Surface Antigen Loss and Transaminase Flares:

Critical Milestones for Achieving Functional Cure

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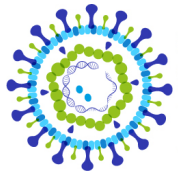
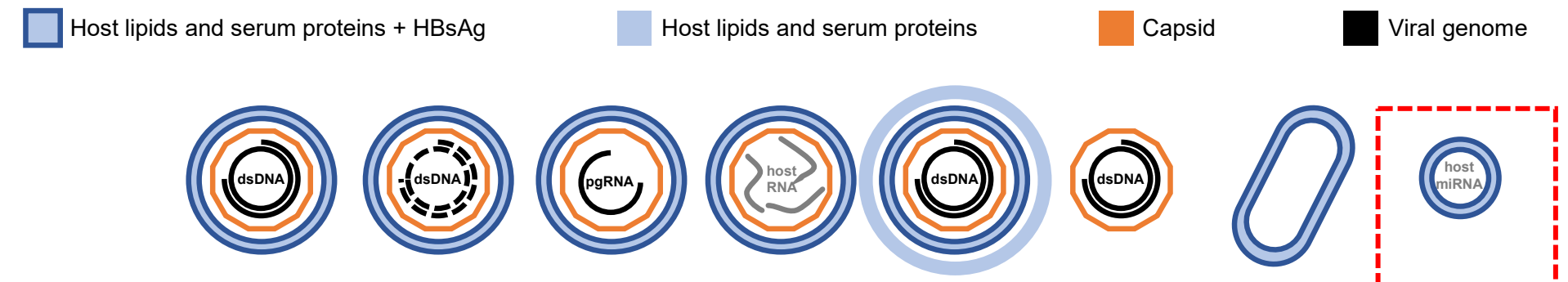


2nd Annual
Chronic HBV Drug Development
From Late Translational to Phase II Clinical Progress
April 25 - 27, 2022, Boston | MA

Particle production in chronic HBV infection



Particle	Dane particle	Quasispecies (mutation)	HBV RNA virion	"Empty" virions	Exosome	"Naked" capsid	SVP (filamentous)	SVP (spherical)
Ratio to Dane particle	1	0.2-0.8	0.001-1	100	unknown	unknown	10	10,000 – 100,000
Size (nM)	44				50-150	27	Ø22	22

99.99% of HBsAg is derived from subviral particles (SVP)

Active cccDNA

? Integrated HBV DNA

Vaillant, ACS Inf Dis 2021; 7: 1351-1368

Production of SVP drives chronicity of HBV infection

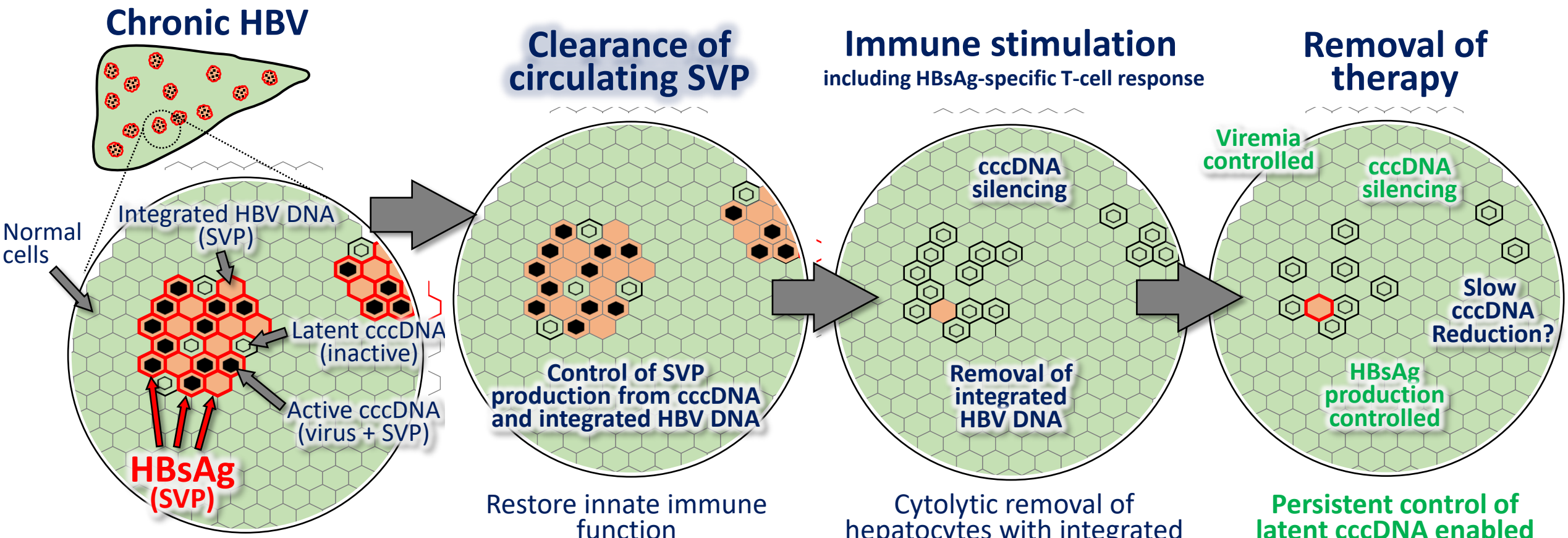
Immunoinhibitory properties of SVP (HBsAg)

Immune function	Target of inhibition	Effect observed
Innate SVP block inactivation of cccDNA	TLR function	*In vitro, in vivo*
	Cytokine signalling	*In vitro*, in humans
	Monocyte and macrophage function	*In vitro*
	Dendritic cell function	*In vitro*
	NK cell function	*In vitro, in vivo,* in humans
Adaptive SVP inhibit clearance of integrated HBV DNA	Sequester anti-HBs	*In vitro*
	HBV specific B-cell function	In humans
	HBV specific CD4+ T-cell function	In humans
	HBV specific T-cell tolerance	*In vitro, in vivo*
	HBV specific T-cell exhaustion	*In vivo*, in humans

Vaillant, ACS Inf Dis 2021; 7: 1351-1368

SVP must be cleared for immune control of infection to be restored

The path to functional cure



HBsAg blocks immune control

Progression of liver disease

Risk of HCC

Restore innate immune function

Exhausted HBsAg-specific T-cell response can be restored

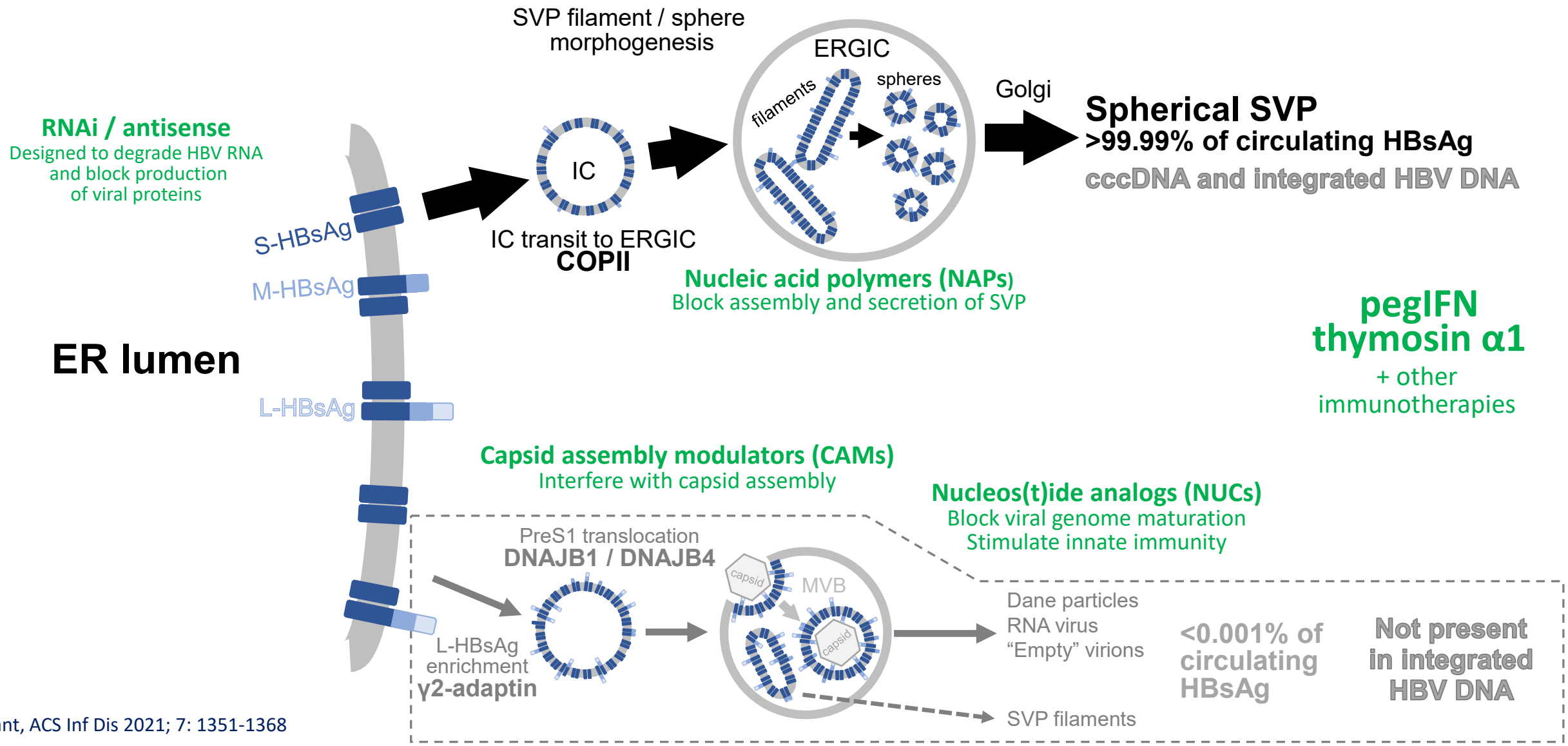
Cytolytic removal of hepatocytes with integrated HBV DNA

Transaminase flares (host mediated) signal removal of integrated HBV DNA

Persistent control of latent cccDNA enabled by elimination of HBsAg production in the liver

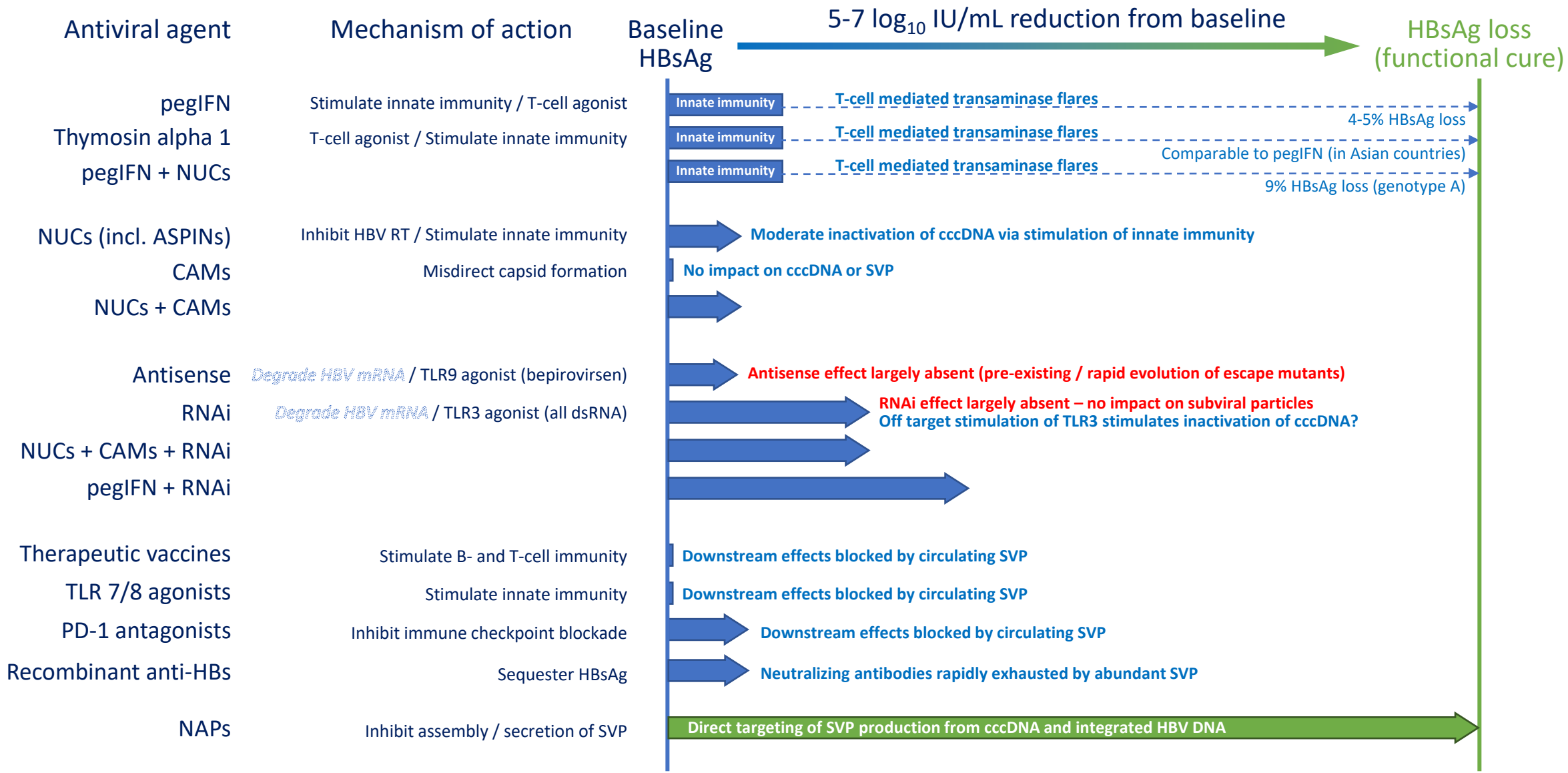
Vaillant, ACS Viruses 2021; 13: 745

Impact of investigational approaches on HBsAg



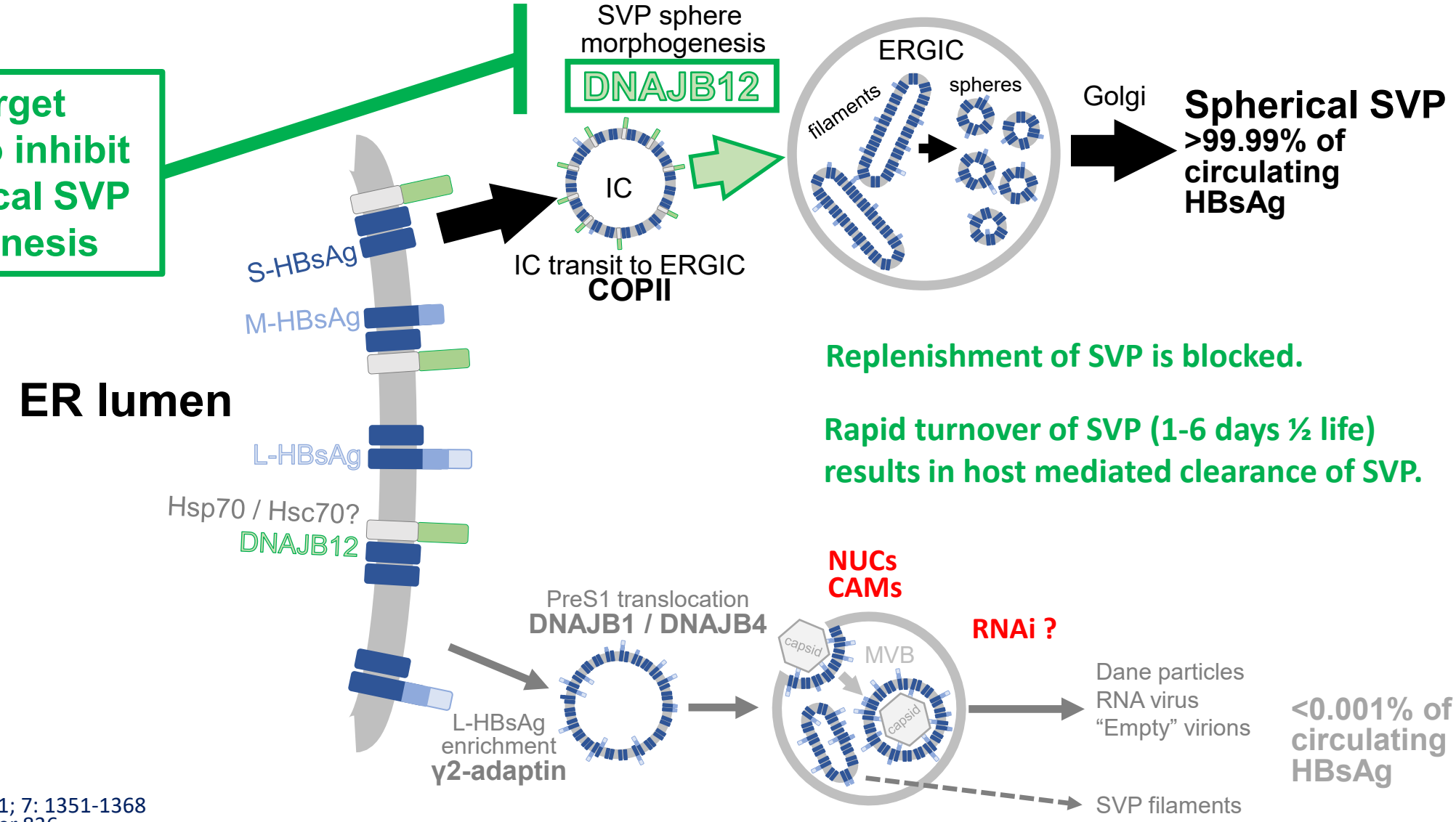
Vaillant, ACS Inf Dis 2021; 7: 1351-1368

HBsAg loss – a critical milestone for functional cure of HBV



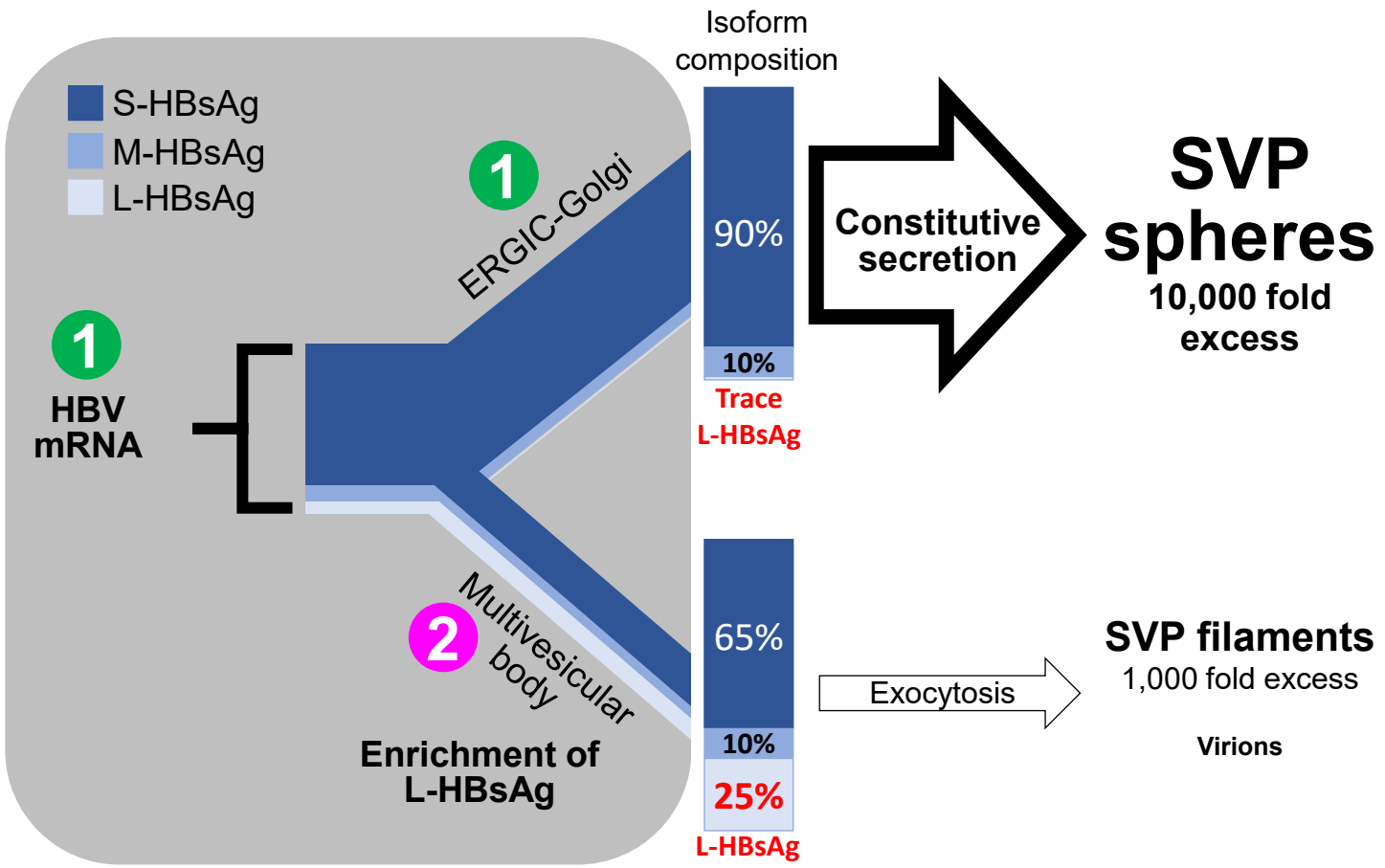
Nucleic acid polymers (NAPs): a unique molecular mechanism in HBV

NAPs target DNAJB12 to inhibit HBV spherical SVP morphogenesis



Vaillant, ACS Infectious Dis 2021; 7: 1351-1368
Boulon et al., AASLD 2021 Poster 836

HBsAg isoform response is dependent on antiviral effect



SVP turn over rapidly
99.99% of HBsAg

1 Selective decline in S-HBsAg

Signals inhibition of spherical SVP release

- HBV mRNA degradation
- Inhibition of SVP assembly / release

2 Selective decline in M/L-HBsAg

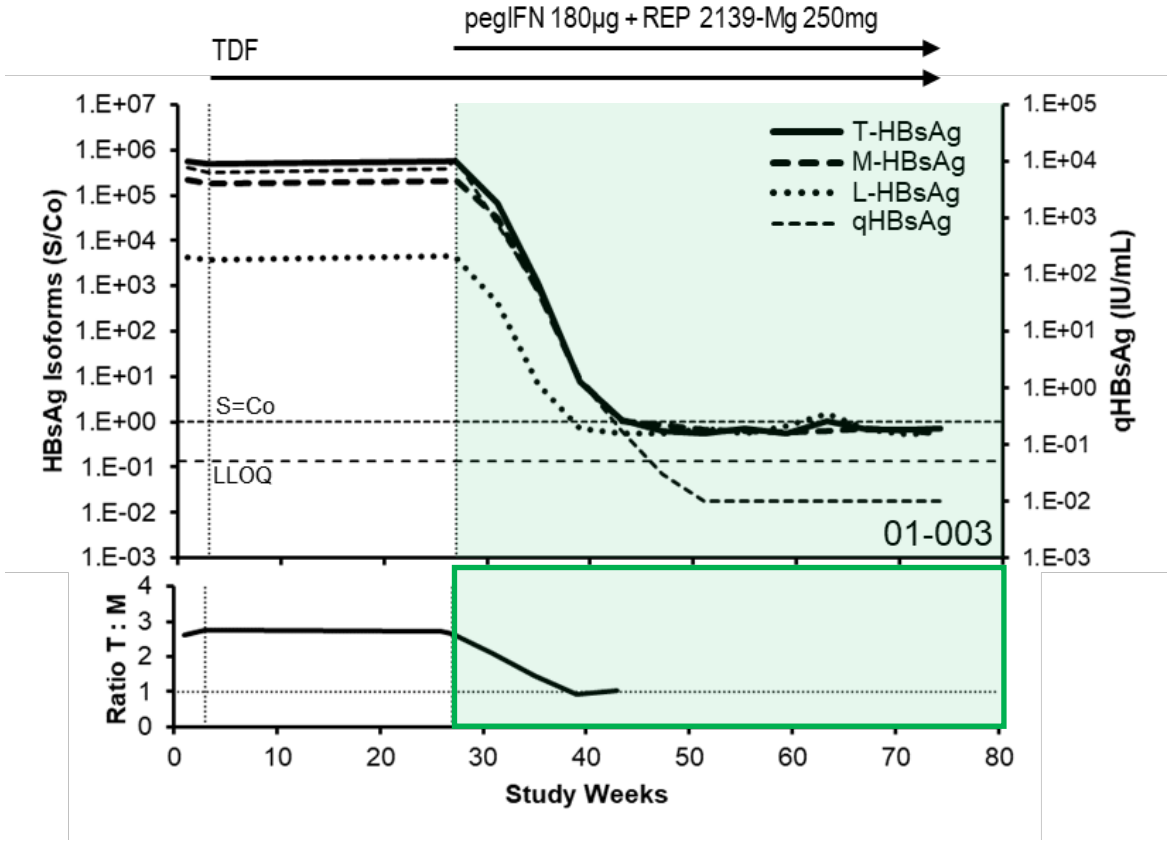
Inhibition of virion assembly?
Inhibition of exocytosis of virions / filaments?
Enhanced autophagy?

Vaillant, ACS Inf Dis 2021; 7: 1351-1368

REP 301 / 401: Validating the NAP mechanism in the clinic

Change in S-HBsAg content: change in ratio of total HBsAg (S+M+L) : preS2 (M+L) over time

HBsAg response



Strong HBsAg declines with NAPs are accompanied by selective decline of S-HBsAg

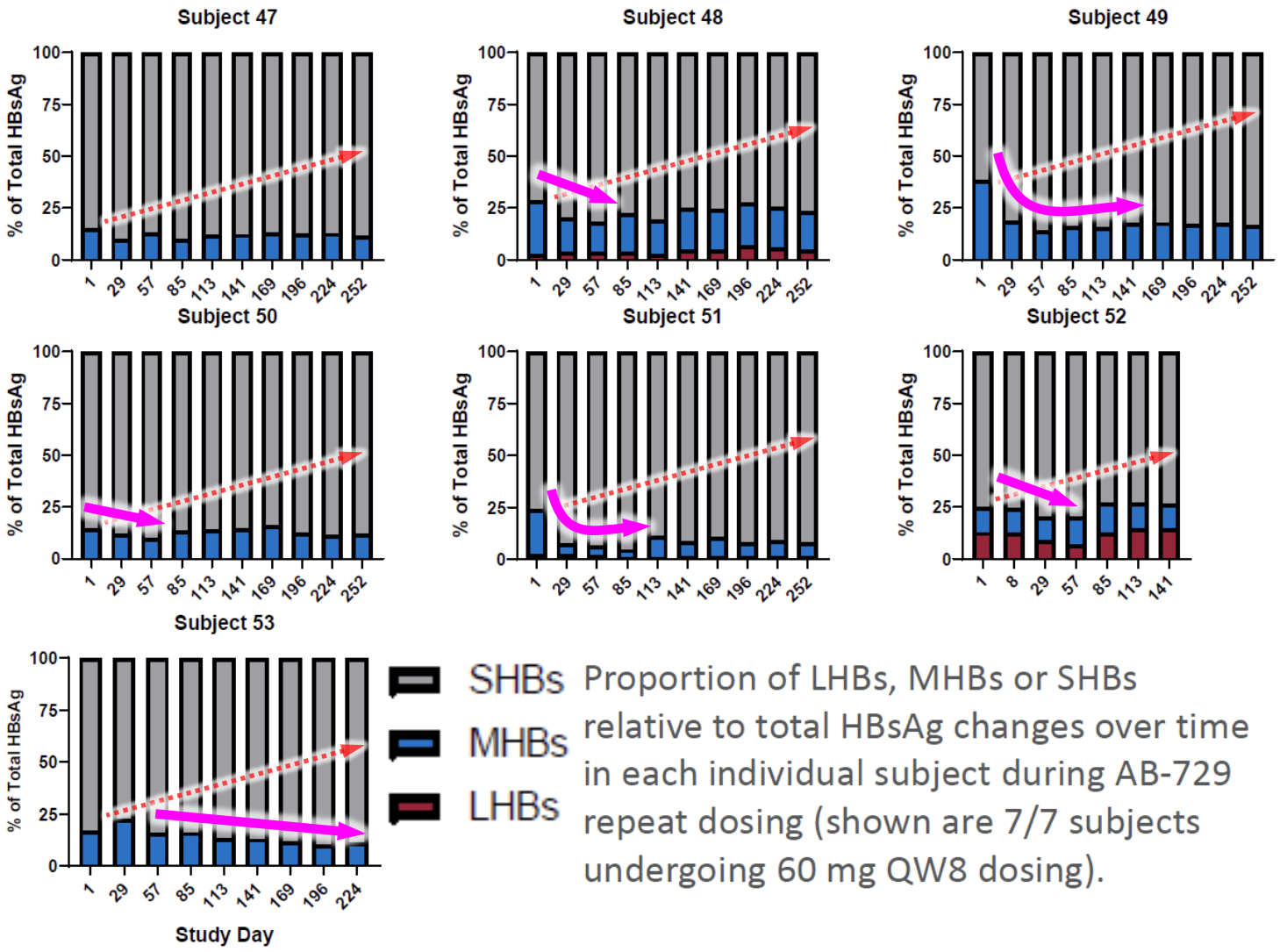
Correlation between selective S-HBsAg clearance during therapy and qHBsAg response
(all 52 participants in REP 301 + REP 401)

qHBsAg response during therapy (decline from baseline)	Total	Selective S-HBsAg decline	p-value
< 2 $\log_{10}$ IU/mL	10	1	< 0.01
> 2 $\log_{10}$ IU/mL	42	39	

Strong HBsAg decline with NAPs is accompanied by clearance of SVP (from cccDNA and integrated HBV DNA)

Bazinet et al., Hepatology Comm 2022; April 2

HBsAg isoform ratio response to AB-729
(identical assay platform used for NAPs)



Proportion of LHBs, MHBs or SHBs relative to total HBsAg changes over time in each individual subject during AB-729 repeat dosing (shown are 7/7 subjects undergoing 60 mg QW8 dosing).

No selective decline in S-HBsAg

SVP sphere production is unaffected

Selective declines in M and L-HBsAg

Selective effects on SVP filaments and virions

TLR3-enhanced autophagy?

Delgado and Deretic, Cell Death Diff 2009; 16: 976-983
Lin et al., Cells 2020; 9: 2101

Inconsistent with mRNA degradation (for all RNAi against HBV)

Thi et al., J Hepatol 2021; 75: S760

What is “potent” HBsAg (SVP) reduction?

0.5 - 1 log_{10} IU/mL reduction from baseline → **Should not be described as “potent”!**

- Rare with CAMs in the absence of NUCs
- Common with NUCs and pegIFN and RNAi
- Consistent with inactivation of cccDNA
- Abundant circulating SVP still present
- Predicts clinical futility for achieving functional cure[1-3]

> 4 log_{10} IU/mL reduction from baseline → **Potent SVP clearance predicting functional cure[4-7]**

- **Associated with strong therapeutic transaminase flares and HBsAg loss**[8-15]
- Allows withdrawal of NUC therapy with sustained virologic control or functional cure[16-19]

- NUCs: <1% per year of therapy[20]
  more likely in GT A[21]

- PegIFN: 6% with 48 weeks of therapy[22]
- PegIFN + NUCs: 9% with 48 weeks of therapy[22]
  Mostly restricted to GT A, more likely with HBeAg positive infection[22,23]

- Rarely observed with RNAi or antisense

- **pegIFN + NUCs + NAPs: 70% with 48 weeks of therapy[24]**
  GT A, GT C and GT D, HBeAg positive or negative[24,25], HBV / HDV co-infected[26]

1. Brunetto et al., Hepatol. 2009; 49: 1141-1150
2. Rijckborst et al., Hepatol. 2010; 52: 454-461
3. Sonneveld et al., Heaptol. 2013; 58: 872-880
4. Wiegand et al., Antiviral Ther. 2008; 13: 547-554
5. Moucari et al., Hepatol. 2009; 49: 1151-1157
6. Marcellin et al., Alimen Pharmacol Ther. 2016; 44: 957-966
7. Ahn et al., Dig Dis Sci. 2018; 63: 3487-3497
8. Wong et al., Liv Int. 2018; 38: 1760-1769
9. Jeng et al., J Viral Hep. 2018; 25: 421-428
10. Nagaoka et al., Hepatol Res. 2016; 46: E89-E99
11. Yano et al., Biomed Rep. 2017; 7: 257-262
12. Hall et al., J Hepatology 2020;73: S69
13. Choi et al., J Hepatology 2020; 73: S866
14. Farag et al., J Hepatology 2020; 73: S877
15. Bazinet et al., J Viral Hepatitis 2021: epub Feb 8
16. Liang et al., Ailment Pharmacol Ther. 2011; 34: 344-352
17. Chan et al., Antiviral Ther. 2011; 16: 1249-1257
18. Lee et al., Clin Mol Hepatol. 2016; 22: 382-389
19. Chen et al., J Viral Hep. 2018; 25: 590-597
20. Chevaliez et al., J Hepatol. 2013; 58: 676-683
21. Marcellin et al., J Hepatol. 2014; 61: 1228-1237
22. Marcellin et al., Gastroenterol. 2016; 150: 134-144
23. Brunetto et al., J Hepatol. 2013; 59: 1153-1159
24. Bazinet et al., Gastroenterology 2020; 158: 2180-2194
25. Al-Mahtab et al., PLoS One 2016; 11: e0156667
26. Bazinet et al., Lancet Gastro Hepatol 2017; 2: 877-889

Transaminase flares:
A key step in establishing functional cure

Transaminase flares are driven by hepatocyte death

ALT / AST: hepatocytes throughout the liver
GGT: hepatocytes lining the sinusoidal epithelium

Non-viral hepatitis (i.e. NASH): signal generalized loss of functional hepatocytes -> **reduced liver function (↑bilirubin, ↓albumin, ↑INR)**

Acute viral hepatitis: loss of functional hepatocytes with spread of infection -> **reduced liver function (NK cell activation)**

Chronic viral hepatitis: liver function acclimates and is maintained in steady state with chronic infection

- **flares signal immune mediated clearance of infected (non-functional) hepatocytes**
- **no change in liver function**
- **when viremia is suppressed, flares are always associated with improved virologic status**

NUCs: HBeAg seroconversion, reduction of circulating HBsAg
pegIFN: SVP clearance, HBsAg seroconversion, functional cure

Host mediated transaminase flares are considered to be T-cell mediated

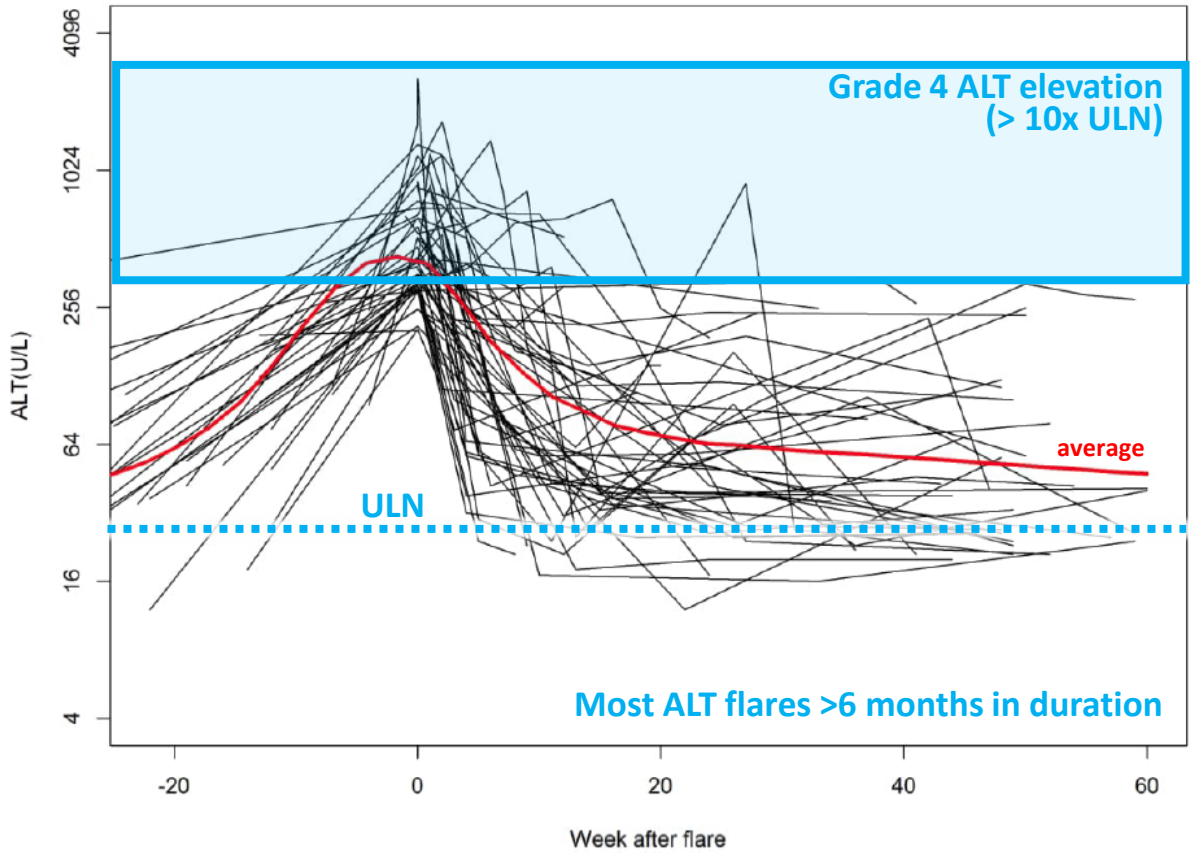
T-cell mediated clearance of infected hepatocytes is crucial to achieve functional cure

Vaillant, ACS Viruses 2021; 13: 745

Transaminase flare geometry in treatment naïve HBeAg+ infection

106 / 1587 surveyed patients experienced ALT flare

No decompensation
No transplantation
No deaths



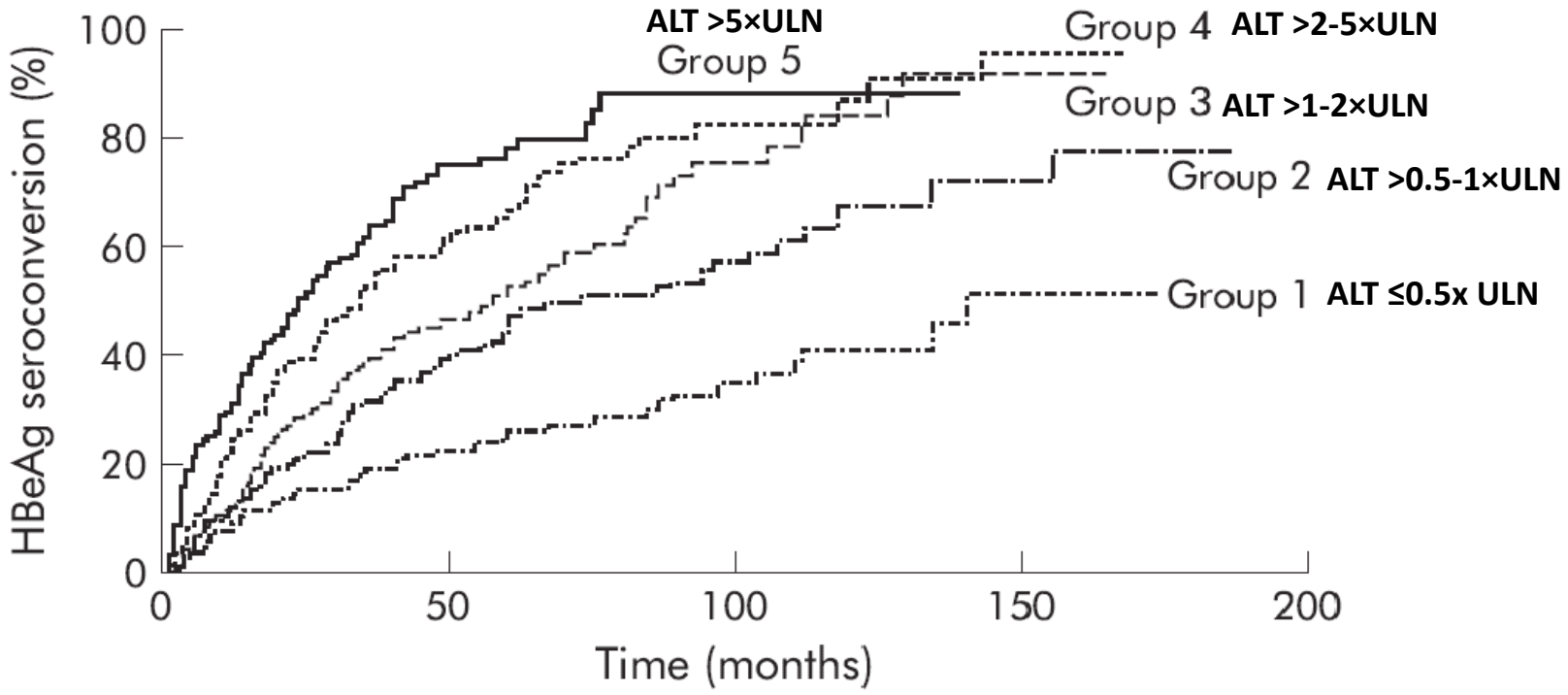
Increased rate of decline of HBV DNA > 1 $\log_{10}$ IU/mL versus no flare.

Increased rate of HBeAg seroconversion versus no flare.

No difference in HBsAg decline between flare and no flare.

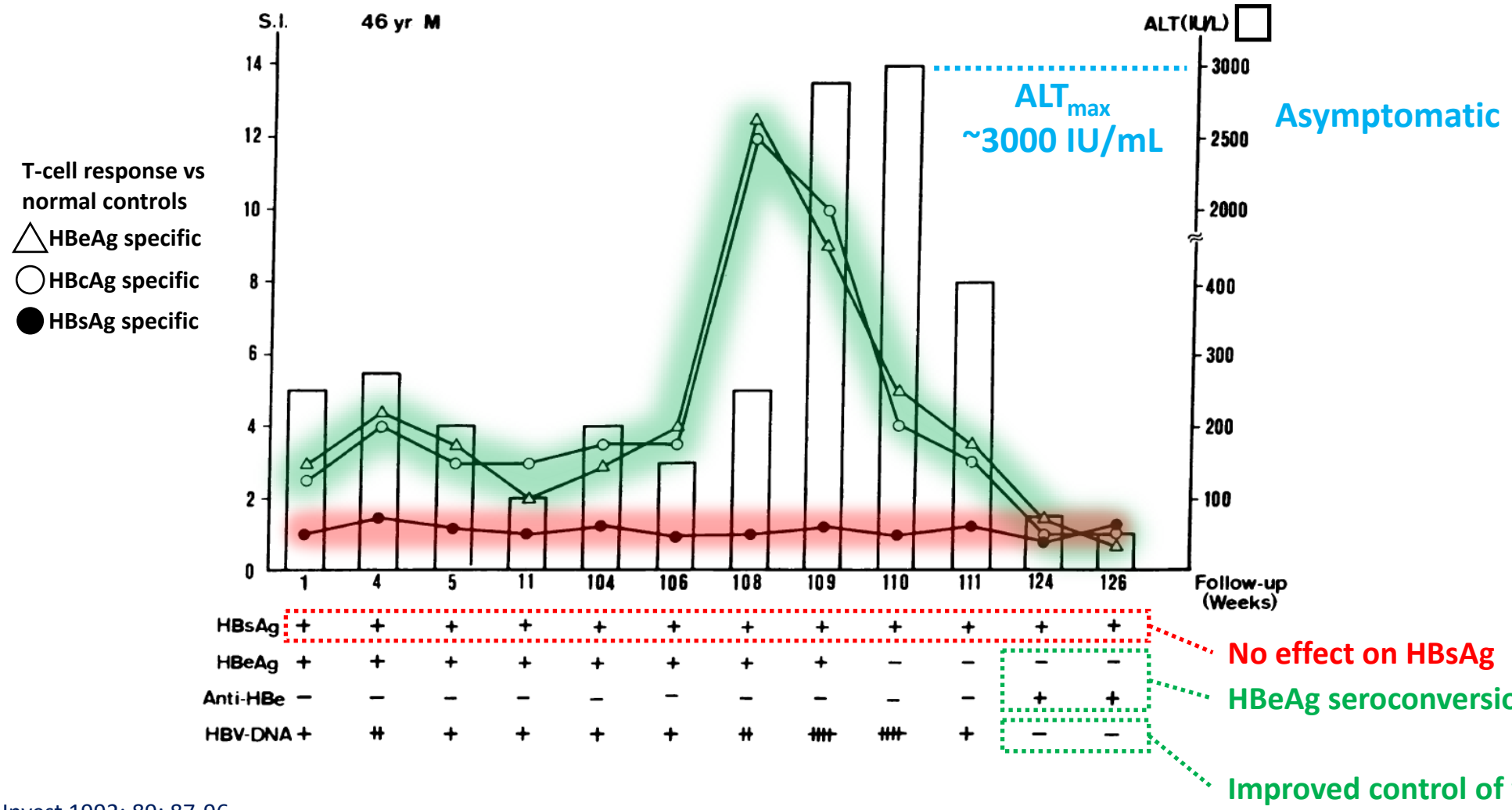
Brahmania et al., Clin Gastroenterol Hepatol. 2019; 17: 2541-2551

Transaminase flare strength in treatment naïve HBeAg+ infection is correlated with increased rates of HBeAg seroconversion



Yuen et al., Gut 2003; 52: 416-419

Transaminase flares are associated with increased core and e-antigen specific T-cell response in naïve patients



Tsai et al., J Clin Invest 1992; 89: 87-96

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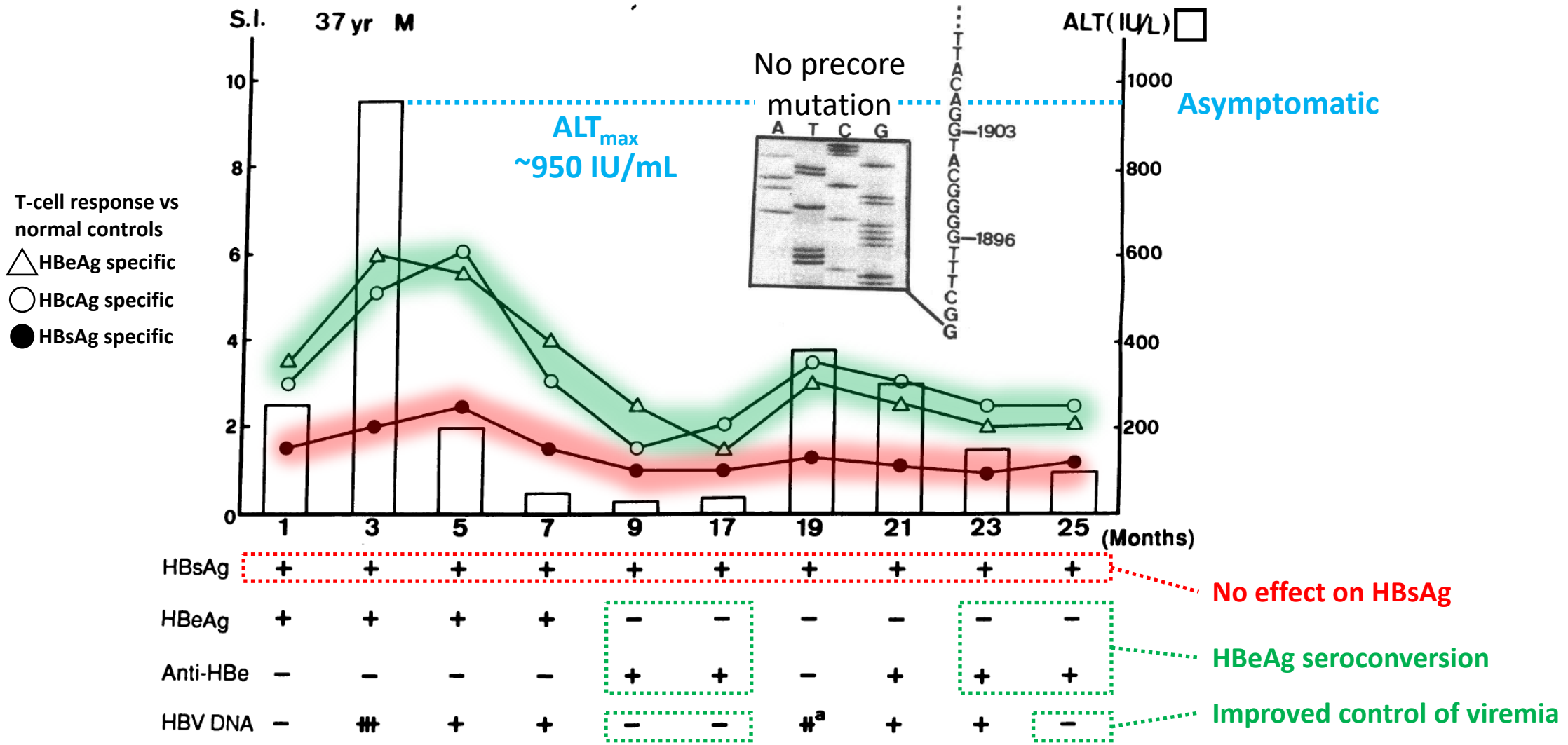
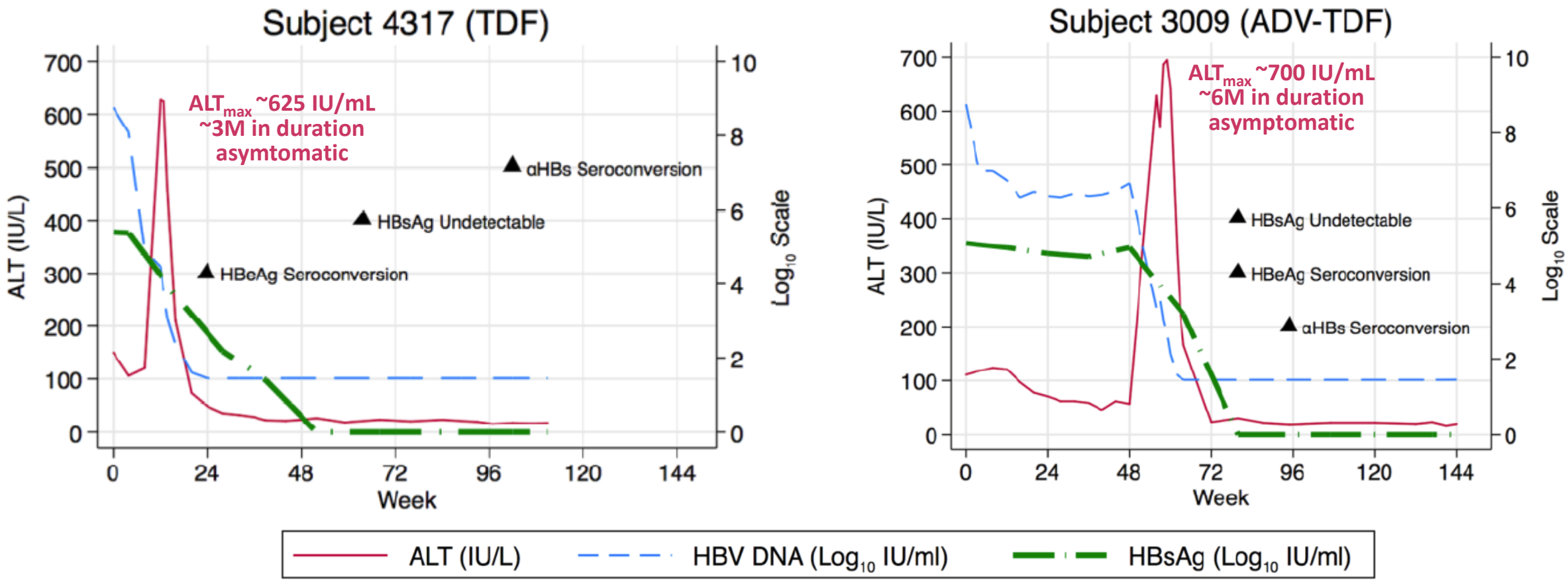


Tsai et al., J Clin Invest 1992; 89: 87-96

Transaminase flares during NUC therapy (genotype A, HBeAg+)

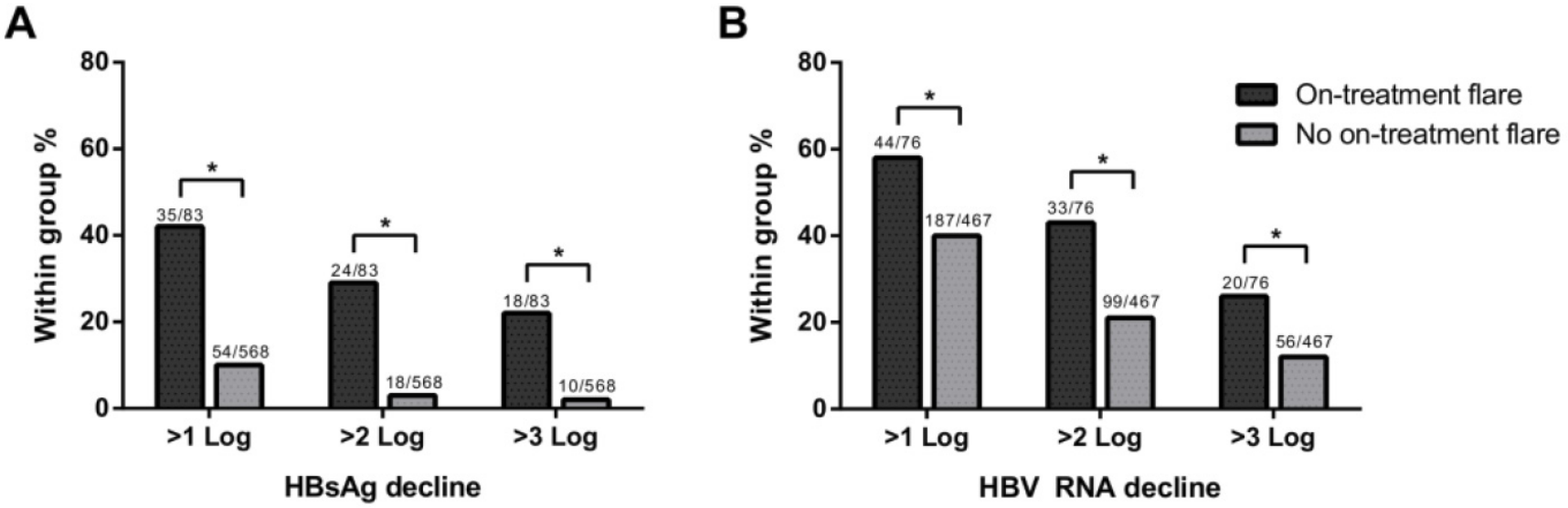


Wong et al., Liver Int. 2018 38: 1760-1769

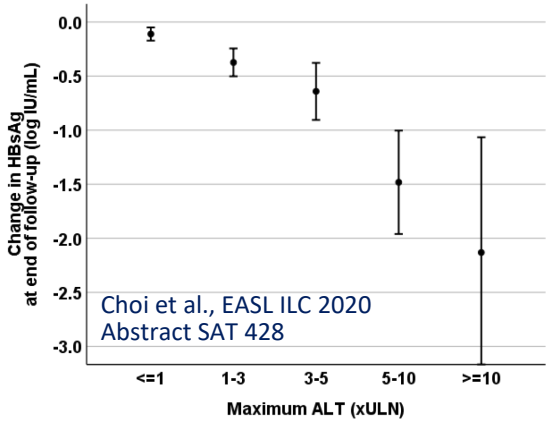
Transaminase flares during NUCs + pegIFN are associated with HBsAg and HBV RNA decline

Median ALT_{max} 7.6x ULN
IQR 6.2-10.5x ULN
Flares in 13% of patients
(Five with two discrete flares)

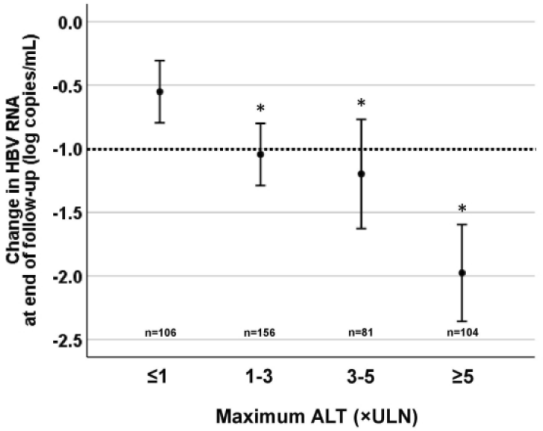
Stronger virologic response in the presence of flares



Stronger virologic response is associated with stronger flares



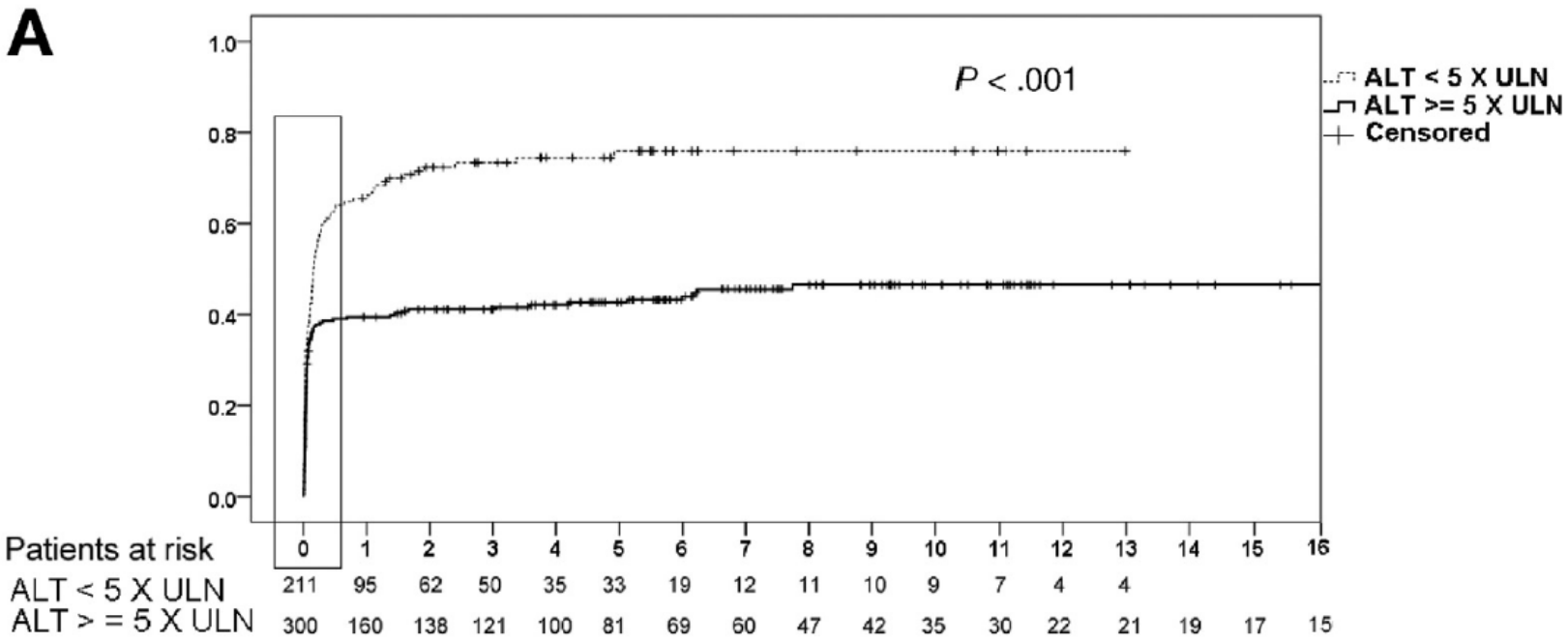
Choi et al., EASL ILC 2020
Abstract SAT 428



Choi et al., J Viral Hepat 2021; 28: 1729-1737

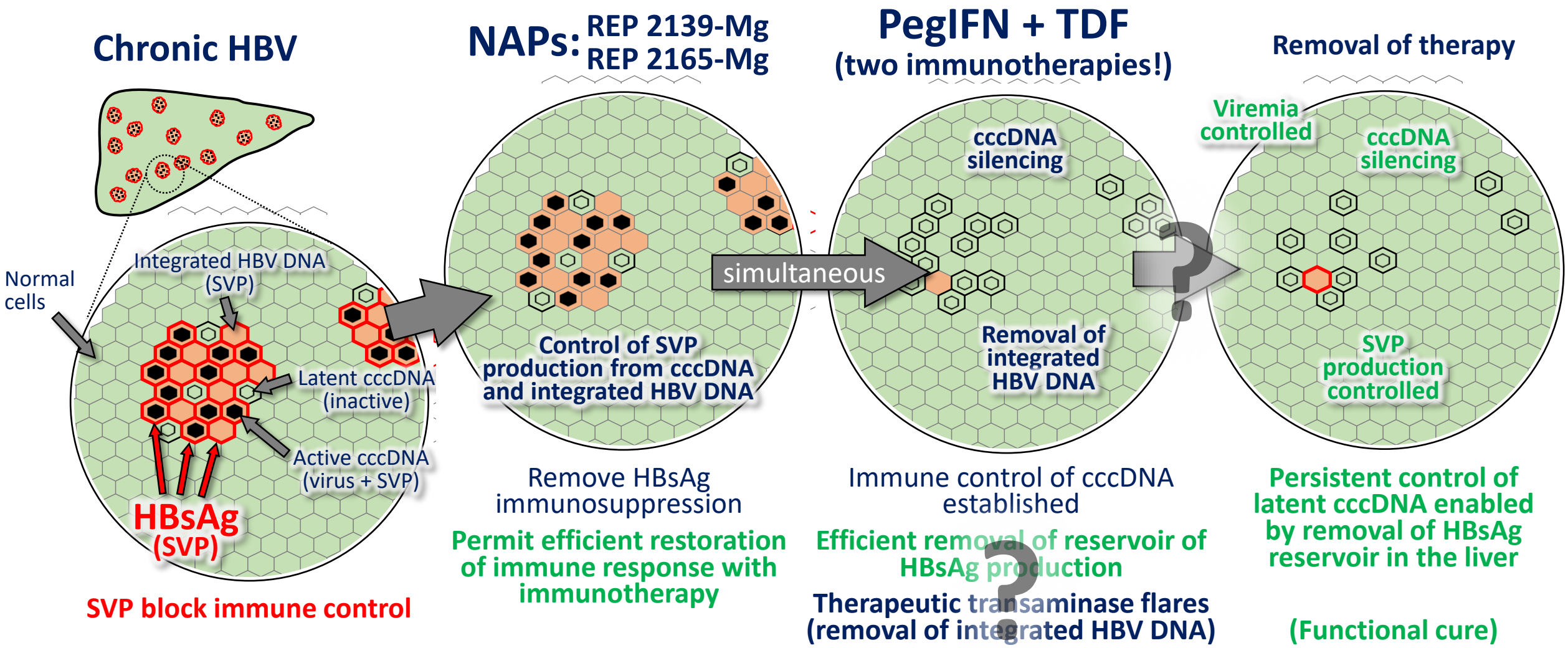
Transaminase flares in chronic HBV infection with decompensated cirrhosis following NUC therapy

Cumulative incidence of mortality or liver transplantation

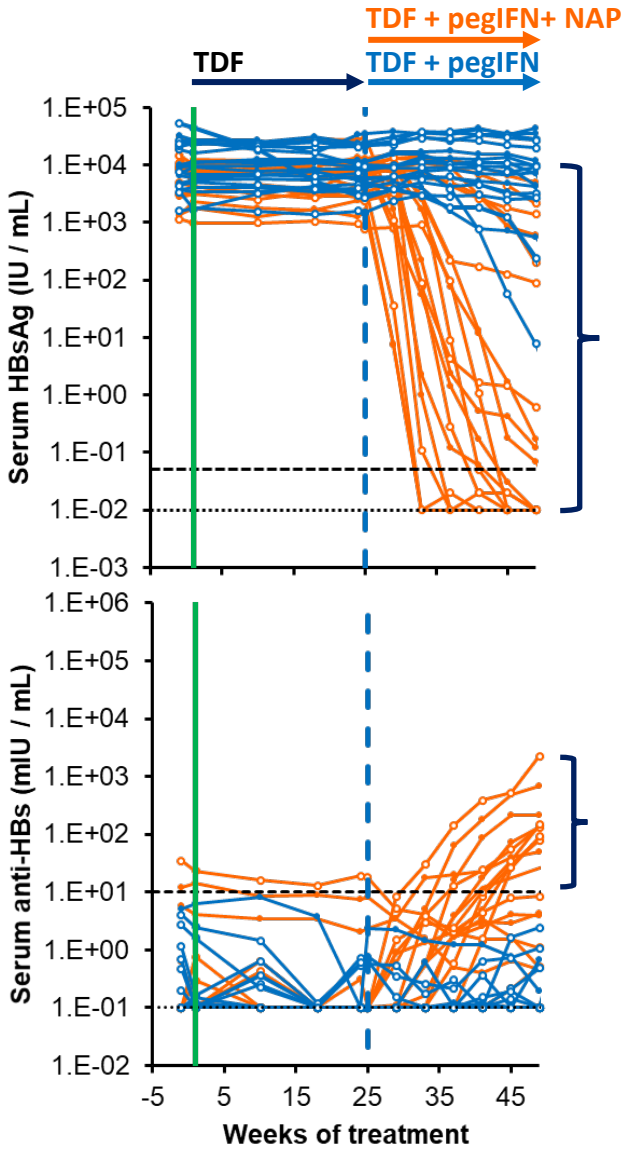


Chang et al., Clin Gastroenterol Hepatol 2020; 18: 2064-2072

REP 401 study:
Putting the pieces of the puzzle together



REP 401 study:
NAPs dramatically improve response to TDF + pegIFN (HBeAg-)



Up to 8 $\log_{10}$ decline from baseline (to < 0.005 IU/mL)
Potent HBsAg decline

Rapid HBsAg seroconversion

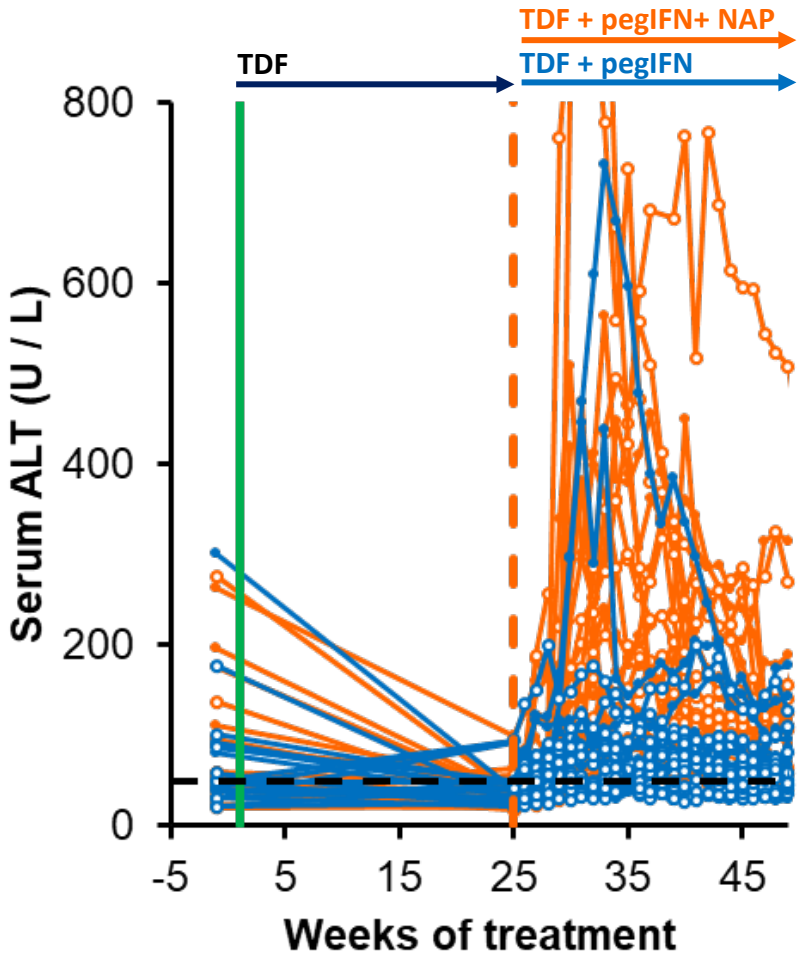
NAP monotherapy:
REP 2055 or REP 2139
Up to 7 $\log_{10}$ HBsAg reduction at 12 weeks
HBsAg seroconversion
Low rates of HBV functional cure

NAPs + TDF + pegIFN
HBsAg < 0.005 IU/mL (60%)
HBsAg seroconversion
Inactivation of cccDNA
Host mediated transaminase flares (95%)
High rates of HBV functional cure (39%)
No further therapy required in 78% of patients

GT D functional cure rate
TDF + pegIFN = 0%
(Marcellin et al, Gastroenterology 2016; 150: 134-144)
NAPs + TDF + pegIFN = 39%

Bazinet et al., Gastroenterol. 2020; 158: 2180-2194
Al-Mahtab et al., PLoS One; 2016; 11: e0156667

REP 401 study:
NAPs dramatically improve response to TDF + pegIFN



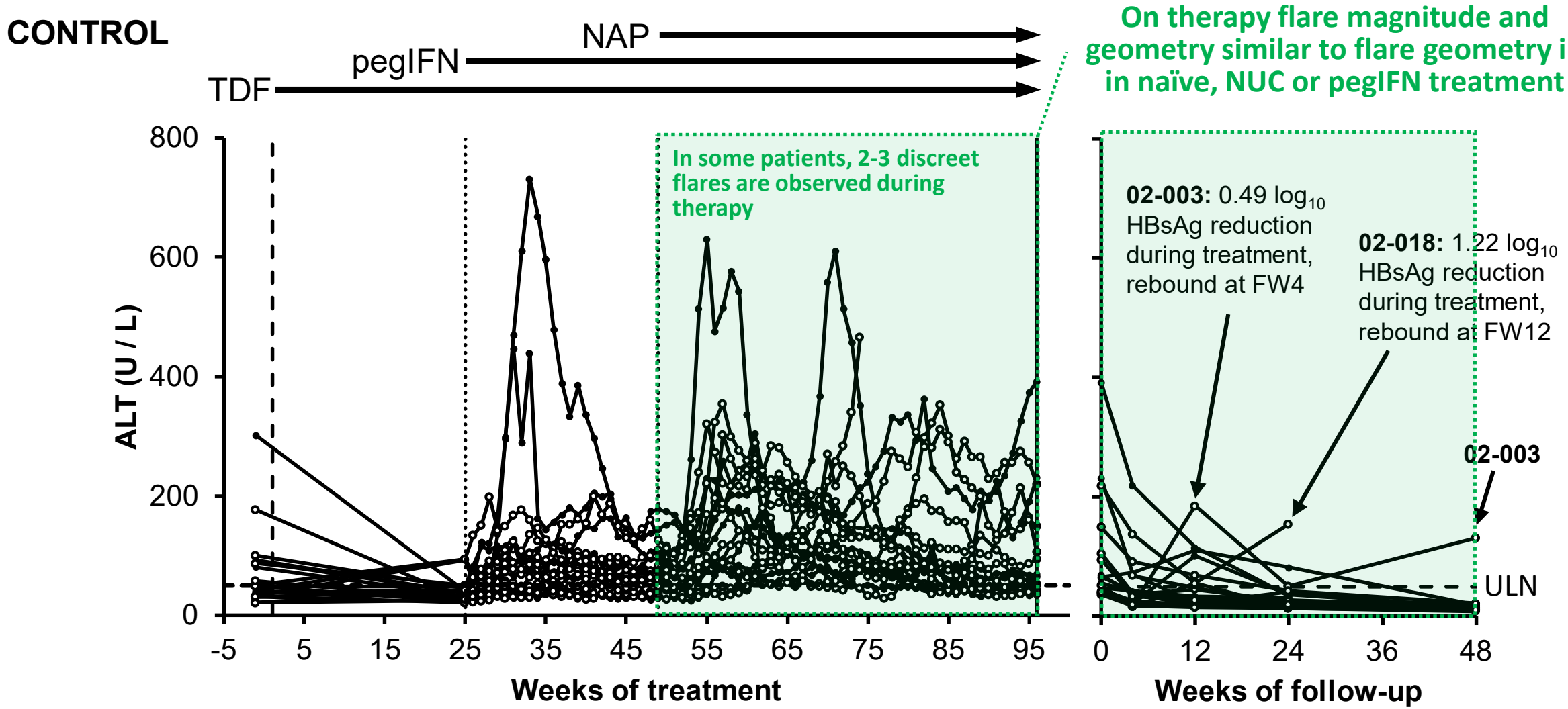
Dramatic increase in host mediated transaminase flares[1]

- **occur in 95% of participants[2]**
- no alteration in liver function / asymptomatic[2]
- correlated with functional cure (when HBsAg is also < 1 IU/mL)[2]
- Signals the removal of cccDNA and integrated HBV DNA[3]

Consistent with the safe and beneficial nature of host mediated transaminase flares during therapy with approved agents, even in cirhottics[4]

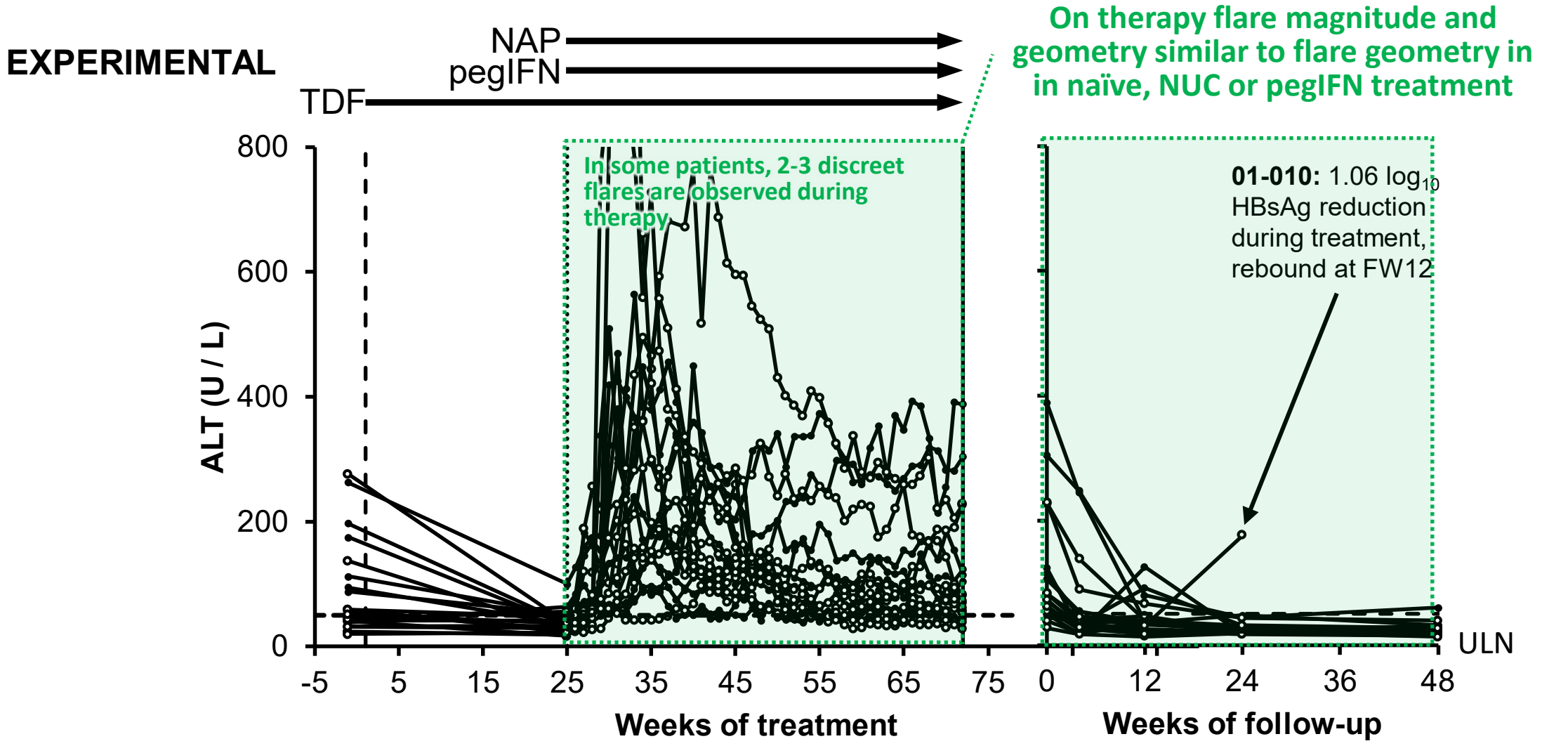
1. Bazinet et al., Gastroenterol. 2020; 158: 2180-2194
2. Bazinet et al., J Viral Hep 2021; 28: 817-825
3. Bazinet et al., Hepatol Comm 2021; 28: 817-825
4. Vaillant, Viruses 2021; 131: 745

REP 401 study ALT flare geometry



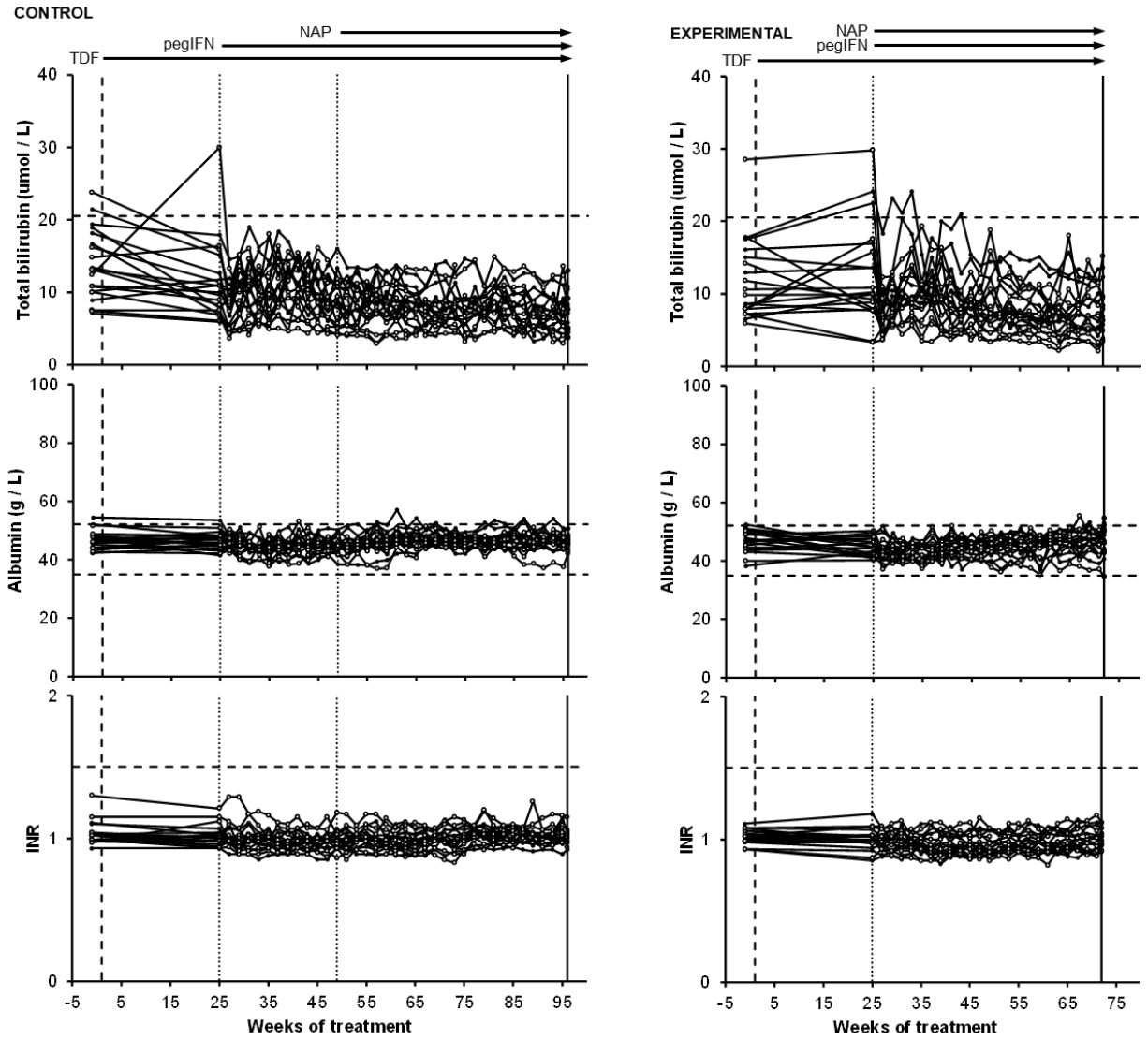
Bazinet et al., Gastroenterol. 2020; 158: 2180-2194 (supplement)

REP 401 study ALT flare geometry



Bazinet et al., Gastroenterol. 2020; 158: 2180-2194 (supplement)

REP 401 study liver synthetic / secretory function



All transaminase flares are host mediated

REP 401 study:
Outcomes after removal of all therapy

Completed treatment and 24-48 weeks of follow-up		36
Clinical response	Normal ALT	89%
	Normal liver median stiffness	56%
HBsAg response	< 1000 IU/mL	72%
	< 1 IU/ml	50%
	≤ LLOQ (0.05 IU/mL)	42%
	Seroconversion	53%
HBV DNA response	≤ 2000 IU/mL	78%
	Target not detected (TND)	47%
Virologic response	Partial cure (Inactive HBV) (HBV DNA ≤ 2000 IU/mL, normal ALT)	39%
	Functional cure (HBsAg < LLOQ, HBV DNA TND, normal ALT)	39%
	Clinical benefit, no therapy required (low risk of progression, reduced risk of HCC)	**78%**

Reversal of liver inflammation / fibrosis

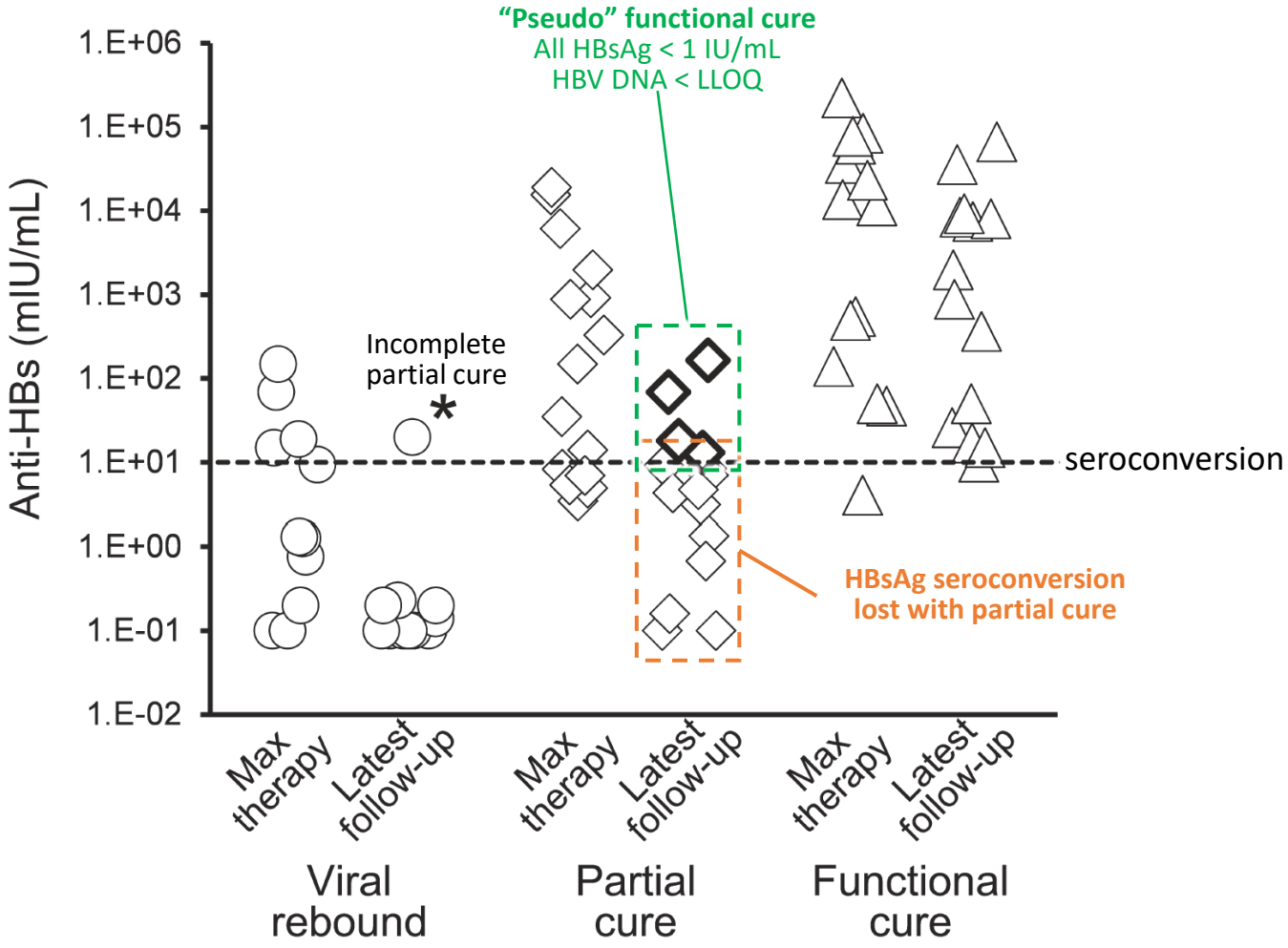
All with:
- **HBsAg < 0.005 IU/mL (ARCHITECT® NEXT)**
- **No HBsAg immunocomplexes**
- **HBV RNA target not detected**
- **HBcrAg < LLOQ**

Efficient silencing of cccDNA

Removal of integrated HBV DNA

Bazinet et al., Gastroenterol. 2020; 158: 2180-2194
Bazinet et al., Hepatol Comm 2021, 5: 1873-1887

REP 401 study:
Anti-HBs response on therapy versus outcome



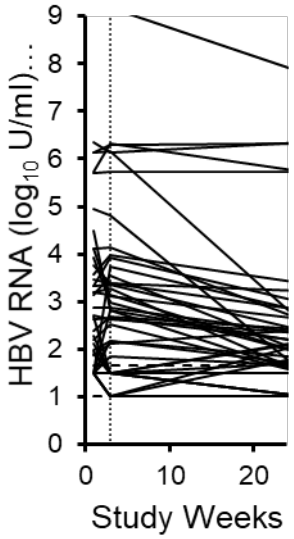
HBsAg seroconversion and anti-HBs elevation during therapy do not predict outcome

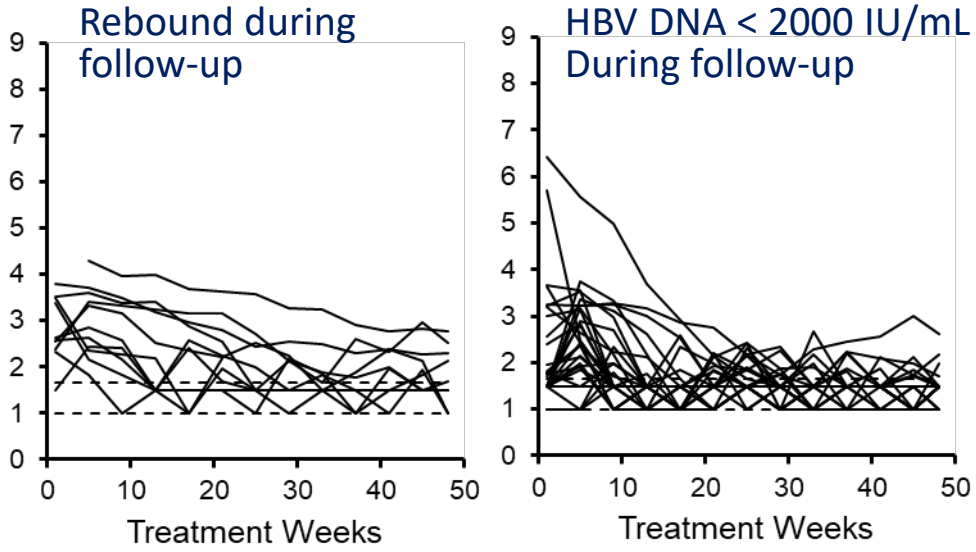
But HBsAg seroconversion persists in functional cure!

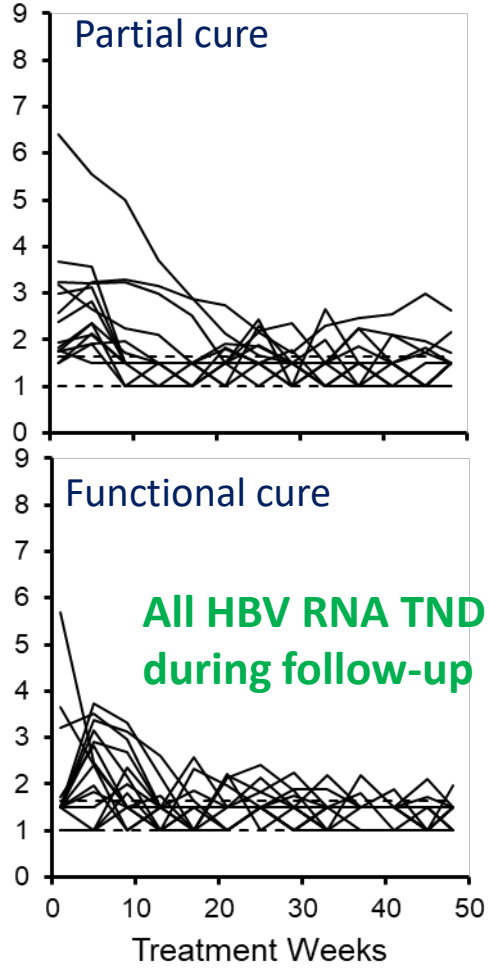
REP 401 study:
HBV RNA response on therapy versus outcome

24 weeks TDF lead-in

48 weeks TDF + pegIFN + NAP







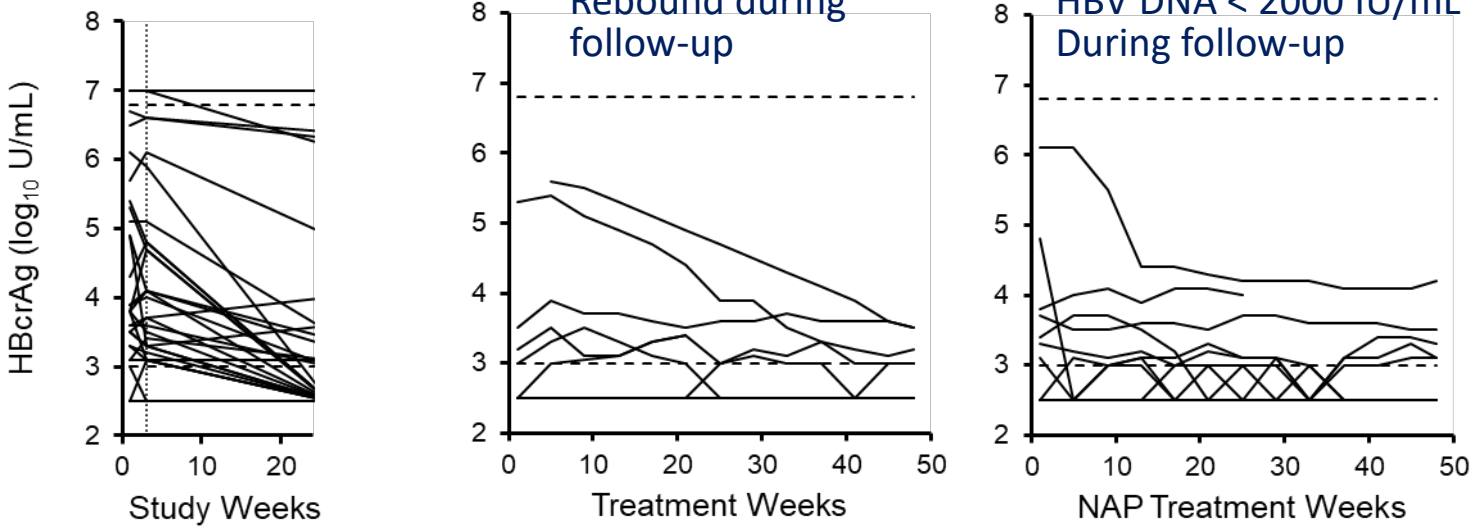
HBV RNA response does not predict clinical outcome

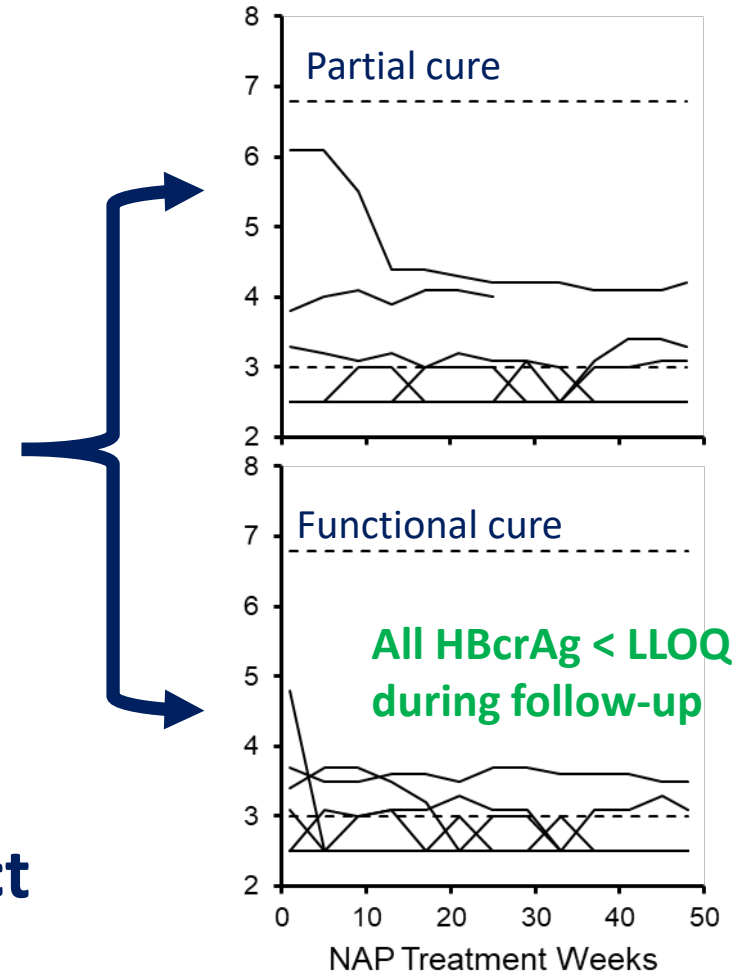
Bazinet et al., Hepatol Comm 2021, 5: 1873-1887

REP 401 study:
HBcrAg response on therapy versus outcome

24 weeks TDF lead-in

48 weeks TDF + pegIFN + NAP



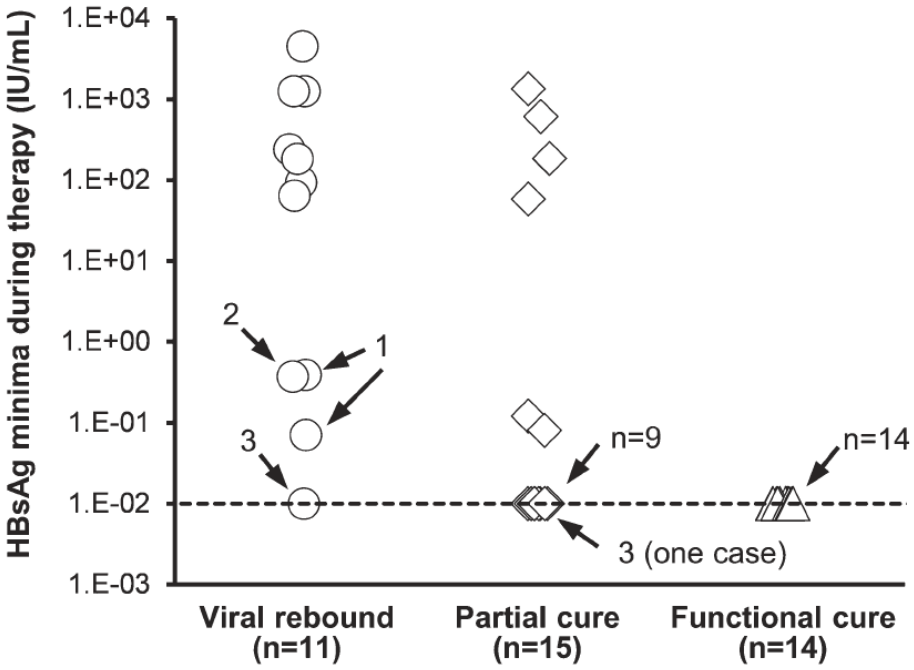


HBcrAg response does not predict clinical outcome

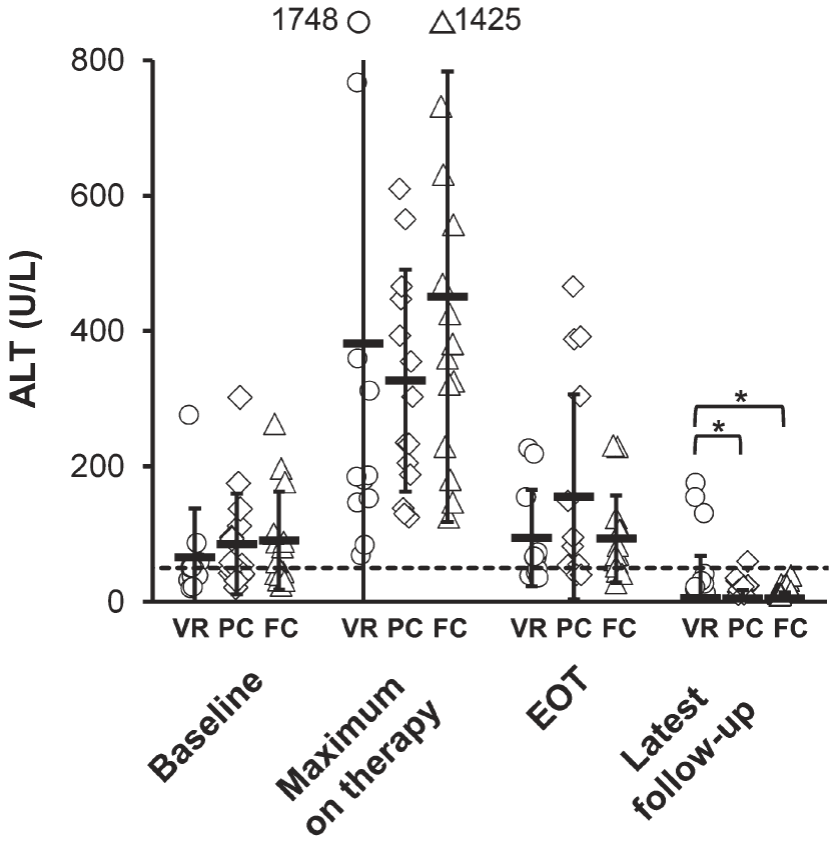
Bazinet et al., Hepatol Comm 2021, 5: 1873-1887

REP 401 study:
Extent of HBsAg clearance during transaminase flares predicts functional cure

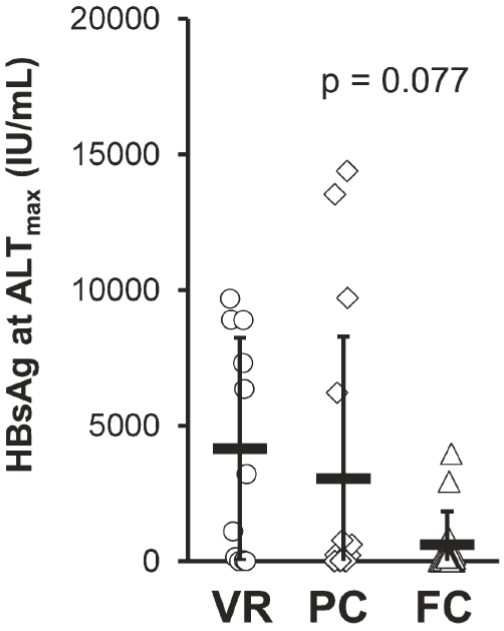
HBsAg loss necessary but insufficient for functional cure



Transaminase flare magnitude does not predict outcome



HBsAg minima during flare predicts outcome



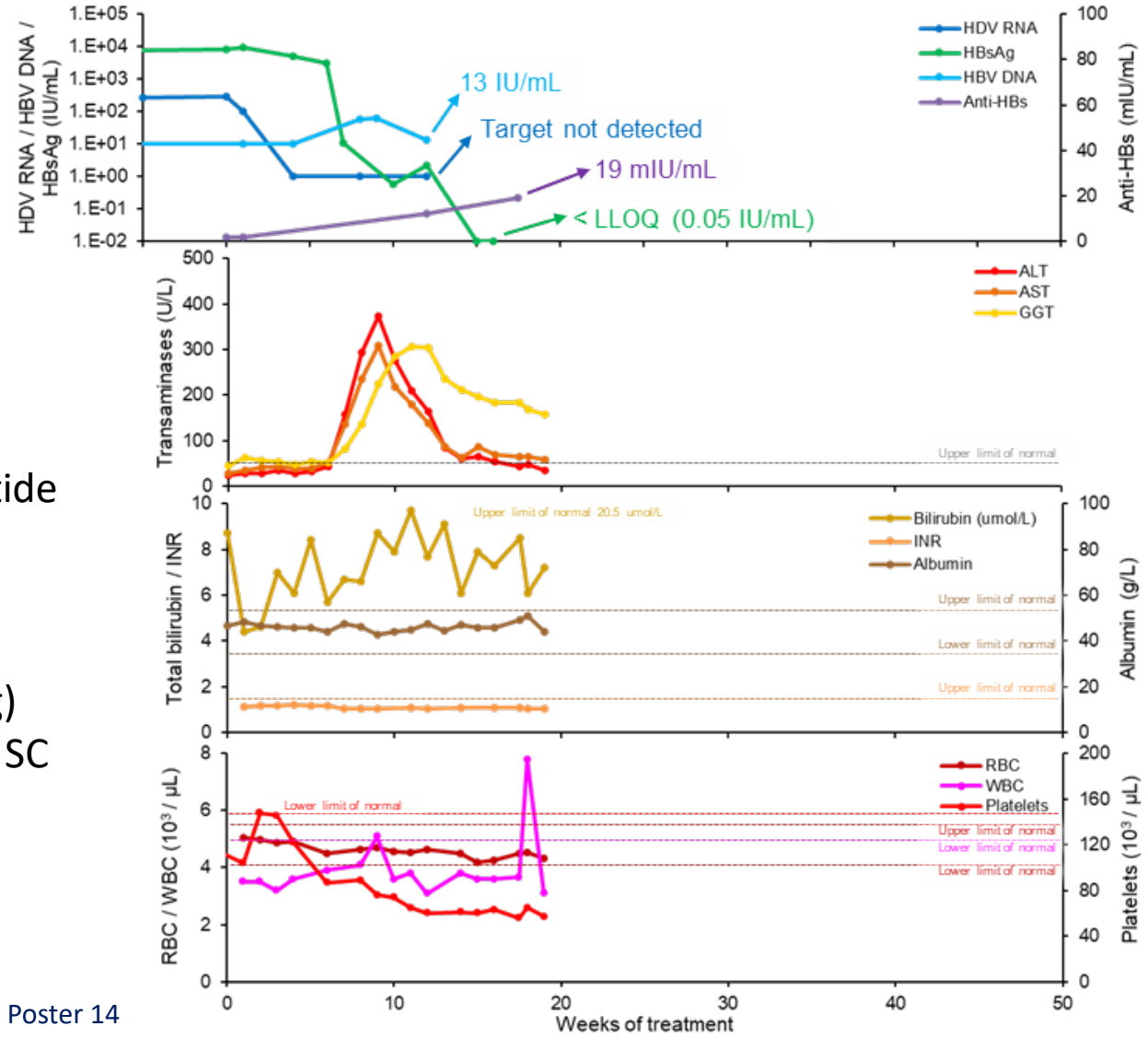
Engagement of HBsAg specific T-cell function is essential to achieve functional cure

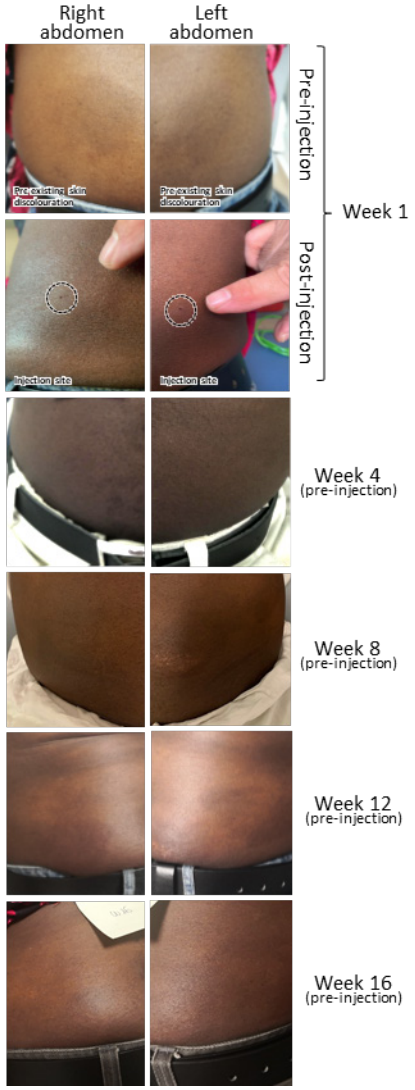
VR = virologic rebound
PC = partial cure
FC = functional cure

Transition of REP 2139-Mg to subcutaneous administration

Senegalese
51 years old male
HBV / HDV (GT5)
Previous failure on:
- TDF + pegIFN
- TDF + pegIFN + bulvertide

Currently receiving:
- TDF
- low dose pegIFN (90ug)
- REP 2139-Mg (250mg) SC (once every week)





Bourlière et al., AASLD 2021 Late Breaking Poster 14

Summary

Subviral particles (SVP): > 99.99% of circulating HBsAg
Prevent immune control and function of immunotherapy
Removal during therapy is essential for functional cure
Poorly targeted by direct acting antivirals (NUCs / CAMs / RNAi / antisense)

Key to Functional Cure

Integrated HBV DNA: Bulk of SVP production in HBeAg negative infection
HBsAg specific T-cell response is required to target efficiently
Therapeutic transaminase flares signal removal of integrated HBV DNA from the liver

Key to Functional Cure

NAPs efficiently target SVP production

Allows efficient, host-mediated clearance of HBsAg
Creates a permissive environment for efficient action of immunotherapy

Reconstitution of HBsAg specific T-cell function is critical

Only approach to target integrated HBV DNA

pegIFN, thymosin alpha1, therapeutic vaccines(?), CAR/TCR T-cells(?)

When used in combination with NAPs:
high rates of asymptomatic host-mediated transaminase flares
high rates of functional cure, silencing of cccDNA and removal of integrated HBV DNA