



Señor

SUPERINTENDENTE DE INDUSTRIA Y COMERCIO

E. S. D.

REF: Expediente 11-114-720

TÍTULO SOLICITADO: TABLETAS PARA TERAPIA DE COMBINACIÓN.

PUBLICACIÓN: Gaceta 640 de 15/03/2012 número de publicación 187.

SOLICITANTE: GILEAD SCIENCES, INC.

APODERADO: ALICIA LLORED A RICAURTE.

TRÁMITE: SUSTENTACION DE OPOSICIÓN

JOSÉ LUIS REYES VILLAMIZAR, mayor de edad, identificado como aparece al pie de mi firma, abogado en ejercicio, titular de la tarjeta profesional número 44655 del Consejo Superior de la Judicatura, obrando en calidad de apoderado de la sociedad **TECNOQUIMICAS S.A.**, en ejercicio de lo dispuesto por el artículo 42 de la Decisión 486 de 2000, muy respetuosamente me permito formular la siguiente

I. PETICIÓN

Sírvase negar el beneficio patentario a la solicitud contenida en el expediente **11-114-720**, por no cumplir con lo dispuesto en la Decisión 486 de 2000, con base en los fundamentos que se expondrán a continuación.

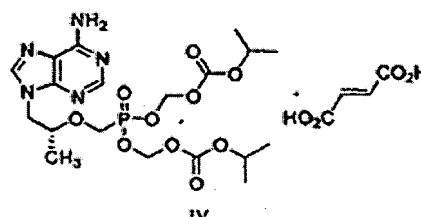
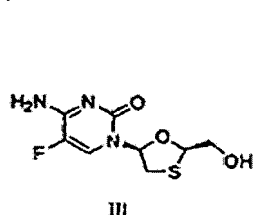
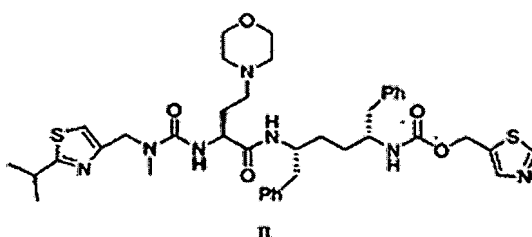
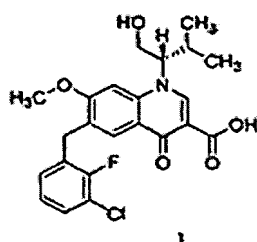
II. LEGÍTIMO INTERÉS

Asiste a **TECNOQUIMICAS S.A.**, legítimo interés para presentar esta oposición, pues la eventual concesión de la solicitud restringiría injustificadamente el empleo

de ciertas sustancias, derivados y procesos que a nuestro juicio carecen de nivel inventivo, en detrimento de sus legítimos intereses comerciales. De igual manera mis clientes están interesados, por su misma condición de industrias farmacéuticas, en evitar la concesión de privilegios de patente que incumplan los requisitos materiales de patentabilidad establecidos en las normas vigentes, y que supongan un riesgo para la libre competencia.

III. RATIFICACIÓN.

La solicitud en curso, que se refiere a formas de dosificación s+olidas (p.e., tabletas) que comprenden un compuesto de Formula I, un compuesto de Formula II, un compuesto de Formula III y una sal de Formula IV



Carece de los requisitos fundamentales para otorgársele el privilegio de patente de invención, particularmente en materia de Novedad y Nivel Inventivo.

IV. COMPLEMENTO DE INFORMACIÓN.

En primer lugar, y teniendo en cuenta que en la reivindicación 1 se hace mención de una combinación farmacológica entre los compuestos de fórmula I, II, III y IV respectivos, es relevante mencionar el concepto de la Doctora en Bioquímica

Silvia Susana Giarcovich¹ en su opinión escrita a cerca de "Asociaciones y Patentes" en donde afirma que las asociaciones farmacológicas están restringidas por ciertos principios y lineamientos preestablecidos por varias entidades, puesto que dichas combinaciones deben estar bajo el precepto de eficacia, seguridad y eficiencia terapéutica, veamos:

"3.3.2. Lineamientos FDA:

A continuación se mencionarán lineamientos que la agencia sanitaria de EEUU ha establecido para el caso de los llamados "*Combination Drug Products*":

3.3.2.1. CFR

El CFR establece la política en relación a las combinaciones de drogas a dosis fija para humanos en 21CFR300.50. Allí se establece que dos o más drogas pueden ser combinadas en una única unidad posológica cuando cada componente hace una contribución al efecto propuesto y la dosis de cada componente es tal que la combinación es segura y efectiva (se consideran casos especiales cuando se busca aumentar la seguridad y eficacia del principal componente o minimizar su potencial abuso).

3.3.2.2. Guía "OTC Combination of Drug Products" [Docket No. 78D-0322]²:

Esta guía establece en detalle la política de la agencia para combinar dos o más ingredientes activos OTC, seguros y eficaces y caracteriza diferentes tipos de asociaciones de OTC:

- Ingredientes activos de diferentes categorías terapéuticas para tratar distintos síntomas simultáneamente: Sólo se acepta esta asociación si cada ingrediente está presente dentro de su rango de seguridad y eficacia.
- Ingredientes activos de la misma categoría terapéutica que tienen distintos mecanismos de acción para tratar los mismos síntomas o condición: Sólo se acepta esta asociación si es mejor o igual que cada ingrediente solo en su dosis terapéutica y también pueden usarse a dosis subterapéuticas si corresponde.
- Ingredientes activos de la misma categoría terapéutica que tienen el mismo mecanismo de acción: Estos no deberían ser combinados a menos que haya alguna ventaja con respecto a los ingredientes simples (mayor eficacia, seguridad, aceptación del paciente o calidad de la formulación).

¹ Profesora Regular Adjunta del Departamento de Farmacología de la Facultad de Farmacia y Bioquímica de la UBA y Directora Técnica y Gerente de Estudios Clínicos de DIFFUCAP-EURAND SACIFI

² Anunciada en el Federal Register (1978), Vol. 43, No. 229.

- Ingredientes considerados adyuvantes, que aumenten o alteren el efecto de otro ingrediente activo, serán, considerados ingredientes activos.

3.3.2.3. Guía Draft "Current GMP for Combination Products":

Este documento provee una guía para la industria y para el *staff* de la FDA acerca de la aplicabilidad de la cGMP para las asociaciones (*combination products*) y define a un *combination product*, de acuerdo al CFR (21CFR3.2(e)), de forma que no sólo involucra drogas sino *devices*.

Un producto combinado es un producto compuesto de cualquier combinación de una droga y un dispositivo; un producto biológico y un dispositivo; una droga y un producto biológico; o una droga, dispositivo, y un producto biológico. Bajo 21 CFR 3.2 (e), un producto de combinación incluye:

- 1. Un producto que comprende dos o más componentes regulados (i.e., droga/dispositivo, biológico/dispositivo, droga/biológico, o droga/dispositivo/biológico) que son combinados físicamente, químicamente, o de otra forma, o mezclados y producidos como una entidad única;*
- 2. Dos o más productos separados, empaquetados juntos en un solo paquete, o como una unidad comprendiendo droga y productos del dispositivo, dispositivo y productos biológicos, o biológico y productos de droga;*
- 3. Una droga, dispositivo, o producto biológico empaquetado separadamente que, de acuerdo a su plan de investigación o el etiquetado propuesto, es destinado para utilizarse únicamente con una droga, dispositivo, o producto biológico aprobada especificada individualmente, dónde al mismo tiempo ambos son deben estar aceptados para la indicación o efecto y donde, luego de la aprobación del producto propuesto, el etiquetado del producto aprobado necesitaría ser cambiado (e.g., para reflejar un cambio en la indicación, forma farmacéutica, dosis, ruta de administración, cambio significativo en la posología); ó*
- 4. Cualquier droga en investigación, dispositivo, o producto biológico empaquetado separadamente que, de acuerdo al etiquetado propuesto es para utilizarse únicamente con otra droga en investigación especificada, dispositivo, o producto biológico, dónde a la vez se requiere que ambos sean aceptados para la misma indicación, uso y efecto.*

Además, dentro de esta guía es muy interesante el concepto de "parte constitutiva de un *combination product*" porque especifica su identidad regulatoria como droga, *device* o producto biológico.

3.3.2.4. Guía Draft "Combination Products Timeliness of Premarket Reviews":

Esta guía provee información a un postulante a reclamar la resolución de una disputa acerca de la duración de la revisión de una postulación referida a un producto combinado. Es una guía referida simplemente a un problema administrativo que demuestra cuán regulado se halla este ámbito que tiene una oficina destinada, la Office of Combination Products.

3.3.3. Lineamientos EMEA: Guía "Fixed Combination Medicinal Products":

Esta guía propone evaluar las potenciales ventajas de la asociación en cuanto a mejoramiento de la relación riesgo/beneficio y a simplificación de la terapia. Asimismo, establece criterios a respetar y requisitos a cumplimentar, tanto generales como particulares. Dentro de los particulares hace especial referencia a:

- La indicación.
- Los estudios farmacodinámicos y farmacocinéticos.
- Eficacia y Seguridad: En este caso diferencia dos escenarios concretos:
 - a) *Cuando la combinación fija corresponde exactamente a combinaciones que están ya en uso difundido, un análisis de los datos bibliográficos bien fundamentados debería ser presentado. Con tal que los datos estén bien documentados, este análisis puede ser útil en reducir la cantidad de ensayos clínicos a ser realizada y podría facilitar la selección de dosis para cada sustancia y el rango de dosis propuesto en la combinación fija.*
 - b) *Cuando la combinación fija es esencialmente nueva (las sustancias activas no son usualmente combinadas, la composición cuantitativa es inusual entre sustancias usualmente combinadas o una sustancia enteramente nueva), los datos necesarios son similares a los de una nueva entidad química en la situación donde la combinación fija será propuesta (primero línea o segunda de la línea de terapia). La existencia de experiencia con las sustancias debería ser tenida en cuenta.*

y especifica Régimen de dosis y Composición, Ensayos Terapéuticos y Aspectos de Seguridad.

- Paquetes combinados de diferentes productos medicinales para administración simultánea o secuencial.

Conclusión:

Las diferentes agencias sanitarias a nivel internacional han regulado fuertemente el registro de asociaciones de forma de garantizar la seguridad y eficacia de las

mismas y la buena calidad en su elaboración. Todo estudio, investigación o análisis derivados de estos requisitos representan procedimientos de rutina y no originales ideas llevadas a la práctica."

Además, se puede considerar que todo efecto sinérgico o terapéutico entre fármacos no se refiere propiamente a una invención, sino que corresponde mejor a la categoría de un descubrimiento, dado que dicho efecto sinérgico o terapéutico conseguido mediante la combinación de fármacos ocurre dentro del organismo y se hace evidente a través de los respectivos ensayos preclínicos y/o clínicos. Así mismo es importante volver a enfatizar en que la combinación de fármacos para tratar ciertas patologías, hace parte de las actividades de la profesión médica, y no de las actividades industriales.

En un segundo aspecto, y esta vez refiriéndonos a las reivindicaciones 6 a 15 y 17, en donde se hace mención explícita a la dosificación de los compuestos I a IV, es importante indicar que esto contraviene lo dispuesto en la Decisión 486 de 2000 que literalmente refiere a que *"No serán patentables (...) d) los métodos terapéuticos o quirúrgicos para el tratamiento humano o animal, así como los métodos de diagnostico aplicados a los seres humanos o a animales."* Dicha mención de dosificaciones tiene el mismo efecto que las reivindicaciones sobre métodos de tratamiento medico, dado que la concentración, en este caso directamente referido a la dosificación de la formulación farmacéutica, no pertenece a la categoría de producto o proceso, sino que hace referencia la forma en la que un producto se utiliza terapéuticamente, y por lo tanto no hace parte de las actividades industriales sino de las actividades médicas. En otras palabras la dosificación o los cambios en la dosificación dentro de un producto farmacéutico no tiene naturaleza inventiva, ya que no cumplen con el estándar de aplicabilidad industrial, dado que dichas modificaciones solo tienen efectos en el organismo (verificables a nivel clínico) y no efectos técnicos.

En un tercer aspecto, y esta vez refiriéndonos a la formulación farmacéutica en si misma y las características técnicas que la definen en el pliego reivindicatorio de la solicitud colombiana en trámite, es importante indicar que los comprimidos multicapa (bicapa para este caso), resultan ser formulaciones convencionales³, lo

³ Vila Jato J.L. Tecnología Farmacéutica. Volumen II. Páginas 126 y 144.

suficientemente descritas en el estado de la técnica como para que cualquier persona medianamente versada en la formulación de productos farmacéuticos, aplicando los respectivos ensayos de preformulación y formulación de rutina, consiga involucrar cualquier principio activo o combinación de principios activos dentro de dichas capas que componen el comprimido.

Lo anterior se hace más evidente si observamos la tabla de la reivindicación 19 en donde claramente, y como cualquier persona medianamente versada en la formulación de productos farmacéuticos debe reconocer, se mencionan excipientes de uso común en la elaboración de comprimidos o tabletas farmacéuticas⁴.

Finalmente la publicación internacional WO 2008/103949 publicada el 28 de agosto de 2008 revela:

In yet another embodiment, the compound of the present invention has an inhibition activity specifically against an isozyme of P450 that is involved in metabolizing anti-viral drugs, *e.g.*, indinavir, nelfinavir, ritonavir, saquinavir etc.

In still yet another embodiment, the compound of the present invention has an inhibition activity specifically against one or more isozymes of P450, but not the other(s). For example, the compound of the present invention can have an inhibition activity specifically against P450 3A, but a reduced, insubstantial, or minimum inhibition activity against another isozyme of P450, *e.g.*, P450 2C9.

(...)

The formulations include those suitable for the foregoing administration routes. The formulations may conveniently be presented in unit dosage form and may be prepared by any of the methods well known in the art of pharmacy.

(...)

Specific embodiments of quaternary combinations comprise, for example,

(...)

⁴ Ibid. Páginas 91 a 105.

disoproxil fumarate/GS-9131/raltegravir, Example S/tenofovir disoproxil

fumarate/GS-9131/rilpivirine, Example S/tenofovir disoproxil

fumarate/emtricitabine/elvitegravir, Example S/tenofovir disoproxil

fumarate/emtricitabine/efavirenz, Example S/tenofovir disoproxil

(...)

En esta anterioridad se revelan formulaciones farmacéuticas tipo tabletas, en forma de dosificación unitaria en la cual pueden ir compuestos tales como elvitegravir (compuesto de fórmula I), un compuesto S (que corresponde al compuesto de fórmula II), emtricitabina (compuesto de fórmula II) y tenofovir (compuesto de fórmula IV), lo que claramente afecta el nivel inventivo de la solicitud, se tiene en cuenta que involucrar dicha combinación farmacológica en una tableta bicapa, haría parte de las habilidades de una persona versada en la formulación de productos farmacéuticos, aplicando el conocimiento técnico del estado de la técnica, tal y como argumentamos en párrafos anteriores.

En este mismo sentido, y atendiendo a las necesidades de los pacientes, tales como obtener un tratamiento individualizado, modulado y sincronizado (como lo menciona por ejemplo en la publicación científica **“Emergence of HIV-1 drug resistance in previously untreated patients initiating combination antiretroviral treatment⁵”**), así como en la presentación de resistencia (como se expone por ejemplo en publicaciones científicas como **“Tenofovir nephrotoxicity: focusing research questions and putting them into clinical context⁶”**, **“Risk of extensive virological failure to the three original antiretroviral drug classes over long-term follow-up from the start of therapy in patients with HIV infection: an observational cohort study⁷”** y **“Antiretroviral treatment of adult HIV infection. Recommendations of the international AIDS society-USA panel⁸”**), para una persona medianamente versada en la farmacología clínica, reunir estos 4 agentes en una sola formulación, resultaría una alternativa obvia para solventar los problemas de una medicación separada.

⁵ V. Von Wyl Et Al. Archives of Internal Medicine. Vol. 167. 2007.

⁶ L. Szczech. The Journal of Infectious Diseases. Vol. 197. 2008.

⁷ Fort he UK collaborative HIV Cohort (CHIC) study Phillips Et. Al. The Lancet Limited. Vol.370. 2007.

⁸ S. HAMmer Et. Al. JAMA. Vol 300. 2008

Por todo lo anterior, le solicito Señor Superintendente, que analice los argumentos citados con el rigor y detenimiento habituales y que decrete, además de la negación de la solicitud en comento, el archivo del citado expediente.

V. ANEXOS.

- ❖ Copia de todos los documentos mencionados en el acápite de Complemento de información.

Señor Superintendente,



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T.P.A. 44.655 del C.S.J.

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(54) Title: MODULATORS OF PHARMACOKINETIC PROPERTIES OF THERAPEUTICS

(57) Abstract: The present application provides for a compound of Formula IV, or a pharmaceutically acceptable salt, solvate, and/or ester thereof, compositions containing such compounds, therapeutic methods that include the administration of such compounds, and therapeutic methods and include the administration of such compounds with at least one additional therapeutic agent.

WO 2008/103949 A1

**MODULATORS OF PHARMACOKINETIC PROPERTIES OF
THERAPEUTICS**

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority to U.S. Provisional Application Ser. No. 60/903,228, entitled "Modulators of Pharmacokinetic Properties of Therapeutics", filed February 23, 2007, and U.S. Provisional Application Ser. No. 60/958,716, entitled "Modulators of Pharmacokinetic Properties of Therapeutics", filed July 6, 2007. The contents of these provisional applications are herein incorporated by reference in their entirety for all purposes.

FIELD OF THE INVENTION

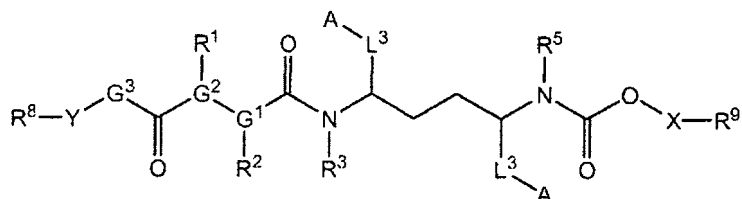
This application relates generally to compounds and pharmaceutical compositions which modify, *e.g.*, improve, the pharmacokinetics of a co-administered drug, and methods of modifying, *e.g.*, improving, the pharmacokinetics of a drug by co-administration of the compounds with the drug.

BACKGROUND OF THE INVENTION

Oxidative metabolism by cytochrome P450 enzymes is one of the primary mechanisms of drug metabolism. It can be difficult to maintain therapeutically effective blood plasma levels of drugs which are rapidly metabolized by cytochrome P450 enzymes. Accordingly, the blood plasma levels of drugs which are susceptible to cytochrome P450 enzyme degradation can be maintained or

What is Claimed:

1. A compound of formula IV,



Formula IV

or a pharmaceutically acceptable salt, solvate, and/or ester thereof, wherein,

each L³ is independently an alkylene or substituted alkylene;

each A is independently an aryl or substituted aryl;

X is heterocyclalkyl;

Y is heterocyclalkyl or alkyl;

G¹ and G² are independently CH or N, with the proviso that G¹ and G² are different;

G³ is -NR⁷- or -O-;

R¹, R³, R⁵, and R⁷ are each independently selected from the group consisting of H, alkyl, substituted alkyl, arylalkyl, and substituted arylalkyl;

R² is independently selected from the group consisting of substituted alkyl, alkoxyalkyl, hydroxyalkyl, trialkylsiloxyalkyl, heterocyclalkyl, substituted heterocyclalkyl, aminoalkyl, substituted aminoalkyl, -alkylene-N(R^a)-C(O)-alkyl, -alkylene-NR^a-C(O)-N(R^a)₂, -alkylene-NR^a-C(=N-R^b)-N(R^a)₂, -alkylene-C(=N-R^b)-N(R^a)₂, -alkylene-C(O)-OH, -alkylene-C(O)-Oalkyl, and -alkylene-C(O)-N(R^c)₂;

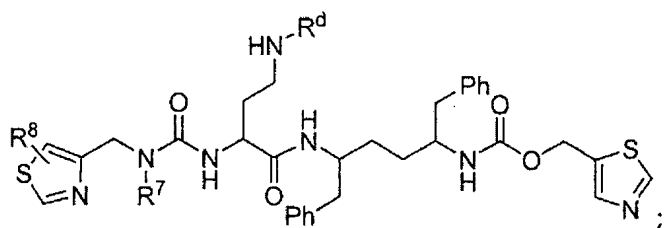
R⁸ and R⁹ are each one or more substituents independently selected from the group consisting of H, alkyl, substituted alkyl, halogen, and -CN;

each R^a is independently selected from the group consisting of H, alkyl, and substituted alkyl;

R^b is selected from the group consisting of H, alkyl, substituted alkyl, CN, and $-S(O_2)\text{-alkyl}$; and

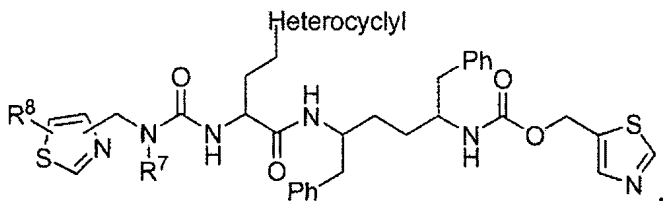
each R^c is independently selected from the group consisting of H, alkyl, substituted alkyl, heterocyclyl, substituted heterocyclyl, $-S(O_2)\text{-alkyl}$, $-S(O_2)\text{-aryl}$, and substituted $-S(O_2)\text{-aryl}$.

2. The compound of claim 1, having the following formula:



wherein R^d is selected from the group consisting of H, haloalkyl, $-C(O)\text{-}N(R^a)_2$, $-C(=N\text{-}R^b)\text{-}N(R^a)_2$, hydroxyalkyl, $-C(N(R^a)_2)=CHNO_2$, $-C(O)\text{-alkyl}$.

3. The compound of claim 1, having the following formula:

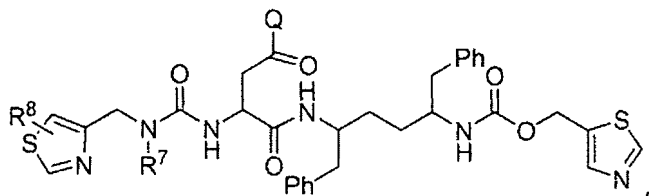


4. The compound of claim 3, wherein said Heterocyclyl is a substituted or unsubstituted heteroaryl.

5. The compound of claim 4, wherein said heteroaryl is selected from the group consisting of triazolyl, pyrrolyl, imidazolyl, pyridyl, pyrazolyl, and imidazolidin-dionyl.

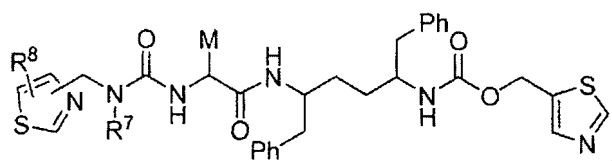
12 x 4

6. The compound of claim 3, wherein said Heterocyclyl is a substituted or unsubstituted heterocycloalkyl.
7. The compound of claim 6, wherein said heterocycloalkyl is selected from the group consisting of pyrrolidinonyl, pyrrolidine-dionyl, oxazolidinonyl, pyrrolidinyl, piperidinyl, piperidinonyl, piperazinyl, thiomorpholinyl, and morpholinyl.
8. The compound of claim 7, wherein said heterocycloalkyl is morpholinyl.
9. The compound of claim 1, having the following formula:



wherein Q is -OH or -N(R^a)₂.

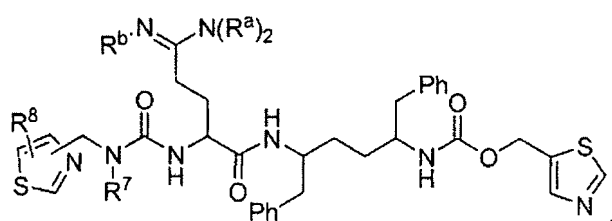
10. The compound of claim 9, wherein Q is -OH.
11. The compound of claim 9, wherein Q is -NHR^a, and R^a is substituted or unsubstituted heterocyclyl or sulfonylalkyl.
12. The compound of claim 1, having the following formula:



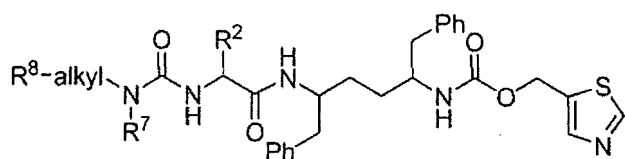
wherein M is substituted alkyl.

13. The compound of claim 12, wherein said substituted alkyl is selected from hydroxyalkyl, substituted hydroxyalkyl, cyanoalkyl, substituted cyanoalkyl, and trialkylsiloxyalkyl.

14. The compound of claim 1, having the following formula:



15. The compound of claim 1, having the following formula:

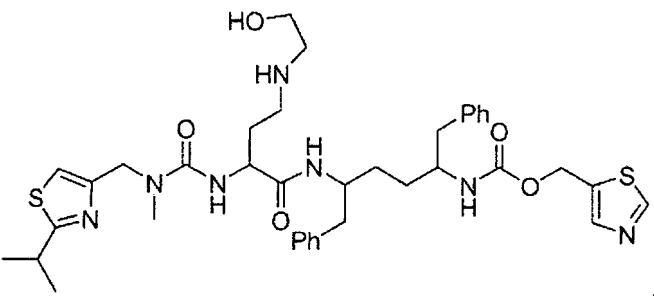
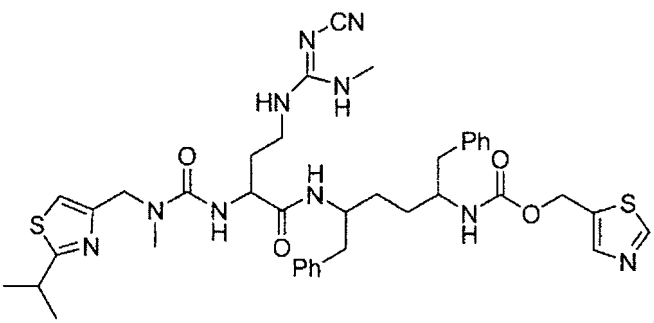
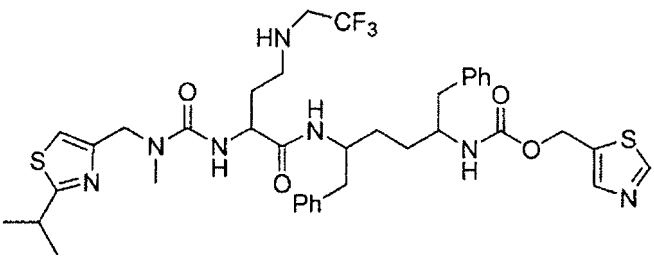
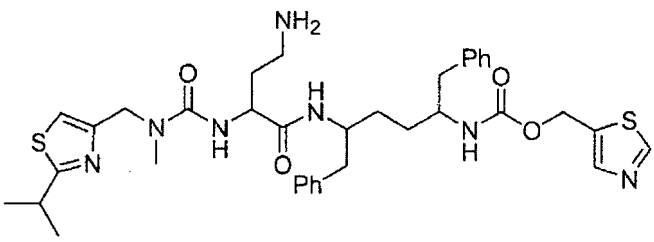
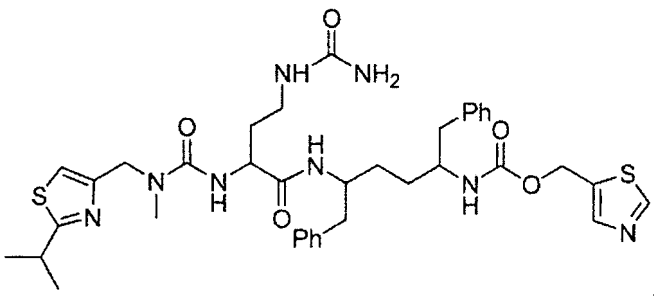


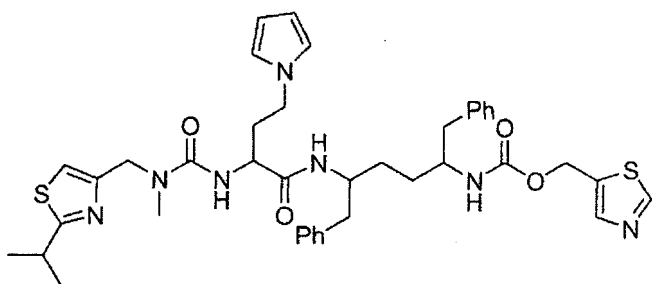
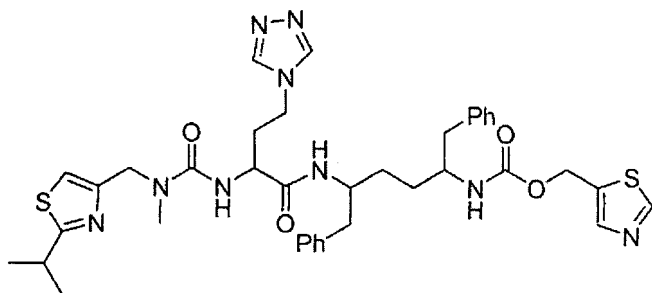
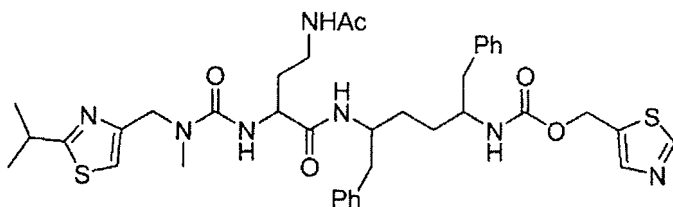
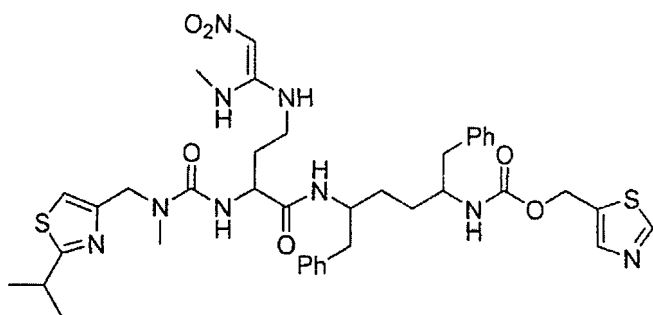
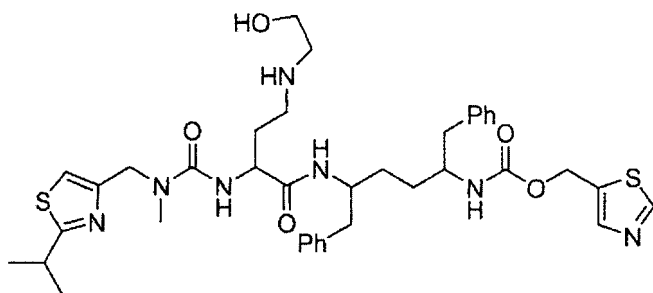
16. The compound of claim 15, wherein R⁸ is H.

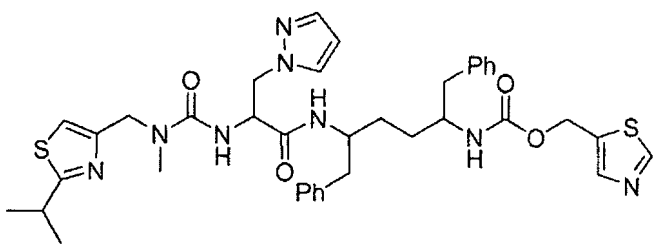
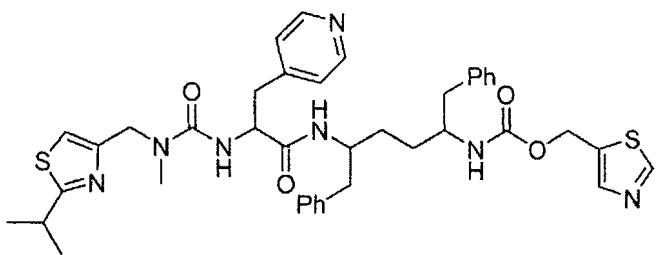
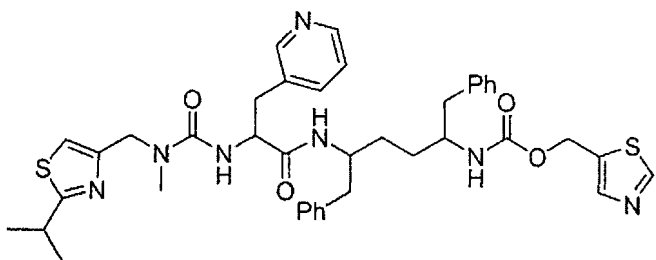
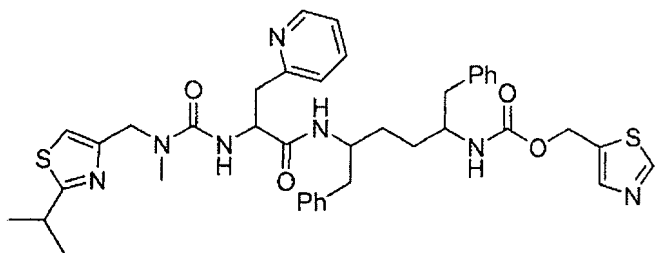
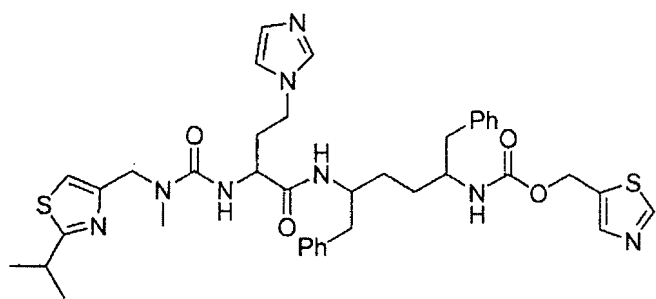
17. The compound of claim 15, wherein R² is aminoalkyl or heterocyclalkyl.

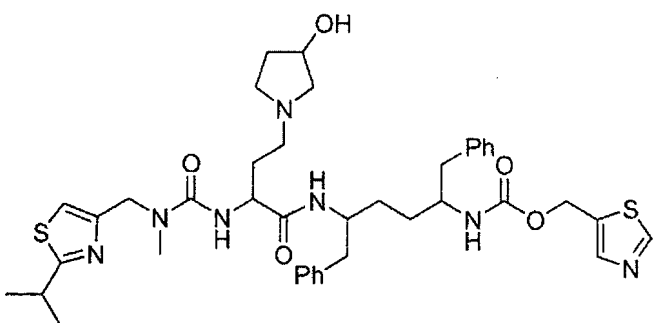
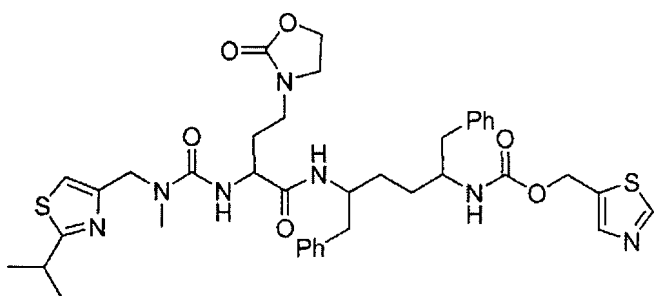
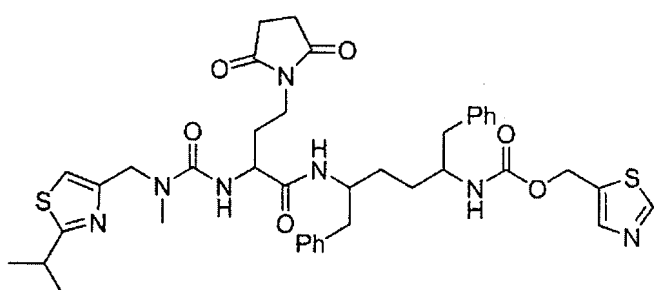
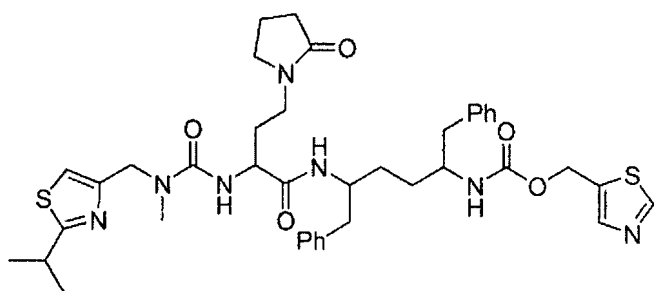
18. The compound of claim 1, selected from the group consisting of:

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V3



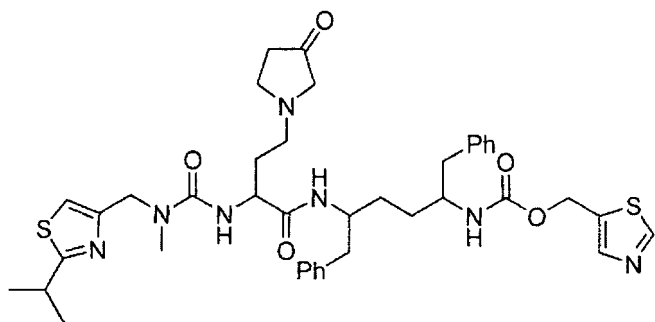
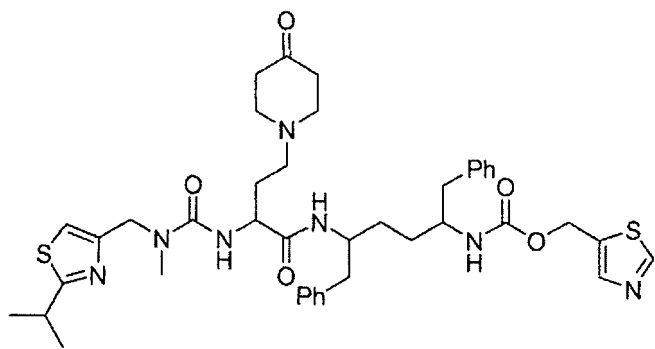
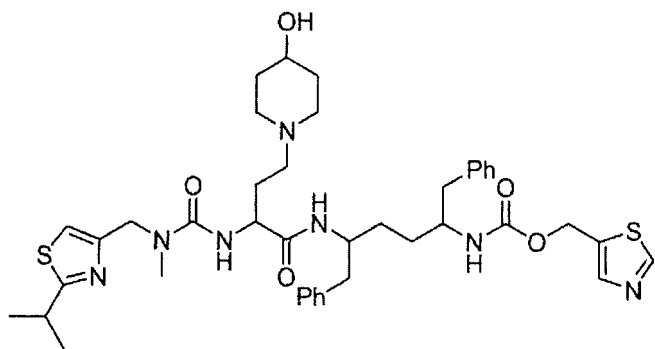


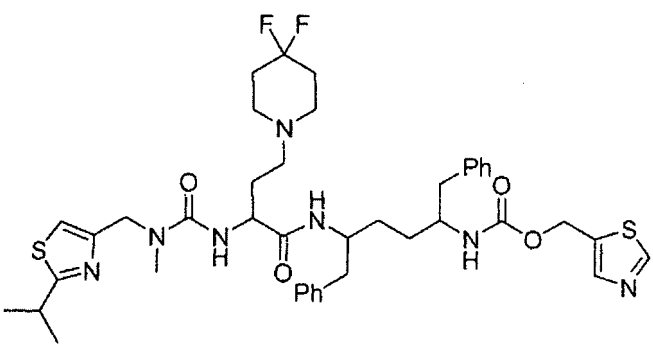
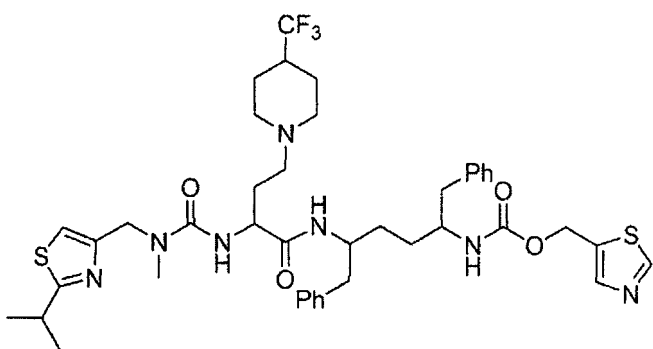
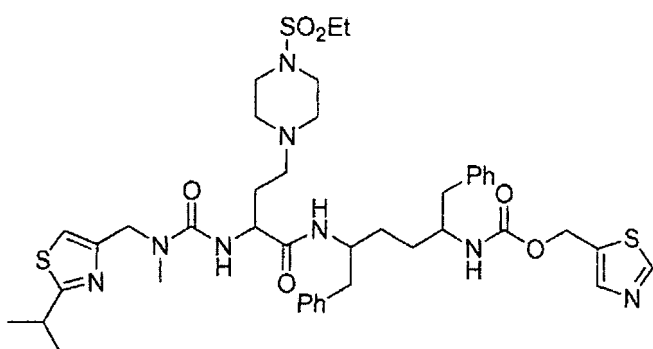
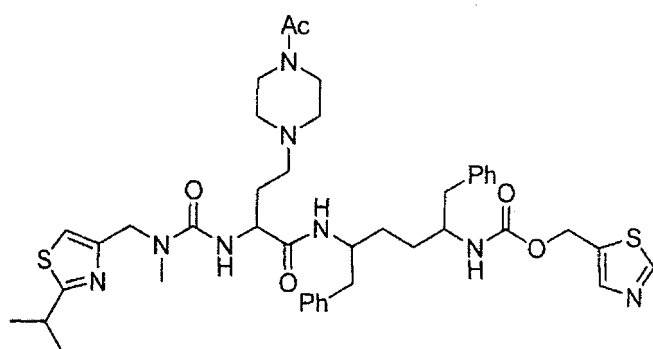




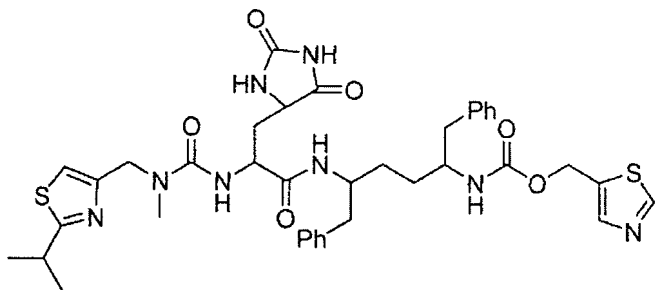
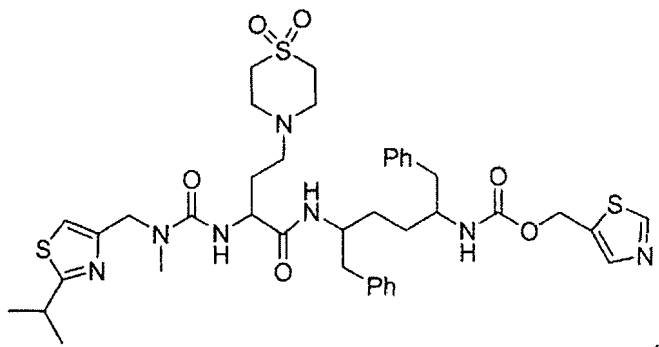
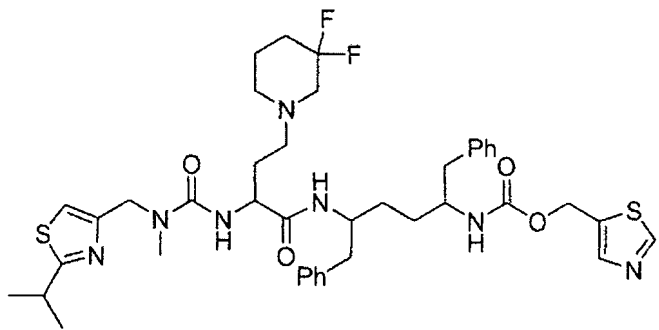
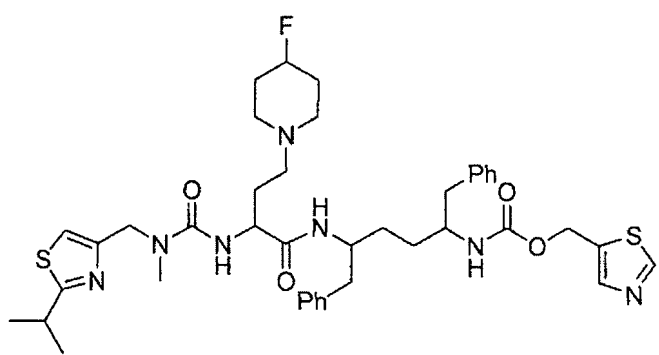
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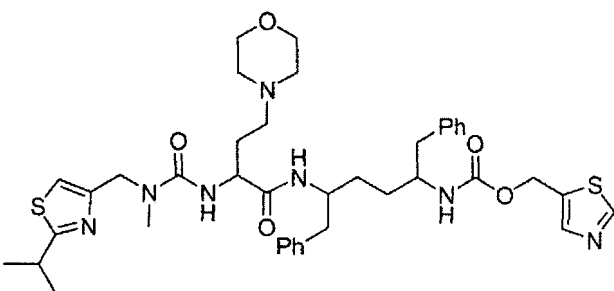
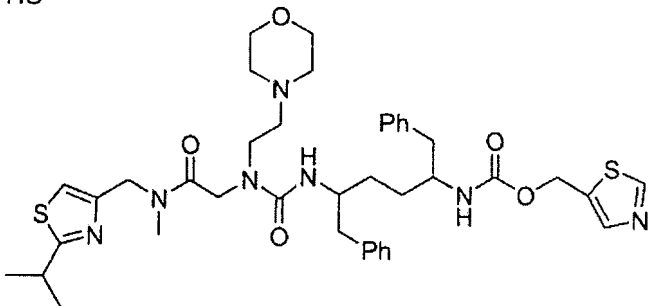
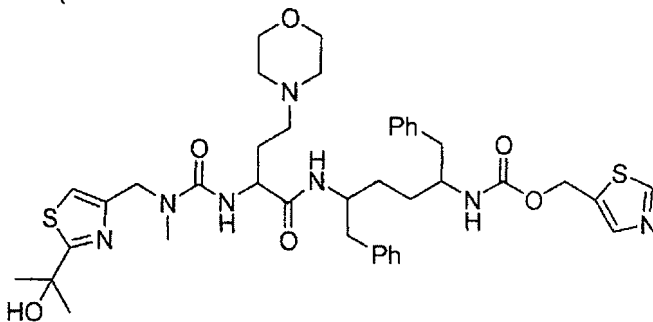
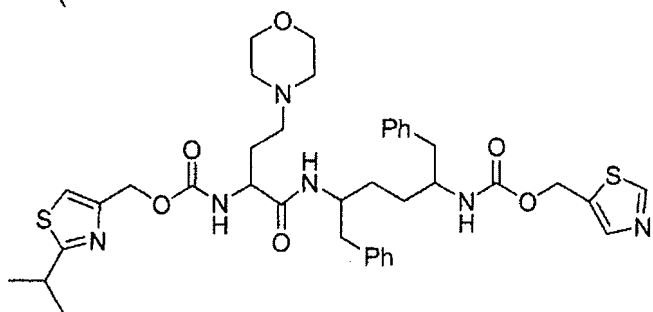
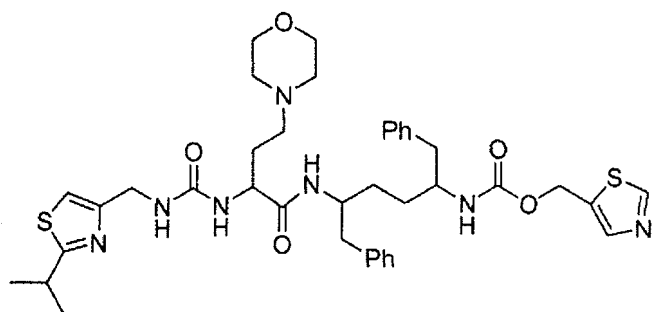
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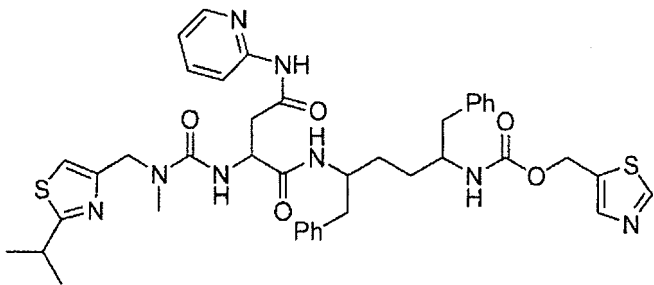
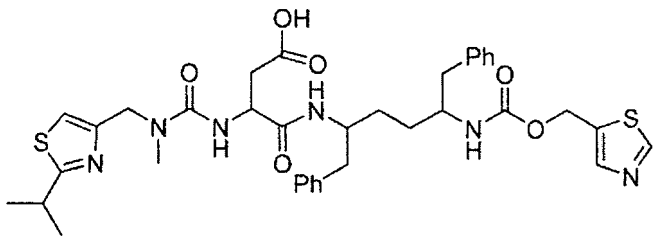
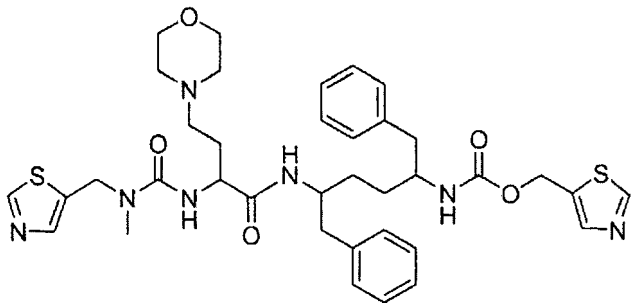
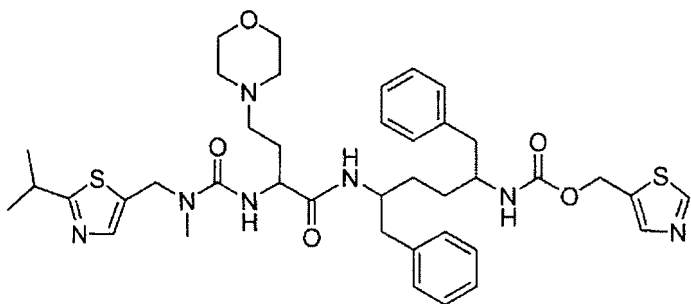
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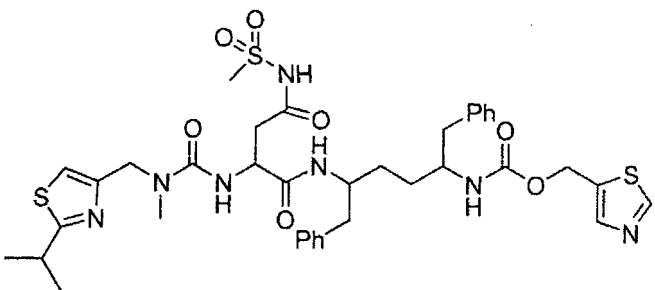
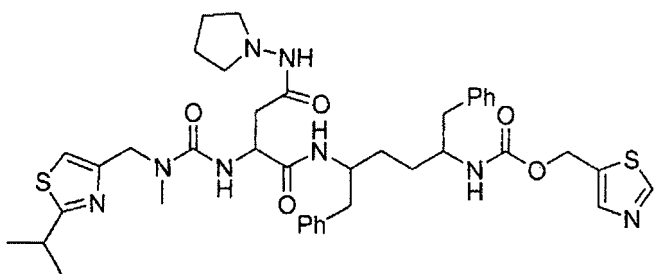
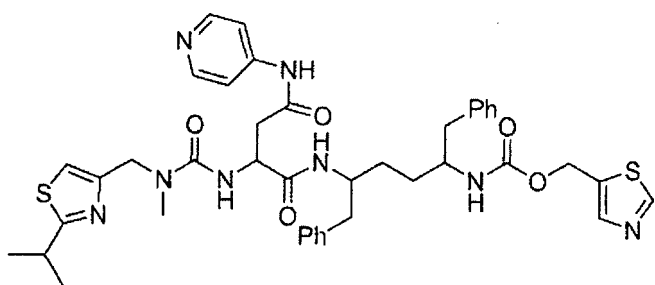
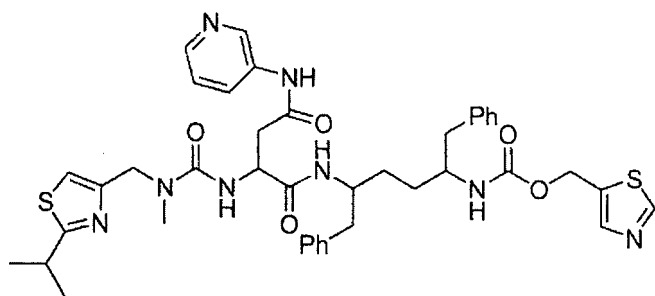
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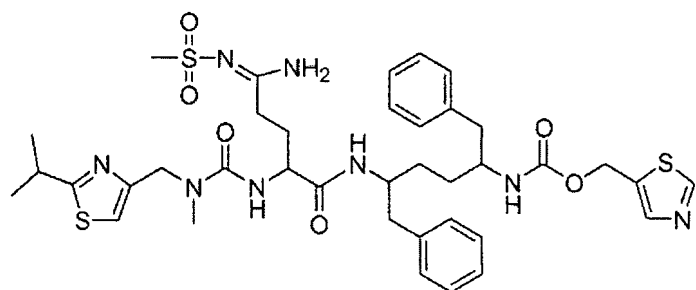
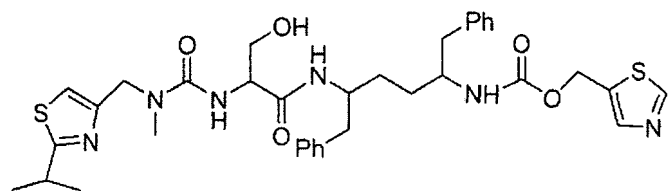
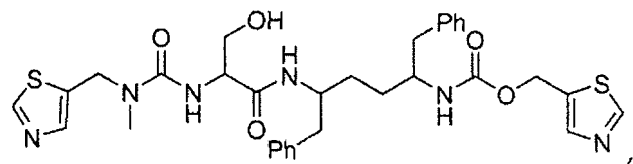
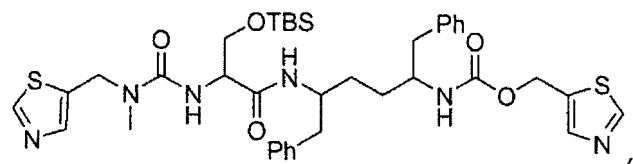
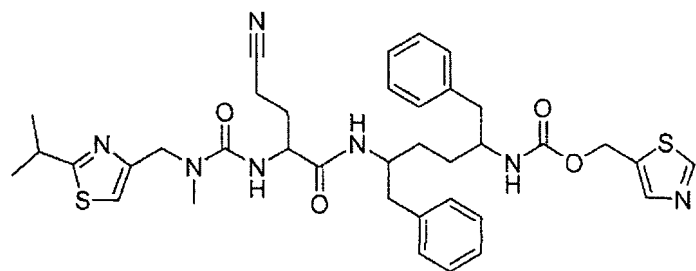
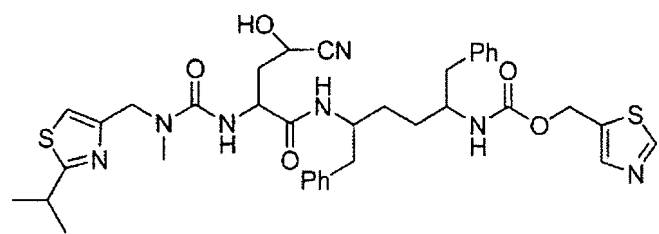


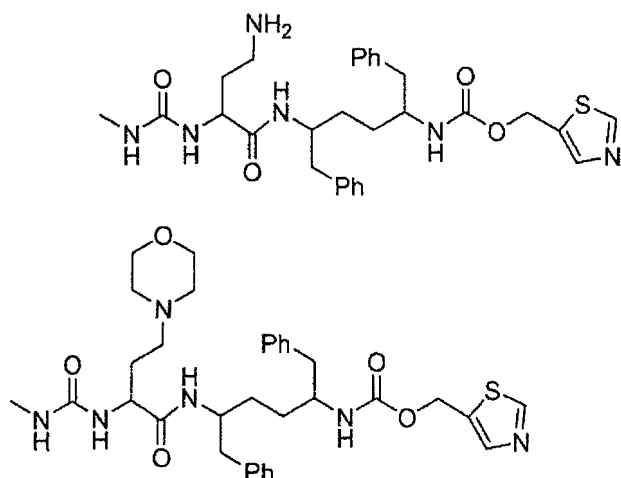
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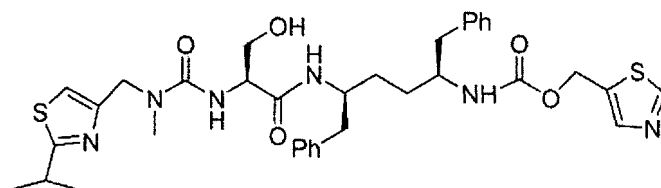


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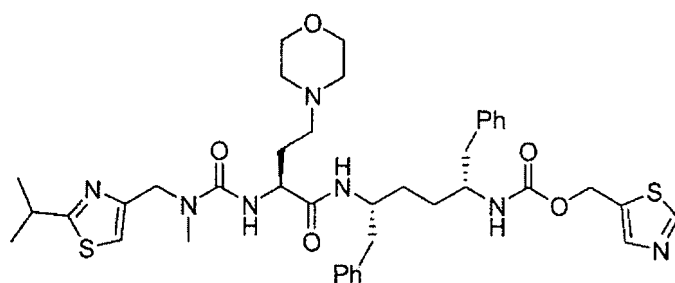


19. A compound which is:



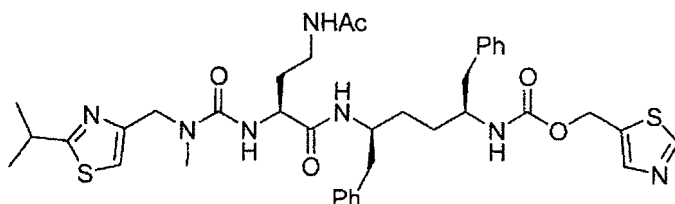
or a pharmaceutically acceptable salt, ester, and/or solvate thereof.

20. A compound which is



or a pharmaceutically acceptable salt, ester, and/or solvate thereof.

21. A compound which is



or a pharmaceutically acceptable salt, ester, and/or solvate thereof.

22. A method for improving the pharmacokinetics of a drug which is metabolized by cytochrome P450 monooxygenase, comprising administering to a patient treated with said drug, a therapeutically effective amount of a compound of claim 1, or a pharmaceutically acceptable salt, solvate, and/or ester thereof.

23. The method of claim 22 wherein said administering comprises administering a therapeutically effective amount of a combination comprising said drug and the compound of Formula IV or a pharmaceutically acceptable salt, solvate, and/or ester of the compound of Formula IV.

24. The method of claim 22, wherein the drug metabolized by cytochrome P450 is an HIV protease inhibiting compound, an HIV non-nucleoside inhibitor of reverse transcriptase, an HIV nucleoside inhibitor of reverse transcriptase, HIV nucleotide inhibitor of reverse transcriptase, an HIV integrase inhibitor, a gp41 inhibitor, a CXCR4 inhibitor, a gp120 inhibitor, a CCR5 inhibitor, capsid polymerization inhibitors, other drugs for treating HIV, an interferon, ribavirin analog, NS3 protease inhibitor, alpha-glucosidase 1 inhibitor, hepatoprotectant, non-nucleoside inhibitor of HCV, NS5a inhibitors, NS5b polymerase inhibitors, other drugs for treating HCV, or mixtures thereof.

25. The method of claim 23, wherein the drug and the compound or pharmaceutically acceptable salt, solvate, and/or ester thereof of claim 1 is administered as a single composition to the patient.

26. The method of claim 22, wherein said improving is increasing blood plasma levels of the drug which is metabolized by cytochrome P450 monooxygenase.
27. A method for inhibiting cytochrome P450 monooxygenase in a patient comprising administering to a patient in need thereof an amount of a compound of claim 1, or a pharmaceutically acceptable salt, solvate, and/or ester thereof, effective to inhibit cytochrome P450 monooxygenase.
28. A method for treating an HIV infection comprising administering to a patient in need thereof a therapeutically effective amount of a compound of claim 1, or a pharmaceutically acceptable salt, solvate, and/or ester thereof, in combination with a therapeutically effective amount of one or more additional therapeutic agents selected from the group consisting of HIV protease inhibiting compounds, HIV non-nucleoside inhibitors of reverse transcriptase, HIV nucleoside inhibitors of reverse transcriptase, HIV nucleotide inhibitors of reverse transcriptase, HIV integrase inhibitors, gp41 inhibitors, CXCR4 inhibitors, gp120 inhibitors, G6PD and NADH-oxidase inhibitors, CCR5 inhibitors, other drugs for treating HIV, and mixtures thereof.
29. The method of claim 28, wherein:
- (1) said HIV protease inhibitors are selected from the group consisting of amprenavir, atazanavir, fosamprenavir, indinavir, lopinavir, ritonavir, nelfinavir, saquinavir, tipranavir, brecanavir, darunavir, TMC-126, TMC-114, mozenavir (DMP-450), JE-2147 (AG1776), L-756423, RO0334649, KNI-272, DPC-681, DPC-684, GW640385X, DG17, PPL-100, DG35, and AG 1859;

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(2) said HIV non-nucleoside inhibitors of reverse transcriptase are selected from the group consisting of capravirine, emivirine, delaviridine, efavirenz, nevirapine, (+) calanolide A, etravirine, GW5634, DPC-083, DPC-961, DPC-963, MIV-150, TMC-120, TMC-278 (rilpivirene), BILR 355 BS, VRX 840773, UK-453061, and RDEA806;

(3) said HIV nucleoside inhibitors of reverse transcriptase are selected from the group consisting of zidovudine, emtricitabine, didanosine, stavudine, zalcitabine, lamivudine, abacavir, amdoxovir, elvucitabine, alovudine, MIV-210, racivir ($\pm$ -FTC), D-d4FC, phosphazide, fozivudine tidoxil, apricitibine (AVX754), amdoxovir, KP-1461, and fosalvudine tidoxil (formerly HDP 99.0003);

(4) said HIV nucleotide inhibitors of reverse transcriptase are selected from the group consisting of tenofovir disoproxil fumarate, GS-9131, and adefovir dipivoxil;

(5) said HIV integrase inhibitors are selected from the group consisting of curcumin, derivatives of curcumin, derivatives of curcumin, chicoric acid, derivatives of chicoric acid, 3,5-dicaffeoylquinic acid, derivatives of 3,5-dicaffeoylquinic acid, aurintricarboxylic acid, derivatives of aurintricarboxylic acid, caffeic acid phenethyl ester, derivatives of caffeic acid phenethyl ester, tyrphostin, derivatives of tyrphostin, quercetin, derivatives of quercetin, S-1360, zintevir (AR-177), L-870812, L-870810, MK-0518 (raltegravir), elvitegravir, BMS-538158, GSK364735C, BMS-707035, MK-2048, and BA 011;

(6) said gp41 inhibitor are selected from the group consisting of enfuvirtide, sifuvirtide, FB006M, and TRI-1144;

(7) said CXCR4 inhibitor is AMD-070;

(8) said entry inhibitor is SP01A;

(9) said gp120 inhibitor is BMS-488043 or BlockAide/ CR;

(10) said G6PD and NADH-oxidase inhibitor is immunitin;

(11) said CCR5 inhibitors are selected from the group consisting of aplaviroc, vicriviroc, maraviroc, PRO-140, INCB15050, PF-232798 (Pfizer), and CCR5mAb004;

(12) said other drugs for treating HIV are selected from the group consisting of BAS-100, SPI-452, REP 9, SP-01A, TNX-355, DES6, ODN-93, ODN-112, VGV-1, PA-457 (bevrimat), Ampligen, HRG214, Cytolin, VGX-410, KD-247, AMZ 0026, CYT 99007A-221 HIV, DEBIO-025, BAY 50-4798, MDX010 (ipilimumab), PBS 119, ALG 889, and PA-1050040 (PA-040).

30. A method for treating an HCV infection comprising administering to a patient in need thereof a therapeutically effective amount of a compound of claim 1, or a pharmaceutically acceptable salt, solvate, and/or ester thereof, in combination with a therapeutically effective amount of one or more additional therapeutic agents selected from the group consisting of interferons, ribavirin analogs, NS3 protease inhibitors, alpha-glucosidase 1 inhibitors, hepatoprotectants, non-nucleoside inhibitors of HCV, and other drugs for treating HCV, or mixtures thereof.

31. The method of claim 30, wherein:

(1) said interferons are selected from the group consisting of pegylated rIFN-alpha 2b, pegylated rIFN-alpha 2a, rIFN-alpha 2b, rIFN-alpha 2a, consensus IFN alpha (infergen), feron, reaferon, intermax alpha, r-IFN-beta, infergen + actimmune, IFN-omega with DUROS, albuferon, locteron, Albuferon, Rebif, Oral interferon alpha, IFNalpha-2b XL, AVI-005, PEG-Infergen, and Pegylated IFN-beta;

(2) said ribavirin analogs are selected from the group consisting of rebetol, copegus, and viramidine (taribavirin);

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(3) said NS5b polymerase inhibitors are selected from the group consisting of NM-283, valopicitabine, R1626, PSI-6130 (R1656), HCV-796, BILB 1941, XTL-2125, MK-0608, NM-107, R7128 (R4048), VCH-759, PF-868554, and GSK625433;

(4) said NS3 protease inhibitor are selected from the group consisting of SCH-503034 (SCH-7), VX-950 (telaprevir), BILN-2065, BMS-605339, and ITMN-191;

(5) said alpha-glucosidase 1 inhibitors are selected from the group consisting of MX-3253 (celgosivir) and UT-231B;

(6) said hepatoprotectants are selected from the group consisting of IDN-6556, ME 3738, LB-84451, and MitoQ;

(7) said non-nucleoside inhibitors of HCV are selected from the group consisting of benzimidazole derivatives, benzo-1,2,4-thiadiazine derivatives, phenylalanine derivatives, A-831, and A-689; and

(8) said other drugs for treating HCV are selected from the group consisting of zadaxin, nitazoxanide (alinea), BIVN-401 (virostat), PYN-17 (altirex), KPE02003002, actilon (CPG-10101), KRN-7000, civacir, GI-5005, ANA-975, XTL-6865, ANA 971, NOV-205, tarvacin, EHC-18, NIM811, DEBIO-025, VGX-410C, EMZ-702, AVI 4065, Bavituximab, Oglufanide, and VX-497 (merimepodib).

32. A pharmaceutical composition comprising a compound of claim 1, or a pharmaceutically acceptable salt, solvate, and/or ester thereof, and a pharmaceutically acceptable carrier or expipient.

33. The pharmaceutical composition of claim 32, further comprising at least one additional therapeutic agent metabolized by cytochrome P450.

34. The pharmaceutical composition of claim 33, wherein the at least one additional therapeutic agent is selected from the group consisting of HIV protease inhibiting compounds, HIV non-nucleoside inhibitors of reverse transcriptase, HIV nucleoside inhibitors of reverse transcriptase, HIV nucleotide inhibitors of reverse transcriptase, HIV integrase inhibitors, gp41 inhibitors, CXCR4 inhibitors, gp120 inhibitors, CCR5 inhibitors, capsid polymerization inhibitors, interferons, ribavirin analogs, NS3 protease inhibitors, alpha-glucosidase 1 inhibitors, hepatoprotectants, non-nucleoside inhibitors of HCV, other drugs for treating HCV, and combinations thereof.

35. The pharmaceutical composition of claim 34, wherein:

(1) said HIV protease inhibitors are selected from the group consisting of amprenavir, atazanavir, fosamprenavir, indinavir, lopinavir, ritonavir, nelfinavir, saquinavir, tipranavir, brecanavir, darunavir, TMC-126, TMC-114, mozenavir (DMP-450), JE-2147 (AG1776), L-756423, RO0334649, KNI-272, DPC-681, DPC-684, GW640385X, DG17, PPL-100, DG35, and AG 1859;

(2) said HIV non-nucleoside inhibitors of reverse transcriptase are selected from the group consisting of capravirine, emivirine, delaviridine, efavirenz, nevirapine, (+) calanolide A, etravirine, GW5634, DPC-083, DPC-961, DPC-963, MIV-150, and TMC-120, TMC-278 (rilpivirene), efavirenz, BILR 355 BS, VRX 840773, UK-453061, and RDEA806;

(3) said HIV nucleoside inhibitors of reverse transcriptase are selected from the group consisting of zidovudine, emtricitabine, didanosine, stavudine, zalcitabine, lamivudine, abacavir, amdoxovir, elvucitabine, alovudine, MIV-210, racivir ($\pm$ -FTC), D-d4FC, emtricitabine, phosphazide, fozivudine tidoxil, apricitibine (AVX754), amdoxovir, KP-1461, and fosalvudine tidoxil (formerly HDP 99.0003), ;

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(4) said HIV nucleotide inhibitors of reverse transcriptase are selected from the group consisting of tenofovir disoproxil fumarate, GS-9131, and adefovir dipivoxil;

(5) said HIV integrase inhibitors are selected from the group consisting of curcumin, derivatives of curcumin, chicoric acid, derivatives of chicoric acid, 3,5-dicaffeoylquinic acid, derivatives of 3,5-dicaffeoylquinic acid, aurintricarboxylic acid, derivatives of aurintricarboxylic acid, caffeic acid phenethyl ester, derivatives of caffeic acid phenethyl ester, tyrphostin, derivatives of tyrphostin, quercetin, derivatives of quercetin, S-1360, zintevir (AR-177), L-870812, and L-870810, MK-0518 (raltegravir), elvitegravir, BMS-538158, GSK364735C, BMS-707035, MK-2048, and BA 011;

(6) said gp41 inhibitor are selected from the group consisting of enfuvirtide, sifuvirtide, FB006M, and TRI-1144;

(7) said CXCR4 inhibitor is AMD-070;

(8) said entry inhibitor is SP01A;

(9) said gp120 inhibitor is BMS-488043 or BlockAide/ CR;

(10) said G6PD and NADH-oxidase inhibitor is immunitin;

(11) said CCR5 inhibitors are selected from the group consisting of aplaviroc, vicriviroc, maraviroc, PRO-140, INCB15050, PF-232798 (Pfizer), and CCR5mAb004;

(12) said other drugs for treating HIV are selected from the group consisting of BAS-100, SPI-452, REP 9, SP-01A, TNX-355, DES6, ODN-93, ODN-112, VGV-1, PA-457 (bevrimat), Ampligen, HRG214, Cytolin, VGX-410, KD-247, AMZ 0026, CYT 99007A-221 HIV, DEBIO-025, BAY 50-4798, MDX010 (ipilimumab), PBS 119, ALG 889, and PA-1050040 (PA-040);

(13) said interferons are selected from the group consisting of pegylated rIFN-alpha 2b, pegylated rIFN-alpha 2a, rIFN-alpha 2b, rIFN-alpha 2a, consensus

IFN alpha (infergen), feron, reaferon, intermax alpha, r-IFN-beta, infergen + actimmune, IFN-omega with DUROS, albuferon, locteron, Albuferon, Rebif, Oral interferon alpha, IFNalpha-2b XL, AVI-005, PEG-Infergen, and Pegylated IFN-beta;

(14) said ribavirin analogs are selected from the group consisting of rebetol, copegus, and viramidine (taribavirin);

(15) said NS5b polymerase inhibitors are selected from the group consisting of NM-283, valopicitabine, R1626, PSI-6130 (R1656), HCV-796, BILB 1941, XTL-2125, MK-0608, NM-107, R7128 (R4048), VCH-759, PF-868554, and GSK625433;

(16) said NS3 protease inhibitor are selected from the group consisting of SCH-503034 (SCH-7), VX-950 (telaprevir), BILN-2065, BMS-605339, and ITMN-191;

(17) said alpha-glucosidase 1 inhibitors are selected from the group consisting of MX-3253 (celgosivir) and UT-231B;

(18) said hepatoprotectants are selected from the group consisting of IDN-6556, ME 3738, LB-84451, and MitoQ;

(19) said non-nucleoside inhibitors of HCV are selected from the group consisting of benzimidazole derivatives, benzo-1,2,4-thiadiazine derivatives, phenylalanine derivatives, A-831, and A-689; and

(20) said other drugs for treating HCV are selected from the group consisting of zadaxin, nitazoxanide (alinea), BIVN-401 (virostat), PYN-17 (altirex), KPE02003002, actilon (CPG-10101), KRN-7000, civacir, GI-5005, ANA-975, XTL-6865, ANA 971, NOV-205, tarvacin, EHC-18, NIM811, DEBIO-025, VGX-410C, EMZ-702, AVI 4065, Bavituximab, Oglufanide, and VX-497 (merimepodib).

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36. A new pharmaceutical composition or use for the preparation of a medicament, comprising a compound of claim 1.
37. A compound of claim 1 as a therapeutic substance.
38. The use of a compound of claim 1 for the manufacture of a medicament for improving the pharmacokinetics of a drug which is metabolized by cytochrome P450 monooxygenase, increasing the blood plasma level of a drug which is metabolized by cytochrome P450 monooxygenase, inhibiting cytochrome P450 monooxygenase, treating an HIV infection, or treating an HCV infection in a patient.
39. The use of claim 38, wherein said drug which is metabolized by cytochrome P450 monooxygenase is an HIV protease inhibiting compounds, HIV non-nucleoside inhibitors of reverse transcriptase, HIV nucleoside inhibitors of reverse transcriptase, HIV nucleotide inhibitors of reverse transcriptase, HIV integrase inhibitors, gp41 inhibitors, CXCR4 inhibitors, gp120 inhibitors, G6PD and NADH-oxidase inhibitors, CCR5 inhibitors, other drugs for treating HIV, an interferon, ribavirin analog, NS3 protease inhibitor, alpha-glucosidase 1 inhibitor, hepatoprotectant, non-nucleoside inhibitor of HCV, and other drugs for treating HCV, or mixtures thereof.
40. The use of claim 39, wherein said medicament is a combination of a compound of claim 1 and one or more additional therapeutic agents selected from the group consisting of HIV protease inhibiting compounds, HIV non-nucleoside inhibitors of reverse transcriptase, HIV nucleoside inhibitors of reverse transcriptase, HIV nucleotide inhibitors of reverse transcriptase, HIV integrase inhibitors, gp41 inhibitors, CXCR4 inhibitors, gp120 inhibitors, G6PD and NADH-

oxidase inhibitors, CCR5 inhibitors, other drugs for treating HIV, interferons, ribavirin analogs, NS5b polymerase inhibitors, NS3 protease inhibitors, alpha-glucosidase 1 inhibitors, hepatoprotectants, non-nucleoside inhibitors of HCV, and other drugs for treating HCV, and mixtures thereof.

41. The use of claim 40, wherein:

(1) said HIV protease inhibitors are selected from the group consisting of amprenavir, atazanavir, fosamprenavir, indinavir, lopinavir, ritonavir, nelfinavir, saquinavir, tipranavir, brexanavir, darunavir, TMC-126, TMC-114, mozenavir (DMP-450), JE-2147 (AG1776), L-756423, RO0334649, KNI-272, DPC-681, DPC-684, GW640385X, DG17, PPL-100, DG35, and AG 1859;

(2) said HIV non-nucleoside inhibitors of reverse transcriptase are selected from the group consisting of capravirine, emivirine, delaviridine, efavirenz, nevirapine, (+) calanolide A, etravirine, GW5634, DPC-083, DPC-961, DPC-963, MIV-150, and TMC-120, TMC-278 (rilpivirene), efavirenz, BILR 355 BS, VRX 840773, UK-453061, and RDEA806;

(3) said HIV nucleoside inhibitors of reverse transcriptase are selected from the group consisting of zidovudine, emtricitabine, didanosine, stavudine, zalcitabine, lamivudine, abacavir, amdoxovir, elvucitabine, alovudine, MIV-210, racivir ($\pm$ -FTC), D-d4FC, emtricitabine, phosphazide, fozivudine tidoxil, apricitibine (AVX754), amdoxovir, KP-1461, and fosaltivudine tidoxil (formerly HDP 99.0003), ;

(4) said HIV nucleotide inhibitors of reverse transcriptase are selected from the group consisting of tenofovir disoproxil fumarate, GS-9131, and adefovir dipivoxil;

(5) said HIV integrase inhibitors are selected from the group consisting of curcumin, derivatives of curcumin, chicoric acid, derivatives of chicoric acid, 3,5-

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dicafeoylquinic acid, derivatives of 3,5-dicafeoylquinic acid, aurintricarboxylic acid, derivatives of aurintricarboxylic acid, caffeic acid phenethyl ester, derivatives of caffeic acid phenethyl ester, tyrphostin, derivatives of tyrphostin, quercetin, derivatives of quercetin, S-1360, zintevir (AR-177), L-870812, and L-870810, MK-0518 (raltegravir), elvitegravir, BMS-538158, GSK364735C, BMS-707035, MK-2048, and BA 011;

(6) said gp41 inhibitor are selected from the group consisting of enfuvirtide, sifuvirtide, FB006M, and TRI-1144;

(7) said CXCR4 inhibitor is AMD-070;

(8) said entry inhibitor is SP01A;

(9) said gp120 inhibitor is BMS-488043 or BlockAide/ CR;

(10) said G6PD and NADH-oxidase inhibitor is immunitin;

(11) said CCR5 inhibitors are selected from the group consisting of aplaviroc, vicriviroc, maraviroc, PRO-140, INCB15050, PF-232798 (Pfizer), and CCR5mAb004;

(12) said other drugs for treating HIV are selected from the group consisting of BAS-100, SPI-452, REP 9, SP-01A, TNX-355, DES6, ODN-93, ODN-112, VGV-1, PA-457 (bevirimat), Ampligen, HRG214, Cytolin, VGX-410, KD-247, AMZ 0026, CYT 99007A-221 HIV, DEBIO-025, BAY 50-4798, MDX010 (ipilimumab), PBS 119, ALG 889, and PA-1050040 (PA-040);

(13) said interferons are selected from the group consisting of pegylated rIFN-alpha 2b, pegylated rIFN-alpha 2a, rIFN-alpha 2b, rIFN-alpha 2a, consensus IFN alpha (infergen), feron, reaferon, intermax alpha, r-IFN-beta, infergen + actimmune, IFN-omega with DUROS, albuferon, locteron, Albuferon, Rebif, Oral interferon alpha, IFNalpha-2b XL, AVI-005, PEG-Infergen, and Pegylated IFN-beta;

(14) said ribavirin analogs are selected from the group consisting of rebetol, copegus, and viramidine (taribavirin);

(15) said NS5b polymerase inhibitors are selected from the group consisting of NM-283, valopicitabine, R1626, PSI-6130 (R1656), HCV-796, BILB 1941, XTL-2125, MK-0608, NM-107, R7128 (R4048), VCH-759, PF-868554, and GSK625433;

(16) said NS3 protease inhibitor are selected from the group consisting of SCH-503034 (SCH-7), VX-950 (telaprevir), BILN-2065, BMS-605339, and ITMN-191;

(17) said alpha-glucosidase 1 inhibitors are selected from the group consisting of MX-3253 (celgosivir) and UT-231B;

(18) said hepatoprotectants are selected from the group consisting of IDN-6556, ME 3738, LB-84451, and MitoQ;

(19) said non-nucleoside inhibitors of HCV are selected from the group consisting of benzimidazole derivatives, benzo-1,2,4-thiadiazine derivatives, phenylalanine derivatives, A-831, and A-689; and

(20) said other drugs for treating HCV are selected from the group consisting of zadaxin, nitazoxanide (alinea), BIVN-401 (virostat), PYN-17 (altirex), KPE02003002, actilon (CPG-10101), KRN-7000, civacir, GI-5005, ANA-975, XTL-6865, ANA 971, NOV-205, tarvacin, EHC-18, NIM811, DEBIO-025, VGX-410C, EMZ-702, AVI 4065, Bavituximab, Oglufanide, and VX-497 (merimepodib).

Emergence of HIV-1 Drug Resistance in Previously Untreated Patients Initiating Combination Antiretroviral Treatment

A Comparison of Different Regimen Types

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Background: Standard first-line combination antiretroviral treatment (cART) against human immunodeficiency virus 1 (HIV-1) contains either a nonnucleoside reverse transcriptase inhibitor (NNRTI) or a ritonavir-boosted protease inhibitor (PI/r). Differences between these regimen types in the extent of the emergence of drug resistance on virological failure and the implications for further treatment options have rarely been assessed.

Methods: We investigated virological outcomes in patients from the Swiss HIV Cohort Study initiating cART between January 1, 1999, and December 31, 2005, with an unboosted PI, a PI/r, or an NNRTI and compared genotypic drug resistance patterns among these groups at treatment failure.

Results: A total of 489 patients started cART with a PI, 518 with a PI/r, and 805 with an NNRTI. A total of 177 virological failures were observed (108 [22%] PI failures, 24 [5%] PI/r failures, and 45 [6%] NNRTI failures). The failure rate was highest in the PI group (10.3 per 100 person-years; 95% confidence interval [CI], 8.5-

12.4). No difference was seen between patients taking a PI/r (2.7; 95% CI, 1.8-4.0) and those taking an NNRTI (2.4; 95% CI, 1.8-3.3). Genotypic test results were available for 142 (80%) of the patients with a virological treatment failure. Resistance mutations were found in 84% (95% CI, 75%-92%) of patients taking a PI, 30% (95% CI, 12%-54%) of patients taking a PI/r, and 66% (95% CI, 49%-80%) of patients taking an NNRTI ($P < .001$). Multidrug resistance occurred almost exclusively as resistance against lamivudine-emtricitabine and the group-specific third drug and was observed in 17% (95% CI, 9%-26%) of patients taking a PI, 10% (95% CI, 0.1%-32%) of patients taking a PI/r, and 50% (95% CI, 33%-67%) of patients taking an NNRTI ($P < .001$).

Conclusions: Regimens that contained a PI/r or an NNRTI exhibited similar potency as first-line regimens. However, the use of a PI/r led to less resistance in case of virological failure, preserving more drug options for the future.

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CURRENT GUIDELINES RECOMMEND the initiation of combination antiretroviral treatment (cART) against human immunodeficiency virus 1 (HIV-1) with 2 nucleoside reverse transcriptase inhibitors (NRTIs) and a nonnucleoside reverse transcriptase inhibitor (NNRTI) or a ritonavir-boosted protease inhibitor (PI/r).¹ These different drug classes vary considerably in the number of mutations required to confer resistance and in the degree of cross-resistance to drugs of the same class. Selection of a single mutation leads to the emergence of high-level resistance against NNRTIs, and resistance usually emerges quickly if treatment failure occurs.^{2,3} In contrast, virological failure with boosted

PIs is associated with a reduced risk of selection of drug resistance mutations.⁴

Few data from observational studies exist on how these differences in genetic barriers, defined as the number of amino acid changes required to confer resistance, and the degree of cross-resistance may drive the emergence of drug resistance and hence affect the success of first-line therapy and beyond.⁵⁻⁷ Moreover, to our knowledge, only 1 head-to-head comparison of boosted PI (lopinavir) vs NNRTI-based (efavirenz) first-line combination therapy has been conducted to date. The AIDS Clinical Trial Group 5142 presented preliminary data on this randomized, open-label trial and observed no difference in rates of primary study end points, which were virological failure and/or treatment dis-

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continuation due to toxic effects.⁸ However, the authors noted trends for a shorter time to those end points in the lopinavir arm and for more resistance accumulation on virological failure in the efavirenz arm.

Our objectives were to study the frequency and patterns of the emergence of viral drug resistance in patients from a large cohort in whom first-line cART failed in a routine clinical setting and to assess the potential impact on future therapies. Therefore, we analyzed patients from the Swiss HIV Cohort Study (SHCS) who were grouped according to their third drug, defined as the additional drug besides NRTIs or lamivudine or emtricitabine in standard 2-class combination therapy (NNRTI, PI, or PI/r). Further analyses were performed to compare the 3 regimen types with regard to virological failure and treatment discontinuation.

METHODS

STUDY DESIGN AND PARTICIPANTS

Included in this analysis were the cART-naïve patients from the SHCS^{9,10} who had initiated antiretroviral treatment between January 1, 1999, when genotyping became available in clinical practice in Switzerland, and December 31, 2005, with either 2 NRTIs and 1 NNRTI or 2 NRTIs and 1 PI (PI/r or unboosted). In case patients experienced an early adverse reaction to the initial treatment, the second cART regimen was considered if the initial regimen was followed for less than 30 days and if no treatment interruption occurred between the first and second regimens. As another inclusion criterion, 1 additional on-treatment HIV RNA measurement after the attainment of undetectable viral load levels of less than 50 copies/mL had to be available, or in patients in whom viral suppression was not achieved, 1 viral load after 180 days or more of continuous therapy. This criterion was required to evaluate our study participants with regard to virological failure while receiving therapy, which was defined as follows: (1) viral rebound with 2 consecutive viral loads greater than 500 copies/mL after previous attainment of undetectable HIV RNA levels, (2) 1 value greater than 500 copies/mL followed by a stop or a modification of the current therapy in patients with previous suppression of viral replication to undetectable levels, or (3) 1 viral load greater than 500 copies/mL after 180 days of continuous treatment in patients without previous suppression of viral load to undetectable levels. The date of the first HIV RNA measurement that indicated a virological failure was considered the failure date.

Drug resistance tests and clinical data collected up until February 1, 2007, were used for this analysis. Resistance tests were included in statistical analyses if the blood sampling was performed on or after the presumed date of virological failure or within 1 month after stopping the initial regimen if the patient continued not to receive treatment. Mean HIV RNA levels at the time of resistance testing were 3.55, 3.33, and 3.23 log copies/mL for the NNRTI group, the PI group, and the PI/r group, respectively (Kruskal-Wallis test, $P = .66$). Resistance data stem from routine clinical testing (60% of all tests) performed by the 4 laboratories in Switzerland authorized by the Federal Office of Public Health and tests specifically performed for this project from frozen repository samples (40%) if specimens were available. All laboratories performed population-based sequencing of the full protease gene and in minimum codons 28 to 225 of the reverse transcriptase gene using commercial assays (Viraseq vs 1, PE Biosystems, Rotkreuz, Switzerland; Viraseq vs 2, Abbott AG, Baar, Switzerland; and vircoTYPE HIV-1 Assay,

Virco Laboratory, Mechelen, Belgium) and in-house methods.¹¹ Resistance data were collected and stored using SmartGene's Integrated Database Network System (version 3.3.0, SmartGene, Zug, Switzerland). The degree of drug resistance was evaluated using the Stanford algorithm¹² version 4.2.6 and a modified version of the Fall 2006 International AIDS Society–USA drug mutation list,¹³ in which the following major mutations were included: NRTIs: M41L, A62V, K65R, D67N, K70E/R, L74V, V75I, F77L, Y115F, F116Y, Q151M, M184V/I, L210W, T215Y/F, K219Q/E, and insertions at codon 69; NNRTIs: L100I, K103N, V106M/A, V108I, Y181C/I, Y188C/L/H, G190S/A, P225H, and P236L; and PIs: D30N, V32I, L33F, M46I/L, I47V/A, G48V, I50V/L, L76V, V82A/F/T/S/L, I84V, N88S, and L90M. Mutations that confer resistance against entry inhibitors were not considered. The 5 resistance categories from the Stanford algorithm were regrouped for this analysis: viruses with a genotypic sensitivity score less than 15 were considered susceptible, those with a genotypic sensitivity score in the range of 15 to 59 were considered to have intermediate resistance, and those with a genotypic sensitivity score greater than 59 were considered to have high resistance. Throughout this study, lamivudine and emtricitabine were considered separate drug classes. In these drugs, the emergence of resistance on failure follows a pattern that is distinct from other NRTIs because only a single mutation at codon 184 is required to confer high resistance.¹⁴

The SHCS was approved by ethics committees of all participating institutions, and written informed consent was obtained from all participants.

STATISTICAL ANALYSES

All statistical analyses were stratified by regimen type (PI group [cART that contained 1 unboosted PI], PI/r group [1 PI/r], or NNRTI group [1 NNRTI]). Patterns of drug resistance were assessed using the Kruskal-Wallis test for continuous data and Fisher exact test for categorical data. Failure rates were analyzed using Kaplan-Meier life tables and univariate and multivariate Cox regression. We performed a competing risk analysis to estimate time receiving first-line therapy, which took into account that some patients were stopping cART because of reasons other than virological failure, such as ART-related toxic effects.^{15,16} The primary event of interest was stopping or modification of a regimen because of virological failure, and stopping or modification of a regimen owing to adverse events (adverse effects and laboratory abnormalities) was considered a competing risk event. For patients not reaching these end points, the analysis was censored at the stop date of the initial cART regimen or the last date of follow-up, whichever occurred first. For the estimation of Cox model parameters, the type of end point (virological failure or adverse event) and year of cART initiation were included as strata. Modeling assumptions were verified using the Schoenfeld residuals method.¹⁷ Reasons for stopping cART are systematically assessed in the SHCS using the Data Collection on Adverse Events of Anti-HIV Drugs reporting scheme.¹⁸

Models were adjusted for baseline demographics and the following potential confounders: HIV RNA viral load and CD4 cell count at therapy initiation as well as adherence.¹⁹ Baseline measurements for HIV RNA and CD4 cell counts were missing for approximately one-fifth of all patients (irrespective of treatment group). Hence, those variables were stratified (<1000, 1000–9999, and $\geq 100\,000$ copies/mL for HIV RNA; <200, 200–349, and ≥ 350 cells/ μ L for CD4 cell count), and additional strata were included for missing values.

All statistical tests were 2-sided. $P < .05$ was considered statistically significant. No adjustments for multiple testing were made. Analyses were performed using Stata SE 9.2 (StataCorp, College Station, Texas).

Table 1. Characteristics of 1812 Patients Initiating cART Between January 1, 1999, and December 31, 2005

Characteristic	No. (%) of Patients ^a		
	PI (n=489)	PI/r (n=518)	NNRTI (n=805)
Age, median (range), y	36 (30-42)	38 (32-45)	38 (32-44)
Male	300 (61)	371 (72)	551 (68)
Transmission category			
Homosexual-bisexual contact	120 (24)	197 (38)	269 (33)
Heterosexual contact	246 (50)	222 (43)	400 (50)
Injection drug use	107 (22)	69 (13)	101 (12)
Other	16 (3)	30 (6)	35 (4)
CDC stage C	89 (18)	120 (23)	157 (20)
Year of cART initiation, median (IQR)	2000 (1999-2001)	2003 (2002-2004)	2002 (2000-2004)
CD4 cell count at cART initiation			
Median (IQR)	198 (108-334)	182 (76.5-304)	209 (124-297)
<200 cells/ μ L	203 (42)	233 (45)	321 (40)
Missing	84 (17)	78 (15)	126 (16)
HIV RNA at cART initiation			
Median log (IQR)	4.76 (4.11-5.20)	5.08 (4.58-5.59)	4.90 (4.38-5.34)
$\geq 100\,000$ copies/mL	133 (27)	234 (45)	285 (35)
Missing	98 (20)	100 (19)	149 (18)
Adherence			
100	116 (74)	332 (81)	459 (77)
95-99	35 (22)	75 (18)	123 (21)
<95	5 (3)	4 (1)	14 (2)
Missing	333 (68)	107 (21)	209 (26)
NRTI			
Zidovudine-lamivudine	316 (65)	359 (69)	552 (69)
Stavudine-lamivudine	96 (20)	21 (4)	52 (6)
Didanosine-stavudine	50 (10)	12 (2)	28 (4)
Tenofovir disoproxil fumarate-emtricitabine	0	28 (5)	26 (3)
Tenofovir-lamivudine	4 (0.8)	60 (12)	102 (13)
Abacavir sulfate-containing combination	10 (2)	15 (3)	14 (2)
Other	13 (3)	23 (4)	31 (4)
Frequency of lamivudine- or emtricitabine-containing backbone combinations	430 (88)	493 (95)	762 (95)
Third drug (PI or NNRTI)			
Nelfinavir mesylate	430 (88)	0 (0)	0
Indinavir sulfate	24 (5)	56 (11)	0
Atazanavir sulfate	21 (4)	46 (9)	0
Lopinavir	0	391 (76)	0
Efavirenz	0	0	736 (91)
Other	14 (3)	25 (5)	69 (9)

Abbreviations: cART, combination antiretroviral treatment; CDC, Centers for Disease Control and Prevention; IQR, interquartile range; NRTI, nucleoside reverse transcriptase inhibitor; NNRTI, nonnucleoside reverse transcriptase inhibitor; PI, protease inhibitor; PI/r, ritonavir-boosted protease inhibitor.

^aData are presented as number (percentage) of patients unless otherwise indicated.

RESULTS

Between January 1, 1999, and December 31, 2005, 2751 study participants in the SHCS initiated therapy with cART, of which 939 patients (34%) were excluded from our primary analyses because of short treatment duration with the same regimen or missing HIV RNA values. Patient characteristics associated with exclusion were following a PI-based therapy, use of injection drugs, female sex, or having higher CD4 cell counts, all characteristics known to be associated with premature therapy discontinuation.^{20,21} The second treatment was considered for 141 patients (44 in the PI group, 48 in the PI/r group, and 49 in the NNRTI group). Of the remaining 1812 patients who contributed 3789 person-years to the competing risk analysis, 489 (27%) initiated cART with unboosted PIs, 518

(29%) with boosted PIs, and 805 (44%) with NNRTIs as the group-specific third drug. A median of 5.7 viral load values per patient per year were obtained.

Baseline characteristics at the time of cART initiation for the 1812 patients are given in **Table 1**. Of note, patients who initiated cART with boosted PIs in general had a lower CD4 cell count and a higher HIV viral load at baseline compared with the PI group and the NNRTI group. Most patients received lamivudine as part of their treatment, ranging from 88% in the unboosted PI group to 95% in the 2 other groups. The most common third drug in the 3 groups was unboosted nelfinavir mesylate (88%), boosted lopinavir (76%), and efavirenz (91%).

In total, 177 patients experienced a virological failure (108 in the unboosted PI group, 24 in the boosted PI/r group, and 45 in the NNRTI group). For those pa-

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Pattern	No. (%) of Patients ^a			P Value
	PI (n=108)	PI/r (n=24)	NNRTI (n=45)	
Resistance test at baseline ^b	64 (59)	14 (58)	34 (76)	
Resistance test at failure	84 (78)	20 (83)	38 (84)	
HIV-1 subtype available	95 (88)	21 (88)	44 (98)	
Subtype B	59 (62)	13 (62)	25 (57)	
Subtype other than B	36 (38)	8 (38)	19 (43)	
No. of IAS-USA mutations, median (IQR)	2 (1-2.5)	0 (0-1)	2 (0-3)	<.001 ^c
No. of drug classes affected (intermediate or high-level resistance against at least 1 drug according to Stanford algorithm), median (IQR)	2 (1-2)	0 (0-1)	2 (0-2)	<.001 ^c
Virus susceptible to all drugs in class according to Stanford algorithm				
NRTI	72 (86)	18 (90)	30 (79)	.46 ^d
Lamivudine or emtricitabine	23 (27)	15 (75)	16 (42)	<.001 ^d
Third drug	40 (48)	18 (90)	20 (53)	<.001 ^d
Drug classes affected (high-level resistance against at least 1 drug per class according to Stanford algorithm)				
By class type				
NRTI	2 (2)	1 (5)	3 (8)	.27 ^d
Lamivudine or emtricitabine	61 (73)	5 (25)	22 (58)	<.001 ^d
Third drug	17 (20)	1 (5)	21 (55)	<.001 ^d
By class combination				
No resistance	18 (21)	15 (75)	13 (34)	
Lamivudine or emtricitabine (1 class)	48 (57)	3 (15)	4 (10)	
NRTI (1 class)	1 (1)	0	0	
Third drug (1 class)	3 (4)	0	2 (5)	
Lamivudine or emtricitabine plus NRTI (2 classes)	0	1 (5)	0	
Lamivudine or emtricitabine plus third drug (2 classes)	13 (16)	1 (5)	16 (42)	
NRTI plus third drug (2 classes)	1 (1)	0	1 (3)	
Lamivudine or emtricitabine plus NRTI plus third drug (3 classes)	0	0	2 (5)	

Abbreviations: cART, combination antiretroviral treatment; HIV-1, human immunodeficiency virus 1; IAS-USA, International AIDS Society–USA; IQR, interquartile range; NRTI, nucleoside reverse transcriptase inhibitor; NNRTI, nonnucleoside reverse transcriptase inhibitor; PI, protease inhibitor; PI/r, ritonavir-boosted protease inhibitor.

^aData are presented as number (percentage) of patients unless otherwise indicated. Percentages may not total 100% because the numbers of patients who had at least 1 resistance mutation, the numbers of patients with NRTI, lamivudine, and third drug mutations, and the numbers of patients without high resistance against NRTI, lamivudine, or the third drug are not shown.

^bThe following baseline resistance mutations were observed: PI group: 1 patient with reverse transcriptase gene (*RT*) 219Q, 1 patient with *RT* 41L and 210W, 1 patient with *RT* 65R, and 1 patient with *PR* 33F; PI/r group: 1 patient with *RT* 103N; and NNRTI group: 1 patient with *RT* 103N.

^cKruskal-Wallis test.

^dFisher exact test.

tients, 112 baseline resistance test results (64 [59%], 14 [58%], and 34 [76%] of patients in the PI, PI/r, and NNRTI groups, respectively; **Table 2**) were available, and pre-existing resistance was detected in 6 samples (5%). Resistance test results at the time of virological failure could be obtained for 142 patients (84 [78%], 20 [83%], and 38 [84%] in the PI, PI/r, and NNRTI groups, respectively) **Table 3**. At least 1 relevant resistance mutation was detected in 71 of the 84 patients (84%; 95% confidence interval [CI], 75%-92%) in the unboosted PI group, 6 of 20 patients (30%; 95% CI, 12%-54%) in the boosted PI/r group, and 25 of 38 (66%; 95% CI, 49%-80%) in the NNRTI group (Fisher exact test, $P < .001$; **Figure 1**). Patients in the unboosted PI and NNRTI groups acquired a median of 2 mutations during treatment (interquartile range [IQR], 1-2.5 and 0-3, respectively), whereas the median number of mutations in the boosted PI group was 0 (IQR, 0-1) (Kruskal-Wallis test, $P < .001$; Figure 1).

A similar pattern was observed when resistance against the 4 drug classes (NRTI, lamivudine or emtricitabine, NNRTI, and PI) was compared using the Stanford algorithm, whereby intermediate or high resistance against 1

or more drugs in a class signified class resistance. We observed that more classes were affected in unboosted PI-based (median, 2; IQR, 1-2) and NNRTI-based (median, 2; IQR, 0-2) regimens compared with boosted PI-based regimens (median, 0; IQR, 0-1; Kruskal-Wallis test, $P < .001$; Figure 1). This difference remained statistically significant when only high-level resistance was considered (data not shown). High-level resistance against lamivudine or emtricitabine was observed in 73% (95% CI, 62%-82%) of the PI group, 25% (95% CI, 9%-49%) of the PI/r group, and 58% (95% CI, 41%-74%) of the NNRTI group (Fisher exact test; $P < .001$). High-level resistance against the respective third drug was most prevalent in the NNRTI group at 55% (95% CI, 38%-71%), followed by the unboosted PI group at 20% (95% CI, 12%-30%) and the boosted PI group at 5% (95% CI, 0%-25%) (Fisher exact test, $P < .001$). Multidrug resistance predominantly occurred as resistance against the third drug and against lamivudine or emtricitabine and was also more frequent in the NNRTI group (50%; 95% CI, 33%-67%) than in the PI group (17%; 95% CI, 9%-26%) or PI/r group (10%; 95% CI, 0.1%-32%, respectively) (Fisher exact test, $P < .001$).

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Table 3. International AIDS Society–USA Mutations in 177 Patients in Whom First-Line cART Failed

Mutation	No. (%) of Patients		
	PI (n=108)	PI/r (n=24)	NNRTI (n=45)
No. tested	84 (78)	20 (83)	38 (84)
NRTI or lamivudine or emtricitabine			
RT gene			
41L	4 (5)	1 (5)	1 (3)
62V	5 (6)	0	1 (3)
65R	0	0	3 (8)
67N	8 (10)	0	1 (3)
70R/E	5 (6)	1 (5)	1 (3)
75I	0	0	1 (3)
115F	0	0	1 (3)
184I/V	61 (73)	5 (25)	22 (58)
210W	0	1 (5)	1 (3)
215F/Y	3 (4)	1 (5)	4 (10)
219E/Q	2 (2)	0	1 (3)
Any TAM1	4 (5)	1 (5)	3 (8)
Any TAM2	12 (14)	1 (5)	2 (5)
Third drug (NNRTI or PI)			
RT gene			
103N	0	0	10 (26)
106A/M	0	0	1 (3)
108I	0	0	3 (8)
181C	0	0	5 (13)
188C	0	0	1 (3)
190A	0	1 (5)	2 (5)
225H	0	0	2 (5)
Protease gene			
30N	18 (21)	0	0
32I	1 (1)	0	0
33F	1 (1)	1 (5) ^a	0
46I/L	11 (13)	2 (10) ^a	1 (3)
82A/F/T/S/L	0	1 (5) ^a	0
84V	1 (1)	0	0
88S	12 (14)	0	0
90M	13 (15)	0	0
Any third drug	44 (52)	2 (10)	18 (47)

Abbreviations: cART, combination antiretroviral treatment; NRTI, nucleoside reverse transcriptase inhibitor; NNRTI, nonnucleoside reverse transcriptase inhibitor; PI, protease inhibitor; PI/r, ritonavir-boosted protease inhibitor; RT, reverse transcriptase; TAM1, any RT gene mutation of the following: 41L, 210W, 215Y; TAM2, any RT gene mutation of the following: 67N, 70R, 215F, 219Q.

^aThe 46L and the 82A mutations were newly selected during therapy with boosted indinavir sulfate. The baseline sequence was available to exclude the presence of transmitted resistance.

We further assessed rates of virological failure and adverse events in the different treatment groups (**Figure 2**). Analyzing the 1812 included patients with respect to virological failure events, we found that crude rates (per 100 person-years) were highest in the unboosted PI group (10.3; 95% CI, 8.5-12.4) and comparable between the boosted PI group (2.7; 95% CI, 1.8-4.0) and the NNRTI group (2.4; 95% CI, 1.8-3.3; log-rank $P < .001$; **Table 4**). A multivariate Cox regression adjusted for potential confounders confirmed this finding. Using the NNRTI group as a reference, hazard ratios were 1.19 (95% CI, 0.71-2.0) for the boosted PI group and 3.83 (95% CI, 2.62-5.59) for the unboosted PI group.

Crude rates for toxic effects related to cART were higher in the PI-based regimens (unboosted PIs: 8.9; 95% CI,

7.3-10.9; boosted PIs: 10.9; 95% CI, 9.0-13.3) compared with 7.8 (95% CI, 6.6-9.2) in the group taking NNRTIs (log-rank test; $P = .03$). When considering both types of events (virological failure and toxic effects) simultaneously, patients taking unboosted PIs had the highest rate at 19.2 (95% CI, 16.7-22.1) followed by boosted PIs (13.6; 95% CI, 11.4-16.3) and NNRTIs (10.3; 95% CI, 8.9-11.8; log-rank test; $P < .001$), which was consistent with results from the multivariate competing risk Cox model. Compared with the NNRTI group, relative hazards for experiencing either a virological failure or a cART-related toxic effect were 1.36 (95% CI, 1.07-1.73) and 1.75 (95% CI, 1.41-2.18) for patients taking boosted PIs and unboosted PIs, respectively. When repeating the competing risk analysis including all patients who had started cART (intent to treat, $n = 2751$), we obtained a lower hazard ratio for the unboosted PI group (1.24; 95% CI, 1.05-1.46) but similar estimates for the boosted PI group (1.28; 95% CI, 1.07-1.52; **Table 4**). Reasons for premature therapy discontinuation of the 939 patients additionally included in the intent-to-treat analysis are shown in **Table 5**.

We also looked at subsequent virological failures in 1001 of 1812 patients (55%; including 141 of 177 patients in whom first-line therapy failed) who initiated a new treatment after stopping first-line therapy and who were not lost to follow-up (107; 6%). We observed that patients in whom first-line therapy had already failed virologically were more likely to experience second-line therapy failure (18 cases; incidence rate, 10.4 per 100 person-years; 95% CI, 6.6-16.5) compared with patients without previous failure (27 patients; incidence rate, 2.4 per 100 person-years; 95% CI, 1.7-3.6; log-rank test; $P < .001$). Because of the small number of cases, this analysis could not be stratified further.

COMMENT

In this study, we observed clinically relevant differences in the emergence of drug resistance for the 3 types of initial regimens. Although cART-containing boosted PIs and NNRTIs appeared to be equivalent with regard to the virological failure rate (ie, 2.7 and 2.4 per 100 person-years, respectively), significantly more resistance emerged in the NNRTI group at virological failure. Virus strains resistant against lamivudine or emtricitabine were present in 58% of patients with virological failure in the NNRTI group, and 55% of all viral samples in this group exhibited resistance against NNRTI. Even more worrisome, multidrug resistance against NNRTIs and at least 1 component of NRTIs and lamivudine or emtricitabine was observed in half of all patients with virological failure in this group (**Table 2**). Nonetheless, NNRTI-containing combinations showed better tolerability than PI-based regimens. Thus, our analysis demonstrates that despite similar potency, boosted PIs are superior to more tolerable NNRTI regimens when comparing the emergence of drug resistance in patients in whom therapy is failing.

The low genetic barrier of NNRTIs and the high potential for cross-resistance in this drug class have long been known from in vitro experiments and clinical trials,^{2,14,22}

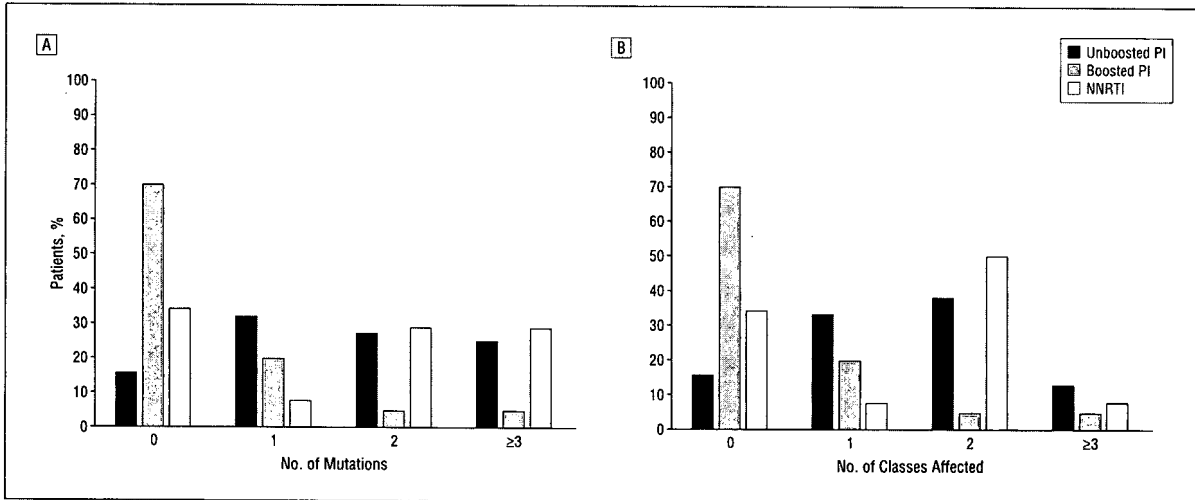


Figure 1. Drug resistance in patients experiencing virological failure of first-line combination antiretroviral treatment for whom genotypic resistance test results could be retrieved (142/177 [80%]). A, Number of International AIDS Society–USA mutations detected (excluding minor protease mutations). B, Frequency of class resistance, defined as at least 1 drug with a genotypic sensitivity score greater than or equal to 15 according to the Stanford algorithm. NNRTI indicates nonnucleoside reverse transcriptase inhibitor; PI, protease inhibitor.

but the impact of those aspects on the emergence of drug resistance in routine clinical practice and the consequences for future therapies have rarely been investigated. In a retrospective cohort analysis, Phillips et al⁶ reported a significantly higher risk of acquiring third drug-specific resistance and multidrug resistance for patients initiating cART with NNRTI compared with boosted PI. Preliminary results from AIDS Clinical Trial Group 5142 also indicated more drug resistance accumulation in NNRTI-based regimens compared with boosted PI regimens.⁸ However, no differences in virological failure and/or stopping because of toxic effects between NNRTI-based and boosted PI-based regimen types were observed.

Our results confirm and extend these previous observations. We found that virological failure of NNRTI-containing regimens led to the emergence of multidrug resistance in half of all cases, 5 times more often than in the boosted PI group. In addition, we observed that almost all cases of multiclass resistance (with 1 exception in the PI group and 1 in the NNRTI group; Table 2) involved the reverse transcriptase mutation *M184V/I*, whereas high-level resistance against NRTIs other than lamivudine or emtricitabine remained rare. Hence, our data demonstrate that NNRTI-based regimens carry a markedly higher risk of losing multiple drug classes in case of failure.

The inclusion of competing risks in our time-to-event analysis has allowed us to gain a more accurate picture of the success of initial cART. The differences in hazard ratios between the competing risk approach and the conventional approach of estimating Cox models imply that the benefits of higher potency of newer regimens compared with unboosted PIs are partially outweighed by the higher frequency of adverse events, especially in boosted PI-containing regimens. This loss of benefit was even more pronounced in the intent-to-treat analysis, in which event rates and relative hazards were no longer different between boosted and unboosted PIs. Further analyses showed that patients following boosted PI regimens had more adverse events related to cART in the first 180 days

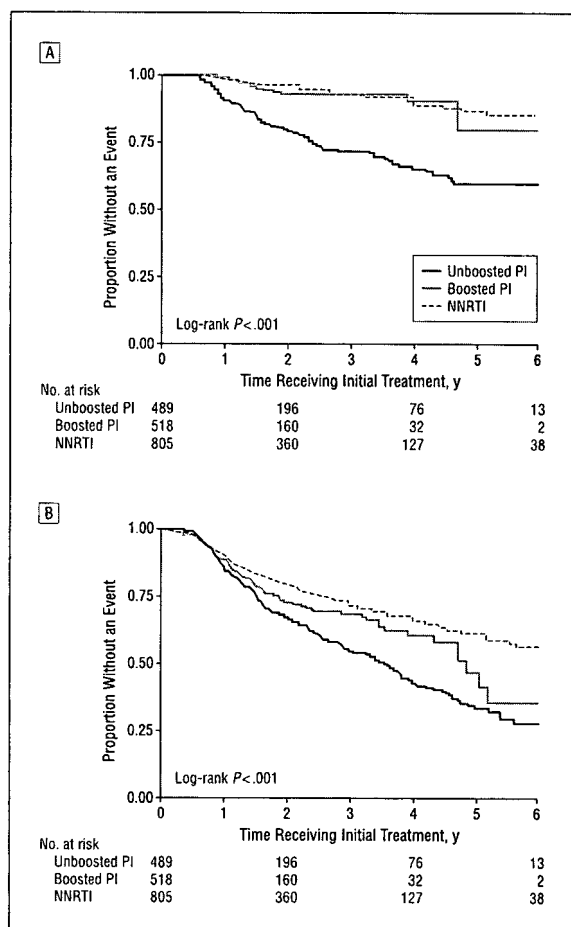


Figure 2. Kaplan-Meier curves for time to first-line therapy discontinuation. A, Therapy discontinuation because of virological failure. B, Therapy discontinuation because of virological failure and combination antiretroviral treatment-related adverse events. NNRTI indicates nonnucleoside reverse transcriptase inhibitor; PI, protease inhibitor. For more detailed group comparisons, see Table 4.

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Table 4. Summary of Time-to-Event Analyses				
Variable	No. (%) of Patients ^a			P Value
	PI	PI/r	NNRTI	
As-treated analysis (n = 1812)	489 (27)	518 (29)	805 (44)	ND
Median time undergoing first-line regimen, d (IQR)	489 (346-1092)	514.5 (327-835)	655 (364-1154)	<.001 ^b
Crude incidence rate of failures per 100 person-years (95% CI)	10.3 (8.5-12.4)	2.7 (1.8-4.0)	2.4 (1.8-3.3)	<.001 ^c
Treatment failures	108 (22)	24 (5)	45 (6)	ND
Crude incidence rate of adverse events per 100 person-years (95% CI)	8.9 (7.3-10.9)	10.9 (9.0-13.3)	7.8 (6.6-9.2)	.03 ^c
Treatment stopped because of cART-related adverse events (all)	94 (19)	98 (19)	144 (18)	ND
Abnormal fat distribution	27 (29)	9 (9)	18 (12)	
Concern of CVD	13 (14)	20 (20)	8 (6)	
Hypersensitivity reaction	1 (1)	1 (1)	2 (1)	
Toxic effects to abdomen or gastrointestinal tract	31 (33)	31 (32)	19 (13)	
Toxic effects to nervous system	9 (10)	3 (3)	64 (44)	
Toxic effects to kidneys	0 (0)	16 (16)	2 (1)	
Hematological toxic effects	1 (1)	9 (9)	11 (8)	
Lactate elevation	0	0	1 (0.7)	
Other toxic effects	12 (13)	9 (9)	19 (13)	
Crude incidence rate of stopping because of virological failure or adverse events per 100 person-years (95% CI)	19.2 (16.7-22.1)	13.6 (11.4-16.3)	10.3 (8.9-11.8)	<.001 ^c
Any event (failures or adverse events)	202 (41)	122 (24)	189 (23)	ND
Relative hazard of stopping cART because of adverse events or virological failure (competing risk analysis) (95% CI) ^d	1.75 (1.41-2.18)	1.36 (1.07-1.73)	1 [Reference]	NA
Intent-to-treat analysis (n=2751)	822 (30)	792 (29)	1137 (41)	NA
Crude incidence rate of stopping because of virological failure or adverse events per 100 person-years (95% CI)	25.0 (22.3-28.0)	25.9 (22.9-29.4)	18.4 (16.5-20.4)	.001 ^c
Any event (failures or adverse events)	294 (36)	249 (31)	358 (32)	NA
Relative hazard of stopping cART because of adverse events or virological failure (competing risk analysis) (95% CI) ^d	1.24 (1.05-1.46)	1.28 (1.07-1.52)	1 [Reference]	NA

Abbreviations: cART, combination antiretroviral treatment; CI, confidence interval; CVD, cardiovascular disease; IQR, interquartile range; NA, not applicable; ND, not done; NNRTI, nonnucleoside reverse transcriptase inhibitor; PI, protease inhibitor; PI/r, ritonavir-boosted protease inhibitor.

^aData are presented as number (percentage) of patients unless otherwise indicated. The on-treatment analysis includes patients with at least 1 additional HIV RNA measurement after the attainment of undetectable HIV viral loads or, if not the case, patients with at least 1 HIV RNA measurement after a minimum of 180 days of continuous treatment with first-line cART. The intent-to-treat analysis includes all patients who initiated cART between January 1, 1999, and December 31, 2005, with combination antiretroviral therapy.

^bKruskal-Wallis test.

^cLog-rank test.

^dCox model adjusted for age, sex, ethnicity, transmission category, baseline CD4 cell counts, and human immunodeficiency virus (HIV) RNA at time of cART initiation.

of therapy (39.4 per 100 person-years; 95% CI, 33.1-46.9) than any of the other groups, with 33.3 (95% CI, 28.7-38.7) and 25.0 (95% CI, 20.3-30.7) in patients taking NNRTIs and PIs, respectively. Overall, NNRTIs were generally better tolerated, and patients following those regimens continued with their initial therapy for a longer period in both the on-treatment analysis and the intent-to-treat analysis.

This study has some limitations. Potentially because of the somewhat shorter follow up-time, relatively few virological failures occurred in the group starting cART with newer regimen types and especially boosted PIs, but our results are largely consistent with other reports on resistance development.⁴ Like all observational studies, this analysis is prone to confounding. The time point of resistance testing might have affected our results because it has been shown that the longer maintenance of failing regimens leads to more resistance.²³ However, sensitivity analyses neither revealed a systematic confounding of our results by the time (per month) between presumed date of virological failure and the sampling date for resistance testing (odds ratio, 1.01; 95% CI, 0.97-

1.06) nor indicated any influence of viral load levels (per log increase) at the time of testing (OR, 0.94; 95% CI, 0.69-1.29).

Furthermore, studies²⁴ have demonstrated detrimental effects of transmitted resistance mutations on treatment success. In the present analysis, pre-cART resistance tests were available for 63% of all patients with a virological failure, and we therefore can not fully exclude potential confounding of our results by baseline resistance.

Another limitation of this study compared with clinical trials is the high variation of drug combinations within the 3 treatment groups. To verify our findings, we limited the time-to-event analyses to the most frequent drugs in the boosted PI group and the NNRTI group (lopinavir [76%] and efavirenz [91%]), which yielded similar results for the comparison of virological failure rates. For the competing risk analysis, however, the previously observed difference in the hazard ratios between boosted PIs and NNRTIs disappeared. A secondary analysis showed that this difference was mainly because of the exclusion of boosted indinavir sulfate, which exhibited

Table 5. Reasons for Premature Therapy Discontinuation in 939 Patients Treated for Less Than 6 Months or With Missing Human Immunodeficiency Virus Viral Load Values and Excluded From the Primary Risk Analyses

Reason	No. (%) of Patients		
	PI (n=333)	PI/r (n=274)	NNRTI (n=332)
Treatment stop because of	92 (28)	127 (46)	169 (51)
cART-related adverse events (all)			
Abnormal fat distribution	1 (1)	2 (2)	1 (0)
Concern of CVD	0	0	2 (1)
Hypersensitivity reaction	5 (5)	9 (7)	26 (15)
Toxicity from abdomen or gastrointestinal tract	27 (29)	55 (43)	28 (17)
Toxic effects to nervous system	4 (4)	3 (2)	44 (26)
Toxic effects to kidneys	2 (2)	12 (9)	3 (2)
Toxic effects to endocrine system	0	0	1 (0.6)
Hematological toxic effects	6 (6)	12 (9)	20 (12)
Other toxic effects (not further specified)	47 (51)	34 (27)	44 (26)
Treatment failure (virological, immunological, or clinical)	33 (10)	6 (2)	11 (3)
Patient's wish or physician's decision (not further specified)	92 (28)	74 (27)	81 (24)
Patient deceased	3 (0.9)	4 (2)	6 (2)
Unknown reason	113 (34)	63 (23)	65 (20)

Abbreviations: cART, combination antiretroviral treatment; CVD, cardiovascular disease; NNRTI, nonnucleoside reverse transcriptase inhibitor; PI, protease inhibitor; PI/r, ritonavir-boosted protease inhibitor.

a far higher rate of treatment discontinuation caused by toxic effects (29.5 per 100 person-years; 95% CI, 20.4-42.7) compared with the remaining third drugs in the boosted PI group (8.7; 95% CI, 6.9-11.1). This finding implies that the apparent tolerability disadvantage of boosted PI compared with the NNRTI regimen might not be as pronounced or even absent with newer, more tolerable combinations that contain boosted atazanavir sulfate or lopinavir.

Previous studies²⁵ have demonstrated associations of adherence and pharmacokinetic drug levels with virological failure and the emergence of drug resistance. Systematic collection of adherence information in the SHCS started in the middle of 2003.¹⁹ Since we do not have complete assessments of adherence for all patients, we performed a sensitivity analysis by confining our time-to-event analyses to patients with at least 1 adherence measure while receiving first-line cART (n=927, data not shown). Of note, our findings were not altered by this, and we are therefore confident that the observed differences in virological failure rates truly reflect drug potency. Therapeutic drug monitoring has been introduced only recently in the SHCS, so systematic analyses of therapeutic drug monitoring are not possible at this time.

In summary, our findings have potentially important clinical implications. Given the risk of severely compromising future therapy options in case of virological failure while taking NNRTIs and the equivalent potency of boosted PI regimens, physicians should critically assess a patient's ability to adhere to NNRTI-based regimens and

to cope with the potential toxic effects, such as adverse effects on the central nervous system. If problems are expected, starting or changing to PI/r therapy most likely represents a safer choice, since a PI/r tends to accumulate less resistance in case of virological failure. Should our findings be confirmed, they should be implemented in treatment guidelines and also be considered for future treatment strategies in developing countries.

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Tenofovir Nephrotoxicity: Focusing Research Questions and Putting Them into Clinical Context

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(See the article by Goicoechea et al., on pages 102–8.)

Multiple reports have indicated that the antiretroviral drug tenofovir has renal toxicity [1–9]. However, beyond simply identifying the existence of this potential toxicity, the key clinical questions that we as providers rely on to assist us in understanding which patients are at highest risk and how often to assess for toxicity have not really been answered. The study by Goicoechea et al. [10] in this issue of the *Journal* takes a key step in helping us to address at least part of this first question. Moreover, this study raises many other points worthy of discussion.

Goicoechea et al. compared kidney function over time among 3 cohorts of patients receiving tenofovir disoproxil fumarate (TDF) plus a ritonavir-boosted protease inhibitor (PI), TDF plus a non-nucleoside reverse-transcriptase inhibitor (NNRTI), and regimens not containing TDF. By use of either the Cockcroft-Gault or the Modification of Diet in Renal Disease equation, a greater decline in kid-

ney function was seen among those persons receiving TDF and a ritonavir-boosted PI than among those in the other 2 groups. Of key interest is that the other 2 groups (i.e., those receiving TDF and an NNRTI and those not receiving TDF) also experienced decrements in their kidney function over time.

Tenofovir toxicity happens. Our understanding of it is in its early stages. Important questions still requiring answers include:

- Who is at risk?
- How frequently is it seen?
- Are there signs earlier than an elevation in creatinine level?
- How is it diagnosed?

Secondary analyses from clinical trials comparing antiretroviral regimens suggest that the risk of tenofovir toxicity is low (~1%–2%) among those enrolled in clinical trials [11, 12]. However, the generalizability of estimates of safety events from trials need to be carefully considered, for 2 reasons. First, clinical trials frequently limit enrollment to subjects who do not have competing risks for outcomes that may not be affected by the therapy. For example, it would make little sense to enroll persons with lung cancer into a trial comparing coronary artery stents. Because renal disease and most risk factors for renal disease could be considered competing [13–15], it is appropriate that such persons might be excluded from the trials, but it is unfortunate in that it likely

results in an underestimate of risk (the extent is unknown at this point). Second, even taken at face value in the populations enrolled in clinical trials, such trials are rarely powered to provide estimates of safety end points. So while these clinical-trial data may comfort us in that there are certain populations at low risk for toxicity, the risks in other groups and in settings in which patients are not monitored as closely as clinical trials will clearly be higher.

Case series documenting toxicity related to tenofovir include both acute renal failure and Fanconi syndrome and have incited much concern about the cumulative risk of tenofovir toxicity in “real life” [1–9]. However, without a true assessment of the denominator (the number of persons receiving a tenofovir-containing regimen), this concern cannot be translated into a measurement of risk and puts us no closer to deriving policies that balance safety with cost and with practicality in screening or in affecting early cases of toxicity.

As a community of providers (academic, private practice, and industry), we need to recognize that we need more information, more guidance, and more answers. We need to assess what data we have, combine them, and provide evidence-based information concerning the risks that our patients experience. We need to recognize that the risk is likely not homogeneous among the universe of pa-

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tients who require antiretroviral therapy but that it probably varies from quite low to high in various subgroups. Goicoechea et al. add substantially to our information on this subject, but we still have so many questions yet to answer.

As a nephrologist, I think the biggest practical problem I face is not having sufficient detail about who is at risk and how to reliably and specifically diagnose a case of tenofovir toxicity. It is clear that persons with HIV infection as a group are aging and are developing increasing numbers of comorbidities. Kidney disease has increased and will likely continue to increase in this population [16]. If we biopsied the kidneys of everyone whose creatinine level rose from 1 to 1.5 mg/dL, the issue that I raised above would not be a concern—whenever a patient experienced such an increase, we would biopsy, know the histology, and treat or change therapy appropriately. But in medicine (particularly nephrology), we “play the odds.” If a person with type 2 diabetes mellitus who had received the diagnosis 5 years ago and had had proteinuria (~4 g) for several years experienced an elevation in creatinine level from 1 to 1.5 mg/dL, the odds suggest that diabetic nephropathy would be the likely cause, and therefore its treatment should be the primary focus. In the circumstance of renal disease in HIV infection, disruption of any successful antiretroviral regimen that is resulting in the suppression of viral replication may not make sense without further investigation to ensure that the rise in creatinine level was not simply the result of progression of the underlying kidney disease. However, the frequency of a case as straightforward as this is clearly much lower than we would like, and, without knowledge of how to diagnose toxicity, we may disrupt some component of successful regimens without adequate cause and miss the opportunity to treat non-medication-related kidney diseases at an early stage.

Having said that, as a nephrologist, tubular toxins never make me happy. I think about my kidneys every time I take a

nonsteroidal anti-inflammatory drug. Yet the attention generated by considerations of tenofovir toxicity can benefit our patients in other ways, including by raising awareness about kidney disease in general. This attention has brought about an enthusiasm to be proactive in assessing kidney health. With that enthusiasm, however, the following will most certainly happen, and we, as a community dedicated to the health of our patients, need to be prepared for it:

- Screening with urine dipsticks will find *more* proteinuria than we expected.
- Regular checks of serum creatinine levels will find *more* kidney disease *earlier*. Some of this kidney dysfunction will be related to tenofovir toxicity, and some will not.

When proteinuria and evidence of decreased kidney function is found, we need to be prepared with the appropriate diagnostic and therapeutic plans. The knowledge that angiotensin-converting enzyme (ACE) inhibitors and angiotensin-receptor blockers benefit most patients with kidney diseases is relatively widespread within most subspecialties of internal medicine (including infectious diseases), but more subtle points—such as how to manage an increase in creatinine level from 1.4 to 1.8 mg/dL after the initiation of an ACE inhibitor or the threshold for blood-pressure and blood-sugar control in someone with kidney disease and hypertension or diabetes mellitus—may not be as well understood.

With the improved survival and resultant aging of persons with HIV infection as a population, we are at a happy but critical juncture in the management of chronic HIV infection. There needs to be a considerable educational cross-pollination between the subspecialties of infectious diseases and nephrology. With the rapid development of newer therapeutic strategies for HIV infection and the changing natural history of treated infection, it is very likely that other medical subspecialties may also need further education to make them more fully informed regarding the risks and

benefits of the components of antiretroviral regimens. Similarly, it must be recognized that providers of care for HIV infection are often the default primary-care providers for their patients and require knowledge that extends beyond infectious diseases. Nephrologists understand this unexpected increase in scope of work, because we are in a similar situation with patients who require dialysis. We sympathize and can help, if asked.

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Risk of extensive virological failure to the three original antiretroviral drug classes over long-term follow-up from the start of therapy in patients with HIV infection: an observational cohort study.

Phillips AN, Leen C, Wilson A, Anderson J, Dunn D, Schwenk A, Orkin C, Hill T, Fisher M, Walsh J, Pillay D, Bansi L, Gazzard B, Easterbrook P, Gilson R, Johnson M, Sabin CA; UK Collaborative HIV Cohort (CHIC) Study.

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Abstract

BACKGROUND: The long-term durability of viral-load suppression provided by the three original antiretroviral drugs is not well characterised. We estimated the proportion of patients who had extensive triple-class failure during long-term follow-up and examined characteristics associated with an increased rate of failure.

METHODS: 7916 patients who started antiretroviral therapy with three or more drugs were followed up from the time that therapy started until the last viral-load measure. Extensive triple-class virological failure was defined by failure of three subclasses of nucleoside reverse transcriptase inhibitors, a non-nucleoside reverse transcriptase inhibitor, and a ritonavir-boosted protease inhibitor.

FINDINGS: 167 patients developed extensive triple-class failure during 27 441 person-years of follow-up. The Kaplan-Meier estimate for the cumulative risk of extensive triple-class failure was 9.2% by 10 years (95% CI 5.0-13.4). There was evidence that this rate has decreased over time (adjusted hazard ratio 0.86 [0.77-0.96] per year more recent; $p=0.006$). Of the 167 patients with extensive triple-class failure, 101 (60%) subsequently had at least one viral load less than 50 copies per mL. The risk of death by 5 years from the time of extensive triple-class failure was 10.6% (2.4-18.8, nine deaths).

INTERPRETATION: We have shown that extensive virological failure of the three main classes of drugs occurs slowly in routine clinical practice. This finding has implications for the planning of treatment programmes in developing countries, where additional drugs outside these classes are unlikely to be available for some time.

Comment in

A wake-up call for global access to salvage HIV drug regimens. [Lancet. 2007]

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Publication Types, MeSH Terms, Substances

Antiretroviral Drug Resistance Testing in Adult HIV-1 Infection: 2008 Recommendations of an International AIDS Society–USA Panel

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Resistance to antiretroviral drugs remains an important limitation to successful human immunodeficiency virus type 1 (HIV-1) therapy. Resistance testing can improve treatment outcomes for infected individuals. The availability of new drugs from various classes, standardization of resistance assays, and the development of viral tropism tests necessitate new guidelines for resistance testing. The International AIDS Society–USA convened a panel of physicians and scientists with expertise in drug-resistant HIV-1, drug management, and patient care to review recently published data and presentations at scientific conferences and to provide updated recommendations. Whenever possible, resistance testing is recommended at the time of HIV infection diagnosis as part of the initial comprehensive patient assessment, as well as in all cases of virologic failure. Tropism testing is recommended whenever the use of chemokine receptor 5 antagonists is contemplated. As the roll out of antiretroviral therapy continues in developing countries, drug resistance monitoring for both subtype B and non-subtype B strains of HIV will become increasingly important.

A panel of the International AIDS Society–USA published recommendations for HIV-1 drug resistance testing in HIV-1–infected adults in 1998, 2000, and 2003 [1–3]. Since the 2003 publication, drug resistance testing has become widespread in the developed world and has been accepted as an important adjunct to the management of patients with detectable plasma viremia who are receiving antiretroviral therapy. Moreover, person-to-person transmission of drug-resistant viruses occurs

in a variety of settings, including between adults and from mother to child [4, 5], indicating that testing for drug resistance before initiating therapy may be useful even for treatment-naïve patients. Novel resistance mutations that confer resistance to older drugs continue to be identified (figure 1), and newer-generation protease inhibitors (PIs) and reverse transcriptase inhibitors have been developed to counteract mutations that confer resistance to the older agents. Approval of agents from new classes, like integrase strand transfer inhibitors (INSTIs) and entry inhibitors, assure that drug resistance testing will become increasingly complex and important in case management in the years ahead. Testing methodologies have improved and are becoming more sensitive, and tests for viral coreceptor use (i.e., tropism) have been introduced.

With increasing patient access to antiretroviral drugs in the developing world, many of the same problems

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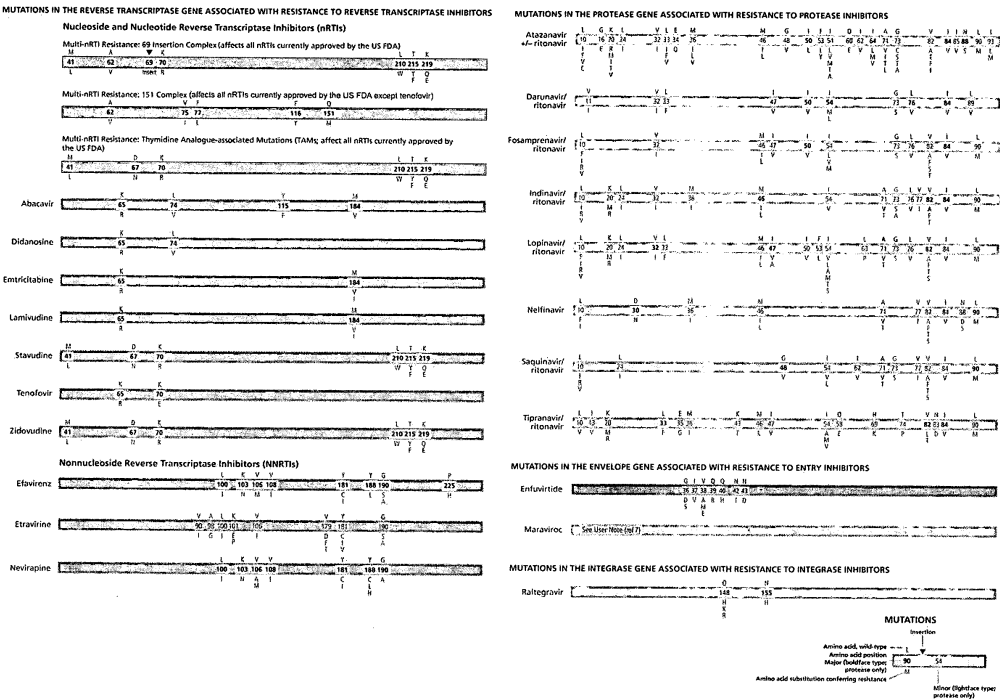


Figure 1. Mutations in HIV-1 that affect susceptibility to antiretroviral drugs, by HIV gene target. The letter above each position is the wild-type amino acid and the letter(s) below each position indicate the substitution(s) that are associated with drug resistance. Reprinted with permission from [6]. Detailed user notes and regular updates are available at the International AIDS Society–USA Web site [7]. FDA, Food and Drug Administration.

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Table 1. Strength of recommendation and quality of evidence rating scale.

Category, grade	Definition
Strength of recommendation	
A	Strong evidence to support the recommendation
B	Moderate evidence to support the recommendation
C	Insufficient evidence to support a recommendation
Quality of evidence	
I ^{a,b}	Evidence from ≥1 randomized, controlled clinical trial
II ^{a,b}	Evidence from nonrandomized clinical trials; cohort or case-control studies
III	Recommendation based on the panel's analysis of accumulated evidence (expert opinion)

^a Peer-reviewed publications.
^b Presented in abstract form at peer-reviewed scientific meetings.

involving drug-resistant virus that are common in the developed world have begun to occur in the developing world, as well. In addition, resistance patterns among the non-subtype B strains of HIV-1, which are circulating more widely in developing countries, may differ from those seen among subtype B strains in North America and Europe. Although resistance testing is not yet widely employed in developing countries because of the costs involved, drug resistance will predictably emerge as antiretroviral therapy is more widely introduced, and the need for appropriate testing will increase. Expert advice is important in the management of patients with drug-resistant infection [8], but because it is not always available, this report will attempt to provide updated recommendations for HIV-1 drug resistance testing and tropism testing in developed countries, with the anticipation that, over time, such recommendations will also prove useful in developing countries.

METHODS

The panel was first convened by the International AIDS Society–USA (which is not related to the worldwide International AIDS Society) in 1997 to develop evidence-based recommendations for the assessment of HIV-1 drug susceptibility and the management of drug-resistant HIV-1 infection in clinical practices in the developed world [1–3]. Panel members are not compensated, and there is a process for panel member rotation. Updated reports are initiated when enough new published or presented information in the field accumulates to warrant revising previous recommendations.

The panel was convened by conference call in mid-2007 and met regularly thereafter to discuss new data published or presented at scientific conferences since its previous report [3]. Topic areas included new information about the prevalence of drug resistance worldwide; new data on mechanisms of resistance by drug class, including INSTI and entry inhibitor classes that have become available since the previous report; developments in assays to determine viral tropism and replication capacity; issues related to non-subtype B HIV-1; and updated recommendations for the clinical use of HIV-1 drug resistance

and tropism testing. Individual panel members were appointed to review topics to be considered. In some cases, pharmaceutical or assay manufacturers were contacted to obtain relevant information in the public domain. Data on file, unpublished observations, personal communications, and other forms of data not previously published or presented in a scientific, public forum were not considered for this report. Discussions of drugs focused on those approved by the US Food and Drug Administration. Clinical recommendations were made by panel consensus.

The quality and strength of the evidence were rated according to a scale (table 1) that was modified in 2006 [9] and originally adapted from published rating scales used by other organizations (e.g., the American Heart Association [10], American Association for the Study of Liver Diseases [11], National Institutes of Health [12], and Infectious Diseases Society of America [13]).

TRANSMISSION AND EPIDEMIOLOGY OF DRUG-RESISTANT HIV-1

Transmission and prevalence of drug resistance in developed countries. Transmission of drug-resistant HIV-1 has been observed in most countries where antiretroviral treatment is available [14–22], and it jeopardizes the success of antiretroviral therapy. Indeed, transmitted drug resistance generally leads to a delay in virologic suppression [18, 23] and to an increased risk of earlier virologic failure [24, 25]. Long-term persistence of transmitted drug resistance in the absence of drug pressure has been documented for many types of mutations [26–30], as have specific revertant mutations for thymidine analogue reverse-transcriptase inhibitor–associated resistance mutations (TAMs) [29]. In contrast with patients with acquired drug-resistant virus that emerged during therapy, patients with transmitted drug-resistant virus do not have a reservoir of drug-susceptible virus. Consequently, transmitted drug-resistant virus can only change to drug-susceptible virus by back mutation [30], and it will do so rapidly only if a substantial fitness benefit occurs with reversion of the drug resistance mutation,

as in the case of the M184V mutation in reverse transcriptase. This mutation can revert relatively early after transmission [26], in contrast with the delayed reversion that occurs with most other mutations [26, 30]. This delayed reversion is different from the reversion associated with acquired drug resistance, in which archived, drug-susceptible wild-type virus reemerges within weeks of withdrawal of the selective pressure of drug treatment [31–34].

The prevalence of newly transmitted drug-resistant HIV-1 strains (primary HIV-1 drug resistance) varies widely with location, transmission risk group, and the sampling time after infection [14, 15, 18, 20–23, 35–37]. Variations in prevalence are multifactorial and reflect different treatment exposures at the population level, potential selection bias caused by non-representative sampling of certain transmission risk groups, different definitions of resistance [38, 39], different sampling times after infection, and different risk behavior and access to therapy among transmission risk groups. A large increase in overall primary resistance, from 13.2% for the period 1995–1998 to 24.1% for the period 2003–2004, was reported in New York, New York, and the rate of transmitted multidrug resistance increased from 2.6% to 9.8% over the same period [36]. A British group also reported high rates of primary resistance in 2003: 19.2% for any drug, 12.4% for nucleoside analogue reverse-transcriptase inhibitors (NRTIs), 8.1% for nonnucleoside analogue reverse-transcriptase inhibitors (NNRTIs), and 6.6% for PIs. High-level resistance was found in 9.3%. In contrast, a representative 10-year transmission surveillance study (1996–2005), conducted by the Swiss HIV Cohort Study, showed considerably lower rates: 7.7% for any drug, 5.5% for NRTIs, 1.9% for NNRTIs, and 2.7% for PIs. Dual- or triple-drug class resistance was observed in only 2% of patients [21]. The rate of transmission, including the transmission of multidrug-resistant virus, was stable over a 10-year period, with the exception of NNRTI-resistant virus transmission, which—as has been reported by other groups—increased in 2005 [16, 18, 20, 36, 40]. These examples demonstrate that specific countries and regions require separate surveillance systems to monitor transmitted HIV drug resistance, because extrapolation from foreign data may be misleading.

The CASCADE study [41] has reported the longest follow-up time for patients with transmitted drug resistance to date; this study found higher initial CD4⁺ T cell counts in patients infected with drug-resistant virus than in patients infected with wild-type virus. This initial higher CD4⁺ T cell count was followed by a faster decrease in CD4⁺ T cell count, such that initial differences in CD4⁺ T cell counts were lost over the 5-year observation period. Thus, the effects of transmitted drug-resistant HIV on the infection's natural history before treatment are not great.

Drug resistance acquired during antiretroviral therapy is

much more common than transmitted drug resistance. Cross-sectional studies involving patients who have been treated but who are viremic yielded probabilities for the presence of at least 1 drug resistance mutation of 76%–90% [42–47]. However, considerable methodologic challenges exist in evaluating such prevalence data. Drug resistance testing can be reliably performed only if plasma HIV-1 RNA levels are >500 copies/mL. This is particularly important if a patient's plasma HIV-1 RNA level was suppressed to below the level of detection before the time that drug resistance testing became available, when patients initially received suboptimal treatment, such as single- or double-NRTI-only regimens. Estimates of prevalence are confounded, because the denominator of all treated patients is often not known, the practice of obtaining drug resistance test results has changed over time, and cross-sectional analyses may underestimate the prevalence of drug resistance [48]. In a recent 10-year longitudinal study from the Swiss HIV Cohort Study, which took into account shifts in population size and in which the denominator of treated subjects was known, the prevalence of drug-resistant virus among antiretroviral therapy-exposed patients was estimated to be 50%–60% in 1999 and decreased to 39%–53% in 2006 [49]. The prevalence of triple drug-resistant virus remained stable at 5%. These lower numbers are likely to be attributable to the improving efficacy of treatment.

Transmission and prevalence of drug resistance in developing countries. Access to antiretroviral drugs in the developing world is increasing rapidly, although only a fraction of individuals who need therapy are currently receiving it. As in the developed world, an increase in transmitted resistance will lag behind an increase in acquired drug resistance. Nevertheless, with (1) >3 million people receiving antiretroviral therapy; (2) treatment failure defined by clinical end points [50]; and (3) limited availability of assays for routine determination of plasma HIV-1 RNA levels and for detecting drug resistance, acquired and transmitted drug resistance in resource-limited settings will present formidable challenges. Even more extensive drug resistance may emerge in this setting than in the developed world [50]. Some studies have already demonstrated the presence of drug-resistant virus in patients with recent infections in developing countries [51]. The World Health Organization is developing a surveillance program to provide early warning of increasing rates of transmitted resistance and to facilitate additional treatment options [52].

Single-dose nevirapine is widely used in the developing world to prevent mother-to-child transmission of HIV-1, but it selects for nevirapine-resistant HIV-1 in 40%–60% of mothers, as detected by population sequencing within 6–8 weeks of administration [53], and this resistance may compromise subsequent response to nevirapine-containing regimens [51, 52, 54, 55]. Children who are born with infection despite nevirapine prophylaxis have a high risk of developing resistance to nevirapine,

which limits their future treatment options [54]. Coadministration of other antiretroviral drugs with nevirapine may reduce the risk of drug-resistant infection in adults and children [56].

A number of characteristics of antiretroviral use in resource-poor settings will affect the level of acquired drug resistance among treated patients. For example, in a Thai cohort receiving a fixed-dose combination of stavudine-lamivudine-nevirapine and infrequent monitoring, virologic failure was associated with more resistance (>90% of isolates had NNRTI- and lamivudine-resistance-associated mutations, and >30% had TAMs) [57] than would be expected in the developed world at the time of first viral rebound [50], although less resistance was observed in cohorts with more-intensive plasma HIV-1 RNA level monitoring [58, 59]. In the absence of real-time viral load monitoring, >55% of patients in Uganda who received zidovudine-lamivudine-abacavir within the Development of Antiretroviral Therapy in Africa study and who had detectable viremia at week 48 had 1–4 TAMs, as well as the M184V mutation [60]. Limited access to antiretroviral drug programs may encourage some infected individuals to share their antiretroviral drugs with others, which may lead to suboptimal dosing. Such undisclosed therapy is likely to be the cause of drug resistance in certain populations entering antiretroviral therapy rollout programs. Pretreatment drug resistant infection was detected in ~10% of a subset of the Development of Antiretroviral Therapy in Africa study recipients in Uganda and Zimbabwe [61].

MECHANISMS OF ANTIRETROVIRAL DRUG RESISTANCE

Since the previous guidelines were published [3], several new drugs have been approved, and novel mechanisms of resistance have been elucidated. This section will review some of these advances.

Reverse-Transcriptase Inhibitors

Etravirine. Etravirine (TMC125) is a second-generation NNRTI that exhibits activity against many viruses that are resistant to first-line NNRTIs. Etravirine has favorable safety, pharmacokinetic, and antiviral activity profiles in heavily treatment-experienced HIV-1-infected patients [62–67]. The impact of pretreatment phenotype and genotype on the virologic response to etravirine at week 24 was examined in the DUET-1 and DUET-2 clinical trials [68–71]. Thirteen baseline HIV-1 reverse-transcriptase mutations were associated with resistance to etravirine in the DUET analyses: V90I, A98G, L100I, K101E/P, V106I, V179D/F, Y181C/I/V, and G190S/A [70, 71]; of note, V179T was also identified in a separate US Food and Drug Administration analysis [72]. In the pooled DUET study results, 70% of subjects had 0 or 1 baseline etravirine mutation, whereas 15% had ≥ 3 baseline etravirine mutations. Notably, the reverse-transcriptase K103N mutation, which is often seen

in virus obtained from patients who experience virologic failure during efavirenz and nevirapine treatment and which confers broad cross-resistance within the first-generation NNRTI class, was not associated with etravirine resistance [72].

Virologic responses were seen in the DUET trials despite the presence of single etravirine mutations [70, 71]. The impact of most of these etravirine mutations depended on the simultaneous presence of Y181C; however, Y181C had an impact only when present with ≥ 1 additional mutation [70, 71, 73]. Having a greater number of baseline etravirine-related mutations was associated with a decreasing virologic response to etravirine, particularly when ≥ 3 mutations were present [70, 74]. The impact of specific etravirine mutational patterns on clinical virologic responses has not been fully defined. No phenotypic “cutoff” levels for clinical responses to etravirine are currently available.

Antagonism among specific reverse-transcriptase mutations. A potentially relevant and mechanistically interesting antagonism among various thymidine analogue NRTIs, TAMs, and the emergence of the tenofovir-associated K65R mutation has been elucidated [75]. TAMs selected by zidovudine or stavudine counteract the selection of the K65R mutation, although TAMs and the K65R mutation do not appear on the same genome because of competing mutational pathways. Thymidine analogue NRTIs, such as zidovudine, may protect against the emergence of the K65R mutation when combined with tenofovir, leading some clinicians to combine these agents. In contrast to HIV-1 drug resistance patterns described for tenofovir and for the triple-NRTI regimen tenofovir-abacavir-lamivudine, which selects for the K65R mutation more frequently, a quadruple-drug regimen of tenofovir plus zidovudine-abacavir-lamivudine in the COL40263 trial selected predominantly for NRTI-associated TAMs in virus from patients in whom therapy had failed [76].

Mutations in the connection and RNase H domains of reverse transcriptase. Mutations in the connection (E312Q, G335C/D, N348I, A360I/V, V365I, T369I, A371V, A376S, and E399D) and RNase H (Q509L) domains of reverse transcriptase are selected by NRTI therapy (in addition to TAMs); these newly recognized mutations, which are located outside of reverse transcriptase regions covered by standard genotype assays, substantially increase resistance to zidovudine when TAMs are also present [77–83]. They can increase cross-resistance to lamivudine, abacavir, and tenofovir (although to a much lesser extent) but do not increase resistance to stavudine or didanosine [77–79]. NNRTIs (mainly nevirapine) are also affected [80, 82].

The Q509L and A371V/Q509L mutations with TAMs impair the formation of RNase H cleavage products, which increases zidovudine-monophosphate excision on RNA/DNA duplexes by reducing template degradation. Q509L and A371V/Q509L also increase the efficiency of excision of short RNA/DNA du-

plexes [81]. The N348I mutation in the reverse-transcriptase connection domain confers dual zidovudine-nevirapine resistance via 2 interrelated mechanisms [82]. First, N348I decreases the ability of nevirapine to inhibit HIV-1 reverse transcriptase; second, N348I substantially decreases the rate of RNase H cleavage, which increases zidovudine-monophosphate excision by reducing RNA/DNA template degradation. Furthermore, the ability of nevirapine to stimulate RNase H is substantially reduced, compared with the wild-type enzyme. The N348I and A360V mutations, in combination with TAMs, decrease the efficiency of RNase H cleavage and increase the amount of rescued reaction product after ATP-dependent excision. Mutations N348I and A360V promote reverse-transcriptase dissociation from an RNase H-competent complex, thereby reducing RNA/DNA template degradation [83]. The N348I mutation occurs relatively frequently and can emerge early during therapy with regimens containing zidovudine and nevirapine [82]. The clinical impact of connection and RNase H domain reverse-transcriptase mutations on virologic response has not been determined.

PIs

Improved understanding of the importance of drug exposure in PI activity and resistance has led to widespread use of low-dose ritonavir boosting to increase drug levels, resulting in more effective competition with viral substrates and reduced impact of single mutations on drug activity. Large cohort data confirm superior virologic suppression with ritonavir-boosted PI-containing regimens, compared with unboosted PI-containing regimens, in drug-naïve patients [50]. The genetic barrier to resistance (i.e., the number of mutations required for resistance to develop combined with difficulty in their selection) is generally greater for ritonavir-boosted PI-containing regimens than it is for unboosted PI-containing regimens. Resistance to ritonavir-boosted PIs requires multiple mutations that vary among PIs, and the degree of resistance depends on the number, as well as the type, of mutations present [50, 73, 84, 85]. The large number of mutations required for resistance makes the selection of resistance to boosted PI regimens uncommon, compared with the selection of resistance to NNRTI-containing regimens, among drug-naïve patients who are experiencing a first regimen failure [50, 86]. Recently approved drugs, such as darunavir and tipranavir, have improved virologic activity in patients harboring PI-resistant HIV-1 [87–89].

Studies relating baseline (i.e., pretreatment) PI susceptibility or mutations to virologic outcome have been performed for boosted PI-containing regimens. Predictions of virologic success can be made by measuring fold-change in susceptibility (phenotype) or number and type of mutations (genotype). Genotypic resistance scores and phenotypic clinical cutoff levels derived from virologic outcome data are now available for dif-

ferent boosted PI-containing regimens [87–93]. Among these regimens, the number and codon positions of PI mutations in the resistance scores vary, with partial overlap among different drugs. For example, the resistance scores of tipranavir-ritonavir and darunavir-ritonavir both include mutations I84V and L33F but differ with respect to many other mutations. Therefore, virus harboring multiple PI mutations will show some degree of reduced susceptibility to all boosted PI regimens, and the clinical usefulness of each regimen may vary greatly. Phenotypic resistance testing may be particularly beneficial in this setting. The extent of both resistance and drug exposure affects PI activity. Thus, considering both parameters might improve predictions of viral inhibition. The inhibitory quotient (i.e., the ratio of the measured plasma minimum concentration value divided by the IC_{50} value or IC_{90} value) characterizes this relationship. Nevertheless, studies evaluating whether assays for drug exposure add to the effectiveness of assays for resistance have yielded conflicting results [94–99]. Inhibitory quotient values better predict PI activity than do resistance or drug levels alone in some, but not all, clinical studies. No randomized studies have yet shown an overall benefit to using inhibitory quotient calculations for PI dose adjustment in place of standard resistance testing.

In addition to mutations in the protease gene associated with PI resistance, mutations in the *gag* cleavage site region, especially p7/p1 and p1/p6, can increase the viral fitness of viruses resistant to PIs [100–103]. These mutations typically occur in conjunction with drug resistance mutations in the protease gene [104–106]. Specific patterns of cleavage-site mutations have been described for certain PIs (e.g., A431V with L24I-M46I/L-I54V-V82A, I437V with I54V-V82F/T/S, L449V with I54M/L/S/T/A, and L449F/R452S/P453L with D30N-I84V). In contrast, mutation P453L and the emergence of V82A were negatively correlated [107]. Mutations in the C-terminal region of the viral *gag* gene (K436E and/or I437T/V), located outside the actual NC/p1 cleavage site, can be selected *in vitro* in the absence of conventional PI resistance mutations, and they can confer resistance by merely changing the substrate of protease and thereby increasing processivity of the enzyme [108]. These mutations were also present in clinical isolates that had reduced susceptibility to PIs but lacked major protease mutations. Whether these cleavage-site mutations are of clinical relevance and should be included in drug resistance assays remains to be determined.

Entry Inhibitors

HIV-1 entry involves the interaction of gp120 with its primary receptor, CD4, followed by binding to 1 of 2 chemokine receptors (chemokine receptor 5 [CCR5] or CXC chemokine receptor 4 [CXCR4]) that serve as coreceptors [109]. Engagement of the coreceptor triggers the assembly of the 2 heptad

repeats (HR-1 and HR-2) in the trimeric gp41 into a 6-helix bundle that leads to the approximation and fusion of the cell and virus membranes [110]. The third variable (V3) loop is the major structural element of gp120 that determines coreceptor recognition and specificity, but regions outside of V3 contribute, as well [111–113].

Enfuvirtide. Enfuvirtide is a synthetic 36-amino acid oligopeptide that inhibits HIV-1 entry by preventing the assembly of HR-1 and HR-2 in the trimeric gp41 into a 6-helix bundle [114]. The drug binds to the trimeric HR-1 complex, thereby inhibiting fusion and blocking virus entry [115]. Resistance to enfuvirtide is mediated by amino acid substitutions within HR-1 at amino acid positions 36–45 of gp41 [116, 117]. The substitutions most frequently associated with resistance to enfuvirtide include G36D/S/V/E, V38A/E/M, Q40H, N42T, and N43D [118–120]. These mutations confer substantially reduced binding of enfuvirtide to HR-1 and a substantial decrease in antiviral activity in vitro [119]. In addition, the N126K and S138A mutations in HR-2 may contribute to reduced susceptibility to enfuvirtide [118]. Viruses carrying enfuvirtide resistance mutations show reduced viral fitness in vitro in the absence of enfuvirtide [121]. Clonal analysis of plasma HIV-1 RNA obtained from patients receiving enfuvirtide in the absence of a fully suppressive antiretroviral regimen showed rapid emergence of enfuvirtide resistance mutations [122]. The earlier emergence of mutants with gp41 substitutions at amino acid positions 36 and 38 suggests that these mutants may have an initial fitness advantage over mutants with substitutions at codons 40 and 43, which tended to emerge later.

Chemokine receptor antagonists. Small-molecule antagonists of the gp120-CCR5 interaction, such as maraviroc and the investigational drug vicriviroc, are allosteric, noncompetitive antagonists that bind to a similar site on CCR5 [123–125]. These drugs are potent inhibitors of HIV-1 [126, 127]. Maraviroc is now approved in the United States for use in treatment-experienced patients with exclusively R5 virus strains. Phase III trials of vicriviroc are under way.

Resistance to CCR5 antagonists selected in vitro is mediated by changes in HIV-1 gp120 that allow the envelope glycoprotein to interact with the drug-bound form of CCR5. A variety of amino acid substitutions associated with maraviroc and vicriviroc resistance have been described throughout the *env* gene; most involve V3, but their effect on drug susceptibility depends on the *env* backbone into which they are introduced [128, 129]. Phenotypically, resistance to the CCR5 antagonists manifests not as a classic rightward shift of the IC_{50} curve but, rather, as a plateau in the maximum achievable suppression of viral replication (figure 2). This plateau, referred to as the percent maximal inhibition, correlates with viral adaptation to use the inhibitor-bound form of CCR5 for entry [129, 130].

Few clinical isolates that are resistant to maraviroc or vicri-

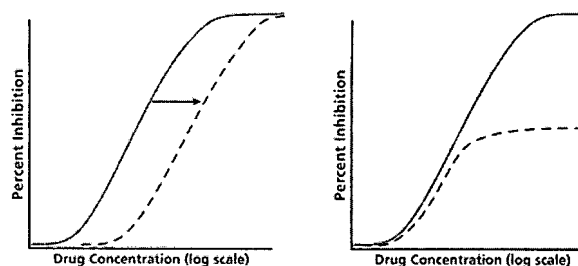


Figure 2. The left panel shows a typical inhibition curve of a susceptible virus (solid line) with a typical competitive inhibitor (e.g., a protease inhibitor). The IC_{50} value of the resistant virus (dotted line) is shifted to the right (arrow). The right panel shows an example of a noncompetitive inhibitor (e.g., a chemokine receptor 5 antagonist). The susceptible virus (solid line) shows a typical inhibition curve, but in this case, the resistant virus (dotted line) reaches a plateau. The maximum achievable percent inhibition is shifted downward (arrow), but the curve does not shift to the right; hence, the IC_{50} value remains unchanged.

viroc have been reported [131–133]. Overall, resistance of R5 virus appears to emerge slowly and is associated with mutations in the V3 loop stem (and possibly elsewhere in the *env* gene), similar to resistance that arises during in vitro passage experiments. The particular mutations observed vary from isolate to isolate. Thus, it is not yet possible to identify resistance to CCR5 antagonists on the basis of specific *env* mutations. Although no signature mutations associated with maraviroc resistance have yet been identified, changes at positions 13 and 26 in the middle of the V3 loop appear to be important [130, 132, 134]. Likewise, data are insufficient to determine the extent of cross-resistance within the class. In clinical trials, virologic failure of CCR5 antagonists has been more frequently attributed to the emergence and outgrowth of CXCR4-using viruses that pre-existed as minority populations before the initiation of CCR5 antagonist therapy.

INSTIs

The INSTIs are a new class of antiretroviral drugs that selectively target the HIV-1 integrase enzyme. Integrase catalyzes numerous steps within the cytoplasm and nucleus of host cells that allow proviral DNA to enter the nucleus and integrate into host cellular DNA. This entire process is required for productive HIV-1 replication. The approved drug raltegravir and other candidate drugs that are in development inhibit the strand transfer reaction.

Resistance to integrase inhibitors can emerge during treatment failure. Phenotypic and genotypic assays have been described that detect HIV-1 integrase resistance [135–137], but plasma-based phenotypic and genotypic assays to detect HIV-1 integrase inhibitor resistance are not yet clinically available. As with PI resistance mutations, INSTI resistance mutations are classified as either major or minor. Major mutations tend

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to be the primary contact residues for drug binding, based on crystal structures, and have an effect on the drug susceptibility phenotype. Major mutations are those selected first in the presence of the drug or those shown at the biochemical or virologic level to lead to an alteration in drug binding or an inhibition of viral activity or viral replication. Minor mutations generally emerge later than major mutations and enhance the degree of resistance or improve replicative fitness of the virus that contains major mutations [73].

Raltegravir is a hydroxypyrimidinone carboxamide derivative of the diketobutanoic acid family. It is approved in the United States and in Europe for use in treatment-experienced HIV-1-infected patients in combination antiretroviral regimens, based on favorable safety, pharmacokinetic, and efficacy parameters [138–143]. In phase II and III studies, virologic failure occurred infrequently and was generally associated with the emergence of resistance mutations [136]. In Merck protocol 005, a phase II study of HIV-1-infected subjects with triple-class drug resistance, genotyping by population sequencing was performed at treatment failure. Phenotyping of patient-derived sequences and site-directed mutants employed single-cycle infection assays on long-terminal repeat-responsive reporter cell lines.

Raltegravir failure was associated with integrase mutations in 2 distinct genetic pathways, defined by ≥ 2 mutations, including (1) a signature (major) mutation at either Q148H/K/R or N155H and (2) ≥ 1 minor mutations unique to each pathway. The major mutations all reduced susceptibility to raltegravir and decreased viral replication capacity. Minor mutations described in the Q148H/K/R pathway included L74M, E138A, E138K, or G140S. These minor mutations consistently enhanced the level of resistance to raltegravir and, when combined with major mutations, improved viral replication capacity in a subset of combinations. The most common mutational pattern in this pathway is Q148H plus G140S. This Q148H-plus-G140S pattern exhibited the greatest decrease of drug susceptibility (>100 -fold phenotypic resistance) and was the fittest variant seen (i.e., it had the highest replication capacity in both infectivity and multiple-cycle replication assays).

Mutations described in the N155H pathway include this major mutation plus 1 of either L74M, E92Q, T97A, E92Q plus T97A, Y143H, G163K/R, V151I, or D232N [73, 136]. Mutations observed in raltegravir protocols were similar to those selected with different integrase inhibitors in cell culture [144, 145]. The impact of specific raltegravir mutational patterns on clinical virologic responses has not been elucidated fully; thus, no phenotypic cutoff levels for clinical response have been determined for raltegravir. The most important prognostic factor that decreased the likelihood of virologic failure and drug resistance was having a genotypic susceptibility score or phenotypic susceptibility score >0 for optimized background reg-

imen; thus, integrase inhibitors should always be paired with other active agents in an HIV-1 treatment regimen [136].

HIV-1 DRUG RESISTANCE ASSAYS

There are 2 general types of resistance assays used in clinical practice: genotypic assays (i.e., HIV-1 gene sequencing to detect mutations that confer HIV-1 drug resistance) and phenotypic assays (i.e., cell culture-based viral replication assays in the absence or presence of drugs). Genotypic testing can be performed with commercial assay kits or in-house protocols. Blinded quality assurance programs indicate a very high concordance between kits and in-house methods used [146–148]. The ability to detect drug-resistance mutations, however, can vary substantially among laboratories [146, 149]. This wide variation results from difficulties in recognizing viral mixtures, particularly in heavily treatment-experienced patient populations [147, 150, 151], sequencing specimens with low viral loads, and testing non-subtype B HIV [149]. Performance also relates to the level of experience among laboratory personnel [149], which suggests that appropriate operator training, certification, and periodic proficiency testing are important for accurate genotyping. Resistance testing laboratories, therefore, need to participate in quality assurance programs [146, 147, 149–152].

Despite numerous studies, appropriate interpretation of genotypic and phenotypic drug resistance testing remains challenging. Results of genotypic tests use lists of predefined drug resistance mutations [73] or classifications by computerized, rules-based algorithms to characterize virus as “susceptible,” “possibly resistant,” or “resistant” to each antiretroviral drug [153–156]. The creation of rule-based algorithms is a difficult and lengthy process, and the algorithms require frequent updating. Algorithms vary considerably in the classification of expected drug activity [150, 151, 156–159]. Differences appear to be lowest for lamivudine and NNRTIs and highest for NRTIs and PIs [150, 151]. The most stringent approach to building algorithms is to evaluate the impact of mutational patterns at the initiation of treatment with a specific drug with regard to treatment response (e.g., decrease of plasma HIV-1 RNA levels according to specific genotypic patterns). Considerable progress has been made in identifying mutational baseline patterns that predict clinical failure for specific drugs. These patterns are currently available for the combinations lopinavir-ritonavir, atazanavir-ritonavir, tipranavir-ritonavir, darunavir-ritonavir, and amprenavir-ritonavir, as well as for zidovudine, stavudine, didanosine, lamivudine, tenofovir, efavirenz, nevirapine, and etravirine [70–72, 91–93, 157, 160–168]. The vast majority of genotypic algorithms are based on data that were obtained using subtype B viruses. Although no large differences exist with regard to interpretation of drug resistance in non-subtype B HIV, discrepancies between genotype and phenotype have been ob-

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served for abacavir and subtype CRF02_AG, atazanavir and subtype C, and NNRTIs and subtype CRF01_AE [169]. HIV-1 proteases in drug-naïve West African patients appear to be generally less sensitive to PIs [170]. More in vivo and in vitro resistance data are clearly needed for non-subtype B HIV.

An alternative approach for the interpretation of genotypic drug resistance information is to correlate genotypic data regarding the plasma HIV-1 RNA of a candidate gene with a large database of paired phenotypes and genotypes [171–174]. Such linkage then permits generation of a “virtual phenotype” by assigning calculated fold-changes in IC_{50} . Although actual and virtual phenotypes show excellent correlation for most drugs, superiority of virtual phenotype over genotype alone could not be demonstrated in predicting clinical response to salvage regimens [173, 175, 176]. Virtual phenotype is an approach to genotype interpretation, and its main limitation is that predictive power depends on the number of matched datasets available. Thus, variation is frequently higher in smaller datasets; consequently, variation is frequently higher for newer drugs. Furthermore, matches are based on preselected codons, not on the entire nucleotide sequence.

Standard phenotypic testing, using recombinant virus assays, is performed by few commercial laboratories. Current assays amplify HIV-1 protease, a part of the HIV-1 reverse transcriptase, as well as the 3'-terminus of *gag*, as a unit from plasma virus, and they generate a recombinant virus pseudovirus with other HIV-1 genes derived from a laboratory construct [177–181]. A comparison between 2 different assays showed an overall concordance of 86.9%, with the highest concordance for PIs (93.4%) and the lowest concordance for NRTIs (79.8%) [150]. However, even within drug classes, concordance can vary widely among specific drugs (e.g., lamivudine has a very high concordance of 93%, but abacavir has a concordance of only 74% [150]). This recombinant technology is being modified so that it can also test for susceptibility to INSTIs, fusion inhibitors, and chemokine receptor antagonists [136, 182].

The results of phenotypic testing are usually presented as the fold-change in susceptibility of the test sample compared with a laboratory control isolate. The initial “technical” cutoff values, representing the interassay variation of cloned virus controls, did not accurately reflect the inherent variation in susceptibility encountered in circulating viruses from drug-naïve patients. The normal distribution of susceptibility to a given drug for wild-type isolates from treatment-naïve individuals (i.e., the “biologic” cutoff) was then adopted. Although clinical cutoffs have been defined for many drugs, the relationship between viral susceptibility and drug response is a continuum in which progressively reduced viral phenotypic susceptibility to a particular drug results in progressively blunted reductions in plasma HIV-1 RNA levels. For practical application, 2 different clinical phenotypic cutoff values should be defined: one above

which clinical responses perceptibly diminish, compared with those of wild-type virus (“intermediate” resistance), and one above which no clinical response can be expected (“full” resistance). Even partial activity can be useful when treatment options are limited [183]. In evaluation of phenotypic cutoffs, drug-specific susceptibility needs to be compared at baseline, before switching to a new drug regimen, and with the drug-specific treatment response (e.g., decrease in plasma HIV-1 RNA levels) that occurs after initiation of new therapy. Since 2003, clinical trial and cohort data have led to a substantial increase in available clinical cutoff values, and such values are now available for most approved drugs.

In addition to standard genotypic and phenotypic testing used in clinical practice, other resistance testing assays may prove to be useful in the future. The allele-specific PCR assay [184–189] and the single-genome [190] and ultra-deep sequencing [191] assays are currently used to investigate the role of minority variants harboring drug resistance that are present below the level of detection by bulk plasma viral population sequencing approaches. Studies involving treatment-naïve and treatment-experienced patients have shown strong associations between the detection of low-frequency drug-resistant variants, particularly those encoding resistance to NNRTIs, and subsequent treatment failure [192–194]. Further studies are required to define the clinically relevant frequency of variants in the virus population. Improvements in assay throughput and reductions in cost are necessary before such assays are available for patient management.

Replication capacity assays, which are designed to measure in vivo fitness of a virus, remain an interesting research tool, but they have not found a role in patient management [195]. A relatively simple and inexpensive alternative for estimating the reduction of in vivo fitness induced by a given nonsuppressive antiretroviral treatment regimen is to determine the differences in plasma HIV-1 RNA levels between pretreatment and on-treatment periods (e.g., for patients lacking fully suppressive treatment options); such information may be useful in optimizing a nonsuppressive treatment regimen [196]. Finally, the cost-effectiveness of using resistance testing assays for treatment-naïve patients and for patients for whom antiretroviral treatment has failed has been demonstrated in various countries [197–202].

VIRAL CORECEPTOR USE TESTING

Phenotypic assays to determine coreceptor use (i.e., tropism testing) require the amplification of *env* sequences from plasma HIV-1 RNA and the construction of viral pseudotypes or infectious recombinant viruses that express the patient-derived *env* sequences along with a reporter gene [182]. These pseudotyped viruses or viral recombinants are then inoculated onto cells that express CD4 along with CCR5 or CXCR4. The pres-

ence of infection is detected by assays that determine reporter-gene activity. HIV-1 isolates that use CCR5 exclusively are termed R5 viruses, those that use only CXCR4 are termed X4 viruses, and those that use both are termed R5/X4, or dual-tropic viruses. Because these assays do not distinguish between the presence of truly dual-tropic viruses and a mixture of R5 and X4 viruses, samples that can infect both CCR5- and CXCR4-expressing cells are often termed dual-mixed viruses. As with commercially available resistance tests, tropism testing generally requires a plasma sample with an HIV-1 level of ≥ 1000 copies/mL. The assay used in most clinical trials of CCR5 antagonists can detect the presence of CXCR4-using virus when they constitute at least 5%–10% of the virus population as minor variants [182]. Assays with improved sensitivity for detection of 0.3% CXCR4-using or dual-mixed virus are now available [203].

Genotypic approaches to determining coreceptor use depend on sequencing the V3 loop and applying one of a variety of predictive algorithms. The 2 most commonly used measures for predicting CXCR4 use include (1) the presence of positively charged amino acids at positions 11 and 25, often referred to as the “11/25 rule,” and (2) the total charge of V3 loop amino acid residues of +5 or greater [204, 205]. Bioinformatic approaches include the use of position-specific scoring matrices [206], neural networks [207], or machine-learning techniques [208]. The heteroduplex tracking assay has also been used to detect the presence of CXCR4-using virus [209]. In this assay, the electrophoretic mobility of PCR-amplified *env* genes is assayed after hybridization to V3-coding sequences from viruses with phenotypically defined coreceptor use. When verified against phenotypic assays, genotypic approaches showed excellent specificity but poor sensitivity for detecting the presence of dual-mixed or CXCR4 viruses in clinical samples [210]. The low sensitivity of these methods is explained, in part, by the extensive heterogeneity of HIV-1 *env* genes in plasma virus populations, which makes it difficult to obtain coherent sequence data with population-based sequencing approaches. Another contributing factor is that not all determinants of viral tropism reside in the V3 loop. For these reasons, genotypic approaches cannot be recommended at present for identifying patients who may be suitable candidates for CCR5 antagonist therapy. Other limitations of genotyping for coreceptor tropism include the inability of population sequencing to detect variants that comprise <25% of the virus population and the lack of interpretation algorithms for sequences that are not from subtype B.

Drug Resistance in Non-Subtype B HIV

Antiretroviral drug design, resistance research, and interpretation systems have been largely based on HIV-1 subtype B viruses, which have historically been the most prevalent subtype

in North America, Western Europe, and Australia. However, subtype B viruses account for only ~12% of the worldwide HIV-1 infections, with subtype C viruses being the most prevalent, accounting for ~50% of cases [211]. An increasing number of individuals who are infected with many of the non-subtype B virus strains now receive antiretroviral therapy because of rollout programs in the resource-limited world and because of increasing migration to the developed world, particularly to countries in Europe. It is essential to appreciate how HIV-1 genetic variation alters the characteristics of drug susceptibility and drug resistance. The differences in the natural polymorphisms between HIV-1 subtype B and non-subtype B viruses have been well documented [212, 213]. The effects of viral subtype on resistance are expressed in 2 broad cases: in genetic routes to, and frequency of, specific mutations and in the algorithms developed for interpreting drug resistance.

Genetic routes to and frequency of specific resistance mutations. Viruses from patients infected with subtypes C, G, or CRF_01 AE for whom a first-line nelfinavir-containing regimen is failing preferentially select the L90M mutation, rather than D30N, which more frequently occurs with subtype B virus [214, 215]. This preferential selection of L90M in protease may stem from methionine polymorphism in these non-subtype B viruses at position 89, rather than from lysine in subtype B [216].

Synonymous differences are also responsible for different resistance mutations. For example, valine at position 106 of reverse transcriptase is coded by a GTG codon in subtype C, in contrast with coding by a GTA codon in subtype B. Thus, subtype C viruses more readily select methionine in the presence of efavirenz (a 1-nucleotide change), whereas subtype B viruses change to an alanine (a 1-nucleotide change) [217]. The V106M mutation is responsible for broad cross-resistance to older NNRTIs [218]. A further example is at reverse transcriptase position 210; subtype F viruses require 2 nucleotide substitutions for L210W to emerge, compared with the 1 mutation required by other subtypes. This difference explains the lower prevalence of L210W in cohorts of patients with subtype F infection [219]. An even more intriguing finding is the increased prevalence of K65R in reverse transcriptase from subtype C virus-infected patients receiving tenofovir or didanosine-containing regimens, compared with the prevalence in reverse transcriptase from patients infected with other virus subtypes. Although synonymous differences at reverse transcriptase position 65 exist, these do not change the number of substitutions required for the K65R mutation. One possible explanation for this is that the longer string of adenines in the reverse-transcriptase gene preceding the codon that codes for K65 in subtype C viruses favors increased slippage of the reverse-transcriptase enzyme during transcription, thus encouraging mutations at position 65 [220, 221].

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Table 2. Summary of clinical situations in which resistance testing is recommended.

Clinical setting	Comments
Before initiation of therapy	
Primary (acute and early) infection	Resistance testing is recommended. Initial therapy may be altered based on resistance test results.
First evaluation of chronic HIV-1 infection	Resistance testing is recommended, including for patients for whom therapy is delayed, because plasma wild-type isolates may replace drug-resistant virus with time in the absence of treatment.
Treatment initiation for chronic HIV-1 infection	Resistance testing is recommended because of a rising prevalence of baseline HIV-1 drug resistance in untreated patients with chronic infection [25], unless preexisting data or stored samples for testing are available.
In antiretroviral-treated patients	
Treatment failure	Resistance testing is recommended. The decision to change therapy should integrate treatment history, new and prior resistance results (if available), and evaluation of adherence and possible drug interactions.
In specific settings	
Pregnancy ^a	Resistance testing is recommended before initiation of therapy to effectively treat the mother and prevent mother-to-child transmission.
Other considerations and general recommendations	Postexposure prophylaxis should consider treatment history and resistance data from the source, when available; A sudden increase in HIV-1 plasma RNA may reflect superinfection, possibly with drug-resistant virus; Plasma samples to be tested for drug resistance should contain at least 500 HIV-1 RNA copies/mL to ensure successful PCR amplification required for all sequencing approaches; It is preferable that the blood sample for resistance testing be obtained while the patient is receiving the failing regimen, if possible; Resistance testing should be performed by laboratories that have appropriate operator training, certification, and periodic proficiency assurance; Genotypic and phenotypic test results should be interpreted by individuals knowledgeable in antiretroviral therapy and drug resistance patterns; Inhibitory quotient testing is not recommended for clinical decision making.

^a If resistance test results are available from before the pregnancy, clinical judgment should guide whether retesting for resistance is necessary.

Drug resistance algorithms for non-subtype B HIV.

The consensus sequence used for many algorithms, against which changes are identified in sequences from tested patients, is a subtype B sequence. This becomes problematic for subtypes in which some “resistance” mutations actually reflect the wild-type consensus for that subtype. This disparity is particularly apparent for minor protease mutations. For instance, 6 subtype-specific polymorphisms in protease (at positions 10, 20, 33, 36, 82, and 93) occur at sites known to be associated with drug resistance in subtype B viruses. These amino acid substitutions may represent the consensus sequence for more than 1 non-subtype B virus, such as M36I in subtypes A, C, D, F, G, CRF01, and CRF02. Most of the polymorphisms are located outside the active site of the protease [222], such as M36I, which is situated in the hinge region of the enzyme flap. Only in subtype G is a polymorphism present in the active site of the enzyme (V82I).

Several reports have suggested similar in vitro susceptibilities to antiretroviral drugs among different group M subtypes [223, 224]. No differences in response to therapy among these subtypes have been reported [195, 225]. The practical implication of these polymorphisms is that resistance algorithms may over-

interpret resistance to specific drugs because of the inclusion of these polymorphisms in the total number of identified resistance mutations. This applies particularly to PIs, for which the total number of mutations is often used to ascribe susceptibility. Therefore, subtype determination should be included in genotypic resistance testing. Because the selection of mutations is incompletely characterized in non-subtype B viruses, it would be beneficial to compare their sequences before treatment and at treatment failure.

CLINICAL APPLICATIONS AND RECOMMENDATIONS

Clinical applications and recommendations are shown in table 2. The essential strategy behind the use of resistance testing for individual patient management is to provide information to assist in the selection of antiretroviral regimens that achieve and maintain virologic suppression—that is, plasma HIV-1 RNA levels below the lower limits of detection of the most sensitive assays available for routine clinical use (50 copies/mL).

Untreated, Established HIV-1 Infection

Because of the prevalence of primary HIV-1 resistance to antiretroviral drugs in developed countries, resistance testing is recommended for all patients at the time of diagnosis of HIV-1 infection as part of the initial, comprehensive assessment [AII]. This recommendation is not restricted to patients for whom initiation of antiretroviral treatment is being considered on the basis of clinical, immunologic, or virologic criteria. The persistence of detectable mutations acquired at the time of HIV-1 infection varies, with some (e.g., the reverse-transcriptase K103N mutation) having the potential to persist for years and others (e.g., the reverse-transcriptase M184V mutation) having a greater potential for reversion [30]. Thus, establishing a drug-susceptibility profile for a patient's virus at the time of diagnosis can be helpful in antiretroviral decision-making years later because of the ability of archived drug-resistant virus to reemerge in cases of suboptimal therapy. Genotypic testing is recommended [AIII] for the initial evaluation, because mutations that may not yet have accumulated enough to affect phenotypic susceptibility can be detected, HIV-1 subtype can be determined, and the cost of genotypic testing is lower than that for phenotypic testing.

Treatment Failure

First or second treatment failure. Because of the high prevalence of infection due to drug-resistant virus among antiretroviral-treated patients with confirmed, detectable plasma virus, drug resistance testing should be performed in all cases of treatment failure [AI] (defined as an insufficient decrease or an increase in plasma HIV-1 RNA level after 1–2 months of treatment or a confirmed viral breakthrough in a patient with previously undetectable virus). However, genotypic and phenotypic resistance assays both have low amplification success rates in specimens with plasma HIV-1 RNA levels <500 copies/mL. Resistance to specific drugs may not always be detected if treatment with the drug has been discontinued before the sample is obtained, because resistant strains are often less fit than wild-type virus and may persist only as undetectable minor subspecies in the absence of drug pressure. Therefore, a specimen for resistance testing should be obtained before treatment has been discontinued or changed, and if treatment has been discontinued, the specimen should be obtained as quickly as possible thereafter. In addition, antiretroviral treatment history is important, along with resistance data, in choosing the subsequent treatment regimen, especially if the treatment has been discontinued for several weeks.

Resistance is not the only cause of treatment failure. Insufficient drug exposure, often resulting from incomplete adherence to therapy, is the most common reason for failure of an initial treatment regimen. However, continuing therapy in the context of treatment failure will often lead to the emergence

and accumulation of additional resistance mutations. This applies particularly to drugs with low genetic barriers to resistance (e.g., the NNRTIs, lamivudine, emtricitabine, enfuvirtide, and raltegravir). Clinical resistance to ritonavir-boosted PIs often emerges at a slower pace, requiring several mutations. Therefore, rapid evaluation and prompt action should occur at the time of treatment failure. For first and second treatment failures, genotypic resistance testing usually suffices, unless the patient had initially acquired multidrug-resistant virus. Current commercially available resistance assays report results for NRTIs, NNRTIs, and PIs. Viral tropism assays that determine whether a patient's virus population is predominantly R5, X4, or dual-mixed are used to indicate whether CCR5 antagonists (e.g., maraviroc) may be an appropriate choice for patients with treatment failure. Tropism determination may be useful in this circumstance [BIII]. It is expected that susceptibility testing for INSTIs (e.g., raltegravir) will be clinically available in the near future.

Multiple treatment failures (advanced treatment failure). In patients who experience multiple treatment failures, the virus is often resistant to NRTIs, NNRTIs, and PIs. Until recently, constructing potent alternative regimens that combined 2 or 3 fully active drugs was often impossible, despite the use of enfuvirtide, because of the high level of intraclass cross-resistance. The increased availability of new drugs—including drugs from existing classes but with low levels of intraclass cross-resistance, such as darunavir and etravirine, and drugs from new classes, such as maraviroc and raltegravir—have made the goal of attaining undetectable viral loads more realistic for patients with numerous treatment failures [9]. In this situation, tropism determination is recommended [AI], and phenotypic testing may be a useful addition to genotypic testing, because the number of mutations and the complexity of mutational interactions may make genotypic interpretation challenging. However, phenotypic testing may not be available because of cost. Genotypic testing is recommended for patients with multiple treatment failures [AI], along with phenotypic testing, if available [AI]. The decision to change therapy and the selection of the new regimen should be discussed with experts who are knowledgeable in antiretroviral therapy, antiretroviral pharmacology, and resistance patterns.

Special Circumstances

Acute and early phase HIV-1 infection. Genotypic resistance testing is recommended for any patient who presents within several months after HIV-1 infection because of the high reported rates of transmitted drug resistance [AII]. If immediate antiretroviral intervention is indicated, the initiation of treatment should not be delayed until this result is available, because the turnaround time may be ≥ 2 weeks; rather, treatment should be modified if the result demonstrates resistance to ≥ 1 com-

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ponent of the regimen. The initial choice of treatment should also take into account the treatment history of the source patient and resistance data for that patient's virus, if available. If treatment is not initiated during the acute or early phases of infection, the resistance test results will still be helpful in the future, because early testing provides the best opportunity to detect transmitted drug resistant virus that has been archived and replaced by more-susceptible virus but which could emerge later during treatment.

Pregnancy. Genotypic resistance testing is recommended for all HIV-1-infected pregnant women with detectable plasma virus, both for their own health and for the health of their infants [AII]. This information will assist with treatment choices for the mother, as well as with choices to prevent mother-to-child HIV-1 transmission (including multidrug-resistant HIV-1 transmission) by selecting a regimen that will be effective and safe for the fetus.

Postexposure prophylaxis. The question of whether to initiate postexposure prophylaxis occurs primarily in 2 settings: accidental exposure of a health care worker and high-risk sexual exposure. In the former circumstance, drug resistance data from the source may be useful in constructing a prophylactic regimen. In the case of high-risk sexual exposure, data on the source are usually not available.

SUMMARY AND FUTURE DIRECTIONS

Antiretroviral drug resistance is present wherever antiretroviral drugs are widely used, and as treatment rollout continues in developing countries, the range of resistance will expand. The incidence, prevalence, and transmission of drug-resistant viruses vary from country to country, and programs for worldwide drug resistance surveillance should increase rapidly to meet the emerging need. In developing countries, methods to simplify specimen collection, storage, and testing should be explored to facilitate better monitoring of individual patients and community patterns.

The approval of several new antiretroviral drugs from different classes since our previous recommendations [3] has increased the complexity of resistance testing and interpretation in the developed world. Resistance testing for entry inhibitors and INSTIs should be incorporated into routine management when such testing becomes available and validated.

Techniques for resistance testing have become more standardized since our previous recommendations and have been widely incorporated into routine management in the developed world. Coreceptor tropism testing has also become available. Future efforts should be made to increase the sensitivity of these assays to better detect minor variants that may be of clinical significance. Moreover, as resistance testing becomes increasingly employed in developing countries, attention should be given to detecting resistance patterns for non-sub-

type B strains of HIV and to establishing algorithms for evaluating their importance.

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