



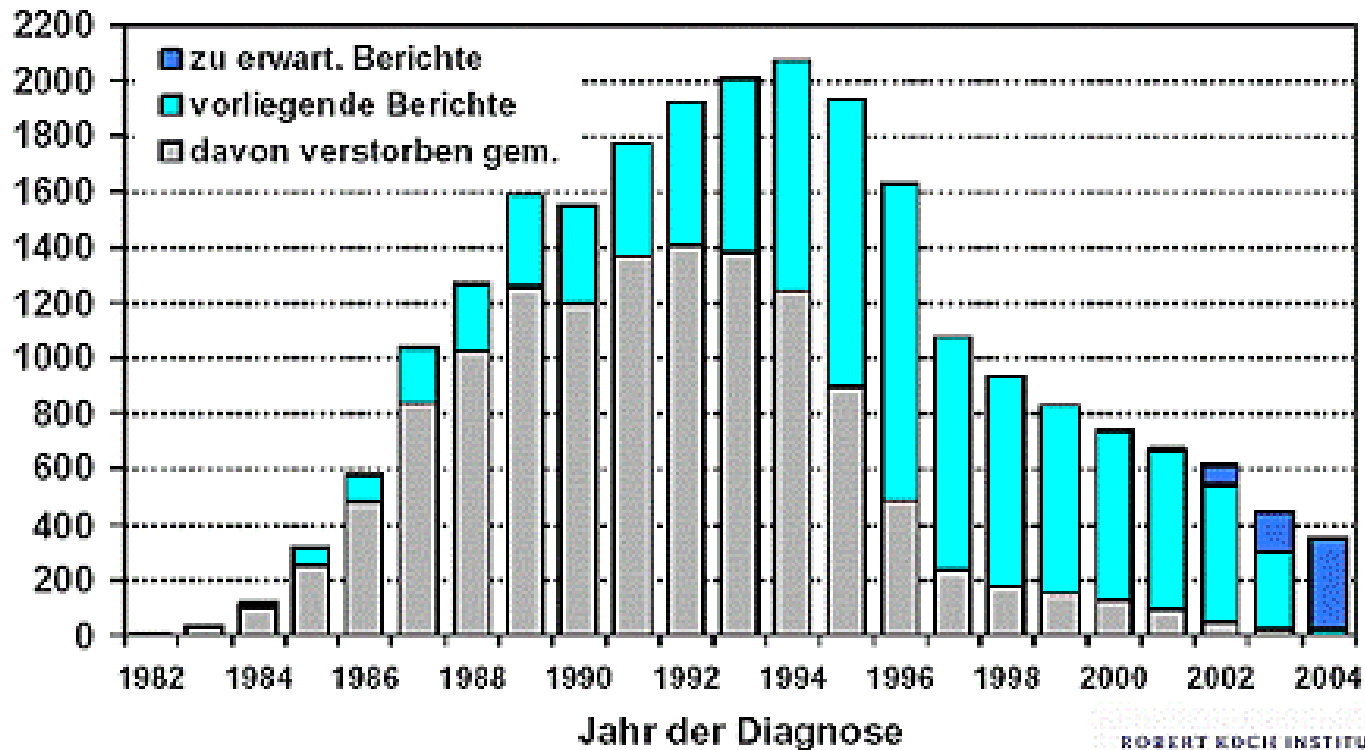
HIV drug resistance

Martin Däumer
Institute of Virology
University of Cologne

AIDS in Deutschland (06/2004)

Verteilung nach Diagnosejahr (N=22.928)

(korrigiert für Meldeverzug; N=23.304)

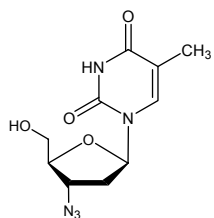


ROBERT KOCH INSTITUT

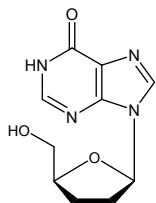




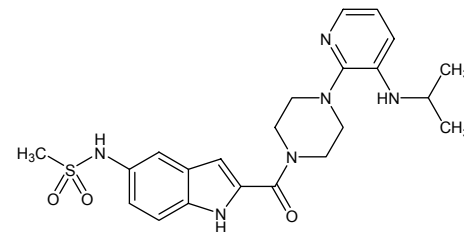
Reverse transcriptase (RT)



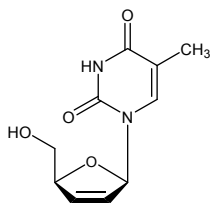
ZDV



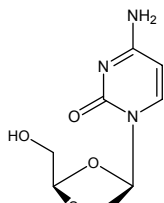
ddl



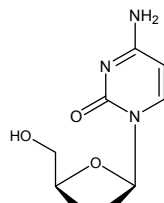
DLV



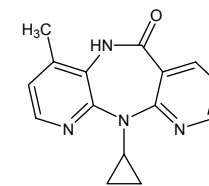
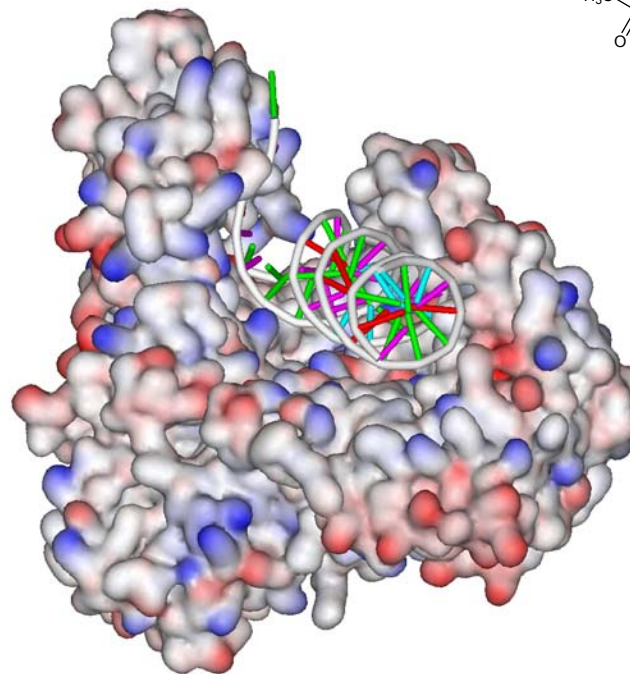
d4T



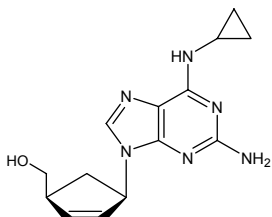
3TC



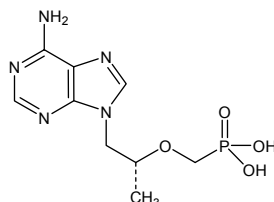
ddC



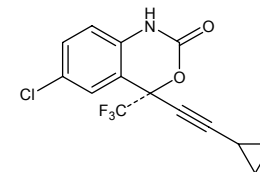
NVP



ABC

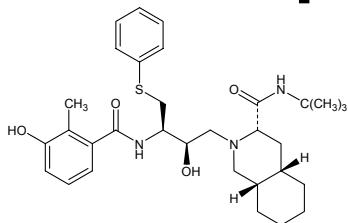


TDF

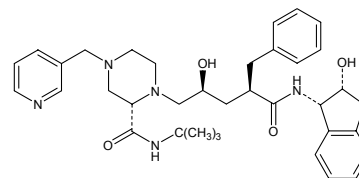


EFV

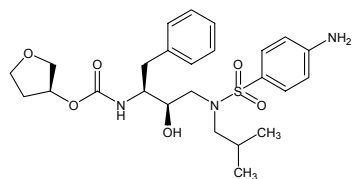
Protease (PR)



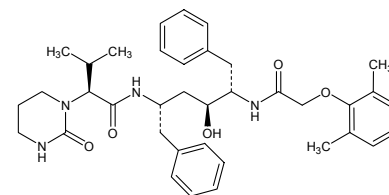
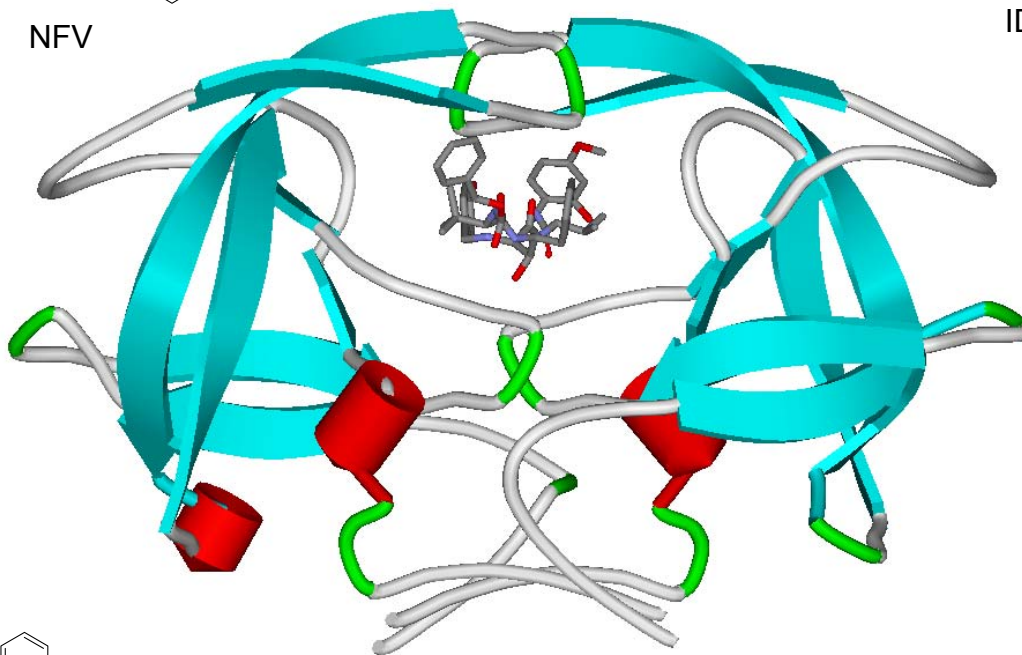
NFV



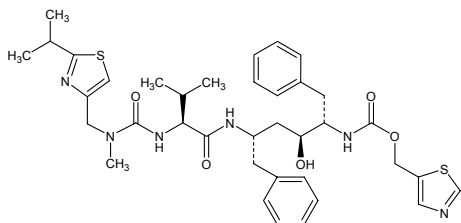
IDV



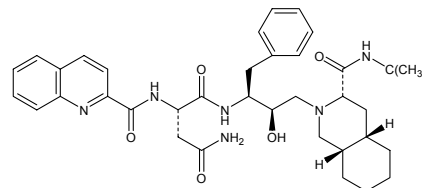
APV



LPV



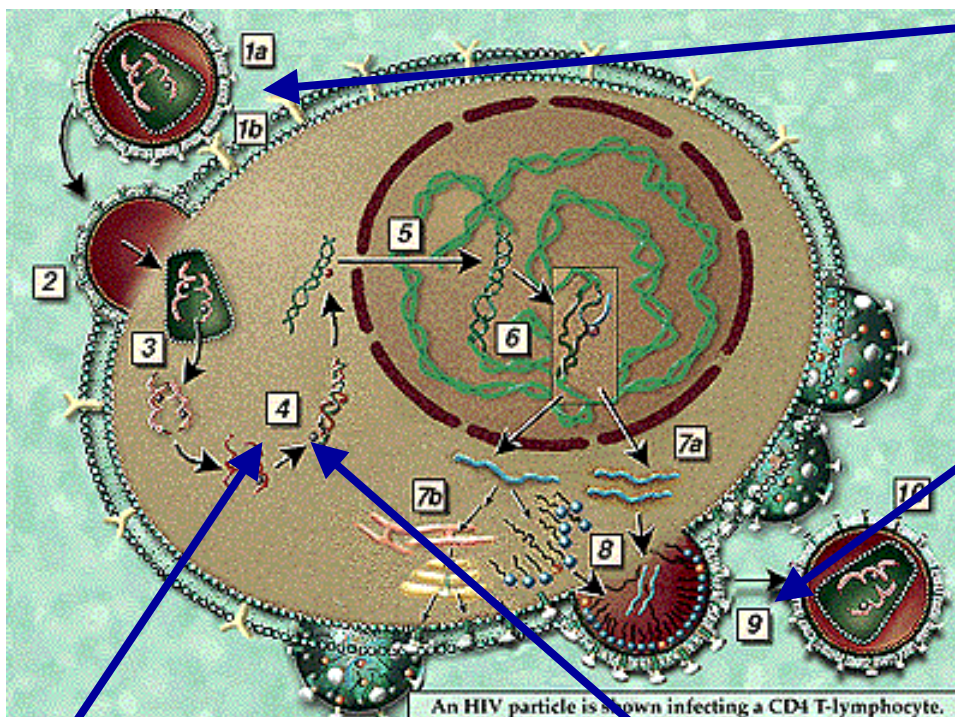
RTV



SQV

Replication cycle and drug targets

1. **Binding & fusion**
2. Entry
3. Uncoating
4. **Reverse transcription**
5. Integration
6. Transcription
7. Translation
8. Assembly & budding
9. **Maturation**



■ Entry inhibitors (EIs)
ENF (T20), MVC

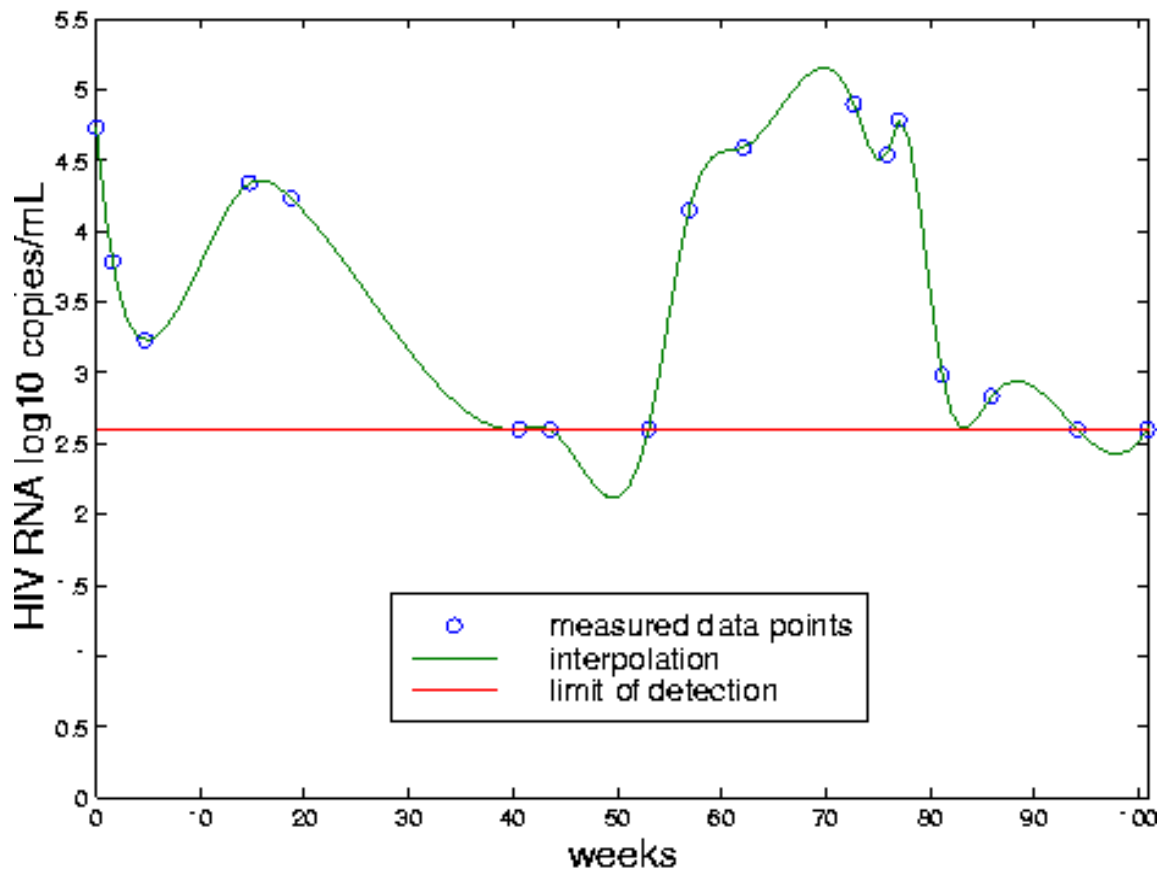
■ Protease inhibitors (PIs)
SQV, IDV, RTV, NFV, APV, LPV, ATV

■ Nucleoside reverse transcriptase inhibitors (NRTIs)
AZT, ddI, ddC, d4T, 3TC, ABC, TDF

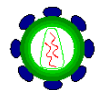
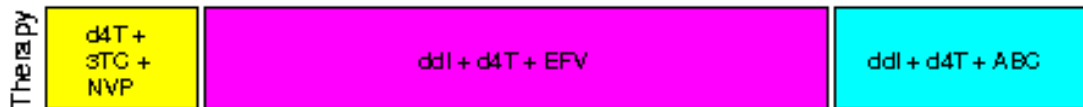
■ Non-nucleoside reverse transcriptase inhibitors (NNRTIs)
EFV, NVP, DLV

Treatment response

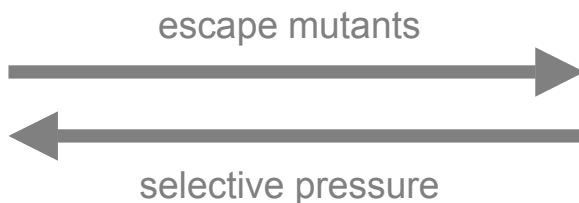
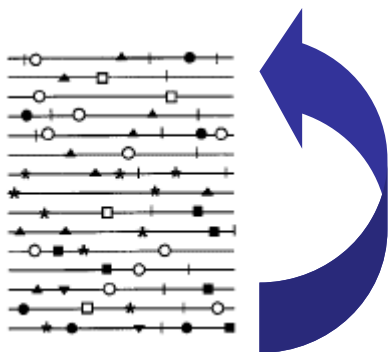
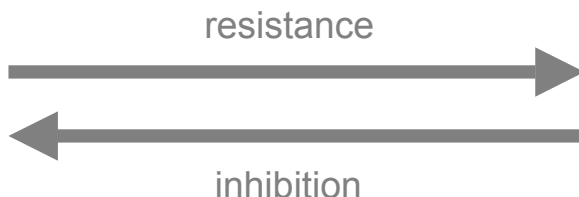
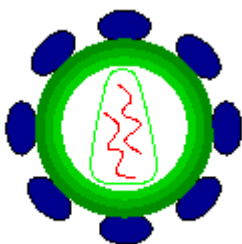
Virus load



- Combination therapy (2-6 drugs)
- Aims at reducing viral load (free virus in blood plasma)
- Lifelong therapy with strong side-effects
- Limited effectiveness due to **drug-resistant** variants



HIV infection: dynamics



Replication dynamics cause a high degree of genetic diversity.

HAART – Highly Active Anti-Retroviral Therapy:
Choose 2-6 drugs from 2 different drug classes.

⇒ viral rebound ⇒ treatment failure

How to measure resistance?

■ Phenotypic Resistance Testing

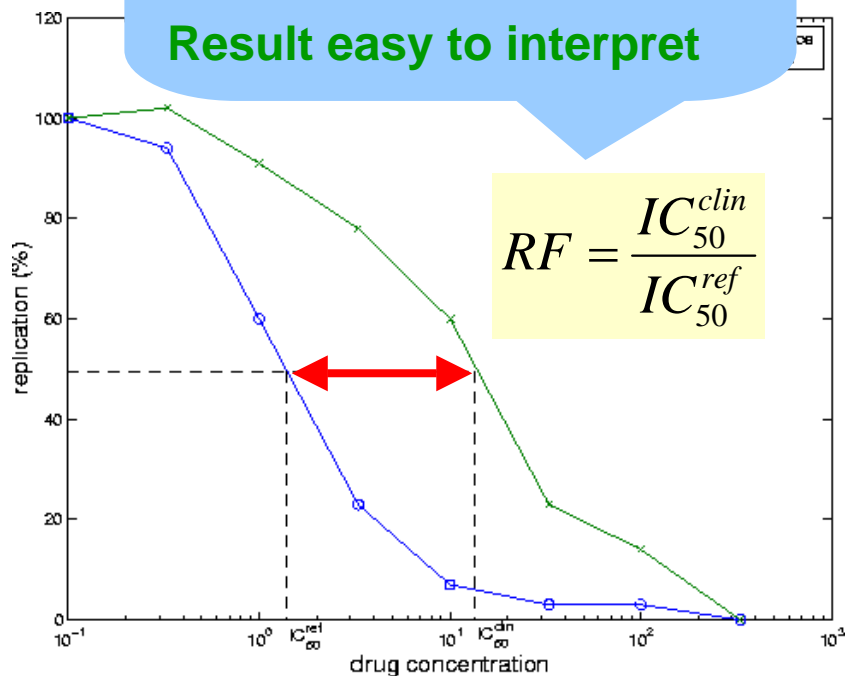
- ▶ measures the amount of drug that is required to inhibit the

Labour intensive

Takes 4-8 weeks,

costs ~1200 €

Result easy to interpret



$$RF = \frac{IC_{50}^{clin}}{IC_{50}^{ref}}$$

■ Genotypic Resistance Testing

- ▶ detects the presence of mutations known to be associated with drug resistance
- ▶ Cycle-sequencing assay

PR: PQITLWQRPLVTVKIGGQL...

RT: PISPIKTVPVRLKPGMDGP...

... ..

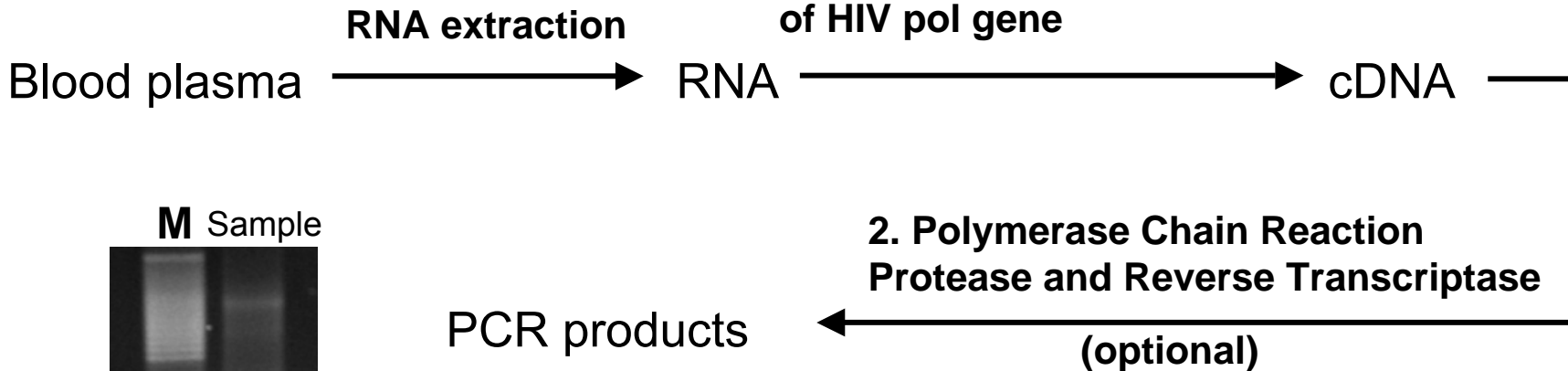
Standardized kits

**Takes a few days,
cheaper: ~360 €**

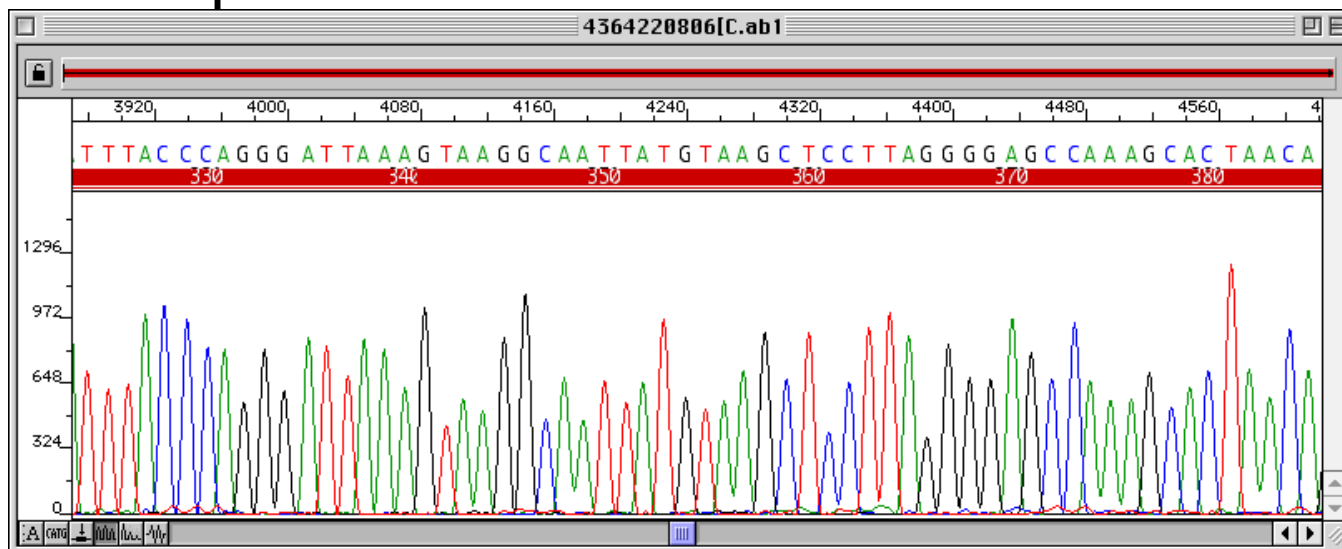
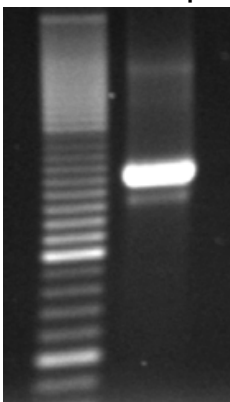
Interpretation challenging

Genotypic resistance testing

Reverse Transcription/
1. Polymerase Chain Reaction
of HIV pol gene



M Sample





„fasta“ file

```
>2006100903 Wed Sep 17 13:38:06 CEST 2003. 1302 bases.  
CCTCAGATCACTCTTTGGCAgCGACCCttCgTCACAATAAAAGTAGGRGG  
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aAGAAATGAATTTGCCaGGAAGRTGGAAACCAAAHTRATAGGGGGAATT  
GGAGGTTTTATGAAAGTAAACAGTATGAGGAAGTACCCATAGaAATCTG  
TGGACATAAGGTTACAGGTACAGTAGTAGTAGGACCTACACCTGCCAACA  
TAATtGGAAGGAATCTGTtGACTCAGCTtGGTTGCACTTTAAaTTTTCCC  
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cCcAaAAGTtAAACAATGGCCATtGACAGAAGAAAAAatAAAAGCATTAG  
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AAGAACAAATCAGTAACAGTACTAGATGTGGGTGATGCATATTTTTTCART  
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TCAaATTTACCAGGGATTAAAGTAAGGCAATTATGCAAACCTCCTTAGAG  
GAACCAAAGCACTGACAGAAGTAGTACCTCTAACGGAAGaAGCAGAGCTA  
GAACTGGCAGAAAACAGGGAGaTTcTgAAAGAACcAGTACATGGAGTGTA  
TTATGaCCCCCTCAAAGAYTTAATAGCAGAAAtAcAGAAGCAGGGGGCAaG  
G
```



Genotype

**2006100903
Protease**

L10F 0
M46I 0
M46L 0
I54M 0
L63P 0
A71V 0
V82A 0
V3I 1
I15V 1
S37N 1
R57K 1
D60E 1
Q61E 1
I62V 1
I72T 1
L76V 1
I93L 1

**2006100903
Reverse Transcriptase**

M41L 0
E44D 0
S68G 0
K103N 0
V118I 0
M184V 0
L210W 0
T215Y 0
A98G 1
D121H 1
I135T 1
D177E 1
I178L 1
E203D 1
Q207E 1
R211K 1
L214F 1
I293V 1



Mutations selected by PIs

**Multi-PI
Resistance:
Accumulation
of Mutations**

L	V	M	I	V	I	L
10	32	46	54	82	84	90
FIRV	I	IL	VML	AFTS	V	M

Indinavir

L	K	L	V	M	M	I	A	G	V	V	I	L
10	20	24	32	36	46	54	71	73	77	82	84	90
IRV	MR	I	I	I	IL	V	VT	SA	I	AFT	V	M

Ritonavir

L	K	V	L	M	M	I	A	V	V	I	L
10	20	32	33	36	46	54	71	77	82	84	90
FIRV	MR	I	F	I	IL	VL	VT	I	AFTS	V	M

Saquinavir

L	G	I	A	G	V	V	I	L
10	48	54	71	73	77	82	84	90
IRV	V	VL	VT	S	I	A	V	M

Nelfinavir

L	D	M	M	A	V	V	I	N	L
10	30	36	46	71	77	82	84	88	90
FI	N	I	IL	VT	I	AFTS	V	DS	M

(Fos)amprenavir

L	V	M	I	I	I	G	I	L
10	32	46	47	50	54	73	84	90
FIRV	I	IL	V	V	LVM	S	V	M

**Lopinavir/
ritonavir**

L	K	L	V	L	M	I	I	F	I	L	A	G	V	I	L
10	20	24	32	33	46	47	50	53	54	63	71	73	82	84	90
FIRV	MR	I	I	F	IL	VA	V	L	VL	P	VT	S	AFTS	V	M

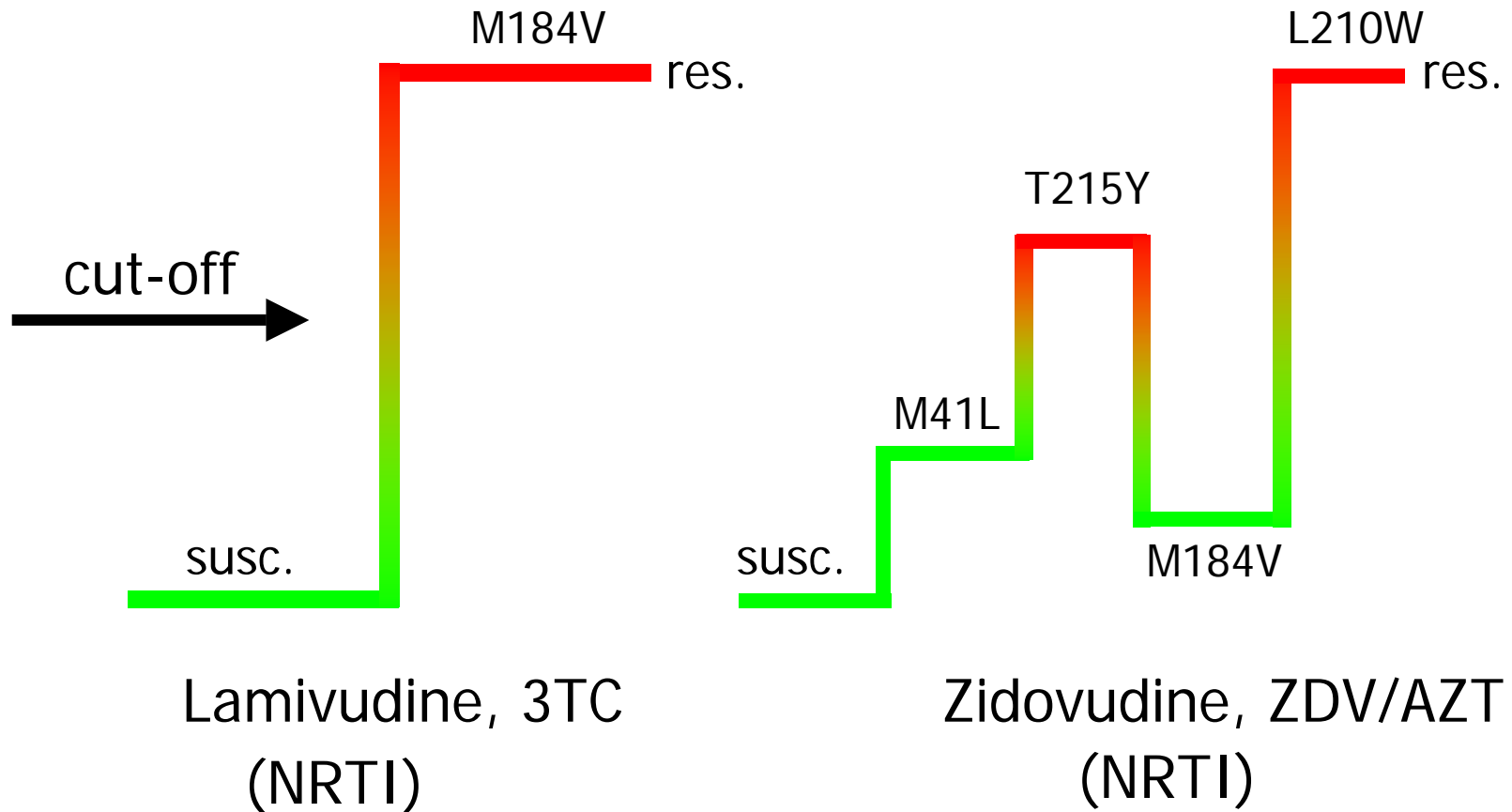
Atazanavir

L	K	L	V	L	M	M	G	I	I	A	G	V	N	L	
10	20	24	32	33	36	46	48	50	54	71	73	82	84	88	90
IFV	RMI	I	I	IF	ILV	I	V	L	L	V	CSTA	A	S	M	

**Tipranavir
/ritonavir**

L	K	L	V	L	M	I	I	V	I	L
10	20	33	46	54	82	84	90			
IV	MLT	IF	I	V	AFLT	V	M			

Defining resistance





Algorithms for genotype interpretation

Stanford HIV Drug Resistance Database

Regainst v6.2



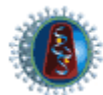
HIV ViroScorer™ v1.0

ANRS AC11

geno2pheno ver3.0



VirtualPhenotype



ViroSeq™ HIV-1 Genotyping System

TRUGENE HIV-1 Genotyping Test

RetroGram™

Decision Support System



Arevir



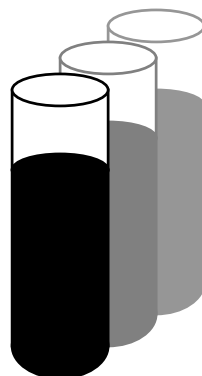
Genotype-phenotype pairs

900 clinical samples

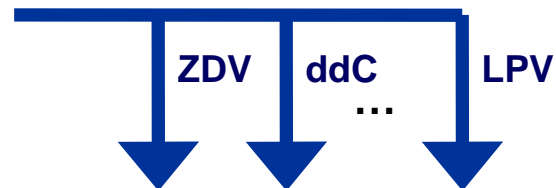
Genotyping



...KVIGSVLIGPTPANIIGRSLM...
...KVVGTVLVGPTPANIIGRNLM...
...KVIGSVLVGHTPSNIIGRNMM...
...KVVGTVLIGPTPVNVIGRNLM...
...

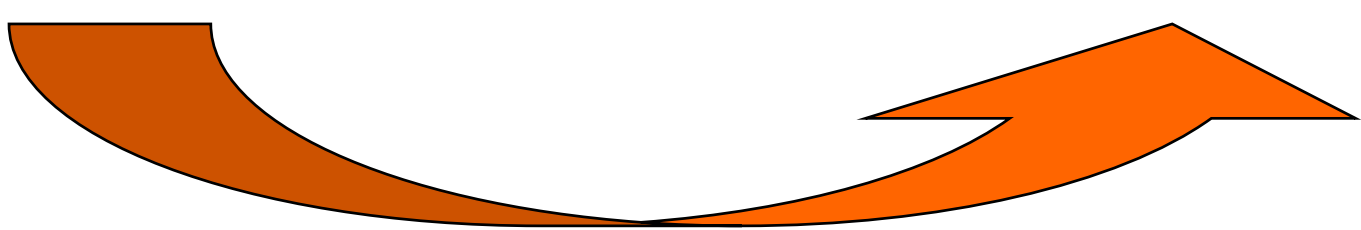


Phenotyping



2.1	5.9	...	1.9
5.3	7.2	...	13.0
406.0	123.3	...	88.7
13.7	24.8	...	2.5

...



Regression model

last update:
February 2003

geno2pheno Version 2.2

[Niko Beerenwinkel](#), [Martin Daumer](#), [Daniel Hoffmann](#), [Rolf Kaiser](#), [Klaus Korn](#), [Thomas Lengauer](#), [Barbara Schmidt](#), [Joachim Selbig](#), [Hauke Walter](#)



- [www.genafor.org](#)
- [Description of output](#)
- [Statistics](#)
- [References](#)
- [Contact](#)



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Netscape: geno2pheno - version 2.1

last update:
February 2003

geno2pheno Version 2.2

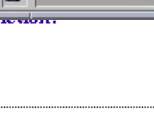
[Niko Beerenwinkel](#), [Martin Daumer](#), [Daniel Hoffmann](#), [Rolf Kaiser](#), [Klaus Korn](#), [Thomas Lengauer](#), [Barbara Schmidt](#), [Joachim Selbig](#), [Hauke Walter](#)



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Netscape: geno2pheno - version 2.1

last update:
February 2003

Phenotype Prediction

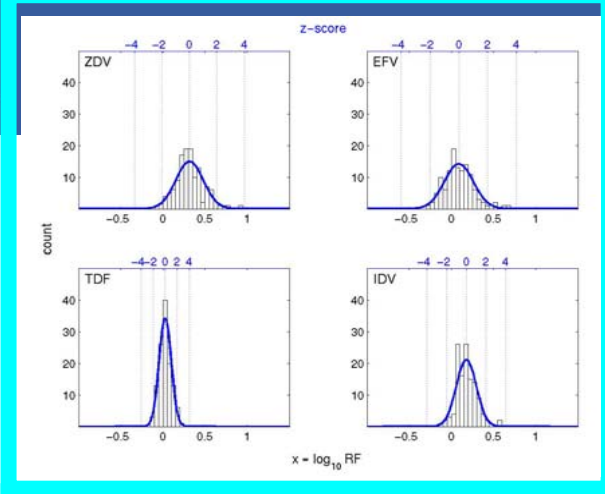
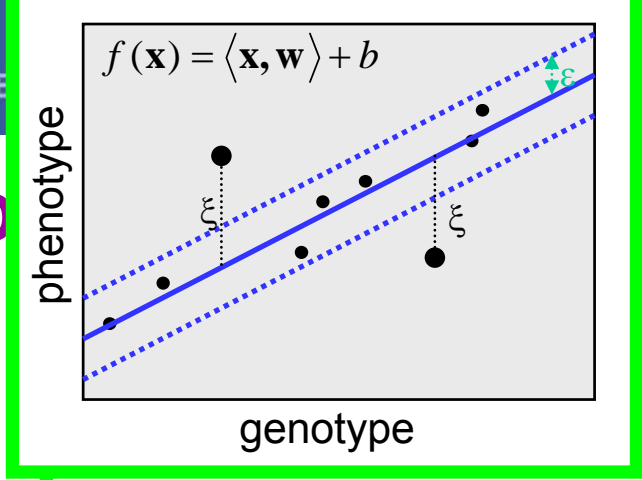
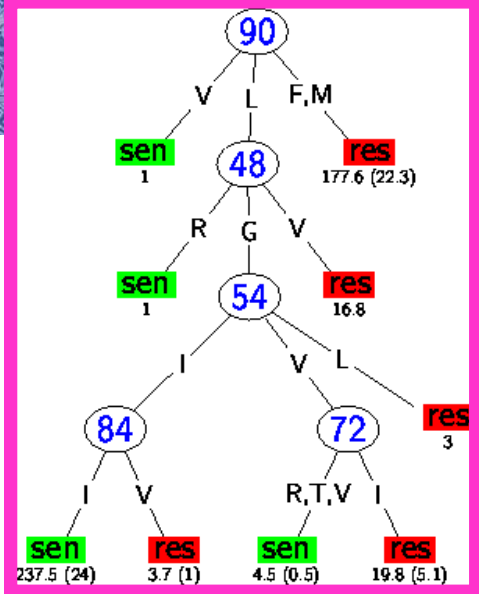
At ambiguous sequence positions (due to a mixed virus population) the resistance associated mutation has been assumed if present.

Drug	Cutoff	Decision tree classification ¹ [confidence factor]	SVM classification ²	Predicted fold-resistance (resistance factor, RF) ²	z-score (number of standard deviations above mean of drug-naive patients) more	Probability score (likelihood of belonging to the resistant subpopulation) more
ZDV	8.5	resistant [0.90]	resistant	423.3	13.3	1
ddc	2.5	susceptible [0.81]	susceptible	2.2	5.1	1
ddI	2.5	resistant [0.52]	resistant	3.8	6.9	0.68
d4T	2.5	resistant [0.74]	susceptible	3.2	7.1	1
3TC	8.5	susceptible [0.80]	resistant	9.7	7.2	0.0045
ABC	2.5	resistant [0.89]	resistant	4.4	9.3	1
TDF	2.5	resistant [0.76]	resistant	7.1	11.7	1
NVP	8.5	resistant [0.74]	resistant	13.1	3.7	0.95
DLV	8.5	resistant [0.74]	resistant	11.9	5.5	1
EFV	8.5	susceptible [0.84]	susceptible	3.4	2.6	0.59
SQV	3.5	resistant [0.88]	resistant	24.8	11.6	1
IDV	3.5	resistant [0.87]	resistant	19.3	8.8	1
RTV	3.5	resistant [0.89]	resistant	25.0	10.7	1
NFV	3.5	resistant [0.93]	resistant	25.1	6.9	1
APV	3.5	susceptible [0.92]	susceptible	5.2	4.0	1
LPV	3.5	susceptible [0.86]	resistant	5.0	5.2	1
ATV	3.5	resistant [0.83]	resistant	15.8	7.4	1

1: based on CS.0, RuleQuest Research Pty Ltd
2: based on LIBSVM, Copyright (c) 2000, Chih-Chung Chang and Chih-Jen Lin

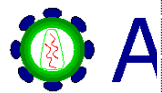
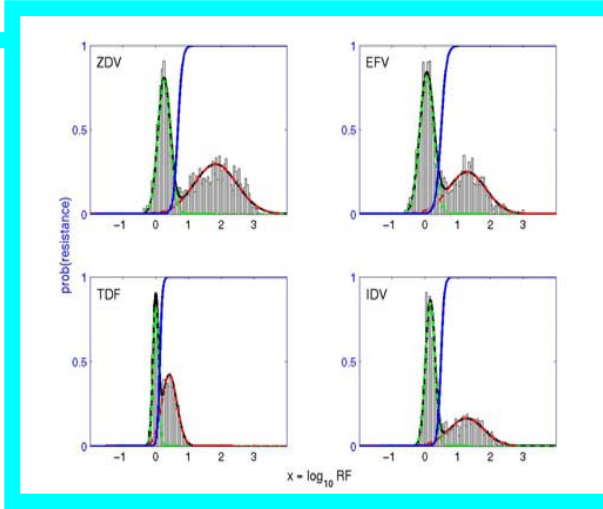
Results

You will



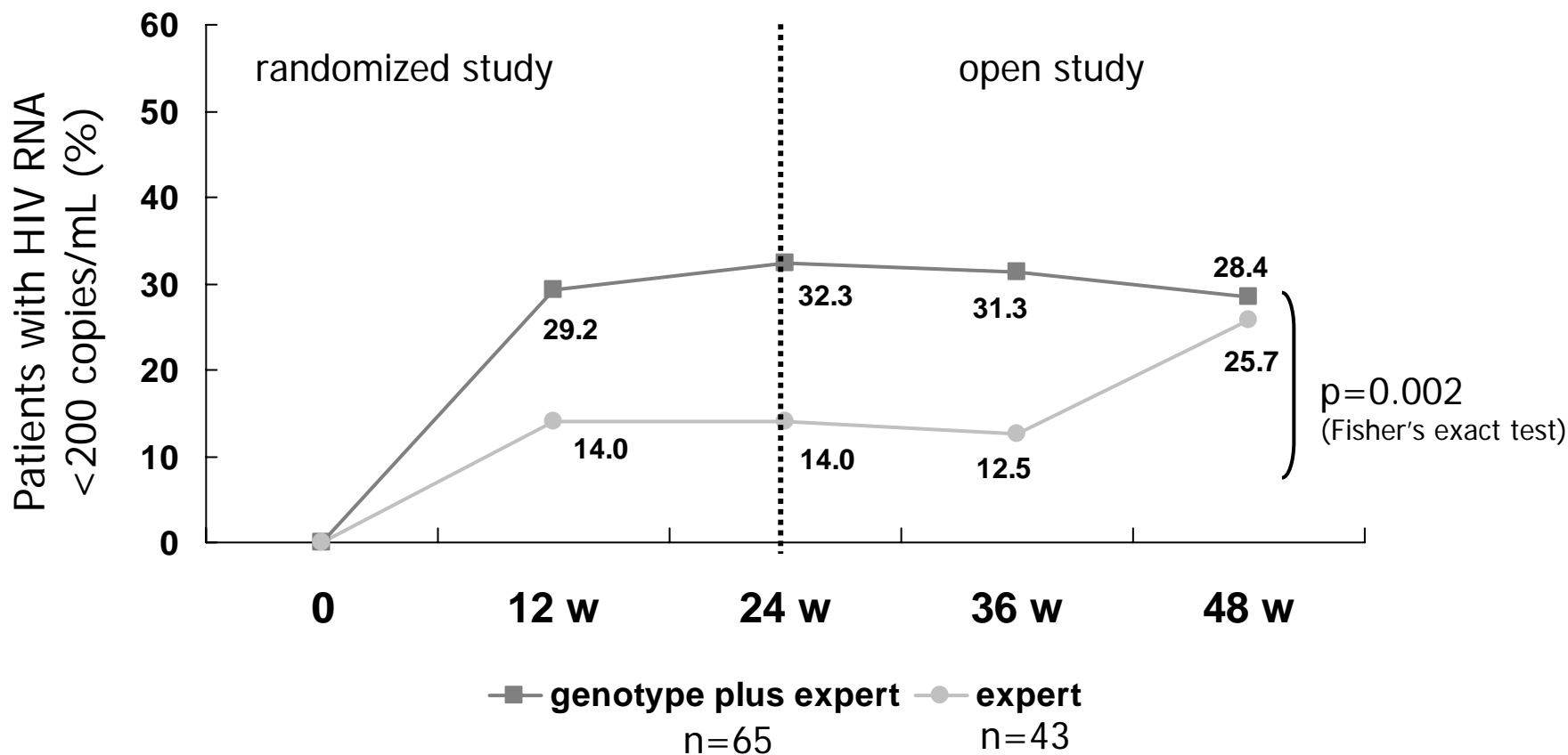
Data Statistics

Drug	Cutoff	Decision tree classification ¹ [confidence factor]	SVM classification ²	Predicted fold-resistance (resistance factor, RF) ²	z-score (number of standard deviations above mean of drug-naïve patients) more	Probability score (likelihood of belonging to the resistant subpopulation) more
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ddC	2.5	susceptible [0.81]	susceptible	2.2	5.1	1
ddI	2.5	resistant [0.52]	resistant	3.8	6.9	0.68
d4T	2.5	resistant [0.74]	susceptible	3.2	7.1	1
3TC	8.5	susceptible [0.80]	resistant	9.7	7.2	0.0045
ABC	2.5	resistant [0.89]	resistant	4.4	9.3	1
TDF	2.5	resistant [0.76]	resistant	7.1	11.7	1
NVP	8.5	resistant [0.74]	resistant	13.1	3.7	0.95
DLV	8.5	resistant [0.74]	resistant	11.9	5.5	1
EFV	8.5	susceptible [0.84]	susceptible	3.4	2.6	0.59
SQV	3.5	resistant [0.88]	resistant	24.8	11.6	1
IDV	3.5	resistant [0.87]	resistant	19.3	8.8	1
RTV	3.5	resistant [0.89]	resistant	25.0	10.7	1
NFV	3.5	resistant [0.93]	resistant	25.1	6.9	1
APV	3.5	susceptible [0.92]	susceptible	5.2	4.0	1
LPV	3.5	susceptible [0.86]	resistant	5.0	5.2	1
ATV	3.5	resistant [0.83]	resistant	15.8	7.4	1





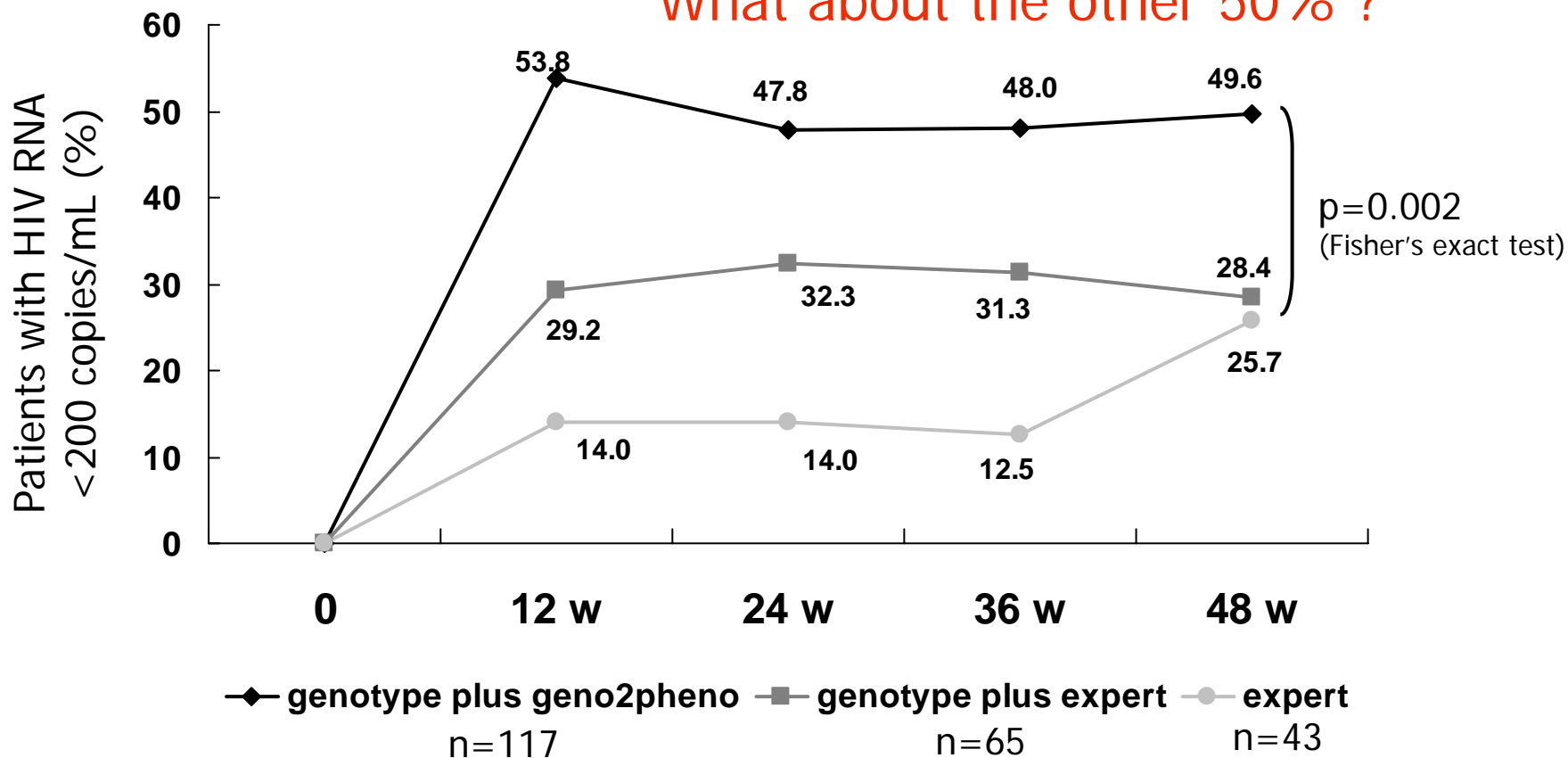
Viradapt study





geno2pheno based therapy switches

What about the other 50% ?





Acknowledgements

Daniel Hoffmann
Joachim Selbig
Rolf Kaiser

ZMB, Bioinformatik, University Duisburg-Essen
MPI of Molecular Plant Physiology, Golm
Institute of Virology, University of Cologne

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MPI für Informatik, Saarbrücken

Melanie Balduin
Saleta Sierra-Aragon
Dörte Hammerschmidt

Institute of Virology, University of Cologne

Barbara Schmidt
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