Neues zur Resistenziologie …

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Berlin AIDS-AK
04.11.2009

Resistenz ist nicht genug

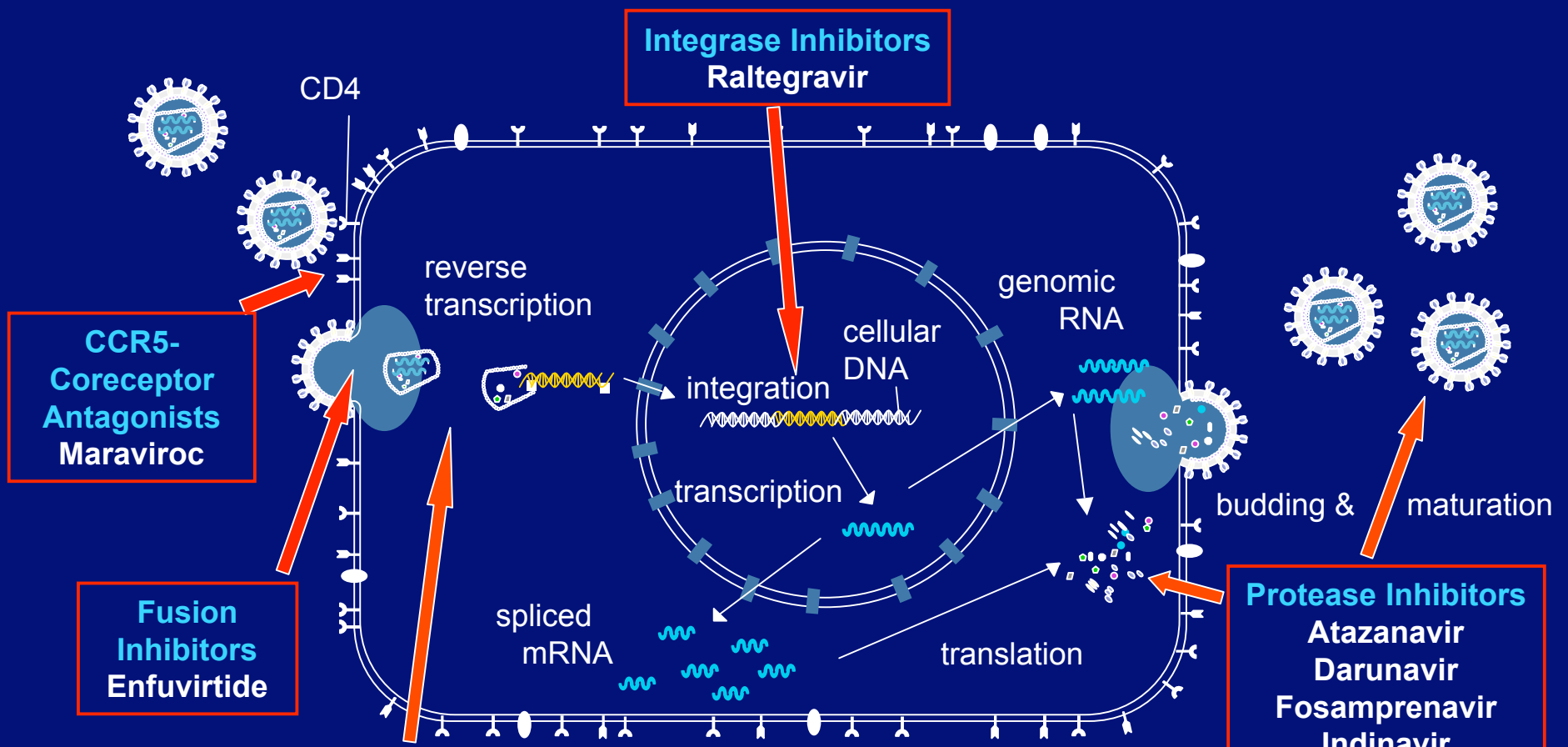
Empfindlichkeit

Aktivität

Potenz

...

Approved Antiretrovirals



Factors influencing antiretroviral therapy response

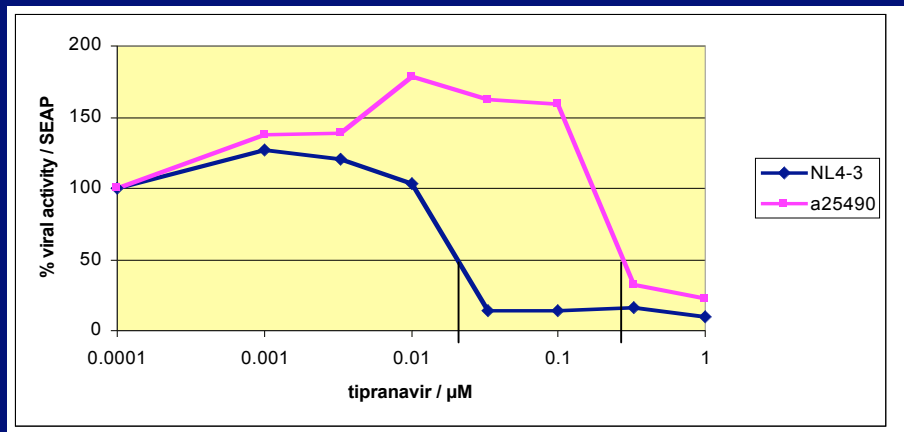
- Virus
  - Resistance (IC50)
  - Coreceptor tropism
  - „Fitness“
    => Pathogenicity

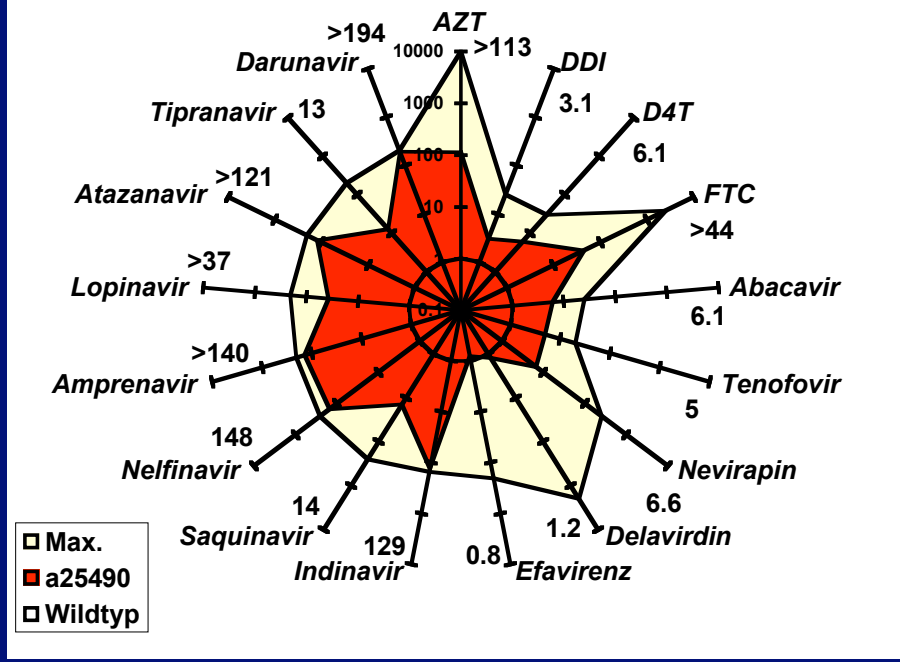
- Drug
  - Potency
  - Plasma level
  - Inhibitory quotient (IQ)
  - Genetic barrier
  - Slope

- Host
  - Immune systeme
    - HLA
  - CCR5-d32
  - Pharmacogenomics

Viral properties influencing therapy response

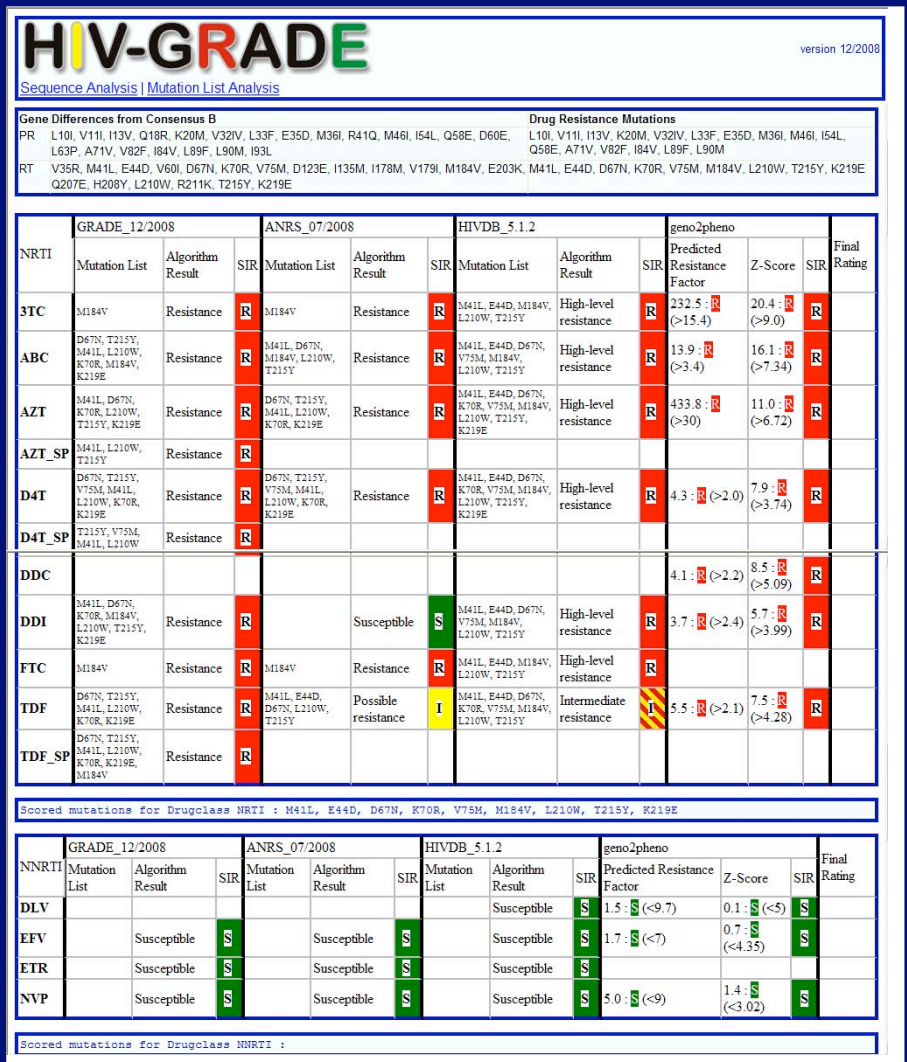
- Viral susceptibility
  - Functional testing
    - IC50
    - Resistance (increase of IC50)
    - Most recent: coreceptor tropism





Viral properties influencing therapy response

- Viral susceptibility
  - Genotyping
    - Mutations associated to drug resistance
    - Mutations associated to coreceptor tropism
      - CCR5
      - CXCR4
    - Need for interpretation systems



HIV-GRADE version 12/2008

Sequence Analysis | Mutation List Analysis

Gene	Differences from Consensus B	Drug Resistance Mutations
PR	L10I, V11I, I13V, Q18R, K20M, V32IV, L33F, E35D, M36I, R41Q, M46I, I54L, Q58E, D60E, L63P, A71V, V82F, I84V, L89F, L90M, I93L	L10I, V11I, I13V, K20M, V32IV, L33F, E35D, M36I, M46I, I54L, Q58E, A71V, V82F, I84V, L89F, L90M
RT	V35R, M41L, E44D, V60I, D67N, K70R, V75M, D123E, I135M, I178M, V179I, M184V, E203K, Q207E, H208Y, L210W, R211K, T215Y, K219E	M41L, E44D, D67N, K70R, V75M, M184V, L210W, T215Y, K219E

NRTI	GRADE_12/2008			ANRS_07/2008			HIVDB_5.1.2			geno2pheno			Final Rating
	Mutation List	Algorithm Result	SIR	Mutation List	Algorithm Result	SIR	Mutation List	Algorithm Result	SIR	Predicted Resistance Factor	Z-Score	SIR	
3TC	M184V	Resistance	R	M184V	Resistance	R	M41L, E44D, M184V, L210W, T215Y	High-level resistance	R	232.5 : R (>15.4)	20.4 : R (>9.0)	R	
ABC	D67N, T215Y, M41L, L210W, K70R, M184V, K219E	Resistance	R	M41L, D67N, M184V, L210W, T215Y	Resistance	R	M41L, E44D, D67N, V75M, M184V, L210W, T215Y	High-level resistance	R	13.9 : R (>3.4)	16.1 : R (>7.34)	R	
AZT	M41L, D67N, K70R, L210W, T215Y, K219E	Resistance	R	D67N, T215Y, M41L, L210W, K70R, K219E	Resistance	R	M41L, E44D, D67N, K70R, V75M, M184V, L210W, T215Y, K219E	High-level resistance	R	433.8 : R (>30)	11.0 : R (>6.72)	R	
AZT_SP	M41L, L210W, T215Y	Resistance	R										
D4T	D67N, T215Y, V75M, M41L, L210W, K70R, K219E	Resistance	R	D67N, T215Y, V75M, M41L, L210W, K70R, K219E	Resistance	R	M41L, E44D, D67N, K70R, V75M, M184V, L210W, T215Y, K219E	High-level resistance	R	4.3 : R (>2.0)	7.9 : R (>3.74)	R	
D4T_SP	T215Y, V75M, M41L, L210W	Resistance	R										
DDC										4.1 : R (>2.2)	8.5 : R (>5.09)	R	
DDI	M41L, D67N, K70R, M184V, L210W, T215Y, K219E	Resistance	R		Susceptible	S	M41L, E44D, D67N, V75M, M184V, L210W, T215Y	High-level resistance	R	3.7 : R (>2.4)	5.7 : R (>3.99)	R	
FTC	M184V	Resistance	R	M184V	Resistance	R	M41L, E44D, M184V, L210W, T215Y	High-level resistance	R				
TDF	D67N, T215Y, M41L, L210W, K70R, K219E	Resistance	R	M41L, E44D, D67N, L210W, T215Y	Possible resistance	I	M41L, E44D, D67N, K70R, V75M, M184V, L210W, T215Y	Intermediate resistance	I	5.5 : R (>2.1)	7.5 : R (>4.28)	R	
TDF_SP	D67N, T215Y, M41L, L210W, K70R, K219E, M184V	Resistance	R										

Scored mutations for Drugclass NRTI : M41L, E44D, D67N, K70R, V75M, M184V, L210W, T215Y, K219E

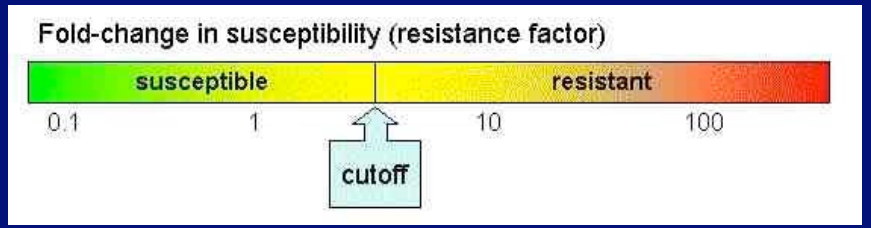
NNRTI	GRADE_12/2008			ANRS_07/2008			HIVDB_5.1.2			geno2pheno			Final Rating
	Mutation List	Algorithm Result	SIR	Mutation List	Algorithm Result	SIR	Mutation List	Algorithm Result	SIR	Predicted Resistance Factor	Z-Score	SIR	
DLV								Susceptible	S	1.5 : S (<9.7)	0.1 : S (<5)	S	
EFV		Susceptible	S		Susceptible	S		Susceptible	S	1.7 : S (<7)	0.7 : S (<4.35)	S	
ETR		Susceptible	S		Susceptible	S		Susceptible	S				
NVP		Susceptible	S		Susceptible	S		Susceptible	S	5.0 : S (<9)	1.4 : S (<3.02)	S	

Scored mutations for Drugclass NNRTI :

geno2pheno

- Viral susceptibility
  - geno2pheno
    - bioinformatically supported predictions
      - Resistance
      - Coreceptor tropism
      - Therapy Optimization (THEO)
        - » Trained on clinical therapy response data
        - » AREVIR DB, EURESIST DB
        - » Including predictions of resistance and genetic barrier

- Resistance predictions by g2p
- Fold resistance (RF)



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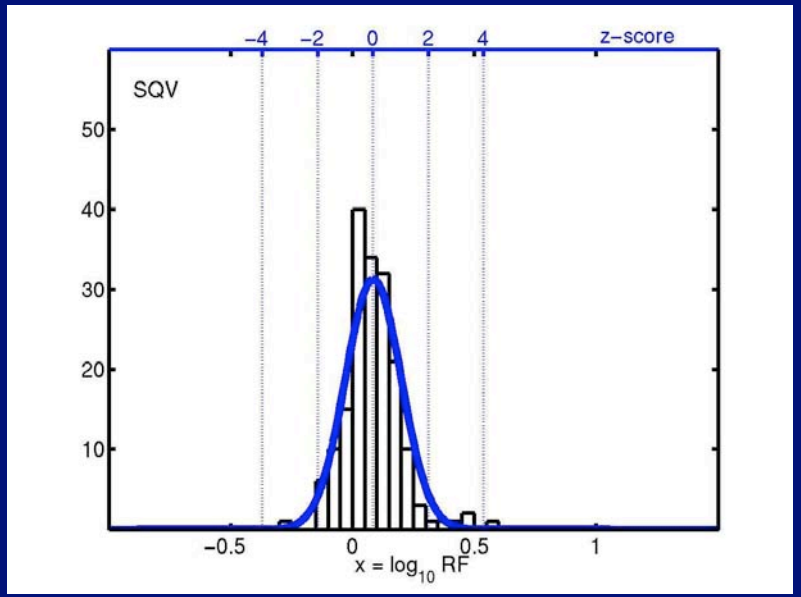

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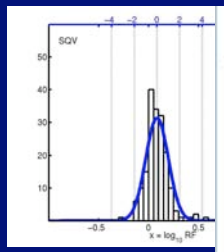

Z-score clinical cutoffs coloring

drug	susceptible	intermediate	resistant
ZDV	z < 4.92	4.92 ≤ z < 6.72	6.72 ≤ z
ddI	z < 2.8	2.8 ≤ z < 3.99	3.99 ≤ z
d4T	z < 1.65	1.65 ≤ z < 3.74	3.74 ≤ z
3TC	z < 5.97	5.97 ≤ z < 9	9 ≤ z
ABC	z < 5.09	5.09 ≤ z < 7.34	7.34 ≤ z
TDF	z < 3	3 ≤ z < 4.3	4.3 ≤ z
NVP	z < 3.02	3.02 ≤ z < 3.02	3.02 ≤ z
EFV	z < 4.35	4.35 ≤ z < 4.35	4.35 ≤ z
SQV/r	z < 5.02	5.02 ≤ z < 8.58	8.58 ≤ z
IDV/r	z < 3.86	3.86 ≤ z < 5.75	5.75 ≤ z
NFV	z < 2.06	2.06 ≤ z < 3.3	3.3 ≤ z
APV/r	z < 6.4	6.4 ≤ z < 6.4	6.4 ≤ z
LPV/r	z < 7.58	7.58 ≤ z < 11.5	11.5 ≤ z
ATV/r	z < 3.18	3.18 ≤ z < 5.95	5.95 ≤ z

geno2pheno

- Viral susceptibility
  - geno2pheno
    - bioinformatically supported predictions
      - Resistance
      - Coreceptor tropism
      - Therapy Optimization (THEO)
        - » Trained on clinical therapy response data
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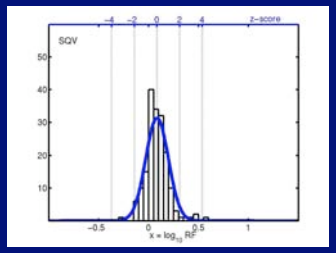
- Resistance predictions by g2p
- Fold resistance (RF)
- Z-score



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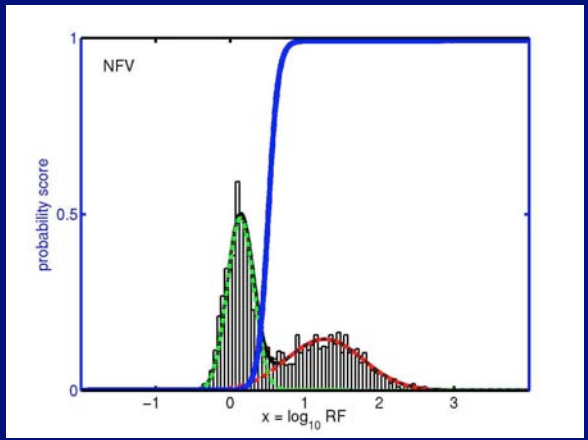
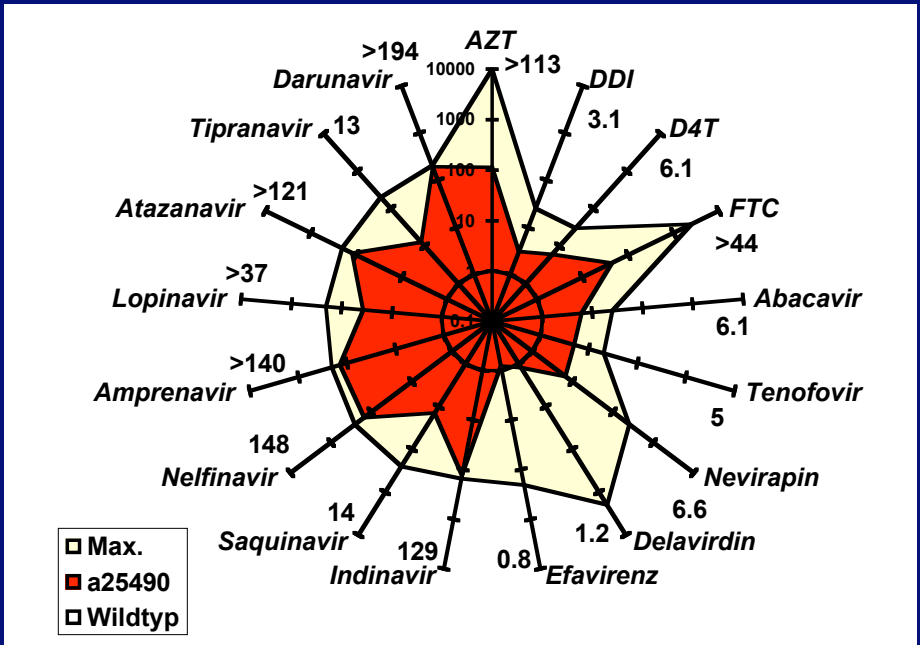
3TC	z < 5.97	5.97 ≤ z < 9	9 ≤ z
ABC	z < 5.09	5.09 ≤ z < 7.34	7.34 ≤ z
TDF	z < 3	3 ≤ z < 4.3	4.3 ≤ z
NVP	z < 3.02	3.02 ≤ z < 3.02	3.02 ≤ z
EFV	z < 4.35	4.35 ≤ z < 4.35	4.35 ≤ z
SQV/r	z < 5.02	5.02 ≤ z < 8.58	8.58 ≤ z
IDV/r	z < 3.86	3.86 ≤ z < 5.75	5.75 ≤ z
NFV	z < 2.06	2.06 ≤ z < 3.3	3.3 ≤ z
APV/r	z < 6.4	6.4 ≤ z < 6.4	6.4 ≤ z
LPV/r	z < 7.58	7.58 ≤ z < 11.5	11.5 ≤ z
ATV/r	z < 3.18	3.18 ≤ z < 5.95	5.95 ≤ z

- Probability score



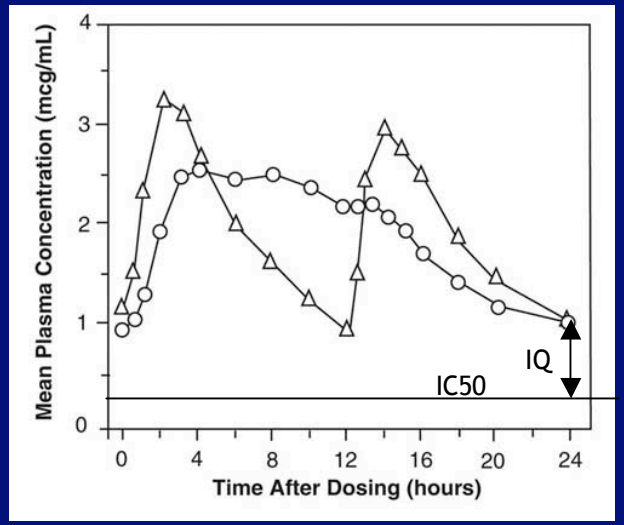
Results from the recombinant virus assay and g2p



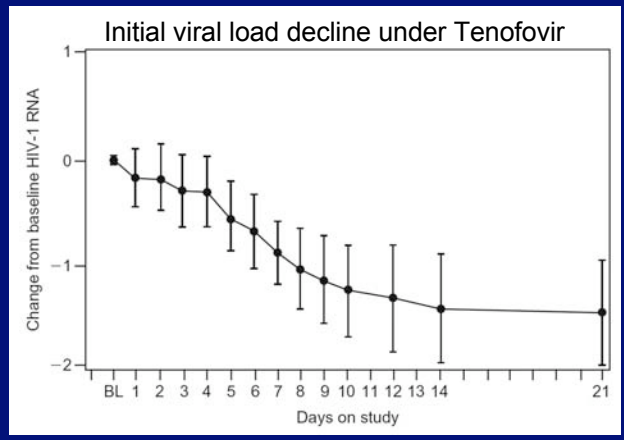
Drug	RF	Z-Score	Prob.Score
ZDV	433.810	10.951	1
ddI	3.742	5.655	1
d4T	4.254	7.856	1
3TC	232.531	20.416	1
ABC	13.895	16.140	1
TDF	5.517	7.527	1
NVP	4.998	1.400	0.19
EFV	1.704	0.710	0.021
SQV/r	52.536	14.004	1
IDV/r	451.450	17.710	1
NFV	652.649	15.826	1
APV/r	689.922	21.083	1
LPV/r	195.898	16.648	1
ATV/r	157.080	13.933	1

Drug properties … potency, what‘s that?

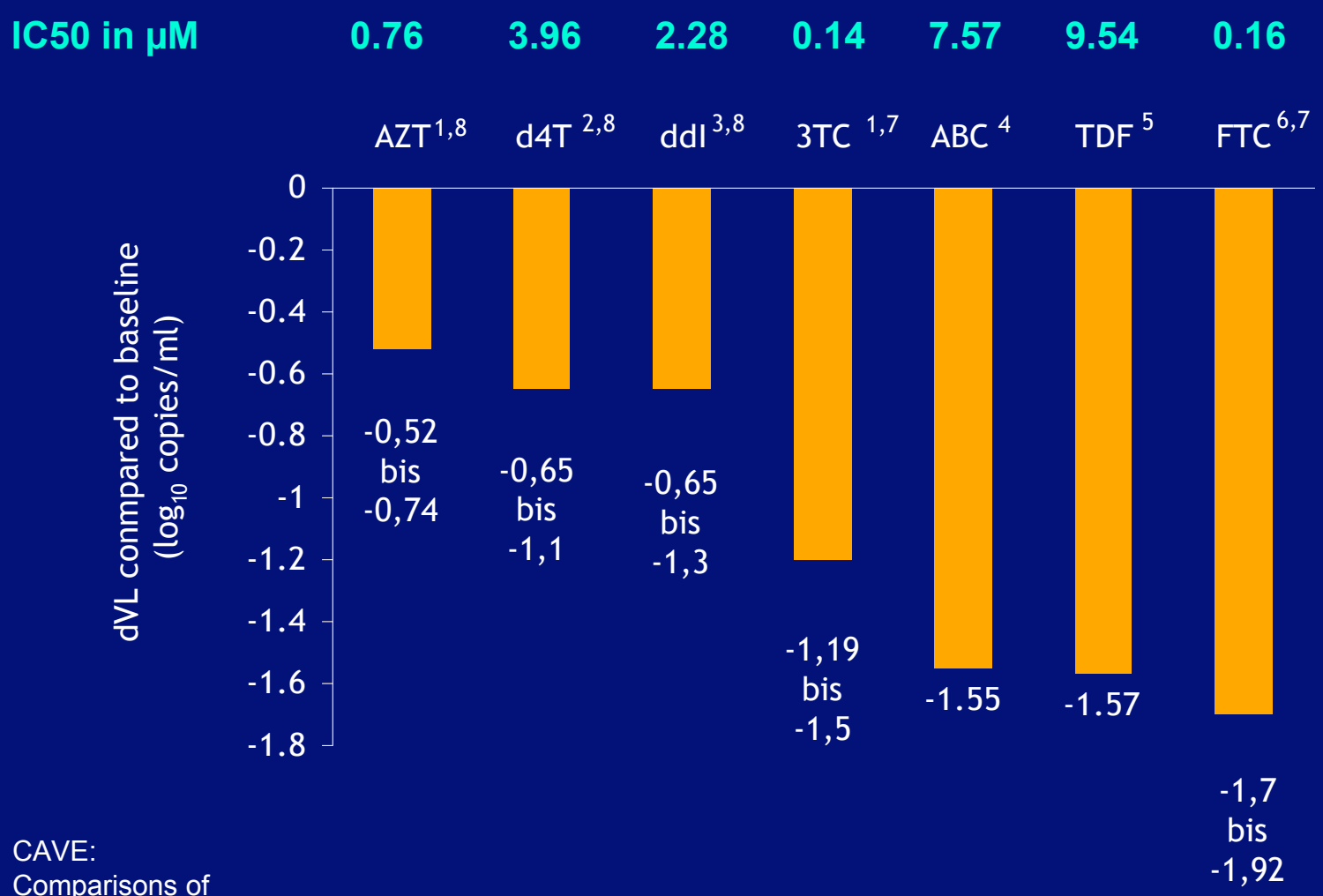
- Drug level
  - Intracellular
    - NRTIs = prodrugs
  - Plasma
    - Cmin => activity
    - Cmax => toxicity
    - IQ = Cmin / IC50



- Potency – are all drugs equally active?
  - IC50
    - Intitial viral load decrease after single drug add-on therapy
    - still:
      - Multiple factors influencing
        - » E.g. drug interactions



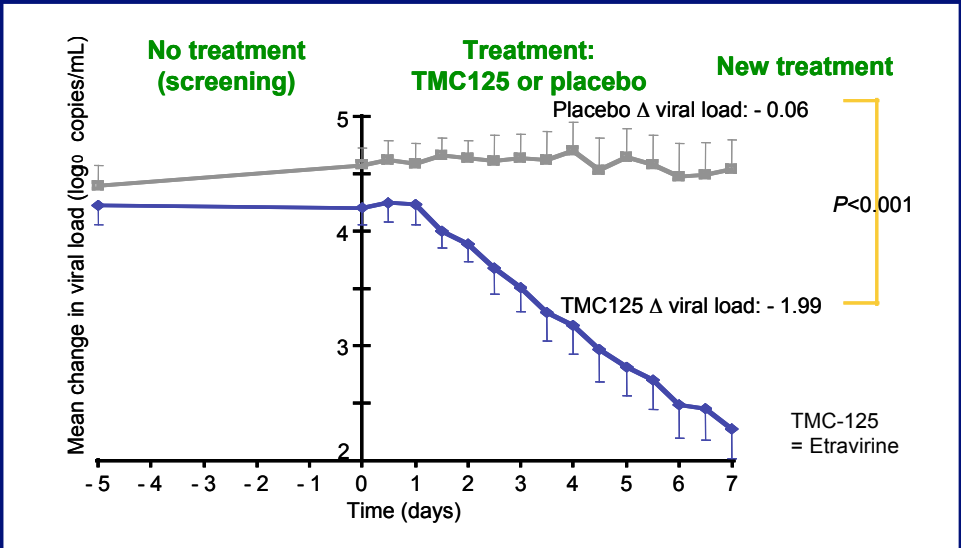
Initial viral load decrease of NRTI

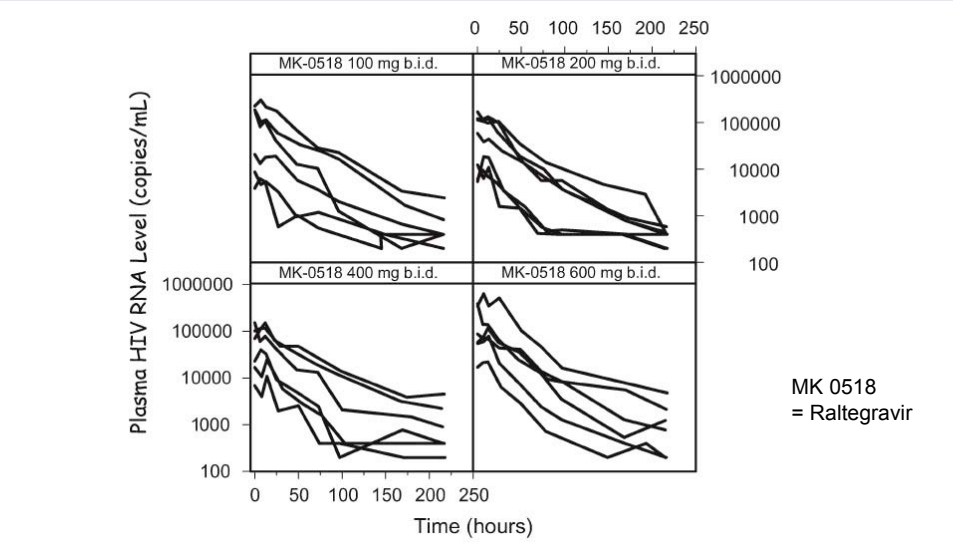


CAVE:
Comparisons of data derived in studies with different designs are not allowed

1 Eron J et al. NEJM 1995; 333:1662-9
2 Acosta EP & Balfour HH. JAIDS 2003; 33:343-348
3 Katzenstein DA et al. NEJM 1996; 335:1091-98
4 Staszewski S et al. AIDS 1998; 12:F197-202
5 Barditch-Crovo P et al. AAC,2001; 2733-1739
6 Rousseau F et al. JAC 2001; 48:507-517
7 Rousseau F et al. JID 2003; 188:1652-58
8 Schnittman S et al. ICAAC 2002. H-160

Initial viral load decreases of recent drugs



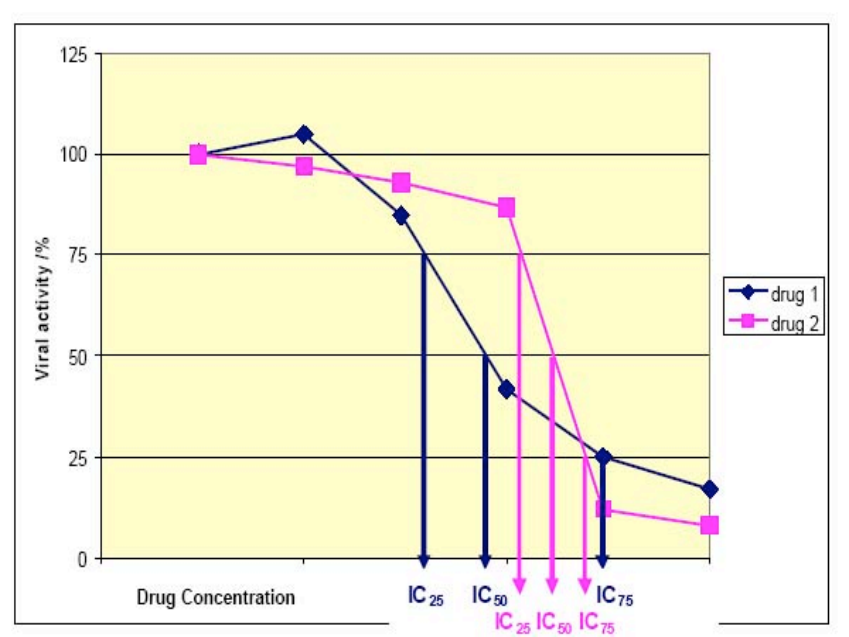


- Etravirine
  - 2 log in 7 days
- Raltegravir
  - **1.9-2.2 log in 10 days**

=> Comparability ?

- **Time**
- **Different backbone activities and interactions**

Slope of dose response curves

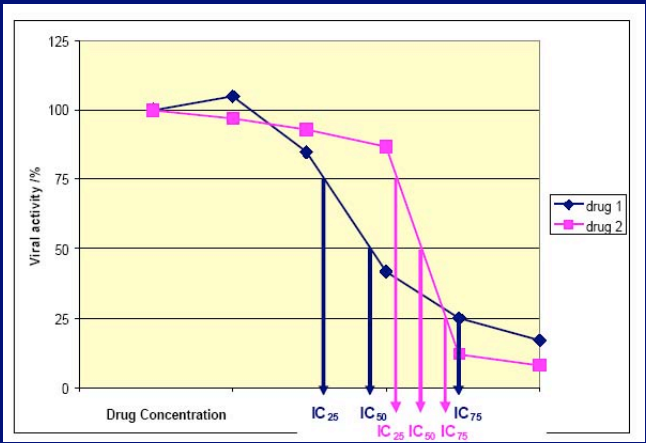


- Determination of
  - IC75 and IC25
  - IC75/IC25
    - Slope
  - The steeper the slope the lower the probability of residual replication in vivo

⇒ Correlation to drug levels ?

The slopes

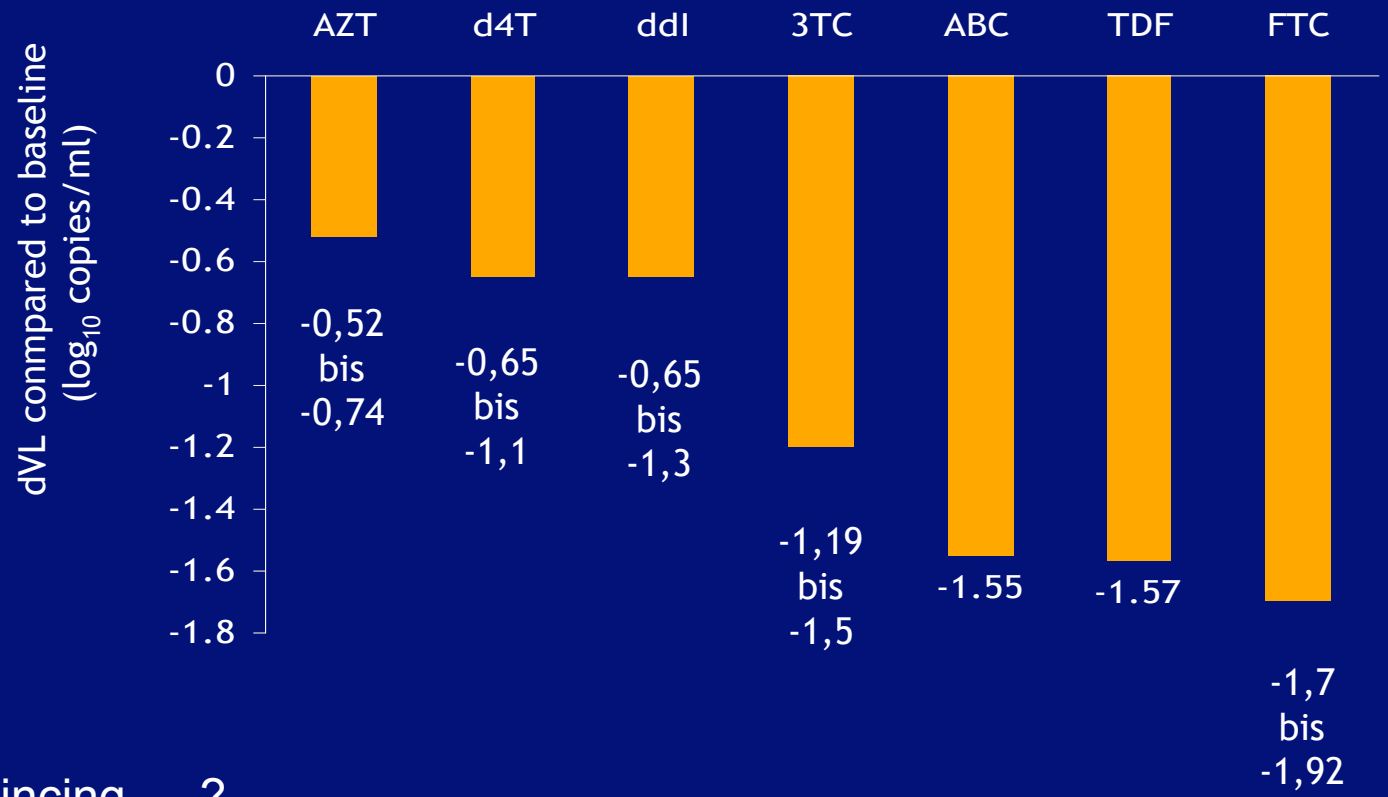
- IC75 / IC25 of 52 independant phenotypic assays
- Data excluded if
  - no inhibition >80%
  - Inhibition >50% in lowest concentration



	ratio IC75 / IC25	quartile 25%	quartile 75%	n
AZT	**27.4**	13.1	60.5	31
d4T	**5.2**	4.1	7.0	45
TDF	**6.1**	4.4	7.6	28
ABC	**5.4**	4.0	7.1	48
ddI	**6.0**	4.1	7.2	41
3TC	**6.1**	4.5	7.0	16
FTC	**3.8**	2.7	5.4	6
NVP	**3.3**	2.8	4.6	42
EFV	**2.6**	2.0	3.8	49
DLV	**3.3**	2.8	4.6	44
ETR	**2.2**	2.0	2.7	17
APV	**3.6**	3.4	4.7	17
ATV	**4.3**	3.5	5.2	17
DRV	**3.3**	2.9	3.3	6
IDV	**3.9**	3.1	4.6	21
LPV	**4.2**	3.3	6.4	13
NFV	**2.5**	2.2	3.2	23
SQV	**4.0**	3.0	6.7	27
TPV	**2.3**	2.0	2.9	12
RAL	**5.8**	5.3	6.6	4

Comparison for NRTI: IC50, slope and initial VL decrease

	AZT	d4T	ddI	3TC	ABC	TDF	FTC
IC50 in µM	0.76	3.96	2.28	0.14	7.57	9.54	0.16
Slope	27.4	5.2	6.0	6.1	5.4	6.1	3.8



Convincing … ?

Drug levels

- How to combine drug level data with slopes?
- Is this all we need ?

Reverse Transkriptase Inhibitoren: Nukleosidanalaoga (NRTIs)

Wirksubstanz	Dosierung (mg/d)	Protein-Bdg (%)	$T_{1/2ig}$ (h)	Spitzenspiegel[4] (mg/l) / [T_{max} (h)]	Talspiegel (mg/l)[4]	Inhibitor. Konz. *in vitro* (µg/l)[5]
Abacavir (ABC)	2x 300	~50	20.5			
Didanosin (ddI)	1x 400	<5	25-40			
Emtricitabin (FTC)	1x 200	<5	39			
Lamivudin (3TC)	1x 300	<35	12			
Stavudin (d4T)	1x 40	<5	3.5			
Zidovudin (ZDV, AZT)	2x 250-300	34-38	3			

Prodrugs, intrazelluläre Konzentration de

Reverse Transkriptase Inhibitoren: Nukleotidanaloga (NtRTIs)

Wirksubstanz	Dosierung (mg/d)	Protein-Bdg (%)	$T_{1/2ig}$ (h)	Spitzenspiegel[4] (mg/l) / [T_{max} (h)]	Talspiegel (mg/l)[4]	Inhibitor. Konz. *in vitro* (µg/l)[5]
Tenofovir (TDF)	1x 300		>24			Prodrug

Reverse Transkriptase Inhibitoren: Nicht-Nukleosidanaloga (NNRTIs)

Wirksubstanz	Dosierung (mg/d)	Protein-Bdg (%)	$T_{1/2}$ (h)[2]	Spitzenspiegel[4] (mg/l) / [T_{max} (h)]	Talspiegel (mg/l)[4]	Inhibitor. Konz. *in vitro* (µg/l)[5]
Efavirenz (EFV)	1x 600	>99	40-55 (- >100[6])	4 ± 1.1 [3-5]	1.7 ± 1	IC_{90} = 0.3-7.9 (frei)
Etravirin (ETV)	2x 200	>99.8	unk	unk	unk	$IC5_{50}$ = 1.4nM (frei)
Nevirapin (NVP)	2x 200	~60	25-30	5.7 [4]	4.5 ± 1.9	IC_{50} = 13-26 (frei)

Integrase Inhibitoren (IIs)

Wirksubstanz	Dosierung (mg/d)	Protein-Bdg (%)	$T_{1/2}$ (h)[2]	Spitzenspiegel[4] (mg/l) / [T_{max} (h)]	Talspiegel (mg/l)[4]	Inhibitor. Konz. *in vitro* (µg/l)[5]
Raltegravir (RGV)	2x 400	~?	~7-12	2.2 (1-4.8) [1-3.3]	.07 (.04-.1)	IC_{95} = 9 ± 7 (frei) IC_{95} = 16 ± 11 (iSerum)

Protease Inhibitoren (PIs)

Wirksubstanz	Dosierung (mg/d)	Protein-Bdg (%)	$T_{1/2}$ (h)[2]	Spitzenspiegel[4] (mg/l) / [T_{max} (h)]	Talspiegel (mg/l)[4]	Inhibitor. Konz. *In vitro* (µg/l)[5]
Atazanavir/**r** (ATV)	1 x 300/100	87	7-12	5.2 ± 3 [1-3]	0.86 ± 0.84	IC_{90} = 8 (frei) IC_{90} = 23 (in Serum)
Darunavir/**r** (DRV)	2x 600/100	95	~15	5.7 ± 1.9 [2.5-4]	3.6 ± 1.2	IC_{50} = 0.7-5 (frei)
Fosamprenavir/**r** (FAPV)	2x 700/100	90	~7	6.8 (5.4-6.9) [~1.5]	2.1 (1.8-2.5)	IC_{50} = 6-21(frei)
Indinavir/**r** (IDV)	2x 800/100	60-65	1.5-2	9 ± 2.9 [0.5-1.1]	0.18 ± 0.13	IC_{95} = 25-100 (frei)
Lopinavir/**r** (LPV)	2x 400/100	98-99	5-6	9.6 ± 4.4 [4-6]	5.5 ± 4	IC_{50} = 3-17 (frei) IC_{50} = 40-180 (iSerum)
Ritonavir (RTV)	2x 600	98-99	3-5	11.2 C 3.6 [3-5]	3.7 ± 2.6	IC_{90} = 70 (frei) IC_{90} = 2100 (in Serum)
Saquinavir$_{hg}$/**r**(SQV	2x 1000/100	98	~4	1.2 (1-1.6) [~2]	0.23 (.17-.3)	IC_{90} = 3-54 (frei)
Tipranavir/**r** (TPV)	2x 500/200	>99.9%	~6	♀ 56 ± 13 [~3] ♂ 46 ± 10 [~3]	♀ 25 ± 14 ♂ 21 ± 10	IC_{50} = 18-42 (frei) IC_{90} = 42-108 (frei)

Unexpected support

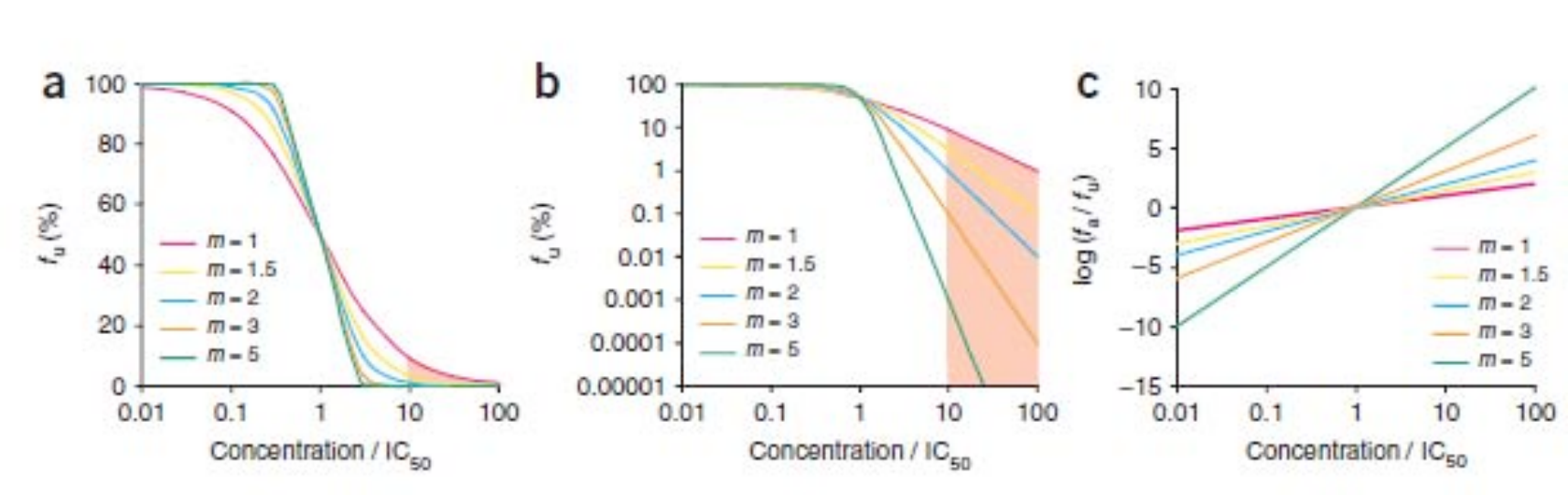
LETTERS

nature medicine

Dose-response curve slope sets class-specific limits on inhibitory potential of anti-HIV drugs

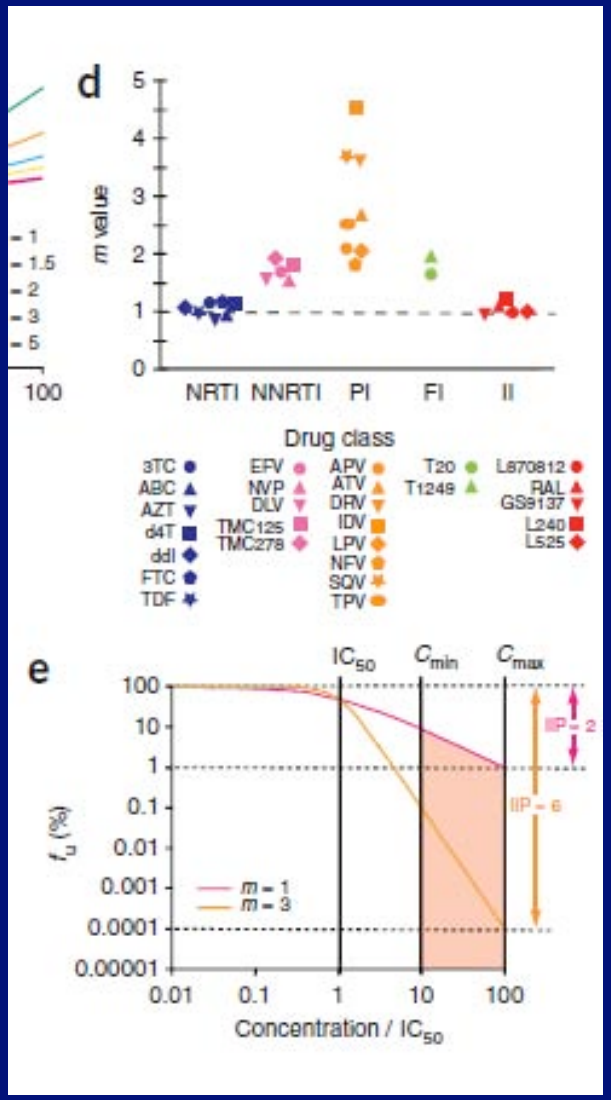
Lin Shen[1,2], Susan Peterson[1], Ahmad R Sedaghat[1], Moira A McMahon[1,2], Marc Callender[1], Haili Zhang[1], Yan Zhou[1], Eleanor Pitt[1], Karen S Anderson[3], Edward P Acosta[4] & Robert F Siliciano[1,5]

Slopes and m-value

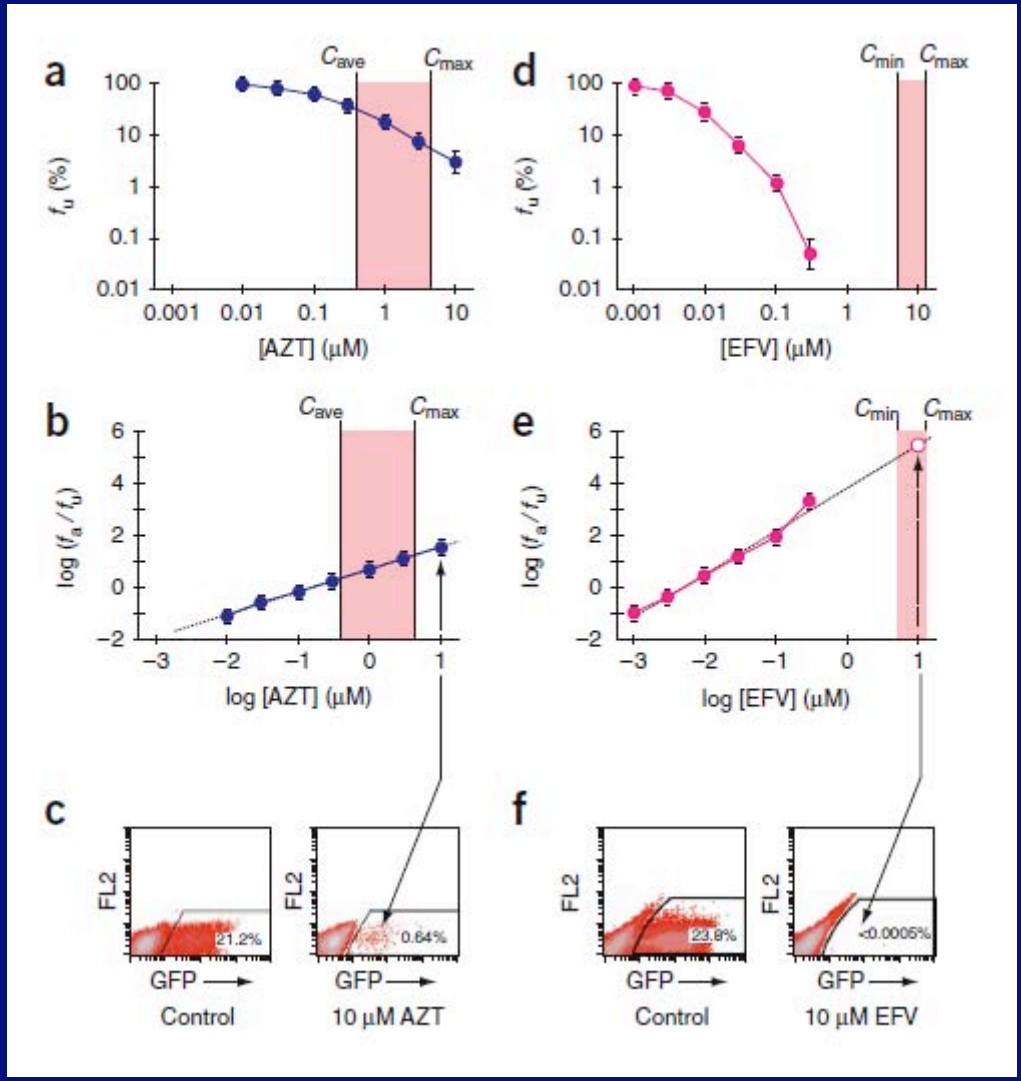


Slopes and m-values

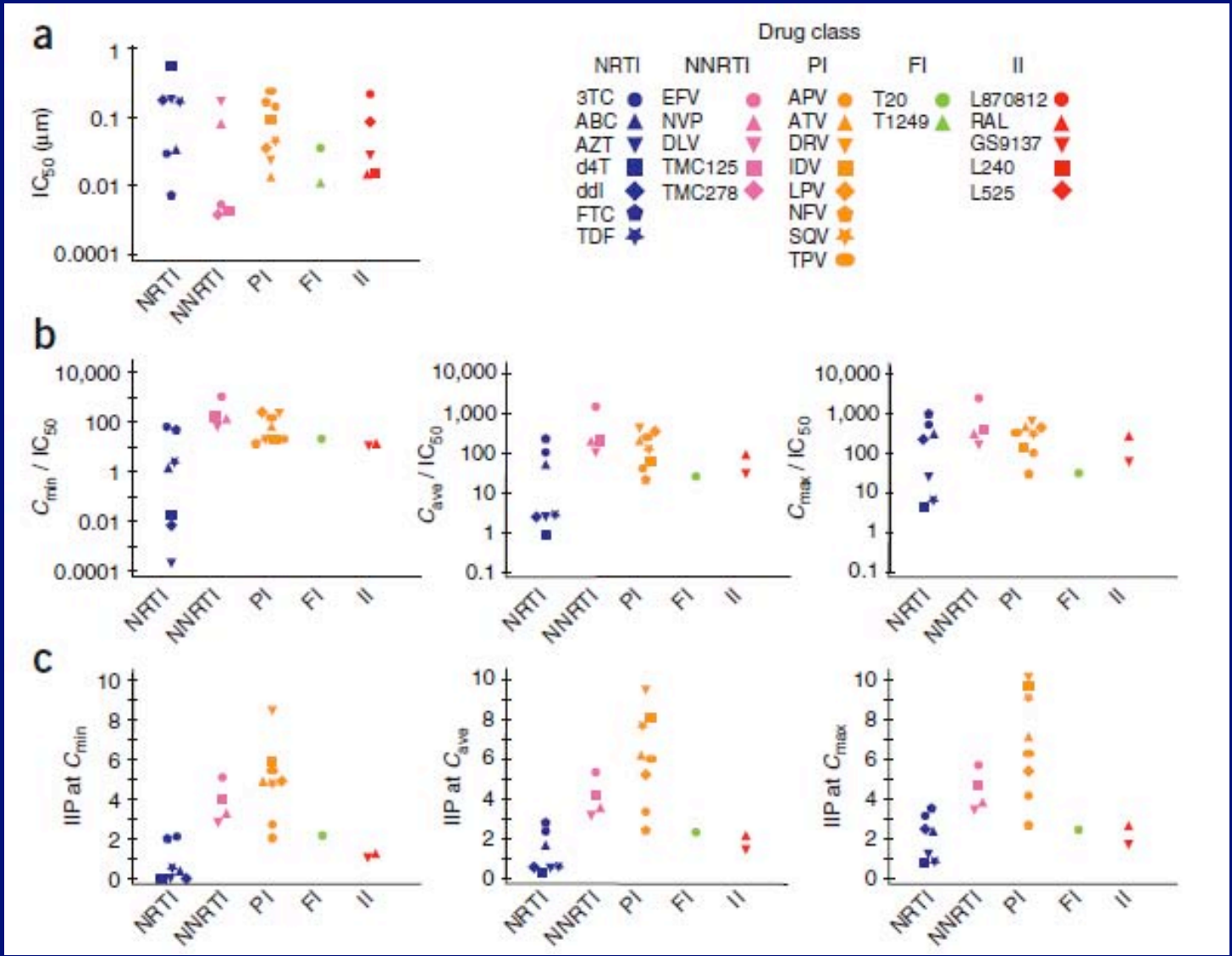
- Drug class specific m-values
- Not fully concordant to clincial response data ?
- What about drug levels in vivo?
  - Maybe also important
  - Instanteous inhibitory potential



Correlation of concentration and percentage of infected cells according to plasma levels

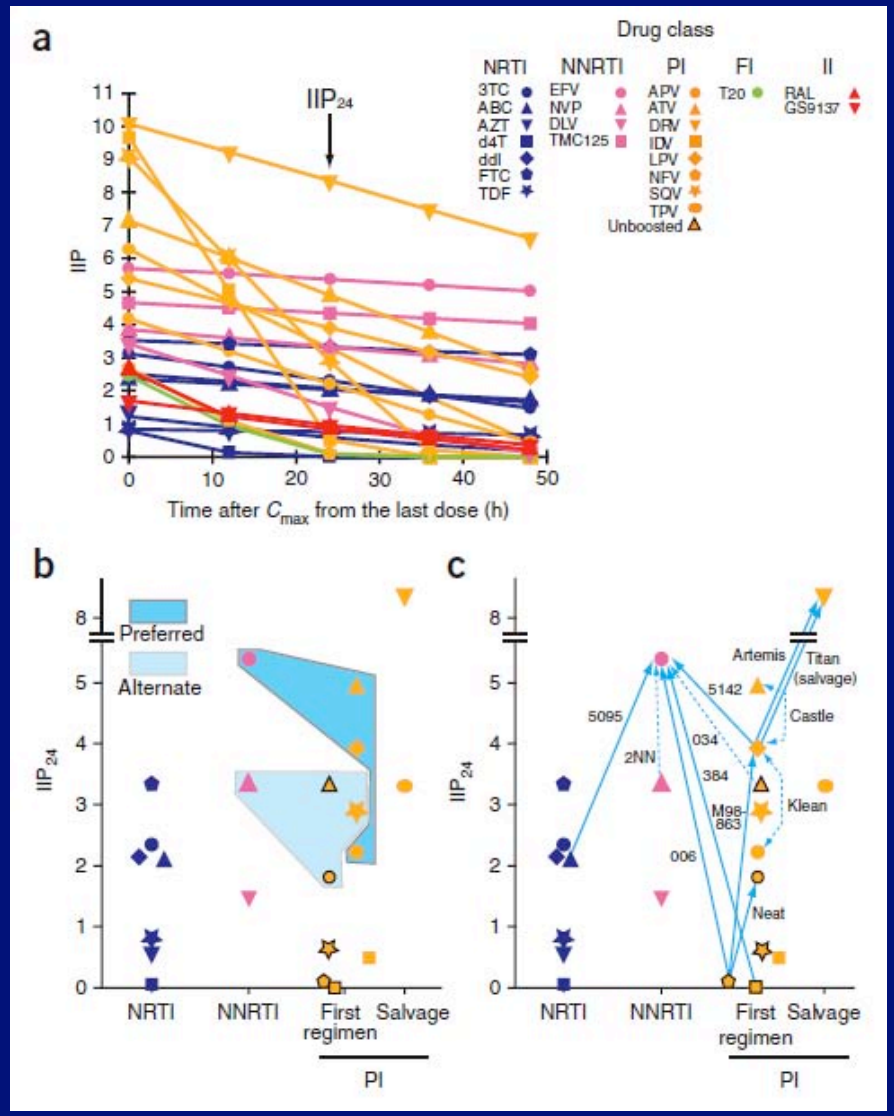


IC50, IQ, IIP in the context of drug levels



Clinical response data and IIP

- IIP24
  - adjusted IIP by halflife (drug level 24h afterlast intake)
- Favorable drugs with highest IIP24
  - According to american treatment guidelines



You Think that was too much? - Try this one!

$$f_{u_{obs}} = f_{u_{act}} \cdot (1 - x) + x = \frac{1}{1 + \left(\frac{D}{IC_{50act}}\right)^{m_{act}}} \cdot (1 - x) + x$$

Our results as far as available

	HWZ	Cmax	C 24h	slope	IC50 (µM)	Cmax / IC50	/ slope	* C (24)	preliminary ranking	
AZT	3	na		27.4	0.556	na				AZT
d4T	3.5	na		5.2	3.997	na				d4T
TDF	50	na		6.1	6.620	na				TDF
ABC	20.5	na		5.4	6.850	na				ABC
ddI	32.5	na		6.0	1.713	na				ddI
3TC	12	na		6.1	0.213	na				3TC
FTC	39	na		3.8	0.098	na				FTC
NVP	27.5	5.7	3.27	3.3	0.055	103	31	103	6	NVP
EFV	47.5	4	3.96	2.6	0.001	2798	1057	4183	2	EFV
DLV	6	na		3.3	0.085					DLV
ETR	41	na		2.2	0.002					ETR
ATV	9.5	6.8	1.35	3.6	0.006	1160	318	428	4	APV
DRV	15	5.2	1.63	4.3	0.003	1516	354	575	3	ATV
FPV	7	5.7	0.83	3.3	0.031	186	57	48	7	DRV
IDV	1.75	9	0.33	3.9	0.035	259	67	22	9	IDV
LPV	5.5	9.6	1.10	4.2	0.011	888	210	231	5	LPV
NFV	3.5	1.9	0.14	2.5	0.030	63	25	3	10	NFV
SQV	4	1.2	0.10	4.0	0.009	133	33	3	10	SQV
TPV	6	50	6.25	2.3	0.012	4008	1711	10692	1	TPV
RAL	9.5	2.2	0.44	5.8	0.006	361	62	27	8	RAL

What‘s wrong with the integrase inhibitors?

- Clinical response:
  - ok
  - E.g. For RAL
    - 83 – 88% mit VL<50 (naiv)
    - 63 % (Benchmark)
  - but: low genetic barrier
- In vitro:
  - Slope = 5.8 ☹
  - IC50 = 6nM ☺
  - IQ = 10 ☹
  - Halflife 10h ☹

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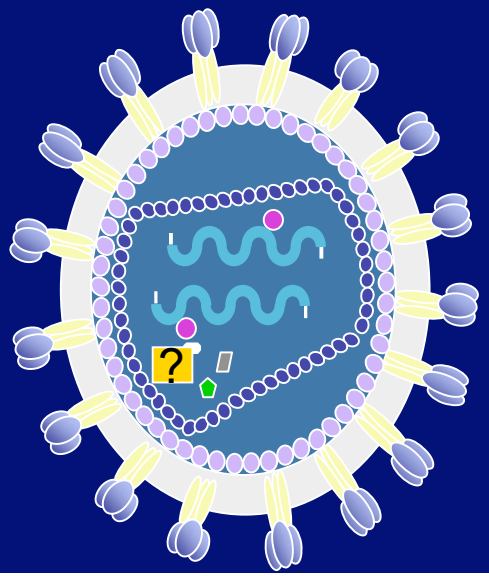
  - High affinity ?

Affinity of raltegravir

- … is reported to be high
- Affinity = how tight is the binding to the target
  - Experimental setting in cell culture (replication)



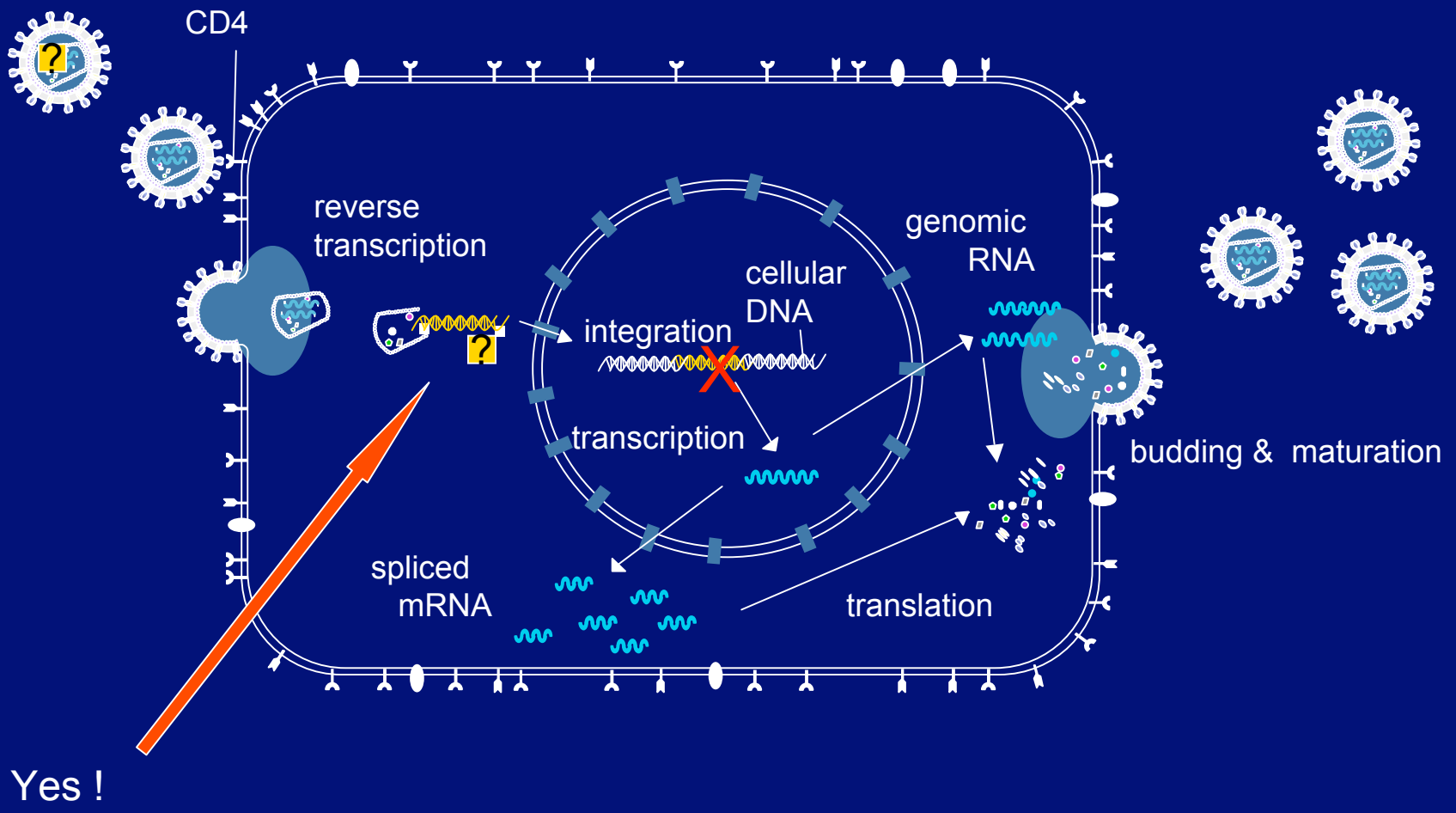
1. Transfection of NL4-3 in the presence of RAL
2. Spin down virus, resuspend in drug-free media

⇒ Virus with RAL inside

3. Infection of CEMx-Luci cells

=> Is binding tight enough to inhibit viral integration ?

Raltegravir is active when added during transfection only



Conclusion for integrase inhibitor activity

- Estimated in vivo time from virus production to integration ~ 12h
  - RAL active 12h after pill intake
  - And of course immediately after pill intake

  - => time shifted activity due to high affinity ?

Discussion

- New criteria for drug activity
  - Slopes
    - Reflects drug vulnerability for residual replication ?
  - Affinity
    - Affinity and halflife playing together the same game ?
  - Intraparticular drug transport
    - Time shifted activity – a new mechanism ?
    - Role for NRTI activity ?

=> Implementation of new parameters in geno2pheno
=> Clinical validation in EURESIST DB
=> .. One day.. Preclinical drug validation possible ?

Thank you for the attention !

- NRC for retroviruses, Erlangen
  - Sabine Wittmann
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