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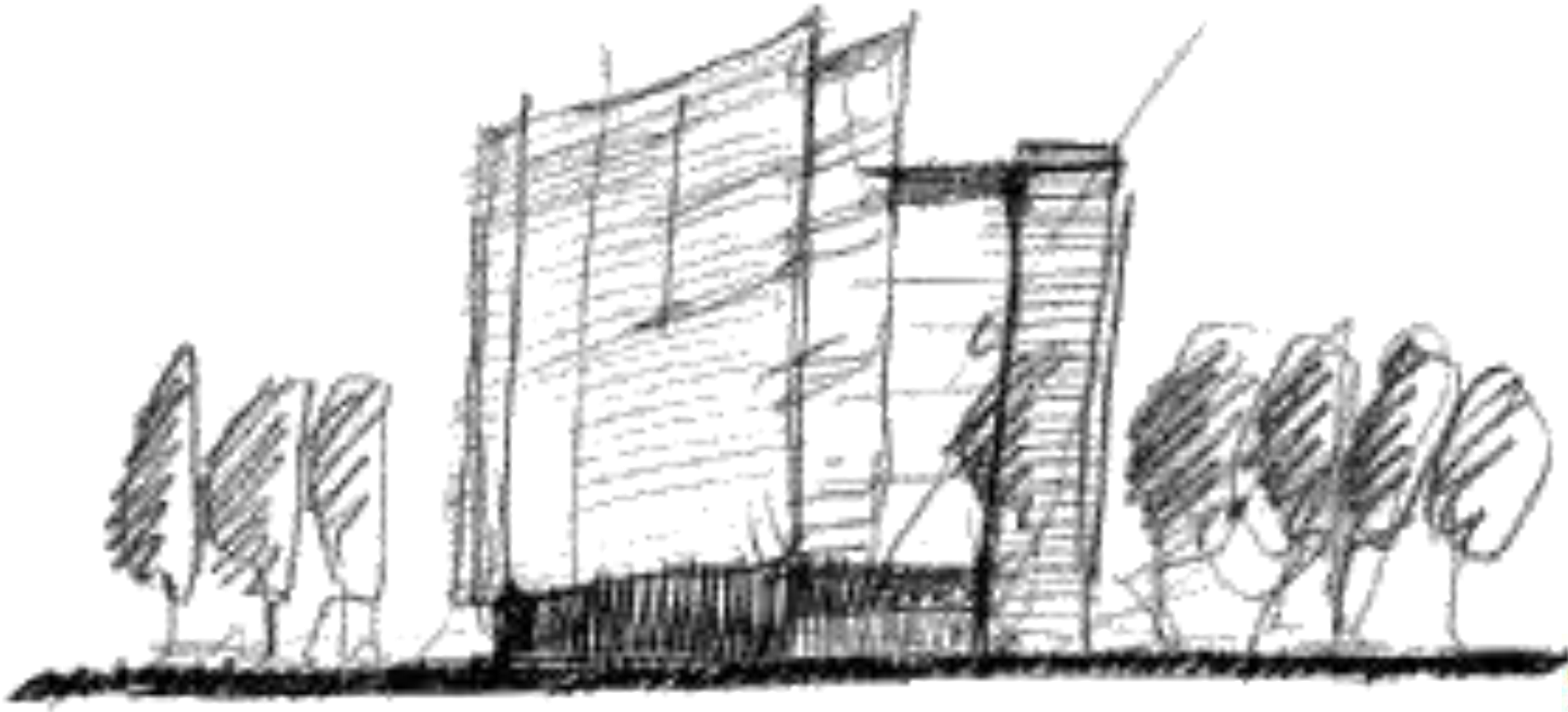
ITALY

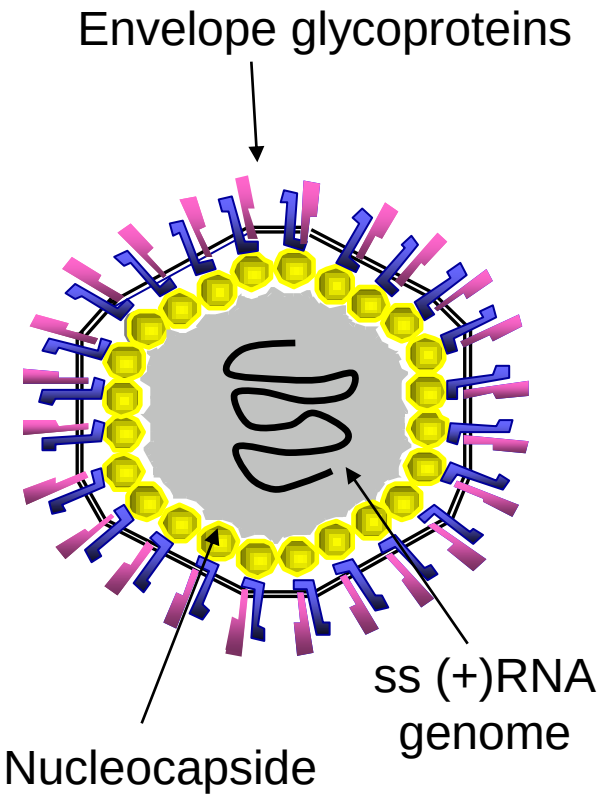
Anti-HCV drug discovery: preclinical studies



Outline of my presentation

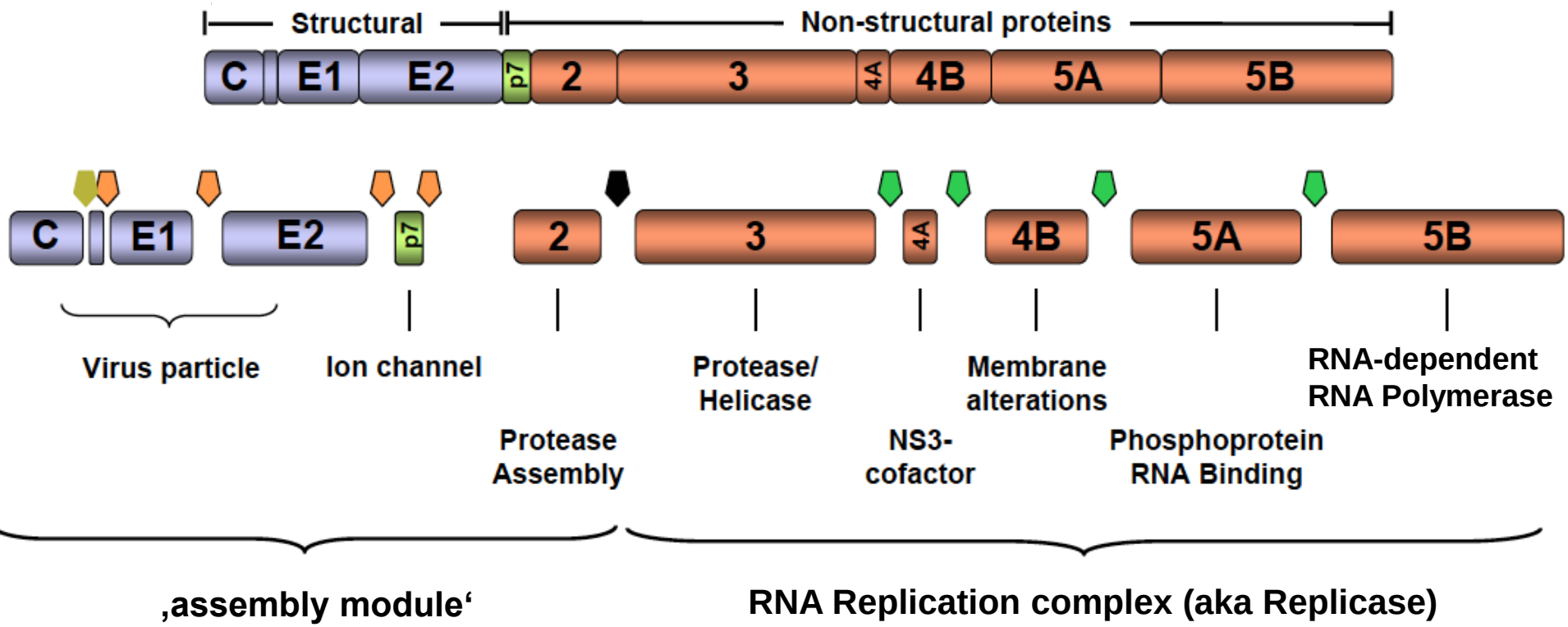
- Introduction to HCV molecular virology
- The HCV Pre-Clinical Tool Box
- Resistance to Direct Acting Antivirals (DAA)

Hepatitis C virus

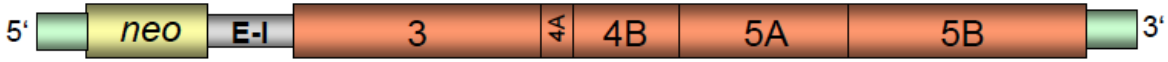


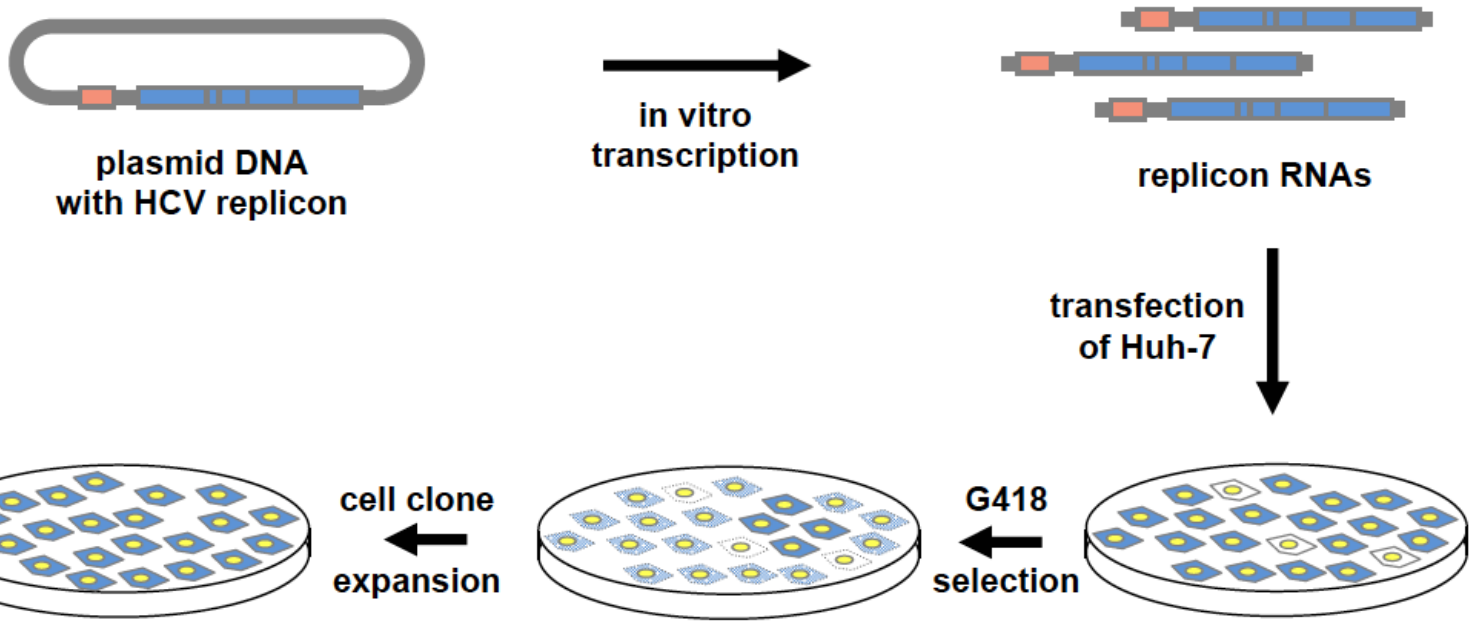
- discovered in 1989
- small (40-60 nm), enveloped virus (family *Flaviviridae*, genus *hepacivirus*)
- (+)-stranded RNA genome (9.6 kb)
- single Open Reading Frame (~3,000 aa)
- Very high sequence variability (7 genotypes, >100 subtypes)

Polyprotein Organization and Functions of HCV Proteins



The HCV Pre-Clinical Tool Box (1): Replicons

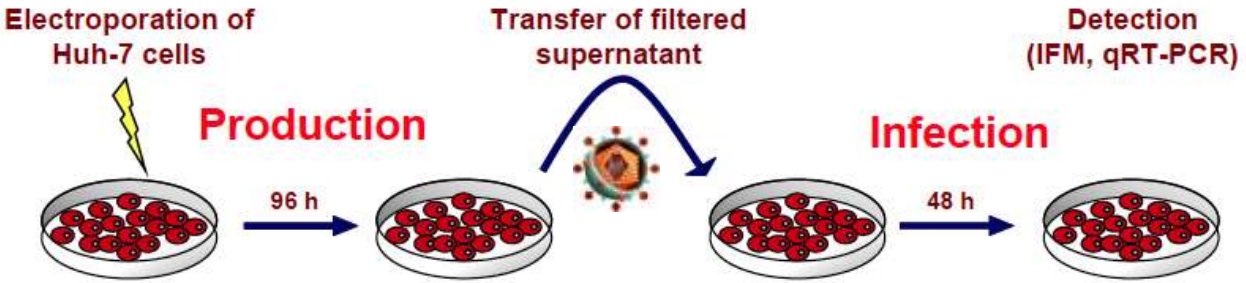




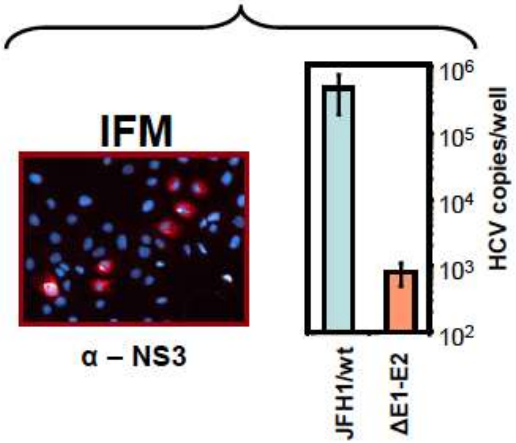
HCV replicons only recapitulate intracellular replication steps

Lohmann et al., Science 1999

The HCV Pre-Clinical Tool Box (2): Cell Culture Grown HCV (HCVcc)



- HCVcc is infectious *in vivo* (chimp; chimeric mouse)
- Virus grown in vivo is infectious in cell culture
- Low virus titres and poor spread in culture



Wakita*, Pietschmann* et al., Nat. Med. 2005; Zhong et al., PNAS 2005

The HCV Pre-Clinical Tool Box (3): Animal Models

Chimpanzee

Mouse with Humanized Liver

Resistance to HCV DAA (Direct Acting Antivirals)

Definition of “Direct-Acting Antivirals” :

Agents that interfere with specific steps in the virus replication cycle through a direct interaction with a viral protein or nucleic acid

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"One definition of a (direct acting) antiviral drug is a drug that selects for resistance"

DD Richman, Hepatology 32:866-867; 2000

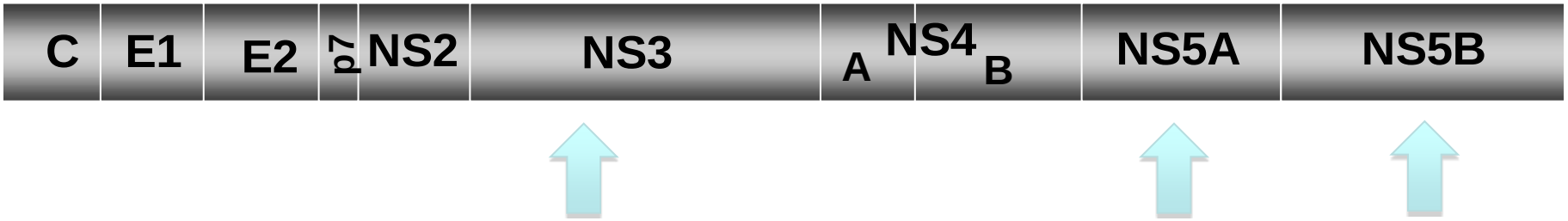
- High genetic diversity of HCV (genotypes, quasispecies)
- Error-prone polymerase
- High mutation rate (>1/10^5 nucleotides/replication cycle)
- High viral production rate (10^{10} infected cells → 10^{12} virions/day)

- All possible single-nucleotide mutations/double-nucleotide mutations, and many 3-nucleotide mutations are generated daily
- ≥ 4 nucleotide mutations needed to control emegence of resistance:

→ **therapeutic efficacy of DAA limited by rapid emergence of drug resistant HCV variants**

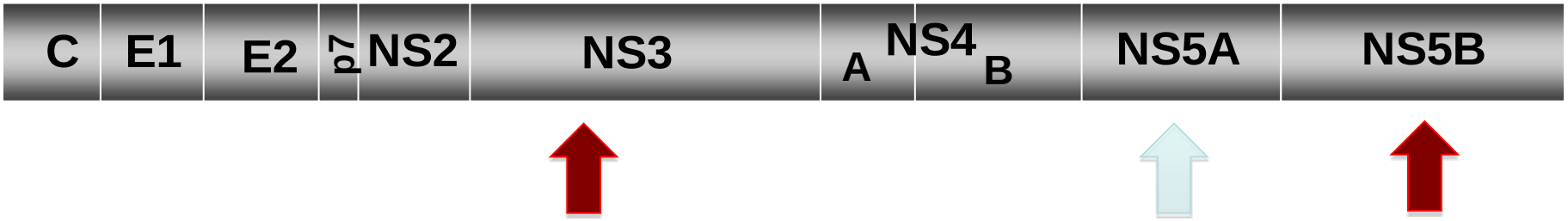
→ **need for combination therapy**

Targets of the most advanced HCV DAAs



- NS3-4A (serine protease)
- NS5A (dual role in RNA replication and virus assembly)
- NS5B (RNA-dependent RNA polymerase)

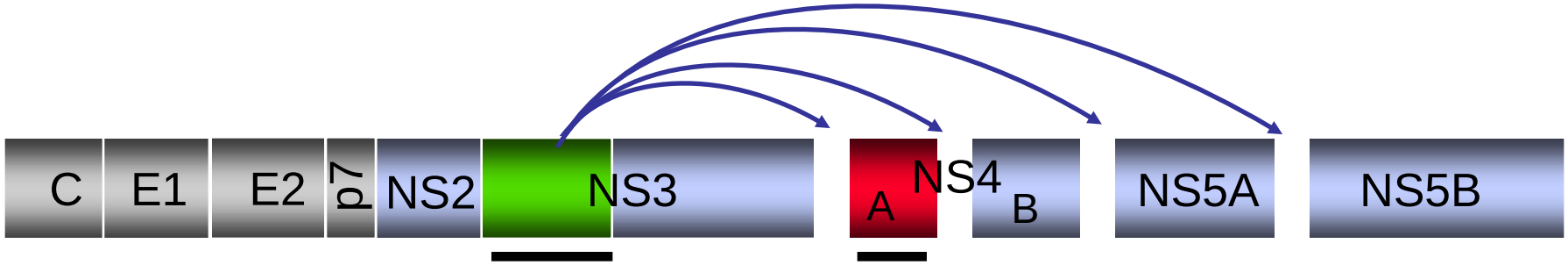
Targets of the most advanced HCV DAAs



- **NS3-4A (serine protease)**
- NS5A (dual role in RNA replication and virus assembly)
- **NS5B (RNA-dependent RNA polymerase)**

The NS3/4A protease polymerase as therapeutic target

The HCV genome is translated as a single polyprotein

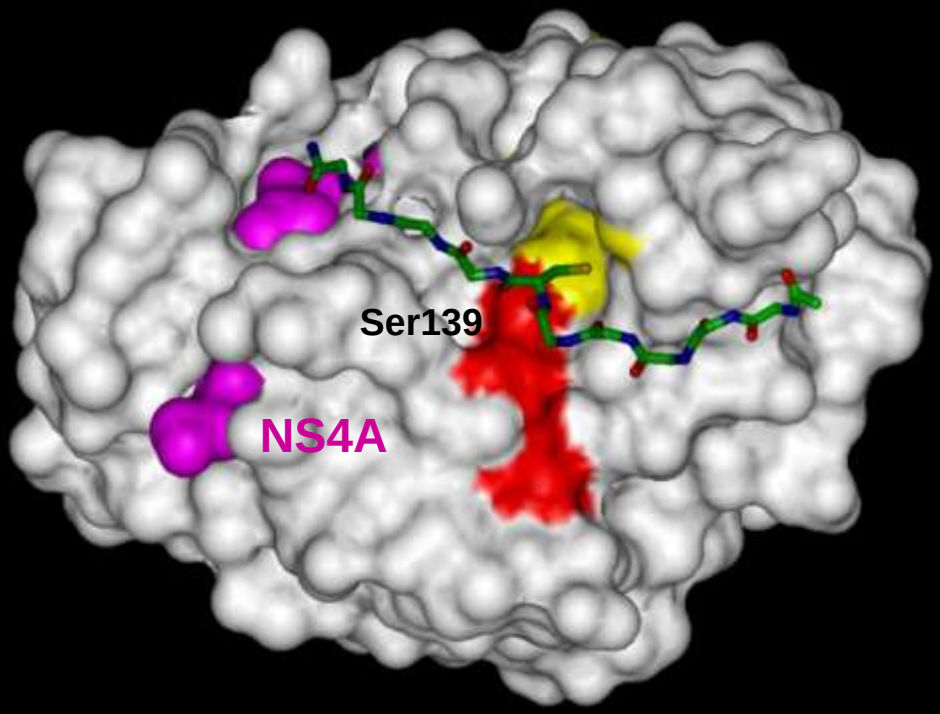


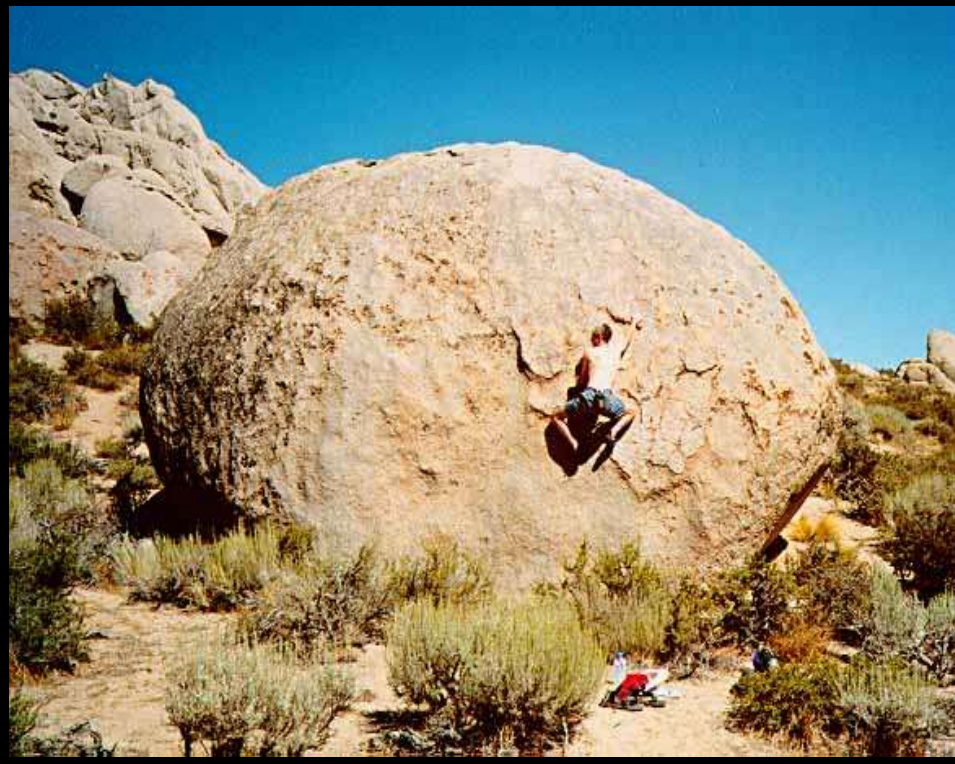
- The NS3/4A protease is responsible for the biogenesis of the nonstructural proteins (components of the RNA replication machinery)
- Inhibits the host innate immune response
  - By cleaving CARDIFF (NFkB/IRF3 activation) the NS3/4A protease shuts down liver cells' natural INTERFERON production

NS3/4A protease inhibitors: a "double-hit"?

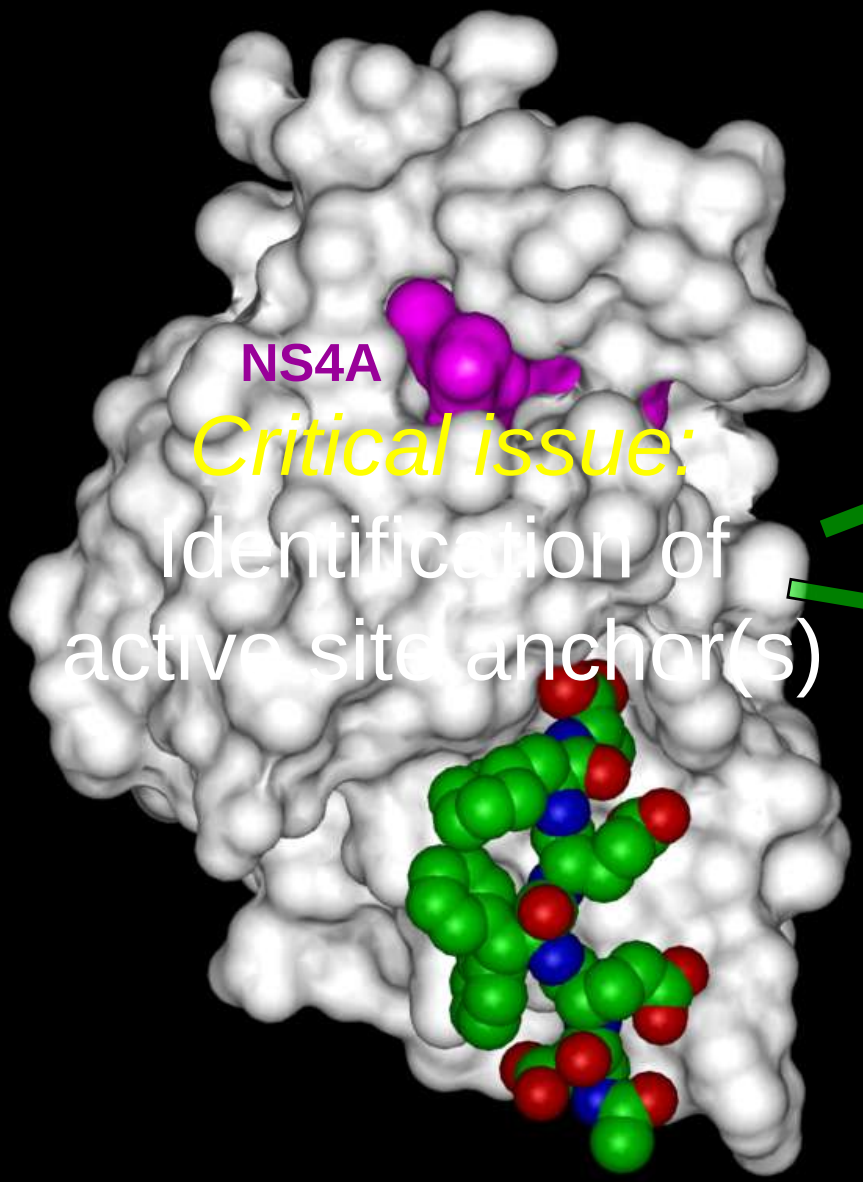
- 1. Block HCV replication
- 2. May restore innate antiviral defenses in HCV-infected cells

HCV NS3/NS4A serine protease: From “undruggable” target to drugs (1996 to 2011)



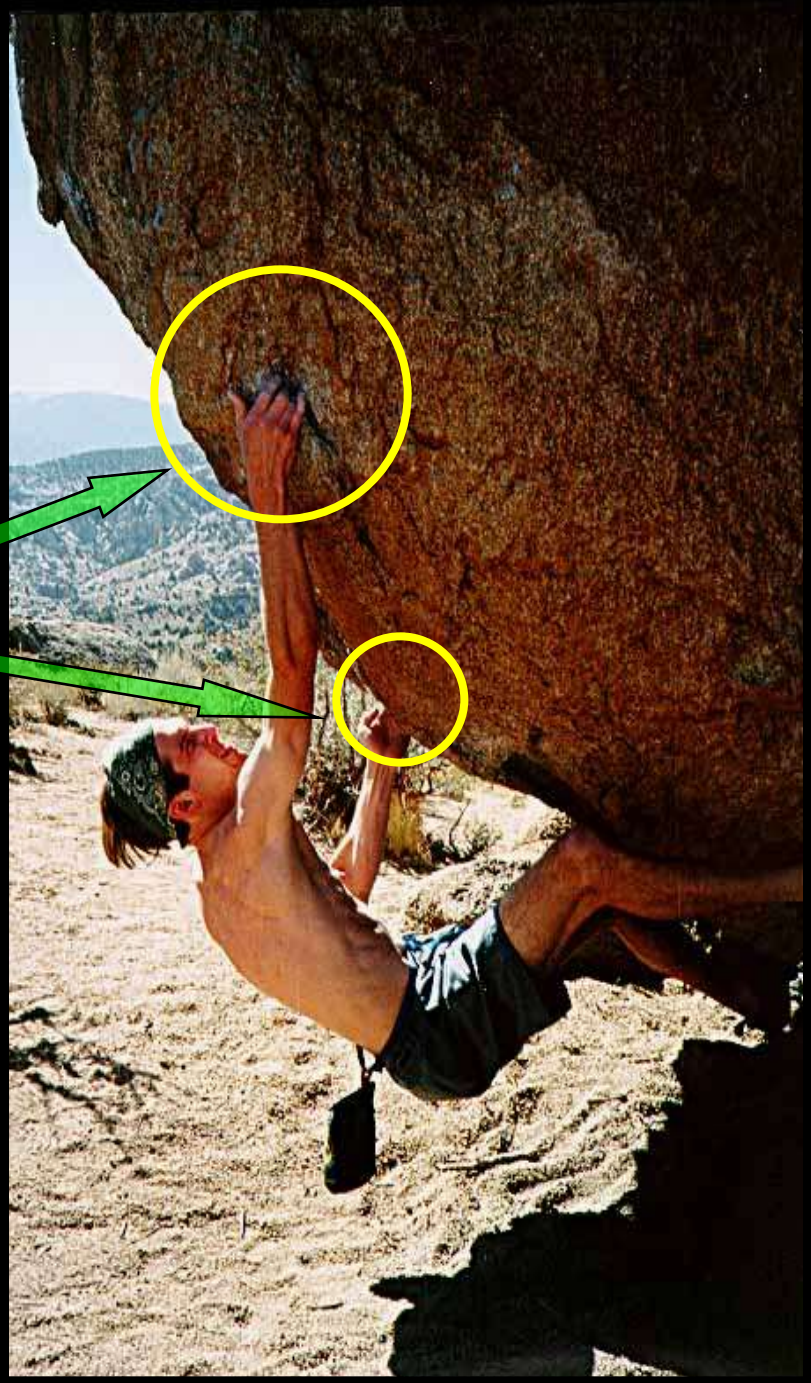


- *Long, shallow and exposed active site - little for inhibitors to grasp*
- *Design of low molecular weight inhibitors is very challenging*



Critical issue:

Identification of active site anchor(s)



HCV NS3/4A Protease

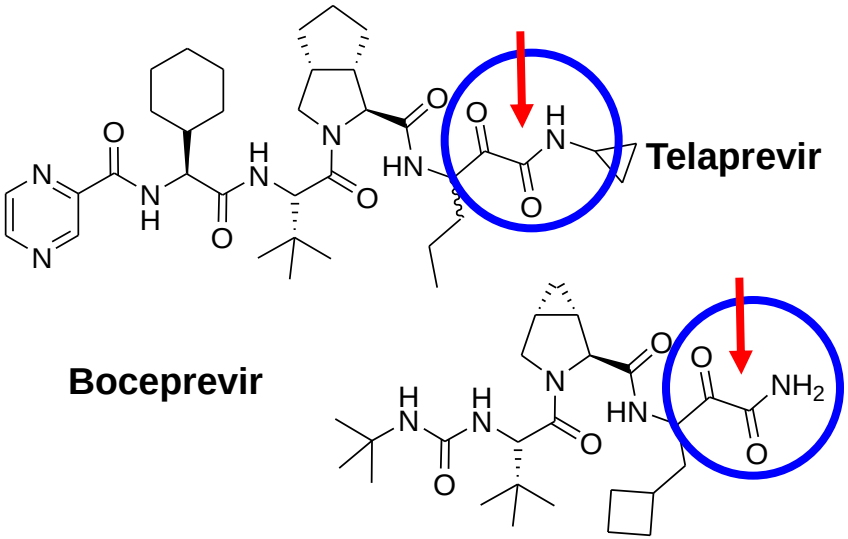
Two classes of protease inhibitors with different active-site anchors

Linear covalent inhibitors

- electrophilic "warhead forms a covalent bond with the enzyme
  - ➢ *substrate-derived α-keto amide peptidomimetic*

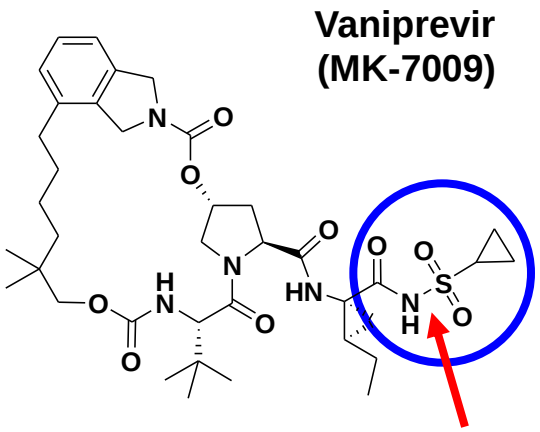
Macrocyclic non-covalent inhibitors

- the NS3/4A protease is auto-inhibited by its product
  - ➢ *product-derived peptidomimetic*



Telaprevir

Boceprevir



Vaniprevir (MK-7009)

NS3-4A protease inhibitors in clinical development

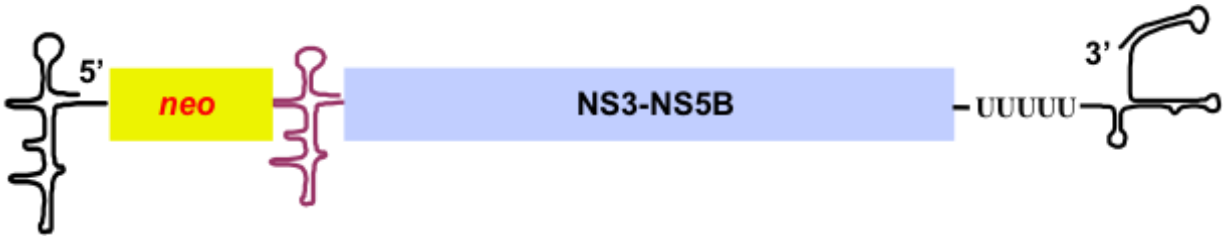
1) Linear covalent inhibitors

Phase 3/FDA Filing:	Telaprevir (VX-950, Vertex/J&J)
	Boceprevir (SCH-503034, Merck)
Phase 1:	VX-985 (Vertex)

2) Macrocyclic non-covalent inhibitors

Phase 2:	Vaniprevir (MK-7009, Merck)
	Danoprevir (R7227/ITMN-191; Intermune/Roche)
	TMC435350 (Tibotec/Medivir)
	BI201335 (Boehringer-Ingelheim)
	GS-9256 (Gilead)
Phase 1:	BMS-650032 (Brystol-Myers Squibb)
	MK-5172 (Merck)

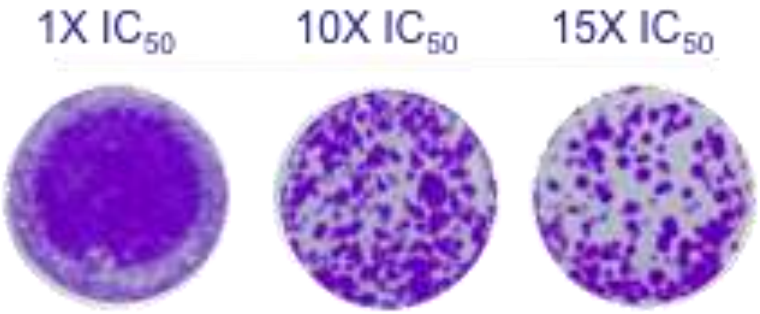
Drug resistant HCV variant can be selected *in vitro* *(selectable replicon system)*



- Culture in the presence of *G418* ***AND*** HCV inhibitors
  - most cells containing wt replicon due to loss of *neomycin* resistance
  - only cells harboring resistant replicons survive and give rise to resistant clones

    → rescue RNA → cDNA sequence → reverse genetics

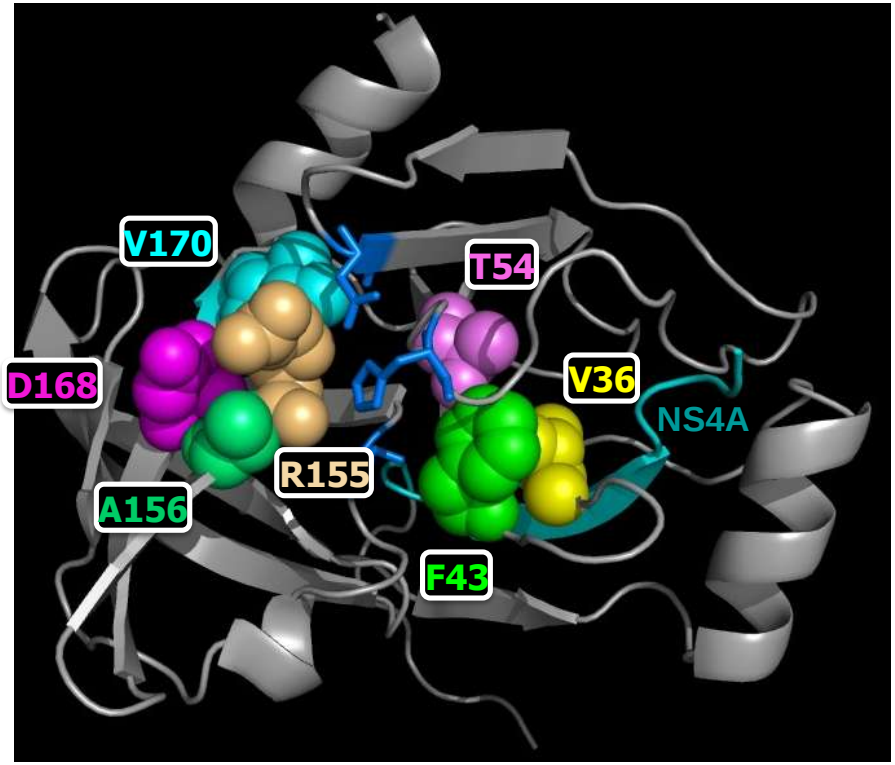
NS3/4A protease inhibitor (PI)



Trozzi et al. *In vitro selection and characterization of hepatitis C virus serine protease variants resistant to an active-site peptide inhibitor* J Virol 2003 77:3669-79

HCV mutants resistant to protease inhibitors readily emerge in cell culture (replicon)

Macrocyclic non-covalent inhibitors		Linear covalent inhibitors	
Ciluprevir (BILN2061)	**Danoprevir** (R7227)	**Telaprevir**	**Boceprevir**
R155 Q **A 156 V/T** D 168 A/V	F43 S T54 A/T **R155 R/K** **A156 S/V** D168 A	T54 A/T **R155 R/K** **A 156 A/S/T/V**	T 54 A/S **R155 K** **A156 S/T** V170A



CONCLUSIONS

- Escape mutants emerge readily to all protease inhibitors
- Low genetic barrier to resistance
  - Single mutations → resistance
  - Moderate to high resistance levels (5-50x)
- Potential for broad cross-resistance to all protease inhibitor classes (R155, A156)

In clinical trials, drug-resistant HCV variants emerge within days of monotherapy with Protease Inhibitors

Replicon

Telaprevir	Boceprevir
T54 A/T R155 R/K A 156 A/S/T/V	T 54 A/S R155 K A156 S/T V170A

Clinical

Telaprevir	Boceprevir
V36 A/M T54 A R155 K/T V36 A/M +R155K/T A156 S/T/V A36A/M+A156V/T	V36 A/L/M F43 C/S V55A T 54 A/S V36M+T54S R155 K/T/P V36A+R155K T54S/A+R155K A156 S T54S+A156S V170A/T/L

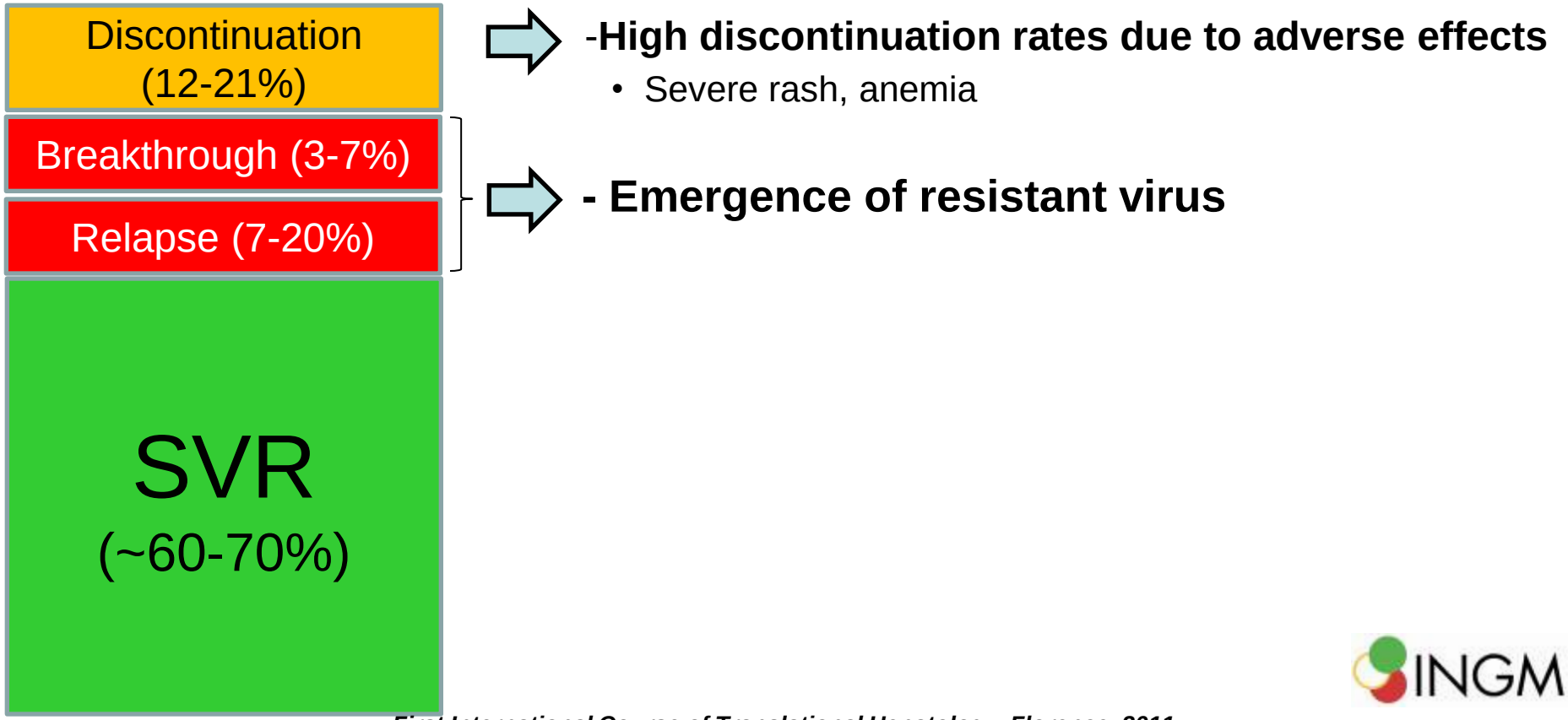
- Broader mutation spectrum *in vivo vs. in vitro*
  - Limited viral diversity in cell culture

    ~10^7 replicon (+) cells in Petri dish *vs.* 10^{10} HCV(+) cells in liver
- Resistance HCV variants are detected in circulation 1 year after stopping treatment
- **Combination therapy is mandatory in order to avoid resistance**

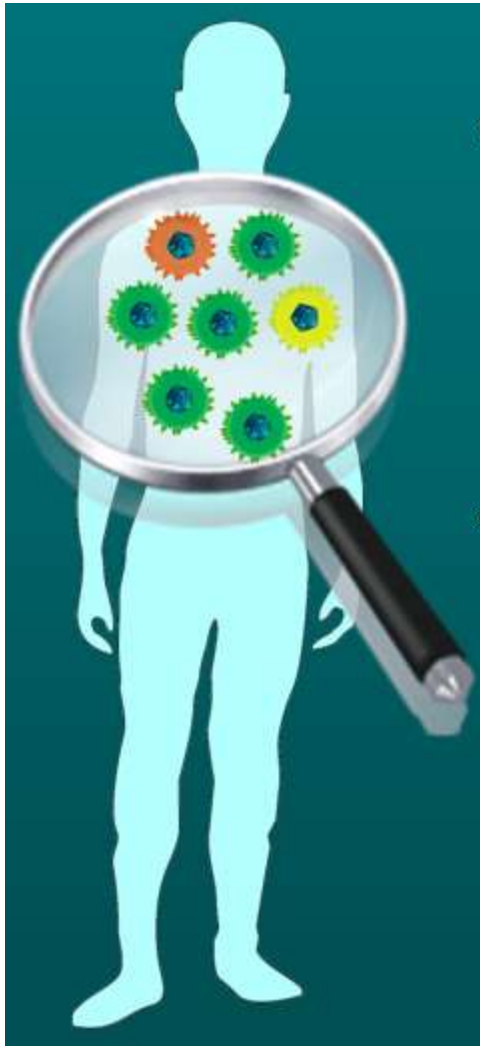
Emergence of resistance is a determinant of failure to triple therapy treatment of HCV1

Critical Factors for Treatment Failure (Telaprevir triple therapy)

PROVE 1. McHutchison JG, et al. N Engl J Med. 2009; PROVE 2. Hézode C, et al. N Engl J Med. 2009



Resistance to Protease Inhibitors: The lessons learned from the lab and from the clinic



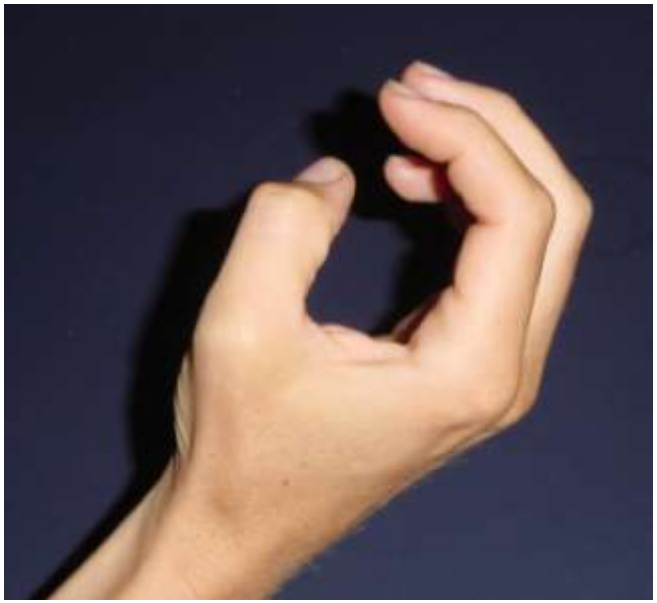
- Restricted genotype spectrum: not active on genotype 3 (naturally resistant variants at aa 168)
- Drug-resistant variants pre-exist at very low levels (~$1/10^4$) prior to initiation of therapy
  - rapidly selected under mono-therapy
  - persist for months/years
- Emergence of resistance can be partially suppressed by combining with PEG-IFN + RBV (triple therapy)
  - Long term viral suppression → significantly increased SVR rates (up to 80+%)
  - Resistant virus found in breakthrough/relapse

The three-dimensional structure of NS5B RdRp

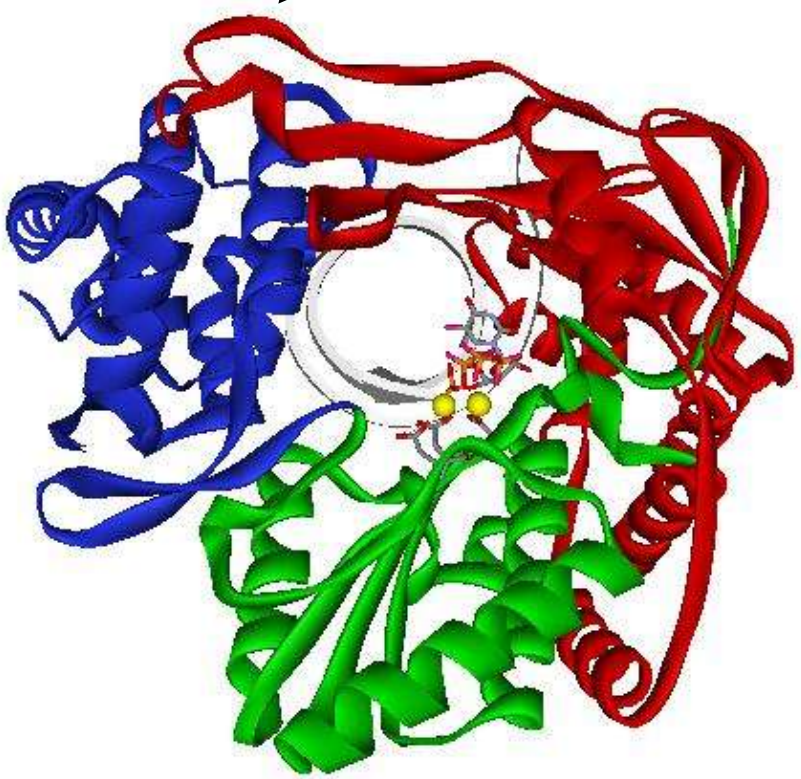
Bressanelli *et al*., (1999) *Proc Natl Acad Sci U S A*

Right-hand shape

"Fully encircled active site"



Finger-tips



Thumb	Palm	Fingers

Inhibitors of HCV NS5B RNA-dependent RNA Polymerase

Two Major Classes of Inhibitors

Non-nucleoside inhibitors (NNIs)

- Allosteric inhibitors
- Several NNI binding sites on the enzyme surface
- Restricted spectrum of action on different HCV genotypes
- Low genetic barrier to resistance

Nucleoside/nucleotide analogues

- Active site inhibitors (chain terminators)
- Equally active on all HCV genotypes
- High genetic barrier to resistance

Inhibitors of HCV NS5B RNA-dependent RNA Polymerase

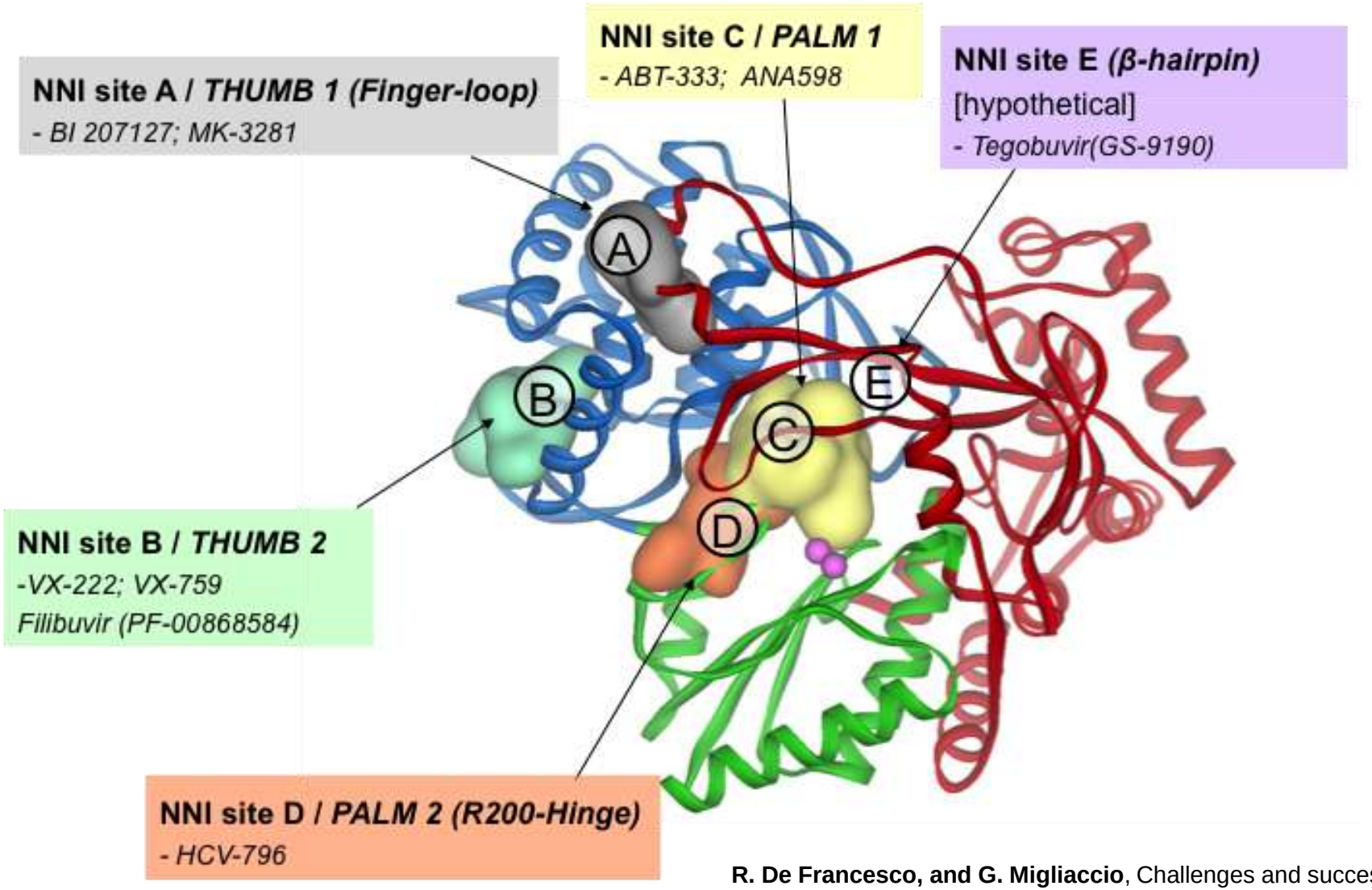
Non-Nucleoside Inhibitors (NNI)

Phase 2:	ANA598 (Anadys)
	Filibuvir/PF-868584 (Pfizer)
	Tegobuvir/GS-9190 (Gilead)
	VX-222 (Vertex)
Phase 1:	BI207127 (Boehringer Ingelheim)
	ABT-333 (Abbott)
	VX-759 (Vertex)

Nucleoside/nucleotide analogs

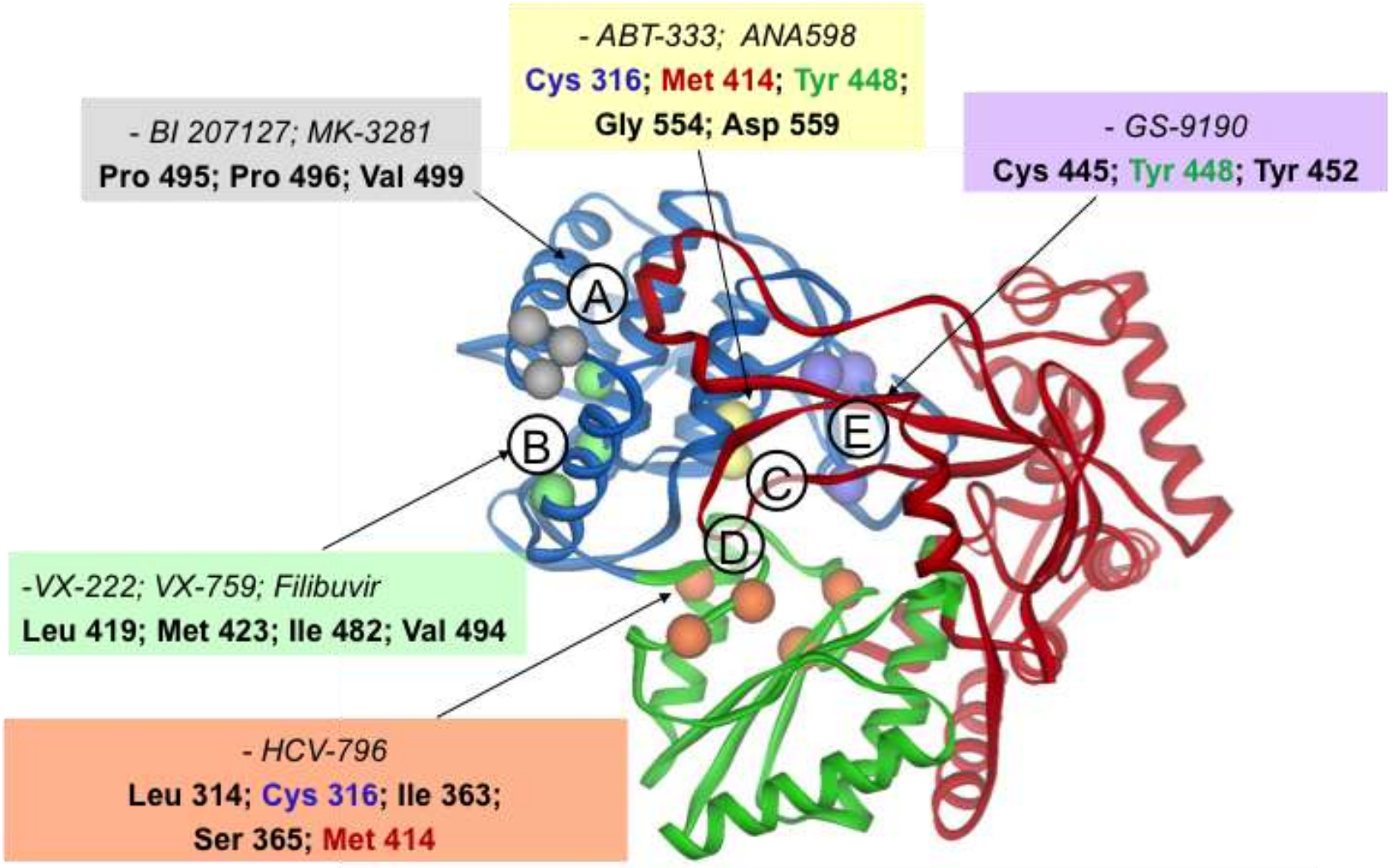
Phase 2:	R7128 (Roche/Pharmasset)
	PSI-7977 (Pharmasset)
Phase 1:	IDX184 (Idenix, *on clinical hold*)
	PSI-938 (Pharmasset)

Five (5) distinct sites for Non-Nucleoside HCV polymerase Inhibitors (NNI)



R. De Francesco, and G. Migliaccio, Challenges and successes in developing new therapies for hepatitis C. **Nature** 436 (2005) 953-60

Drug-resistant variants to polymerase non-nucleoside inhibitors are readily selected *in vitro* and in patients



•*Cross-resistance among different NNI classes*

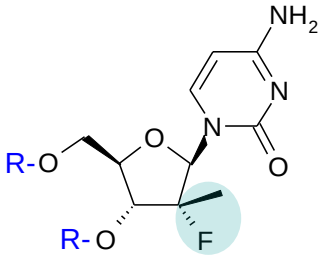
Therapeutic efficacy of non-nucleoside HCV polymerase inhibitors will be severely limited by drug-resistance

- Very low genetic barrier to resistance *in vitro* and *in vivo*
- Emergence of resistance observed during monotherapy or combination therapy
- Cross-resistance among different NNI classes
- Restricted spectrum of action (most active on genotype 1b)
- Resistant variants/polymorphisms pre-exist in patient population (*e.g.*, **C316Y→ N** in genotype 1b)

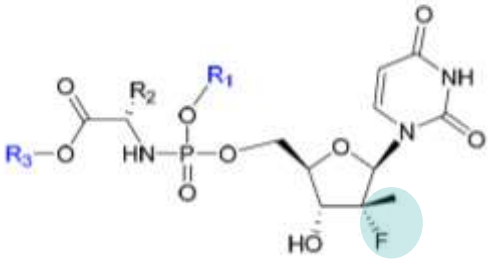
Inhibitors of HCV NS5B RNA-dependent RNA Polymerase

2’-C-modified-nucleos(t)ide analogues

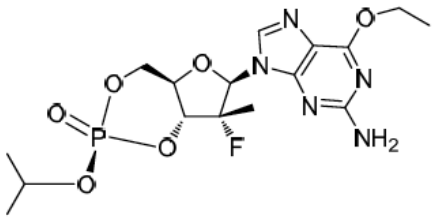
Mericitabine/R7128 (Roche)
Prodrug of 2’-deoxy-2’-fluoro-2’-C-methylcytidine



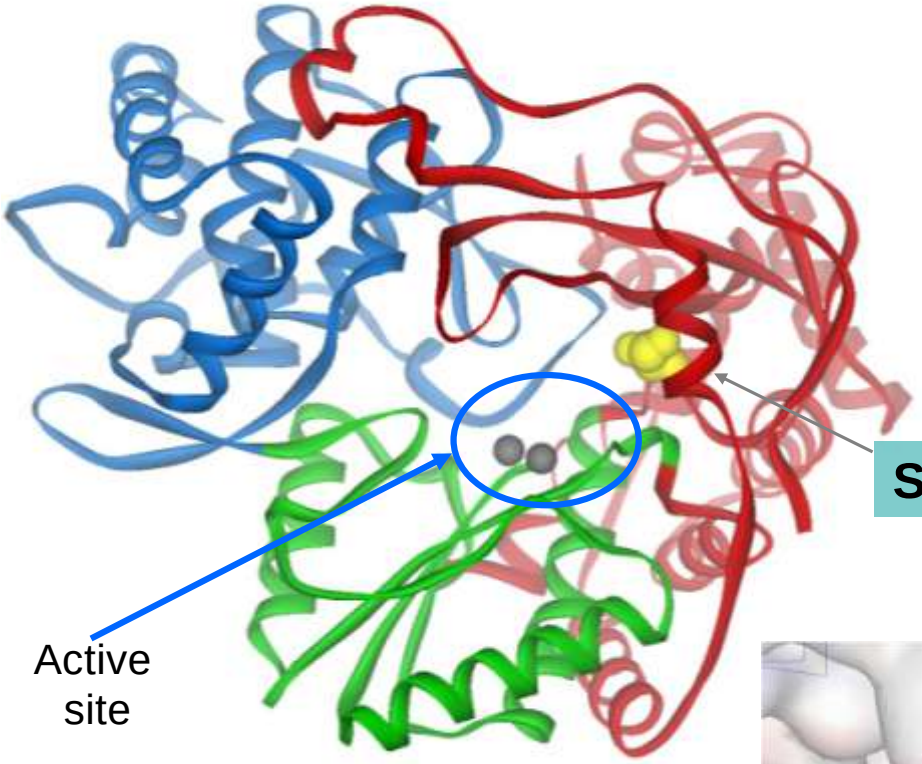
PSI-7977 (Pharmasset)
Phosphoramidate prodrug of 2'-deoxy-2'-fluoro-2'-C-methyluridine



PSI-938 (Pharmasset)
Phosphoramidate prodrug of 2'-deoxy-2'-fluoro-2'-C-methylguanosine



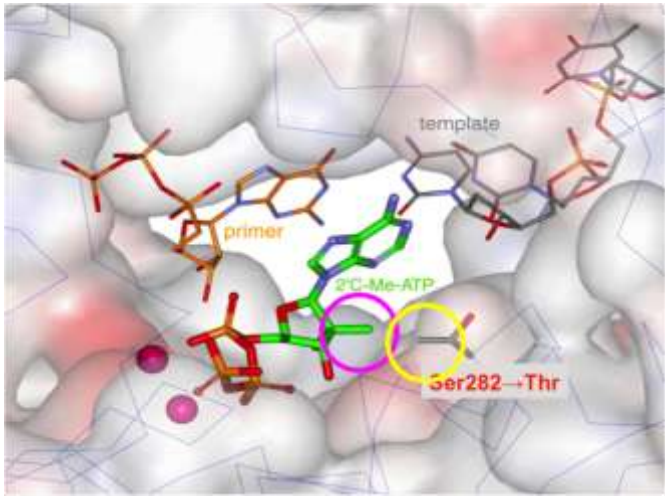
Drug-resistance to 2'-C-methyl nucleoside inhibitors can be selected in vitro (replicon system)



Active site

Ser 282→Thr

- Low to moderate resistance levels (2-10x)
- Low viral fitness
- Higher barrier to resistance *vs.* PIs or NNIs



(Migliaccio et al., J Biol Chem 2003;278:49164-70)

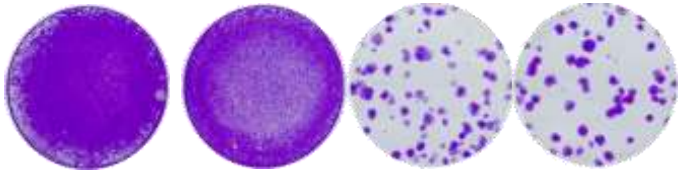
HCV presents a higher barrier to resistance to nucleoside analogs than to non-nucleoside polymerase or protease inhibitors

McCown *et al*, (2008) Antimicrob Agents Chemother

NS5B non-nucleoside polymerase inhibitor (NNI)

HCV-796

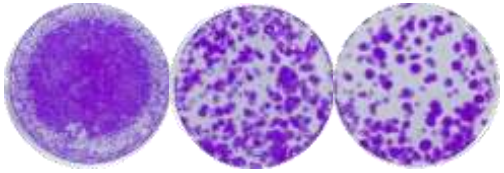
Untreated 1X IC_{50} 10X IC_{50} 15X IC_{50}



NS3/4A protease inhibitor (PI)

Telaprevir

1X IC_{50} 10X IC_{50} 15X IC_{50}



NS5B nucleoside analog polymerase inhibitor (NI)

Mericitabine/R7128

1X IC_{50} 10X IC_{50} 15X IC_{50}

- Resistant colonies selected for HCV-796 (NNI)
  - C316Y and S365S/A
- Resistant colonies selected for Telaprevir (PI)
  - A156T/S and T54T/A
- Treatment with Mericitabine (nucleoside analog) resulted in clearance of the replicon after 3 week selection
  - No resistance mutations detected

High barrier to resistance to nucleoside analog observed *in vivo*

• Mericitabine, PSI-7977, PSI-938:

No Evidence of Viral Resistance After 14 Days of Monotherapy

•1000 mg BID Mericitabine + PEG-IFN/RV (PROPEL Study) resulted in 83% cEVR

No resistant variants detected at baseline or during treatment (12 wks)

EASL 2011 (Berlin):

•D.R. Nelson et al. (Late breaker 1372)
ONCE DAILY PSI-7977 PLUS PEG-IFN/RBV IN HCV GT1: 98% RAPID VIROLOGIC RESPONSE, COMPLETE EARLY VIROLOGIC RESPONSE: THE PROTON STUDY

•E. Lawitz et al. (Late breaker 1370)
ONCE DAILY DUAL-NUCLEOTIDE COMBINATION OF PSI-938 AND PSI-7977 PROVIDES 94% HCV RNA < LOD AT DAY 14: FIRST PURINE/PYRIMIDINE CLINICAL COMBINATION DATA (THE NUCLEAR STUDY)

Resistance to HCV Direct-Acting Antivirals

KEY POINTS

– Higher genetic barrier to viral resistance observed with nucleosides *versus* non-nucleosides, NS3-4A protease or NS5A inhibitors

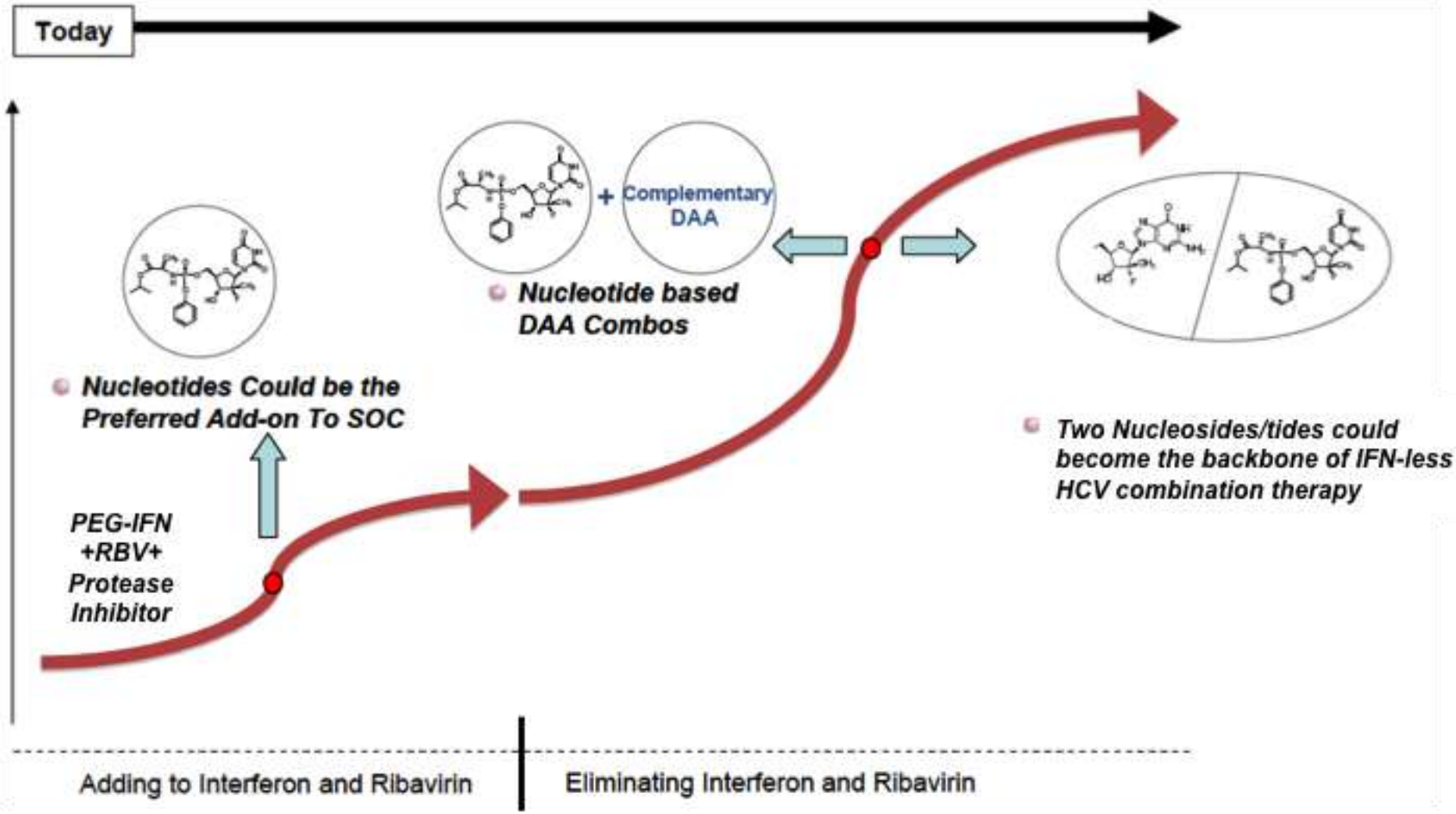
- Non-Nucleoside Pol Inhibitors, Protease Inhibitors, NS5A Inhibitors: frequent mutants, high resistance, good replication fitness → LOW BARRIER TO RESISTANCE

- Nucleosides: infrequent mutants, low to moderate resistance, poor replication fitness → HIGH BARRIER TO RESISTANCE

- Combination of pegIFN/RBV with a “low-barrier” DAA can only partially suppress the emergence of resistance (especially in IL28B TT/CT)

- Need to include one or more “high-barrier” DAA in combination therapy

EVOLUTION OF ANTI_HCV THERAPY (MY VIEW)



Modified from www.pharmasset.com

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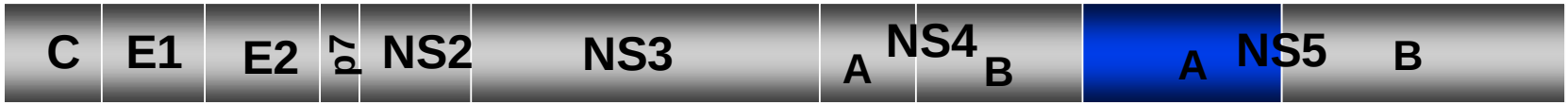
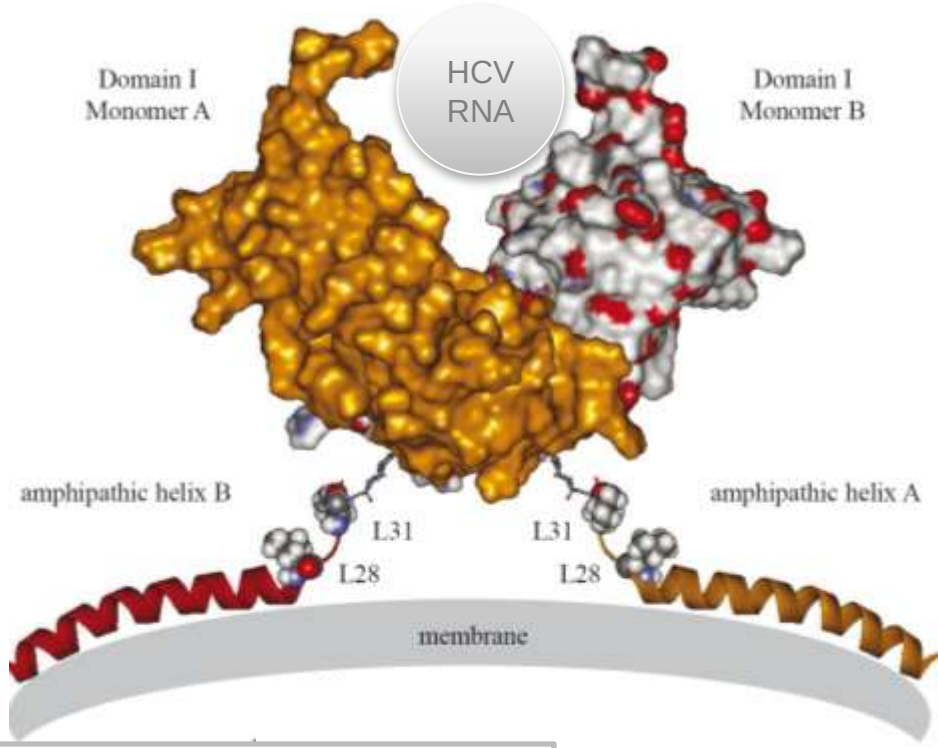
Thanks to CM Rice (Rockefeller U) and R Barteschlager (U. of Heidelberg) for providing slides/images

NS5A-targeting agents in clinical development



- Dimeric phosphoprotein
- Replicase component (RNA binding)
- Implicated in virus assembly



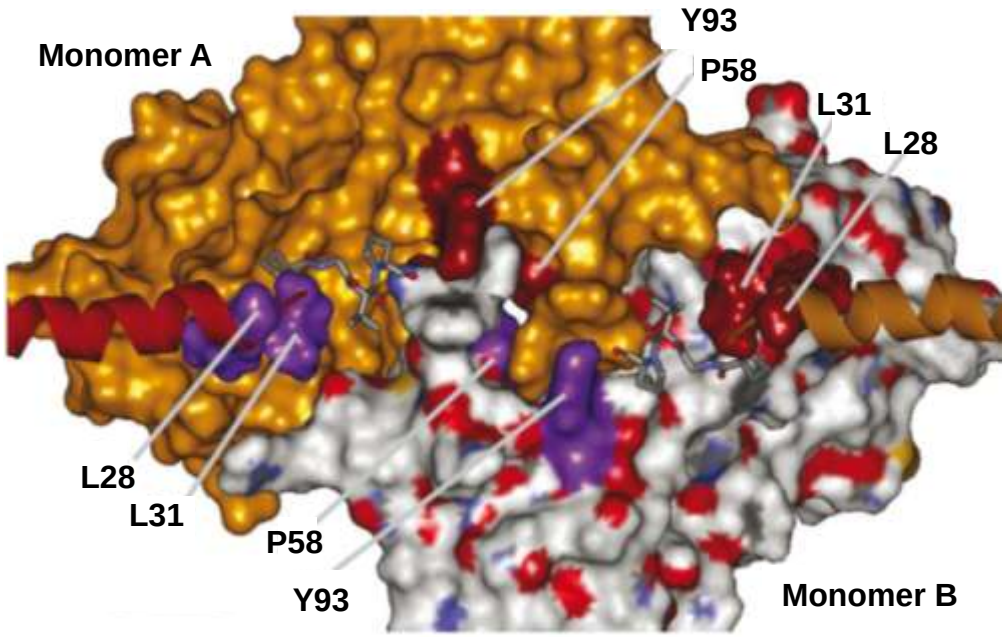
Phase 2	BMS-790052 (Brystol Myers Squibb)
Phase 1	AZD2836 (Astra Zeneca/Arrow)
	AZD7295 (Astra Zeneca/Arrow)

Picture from: Schmitz U, Tan SL. "NS5A--from obscurity to new target for HCV therapy", Recent Pat Antiinfect Drug Discov. 2008

Drug-resistant variants to NS5A inhibitors are readily selected *in vitro* (replicon system)

– Mutations conferring resistance map to the NS5A domain I dimer interface

M28T (1a); L28V (1b)
L31V/F/M
P58L
Y93H/C/W



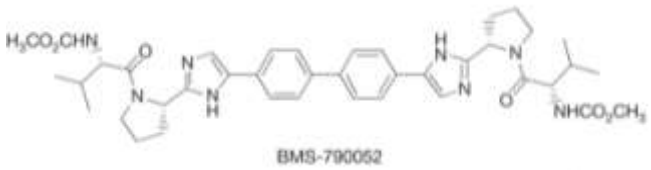
– Single AA mutation sufficient for high-level resistance to class

– Resistant variants maintain good replication fitness

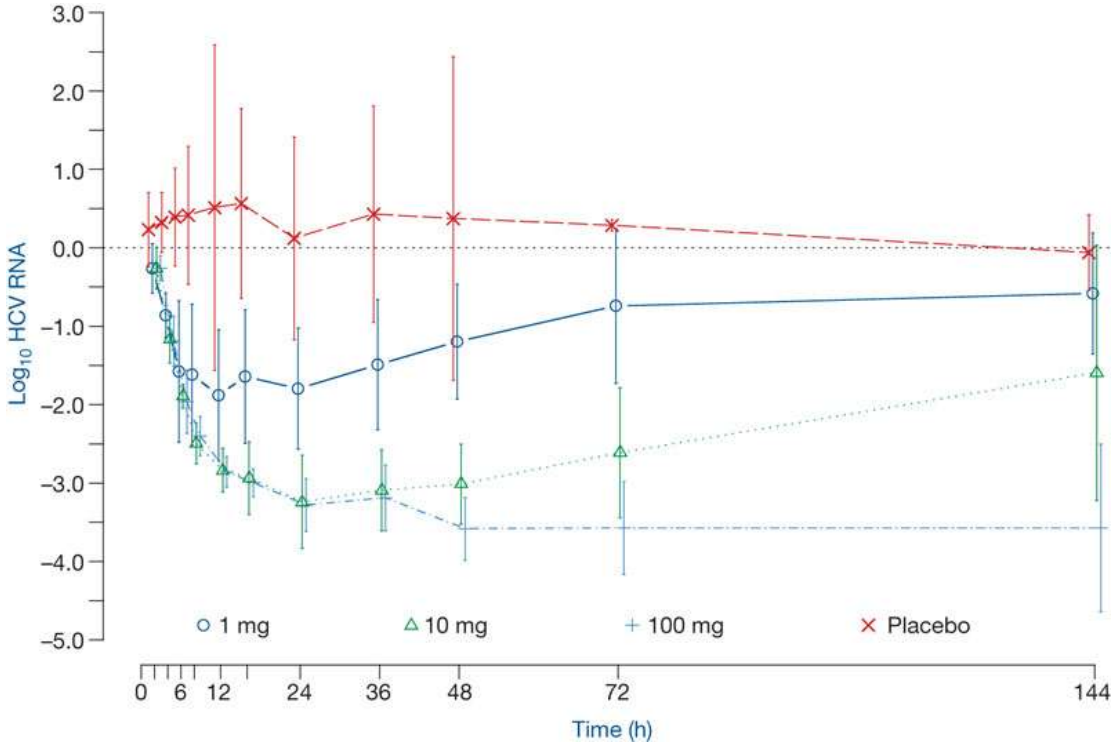
→ Low genetic barrier to resistance

Reviewed in: Schmitz U, Tan SL. "NS5A--from obscurity to new target for HCV therapy", Recent Pat Antiinfect Drug Discov. 2008

Discovery of the most potent DAA so far: BMS-790052



- Replicon EC_{50} : 10-50 pM
- Active across HCV 1-6



- Profound and long term viral suppression is achieved after single oral doses of BMS-790052
- Resistant variants detected in treated patients (M28T, Q30H/R, L31M, Y93H) were accurately predicted by replicon *in vitro* studies

*M Gao et al. Nature **465**, 96-100 (2010)*

HCV NS5A inhibitors

SUMMARY

- Drug-resistant variants to NS5A inhibitors are readily selected *in vitro*
  - Mutations may confer high-level resistance and retain good replication capacity

    → *Low genetic barrier to resistance in the replicon system (in vitro)*
- Design of highly potent (pM) and efficacious NS5A inhibitor (BMS-790052)
  - Activity across major genotypes
  - Suppression of viremia for a week after a single dose as monotherapy in P.O.C. study
  - Significant activity (nM) retained against resistant mutants
  - Resistance variants observed in patients