and three different clinical HIV-1 isolates harboring the M184V substitution (isolates 3350, 4742, and 4205) were used to establish initial infections in 2 X 10^6 peripheral blood mononuclear cells (PBMCs) at a multiplicity of infection of 0.01, based on 50% tissue culture infectious doses, in 24-well plates in the presence or absence of ddC, ddI, d4T, 3TC, SPD754, or ABC (Johnson and Byington, 1994; Salomon et al., 1994).

Clinical isolate 4205 displayed resistance to multiple NRTIs and contained several mutations for resistance to thymidine analogues (i.e., D67N, T215F and K219Q) as well as M184V. This isolate also harbored mutations in the RT gene at A98G, T69D, V118I, and R211K in addition to L63P in the protease gene, but did not harbor primary mutations associated with resistance to protease inhibitors. Two other clinical isolates, i.e., 3350 and 4742, a subtype C variant, contained only M184V as a drug resistance-associated mutation. In some experiments, cells were infected with alternating 1:9 or equal (i.e., 5:5) mixtures of the different HIV-1 isolates used. After seven days, the cells were resuspended in their original culture medium, and 500 µL of supernatant and cell mixture were transferred to a new well with 2 X 10^6 PBMCs and fresh solutions of the NRTIs. Thereafter, new rounds of infection were initiated by serial passage of the viral cultures at weekly intervals for the duration of the experiments (i.e., 9, 10 or 26 weeks).

3.3.2. Genotypic analysis

Proviral DNA was extracted and amplified from viral cultures of the HIV-1 isolates in preparation for genotypic analysis. The TRUGENE® HIV-1 Genotyping Test was used in conjunction with the OpenGene® automated DNA sequencing system (Bayer Diagnostics Inc., Toronto, Ontario, Canada) to sequence the RT and protease regions of HIV-1 (Salomon et al., 2000). The sensitivity of this genotypic assay for the detection of minority viral species in mixtures is reported by the manufacturer to be 20% (OpenGene® System User Manual).

In some experiments, the line probe assay (LiPA; LiPA HIV-1 RT; Innogenetics Inc., Norcross, Ga.) was used as described previously to detect WT and drug resistance-related codons at position 184 in the RT gene (Stuyver et al., 1997; Servais et al., 2001a). The intensities of the reactive LiPA bands were scored to estimate the percentage of WT or M184V species in mixtures of different viruses whose proportions were predetermined. Previous studies have shown that semiquantitative interpretation of LiPA band signals is possible for various RT codons in order to determine the relative presence of both WT and mutant viruses in clinical samples (Sheridan et al., 1998; Villahermosa et al., 1998). The basis for the LiPA technology involves reverse hybridization of a biotin-labeled PCR fragment that encodes the HIV-1 RT or protease gene region of interest to immobilized oligonucleotides that are embedded within nitrocellulose membrane test strips (Van Laethem et al., 1999; Servais et al.,

2001a). The presence of WT or drug-resistant variants is determined using a biotin-streptavidin colorimetric read-out in which the relative amount of either species is proportional to the intensity of the bands (Descamps et al., 1998; Rusconi et al., 2000), enabling this test to detect individual genotypes among populations with mixtures of genotypes (Descamps et al., 1998; Van Laethem et al., 1999; Rusconi et al., 2000; Servais et al., 2001a; Halfon et al., 2003). LiPA can often reveal the presence of minority species that constitute as little as 1% of the viral population, whereas the cutoff for detection of majority species using DNA sequencing ranges from 10% to 30% (Stuyver et al., 1997; Descamps et al., 1998; Van Laethem et al., 1999; Rusconi et al., 2000; Servais et al., 2001a; Halfon et al., 2003).

3.3.3. Phenotypic resistance testing

The drug susceptibility of HIV-1 clinical isolates was measured by determining the extent to which select NRTIs inhibited replication in tissue culture (Salomon et al., 2000). Briefly, infected PBMCs were grown in 96-well culture plates in both the absence and presence of various concentrations of NRTIs. After 7 days, RT assays were performed with culture fluids, in conjunction with Prism analytic software (GraphPad Prism version 3.03 for Windows; GraphPad Software, San Diego Calif.), to determine the IC_{50}s (Salomon et al., 2000). The IC_{50}s obtained for isolates were compared to the IC_{50}s previously determined for WT viruses in order to compute the fold-resistance to the NRTIs tested. In instances in which more than one measurement of the IC_{50}

was performed, the results are expressed as the mean $IC_{50} \pm$ the standard deviation (S.D.). The antiretroviral drugs used for phenotypic testing included ZDV, 3TC, ABC, ddI, and d4T.

The following NRTIs were kindly provided by various pharmaceutical companies: ABC, ZDV and 3TC, GlaxoSmithKline; SDP-754 [(-)-2'-deoxy-3'-oxa-4'-thiocytidine], Shire Biochem; ddI and d4T, Bristol-Myers Squibb; and ddC, Roche.

3.4. Results

The *in vitro* sensitivities of multinucleoside-resistant HIV-1 strain 4205 and two 3TC-resistant viruses, i.e., isolates 3350 and 4742, were studied with a panel of NRTIs that included ZDV, 3TC, ddI, d4T, and ABC. Table 1 shows that clinical isolate 4205 was highly resistant to 3TC (>1,000-fold), ZDV (59-fold) and ABC (50-fold) compared with the wild-type, while also displaying significantly reduced susceptibility to both ddI (10-fold) and d4T (12-fold) due to the presence of several mutations for resistance to nucleoside analogues. Clinical isolates 3350 and 4742 were, as expected, highly resistant to 3TC (>1,000-fold). Both of these viruses were resistant to each of ddI, d4T, and ABC while they retained their sensitivities to ZDV.

The effect of various concentrations of select NRTIs or the absence of drug on maintenance of the M184V substitution in culture was examined with these isolates for 26 weeks. The concentrations of ddI and ABC that were used were one, two, and five times their respective IC_{50}s; and the isolates were assessed for the presence of the M184V mutation in the RT gene after 26 weeks. For clinical isolate 4205, the results in Table 2 show that the absence of drug pressure resulted in reversion to the WT, i.e., M184, during this time, while exposure to ABC at the IC_{50} (i.e., 0.2 μM) did not provide sufficient drug pressure to fully maintain M184V, with M184 and 184V being detected as a mixture after 26 weeks. However, higher concentrations of ABC (i.e., two and five times the IC_{50}) were able to preserve the M184V mutation over 26 weeks. In this experiment, all

Table 1: IC_{50} of various NRTIs for clinical isolates and fold-resistance of clinical isolates to the various NRTIs

	IC_{50} [μM] (fold-resistance)[a]		
Drug	**Isolate 3350**	**Isolate 4742**	**Isolate 4205**
ZDV	0.01 ± 0.01 (0.4)	0.01 ± 0.005 (0.5)	1.7 ± 0.5 (59)
3TC	>100 (>1,000) [b]	>100 (>1,000) [b]	>100 (>1,000) [b]
ddI	>20 and <80 (8-32) [b]	25.4 ± 4.2 (10)	25.2 ± 0.5 (10)
d4T	0.6 ± 0.2 (12)	0.3 ± 0.08 (6)	0.5 ± 0.3 (12)
ABC	9.9 ± 2.1 (40)	4.5 ± 0.9 (18)	12.4 ± 11 (50)

[a] Standard deviations are included for all studies except those for which a broad range of IC_{50} was obtained (n = 2). Isolates 3350 and 4742 (a subtype C isolate) contained the M184V mutation. Isolate 4205 contained the A98G, D67N, T69D, V118I, M184V, R211K, T215Y, K219Q, and L63P mutations.

[b] In these instances, a broad range of IC_{50} was obtained, as indicated (n = 2).

concentrations of ddI evaluated (i.e., 2.5, 5.0, and 10.0 μM) were able to maintain M184V over the 26 weeks, and genotypic analysis after that time did not reveal other changes in the RT gene.

We also investigated the effects of two concentrations of 3TC on the maintenance of M184V in culture using two different clinical isolates. The data of Table 2 show that M184V was preserved during 26 weeks when either the IC_{50} of 3TC (0.1 μM) or a subtherapeutic concentration of 3TC (i.e., 0.05 μM) was used.

The effect of antiretroviral pressure on the selection or preservation of M184V was also investigated in cultures in which infection of PBMCs was initiated with mixtures of WT HXB2D cloned virus and clinical isolate 3350 that contained M184V. The concentrations of the viral inocula were based on the p24 values for viruses that had been grown in PBMCs as described (Johnson and Byington, 1994; Salomon et al., 1994); and the ratio of viruses used was 1:1, 5:5, or 1:9.

The data in Table 3 show that ddC at a concentration of 0.05 μM, i.e., the IC_{50}, was not able to select for M184V in cultures of WT HXB2D but show that this concentration of drug was able to maintain M184V over 9 weeks in all of the cultures with dual infections with HXB2D and clinical isolate 3350. Control cultures containing only isolate 3350 reverted or deselected to either a mixed

Table 2: Effects of different NRTIs on maintenance of the M184V mutation in tissue culture

			Genotype obtained with the following drugs at the indicated concn:							
			3TC		ABC				ddI	
Virus	**Wk**	**No Drug**	**0.1 µM**	**0.05 µM**	**0.2 µM**	**0.4 µM**	**1.0 µM**	**2.5 µM**	**5.0 µM**	**10.0 µM**
Isolate 4205	26	M184			184M/V	184V	184V	184V	184V	184V
Isolate 3350	6		184V	184V						
	13		184V	184V						
	28		184V	184V						
Isolate 4742	13		184V	184V						
	19		184V	184V						
	28		184V	184V						

184M-184V genotype or the WT during this period in the absence of drug pressure.

In 1:9 mixtures of WT HXB2D and isolate 3350, 0.05 μM ddC was more effective than 0.006μM ddC at maintaining M184V over 9 weeks. In the latter case, mixtures of both WT viruses and viruses with the M184V mutation were found. Reversion to M184 with 0.006 μM ddC was also observed in cultures containing equal mixtures of both viruses. In contrast, at 0.05 μM either ddC or 3TC provided adequate pressure to maintain M184V. In cultures infected with 9:1 mixtures of WT virus and isolate 3350, M184V was effectively selected and/or maintained with 0.05 μM ddC, indicating the importance of the concentration of ddC used.

The effectiveness of ABC and ddI in selection and/or maintenance of M184V were also evaluated using different mixtures of infecting viruses in tissue culture. The results in Table 4 show that treatment with 3.0 μM ddI for 10 weeks was unable to select for the M184V mutation in the HXB2D WT strain. In contrast, 3.0 μM SPD754 (60% 184V) and 1.28 μM ABC (50% 184V) were only partially selective for this mutation over this time. However, selection of M184V did occur with 1.28 μM 3TC. In the absence of drug pressure, reversion to WT M184 was much more efficient with the M184V clone (isolate 3350) than with the multinucleoside-resistant HIV-1 variant (isolate 4205). In the latter case, LiPA

Table 3: Effect of 9 weeks of antiretroviral drug pressure on maintenance of M184V in mixtures of HXB2D and clinical isolate 3350, an M184V-containing HIV-1 variant

Virus mixture (HXB2D:3350)	**Genotype at RT codon 184 after 9 wk of treatment with:**			
	No drug	**ddC (0.05 μM)**	**ddC (0.006 μM)**	**3TC (0.05 μM)**
HXB2D Only		M184		
1:9	M184, M184 [a]	184V, 184V [a]	184M/V	184V
5:5	M184	184V, 184V [a]	M184	184V
9:1	M184	184V	M184	184V
3350 Only	184M/V, M184 [a]	184V	184V	

[a] Results of two replicate experiments.

testing revealed that the frequency of M184V codons was 70% after 10 weeks without treatment with antiretroviral drugs.

When M184V-containing species were already present in the infecting viral population, all of the NRTIs used were capable of preserving the M184V mutation in various mixtures of HXB2D and isolate 4205. However, in mixtures of HXB2D and isolate 3350, 3.0 μM ddI was not able to maintain the presence of M184V to the same extent as 1.28 μM either ABC or 3TC after 10 weeks of tissue culture in the presence of these drugs. In this study, the use of 3.0 μM SPD754 also provided adequate drug pressure to preserve the M184V mutation in all mixtures of HXB2D and isolate 3350.

Table 4: Selection and/or maintenance of the M184V mutation during 10 weeks of antiretroviral drug exposure in mixtures of HIV-1 isolates

Virus mixture	Ratio	LiPA genotype at codon 184 and % of isolates with the genotype after 10 wk of treatment with:				
		No drug	ABC (1.28 μM)	ddI (3.0 μM)	3TC (1.28 μM)	SPD754 (3.0 μM)
HXB2D Only		M184	184V (50%)	M184	184V (100%)	184V (60%)
HXB2D:3350	9:1	M184	184V (100%)	M184	184V (100%)	184V (100%)
HXB2D:3350	5:5	M184	184V (95%)	184V (60%)	184V (100%)	184V (100%)
HXB2D:3350	1:9	184V (2%)	184V (100%)	184V (70%)	184V (100%)	184V (100%)
3350 Only		M184	184V (55%)	184V (100%)	184V (100%)	184V (100%)
HXB2D:4205	9:1	184V (50%)	184V (100%)	184V (100%)	184V (100%)	184V (100%)
HXB2D:4205	5:5	184V (60%)	184V (100%)	184V (100%)	184V (100%)	184V (100%)
HXB2D:4205	1:9	184V (50%)	184V (100%)	184V (100%)	184V (100%)	184V (100%)
4205 Only		184V (70%)	184V (100%)	184V (100%)	184V (100%)	184V (100%)

3.5. Discussion

The M184V substitution in HIV-1 RT is rapidly selected in tissue culture following serial passage of WT virus in the presence of increasing concentrations of 3TC and is associated with the development of high-level phenotypic resistance (i.e., >500-fold) to this antiviral agent (Boucher et al., 1993; Gao et al., 1993; Tisdale et al., 1993). M184V is also rapidly selected *in vivo* and can be frequently detected in antiretroviral-naïve patients experiencing plasma HIV-1 RNA rebounds following initiation of therapy with 3TC-containing regimens (Kavlick et al., 1995; Larder et al., 1995; Schuurman et al., 1995; Wainberg et al., 1995; Kuritzkes et al., 1996; Wainberg et al., 1996a).

M184V is known to impart a fitness disadvantage to HIV-1. In the absence of selection pressure for this mutation, the replication capacity of viruses harboring M184V may be reduced by 3% to 10% (Sharma and Crumpacker, 1999; Devereux et al., 2001; Quiñones-Mateu et al., 2001) to as much as 48% to 57% in comparison to that of WT HIV-1 (Miller et al., 2003), depending on the viral strain and laboratory methods used. In addition, infection with viruses containing M184V often results in lower levels of viremia in plasma as a result of their diminished replication competence (Catucci et al., 1999). The rapid disappearance of M184V has been documented in treatment-experienced patients in whom selection pressure for this mutation was removed (Zaccarelli et al., 2003), and this has been shown to be frequently associated with a sharp rebound of plasma viremia, as was the case following the removal of 3TC from a triple-

drug induction regimen (Descamps et al., 2000) and, more recently, in therapy-experienced patients undergoing partial treatment interruption in which NRTIs were discontinued from a regimen that initially contained both these drugs and protease inhibitors (Deeks et al., 2003). Moreover, virological and immunological discordance in which CD4 cell counts are stable or increased, despite persistent plasma viremia (i.e., HIV-1 RNA levels, >10,000 copies/ml), has been reported in a subset of heavily pre-treated patients who were infected with isolates with the M184V substitution and who experienced virological failure during antiretroviral therapy (Nicastri et al., 2003).

However, the selection or maintenance of M184V by NRTIs other than 3TC has not been extensively investigated. By using a multinucleoside-resistant viral isolate that harbored M184V, in addition to several other mutations for resistance to nucleoside analogues, both ABC (0.4 and 1.0 μM) and ddI (2.5, 5.0, and 10.0 μM) effectively maintained M184V over a period of 26 weeks. Similarly, treatment of an M184V-containing clinical isolate with ddC (0.05 or 0.006 μM) for 9 weeks also maintained M184V *in vitro*. Exposure to 0.05 μM ddC for 9 weeks did not select for M184V with HIV-1 HXB2D, a laboratory WT clone.

LiPA was used instead of DNA sequencing for some experiments (Table 4) in order to detect the proportions of WT and M184V variants in primary cell cultures infected with different mixtures of these viruses. In our study, HIV-1

genotyping by LiPA revealed a considerably lower proportion of M184V-substituted codons in the 9:1 (0% 184V), 5:5 (60% 184V) and 1:9 (70%184V) mixtures of HXB2D and isolate 3350 that were maintained with 3.0μM ddI for 10 weeks than in cell cultures exposed to 1.28 μM either ABC or 3TC for the same length of time. Although the relative frequency of mutants with M184V maintained by ddI in the viral mixtures was observed to increase contemporaneously with the dilution of WT species, our data suggest that ddI may not be able to provide selection pressure to the same degrees as ABC and 3TC to preserve M184V, especially when the latter constitutes a minority species in the viral population.

Genotypic analysis indicated that isolate 4205 contained V118I. This mutation, when it is present either alone or together with E44A/D, can confer moderate phenotypic resistance to 3TC (Hertogs et al., 2000; Romano et al., 2002). In addition, data from other studies (Brenner et al., 2003) suggest that V118I may also mediate a negative compensatory effect in regard to M184V that could possibly improve the replicative fitness of multi-drug-resistance HIV-1 variants that also harbor the M184V substitution in the RT gene. It is possible that V118I may have imparted a competitive advantage to isolate 4205 that allowed this virus to replicate to high levels and persist for 10 weeks in mixed viral populations that also contained WT species.

The M184V substitution in the RT gene confers low-level cross-resistance to structurally unrelated NRTIs, such as ABC, ddC, and ddI in tissue culture (Gao et al., 1993; Miller V. et al., 1998; Miller and Larder, 2001). However, the presence of M184V, which was detected in 60% of 3TC-experienced patients with virological failure, did not compromise the virological outcomes during subsequent treatment with new regimens that included d4T plus ddI (Winters et al., 2003). Furthermore, M184V was detected in only 27% of these patients, whereas it was detected in 100% of the patients who received an alternate regimen that included d4T plus 3TC (Winters et al., 2003). Similar results were reported in another study (Eron et al., 2002), in which virological responses during 8 weeks of treatment with ddI or ddI-hydroxyurea were not adversely affected by the presence of M184V. Thus, clinical studies suggest that the presence of the M184V mutation does not appear to adversely affect virological response to ddI-containing regimens. Moreover, our LiPA results with ddI are consistent with the clinical data that show that treatment with this drug is relatively ineffective at maintaining M184V in clinical samples (Svedhem et al., 2002; Winters et al., 2003).

One important limitation of this work is that we have not carried out these studies using isogenically matched WT and M184V-containing clinical variants. Consequently, the results obtained could conceivably have been affected by differences in alleles in a variety of viral genes that might have affected viral fitness or the ability of other mutations to compensate for the effects of M184V.

The construction of several pairs of matched clinical isolates containing multiple resistance-associated mutations is in progress. The fact that a variety of both clinical and laboratory-generated viruses have been used in this study and the fact that consistent results have been obtained with each type make it unlikely that the results that will be ultimately obtained with isogenic pairs of viruses will be different from those described here.

We have demonstrated that a variety of drugs, including ABC, the novel nucleoside analogue SPD754, and a subtherapeutic concentration of 3TC (i.e., 0.05 μM) can be used in tissue culture to maintain the M184V substitution in RT. These findings may have important clinical implications, insofar as the presence of this mutation has been shown in certain circumstances to be associated with improved therapeutic outcomes. It should be noted that a recent clinical study in which 3TC was either maintained or discontinued in a second-line regimen in which patients otherwise received three effective new drugs did not show virological benefit for the maintenance of M184V (Dragsted et al., 2004). However, this study was not sufficiently powered to reveal small differences between the arms in regard to the levels of plasma viremia, since all of the patients had received new active drugs. Prospective clinical trials are still warranted to investigate whether the maintenance of M184V can confer clinical benefit.

3.6. Acknowledgments

This work was sponsored by the Canadian Institutes for Health Research (CIHR), the Canadian Foundation for AIDS Research (CANFAR), and the Fonds de la Recherche en Santé du Québec (FRSQ).

CHAPTER 4

Final Conclusion and Summary

We have investigated the effectiveness of various NRTIs that are structurally-unrelated to 3TC in maintenance of the M184V substitution in HIV-1 RT in 3TC-resistant viruses that contained M184V alone or in a background that included other NAMs. Overall, the results of our study showed that each of ABC, ddI, the novel cytosine analogue SPD754, and a subtherapeutic concentration of 3TC (i.e., 0.05 μM) were able to provide adequate pharmacological pressure in tissue culture needed to maintain the M184V mutation in primary cells infected with a multi-NRTI resistant HIV-1 variant (i.e., isolate 4205). However, our analysis of genotypic data obtained with the LiPA test also suggests that ddI may not be able to preserve M184V as effectively as the other drugs tested, especially when M184V represents a minority species in the replicating viral pool (i.e., 10% to 50%) and in the absence of additional mutations in the RT gene. Comparatively, subtherapeutic 3TC was able to maintain the M184V substitution for up to 28 weeks in tissue culture in a clinical isolate (i.e., isolate 3350) in which no other NAMs were present. In another study from our laboratory, treatment with 0.1 μM 3TC (the IC_{50}) also maintained the presence of the M184V mutation in this clinical isolate for 20 weeks (Diallo et al., 2002). Furthermore, and in accord with these findings, it was observed that ddI could not initially select the M184V mutation in a wild-type HIV-1 laboratory strain (i.e., HXB2D) while 3TC, and to a lesser extent, SPD754 and ABC were able to do so. This suggests that ddI by itself may not exert significant pharmacological pressure for M184V unless additional mutations are also present in the RT gene.

The observation that ddI was able to maintain M184V in a multi-NRTI resistant isolate but could not do so to the same extent as ABC, SPD754 and 3TC in another virus that contained only M184V underscores the importance of differences in viral replication capacity on the emergence of drug resistance and outcomes of antiretroviral therapy. In the complete absence of pharmacological pressure, M184V was detected as a majority species (i.e., 70%) in PBMCs infected with isolate 4205. Additionally, the persistence of M184V-variants in mixed genotypic populations (i.e., 50% to 60%) was relatively unaffected by increasing dilution with wild-type species. This finding is not entirely unexpected and is consistent with previous reports that multi-drug resistant HIV-1 variants that contain resistance-conferring mutations to two or three classes of ARVs are able to establish persistent infections, and moreover, that these viruses can remain genotypically stable in tissue culture for extended periods without drug pressure in spite of their significant replication disadvantage compared to drug-sensitive species (Brenner at al., 2002; Simon et al., 2003).

However, this was not the case for isolate 3350 where the presence of M184V species could not be maintained in tissue culture without the gain in viral fitness imparted by drug pressure. Genotypic analysis of isolate 4205 detected the V118I mutation in the RT gene which, when present alone or together with another mutation, E44A/D, can confer moderate phenotypic resistance to 3TC in the absence of M184V (Hertogs et al., 2000). In addition, V118I is thought to develop as a compensatory mutation in multi-NRTI resistant HIV-1 that may

partially offset the impairment in replication capacity associated with M184V (Brenner et al., 2003). In our study, the V118I mutation may have conferred a competitive replication advantage to isolate 4205 that allowed this virus to persist during 10 weeks in mixed genotypic populations that also contained considerable proportions of wild-type species. Although ddI infrequently selects for M184V in tissue culture, pressure provided by this drug in viral mixtures containing significant levels of pre-existing M184V species may have further amplified the replication capacity differential between wild-type and M184V species, thereby allowing the latter to replicate to even higher levels as confirmed by our LiPA results. Therefore, in the context of the multi-NRTI resistant isolate 4205, all of the drugs studied (i.e., ddI, ABC, SPD754 and 3TC) possessed similar ability to maintain the M184V mutation in tissue culture because of the presence of compensatory mutations such as V118I that can antagonize the effect of M184V.

Our data may also provide a laboratory basis for explaining the differential rates of persistence for the M184V substitution following virological failure of antiretroviral combination therapy regimens that include NRTIs such as ABC or ddI, subsequent to selection of M184V during earlier treatment with 3TC. In one study, M184V was present in 95% of patients that experienced initial virological failure with a 3TC-containing regimen (i.e., 54/57 patients), but could be detected in only 67% of patients who had switched to a new regimen (i.e., 38/57 patients), including 12% (n = 7) and 3.5% (n = 2) of patients in whom 3TC had been switched to ddI and ABC, respectively. Overall, the M184V mutation was

maintained in fewer than 50% (n = 7) of all patients (n = 17) in which 3TC had been substituted by ddI (Svedhem et al., 2002). Consistent results have been reported in other studies performed with NRTI-experienced patients who had experienced virological failure on a 3TC-containing regimen in which the prevalence of the M184V mutation was found to be reduced considerably following substitution of 3TC by ddI in a second line regimen in comparison to those cases in which 3TC was retained (Eron et al., 2002; Gazzard et al., 2002; Winters et al., 2003). Taken collectively, the results of several clinical trials indicate that the appearance of the M184V mutation in NRTI-experienced patients does not prejudice treatment outcomes to ddI, and moreover, that ddI is relatively ineffective at preserving M184V compared to 3TC. The latter conclusion seems substantiated on the basis of findings obtained in this study with the LiPA test, which provides a better indication of the presence of minority viral populations in genotypic mixtures than does population-based sequencing such as TruGene technology.

The M184V substitution in HIV-1 RT has been shown to mediate several changes in RT enzymatic activity that are thought to explain the residual antiviral activity of 3TC following the development of high levels of phenotypic resistance to this drug (Petrella and Wainberg, 2002; Diallo et al., 2003c; Turner et al., 2004). However, the relative contribution and durability of each of these alterations of RT function may not be the same at all times. For instance, some of these effects such as enhanced levels of pyrophosphorolysis and/or nucleotide-

dependent primer unblocking, both of which play a role in regard to restoration of antiviral susceptibility to ZDV in the setting of ZDV resistance (and hypersusceptibility to ZDV and TDF) may occur only transiently and not be of equal therapeutic consequence in all circumstances. It has been shown that pressure from ZDV can accelerate reversion of M184V variants *in vitro* (Rusconi et al., 1998; Diallo et al., 2002), and, although the emergence of M184V may be advantageous for some drugs such as ZDV, it is not known if the use of the latter NRTI in therapeutic regimens may also contribute to the loss or reduced persistence of M184V in viral isolates over time.

As discussed previously, the viral fitness deficit conferred by the M184V mutation is also a consequence of multiple alterations of RT function, e.g., reduced enzyme processivity (Sharma and Crumpacker, 1999; Naeger et al., 2001a; Wainberg, 2004b), and delayed initiation of reverse transcription (Diallo et al., 2003b), and is considered by many to represent a mitigating factor in regard to virological and immunological outcomes of antiretroviral therapy. M184V-mediated reductions of plasma HIV-1 viremia and/or augmented CD4 cell counts have been documented in early clinical trials of 3TC monotherapy (Eron, 1996; Kuritzkes et al., 1996) and in a small number of studies in which treatment with 3TC was intentionally stopped as part of a therapeutic strategy to assess the effectiveness of a simpler maintenance regimen e.g., the Trilège study (Pialoux et al., 1998; Descamps et al., 2000). Another trial involving partial treatment interruption (PTI) in which either PIs, or alternatively, NRTIs were discontinued

from a regimen that initially had contained both these drugs (Deeks et al., 2003) showed a benefit for continuation of 3TC. More recently, the potential benefit of sustained drug pressure to preserve the M184V mutation as a means of preventing immunological failure (i.e., CD4 cell count <350 cells/ mm^3) was further investigated in a small unblinded pilot study (i.e., E-184V study) (Castagna et al., 2004). In this trial, 50 HIV-infected patients with persistent viremia (i.e., plasma HIV-1 RNA >1,000 copies/ml) and relatively high CD4 cell counts (i.e., 500 cells/mm^3) were randomized to either a complete treatment interruption (TI) or montherapy with 3TC for 48 weeks. A preliminary analysis performed on 8/22 patients and 9/18 patients that completed 24 weeks of follow-up in the TI and 3TC monotherapy groups, respectively, showed that treatment with 3TC resulted in lower levels of HIV-1 plasma viremia while also conferring a protective effect in regard to the CD4 cell count compared to the TI arm. Although based on a relatively small sample size, the findings of the E-184V study are in agreement with those of related studies that collectively suggest that maintenance of the M184V mutation with drugs such as 3TC in patients with virological failure is associated with improved virological and/or immunological outcomes in certain clinical settings.

Interestingly, studies have shown that other inhibitors of HIV-1 RT in addition to 3TC may also manifest residual antiviral activity in extensively pre-treated patients (Maldarelli et al., 2003). The discontinuation of d4T, but not ddI or EFV, from combination therapy regimens in patients with virological failure

(i.e., HIV-1 RNA>5,000 copies/ml) in whom baseline viral isolates harbored several NAMs that predict resistance to d4T, was observed to elicit a further increase in levels of plasma viremia that later decreased in most patients electing to resume treatment with this drug. These findings suggest that continued treatment with d4T, like that with 3TC, may be of benefit to HIV-1-infected patients in need of salvage antiretroviral therapy despite the presence of resistance-conferring mutations to these drugs.

In summary, we have established a rationale in support of the need for additional clinical studies to further elucidate the potential benefit of maintenance of the M184V substitution as an adjunct therapeutic intervention in patients experiencing virological failure on a regimen that had previously selected for this mutation. The basis for this has been largely inferred from both laboratory studies that have documented a diverse array of M184V-mediated alterations of RT activity, some of which are associated with impairment of viral fitness, and additionally, from limited clinical trials of treatment interruption in antiretroviral therapy-experienced patients in whom an observed improvement in virological and/or immunological responses was positively correlated with the continued presence of M184V in the viral isolates from these patients. However, only one study, (see below), i.e., the COLATE Trial, has examined the therapeutic relevance of preserving the M184V mutation in treatment-experienced patients with detectable levels of HIV-1 plasma viremia in a prospective manner.

Therefore, additional randomized controlled clinical trials (RCTs) to validate this concept are warranted.

The COLATE Trial (Dragsted at al., 2004) was an open-label, multi-centered, randomized study to evaluate the virological efficacy and safety of continued treatment with 3TC versus discontinuation of 3TC in a second-line or subsequent regimen in patients with HIV-1 infection with incomplete viral suppression (i.e., HIV-1 RNA >1,000 copies/ml) who had received treatment with an initial 3TC-containing regimen. Eligible patients with genotypic evidence of the M184V mutation in their baseline viral isolates received treatment with a new regimen that contained three new active ARVs other than 3TC for 48 weeks. In addition, patients were also stratified according to whether they experienced virological failure on their first (i.e., stratum A) or a later regimen (i.e., stratum B). The primary study endpoint was reduction of $\log_{10}$ plasma HIV-1 RNA after 48 weeks of treatment as determined using the area under the curve over time minus changes from baseline method (i.e., AAUCMB); secondary virological endpoints included the proportion of patients with plasma HIV-1 RNA less than 400 copies/ml and 50 copies/ml, and, the time to protocol-defined virological failure which required plasma HIV-1 RNA levels to have either decreased by <0.5 $\log_{10}$ from baseline, or alternatively, increased >1.0 $\log_{10}$ relative to the documented viral load nadir. The differences in the CD4 cell count change from baseline or the time required for an increase of >100 CD4 cells/ml after 48 weeks of therapy were also assessed between the two treatment groups.

As originally planned, 160 patients needed to be randomized into the COLATE Trial in order to detect statistically significant (i.e., $p \leq 0.05$) differences of >0.5 $\log_{10}$ in AAUCMB between the two treatment groups with a power of 90%. However, because of an unexpectedly long patient enrollment period, an interim statistical analysis was requested by the COLATE Trial's data safety monitoring board (DSMB) in order to ascertain the continued validity of this study. An intention-to-treat statistical analysis was performed using data that were available from 54 (83%) and 60 patients (91%) from the control (i.e., no 3TC) and 3TC groups, respectively, that had completed 48 weeks of treatment on their originally assigned regimen. No significant differences in the primary study endpoint could be shown between patients who had discontinued 3TC (AAUCMB = -1.4) versus those who had received treatment with 3TC (given as 150 mg twice daily) in their new regimen (AAUCMB = -1.5). Neither did this analysis show statistically significant differences with respect to the secondary endpoints or the proportions of patients who experienced grade 3 or 4 adverse events between the two arms of the study. Furthermore, in those cases in which a sequence could be obtained, genotypic analysis revealed that the M184V mutation was maintained in viral isolates from patients who had continued treatment with 3TC but that reversions to wild-type had commonly occurred between 12 to 24 weeks in the patients who discontinued the 3TC.

Several ideas have been proposed to explain the failure of the COLATE Trial to demonstrate a benefit of continued 3TC use in patients who experienced

virological failure with the M184V mutation. Firstly, COLATE was not powered to detect plasma HIV-1 RNA differences <0.5 $\log_{10}$ between the two treatment groups, and, moreover, the study's statistical power was further diminished as a result of this trial's inability to attain its target enrollment of 160 patients. These limitations may be especially relevant in view of the fact that both of the randomized treatments produced excellent suppression of plasma HIV-1 RNA, which would have precluded detection of a more modest 3TC-mediated therapeutic effect (i.e., <0.5 $\log_{10}$). Further dilution of a potential 3TC effect may also have occurred because of the high proportion of stratum A patients (n = 55) enrolled early in the trial, because these patients would have had a better chance of becoming aviremic compared to the more therapy-experienced stratum B patients (n = 76), independent of the randomized treatment that they received. In accordance with this finding, it would be of interest to study more heavily-treated patients in future prospective clinical trials to test the M184V benefit hypothesis. Also, the adoption of an open-label study design in COLATE may have rendered the trial more susceptible to investigator bias. Of course, this problem might have been eliminated by blinding the 3TC intervention group with the use of matching placebo, as is generally the standard for RCTs.

In planning new trials, consideration should also be given to assure that proper study conditions are maintained so that an M184V-associated therapeutic benefit can be realized. This requirement could entail not only the recruitment of more heavily treatment-experienced patients but also the concomitant use of drugs

such as ZDV or TDF in 3TC-containing regimens since, as previously discussed, the presence of the M184V mutation in the RT gene has been shown to be advantageous, even if only transiently, in regard to the antiviral activity of both of these ARVs. Another strategy that may also have merit involves monitoring the replication capacity (RC) of viral isolates obtained at baseline and at the end of the treatment period from both patients with undetectable levels of plasma HIV-1 RNA as well as in patients who experience virological failure on a new antiretroviral regimen. The correlation of changes observed in RC with genotypic analysis of the HIV-1 RT and PR genes may be of assistance with respect to the identification of confounding factors (e.g., compensatory mutations), that can interfere with the detection of a 3TC-mediated effect regarding virological and/or immunological responses.

Finally, it is important to remember that COLATE was the first RCT conducted to test the M184V benefit hypotheses. The trial's equivocal but nonetheless disappointing outcome can be considered to be as much a reflection of this study's inherent flaws and limitations as it is of the technical complexity of clinically validating the study concept. Therefore, additional verification of the therapeutic relevance of the M184V substitution in HIV-1 RT remains an important goal that, ultimately, may lead to the development of complementary treatment strategies for salvage therapy patients for whom current therapeutic options are limited.

CONTRIBUTION TO ORIGINAL KNOWLEDGE

The research results and concepts presented in this doctoral dissertation are based on manuscripts that have already been published in two refereed scientific journals. This work was performed under the supervision of Dr. Mark A. Wainberg and in collaboration with the co-authors identified in the title pages for chapters 2 and 3 of this thesis. The originality of this research and my contributions to the scientific community are summarized as follows.

Chapter 2: The development and presence of the M184V mutation within the highly conserved YMDD motif of HIV-1 RT, located close to the polymerase catalytic site has been shown to be associated with multiple alterations of RT enzymatic function (i.e., decreased RT processivity, reduced nucleotide-dependent primer unblocking, increased fidelity, hypersusceptibility to ZDV and TDF, impaired viral fitness, and delayed appearance of mutations in the HIV-1 *pol* gene that are responsible for resistance to certain NRTIs, and possibly, PIs) that may be of therapeutic consequence. However, the potential clinical benefit conferred by maintenance of the M184V mutation has not been adequately investigated in prospective fashion in randomized controlled clinical trials. We have therefore conducted a systematic review of the recent HIV/AIDS clinical trials literature in order to identify and evaluate circumstances in which the presence of the M184V mutation in viral isolates from patients with persistent and/or rebounding plasma HIV-1 viremia was positively correlated with improved virological and/or immunological outcomes in different clinical settings. Taken together with the aforementioned M184V-mediated alterations of RT biochemical

activity, the results of our survey provide a sound scientific rationale for further investigation of the therapeutic maintenance of the M184V mutation in second-line or subsequent regimens in patients with chronic HIV-1 infection in whom this mutation was previously selected by an initial 3TC or other NRTI-containing regimen.

Chapter 3: The M184V substitution in HIV-1 RT is rapidly selected *in vitro* by 3TC and a related nucleoside analogue, FTC, while conferring high-level phenotypic resistance to both of these drugs. However, the relative effectiveness of NRTIs that are structurally-unrelated to 3TC at maintaining the M184V mutation in tissue culture has not been extensively documented. Therefore, we studied the maintenance of the M184V mutation by a diverse set of NRTIs that included 3TC, ABC, ddC, ddI, and the novel nucleoside analogue SPD754 in viral isolates that contained M184V alone or in a background that also included several other resistance-conferring mutations in the HIV-1 RT gene. Our study showed that ddI, unlike 3TC, ABC and SPD754, was unable to select the M184V mutation in a wild-type HIV-1 clonal virus (i.e., HXB2D). Moreover, it was also observed that ddI was not as effective as the other NRTIs tested at preserving the M184V mutation in mixtures of HXB2D and an M184V-clinical isolate in which no other drug resistance mutations were present (i.e., isolate 3350), especially when the latter constituted a minority species in the replicating viral population. However, all of the drugs studied, including ddI, displayed similar capacity to maintain the M184V mutation in mixtures of HXB2D and a multi-NRTI-resistant

clinical isolate (i.e., isolate 4205). In addition, a sub therapeutic level of 3TC (i.e., 0.05 μM) was also able to maintain the M184V mutation during 28 weeks in HIV-1 subtype B (i.e., isolate 3350) and C (i.e., isolate 4742) variants that did not contain any drug resistance mutations other than M184V. Collectively, these findings may be helpful in providing guidance for choice of antiretroviral regimens when planning clinical trials for therapeutic maintenance of the M184V mutation.

Chapter 4: Despite the availability of concordant data from laboratory and clinical studies suggesting that therapeutic maintenance of the M184V mutation might be of clinical benefit, this subject has not been thoroughly investigated in randomized controlled clinical trials with the exception of the COLATE Trial. The COLATE Trial's equivocal outcome has been analyzed in relation to important aspects of the study design and inherent technical limitations that may have biased this trial's overall conclusion. We have made several recommendations to improve future studies regarding M184V-mediated effects on virological and/or immunological responses in NRTI-experienced patients with HIV-1 infection. As discussed herein, these potential improvements may include: investigation of more heavily pre-treated subjects, combined use of drugs such as ZDV or TDF in conjunction with 3TC in second-line or later regimens designed to preserve the M184V mutation, and monitoring changes in the baseline replication capacity of viral isolates from both the 3TC-treated and control study

groups in order to detect the antagonistic effects of compensatory mutations in regard to M184V and/or other non-M184V-mediated alterations of viral fitness.

REFERENCES

Ait-Khaled M., A. Rakik, P. Griffin, A. Cutrell, M.A. Fischl, N. Clumeck, S.B. Greenberg, R. Rubio, B.S. Peters, F. Pulido, J. Gould, G. Pearce, W. Spreen, M. Tisdale, and S. Lafon; CNA3003 International Study Team. 2002a. Mutations in HIV-1 reverse transcriptase during therapy with abacavir, lamivudine and zidovudine in HIV-1-infected adults with no prior antiretroviral therapy. Antivir. Ther. 7: 43-51.

Ait-Khaled M., C. Stone, G. Amphlett, B. Clotet, S. Staszewski, C. Katlama, and M. Tisdale; CNA3002 International Study Team. 2002b. M184V is associated with a low incidence of thymidine analogue mutations and low phenotypic resistance to zidovudine and stavudine. AIDS 16: 1686-1689.

Anderson K.S. 2001. The molecular basis of inhibition and toxicity of modified cytosine analogues targeting HIV-1 reverse transcriptase. Antiviral Chem. Chemother. 12: 13-17.

Anderson K.S. 2002. Perspectives on the molecular mechanism of inhibition and toxicity of nucleoside analogs that target HIV-1 reverse transcriptase. Biochim. Biophys. Acta 1587: 296-299.

Antinori A., G. Liuzzi, A. Cingolani, A. Bertoli, S. Di Giambenedetto, M.P. Trotta, M.G. Rizzo, E. Girardi, A. De Luca, and C.F. Perno. 2001. Drug-resistant mutants of HIV-1 in patients exhibiting increasing CD4 cell count despite virological failure of highly active antiretroviral therapy. AIDS 15: 2325-2327.

Arens M. 2001. Clinically relevant sequence-based genotyping of HBV, HCV, CMV, and HIV. J. Clin. Virol. 22: 11-29.

Arion D., N. Kaushik, S. McCormick, G. Borkow, and M.A. Parniak. 1998. Phenotypic mechanism of HIV-1 resistance to 3'-azido-3'-deoxythymidine (AZT): increased polymerization processivity and enhanced sensitivity to pyrophosphate of the mutant viral reverse transcriptase. Biochemistry 37: 15908-15917.

Bacheler L., S. Jeffrey, G. Hanna, R. D'Aquila, L. Wallace, K. Logue, B. Cordova, K. Hertogs, B. Larder, R. Buckery, D. Baker, K. Gallagher, H. Scarnati, R. Tritch, and C. Rizzo. 2001. Genotypic correlates of phenotypic resistance to efavirenz in virus isolates from patients failing nonnucleoside reverse transcriptase inhibitor therapy. J. Virol. 75: 4999-5008.

Back N.K., and B. Berkhout. 1997. Limiting deoxynucleoside triphosphate concentrations emphasize the processivity defect of lamivudine-resistant variants of human immunodeficiency virus type 1 reverse transcriptase. Antimicrob. Agents Chemother. 41: 2484-2491.

Back N.K., M. Nijhuis, W. Keulen, C.A. Boucher, B.O. Oude Essink, A.B. van Kuilenburg, A.H. van Gennip, and B. Berkhout. 1996. Reduced replication of 3TC-resistant HIV-1 variants in primary cells due to a processivity defect of the reverse transcriptase enzyme. EMBO J. 15: 4040-4049.

Badri S.M., O.M. Adeyemi, B.E. Max, B.M. Zagorski, and D.E. Barker. 2003. How does expert advice impact genotypic resistance testing in clinical practice? Clin. Infect. Dis. 37: 708-713.

Bally F., R. Martinez, S. Peters, P. Sudre, and A. Telenti. 2000. Polymorphism of HIV type 1 gag p7/p1 and p1/p6 cleavage sites: clinical significance and implications for resistance to protease inhibitors. AIDS Res. Hum. Retroviruses 16: 1209-1213.

Balotta C., A. Berlusconi, A. Pan, M. Violin, C. Riva, M.C. Colombo, A. Gori, L. Papagno, S. Corvasce, R. Mazzucchelli, G. Facchi, R. Velleca, G. Saporetti, M. Galli, S. Rusconi, and M. Moroni. 2000. Prevalence of transmitted nucleoside analogue-resistant HIV-1 strains and pre-existing mutations in pol reverse transcriptase and protease region: outcome after treatment in recently infected individuals. Antivir. Ther. 5: 7-14.

Balzarini J., A. Karlsson, M.J. Perez-Perez, M.J. Camarasa, W.G. Tarpley, and E. De Clercq. 1993. Treatment of human immunodeficiency virus type 1 (HIV-1)-infected cells with combinations of HIV-1-specific inhibitors results in different resistance pattern than does treatment with single-drug therapy. J. Virol. 67: 5353-5359.

Bates M., T. Wrin, W. Huang, C. Petropoulos, and N. Hellmann. 2003. Practical applications of viral fitness in clinical practice. Curr. Opin. Infect. Dis. 16: 11-18.

Baxter J.D., D.L. Mayers, D.N. Wentworth, J.D. Neaton, M.L. Hoover, M.A. Winters, S.B. Mannheimer, M.A. Thompson, D.I. Abrams, B.J. Brizz, J.P. Ioannidis, and T.C. Merigan. 2000. A randomized study of antiretroviral management based on plasma genotypic antiretroviral resistance testing in patients failing therapy. CPCRA 046 Study Team for the Terry Beirn Community Programs for Clinical Research on AIDS. AIDS 14: F83-F93.

Bean P. 2002. The multiple benefits associated with HIV drug-resistance mutation M184V. Am. Clin. Lab. 21: 16-18.

Berkhout B. 1999. HIV-1 evolution under pressure of protease inhibitors: climbing the stairs of viral fitness. J. Biomed. Sci. 6: 298-305.

Bhat T.N., E.T. Baldwin, B. Liu, Y.S. Cheng, and J.W. Erickson. 1994. Crystal structure of a tethered dimer of HIV-1 proteinase complexed with an inhibitor. Nat. Struct. Biol. 1: 552-556.

Bocket L., Y. Yazdanpanah, F. Ajana, Y. Gerard, N. Viget, A. Goffard, I. Alcarez, P. Wattre, and Y. Mouton. 2003. Thymidine analogue mutations emergence in antiretroviral-naive patients on triple therapy including either zidovudine or stavudine. Antivir. Ther. 8: S154.

Bongiovanni M., T. Bini, F. Adorni, P. Meraviglia, A. Capetti, F. Tordato, P. Cicconi, E. Chiesa, L. Cordier, A. Cargnel, S. Landonio, S. Rusconi, and A. d'Arminio Monforte. 2003. Virological success of lopinavir/ritonavir salvage regimen is affected by an increasing number of lopinavir/ritonavir-related mutations. Antivir. Ther. 8: 209-214.

Boucher C.A., N. Cammack, P. Schipper, R. Schuurman, P. Rouse, M.A. Wainberg, and J.M. Cameron. 1993. High-level resistance to (-) enantiomeric 2'-deoxy-3'-thiacytidine in vitro is due to one amino acid substitution in the catalytic site of human immunodeficiency virus type 1 reverse transcriptase. Antimicrob. Agents Chemother. 37: 2231-2234.

Boyer P.L., and S.H. Hughes. 1995. Analysis of mutations at position 184 in reverse transcriptase of human immunodeficiency virus type 1. Antimicrob. Agents Chemother. 39: 1624-1628.

Boyer P.L., S.G. Sarafianos, E. Arnold, and S.H. Hughes. 2001. Selective excision of AZTMP by drug-resistant human immunodeficiency virus reverse transcriptase. J. Virol. 75: 4832-4842.

Boyer P.L., S.G. Sarafianos, E. Arnold, and S.H. Hughes. 2002a. Nucleoside analog resistance caused by insertions in the fingers of human immunodeficiency virus type 1 reverse transcriptase involves ATP-mediated excision. J. Virol. 76: 9143-9151.

Boyer P.L., S.G. Sarafianos, E. Arnold, and S.H. Hughes. 2002b. The M184V mutation reduces the selective excision of zidovudine 5'-monophosphate (AZTMP) by the reverse transcriptase of human immunodeficiency virus type 1. J. Virol. 76: 3248-3256.

Brenner B.G., J.P Routy, M. Petrella, D. Moisi, M. Oliveira, M. Detorio, B. Spira, V. Essabag, B. Conway, R. Lalonde, R.P. Sekaly, and M.A. Wainberg. 2002. Persistence and fitness of multidrug-resistant human ummunodeficiency virus type 1 acquired in primary infection. J. Virol. 76: 1753–1761.

Brenner B.G., M. Oliveira, V. Micheli, D. Moisi, A. Cargnel, M. Petrella, and M.A. Wainberg. 2003. Implications of lamivudine use and the M184V mutation in V118I-related multidrug resistance. Antivir. Ther. 8 : S155.

Brinkman K., J.A. Smeitink, J.A. Romijn, and P. Reiss. 1999. Mitochondrial toxicity induced by nucleoside analogue reverse transcriptase inhibitors is a key factor in the pathogenesis of antiretroviral therapy related lipodystrophy. Lancet 354: 1112-1115.

Bunn P.A. Jr. 2001. Triplet combination chemotherapy and targeted therapy regimens. Oncology (Huntingt.) 15: 26-32.

Burger D.M., R.M. Hoetelmans, P.P. Koopmans, P.L. Meenhorst, J.W. Mulder, Y.A. Hekster, and J.H. Beijnen. 1997. Clinically relevant drug interactions with antiretroviral agents. Antivir. Ther. 2: 149-165.

Byrnes V.W., V.V. Sardana, W.A. Schleif, J.H. Condra, J.A. Waterbury, J.A. Wolfgang, W.J. Long, C.L. Schneider, A.J Schlabach, B.S. Wolanski, et al. 1993. Comprehensive mutant enzyme and viral variant assessment of human immunodeficiency virus type 1 reverse transcriptase resistance to non-nucleoside inhibitors. Antimcirob. Agents Chemother. 37: 1576-1579.

CAESAR Coordinating Committee. 1997. Randomised trial of addition of lamivudine or lamivudine plus loviride to zidovudine-containing regimens for patients with HIV-1 infection: the CAESAR trial. Lancet 349: 1413-1421.

Caliendo A.M., A. Savara, D. An, K. DeVore, J.C. Kaplan, and R.T. D'Aquila. 1996. Effects of zidovudine-selected human immunodeficiency virus type 1 reverse transcriptase amino acid substitutions on processive DNA synthesis and viral replication. J. Virol. 70: 2146-2153.

Cameron D.W., M. Heath-Chiozzi, S. Danner, C. Cohen, S. Kravcik, C. Maurath, E. Sun, D. Henry, R. Rode, A. Potthoff, and J. Leonard. 1998. Randomised placebo-controlled trial of ritonavir in advanced HIV-1 disease. The Advanced HIV Disease Ritonavir Study Group. Lancet 351: 543-549.

Campbell T.B., R.K. Young, S.C. Johnson, E.R. Lanier, and D.R. Kuritzkes. 2003. Antiviral activity of lamivudine in persons infected with HIV-1 that has M184V and multiple thymidine analogue mutations. Antivir. Ther. 8: S156.

Carr A., and D.A. Cooper. 2000. Adverse effects of antiretroviral therapy. Lancet 356: 1423-1430.

Castagna A., A. Danise, E. Carini, E. Boeri, L. Galli, N. Gianotti, A. De Bona, H. Hasson, M. Guffanti, E. Seminari, M. Clementi, and A. Lazzarin. 2004. E-184V. Pilot study to evaluate immunological response to lamivudine monotherapy vs treatment interruption in failing HIV-1 infected subjects, harbouring the M184V mutation. Fifteenth International AIDS Conference, Bangkok, Thailand. Abstract WeOrB1286.

Catucci M., G. Venturi, L. Romano, M.L. Riccio, A. De Milito, P.E. Valensin, and M. Zazzi. 1999. Development and significance of the HIV-1 reverse transcriptase M184V mutation during combination therapy with lamivudine, zidovudine, and protease inhibitors. J. Acquir. Immune Defic. Syndr. 21: 203-208.

Cervia J.S., and M.A. Smith. 2003. Enfuvirtide (T-20): a novel human immunodeficiency virus type 1 fusion inhibitor. Clin. Infect. Dis. 37: 1102-1106.

Chaix C., C. Grenier-Sennelier, P. Clevenbergh, J. Durant, J.M. Schapiro, P. Dellamonica, and I. Durand-Zaleski. 2000. Economic evaluation of drug resistance genotyping for the adaptation of treatment in HIV-infected patients in the VIRADAPT study. J. Acquir. Immune Defic. Syndr. 24: 227-231.

Chesney M.A., J. Ickovics, F.M. Hecht, G. Sikipa, and J. Rabkin. 1999. Adherence: a necessity for successful HIV combination therapy. J. AIDS 13: S271-S278.

Chong K., P. Pagano, and R. Hinshaw. 1994. Bisheteroarylpiperazine reverse transcriptase inhibitor in combination with 3'-azido-3'-deoxythymidine or 2', 3'-dideoxycytidine synergistically inhibits human immunodeficiency virus type 1 replication *in vitro*. Antimicrob. Agents Chemother. 38: 288-293.

Clavel F., and A.J. Hance. 2004. HIV drug resistance. N. Engl. J. Med. 350: 1023-1035.

Clemente J.C., R. Hemrajani, L.E. Blum, M.M. Goodenow, and B.M. Dunn. 2003. Secondary mutations M36I and A71V in the human immunodeficiency virus type 1 protease can provide an advantage for the emergence of the primary mutation D30N. Biochemistry 42: 15029-15035.

Coffin J.M. 1995. HIV population dynamics in vivo: implication for genetic variation, pathogenesis, and therapy. Science 267: 483-489.

Colgrove R. and A. Japour. 1999. A combinatorial ledge: reverse transcriptase fidelity, total body viral burden, and the implications of multiple-drug HIV therapy for the evolution of antiviral resistance. Antiviral Res. 41: 45-56.

Collier A.C., R.W. Coombs, D.A. Schoenfeld, R. Bassett, A. Baruch, and L. Corey. 1996a. Combination therapy with zidovudine, didanosine and saquinavir. Antiviral Res. 29: 99.

Collier A.C., R.W. Coombs, D.A. Schoenfeld, R.L. Bassett, J. Timpone, A. Baruch, M. Jones, K. Facey, C. Whitacre, V.J. McAuliffe, H.M. Friedman, T.C. Merigan, R.C. Reichman, C. Hooper, and L. Corey. 1996b. Treatment of human immunodeficiency virus infection with saquinavir, zidovudine, and zalcitabine. AIDS Clinical Trials Group. N. Engl. J. Med. 334: 1011-1017.

Condra J. 1998. Virologic and clinical implications of resistance to HIV-1 protease inhibitors. Drug Resist. Update. 1: 292-299.

Côté H.C., Z.L. Brumme, K.J. Craib, C.S. Alexander, B. Wynhoven, L. Ting, H. Wong, M. Harris, P.R. Harrigan, M.V. O'Shaughnessy, and J.S. Montaner. 2002. Changes in peripheral blood mitochondrial DNA among HIV-infected patients experiencing nucleoside toxicity. N. Engl. J. Med. 346: 811-820.

Davies J.F. 2nd., Z. Hostomska, Z. Hostomsky, S.R. Jordan, and D.A. Matthews. 1991. Crystal structure of the ribonuclease H domain of HIV-1 reverse transcriptase. Science 252: 88-95.

De Clercq E. 1992. HIV inhibitors targeted at the reverse transcriptase. AIDS Res. Hum. Retroviruses 8: 119-134.

De Jong M.D., R. Schuurman, J.M. Lange, and C.A. Boucher. 1996. Replication of a pre-existing resistant HIV-1 subpopulation in vivo after introduction of a strong selective drug pressure. Antivir. Ther. 1: 33-41.

De Maat M.M., G.C. Ekhart, A.D. Huitema, C.H. Koks, J.W. Mulder, and J.H. Beijnen. 2003. Drug interactions between antiretroviral drugs and comedicated agents. Clin. Pharmacokinet. 42: 223-282.

De Mendoza C., O. Gallego, and V. Soriano. 2002. Mechanisms of resistance to antiretroviral drugs - clinical implications. AIDS Rev. 4: 64-82.

Debouck C. 1992. The HIV-1 protease as a therapeutic target for AIDS. AIDS Res. Hum. Retroviruses 8: 153-164.

Deeks S.G. 1999. Failure of HIV-1 protease inhibitors to fully suppress viral replication. Implications for salvage therapy. Adv. Exp. Med. Biol. 458: 175-182.

Deeks S.G. 2001. International perspectives on antiretroviral resistance. Nonnucleoside reverse transcriptase inhibitor resistance. J. Acquir. Immune Defic. Syndr. 26: S25-S33.

Deeks S.G., J.N. Martin, R. Hoh, T. Wrin, C. Petropoulos, and R.M. Grant. 2003. Continued reverse transcriptase inhibitor therapy is sufficient to maintain short-term partial suppression of multi-drug resistant viremia. Tenth Conference on Retroviruses and Opportunistic Infections, CROI, Boston, MA, USA. Abstract 640.

Deeks S.G., T. Wrin, T. Liegler, R. Hoh, M. Hayden, J.D. Barbour, N.S. Hellmann, C.J. Petropoulos, J.M. McCune, M.K. Hellerstein, and R.M. Grant. 2001. Virologic and immunologic consequences of discontinuing combination antiretroviral-drug therapy in HIV-infected patients with detectable viremia. N. Engl. J. Med. 344: 472-480.

Delaugerre C., M. Mouroux, A. Yvon-Groussin, A. Simon, F. Angleraud, J.M. Huraux, H. Agut, C. Katlama, and V. Calvez. 2001. Prevalence and conditions of selection of E44D/A and V118I human immunodeficiency virus type 1 reverse transcriptase mutations in clinical practice. Antimicrob. Agents Chemother. 45: 946-948.

Demeter L., and R. Haubrich. 2001. International perspectives on antiretroviral resistance. Phenotypic and genotypic resistance assays: methodology, reliability, and interpretations. J. Acquir. Immune Defic. Syndr. 26: S3-S9.

Descamps D., P. Flandre, V. Calvez, G. Peytavin, V. Meiffredy, G. Collin, C. Delaugerre, S. Robert-Delmas, B. Bazin, J.P. Aboulker, G. Pialoux, F. Raffi, and F. Brun-Vezinet. 2000. Mechanisms of virologic failure in previously untreated HIV-infected patients from a trial of induction-maintenance therapy. Trilege (Agence Nationale de Recherches sur le SIDA 072) Study Team. JAMA 283: 205-211.

Descamps D., V. Calvez, G. Collin, A. Cecille, C. Apetrei, F. Damond, C. Katlama, S. Matheron, J.M. Huraux, and F. Brun-Vezinet. 1998. Line probe assay for detection of human immunodeficiency virus type 1 mutations conferring resistance to nucleoside inhibitors of reverse transcriptase: comparison with sequence analysis. J. Clin. Microbiol. 36: 2143-2145.

Deval J., K.L. White, M.D. Miller, N.T. Parkin, J. Courcambeck, P. Halfon, B. Selmi, J. Boretto, and B. Canard. 2004. Mechanistic basis for reduced viral and enzymatic fitness of HIV-1 reverse transcriptase containing both K65R and M184V mutations. J. Biol. Chem. 279: 509-516.

Devereux H.L., V.C. Emery, M.A. Johnson, and C. Loveday. 2001. Replicative fitness in vivo of HIV-1 variants with multiple drug resistance-associated mutations. J. Med. Virol. 65: 218-224.

Di Perri G., and S. Bonora. 2004. Which agents should we use for the treatment of multidrug-resistant Mycobacterium tuberculosis? J. Antimicrob. Chemother. 54: 593-602.

Diallo K., M. Oliveira, D. Moisi, B. Brenner, M.A. Wainberg, and M. Götte. 2002. Pressure of zidovudine accelerates the reversion of lamivudine resistance-conferring M184V mutation in the reverse transcriptase of human immunodeficiency virus type 1. Antimicrob. Agents Chemother. 46: 2254-2256.

Diallo K., B. Brenner, M. Oliveira, D. Moisi, M. Detorio, M. Götte, and M.A. Wainberg. 2003a. The M184V substitution in human immunodeficiency virus type 1 reverse transcriptase delays the development of resistance to amprenavir and efavirenz in subtype B and C clinical isolates. Antimicrob. Agents Chemother. 47: 2376-2379.

Diallo K., B. Marchand, X. Wei, L. Cellai, M. Götte, and M.A. Wainberg. 2003b. Diminished RNA primer usage associated with the L74V and M184V mutations in the reverse transcriptase of human immunodeficiency virus type 1 provides a possible mechanism for diminished viral replication capacity. J. Virol. 77: 8621-8632.

Diallo K., M. Götte, and M.A. Wainberg. 2003c. Molecular impact of the M184V mutation in human immunodeficiency virus type 1 reverse transcriptase. Antimicrob. Agents Chemother. 47: 3377-3383.

Dienstag J.L., E.R. Schiff, T.L. Wright, R.P. Perrillo, H.W. Hann, Z. Goodman, L. Crowther, L.D. Condreay, M. Woessner, M. Rubin, and N.A. Brown. 1999. Lamivudine as initial treatment for chronic hepatitis B in the United States. N. Engl. J. Med. 341: 1256-1263.

Ding J., K. Das, H. Moereels, L. Koymans, K. Andries, P.A. Janssen, S.H. Hughes, and E. Arnold. 1995. Structure of HIV-1 RT/TIBO R 86183 complex reveals similarity in the binding of diverse non-nucleoside inhibitors. Nat. Struct. Biol. 2: 407-415.

Doualla-Bell F., D. Turner, H. Loemba, M. Petrella, B. Brenner, and M.A. Wainberg. 2004. HIV drug resistance and optimization of antiviral treatment in resource-poor countries. Med. Sci. 20: 882-886.

Dragsted U., Z. Fox, L. Mathiesen, C. Katlama, M. Youle, J. Gerstoft, J.N. Bruun, and J.D. Lundgren for the COLATE Trial group. 2004. Final week 48 analysis of a phase 4, randomised, open-label, multi-center trial to evaluate safety and efficacy of continued lamivudine twice daily versus discontinuation of lamivudine in HIV-1-infected adults with virological failure on ongoing combination treatments containing lamivudine: The COLATE Trial. Eleventh Conference on Retroviruses and Opportunistic Infections, CROI, San Francisco, CA, USA. Abstract 549.

Drosopoulos W.C., L.F. Rezende, M.A. Wainberg, and V.R. Prasad. 1998. Virtues of being faithful: can we limit the genetic variation in human immunodeficiency virus? J. Mol. Med. 76: 604-612.

Dueweke T.J., T. Pushkarskaya, S.M. Poppe, S.M. Swaney, J.Q. Zhao, I.S. Chen, M. Stevenson, and W.G. Tarpley. 1993. A mutation in reverse transcriptase of bis (hetroary) piperazine-resistant human immunodeficiency virus type 1 that confers increased sensitivity to other non-nucleoside inhibitors. Proc. Natl. Acad. Sci. USA 90: 4713-4717.

Dunne A.L., F.M. Mitchell, S.K. Coberly, N.S. Hellmann, J. Hoy, A. Mijch, C.J. Petropoulos, J. Mills, and S.M. Crowe. 2001. Comparison of genotyping and phenotyping methods for determining susceptibility of HIV-1 to antiretroviral drugs. AIDS 15: 1471-1475.

Durant J., P. Clevenbergh, P. Halfon, P. Delgiudice, S. Porsin, P. Simonet, N. Montagne, C.A. Boucher, J.M. Schapiro, and P. Dellamonica. 1999. Drug-resistance genotyping in HIV-1 therapy: the VIRADAPT randomised controlled trial. Lancet 353: 2195-2199.

Dybul M., A.S. Fauci, J.G. Bartlett, J.E. Kaplan, and A.K. Pau; Panel on Clinical Practices for Treatment of HIV. 2002. Guidelines for using antiretroviral agents among HIV-infected adults and adolescents. Ann. Intern. Med. 137: 381-433.

Dykes C., K. Fox, A. Lloyd, M. Chiulli, E. Morse, and L.M. Demeter. 2001. Impact of clinical reverse transcriptase sequences on the replication capacity of HIV-1 drug-resistant mutants. Virology 285: 193-203.

Eliopoulos G.M., and R.C. Jr. Moellering. 1982. Antibiotic synergism and antimicrobial combinations in clinical infections. Rev. Infect. Dis. 4: 282-293.

Eron J., R.J. Bosch, L. Petch, S. Fiscus, and I. Frank for the AACTG 307 Protocol Team. 2002. Antiretroviral activity of didanosine in lamivudine-experienced subjects in comparison to activity in subjects who were lamivudine-naive. Antivir. Ther. 7: S102.

Eron J.J. Jr. 1996. The treatment of antiretroviral-naive subjects with the 3TC/zidovudine combination: a review of North American (NUCA 3001) and European (NUCB 3001) trials. AIDS 10: S11-S19.

Eron J.J., S.L. Benoit, J. Jemsek, R.D. MacArthur, J. Santana, J.B. Quinn, D.R. Kuritzkes, M.A. Fallon, and M. Rubin. 1995. Treatment with lamivudine, zidovudine, or both in HIV-positive patients with 200 to 500 CD4+ cells per cubic millimeter. North American HIV Working Party. N. Engl. J. Med. 333: 1662-1669.

Esnouf R., J. Ren, C. Ross, Y. Jones, D. Stammers, and D. Stuart. 1995. Mechanism of inhibition of HIV-1 reverse transcriptase by non-nucleoside inhibitors. Nat. Struct. Biol. 2: 303-308.

Fan W., K.R. Johnson, and M.C. 3rd. Miller. 1998. In vitro evaluation of combination chemotherapy against human tumor cells. Oncol. Rep. 5: 1035-1042.

Feng J.Y., and K.S. Anderson. 1999. Mechanistic studies examining the efficiency and fidelity of DNA synthesis by the 3TC-resistant mutant (184V) of HIV-1 reverse transcriptase. Biochemistry 38: 9440-9448.

Fischer K.P., K.S. Gutfreund, and D.L. Tyrrell. 2001. Lamivudine resistance in hepatitis B: mechanisms and clinical implications. Drug Resist. Update. 4: 118-128.

Fischer M., R. Hafner, C. Schneider, A. Trkola, B. Joos, H. Joller, B. Hirschel, R. Weber, and H.F. Gunthard; Swiss HIV Cohort Study. 2003. HIV RNA in plasma rebounds within days during structured treatment interruptions. AIDS 17: 195-199.

Fischl M.A. 1999. Antiretroviral therapy in 1999 for antiretroviral-naive individuals with HIV infection. AIDS 13: S49-S59.

Fischl M.A., D.D. Richman, M.H. Grieco, M.S. Gottlieb, P.A. Volberding, O.L. Laskin, J.M. Leedom, J.E. Groopman, D. Mildvan, R.T. Schooley, et al. 1987. The efficacy of azidothymidine (AZT) in the treatment of patients with AIDS and AIDS-related complex. A double-blind, placebo-controlled trial. N. Engl. J. Med. 317: 185-191.

Flandre P., D. Descamps, V. Joly, V. Meiffredy, C. Tamalet, J. Izopet, J.P. Aboulker, and F. Brun-Vezinet. 2003. Predictive factors and selection of thymidine analogue mutations by nucleoside reverse transcriptase inhibitors according to initial regimen received. Antivir. Ther. 8: 65-72.

Flandre P., F. Raffi, D. Descamps, V. Calvez, G. Peytavin, V. Meiffredy, M. Harel, S. Hazebrouck, G. Pialoux, J.P. Aboulker, and F. Brun-Vezinet. 2002. Final analysis of the Trilege induction-maintenance trial: results at 18 months. AIDS 16: 561-568.

Fletcher R.S., D. Arion, G. Borkow, M.A. Wainberg, G.I. Dmitrienko, and M.A. Parniak. 1995. Synergistic inhibition of HIV-1 reverse transcriptase DNA polymerase activity and virus replication *in vitro* by combinations of carboxanilide non-nucleoside compounds. Biochemistry 34: 10106-10112.

Frei E. 3rd. 1985. Curative cancer chemotherapy. Cancer Res. 45: 6523-6537.

Frieden T.R., T.R. Sterling, S.S. Munsiff, C.J. Watt, and C. Dye. 2003. Tuberculosis. Lancet 362: 887-899.

Frost S.D., M. Nijhuis, R. Schuurman, C.A. Boucher, and A.J. Brown. 2000. Evolution of lamivudine resistance in human immunodeficiency virus type 1-infected individuals: the relative roles of drift and selection. J. Virol. 74: 6262-6268.

Fung H.B., E.A. Stone, and F.J. Piacenti. 2002. Tenofovir disoproxil fumarate: a nucleotide reverse transcriptase inhibitor for the treatment of HIV infection. Clin. Ther. 24: 1515-1548.

Furman P.A., and D.W. Barry. 1988. Spectrum of antiviral activity and mechanism of action of zidovudine. An overview. Am. J. Med. 85: 176-181.

Furman P.A., G.R. Painter, and K.S. Anderson. 2000. An analysis of the catalytic cycle of HIV-1 reverse transcriptase: opportunities for chemotherapeutic intervention based on enzyme inhibition. Curr. Pharm. Des. 6: 547-567.

Furman P.A., J.A. Fyfe, M.H. St. Clair, K. Weinhold, J.L. Rideout, G.A. Freeman, S.N. Lehrman, D.P. Bolognesi, S. Broder, H. Mitsuya, et al. 1986. Phosphorylation of 3'-azido-3' deoxythymidine and selective interactions of the 5'-triphosphate with human immunodeficiency virus reverse transcriptase. Proc. Natl. Acad. Sci. USA 83: 8333-8337.

Gallant J.E. 2004. HIV counseling, testing, and referral. Am. Fam. Physician 70: 295-302.

Gao H.Q., P.L. Boyer, S.G. Sarafianos, E. Arnold, and S.H. Hughes. 2000. The role of steric hindrance in 3TC resistance of human immunodeficiency virus type-1 reverse transcriptase. J. Mol. Biol. 300: 403-418.

Gao Q., Z. Gu, M.A. Parniak, J. Cameron, N. Cammack, C. Boucher, and M.A. Wainberg. 1993. The same mutation that encodes low-level human immunodeficiency virus type 1 resistance to 2',3'-dideoxyinosine and 2',3'-dideoxycytidine confers high-level resistance to the (-) enantiomer of 2',3'-dideoxy-3'-thiacytidine. Antimicrob. Agents Chemother. 37: 1390-1392.

Gartland M., and The AVANTI Study Group. 2001. AVANTI 3: a randomized, double-blind trial to compare the efficacy and safety of lamivudine plus zidovudine versus lamivudine plus zidovudine plus nelfinavir in HIV-1-infected antiretroviral-naive patients. Antivir. Ther. 6: 127-134.

Gatanaga H., Y. Suzuki, H. Tsang, K. Yoshimura, M.F. Kavlick, K. Nagashima, R.J. Gorelick, S. Mardy, C. Tang, M.F. Summers, and H. Mitsuya. 2002. Amino acid substitutions in Gag protein at non-cleavage sites are indispensable for the development of a high multitude of HIV-1 resistance against protease inhibitors. J. Biol. Chem. 277: 5952-5961.

Gazzard B.G., A. Pozniak, M. Peeters, R. Hoetelmans, and N.M. Graham. 2002. Influence of the M184V mutation on virologic outcome of highly active antiretroviral therapy with or without ddI. Fourteenth International AIDS Conference, Barcelona, Spain. Abstract MoPeB3144.

Ghosn J., J.P. Viard, C. Katlama, M. de Almeida, R. Tubiana, F. Letourneur, L. Aaron, C. Goujard, D. Salmon, M. Leruez-Ville, C. Rouzioux, M.L. Chaix. 2004. Evidence of genotypic resistance diversity of archived and circulating viral strains in blood and semen of pre-treated HIV-infected men. AIDS 18: 447-457.

Goody R.S., B. Muller, and T. Restle. 1991. Factors contributing to the inhibition of HIV reverse transcriptase by chain-terminating nucleotides in vitro and in vivo. FEBS Lett. 291: 1-5.

Götte M., and M.A. Wainberg. 2000. Biochemical mechanisms involved in overcoming HIV resistance to nucleoside inhibitors of reverse transcriptase. Drug Resist. Update. 3: 30-38.

Götte M., D. Arion, M.A. Parniak, and M.A. Wainberg. 2000. The M184V mutation in the reverse transcriptase of human immunodeficiency virus type 1 impairs rescue of chain-terminated DNA synthesis. J. Virol. 74: 3579-3585.

Gottfredsson M., and P.R. Bohjanen. 1997. Human immunodeficiency virus type I as a target for gene therapy. Front. Biosci. 2: 619-634.

Gray N.M., C.L. Marr, C.R. Penn, J.M. Cameron, and R.C. Bethell. 1995. The intracellular phosphorylation of (-)-2'-deoxy-3'-thiacytidine (3TC) and the incorporation of 3TC 5'-monophosphate into DNA by HIV-1 reverse transcriptase and human DNA polymerase gamma. Biochem. Pharmacol. 50: 1043-1051.

Gu Z., Q. Gao, X. Li, M.A. Parniak, and M.A. Wainberg. 1992. Novel mutation in the human immunodeficiency virus type 1 reverse transcriptase gene that encodes cross-resistance to 2',3'-dideoxyinosine and 2',3'-dideoxycytidine. J. Virol. 66: 7128-7135.

Gu Z., Y. Quan, Z. Li, E.J. Arts, and M.A. Wainberg. 1995. Effects of non-nucleosides inhibitors of human immunodeficiency virus type 1 in cell-free recombinant reverse transcriptase assay. J. Biol. Chem. 270: 31046-31051.

Gulick R.M., J.W. Mellors, D. Havlir, J.J. Eron, C. Gonzalez, D. McMahon, D.D. Richman, F.T. Valentine, L. Jonas, A. Meibohm, E.A. Emini, and J.A. Chodakewitz. 1997. Treatment with indinavir, zidovudine, and lamivudine in adults with human immunodeficiency virus infection and prior antiretroviral therapy. N. Engl. J. Med. 337: 734-739.

Hadziyannis S.J., N.C. Tassopoulos, E.J. Heathcote, T.T. Chang, G. Kitis, M. Rizzetto, P. Marcellin, S.G. Lim, Z. Goodman, M.S. Wulfsohn, S. Xiong, J. Fry, and C.L. Brosgart; Adefovir Dipivoxil 438 Study Group. 2003. Adefovir dipivoxil for the treatment of hepatitis B e antigen-negative chronic hepatitis B. N. Engl. J. Med. 348: 800-807.

Halfon P., J. Durant, P. Clevenbergh, H. Carsenti, L. Celis, H. Khiri, K. De Smet, A. De Brauwer, F. Hulstaert, and P. Dellamonica. 2003. Kinetics of disappearance of resistance mutations and reappearance of wild-type during structured treatment interruptions. AIDS 17: 1351-1361.

Hamers F.F., and A.M. Downs. 2004. The changing face of the HIV epidemic in western Europe: what are the implications for public health policies? Lancet 364: 83-94.

Hanna G.J., and R.T. D'Aquila. 2001. Clinical use of genotypic and phenotypic drug resistance testing to monitor antiretroviral chemotherapy. Clin. Infect. Dis. 32: 774-782.

Harrigan P.R., C. Stone, P. Griffin, I. Najera, S. Bloor, S. Kemp, M. Tisdale, and B. Larder. 2000. Resistance profile of the human immunodeficiency virus type 1 reverse transcriptase inhibitor abacavir (1592U89) after monotherapy and combination therapy. CNA2001 Investigative Group. J. Infect. Dis. 181: 912-920.

Harrigan P.R., S. Bloor, and B.A. Larder. 1998. Relative Replicative Fitness of Zidovudine-Resistant Human Immunodeficiency Virus Type 1 Isolates In Vitro. J. Virol. 72: 3773–3778.

Hart G.J., D.C. Orr, C.R. Penn, H.T. Figueiredo, N.M. Gray, R.E. Boehme, and J.M. Cameron. 1992. Effects of (-) 2'-deoxy-3'-thiacytidine (3TC) 5'-triphosphate on human immunodeficiency virus reverse transcriptase and mammalian DNA polymerases alpha, beta and gamma. Antimicrob. Agents Chemother. 36: 1688-1694.

Havlir D.V., C. Tierney, G.H. Friedland, R.B. Pollard, L. Smeaton, J.-P. Sommadossi, L. Fox, H. Kessler, K.H. Fife, and D.D. Richman. 2000. In vivo antagonism with zidovudine plus stavudine combination therapy. J. Infect. Dis. 182: 321-325.

Hertogs K., S. Bloor, V. De Vroey, C. van Den Eynde, P. Dehertogh, A. van Cauwenberge, M. Sturmer, T. Alcorn, S. Wegner, M. van Houtte, V. Miller, and B.A. Larder. 2000. A novel human immunodeficiency virus type 1 reverse transcriptase mutational pattern confers phenotypic lamivudine resistance in the absence of mutation 184V. Antimicrob. Agents Chemother. 44: 568-573.

Ho H.-T., and M.J.M. Hitchcock. 1989. Cellular pharmacology of 2', 3'-dideoxy-2', 3'-didehydrothymidine, a nucleoside analog active against human immunodeficiency virus. Antimicrob. Agents Chemother. 33: 844-849.

Hogg R.S., K.V. Heath, B. Yip, K.J. Craib, M.V. O'Shaughnessy, M.T. Schechter, and J.S. Montaner. 1998. Improved survival among HIV-infected individuals following initiation of antiretroviral therapy. JAMA 279: 450-454.

Hoggard P.G., J. Lloyd, S.H. Khoo, M.G. Barry, L. Dann, S.E. Gibbons, E.G. Wilkins, C. Loveday, and D.J. Back. 2001. Zidovudine phosphorylation determined sequentially over 12 months in human immunodeficiency virus-infected patients with or without previous exposure to antiretroviral agents. Antimicrob. Agents Chemother. 45: 976-980.

Hoggard P.G., S. Kewn, M.G. Barry, S.H. Khoo, and D.J. Back. 1997. Effects of drugs on 2', 3'-dideoxy-2', 3'-didehydrothymidine phosphorylation in vitro. Antimicrob. Agents Chemother. 41: 1231-1236.

Hortobagyi G.N. 1997. Chemotherapy of breast cancer: a historical perspective. Semin. Oncol. 24: S17-1-S17-4.

Huang H., R. Chopra, G.L. Verdine, and S.C. Harrison. 1998. Structure of a covalently trapped catalytic complex of HIV-1 reverse transcriptase: implications for drug resistance. Science 282: 1669-1675.

Inouye P., E. Cherry, M. Hsu, S. Zolla-Pazner, and M.A. Wainberg. 1998. Neutralizing antibodies directed against the V3 loop select for different escape variants in a virus with mutated reverse transcriptase (M184V) than in wild-type human immunodeficiency virus type 1. AIDS Res. Hum. Retroviruses 14: 735-740.

Isel C., C. Ehresmann, P. Walter, B. Ehresmann, and R. Marquet. 2001. The emergence of different resistance mechanisms toward nucleoside inhibitors is explained by the properties of the wild type HIV-1 reverse transcriptase. J. Biol. Chem. 276: 48725-48732.

Jacobo-Molina A., and E. Arnold. 1991. HIV reverse transcriptase structure-function relationships. Biochemistry 30: 6351-6356.

Jacobo-Molina A., J. Ding, R.G. Nanni, A.D. Clark Jr., X. Lu, C. Tantillo, R.L. Williams, G. Kamer, A.L. Ferris, P. Clark, et al. 1993. Crystal structure of human immunodeficiency virus type 1 reverse transcriptase complexed with double-stranded DNA at 3.0 A resolution shows bent DNA. Proc. Natl. Acad. Sci. USA 90: 6320-6324.

Jain R.G., E.S. Furfine, L. Pedneault, A.J. White, and J.M. Lenhard. 2001. Metabolic complications associated with antiretroviral therapy. Antivir. Res. 51: 151-177.

Johnson V., and R. Byington. 1994. Quantitative assays for virus infectivity, p. 71-76. *In* ACTG virology manual for HIV laboratories. National Institute for Allergy and Infectious Diseases, Bethesda, Md.

Johnson V.A., F. Brun-Vezinet, B. Clotet, B. Conway, R.T. D'Aquila, L.M. Demeter, D.R. Kuritzkes, D. Pillay, J.M. Schapiro, A. Telenti, and D.D. Richman. 2004. Update of the drug resistance mutations in HIV-1: 2004. Top. HIV Med. 12: 119-124.

Johnston M.I., and D.F. Hoth. 1993. Present status and future prospects for HIV therapies. Science 260: 1286-1293.

Jonas M.M., J. Mizerski, I.B. Badia, J.A. Areias, K.B. Schwarz, N.R. Little, M.J. Greensmith, S.D. Gardner, M.S. Bell, and E.M. Sokal; International Pediatric Lamivudine Investigator Group. 2002. Clinical trial of lamivudine in children with chronic hepatitis B. N. Engl. J. Med. 346: 1706-1713.

Jonckheere H., M. Witvrouw, E. De Clercq, and J. Anne. 1998. Lamivudine resistance of HIV type 1 does not delay development of resistance to nonnucleoside HIV type 1-specific reverse transcriptase inhibitors as compared with wild-type HIV type 1. AIDS Res. Hum. Retroviruses 14: 249-253.

Karayiannis P. 2003. Hepatitis B virus: old, new and future approaches to antiviral treatment. J. Antimicrob. Chemother. 51: 761-785.

Katz R.A., and A.M. Skalka. 1994. The retroviral enzymes. Annu. Rev. Biochem. 63: 133-173.

Kavlick M.F., T. Shirasaka, E. Kojima, J.M. Pluda, F. Hui Jr., R. Yarchoan, and H. Mitsuya. 1995. Genotypic and phenotypic characterization of HIV-1 isolated from patients receiving (--)-2', 3'-dideoxy-3'-thiacytidine. Antiviral Res. 28: 133-146.

Keulen W., A. van Wijk, R. Schuurman, B. Berkhout, and C.A. Boucher. 1999. Increased polymerase fidelity of lamivudine-resistant HIV-1 variants does not limit their evolutionary potential. AIDS 13: 1343-1349.

Keulen W., N.K. Back, A. van Wijk, C.A. Boucher, and B. Berkhout. 1997. Initial appearance of the 184Ile variant in lamivudine-treated patients is caused by the mutational bias of human immunodeficiency virus type 1 reverse transcriptase. J. Virol. 71: 3346-3350.

Kijak G.H., V. Simon, P. Balfe, J. Vanderhoeven, S.E. Pampuro, C. Zala, C. Ochoa, P. Cahn, M .Markowitz, and H. Salomon. 2002. Origin of human immunodeficiency virus type 1 quasispecies emerging after antiretroviral treatment interruption in patients with therapeutic failure. J. Virol. 76: 7000-7009.

Kohl N.E., E.A. Emini, W.A. Schleif, L.J. Davis, J.C. Heimbach, R.A. Dixon, E.M. Scolnick, and I.S. Sigal. 1988. Active human immunodeficiency virus protease is required for viral infectivity. Proc. Natl. Acad. Sci. USA 85: 4686-4690.

Kohlstaedt L.A., J. Wang, J.M. Friedman, P.A. Rice, and T.A. Steitz. 1992. Crystal structure at 3.5 A resolution of HIV-1 reverse transcriptase complexed with an inhibitor. Science 256: 1783-1790.

Kosalaraksa P., M.F. Kavlick, V. Maroun, R. Le, and H. Mitsuya. 1999. Comparative fitness of multi-dideoxynucleoside-resistant human immunodeficiency virus type 1 (HIV-1) in an in vitro competitive HIV-1 replication assay. J. Virol. 73: 5356–5363.

Kuritzkes D. 2002. Drug resistance. Navigating resistance pathways. AIDS Read. 12: 395-407.

Kuritzkes D.R. 2004a. Extending antiretroviral therapy to resource-poor settings: implications for drug resistance. AIDS 18: S45-S48.

Kuritzkes D.R. 2004b. Preventing and managing antiretroviral drug resistance. AIDS Patient Care STDS. 18: 259-273.

Kuritzkes D.R., J.B. Quinn, S.L. Benoit, D.L. Shugarts, A. Griffin, M. Bakhtiari, D. Poticha, J.J. Eron, M.A. Fallon, and M. Rubin. 1996. Drug resistance and virologic response in NUCA 3001, a randomized trial of lamivudine (3TC) versus zidovudine (ZDV) versus ZDV plus 3TC in previously untreated patients. AIDS 10: 975-981.

Kuritzkes D.R., R.L. Bassett, R.K. Young, H. Barrett, J.L. Koel, J.D. Hazelwood, and V.A. Johnson for ACTG 306 and 370 Protocol Teams. 2002. Rate of emergence of thymidine analogue resistance mutations in HIV-1 reverse transcriptase selected by stavudine or zidovudine-based regimens in treatment-naïve patients. Antivir. Ther. 7: S31.

Kuritzkes D.R., R.L. Bassett, V.A. Johnson, I.C. Marschner, J.J. Eron, J.P. Sommadossi, E.P. Acosta, R.L. Murphy, K. Fife, K. Wood, D. Bell, A. Martinez, and C.B. Pettinelli. 2000. Continued lamivudine versus delavirdine in combination with indinavir and zidovudine or stavudine in lamivudine-experienced patients: results of Adult AIDS Clinical Trials Group protocol 370. AIDS 14: 1553-1561.

Lalezari J.P., E. DeJesus, D.W. Northfelt, G. Richmond, P. Wolfe, R. Haubrich, D. Henry, W. Powderly, S. Becker, M. Thompson, F. Valentine, D. Wright, M. Carlson, S. Riddler, F.F. Haas, R. DeMasi, P.R. Sista, M. Salgo, and J. Delehanty. 2003a. A controlled Phase II trial assessing three doses of enfuvirtide (T-20) in combination with abacavir, amprenavir, ritonavir and efavirenz in non-nucleoside reverse transcriptase inhibitor-naive HIV-infected adults. Antivir. Ther. 8: 279-287.

Lalezari J.P., J.J. Eron, M. Carlson, C. Cohen, E. DeJesus, R.C. Arduino, J.E. Gallant, P. Volberding, R.L. Murphy, F. Valentine, E.L. Nelson, P.R. Sista, A. Dusek, and J.M. Kilby. 2003b. A phase II clinical study of the long-term safety and antiviral activity of enfuvirtide-based antiretroviral therapy. AIDS 17: 691-698.

Lalezari J.P., K. Henry, M. O'Hearn, J.S. Montaner, P.J. Piliero, B. Trottier, S. Walmsley, C. Cohen, D.R. Kuritzkes, J.J. Jr. Eron, J. Chung, R. DeMasi, L. Donatacci, C. Drobnes, J. Delehanty, and M. Salgo; TORO 1 Study Group. 2003c. Enfuvirtide, an HIV-1 fusion inhibitor, for drug-resistant HIV infection in North and South America. N. Engl. J. Med. 348: 2175-2185.

Lam P.Y., P.K. Jadhav, C.J. Eyermann, C.N. Hodge, Y. Ru, L.T. Bacheler, J.L. Meek, M.J. Otto, M.M. Rayner, Y.N. Wong, et al. 1994. Rational design of potent, bioavailable, nonpeptide cyclic ureas as HIV protease inhibitors. Science 263: 380-384.

Lange J. 2001. A rational approach to the selection and sequencing of nucleoside/nucleotide analogues: a new paradigm. Antivir. Ther. 6: 45-54.

Lange J.M. Triple combinations: present and future. 1995. J. Acquir. Immune Defic. Syndr. Hum. Retrovirol. 10: S77-S82.

Larder B.A. 1995. Viral resistance and the selection of antiretroviral combinations. J. Acquir. Immune Defic. Syndr. Hum. Retrovirol. 10: S28-S33.

Larder B.A., S. Bloor, S.D. Kemp, K. Hertogs, R.L. Desmet, V. Miller, M. Sturmer, S. Staszewski, J. Ren, D.K. Stammers, D.I. Stuart, and R. Pauwels. 1999. A family of insertion mutations between codons 67 and 70 of human immunodeficiency virus type 1 reverse transcriptase confer multinucleoside analog resistance. Antimicrob. Agents Chemother. 43: 1961-1967.

Larder B.A., S.D. Kemp, and P.R. Harrigan. 1995. Potential mechanism for sustained antiretroviral efficacy of AZT-3TC combination therapy. Science 269: 696-699.

Lazzarin A., B. Clotet, D. Cooper, J. Reynes, K. Arasteh, M. Nelson, C. Katlama, H.J. Stellbrink, J.F. Delfraissy, J. Lange, L. Huson, R. DeMasi, C. Wat, J. Delehanty, C. Drobnes, and M. Salgo; TORO 2 Study Group. 2003. Efficacy of enfuvirtide in patients infected with drug-resistant HIV-1 in Europe and Australia. N. Engl. J. Med. 348: 2186-2195.

Lederman M.M. 2001. Immune restoration and CD4+ T-cell function with antiretroviral therapies. AIDS 15: S11-S15.

Lee K., and C. K. Chu. 2001. Molecular modeling approach to understanding the mode of action of L-nucleosides as antiviral agents. Antimicrob. Agents Chemother. 45: 138-44.

Lee K., Y. Chong, and C.K. Chu. 2001. Understanding the mode of action of L-nucleosides as antiviral agents: a molecular modeling approach. Nucleosides Nucleotides Nucleic Acids 20: 385-388.

Lerma J.G., and W. Heneine. 2001. Resistance of human immunodeficiency virus type 1 to reverse transcriptase and protease inhibitors: genotypic and phenotypic testing. J. Clin. Virol. 21: 197-212.

Liaw Y.F. 2001. Impact of YMDD mutations during lamivudine therapy in patients with chronic hepatitis B. Antiviral Chem. Chemother. 12: 67-71.

Liegler T.J., M.S. Hayden, K.H. Lee, R. Hoh, S.G. Deeks, and R.M. Grant. 2001. Protease inhibitor-resistant HIV-1 from patients with preserved CD4 cell counts is cytopathic in activated CD4 T lymphocytes. AIDS 15: 179-184.

Little S.J., S. Holte, J.P. Routy, E.S. Daar, M. Markowitz, A.C. Collier, R.A. Koup, J.W. Mellors, E. Connick, B. Conway, M. Kilby, L. Wang, J.M. Whitcomb, N.S. Hellmann, and D.D. Richman. 2002. Antiretroviral-drug resistance among patients recently infected with HIV. N. Engl. J. Med. 347: 385-394.

Lorenzi P., M. Opravil, B. Hirschel, J.P. Chave, H.J. Furrer, H. Sax, T.V. Perneger, L. Perrin, L. Kaiser, and S. Yerly. 1999. Impact of drug resistance mutations on virologic response to salvage therapy. Swiss HIV Cohort Study. AIDS 13: F17-F21.

Louie M., and M. Markowitz. 2002. Goals and milestones during treatment of HIV-1 infection with antiretroviral therapy: a pathogenesis-based perspective. Antiviral. Res. 55: 15-25.

Louie M., C. Hogan, M. Di Mascio, A. Hurley, V. Simon, J. Rooney, N. Ruiz, S. Brun, E. Sun, A.S. Perelson, D.D. Ho, and M. Markowitz. 2003. Determining the relative efficacy of highly active antiretroviral therapy. J. Infect. Dis. 187: 896-900.

Loveday C. 2001. International perspectives on antiretroviral resistance. Nucleoside reverse transcriptase inhibitor resistance. J. Acquir. Immune Defic. Syndr. 26: S10-S24.

Maguire M., M. Gartland, S. Moore, A. Hill, M. Tisdale, R. Harrigan, and J.P. Kleim. 2000. Absence of zidovudine resistance in antiretroviral-naive patients following zidovudine/lamivudine/protease inhibitor combination therapy: virological evaluation of the AVANTI 2 and AVANTI 3 studies. AIDS 14: 1195-1201.

Maldarelli F., S. Palmer, M. Kearney, J. Falloon, R.T. Davey, A. Powers, S. Vogel, A. Pau, R. Dewar, J.A. Metcalf, J. Mellors, and J. Coffin. 2003. Short duration single drug discontinuation to assess the activity of individual drugs in patients failing antiretroviral therapy. Antivir. Ther. 8: S149.

Marcelin A.G., M. Wirden, C. Delaugerre, P. Viegas, A. Simon, C. Katlama, and V. Calvez. 2002. Selection of thymidine analogue mutations by nucleoside reverse transcriptase inhibitors occurs step by step and through two different pathways. Antivir. Ther. 7: S34.

Marcellin P., T.T. Chang, S.G. Lim, M.J. Tong, W. Sievert, M.L. Shiffman, L. Jeffers, Z. Goodman, M.S. Wulfsohn, S. Xiong, J. Fry, and C.L. Brosgart; Adefovir Dipivoxil 437 Study Group. 2003. Adefovir dipivoxil for the treatment of hepatitis B e antigen-positive chronic hepatitis B. N. Engl. J. Med. 348: 808-816.

Margot N.A., E. Isaacson, I. McGowan, A.K. Cheng, R.T. Schooley, and M.D. Miller. 2002. Genotypic and phenotypic analyses of HIV-1 in antiretroviral-experienced patients treated with tenofovir DF. AIDS 16: 1227-1235.

Markowitz M., M. Conant, A. Hurley, R. Schluger, M. Duran, J. Peterkin, S. Chapman, A. Patick, A. Hendricks, G.J. Yuen, W. Hoskins, N. Clendeninn, and D.D. Ho. 1998. A preliminary evaluation of nelfinavir mesylate, an inhibitor of human immunodeficiency virus (HIV)-1 protease, to treat HIV infection. J. Infect. Dis. 177: 1533-1540.

Martinez-Picado J., K. Morales-Lopetegi, T. Wrin, J.G. Prado, S.D. Frost, C.J. Petropoulos, B. Clotet, and L. Ruiz. 2002. Selection of drug-resistant HIV-1 mutants in response to repeated structured treatment interruptions. AIDS 16: 895-899.

Masquelier B., D. Descamps, I. Carriere, F. Ferchal, G. Collin, M. Denayrolles, A. Ruffault, B. Chanzy, J. Izopet, C. Buffet-Janvresse, M.P. Schmitt, E. Race, H.J. Fleury, J.P. Aboulker, P. Yeni, amd F. Brun-Vezinet. 1999. Zidovudine resensitization and dual HIV-1 resistance to zidovudine and lamivudine in the delta lamivudine roll-over study. Antivir. Ther. 4: 69-77.

Mathez D., P. Bagnarelli, I. Gorin, C. Katlama, G. Pialoux, G. Saimot, P. Tubiana, P. De Truchis, J.P. Chauvin, R. Mills, R. Rode, M. Clementi, and J. Leibowitch. 1997. Reductions in viral load and increases in T lymphocyte numbers in treatment-naive patients with advanced HIV-1 infection treated with ritonavir, zidovudine and zalcitabine triple therapy. Antivir. Ther. 2: 175-183.

Mayers D. 1996. Rational approaches to resistance: nucleoside analogues. AIDS 10: S9-S13.

Mayers D.L. 1997. Prevalence and incidence of resistance to zidovudine and other antiretroviral drugs. Am. J. Med. 102: 70-75.

Melegari M., P.P. Scaglioni, and J.R. Wands. 1998. Hepatitis B virus mutants associated with 3TC and famciclovir administration are replication defective. Hepatology 27: 628-633.

Menéndez-Arias L. 2002. Molecular basis of fidelity of DNA synthesis and nucleotide specificity of retroviral reverse transcriptases. Prog. Nucleic Acid Res. Mol. Biol. 71: 91-147.

Menzo S., A. Monachetti, C. Balotta, S. Corvasce, S. Rusconi, S. Paolucci, F. Baldanti, P. Bagnarelli, and M. Clementi. 2003. Processivity and drug-dependence of HIV-1 protease: determinants of viral fitness in variants resistant to protease inhibitors. AIDS 17: 663-671.

Merrill D.P., M. Moonis, T.C. Chou, and M.S. Hirsch. 1996. Lamivudine or stavudine in two- and three-drug combinations against human immunodeficiency virus type 1 replication in vitro. J. Infect. Dis. 173: 355-364.

Metzner K.J., S. Bonhoeffer, M. Fischer, R. Karanicolas, K. Allers, B. Joos, R. Weber, B. Hirschel, L.G. Kostrikis, and H.F. Gunthard; The Swiss HIV Cohort Study. 2003. Emergence of minor populations of human immunodeficiency virus type 1 carrying the M184V and L90M mutations in subjects undergoing structured treatment interruptions. J. Infect. Dis. 188: 1433-1443.

Meyer P., I. Pfeifer, S. Matsuura et al. 2000. Effects of M41L and T215Y mutations in HIV-1 reverse transcriptase on removal of chain-terminators from blocked primer/templates. Antivir. Ther. 5: 14.

Meyer P.R., S.E. Matsuura, A.A. Tolun, I. Pfeifer, A.G. So, J.W. Mellors, and W.A. Scott. 2002. Effects of specific zidovudine resistance mutations and substrate structure on nucleotide-dependent primer unblocking by human immunodeficiency virus type 1 reverse transcriptase. Antimicrob. Agents Chemother. 46: 1540-1545.

Meyer P.R., S.E. Matsuura, A.G. So, and W.A. Scott. 1998. Unblocking of chain-terminated primer by HIV-1 reverse transcriptase through a nucleotide-dependent mechanism. Proc. Natl. Acad. Sci. USA 95: 13471-13476.

Meyer P.R., S.E. Matsuura, A.M. Mian, A.G. So, and W.A. Scott. 1999. A mechanism of AZT resistance: an increase in nucleotide-dependent primer unblocking by mutant HIV-1 reverse transcriptase. Mol. Cell 4: 35-43.

Meynard J.L., M. Vray, L. Morand-Joubert, E. Race, D. Descamps, G. Peytavin, S. Matheron, C. Lamotte, S. Guiramand, D. Costagliola, F. Brun-Vezinet, F. Clavel, and P.M. Girard, the Narval Trial Group. 2002. Phenotypic or genotypic resistance testing for choosing antiretroviral therapy after treatment failure: a randomized trial. AIDS 16: 727-736.

Miller M., N. Margot, and B. Lu. 2002a. Effect of baseline nucleoside-associated resistance on response to tenofovir DF (TDF) therapy: integrated analyses of studies 902 and 907. Ninth Conference on Retroviruses and Opportunistic Infections, CROI, Seattle, WA, USA. Abstract 43.

Miller M.D., K.E. Anton, A.S. Mulato, P.D. Lamy, and J.M. Cherrington. 1999. Human immunodeficiency virus type 1 expressing the lamivudine-associated M184V mutation in reverse transcriptase shows increased susceptibility to adefovir and decreased replication capability in vitro. J. Infect. Dis. 179: 92-100.

Miller M.D., K.L. White, C.J. Petropoulos, and N.T. Parkin. 2003. Decreased replication capacity of HIV-1 clinical isolates containing K65R or M184V mutations. Tenth Conference on Retroviruses and Opportunistic Infections, CROI, Boston, MA, USA. Abstract 616.

Miller M.D., L. Zhong, S. Chen, N.A Margot, and M. Wulfsohn. 2002b. Multivariate analysis of antiviral response to tenofovir DF therapy in antiretroviral-experienced patients. Antivir. Ther. 7: S12.

Miller M.D., N.A. Margot, K. Hertogs, B. Larder, and V. Miller. 2001. Antiviral activity of tenofovir (PMPA) against nucleoside-resistant clinical HIV samples. Nucleosides Nucleotides Nucleic Acids 20: 1025-1028.

Miller M.D., P.D. Lamy, M.D. Fuller, A.S. Mulato, N.A. Margot, T. Cihlar, and J.M. Cherrington. 1998. Human immunodeficiency virus type 1 reverse transcriptase expressing the K70E mutation exhibits a decrease in specific activity and processivity. Mol. Pharmacol. 54: 291-297.

Miller V. 2001. International perspectives on antiretroviral resistance. Resistance to protease inhibitors. J. Acquir. Immune Defic. Syndr. 26: S34-S50.

Miller V., and B. A. Larder. 2001. Mutational patterns in the HIV genome and cross-resistance following nucleoside and nucleotide analogue drug exposure. Antivir. Ther. 6: 25-44.

Miller V., M. Ait-Khaled, C. Stone, P. Griffin, D. Mesogiti, A. Cutrell, R. Harrigan, S. Staszewski, C. Katlama, G. Pearce, and M. Tisdale. 2000. HIV-1 reverse transcriptase (RT) genotype and susceptibility to RT inhibitors during abacavir monotherapy and combination therapy. AIDS 14: 163-171.

Miller V., M. Sturmer, S. Staszewski, B. Groschel, K. Hertogs, M.P. de Bethune, R. Pauwels, P.R. Harrigan, S. Bloor, S.D. Kemp, and B.A. Larder. 1998. The M184V mutation in HIV-1 reverse transcriptase (RT) conferring lamivudine resistance does not result in broad cross-resistance to nucleoside analogue RT inhibitors. AIDS 12: 705-712.

Miller V., T. Stark, A.E. Loeliger, and J.M. Lange. 2002. The impact of the M184V substitution in HIV-1 reverse transcriptase on treatment response. HIV Med. 3: 135-145.

Mitsuya H., and S. Broder. 1986. Inhibition of the in vitro infectivity and cytopathic effect of human T-lymphotrophic virus type III/lymphadenopathy-associated virus (HTLV-III/LAV) by 2', 3'-dideoxynucleosides. Proc. Natl. Acad. Sci. USA 83: 1911-1915.

Mitsuya H., R.F. Jarrett, M. Matsukura, F. Di Marzo Veronese, A.L. DeVico, M.G. Sarngadharan, D.G. Johns, M.S. Reitz, and S. Broder. 1987. Long-term inhibition of human T-lymphotropic virus type III/lymphadenopathy-associated virus (human immunodeficiency virus) DNA synthesis and RNA expression in T cells protected by 2', 3'-dideoxynucleosides in vitro. Proc. Natl. Acad. Sci. USA 84: 2033-2037.

Mocroft A., C. Katlama, A.M. Johnson, C. Pradier, F. Antunes, F. Mulcahy, A. Chiesi, A.N. Phillips, O. Kirk, and J.D. Lundgren. 2000. AIDS across Europe, 1994-98: the EuroSIDA study. Lancet 356: 291-296.

Molina J.M., G. Chene, F. Ferchal, V. Journot, I. Pellegrin, M.N. Sombardier, C. Rancinan, L. Cotte, I. Madelaine, T. Debord, and J.M. Decazes. 1999. The ALBI trial: a randomized controlled trial comparing stavudine plus didanosine with zidovudine plus lamivudine and a regimen alternating both combinations in previously untreated patients infected with human immunodeficiency virus. J. Infect. Dis. 180: 351-358.

Molla A., M. Korneyeva, Q. Gao, S. Vasavanonda, P.J. Schipper, H.M. Mo, M. Markowitz, T. Chernyavskiy, P. Niu, N. Lyons, A. Hsu, G.R. Granneman, D.D. Ho, C.A. Boucher, J.M. Leonard, D.W. Norbeck, and D.J. Kempf. 1996. Ordered accumulation of mutations in HIV protease confers resistance to ritonavir. Nat. Med. 2: 760-766.

Montaner J.S., R. DeMasi, and A.M. Hill. 1998. The effects of lamivudine treatment on HIV-1 disease progression are highly correlated with plasma HIV-1 RNA and CD4 cell count. AIDS 12: F23-F28.

Montes B., and M. Segondy. 2002. Prevalence of the mutational pattern E44D/A and/or V118I in the reverse transcriptase (RT) gene of HIV-1 in relation to treatment with nucleoside analogue RT inhibitors. J. Med. Virol. 66: 299-303.

Moore J.P., and M. Stevenson. 2000. New targets for inhibitors of HIV-1 replication. Nature Rev. Mol. Cell Biol. 1: 40-49.

Mouroux M., D. Descamps, J. Izopet, A. Yvon, C. Delaugerre, S. Matheron, A. Coutellier, M.A. Valantin, M. Bonmarchand, H. Agut, P. Massip, D. Costagliola, C. Katlama, F. Brun-Vezinet, and V. Calvez. 2001. Low-rate emergence of thymidine analogue mutations and multi-drug resistance mutations in the HIV-1 reverse transcriptase gene in therapy-naive patients receiving stavudine plus lamivudine combination therapy. Antivir. Ther. 6: 179-183.

Moyle G. 2001. Resistance and cross-resistance to abacavir. HIV Med. 2: 154-162.

Murphy R. 1999. New antiretroviral drugs part I: PIs. AIDS Clin. Care 11: 35-37.

Naeger L.K., N.A. Margot, and M.D. Miller. 2001a. Increased drug susceptibility of HIV-1 reverse transcriptase mutants containing M184V and zidovudine-associated mutations: analysis of enzyme processivity, chain-terminator removal and viral replication. Antivir. Ther. 6: 115-126.

Naeger L.K., N.A. Margot, and M.D. Miller. 2001b. Tenofovir (PMPA) is less susceptible to pyrophosphorolysis and nucleotide-dependent chain-terminator removal than zidovudine or stavudine. Nucleosides Nucleotides Nucleic Acids 20: 635-639.

Naeger L.K., N.A. Margot, and M.D. Miller. 2002. ATP-dependent removal of nucleoside reverse transcriptase inhibitors by human immunodeficiency virus type 1 reverse transcriptase. Antimicrob. Agents Chemother. 46: 2179-2184.

Navia M.A., and B.M. McKeever. 1990. A role for the aspartyl protease from the human immunodeficiency virus type 1 (HIV-1) in the orchestration of virus assembly. Ann. NY Acad. Sci. 616: 73-85.

Navia M.A., P.M. Fitzgerald, B.M. McKeever, C.T. Leu, J.C. Heimbach, W.K. Herber, I.S. Sigal, P.L. Darke, and J.P. Springer. 1989. Three-dimensional structure of aspartyl protease from human immunodeficiency virus HIV-1. Nature 337: 615-620.

Nicastri E., L. Sarmati, G. d'Ettorre, S.G. Parisi, L. Palmisano, C. Galluzzo, M. Montano, I. Uccella, R. Amici, F. Gatti, V. Vullo, E. Concia, S. Vella, and M. Andreoni. 2003. High prevalence of M184 mutation among patients with viroimmunologic discordant responses to highly active antiretroviral therapy and outcomes after change of therapy guided by genotypic analysis. J. Clin. Microbiol. 41: 3007-3012.

Nijhuis M., R. Schuurman, D. de Jong, J. Erickson, E. Gustchina, J. Albert, P. Schipper, S. Gulnik, and C.A. Boucher. 1999. Increased fitness of drug resistant HIV-1 protease as a result of acquisition of compensatory mutations during suboptimal therapy. AIDS 13: 2349-2359.

Nijhuis M., S. Deeks, and C. Boucher. 2001. Implications of antiretroviral resistance on viral fitness. Curr. Opin. Infect. Dis. 14: 23-28.

Nikolenko G.N., S. Palmer, F. Maldarelli, J.W. Mellors, J.M. Coffin, and V.K. Pathak. 2005. Mechanism for nucleoside analog-mediated abrogation of HIV-1 replication: Balance between RNase H activity and nucleotide excision. Proc. Natl. Acad. Sci. USA 102: 2093-2098.

Nunberg J.H., W.A. Schleif, E.J. Boots, J.A. O'Brien, J.C. Quintero, J.M. Hoffman, E.A. Emini, and M.E. Goldman. 1991. Viral resistance to human immunodeficiency virus type 1-specific pyridinone reverse transcriptase inhibitors. J. Virol. 65: 4887-4892.

Oude Essink B.B., N.K. Back, and B. Berkhout. 1997. Increased polymerase fidelity of the 3TC-resistant variants of HIV-1 reverse transcriptase. Nucleic Acids Res. 25: 3212-3217.

Overbaugh J., and C.R. Bangham. 2001. Selection forces and constraints on retroviral sequence variation. Science 292: 1106-1109.

Palella F.J. Jr., K.M. Delaney, A.C. Moorman, M.O. Loveless, J. Fuhrer, G.A. Satten, D.J. Aschman, and S.D. Holmberg. 1998. Declining morbidity and mortality among patients with advanced human immunodeficiency virus infection. N. Engl. J. Med. 338: 853-860.

Papatheodoridis G.V., E. Dimou, and V. Papadimitropoulos. 2002. Nucleoside analogues for chronic hepatitis B: antiviral efficacy and viral resistance. Am. J. Gastroenterol. 97: 1618-1628.

Parkin N.T., N.S. Hellmann, J.M. Whitcomb, L. Kiss, C. Chappey, and C.J. Petropoulos. 2004. Natural variation of drug susceptibility in wild-type human immunodeficiency virus type 1. Antimicrob. Agents Chemother. 48: 437-443.

Patick A.K., M. Duran, Y. Cao, D. Shugarts, M.R. Keller, E. Mazabel, M. Knowles, S. Chapman, D.R. Kuritzkes, and M. Markowitz. 1998. Genotypic and phenotypic characterization of human immunodeficiency virus type 1 variants isolated from patients treated with the protease inhibitor nelfinavir. Antimicrob. Agents Chemother. 42: 2637-2644.

Perry C.M., and D. Faulds. 1997. Lamivudine. A review of its antiviral activity, pharmacokinetic properties and therapeutic efficacy in the management of HIV infection. Drugs 53: 657-680.

Peter K., and J.G. Gambertoglio. 1998. Intracellular phosphorylation of zidovudine (ZDV) and other nucleoside reverse transcriptase inhibitors (RTI) used for human immunodeficiency virus (HIV) infection. Pharm. Res. 15: 819-825.

Petrella M., and M. A. Wainberg. 2002. Might the M184V substitution in HIV-1 RT confer clinical benefit? AIDS Rev. 4: 224-232.

Petrella M., B. Brenner, H. Loemba, and M.A. Wainberg. 2001. HIV drug resistance and implications for the introduction of antiretroviral therapy in resource-poor countries. Drug Resist. Update. 4: 339-346.

Petropoulos C.J., N.T. Parkin, K.L. Limoli, Y.S. Lie, T. Wrin, W. Huang, H. Tian, D. Smith, G.A. Winslow, D.J. Capon, and J.M. Whitcomb. 2000. A novel phenotypic drug susceptibility assay for human immunodeficiency virus type 1. Antimicrob. Agents Chemother. 44: 920-928.

Pialoux G., F. Raffi, F. Brun-Vezinet, V. Meiffredy, P. Flandre, J.A. Gastaut, P. Dellamonica, P. Yeni, J.F. Delfraissy, and J.P. Aboulker. 1998. A randomized trial of three maintenance regimens given after three months of induction therapy with zidovudine, lamivudine, and indinavir in previously untreated HIV-1-infected patients. Trilege (Agence Nationale de Recherches sur le SIDA 072) Study Team. N. Engl. J. Med. 339: 1269-1276.

Picard V., E. Angelini, A. Maillard, E. Race, F. Clavel, G. Chene, F. Ferchal, and J.M. Molina. 2001. Comparison of genotypic and phenotypic resistance patterns of human immunodeficiency virus type 1 isolates from patients treated with stavudine and didanosine or zidovudine and lamivudine. J. Infect. Dis. 184: 781-784.

Picchio G.R., H. Valdez, R. Sabbe, A.L. Landay, D.R. Kuritzkes, M.M. Lederman, and D.E. Mosier. 2000. Altered viral fitness of HIV-1 following failure of protease inhibitor-based therapy. J. Acquir. Immune Defic. Syndr. 25: 289-295.

Pluda J.M., T.P. Cooley, J.S. Montaner, L.E. Shay, N.E. Reinhalter, S.N. Warthan, J. Ruedy, H.M. Hirst, C.A. Vicary, J.B. Quinn, et al. 1995. A phase I/II study of 2'-deoxy-3'-thiacytidine (lamivudine) in patients with advanced human immunodeficiency virus infection. J. Infect. Dis. 171: 1438-1447.

Press N., R. Woods, J. Raboud, P.R. Harrigan, and J.G.S. Montaner for the INCAS AVANTI and CNAAB3005 Study Teams. 2002. The effect of different HAART regimens on slope of viral rebound in HIV-infected patients failing therapy. Ninth Conference on Retroviruses and Opportunistic Infections, CROI, Seattle, WA, USA. Abstract 419-W.

Preston B.D., and J.P. Dougherty. 1996. Mechanisms of retroviral mutation. Trends Microbiol. 4: 16-21.

Quan Y., B.G. Brenner, M. Oliveira, and M.A. Wainberg. 2003. Lamivudine can exert a modest antiviral effect against human immunodeficiency virus type 1 containing the M184V mutation. Antimicrob. Agents Chemother. 47: 747-754.

Quan Y., Z. Gu, X. Li, C. Liang, M.A. Parniak, and M.A. Wainberg. 1998. Endogenous reverse transcriptase assays reveal synergy between combinations of the M184V and other drug resistance-conferring mutations in interactions with nucleoside analog triphosphates. J. Mol. Biol. 277: 237-247.

Quan Y., Z. Gu, X. Li, Z. Li, C.D. Morrow, and M.A. Wainberg. 1996. Endogenous reverse transcription assays reveal high-level resistance to the triphosphate of (-) 2'-dideoxy-3'-thiacytidine by mutated M184V human immunodeficiency virus type 1. J. Virol. 70: 5642-5645.

Quiñones-Mateu M.E., and E.J. Arts. 2002. Fitness of drug resistant HIV-1: methodology and clinical implications. Drug Resist. Update. 5: 224-233.

Quiñones-Mateu M.E., J. Weber, H.R. Rangel, and B. Chakraborty. 2001. HIV-1 fitness and antiretroviral drug resistance. AIDS Rev. 3: 223-242.

Quiñones-Mateu M.E., S.C. Ball, A.J. Marozsan, V.S. Torre, J.L. Albright, G. Vanham, G. Van Der Groen, R.L. Colebunders, and E.J. Arts. 2000. A dual infection/competition assay shows a correlation between ex vivo human immunodeficiency virus type 1 fitness and disease progression. J. Virol. 74: 9222–9233.

Quirós-Roldán E., M. Airoldi, F. Moretti, C. Fausti, A. Pan, S. Casari, C. Torti, F. Castelli, and G. Carosi. 2002. Genotype resistance profiles in patients failing an NNRTI-containing regimen, and modifications after stopping NNRTI therapy. J. Clin. Lab. Anal. 16: 76-78.

Quirós-Roldán E., S. Signorini, F. Castelli, C. Torti, A. Patroni, M. Airoldi, and G. Carosi. 2001. Analysis of HIV-1 mutation patterns in patients failing antiretroviral therapy. J. Clin. Lab. Anal. 15: 43-46.

Ray A.S., Z. Yang, J. Shi, A. Hobbs, R.F. Schinazi, C.K. Chu, and K.S. Anderson. 2002. Insights into the molecular mechanism of inhibition and drug resistance for HIV-1 RT with carbovir triphosphate. Biochemistry 41: 5150-5162.

Rezende L.F., W.C. Drosopoulos, and V.R. Prasad. 1998. The influence of 3TC resistance mutation M184I on the fidelity and error specificity of human immunodeficiency virus type 1 reverse transcriptase. Nucleic Acids Res. 26: 3066-3072.

Richman D., C.K. Shih, I. Lowy, J. Rose, P. Prodanovich, S. Goff, and J. Griffin. 1991. Human immunodeficiency virus type 1 mutants resistant to non-nucleoside inhibitors of reverse transcriptase arise in cell culture. Proc. Natl. Acad. Sci. USA 88: 11241-11245.

Richman D.D. 2001. Antiretroviral activity of emtricitabine, a potent nucleoside reverse transcriptase inhibitor. Antivir. Ther. 6: 83-88.

Richman D.D., S.C. Morton, T. Wrin, N. Hellmann, S. Berry, M.F. Shapiro, and S.A. Bozzette. 2004. The prevalence of antiretroviral drug resistance in the United States. AIDS 18: 1393-1401.

Rizzetto M. 2002. Efficacy of lamivudine in HBeAg-negative chronic hepatitis B. J. Med. Virol. 66: 435-451.

Roberts J.D., K. Bebenek, and T.A. Kunkel. 1988. The accuracy of reverse transcriptase from HIV-1. Science 242: 1171-1173.

Roberts N.A., J.A. Martin, D. Kinchington, A.V. Broadhurst, J.C. Craig, I.B. Duncan, S.A. Galpin, B.K. Handa, J. Kay, A. Krohn, et al. 1990. Rational design of peptide-based HIV proteinase inhibitors. Science 248: 358-361.

Roge B.T., T.L. Katzenstein, N. Obel, H. Nielsen, O. Kirk, C. Pedersen, L. Mathiesen, J. Lundgren, and J. Gerstoft. 2003. K65R with and without S68: a new resistance profile in vivo detected in most patients failing abacavir, didanosine and stavudine. Antivir. Ther. 8: 173-182.

Roman F., D. Gonzalez, C. Lambert, S. Deroo, A. Fischer, T. Baurith, T. Staub, R. Boulme, V. Arendt, F. Schneider, R. Hemmer, and J.C. Schmit. 2003. Uncommon mutations at residue positions critical for enfuvirtide (T-20) resistance in enfuvirtide-naive patients infected with subtype B and non-B HIV-1 strains. J. Acquir. Immune Defic. Syndr. 33: 134-139.

Romano L., G. Venturi, S. Bloor, R. Harrigan, B.A. Larder, J.C. Major, and M. Zazzi. 2002. Broad nucleoside-analogue resistance implications for human immunodeficiency virus type 1 reverse-transcriptase mutations at codons 44 and 118. J. Infect. Dis. 185: 898-904.

Ross L., A. Scarsella, S. Raffanti, K. Henry, S. Becker, R. Fisher, Q. Liao, A. Hirani, N. Graham, M. St. Clair, and J. Hernandez; NZT40012 Study Team. 2001a. Thymidine analog and multinucleoside resistance mutations are associated with decreased phenotypic susceptibility to stavudine in HIV type 1 isolated from zidovudine-naive patients experiencing viremia on stavudine-containing regimens. AIDS Res. Hum. Retroviruses 17: 1107-1115.

Ross L., K. Henry, D. Paar, P. Salvato, M. Shaefer, R. Fisher, Q. Liao, and M. St. Clair. 2001b. Thymidine-analog and multi-nucleoside resistance mutations are observed in both zidovudine-naive and zidovudine-experienced subjects with viremia after treatment with stavudine-containing regimens. J. Hum. Virol. 4: 217-222.

Rousseau M.N., L. Vergne, B. Montes, M. Peeters, J. Reynes, E. Delaporte, and M. Segondy. 2001. Patterns of resistance mutations to antiretroviral drugs in extensively treated HIV-1-infected patients with failure of highly active antiretroviral therapy. J. Acquir. Immune Defic. Syndr. 26: 36-43.

Rusconi S., M.P. De Pasquale, L. Milazzo, S. Kurtagic, E. Bulgheroni, P. Citterio, M. Galazzi, S. La Seta Catamancio, and M. Galli. 1998. Loss of lamivudine resistance in a zidovudine and lamivudine dual-resistant HIV-1 isolate after discontinuation of in vitro lamivudine drug pressure. Antivir. Ther. 3: 203-207.

Rusconi S., S. La Seta Catamancio, F. Sheridan, and D. Parker. 2000. A genotypic analysis of patients receiving zidovudine with lamivudine, didanosine or zalcitabine dual therapy using the LiPA point mutation assay to detect genotypic variation at codons 41, 69, 70, 74, 184 and 215. J. Clin. Virol. 19: 135-142.

Salomon H., A. Belmonte, K. Nguyen, Z. Gu, M. Gelfand, and M.A. Wainberg. 1994. Comparison of cord blood and peripheral blood mononuclear cells as targets for viral isolation and drug sensitivity studies involving human immunodeficiency virus type 1. J. Clin. Microbiol. 32: 2000–2002.

Salomon, H., M.A. Wainberg, B. Brenner, Y. Quan, D. Rouleau, P. Cote, R. LeBlanc, E. Lefebvre, B. Spira, C. Tsoukas, R.P. Sekaly, B. Conway, D. Mayers, and J.P. Routy. 2000. Prevalence of HIV-1 resistant to antiretroviral drugs in 81 individuals newly infected by sexual contact or injecting drug use. Investigators of the Quebec Primary Infection Study. AIDS 14: F17-23.

Sardana V.V., E.A. Emini, L. Gotlib, D.J. Graham, D.W. Lineberger, W.J. Long, A.J. Schlabach, J.A. Wolfgang, and J.H. Condra. 1992. Functional analysis of HIV-1 reverse transcriptase amino acids involved in resistance to multiple non-nucleoside inhibitors. J. Biol. Chem. 267: 17526-17530.

Scarlatti G. 2004. Mother-to-child transmission of HIV-1: advances and controversies of the twentieth centuries. AIDS Rev. 6: 67-78.

Schatz O., J. Mous, and S.F. Le Grice. 1990. HIV-1 RT-associated ribonuclease H displays both endonuclease and 3'----5' exonuclease activity. EMBO J. 9: 1171-1176.

Schinazi R.F., R.M. Lloyd Jr., M.H. Nguyen, D.L. Cannon, A. McMillan, N. Ilksoy, C.K. Chu, D.C. Liotta, H.Z. Bazmi, and J.W. Mellors. 1993. Characterization of human immunodeficiency viruses resistant to oxathiolane-cytosine nucleosides. Antimicrob. Agents Chemother. 37: 875-881.

Schinazi R.F., S. Schlueter-Wirtz, and L. Stuyver. 2001. Early detection of mixed mutations selected by antiretroviral agents in HIV-infected primary human lymphocytes. Antiviral Chem. Chemother. 12: 61-65.

Schmidt B., K. Korn, and H. Walter. 2002. Technologies for measuring HIV-1 drug resistance. HIV Clin. Trials 3: 227-236.

Schneider B., R. Sarfati, D. Deville-Bonne, and M. Veron. 2000. Role of nucleoside diphosphate kinase in the activation of anti-HIV nucleoside analogs. Bioenerg. Biomembr. 32: 317-324.

Schooley R.T., P. Ruane, R.A. Myers, G. Beall, H. Lampiris, D. Berger, S.S. Chen, M.D. Miller, E. Isaacson, and A.K. Cheng; Study 902 Team. 2002. Tenofovir DF in antiretroviral-experienced patients: results from a 48-week, randomized, double-blind study. AIDS 16: 1257-1263.

Schuurman R., M. Nijhuis, R. van Leeuwen, P. Schipper, D. de Jong, P. Collis, S.A. Danner, J. Mulder, C. Loveday, C. Christopherson, et al. 1995. Rapid changes in human immunodeficiency virus type 1 RNA load and appearance of drug-resistant virus populations in persons treated with lamivudine (3TC). J. Infect. Dis. 171: 1411-1419.

Schweighardt B., G.M. Ortiz, R.M. Grant, M. Wellons, G.D. Miralles, L.G. Kostrikis, J.A. Bartlett, and D.F. Nixon. 2002. Emergence of drug-resistant HIV-1 variants in patients undergoing structured treatment interruptions. AIDS 16: 2342-2344.

Servais J., C. Lambert, E. Fontaine, J.M. Plesseria, I. Robert, V. Arendt, T. Staub, F. Schneider, R. Hemmer, G. Burtonboy, and J.C. Schmit. 2001a. Comparison of DNA sequencing and a line probe assay for detection of a human immunodeficiency virus type 1 drug resistance mutations in patients failing highly active antiretroviral therapy. J. Clin. Microbiol. 39: 454-459.

Servais J., J.M. Plesseria, C. Lambert, E. Fontaine, I. Robert, V. Arendt, T. Staub, F. Schneide, R. Hemme, and J.C. Schmit. 2001b. Genotypic correlates of resistance to HIV-1 protease inhibitors on longitudinal data: the role of secondary mutations. Antivir. Ther. 6: 239-248.

Sharma P. L., and C. S. Crumpacker. 1999. Decreased processivity of human immunodeficiency virus type 1 reverse transcriptase (RT) containing didanosine-selected mutation Leu74Val: a comparative analysis of RT variants Leu74Val and lamivudine-selected Met184Val. J. Virol. 73: 8448-8456.

Sheridan F., D. Parker, R. Schuurman, L. De Graaf, J. Tijnagel, and C. Boucher. 1998. Semi-quantitative HIV-1 mutation analysis using the Murex-Innogenetics Lipa HIV-1 RT assay. Twelfth World AIDS Conference, Geneva, Switzerland. Abstract 41225.

Shirasaka T., M.F. Kavlick, T. Ueno, W.Y. Gao, E. Kojima, M.L. Alcaide, S. Chokekijchai, B.M. Roy, E. Arnold, R. Yarchoan, et al. 1995. Emergence of human immunodeficiency virus type 1 variants with resistance to multiple dideoxynucleosides in patients receiving therapy with dideoxynucleosides. Proc. Natl. Acad. Sci. USA 92: 2398-2402.

Shulman N.S., M.D. Hughes, M.A. Winters, R.W. Shafer, A.R. Zolopa, N.S. Hellmann, M. Bates, J.M. Whitcomb, and D.A. Katzenstein. 2002. Subtle decreases in stavudine phenotypic susceptibility predict poor virologic response to stavudine monotherapy in zidovudine-experienced patients. J. Acquir. Immune Defic. Syndr. 31: 121-127.

Siliciano J.D., and R.F. Siliciano. 2004. A long-term latent reservoir for HIV-1: discovery and clinical implications. J. Antimicrob. Chemother. 54: 6-9.

Simon V., N. Padte, D. Murray, J. Vanderhoeven, T. Wrin, N. Parkin, M. Di Mascio, and M. Markowitz. 2003. Infectivity and replication capacity of drug-resistant human immunodeficiency virus type 1 variants isolated during primary infection. J. Virol. 77: 7736-7745.

Smith T.F. 2001. Susceptibility testing. Viral pathogens. Infect. Dis. Clin. North Am. 15: 1263-1294.

Snider D.E. Jr., D.L. Cohn, P.T. Davidson, E.S. Hershfield, M.H. Smith, and F.D. Jr. Sutton. 1985. Standard therapy for tuberculosis 1985. Chest 87: S117-S124.

Soriano V., and C. de Mendoza. 2002. Genetic mechanisms of resistance to NRTI and NNRTI. HIV Clin. Trials 3: 237-248.

Spence R.A., W.M. Kati, K.S. Anderson, and K.A. Johnson. 1995. Mechanism of inhibition of HIV-1 reverse transcriptase by non-nucleoside inhibitors. Science 267: 988-992.

Squires K., G. Pierone, D. Berger, C. Steinhart, N. Bellos, S.L. Becker, S.S. Chen, M.D. Miller, D.F. Coakley, and A. Cheng for the Study 907 Team. 2002. Tenofovir DF: a 48-week final analysis from a phase III randomized, double blind placebo controlled study in antiretroviral experienced patients. Ninth Conference on Retroviruses and Opportunistic Infections, CROI, Seattle, WA, USA. Abstract 413-W.

Squires K.E. 2001. An introduction to nucleoside and nucleotide analogues. Antivir. Ther. 6: 1-14.

Staszewski S. 1995. Zidovudine and lamivudine: results of phase III studies. J. Acquir. Immune Defic. Syndr. Hum. Retrovirol. 10: S57.

Stein D.S., and K.H. Moore. 2001. Phosphorylation of nucleoside analog antiretrovirals: a review for clinicians. Pharmacotherapy 21: 11-34.

Stoddart C.A., T.J. Liegler, F. Mammano, V.D. Linquist-Stepps, M.S. Hayden, S.G. Deeks, R.M. Grant, F. Clavel, and J.M. McCune. 2001. Impaired replication of protease inhibitor-resistant HIV-1 in human thymus. Nature Med. 7: 712-718.

Stuyver L., A. Wyseur, A. Rombout, J. Louwagie, T. Scarcez, C. Verhofstede, D. Rimland, R.F. Schinazi, and R. Rossau. 1997. Line probe assay for rapid detection of drug-selected mutations in the human immunodeficiency virus type 1 reverse transcriptase gene. Antimicrob. Agents Chemother. 41: 284-291.

Sugiura W., Z. Matsuda, Y. Yokomaku, K. Hertogs, B. Larder, T. Oishi, A. Okano, T. Shiino, M. Tatsumi, M. Matsuda, H. Abumi, N. Takata, S. Shirahata, K. Yamada, H. Yoshikura, and Y. Nagai. 2002. Interference between D30N and L90M in selection and development of protease inhibitor-resistant human immunodeficiency virus type 1. Antimicrob. Agents Chemother. 46: 708-715.

Svedhem V., A. Lindkvist, K. Lidman, and A. Sonnerborg. 2002. Persistence of earlier HIV-1 drug resistance mutations at new treatment failure. J. Med. Virol. 68: 473-478.

Tapper M.L., C. Flexner, J.J. Eron, and J.M. Molina. 2004. Simplifying antiretroviral therapy. AIDS Read. 14: 355-371.

Tarrago-Litvak L., M.L. Andreola, G.A. Nevinsky, L. Sarih-Cottin, and S. Litvak. 1994. The reverse transcriptase of HIV-1: from enzymology to therapeutic intervention. FASEB J. 8: 497-503.

The AVANTI Study Group. 2000. AVANTI 2. Randomized, double-blind trial to evaluate the efficacy and safety of zidovudine plus lamivudine versus zidovudine plus lamivudine plus indinavir in HIV-infected antiretroviral-naive patients. AIDS 14: 367-374.

Tisdale M., S.D. Kemp, N.R. Parry, and B.A. Larder. 1993. Rapid *in vitro* selection of human immunodeficiency virus type 1 resistant to 3'-thiacytidine inhibitors due to a mutation in the YMDD region of reverse transcriptase. Proc. Natl. Acad. Sci USA 90: 5653-5656.

Tisdale M., T. Alnadaf, and D. Cousens. 1997. Combination of mutations in human immunodeficiency virus type 1 reverse transcriptase required for resistance to the carbocyclic nucleoside 1592U89. Antimicrob. Agents Chemother. 41: 1094-1098.

Tomaras G.D., and M.L. Greenberg. 2001. Mechanisms for HIV-1 entry: current strategies to interfere with this step. Curr. Infect. Dis. Rep. 3: 93-99.

Torre D., and R. Tambini. 2002. Antiretroviral drug resistance testing in patients with HIV-1 infection: a meta-analysis study. HIV Clin. Trials 3: 1-8.

Tremblay C.L., D.L. Poulin, J.L. Hicks, S. Selliah, A. Chamberland, F. Giguel, C.S. Kollmann, T.C. Chou, H. Dong, and M.S. Hirsch. 2003. Favorable interactions between enfuvirtide and 1-beta-D-2, 6-diaminopurine dioxolane in vitro. Antimicrob. Agents Chemother. 47: 3644-3646.

Tural C., L. Ruiz, C. Holtzer, J. Schapiro, P. Viciana, J. Gonzalez, P. Domingo, C. Boucher, C. Rey-Joly, and B. Clotet; Havana Study Group. 2002. Clinical utility of HIV-1 genotyping and expert advice: the Havana trial. AIDS 6: 209-218.

Turner D., J.M. Schapiro, B.G. Brenner, and M.A. Wainberg. 2004. The influence of protease inhibitor resistance profiles on selection of HIV therapy in treatment-naive patients. Antivir. Ther. 9: 301-314.

Tyndall M.W. 2003. Impact of supervised injection facilities on community HIV levels: a public health perspective. Expert Rev. Anti. Infect. Ther. 1: 543-549.

Ueno T., T. Shirasaka, and H. Mitsuya. 1995. Enzymatic characterization of human immunodeficiency virus type 1 reverse transcriptase resistant to multiple 2', 3'-dideoxynucleoside 5'-triphosphates. J. Biol. Chem. 270: 23605-23611.

UNAIDS/WHO. 2004. AIDS epidemic update. Joint United Nations Programme on HIV/AIDS (UNAIDS) and World health Organization (WHO). Geneva, Switzerland.

Valenti W.M. 2004. Expanding role of conformulations in the treatment of HIV infection: impact of fixed-dose combinations. AIDS Read 14: 541-550.

Van Houtte M., P. Lecocq, and L. Bacheler. 2003. Prevalence and quantitative phenotypic resistance patterns of specific nucleoside analogue mutation combinations and of mutations 44 and 118 in reverse transcriptase in a large dataset of recent HIV-1 clinical isolates. Antivir. Ther. 8: S42.

Van Laethem K., K. Van Vaerenbergh, J.C. Schmit, S. Sprecher, P. Hermans, V. De Vroey, R. Schuurman, T. Harrer, M. Witvrouw, E. Van Wijngaerden, L. Stuyver, M. Van Ranst, J. Desmyter, E. De Clercq, and A.M. Vandamme. 1999. Phenotypic assays and sequencing are less sensitive than point mutation assays for detection of resistance in mixed HIV-1 genotypic populations. J. Acquir. Immune Defic. Syndr. Hum. Retrovirol. 22: 107-118.

Van Rompay K.K., T.B. Matthews, J. Higgins, D.R. Canfield, R.P. Tarara, M.A. Wainberg, R.F. Schinazi, N.C. Pedersen, and T.W. North. 2002. Virulence and reduced fitness of simian immunodeficiency virus with the M184V mutation in reverse transcriptase. J. Virol. 76: 6083-6092.

Van Vaerenbergh K., K. Van Laethem, J. Albert, C.A. Boucher, B. Clotet, M. Floridia, J. Gerstoft, B. Hejdeman, C. Nielsen, C. Pannecouque, L. Perrin, M.F. Pirillo, L. Ruiz, J.C. Schmit, F. Schneider, A. Schoolmeester, R. Schuurman, H.J. Stellbrink, L. Stuyver, J. Van Lunzen, B. Van Remoortel, E. Van Wijngaerden, S. Vella, M. Witvrouw, S. Yerly, E. De Clercq, J. Destmyer, and A.M. Vandamme. 2000. Prevalence and characteristics of multinucleoside-resistant human immunodeficiency virus type 1 among European patients receiving combinations of nucleoside analogues. Antimicrob. Agents Chemother. 44: 2109-2117.

Vandamme A.M., Z. Debyser, R. Pauwels, K. De Vreese, P. Goubau, M. Youle, B. Gazzard, P.A. Stoffels, G.F. Cauwenbergh, J. Anne, et al. 1994. Characterization of HIV-1 strains isolated from patients treated with TIBO R82913. AIDS Res. Hum. Retroviruses 10: 39-46.

Villahermosa M.L., G. Contreras, L. Perez-Alvarez, F. Bru, L. Medrano, E. Delgado, C. Colomo, M. Thomson, and R. Najera. 1998. Evaluation of mixtures of wild-type HIV-1 and HIV-1 with resistance point mutations against reverse transcriptase inhibitors. Antivir. Ther. 3: 221-227.

Vittinghoff E., S. Scheer, P. O'Malley, G. Colfax, S.D. Holmberg, and S.P. Buchbinder. 1999. Combination antiretroviral therapy and recent declines in AIDS incidence and mortality. J. Infect. Dis. 179: 717-720.

Wainberg M.A. 1997. Increased fidelity of drug-selected M184V mutated HIV-1 reverse transcriptase as the basis for the effectiveness of 3TC in HIV clinical trials. Leukemia 11: 85-88.

Wainberg M.A. 2004a. HIV-1 subtype distribution and the problem of drug resistance. AIDS 18: S63-S68.

Wainberg M.A. 2004b. The impact of the M184V substitution on drug resistance and viral fitness. Expert Rev. Anti. Infect. Ther. 2: 147-151.

Wainberg M.A., and MA Parniak. 1997. Is HIV resistance to 3TC of clinical benefit? International Antiviral News 5: 3-5.

Wainberg M.A., H. Salomon, Z. Gu, J.S. Montaner, T.P. Cooley, R. McCaffrey, J. Ruedy, H.M. Hirst, N. Cammack, J. Cameron, et al. 1995. Development of HIV-1 resistance to (-) 2'-deoxy-3'-thiacytidine in patients with AIDS or advanced AIDS-related complex. AIDS 9: 351-357.

Wainberg M.A., L. Lewis, H. Salomon, Z. Gu, A. Keller, N. Cammack, J. Goldsmith, J. Church, B. Spira, S. Wheeler, and P. Pizzo. 1996a. Resistance to (-)-2', 3'-dideoxy-3'-thiacytidine (3TC) in HIV-1 isolated from paediatric patients. Antivir. Ther. 1: 98-104.

Wainberg M.A., M. Hsu, Z. Gu, G. Borkow, and M.A. Parniak. 1996b. Effectiveness of 3TC in HIV clinical trials may be due in part to the M184V substitution in 3TC-resistant HIV-1 reverse transcriptase. AIDS 10: S3-S10.

Wainberg M.A., W.C. Drosopoulos, H. Salomon, M. Hsu, G. Borkow, M. Parniak, Z. Gu, Q. Song, J. Manne, S. Islam, G. Castriota, and V.R. Prasad. 1996c. Enhanced fidelity of 3TC-selected mutant HIV-1 reverse transcriptase. Science 271: 1282-1285.

Wainberg M.A., M.D. Miller, Y. Quan, H. Salomon, A.S. Mulato, P.D. Lamy, N.A. Margot, K.E. Anton, and J.M. Cherrington. 1999. In vitro selection and characterization of HIV-1 with reduced susceptibility to PMPA. Antivir. Ther. 4: 87-94.

Walter H., B. Schmidt, M. Werwein, E. Schwingel, and K. Korn. 2002. Prediction of abacavir resistance from genotypic data: impact of zidovudine and lamivudine resistance in vitro and in vivo. Antimicrob. Agents Chemother. 46: 89-94.

Weber J., H.R. Rangel, B. Chakraborty, M.L. Marotta, H. Valdez, K. Fransen, E. Florence, E. Connick, K.Y. Smith, R.L. Colebunders, A. Landay, D.R. Kuritzkes, M.M. Lederman, G. Vanham, and M.E. Quinones-Mateu. 2003. Role of baseline pol genotype in HIV-1 fitness evolution. J. Acquir. Immune Defic. Syndr. 33: 448-460.

Wei X., J.M. Decker, H. Liu, Z. Zhang, R.B. Arani, J.M. Kilby, M.S. Saag, X. Wu, G.M. Shaw, and J.C. Kappes. 2002. Emergence of resistant human immunodeficiency virus type 1 in patients receiving fusion inhibitor (T-20) monotherapy. Antimicrob. Agents Chemother. 46: 1896-1905.

Weinstein M.C., S.J. Goldie, E. Losina, C.J. Cohen, J.D. Baxter, H. Zhang, A.D. Kimmel, and K.A. Freedberg. 2001. Use of genotypic resistance testing to guide HIV therapy: clinical impact and cost-effectiveness. Ann. Intern. Med. 134: 440-450.

Whitehurst N., C. Chappey, C. Petropoulos, N. Parkin, and A. Gamarnik. 2003. Polymorphisms in p1-p6/p6* of HIV type 1 can delay protease autoprocessing and increase drug susceptibility. AIDS Res. Hum. Retroviruses 19: 779-784.

Whitney J.B., M. Oliveira, M. Detorio, Y. Guan, and M.A. Wainberg. 2002. The M184V mutation in reverse transcriptase can delay reversion of attenuated variants of simian immunodeficiency virus. J. Virol. 76: 8958-8962.

Winston A., S. Mandalia, D. Pillay, B. Gazzard, and A. Pozniak. 2002. The prevalence and determinants of the K65R mutation in HIV-1 reverse transcriptase in tenofovir-naive patients. AIDS 16: 2087-2089.

Winters M.A., J.D. Baxter, D.L. Mayers, D.N. Wentworth, M.L. Hoover, J.D. Neaton, and T.C. Merigan. 2000. Frequency of antiretroviral drug resistance mutations in HIV-1 strains from patients failing triple drug regimens. The Terry Beirn Community Programs for Clinical Research on AIDS. Antivir. Ther. 5: 57-63.

Winters M.A., R.J. Bosch, M.A. Albrecht, and D.A. Katzenstein for the ACTG 364 Study Team. 2002. Clinical impact of the M184V mutation on switching to didanosine or maintaining lamivudine treatment in nucleoside-experienced patients. Antivir. Ther. 7: S101.

Winters M.A., R.J. Bosch, M.A. Albrecht, and D.A. Katzenstein; AIDS Clinical Trials Group 364 Study Team. 2003. Clinical impact of the M184V mutation on switching to didanosine or maintaining lamivudine treatment in nucleoside reverse-transcriptase inhibitor-experienced patients. J. Infect. Dis. 188: 537-540.

Winters M.A., R.W. Shafer, R.A. Jellinger, G. Mamtora, T. Gingeras, and T.C. Merigan. 1997. Human immunodeficiency virus type 1 reverse transcriptase genotype and drug susceptibility changes in infected individuals receiving dideoxyinosine monotherapy for 1 to 2 years. Antimicrob. Agents Chemother. 41: 757-762.

Wlodawer A. 2002. Rational approach to AIDS drug design through structural biology. Annu. Rev. Med. 53: 595-614.

Wlodawer A., and J. Vondrasek. 1998. Inhibitors of HIV-1 protease: a major success of structure-assisted drug design. Annu. Rev. Biophys. Biomol. Struct. 27: 249-284.

Wu J.C., T.C. Warren, J. Adams, J. Proudfoot, J. Skiles, P. Raghavan, C. Perry, I. Potocki, P.R. Farina, and P.M. Grob. 1991. A novel dipyridodiazepinone inhibitor of HIV-1 reverse transcriptase acts through a nonsubstrate binding site. Biochemistry 30: 2022-2026.

Yeni P.G., S.M. Hammer, C.C. Carpenter, D.A. Cooper, M.A. Fischl, J.M. Gatell, B.G. Gazzard, M.S. Hirsch, D.M. Jacobsen, D.A. Katzenstein, J.S. Montaner, D.D. Richman, M.S. Saag, M. Schechter, R.T. Schooley, M.A. Thompson, S. Vella, and P.A.Volberding. 2002. Antiretroviral treatment for adult HIV infection in 2002: updated recommendations of the International AIDS Society-USA Panel. JAMA 288: 222-235.

Yeni P.G., S.M. Hammer, M.S. Hirsch, M.S. Saag, M. Schechter, C.C. Carpenter, M.A. Fischl, J.M. Gatell, B.G. Gazzard, D.M. Jacobsen, D.A. Katzenstein, J.S. Montaner, D.D. Richman, R.T. Schooley, M.A. Thompson, S. Vella, and P.A. Volberding. 2004. Treatment for adult HIV infection: 2004 recommendations of the International AIDS Society-USA Panel. JAMA 292: 251-265.

Youree B.E., and R.T. D'Aquila. 2002. Antiretroviral resistance testing for clinical management. AIDS Rev. 4: 3-12.

Yung L., and D. Linch. 2003. Hodgkin's lymphoma. Lancet 361: 943-951.

Zaccarelli M., C.F. Perno, F. Forbici, A. Cingolani, G. Liuzzi, A. Bertoli, M.P. Trotta, M. C. Bellocchi, S. Di Giambenedetto, V. Tozzi, C. Gori, R. D'Arrigo, P. De Longis, P. Noto, E. Girardi, A. De Luca, and A. Antinori. 2003. Using a database of HIV patients undergoing genotypic resistance test after HAART failure to understand the dynamics of M184V mutation. Antivir. Ther. 8: 51-56.

Zhang H., Y. Zhou, C. Alcock, T. Kiefer, D. Monie, J. Siliciano, Q. Li, P. Pham, J. Cofrancesco, D. Persaud, and R.F. Siliciano. 2004. Novel single-cell-level phenotypic assay for residual drug susceptibility and reduced replication capacity of drug-resistant human immunodeficiency virus type 1. J. Virol. 78: 1718-1729.

Zhang Y.M., H. Imamichi, T. Imamichi, H.C. Lane, J. Falloon, M.B. Vasudevachari, and N.P. Salzman. 1997. Drug resistance during indinavir therapy is caused by mutations in the protease gene and in its Gag substrate cleavage sites. J. Virol. 71: 6662-6670.