



**MOLECULAR
VIROLOGY**
HEIDELBERG

Medical Faculty Heidelberg

Hepatitis C virus: from bench to bedside

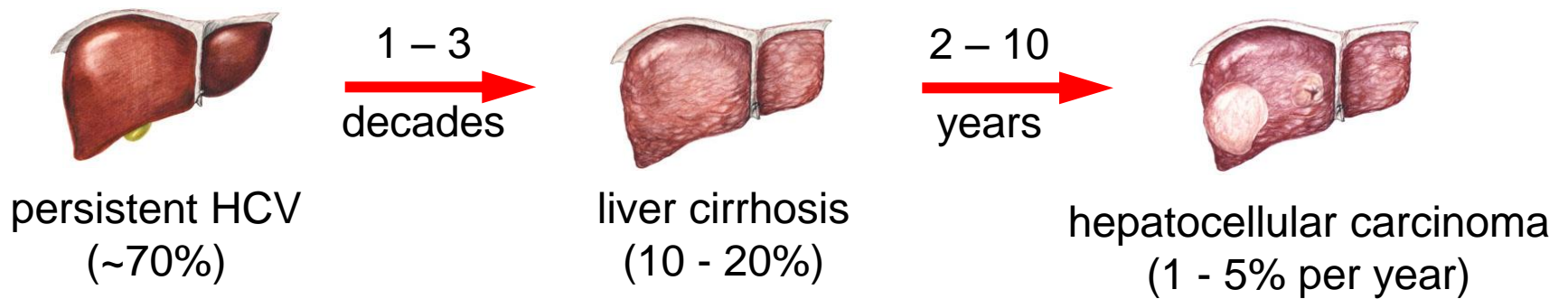
Ralf Bartenschlager

Heidelberg University



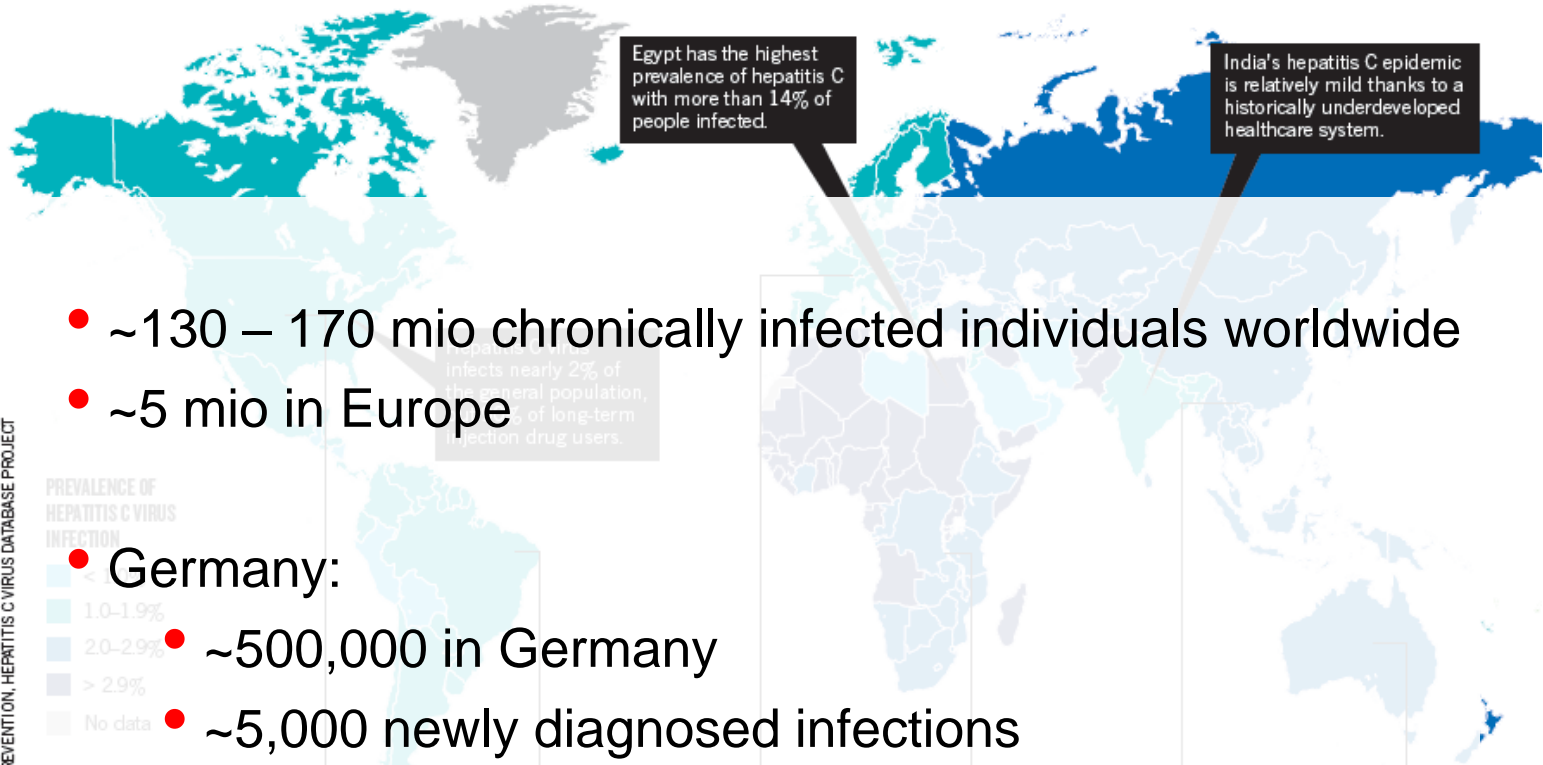
Hepatitis C

Cofactors (EtOH, obesity, age, sex)



- ~25% of all HCC cases
- ~25% of LTx

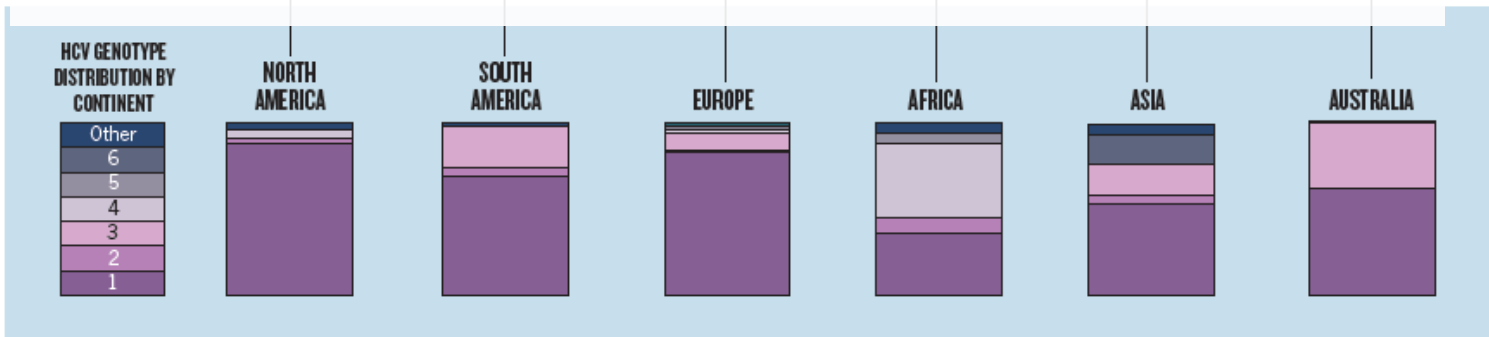
HCV prevalence and distribution of genotypes



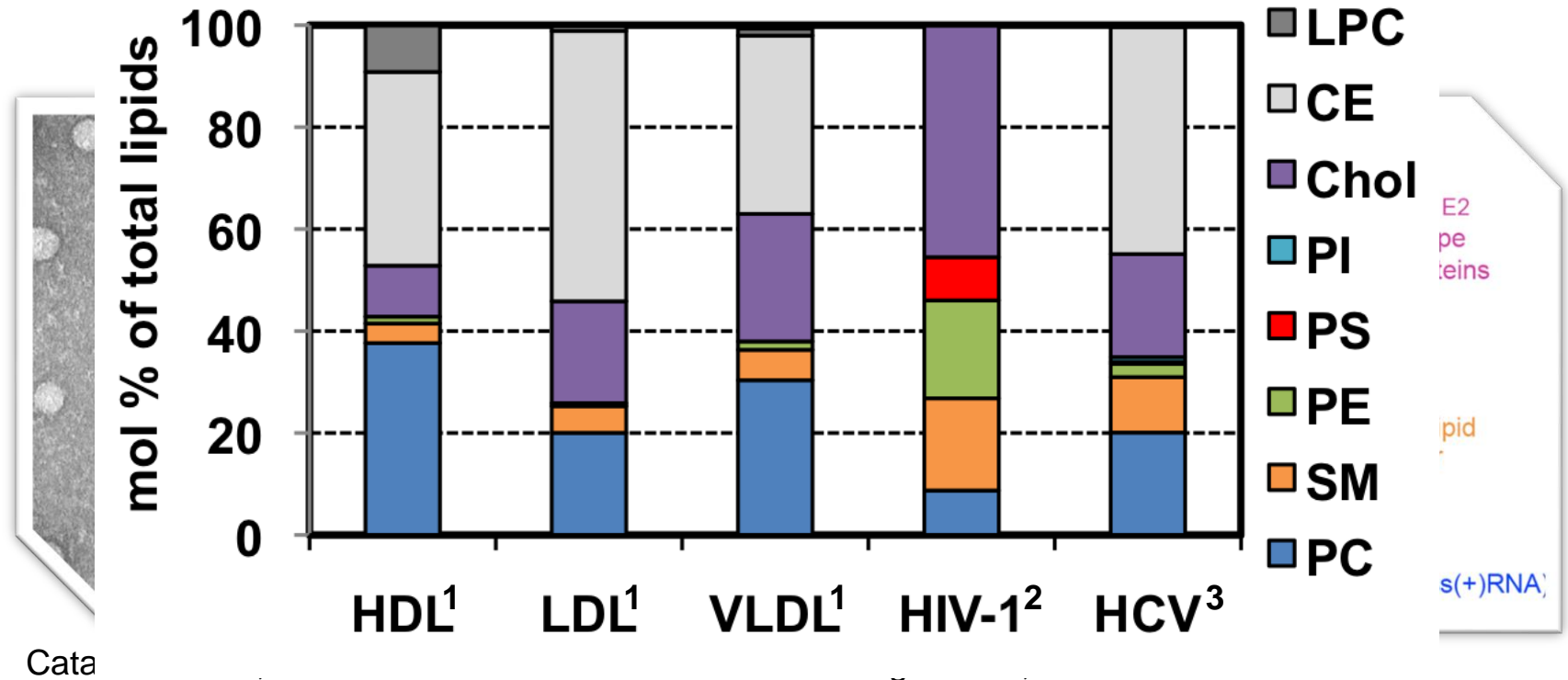
- ~130 – 170 mio chronically infected individuals worldwide
- ~5 mio in Europe

- Germany:
 - ~500,000 in Germany
 - ~5,000 newly diagnosed infections

SOURCES: THE US CENTER FOR DISEASE CONTROL AND PREVENTION, HEPATITIS C VIRUS DATABASE PROJECT



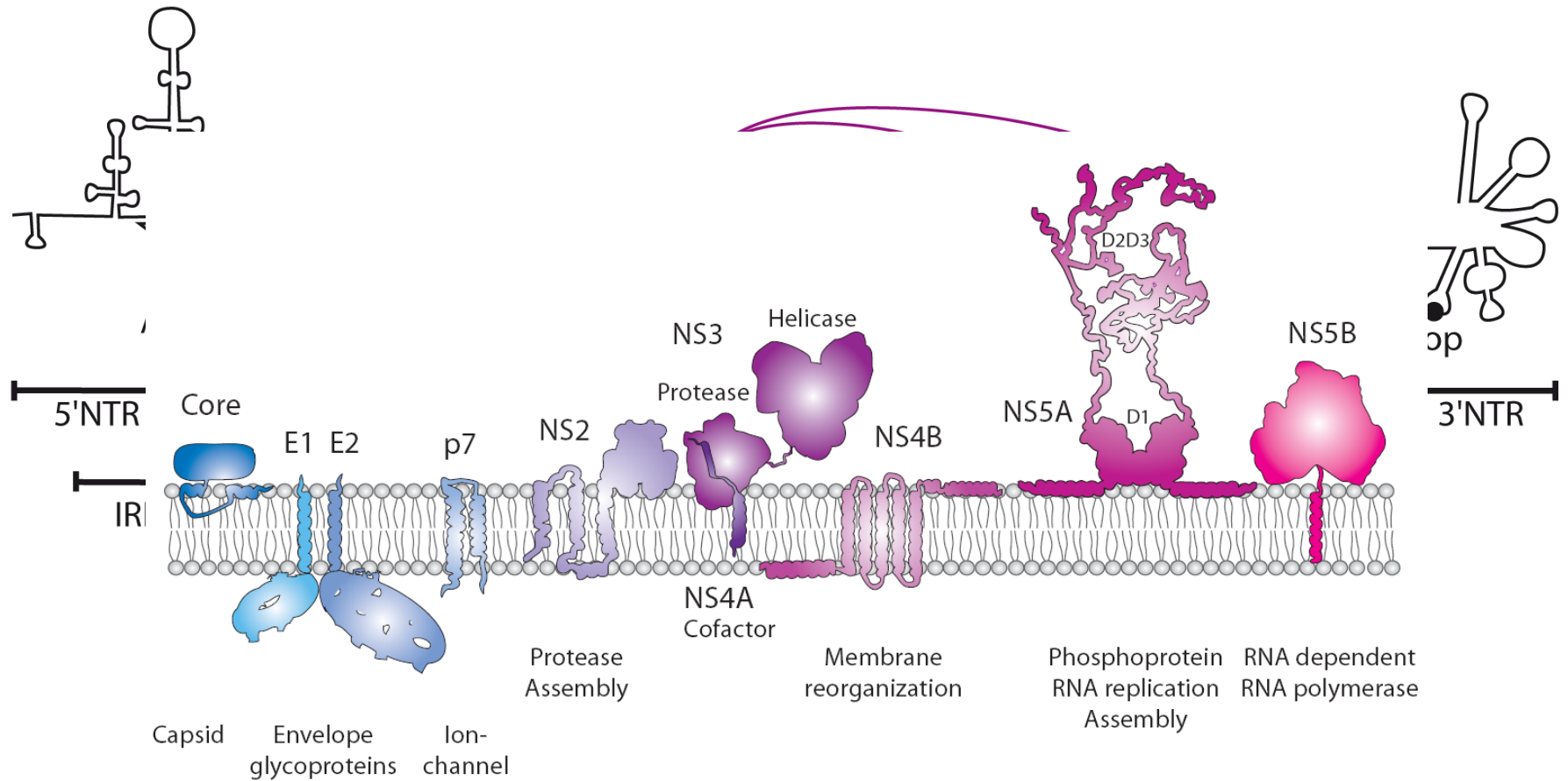
Hepatitis C virus particle properties



¹Wiesner et al., J Lipid Res 2008; ²Brügger et al., PNAS 2006; ³Merz, Long et al., JBC 2010

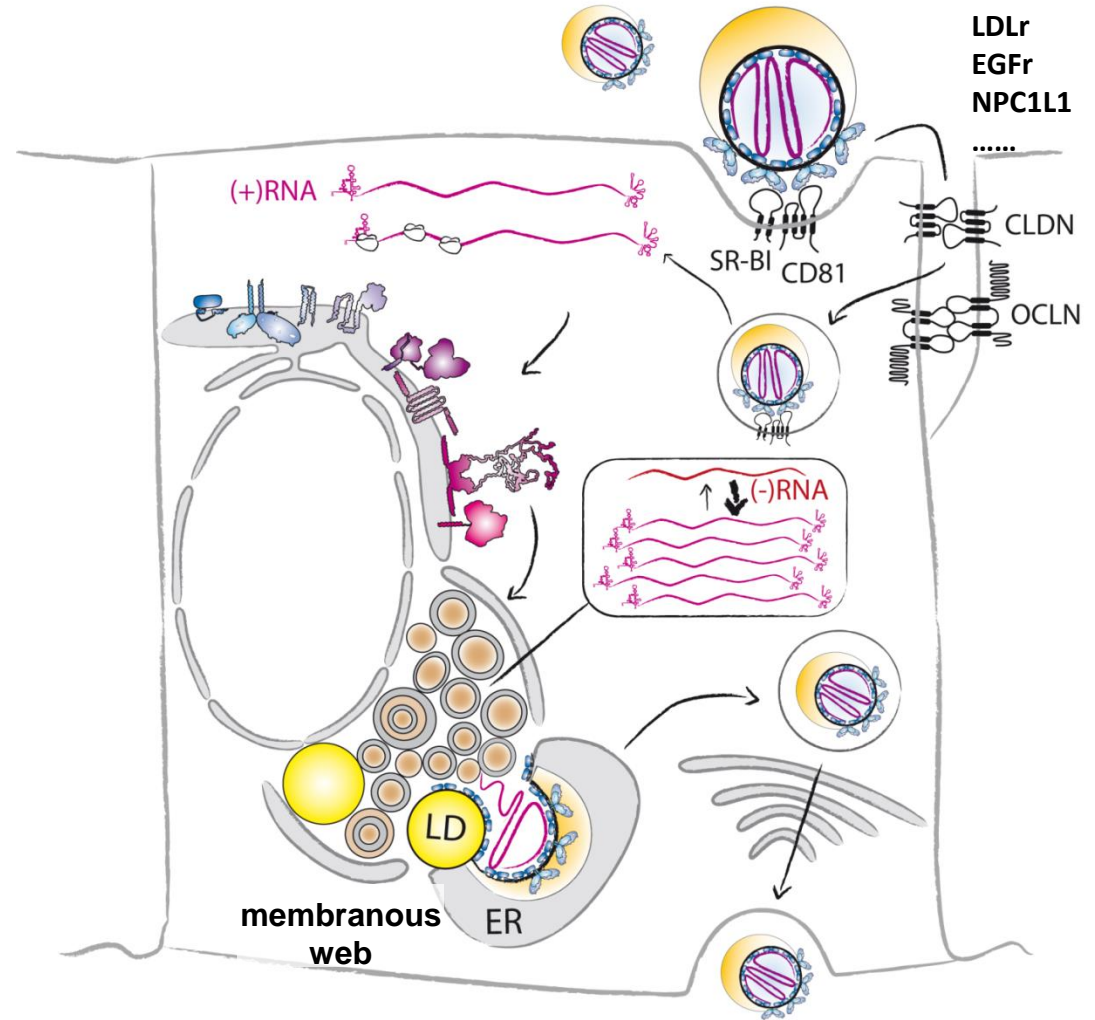


HCV genome organization



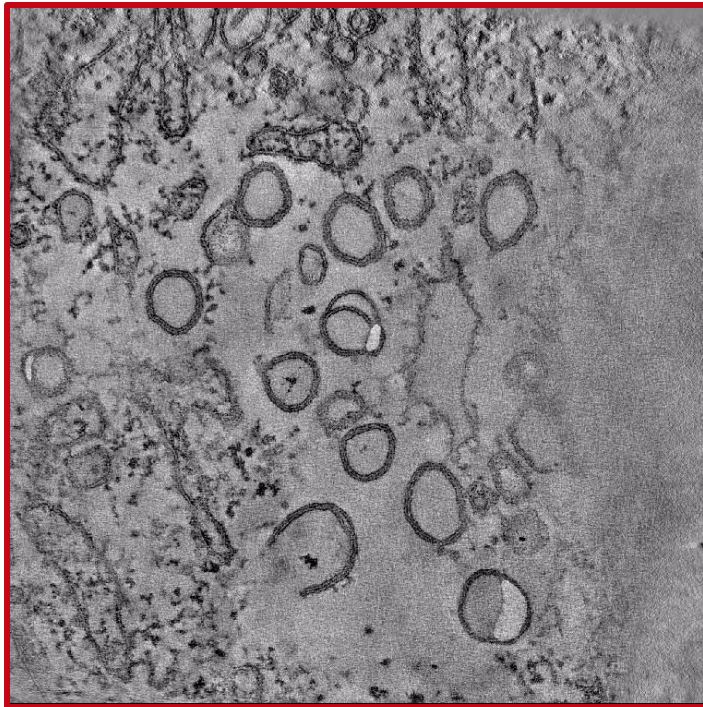
Bartenschlager et al., NRM 2013

HCV replication cycle

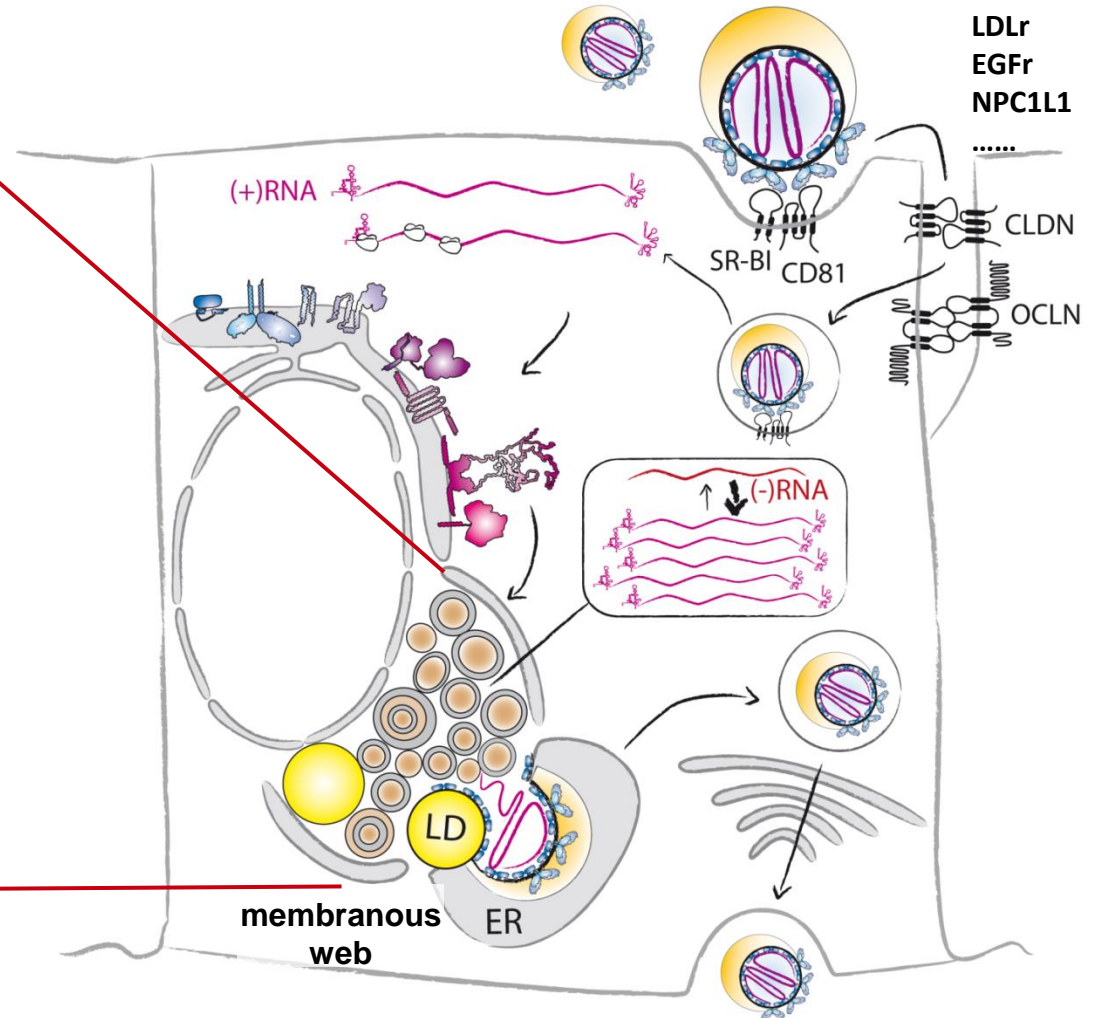


Bartenschlager et al., NRM 2013

HCV replication cycle



Romero-Brey et al., Plos Path 2012



Bartenschlager et al., NRM 2013

HCV replication cycle: Basic parameters

Implications for HCV-specific antiviral therapy:

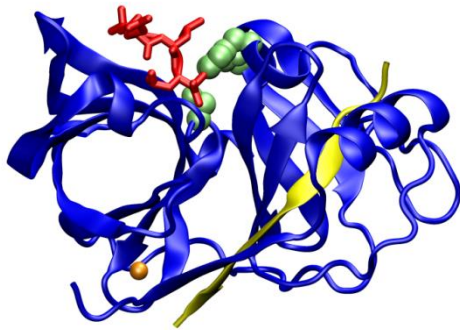
Parameter	Value
Virus production/day	10^{12}
T1/2 virus particle	~45 min
Virus variants/day	10^{12}

**High elimination rate should be possible
when using efficient inhibitors**



Viral targets for therapy of chronic hepatitis C

NS3/4A protease



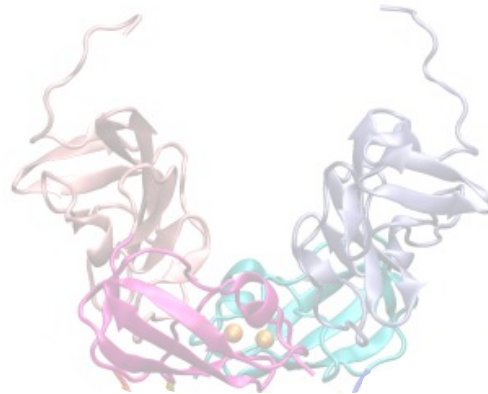
Cleavage of viral polyprotein

Inhibition of IFN system



Protease inhibitors (...**previr**)

NS5A



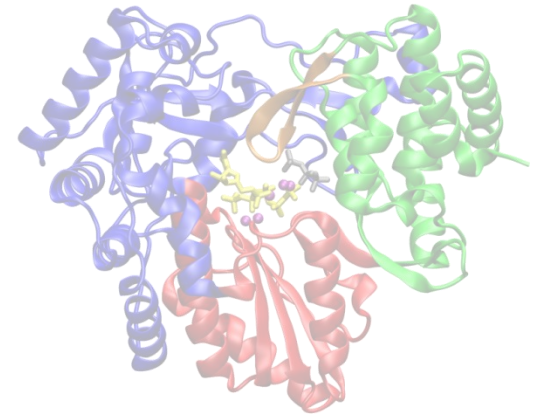
RNA replication

Assembly of HCV particles



NS5A inhibitors (...**asvir**)

NS5B RdRp



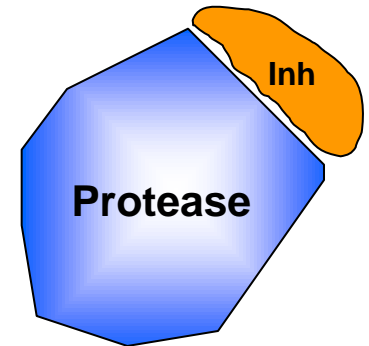
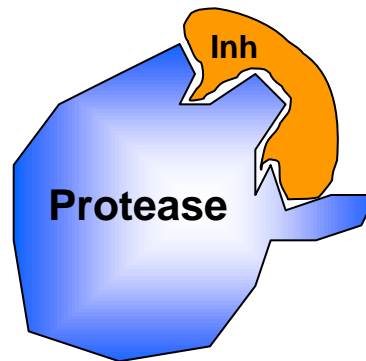
RNA-dependent RNA polymerase



NS5B inhibitors (...**buvir**)

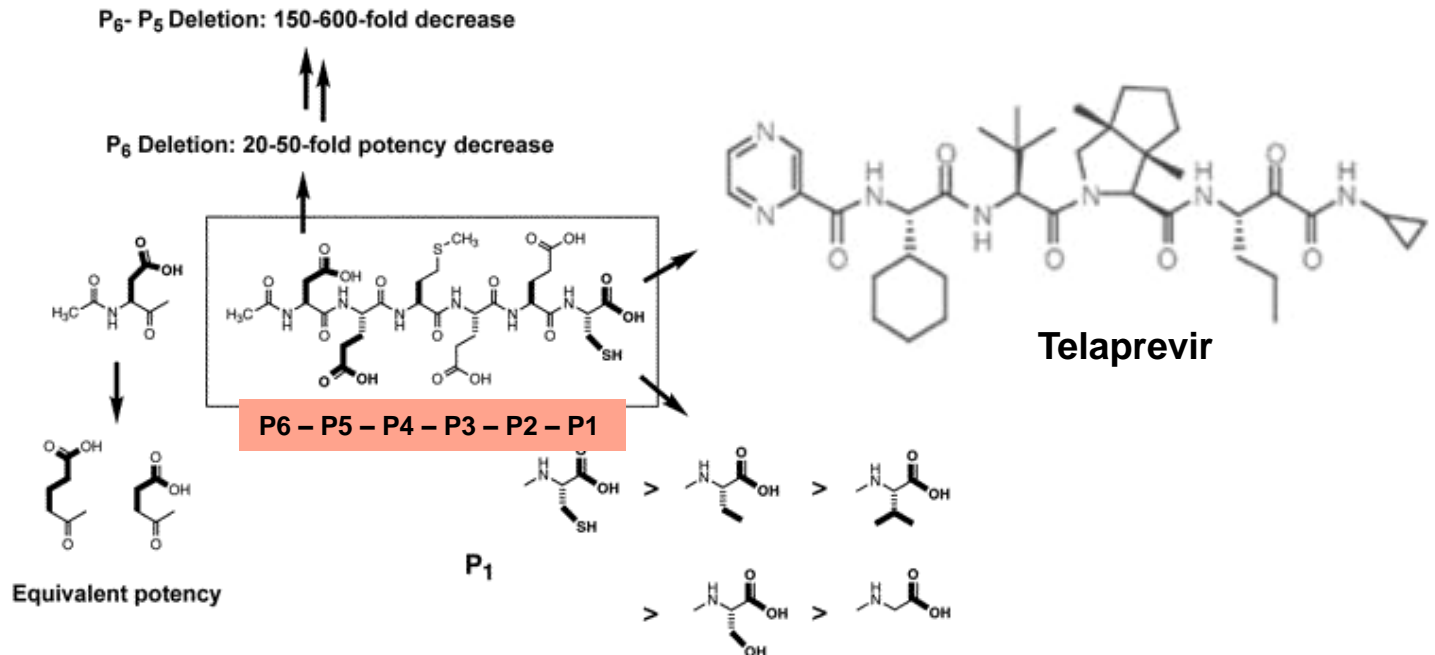
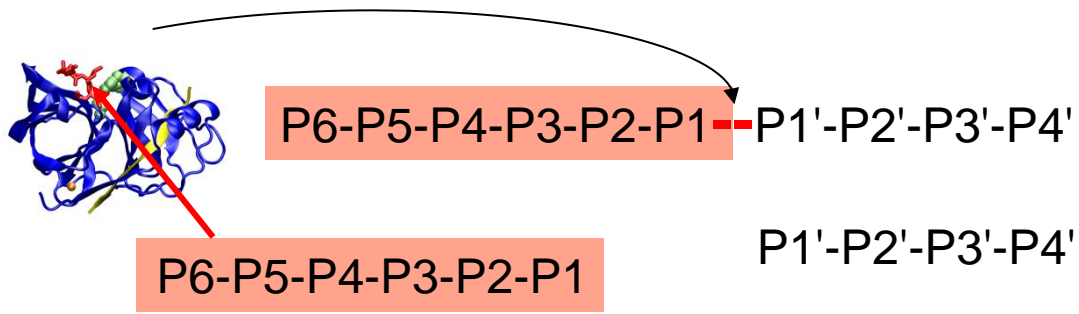
HCV NS3 protease

- chymotrypsin-like enzyme
- requires viral cofactor (NS4A)
- low substrate specificity
- flat , shallow substrate binding pocket



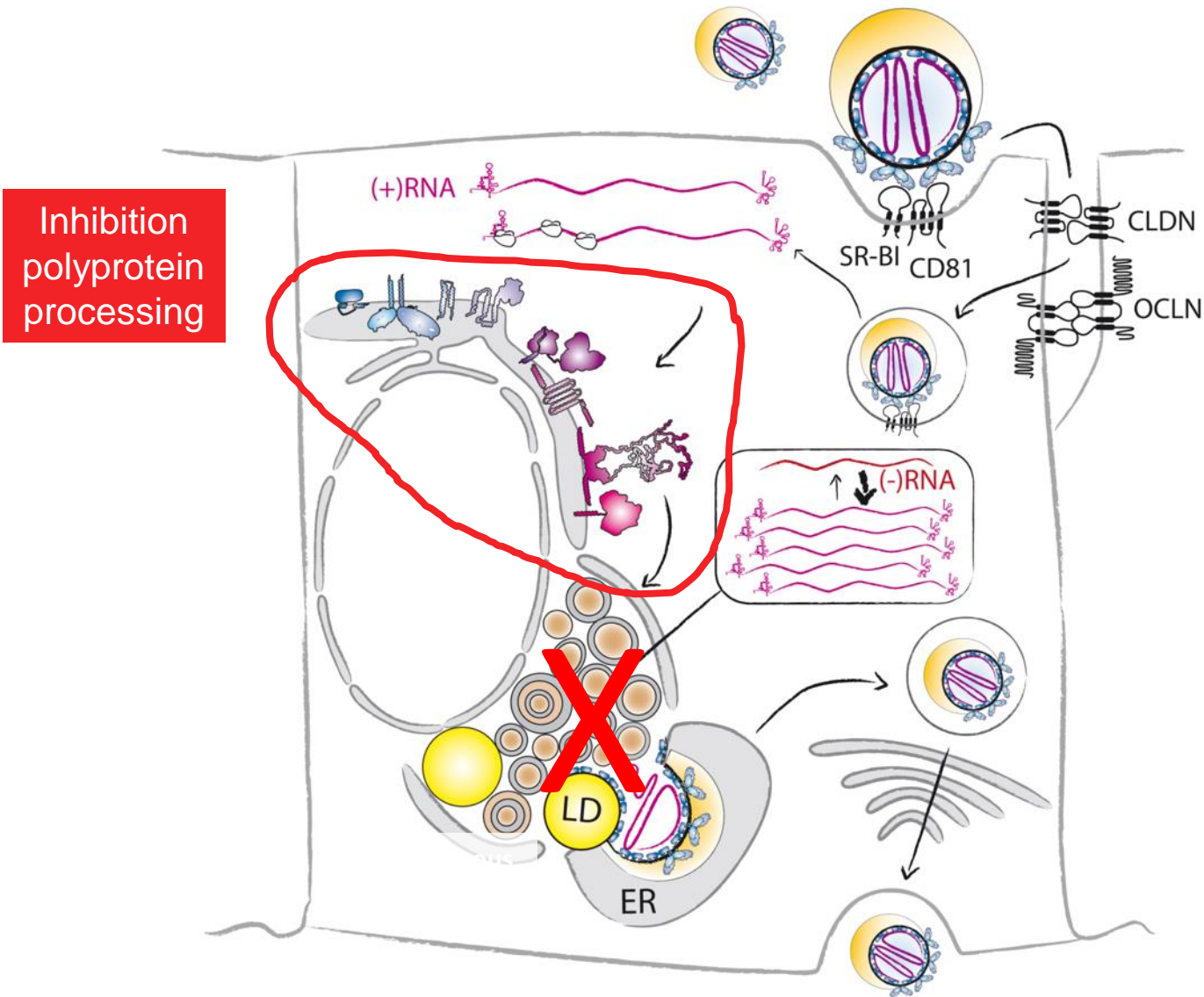


Development of HCV-specific protease inhibitors



Ingallinella et al., Biochemistry 1998

Impact of NS3 protease inhibitors on HCV replication cycle





(Cross) resistance against NS3/4A protease inhibitors

	V36A/M	T54A	V55A	Q80/RK	R155K/TQ	A156S	A156V/T	D168A/V/T/H	V179A
Telaprevir (linear)	Red	Red	Light Blue	Light Blue	Red	Light Blue	Red	Light Blue	Light Blue
Bosoniprevir (linear)	Red	Red	Light Blue	Light Blue	Red	Light Blue	Red	Light Blue	Light Blue
SCH900518 (linear)	Red	Red	Light Blue	Light Blue	Red	Light Blue	Red	Light Blue	Light Blue
BI-201335 (linear)	Light Blue	Light Blue	Light Blue	Light Blue	Red	Light Blue	Red	Light Blue	Light Blue
ITMN191 (macrocyclic)	Light Blue	Light Blue	Light Blue	Light Blue	Red	Light Blue	Red	Light Blue	Light Blue
MK7009 (macrocyclic)	Light Blue	Red	Light Blue	Light Blue	Red	Light Blue	Red	Light Blue	Light Blue
TMC435350 (macrocyclic)	Light Blue	Light Blue	Light Blue	Light Blue	Red	Light Blue	Red	Light Blue	Light Blue
BI-201335 (linear)	Light Blue	Light Blue	Light Blue	Light Blue	Red	Light Blue	Red	Light Blue	Light Blue
MK5172 (macrocyclic)	Light Blue	Light Blue	Light Blue	Light Blue	Red	Light Blue	Red	Light Blue	Light Blue
GS-9256 (macrocyclic)	Light Blue	Light Blue	Light Blue	Light Blue	Red	Light Blue	Red	Light Blue	Light Blue
ABT 450 (macrocyclic)	Light Blue	Light Blue	Light Blue	Light Blue	Red	Light Blue	Red	Light Blue	Light Blue
BMS-791325 (macrocyclic)	Light Blue	Light Blue	Light Blue	Light Blue	Red	Light Blue	Red	Light Blue	Light Blue

Rapid selection for resistance with 1st generation PIs

Higher resistance barrier with 2nd/3rd generation PIs

Yet:

combination therapy

*Mutations associated with resistance in vitro only

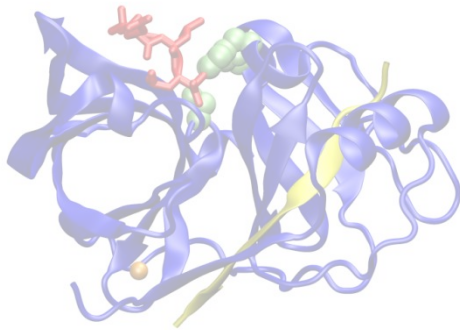
EC50 > 4-fold

Halfon & Locarnini, J. Hepatol. 2011



Viral targets for therapy of chronic hepatitis C

NS3/4A protease

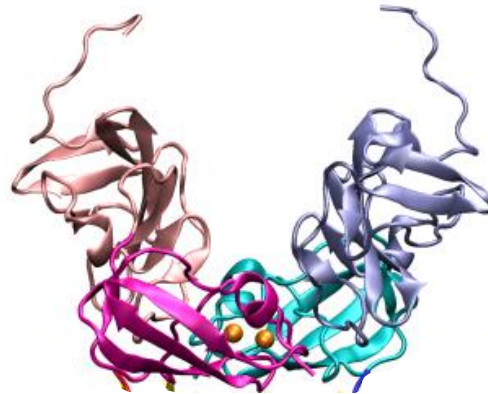


Cleavage of viral polyprotein
Inhibition of IFN system



Protease inhibitors (...*previr*)

NS5A

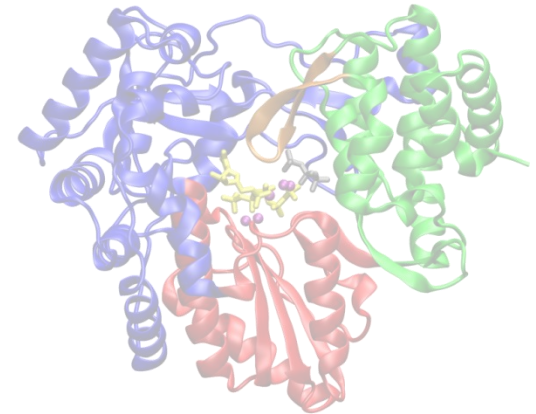


RNA replication
Assembly of HCV particles



NS5A inhibitors (...*asvir*)

NS5B RdRp



RNA-dependent RNA polymerase



NS5B inhibitors (...*buvir*)



Daclatasvir

What is the mode-of-action?

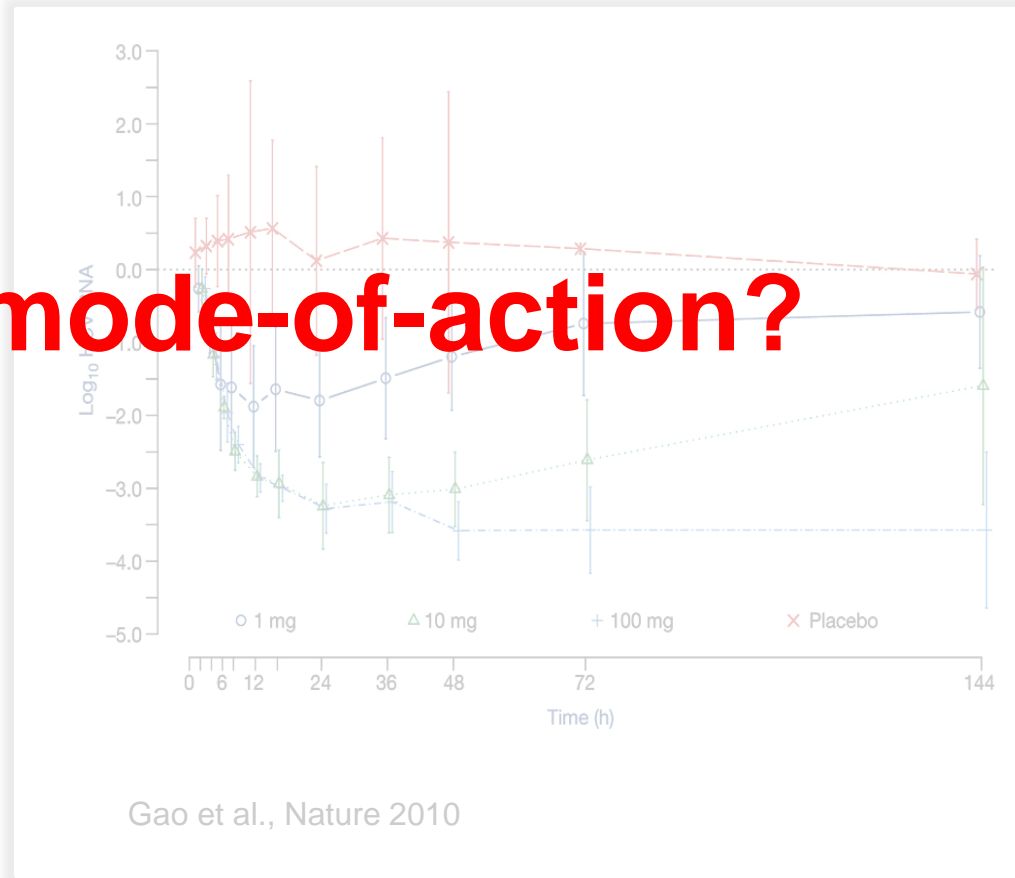
Identified by HCV drug HCV resistance

Picomolar activity on HCV genotype 1

Resistance mutations in NS5A domain I
(e.g. L31V, Y93H)

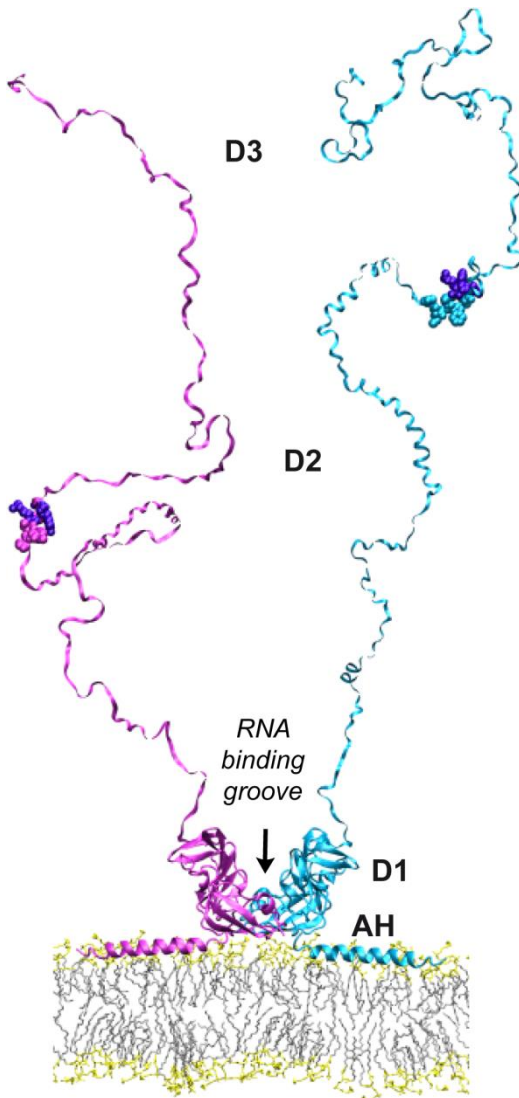


target NS5A



Gao et al., Nature 2010

Properties of NS5A



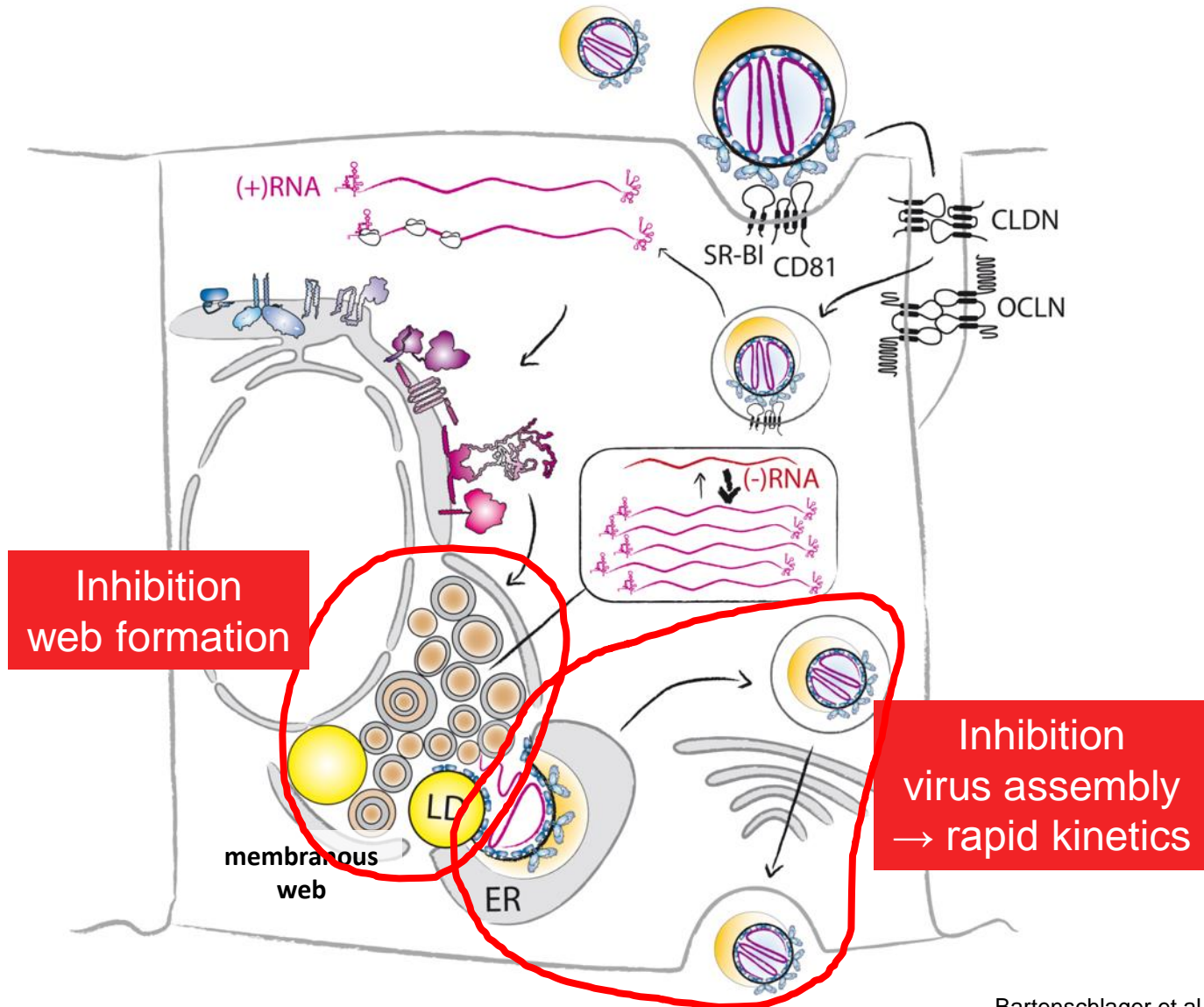
Bartenschlager et al., NRM 2013

- RNA-binding phosphoprotein
- required for RNA replication (membr. web formation)
- required for assembly of infectious virions
- required to block the innate immune response
- required for



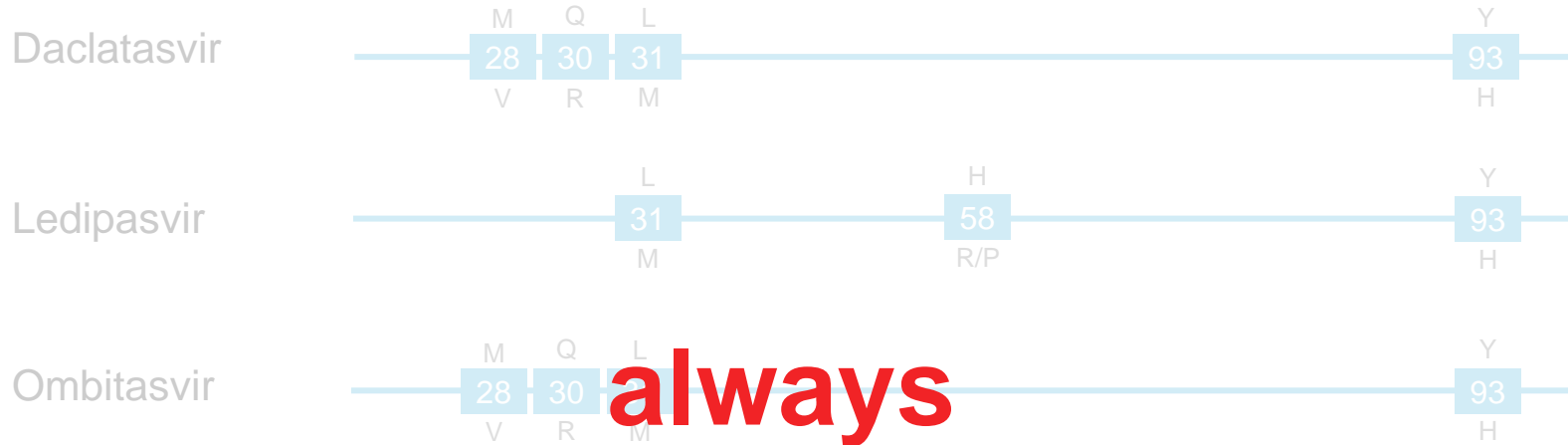
Tellinghuisen 2004, 2005, 2008; Neddermann 2004; Appel 2005, 2008; Moradpour 2005; Liang 2007; Hanouille 2009; Hwang 2010; Verdegem 2011; Lim 2012

Impact of NS5A inhibitors on HCV replication cycle



Bartenschlager et al., NRM 2013

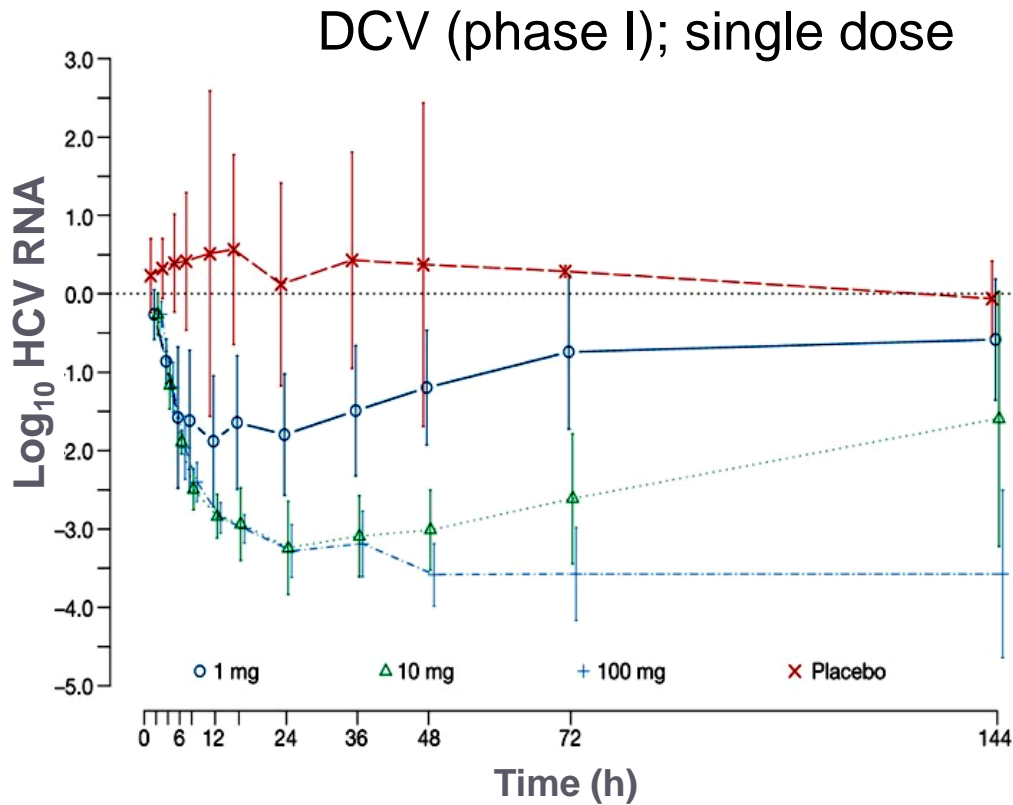
Prevalence of natural NS5A-inhibitor resistance variants in DAA-naive patients



NS5A majority amino acid		Majority amino acid prevalence		Observed variant(s) (%)		Variants not observed
1a	1b	1a	1b	1a (n=538)	1b (n=239)	
L23	L23	100	100			F
M28	L28	96,5	99,2	T (0,37)		
Q30	R30 ^a	98,7	92,9	H (1,3), R(0,74)	H (0,42)	E
L31	L31	99,3	94,6	M (0,93)	M (6,28)	F,V
P32	P32	100	100			L
Q58	P58	100	92,9	S (0,19)	S (3,35)	
Y93	Y93	98,7	97,5	C (0,37), N (0,37)	H (3,77)	

^a The majority amino acid in subtype 1b (R30) is the resistant variant (Q30R) in subtype 1a

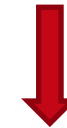
High antiviral activity of NS5A inhibitors and clinical implications



Extremely rapid reduction
of virus production



Reduced number
of virus variants



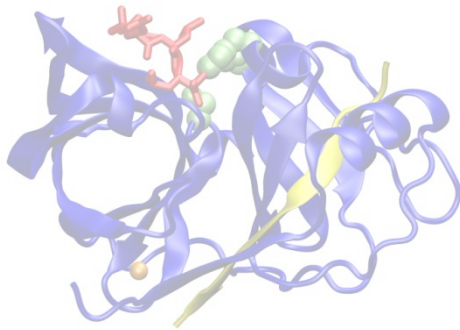
Reduced number
of new resistance variants

Gao et al., Nature 2010



Viral targets for therapy of chronic hepatitis C

NS3/4A protease



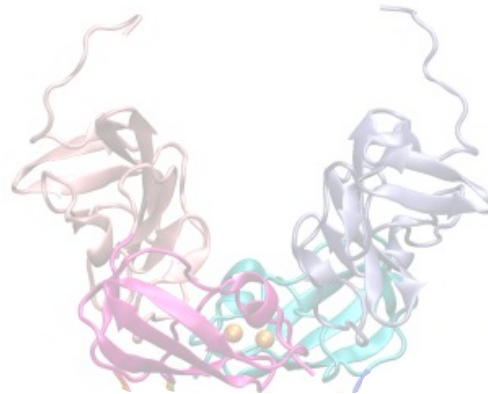
Cleavage of viral polyprotein

Inhibition of IFN system



Protease inhibitors (...previr)

NS5A



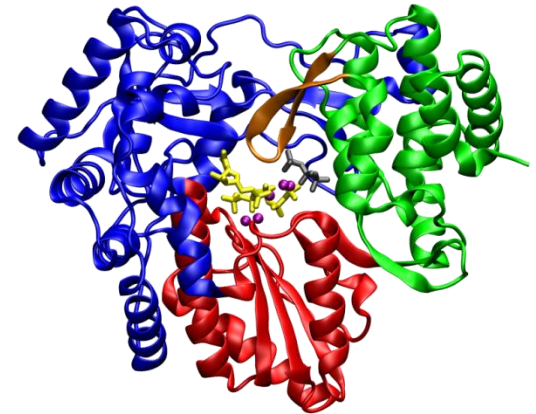
RNA replication

Assembly of HCV particles



NS5A inhibitors (...asvir)

NS5B RdRp

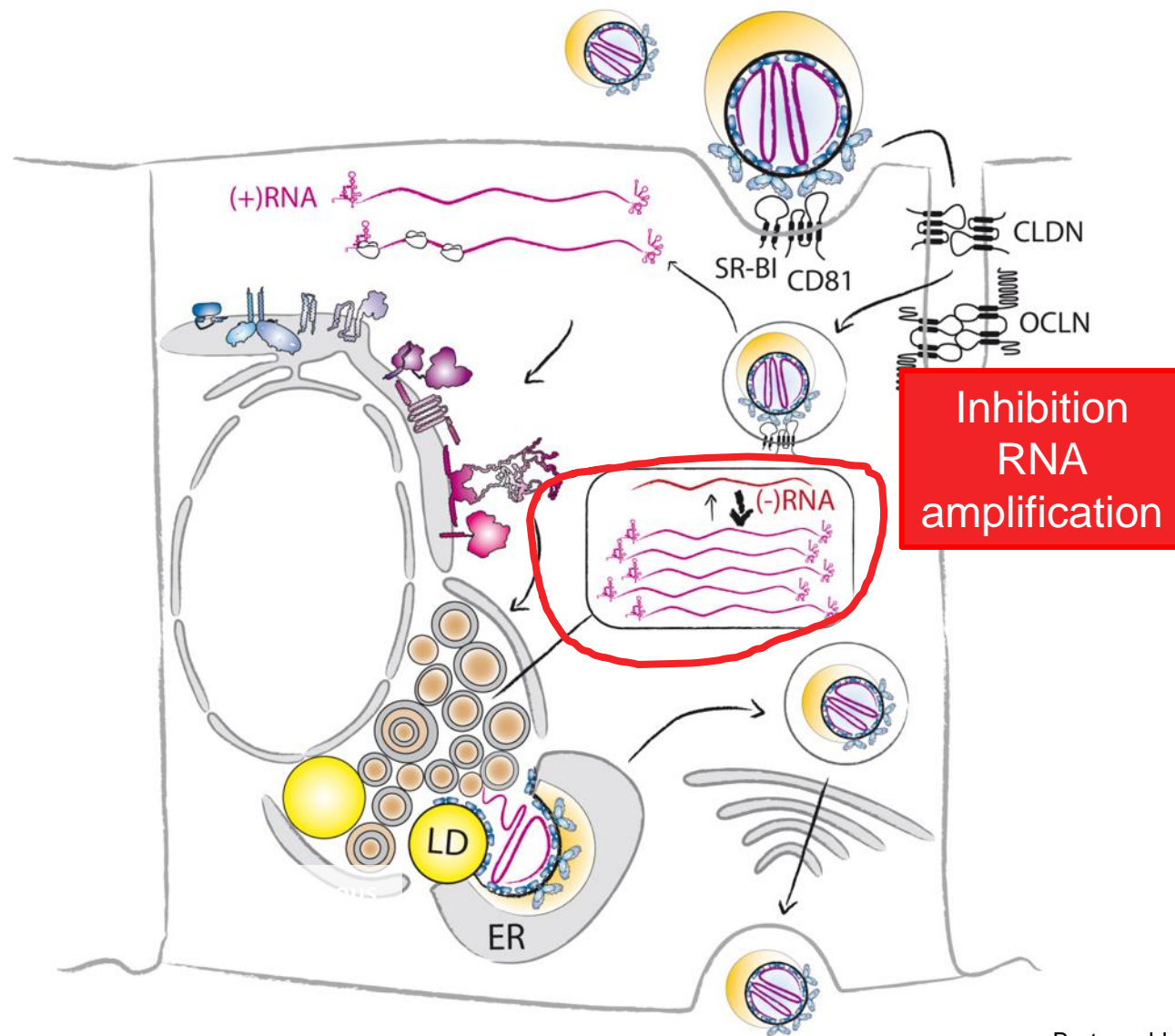


RNA-dependent RNA polymerase



NS5B inhibitors (...buvir)

Impact of NS5B polymerase inhibitors on HCV replication cycle



Bartenschlager et al., NRM 2013

Nucleosidic and non-nucleosidic NS5B-specific drugs

Non-nucleosidic Inhibitors

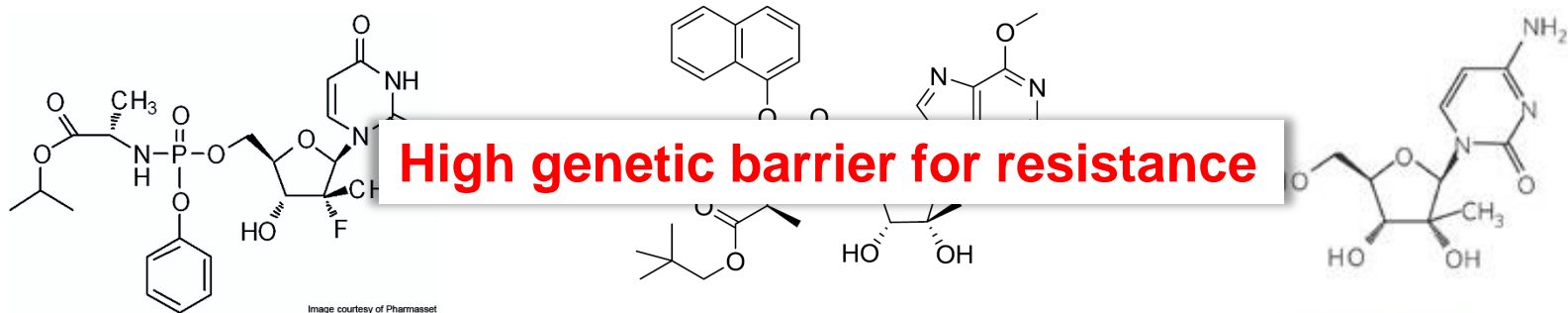


Benzimidazole derivative

Thiophene derivative

Dasabuvir

Nucleosidic Inhibitors



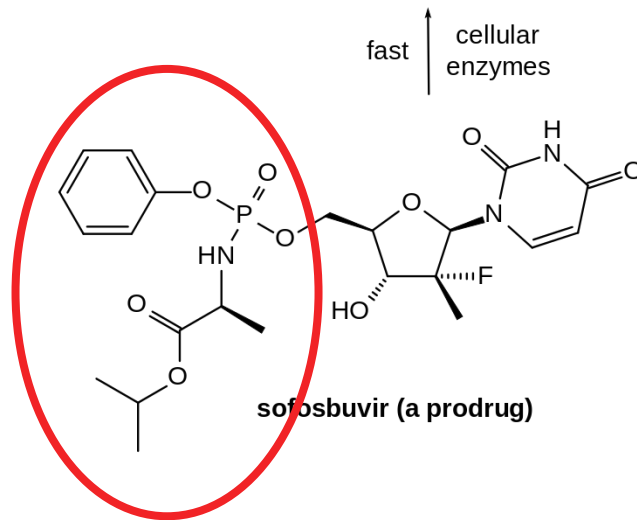
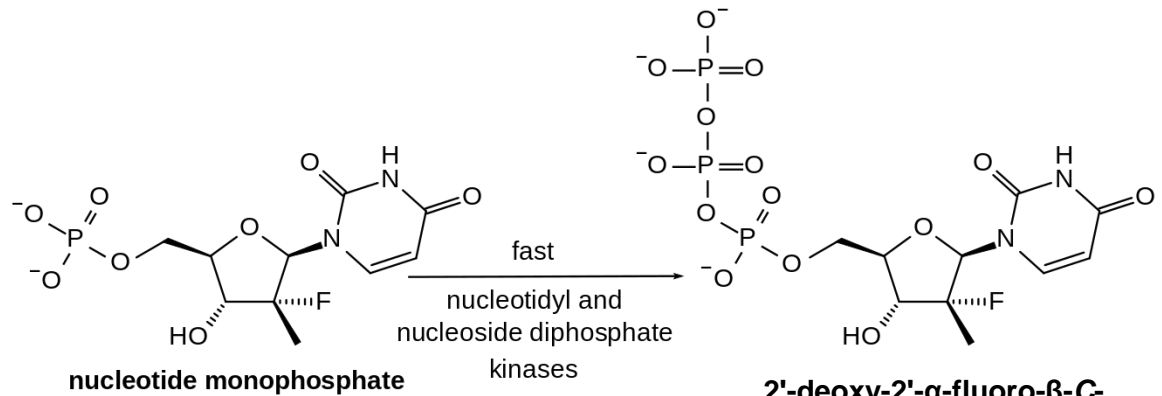
PSI-7977 (Sofosbuvir)

INX-189

2'-C-methyl cytidine

Image courtesy of Pharmasset

Properties of Sofosbuvir

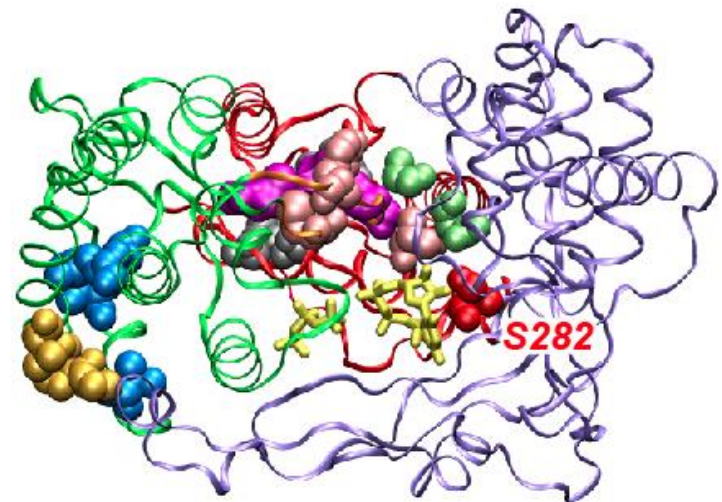
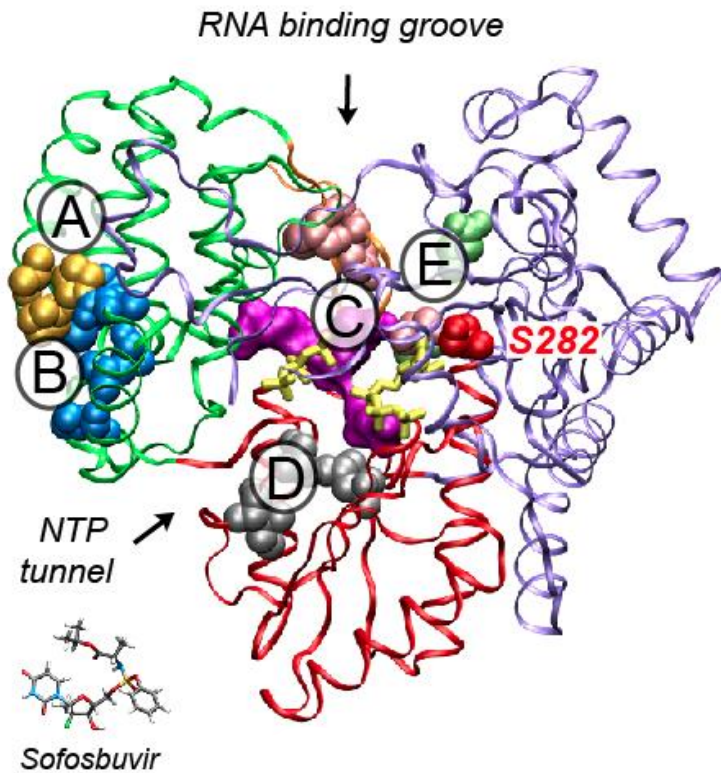


<http://en.wikipedia.org/wiki/Sofosbuvir>

Positions of resistance mutations in NS5B

A – E = Resistance positions with NNIs

S282 = Resistance against NIs



Bartenschlager et al., NRM 2013



Factors of antiviral therapy success

	HCV
Mutation rate/d	10e-5
Virus production/d	10e12
Variants/d	10e12
Persistence reservoir	No
Antiviral potency	(very) high
Genetic barrier	low to very high
Replication fitness	very low to very high

Acknowledgements

AG Bartenschlager (past & present members)

AG Lohmann

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AG Binder

AG Urban

collaborations (few selected)

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J. Briggs, Y. Schwab, C. Antony (EMBL, HD)

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