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Boosting innate and adaptive immunity for HBV cure

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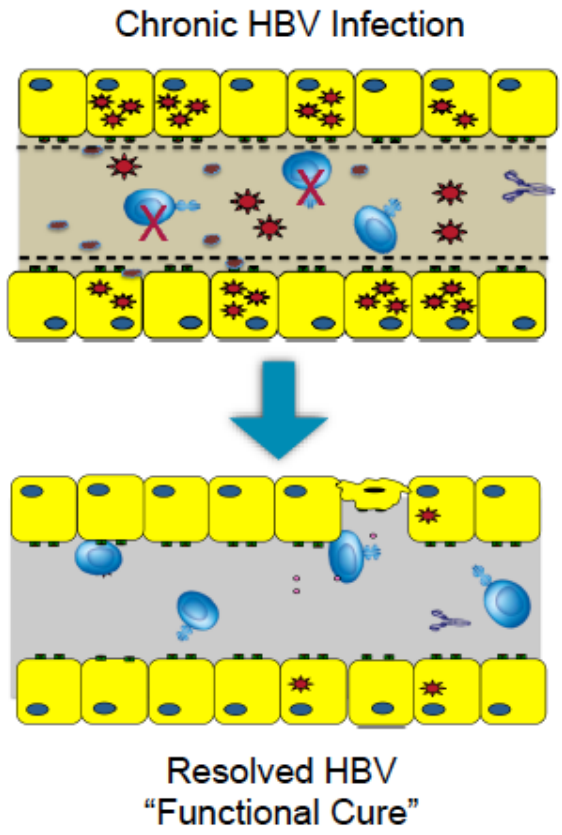
3. Hospices Civils de Lyon, Service d'Hépato-Gastroentérologie

Disclosure

Relations that could be relevant for the meeting	Company names
Sponsorship or refund funds	Jansen, Gilead, MSD, Roche, Intercept
Payment or other financial remuneration (Advisory Committees or Review Panels) (Research Projects)	 Gilead, Galapagos, Assembly Pharma BMS, Contravir, Evotec/Sanofi
Shareholder rights	NA
Other relations (Speaking and Teaching) (Advisory Committees or Review Panels)	 Gilead, MSD, Roche, Jansen Jansen, Roche, Arbutus, Evotec/Sanofi

Do we need anti viral immunity to cure HBV?



no

CpAMs
"Capsid inhibitors"

Entry inhibitors

If cccDNA half life is shorter than presumed

You may think of it as « intensification »

Inhibitors of HBsAg release

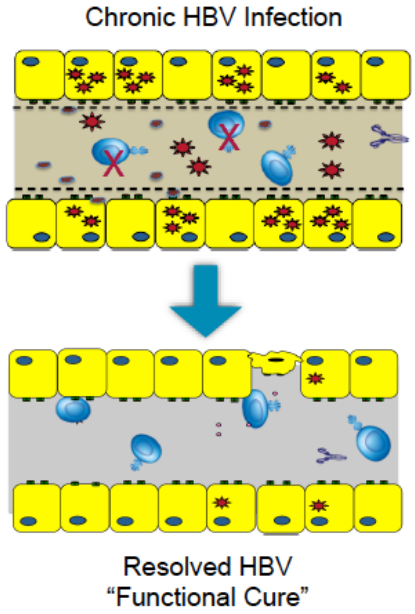
RNA interference

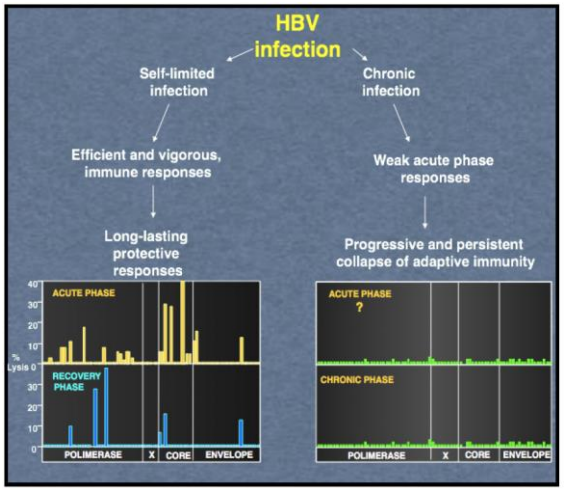
RNA destabilizers

If lowering HBsAg is sufficient to restore immune responses

Adapted from: Bertoletti A SHC 2019

Do we have evidences that immunity can control HBV ?





- Functional dichotomy of adaptive immunity in chronic versus resolved patients
- Immune suppression (T and B cells) causes HBV reactivation
- Transplantation of HBV primed bone marrow causes HBV functional control in CHB patients

  Lau et al. Hepatology 1997
  Ilan et al. Gastroenterology 1993

- HBsAg+ liver became HBsAg negative after transplantation in resolved HBV.

  Loggi et al. J Hepatol 2009

Adapted from: Bertoletti A SHC 2019

Restoration of antiviral immunity

Background

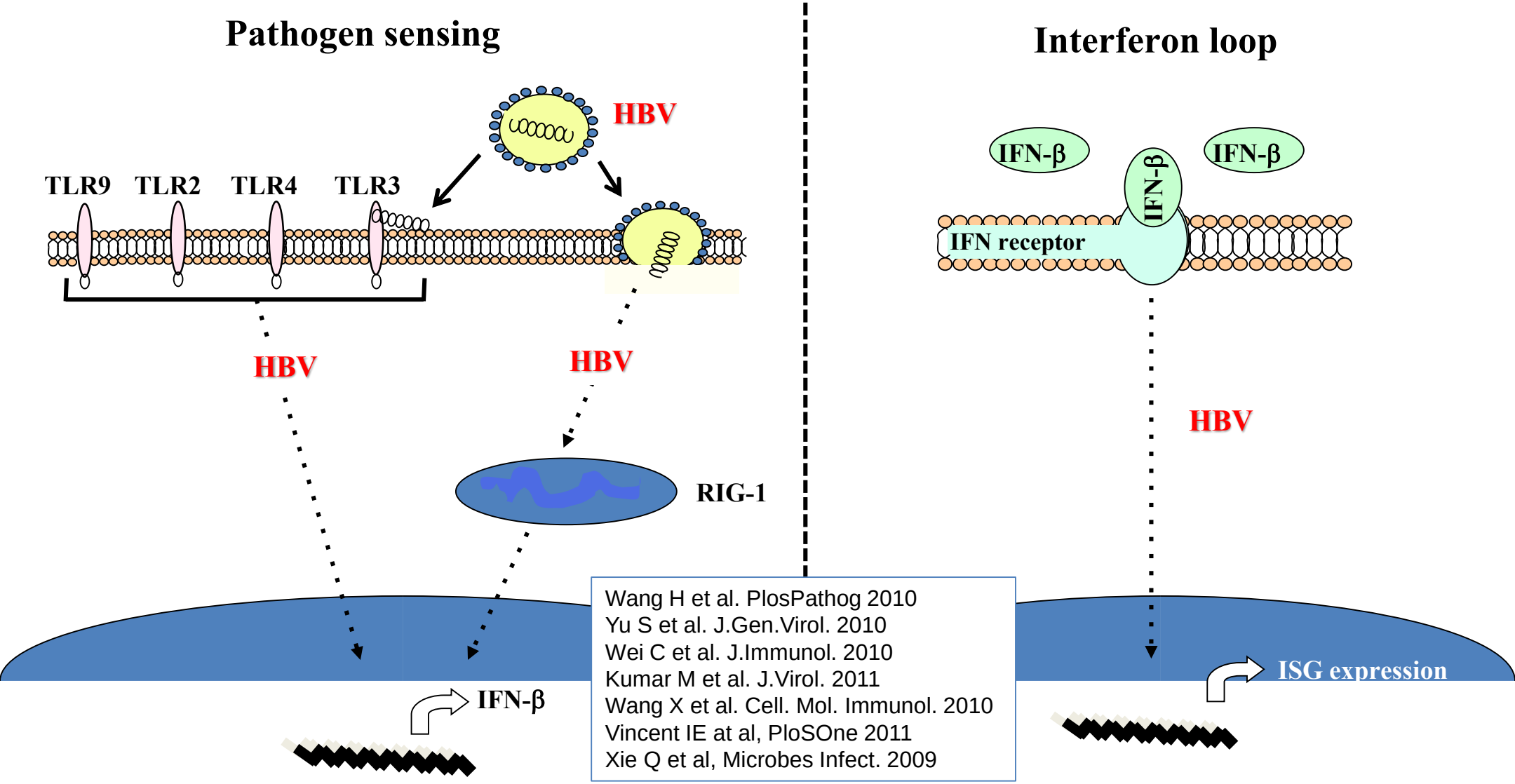
1. **HBV is recognized by the innate immunity but it is poorly sensed and is a weak inducer of innate pro-inflammatory cytokines**

2. **HBV-specific T cells in chronic infection are deeply dysfunctional**

3. **NK cells in chronic HBV infection seem to be impaired in their anti-viral capacity**

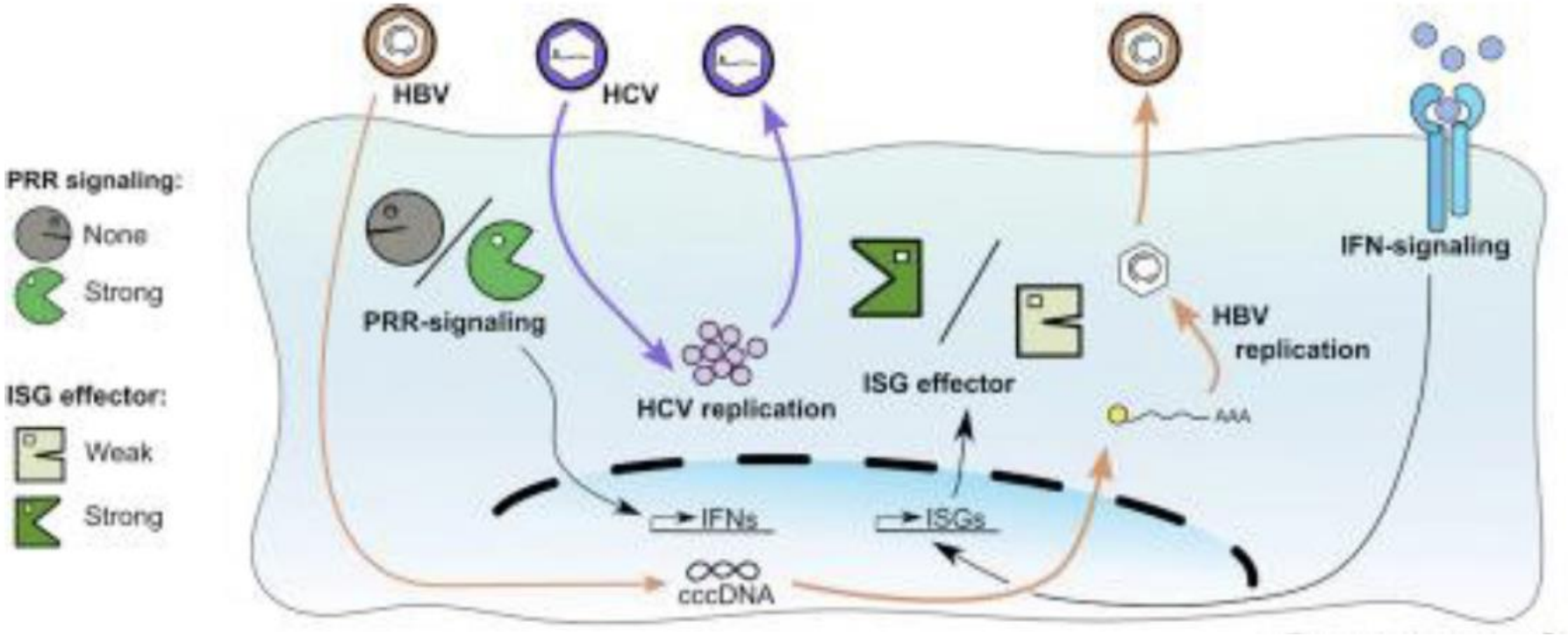
4. **Expansion of HBsAg-specific atypical memory B cells in CHB**

Limited HBV sensing is combined with active suppression of innate responses



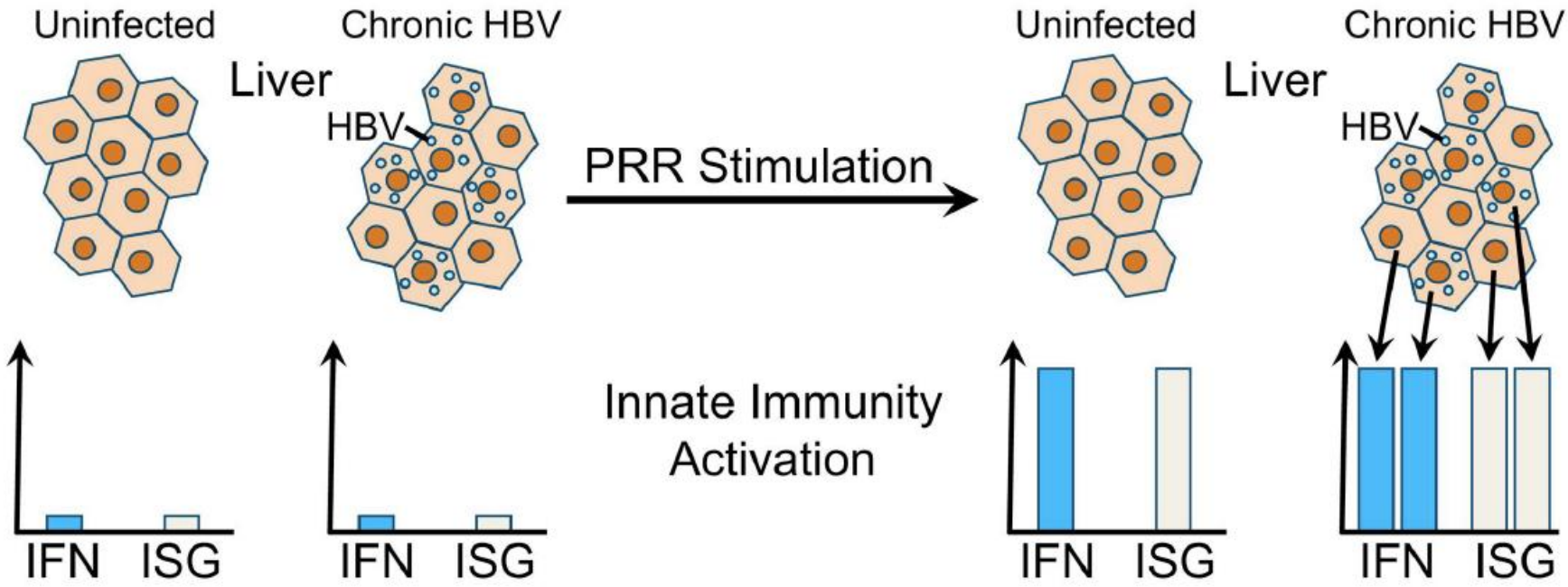
Wang H et al. PlosPathog 2010
Yu S et al. J.Gen.Virol. 2010
Wei C et al. J.Immunol. 2010
Kumar M et al. J.Virol. 2011
Wang X et al. Cell. Mol. Immunol. 2010
Vincent IE at al, PloSOne 2011
Xie Q et al, Microbes Infect. 2009

HBV by-passes the innate immune response and does not protect HCV from antiviral activity of interferon



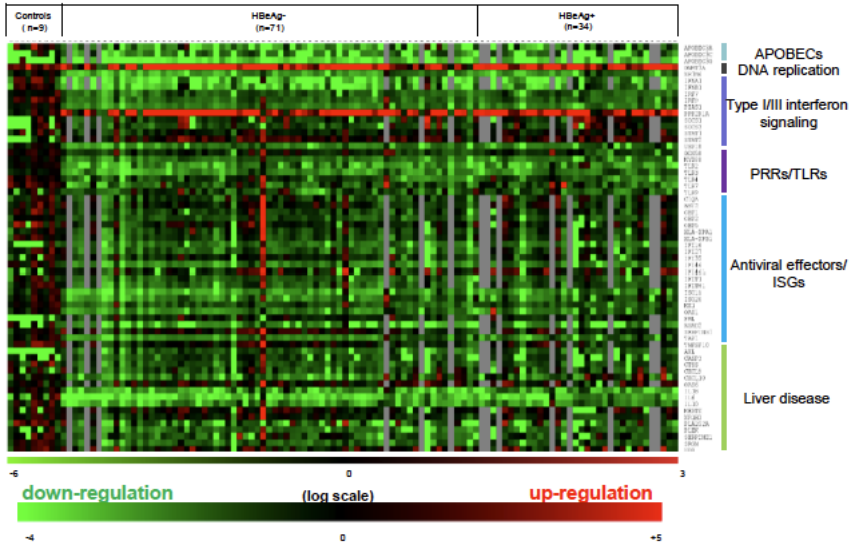
Mutz et al, Gastroenterology 2018

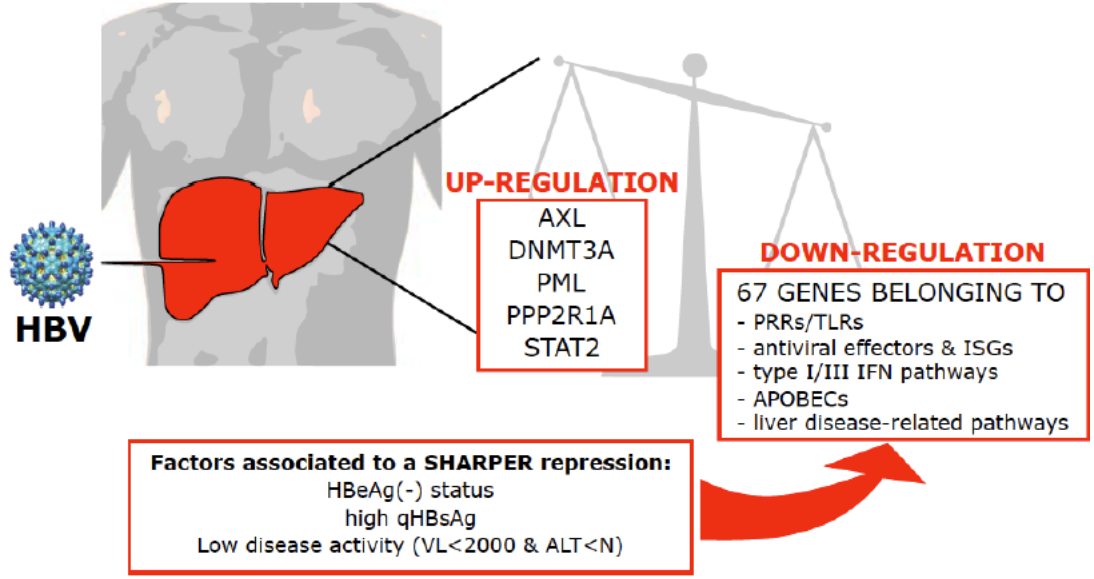
Hepatitis B virus does not interfere with innate immune responses in the human liver



Suslov et al, Gastroenterology 2018

Suppression of intrahepatic ISG expression in CHB patients: role of high HBsAg levels





Lebossé et al, J Hepatol 2017

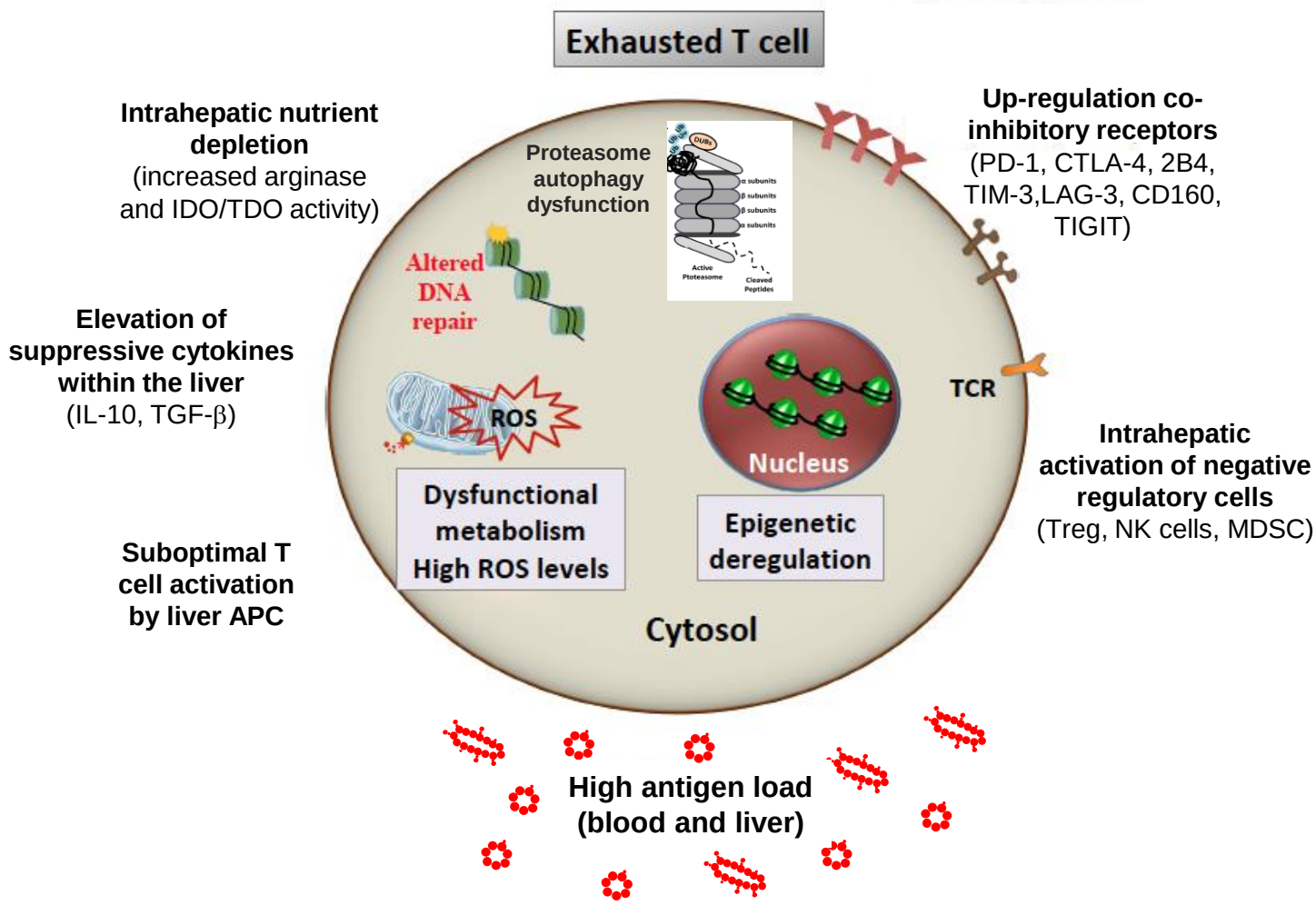
1. HBV is recognized by the innate immunity but it is poorly sensed and is a weak inducer of innate pro-inflammatory cytokines

2. **HBV-specific T cells in chronic infection are deeply dysfunctional**

3. NK cells in chronic HBV infection seem to be impaired in their anti-viral capacity

4. Expansion of HBsAg-specific atypical memory B cells in CHB

HBV-specific T cells are dysfunctional in chronic HBV infection



HBV specific CD8 T cell responses in humans

- **Hepatic microenvironment**

  Arginase, IDO, IL-10, TGFβ

  Tolerogenic antigen presenting cells

- **Local induction of regulatory cells**

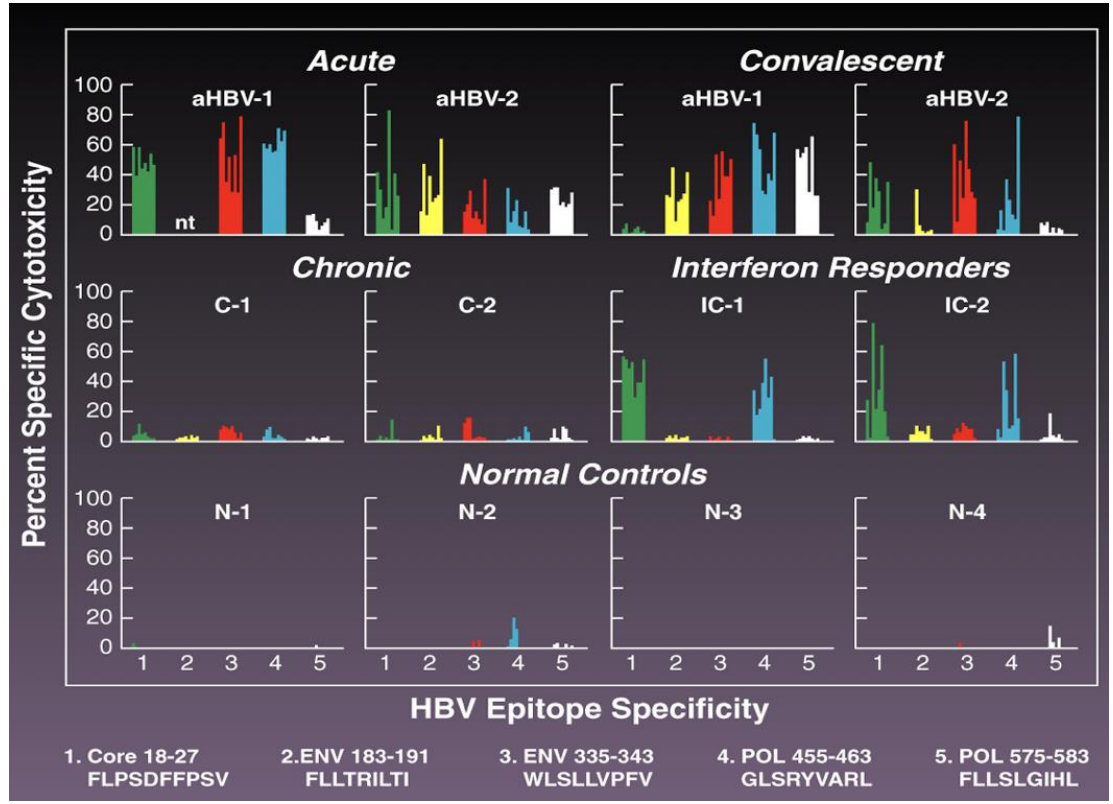
  T regs by LSECs, stellate cells and DC

  MDSCs by stellate cells

- **Attrition of T cell responses**

  Functional exhaustion/up-regulation co-inhibitory receptors (effector T cells)

  T cell killing by NK cells



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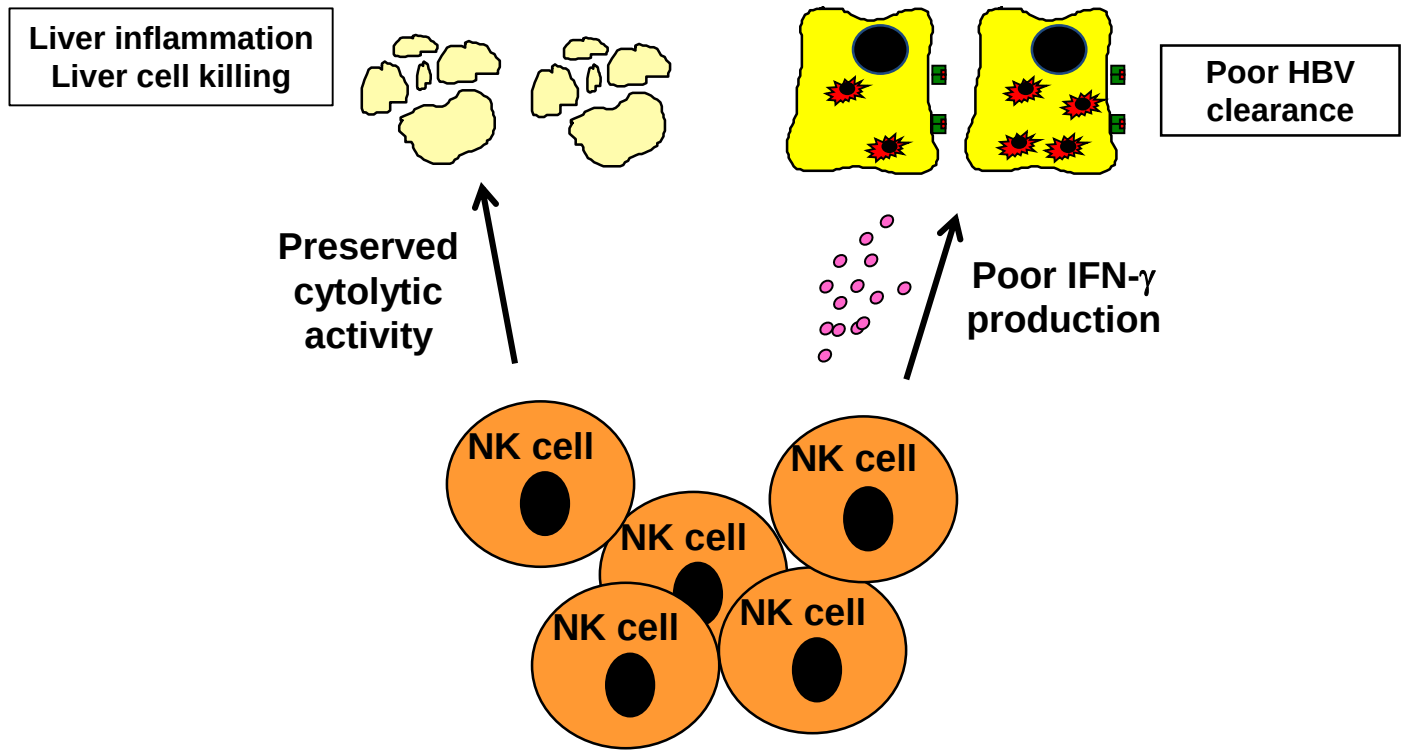
NK cell functional dichotomy in chronic HBV infection:

more pathogenic than protective



Boni C et al Hepatology 2015
Peppa D et al J Exp Med 2013
Maini M et al Front Immunol 2013

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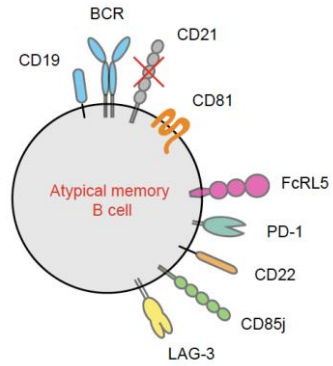
HBsAg specific atypical memory B cells in CHB



- HBsAg AtMBCs > AtMBCs HBcAg
- enriched for PD-1 and T-bet
- Impaired cytokine production
- Impaired plasma cell differentiation
- Defective antibody response

Gastroenterology 2018;154:2222–2236

Dysregulated Response of Follicular Helper T Cells to Hepatitis B Surface Antigen Promotes HBV Persistence in Mice and Associates With Outcomes of Patients

Xiaowen Wang,[1,2,*] **Qingyang Dong**,[1,*] **Qian Li**,[3,4,*] Yuanyuan Li,[5] Dianyuan Zhao,[1] Jinjie Sun,[6] Junliang Fu,[5] Fanping Meng,[5] Hu Lin,[5] Junjie Luan,[5] Biao Liu,[1] Min Wang,[1] Fu-Sheng Wang,[5] Fuchu He,[1,2,4] and Li Tang[1,3]

JCI The Journal of Clinical Investigation

Circulating and intrahepatic antiviral B cells are defective in hepatitis B

Alice R. Burton, … , Nadege Pelletier, Mala K. Maini

JCI The Journal of Clinical Investigation 2018

PD-1 blockade partially recovers dysfunctional virus–specific B cells in chronic hepatitis B infection

Loghman Salimzadeh, … , Patrick T.F. Kennedy, Antonio Bertoletti

Targeting Innate Immunity

- **TLR-7 agonists** : target pDCs; induction of endogenous type I IFN
  [Direct antiviral effect and restoration of anti-viral immunity]

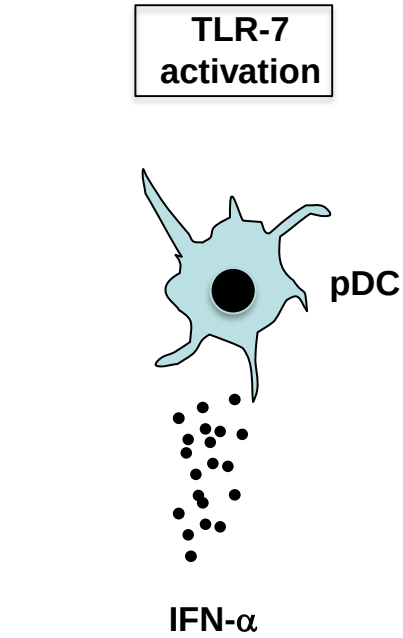
- **TLR-8 agonists** : target monocytes CD161^{bright} / MAIT / Nk^{bright} cells
  [Induction of IL12, IL18, IFNγ]

- **RIG-I agonists** : restoration of endogenous IFN production & interference on Polymerase/pgRNA interaction

Immunomodulators					
Drug	Company	Target	Formulation	Delivery	Stage
GS-9620	Gilead Sciences	TLR-7 agonist	Small molecule	Oral	Phase 2
GS-9688	Gilead Sciences	TLR-8 agonist	Small molecule	Oral	Phase 1
Inarigivir (SB9200)	Spring Bank Pharmaceuticals	RIG-I/NOD agonist	Small molecule	Oral	Phase 2
RO6864018 (RG7795, ANA773)	Roche	TLR-7 agonist	Small molecule	Oral	Phase 2
AIC 649	AiCuris	TLR-9 agonist	Inactivated parapoxvirus ovis (iPPVO) particle	Infusion	Phase 1

Gehring et al, Gastroenterology 2019

Manipulation strategies for innate immunity



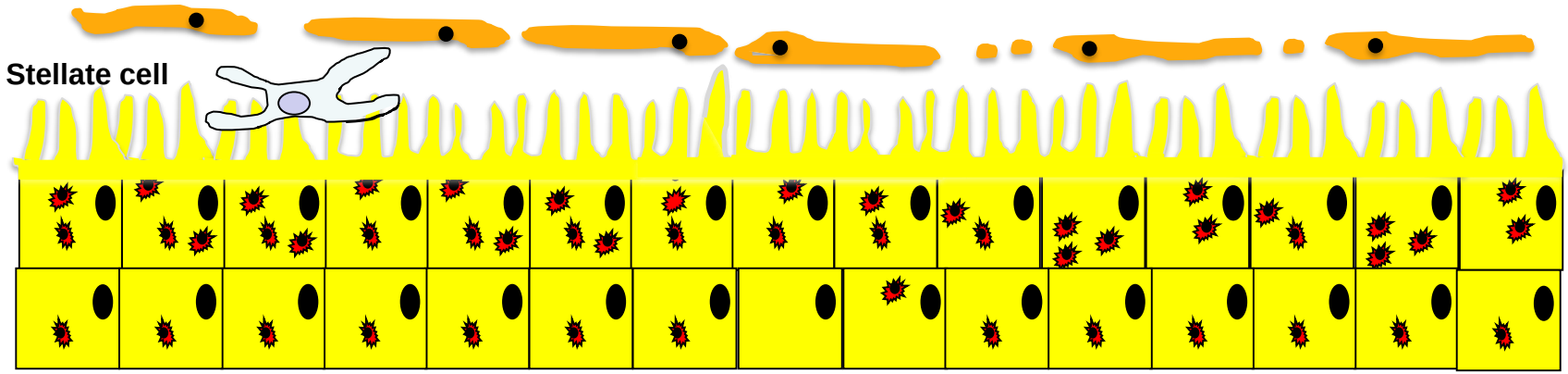
TLR7 agonist GS-9620 :

- ***prolonged suppression of hepatitis B virus in chronically infected chimpanzees***

Lanford et al, Gastroenterology 2013

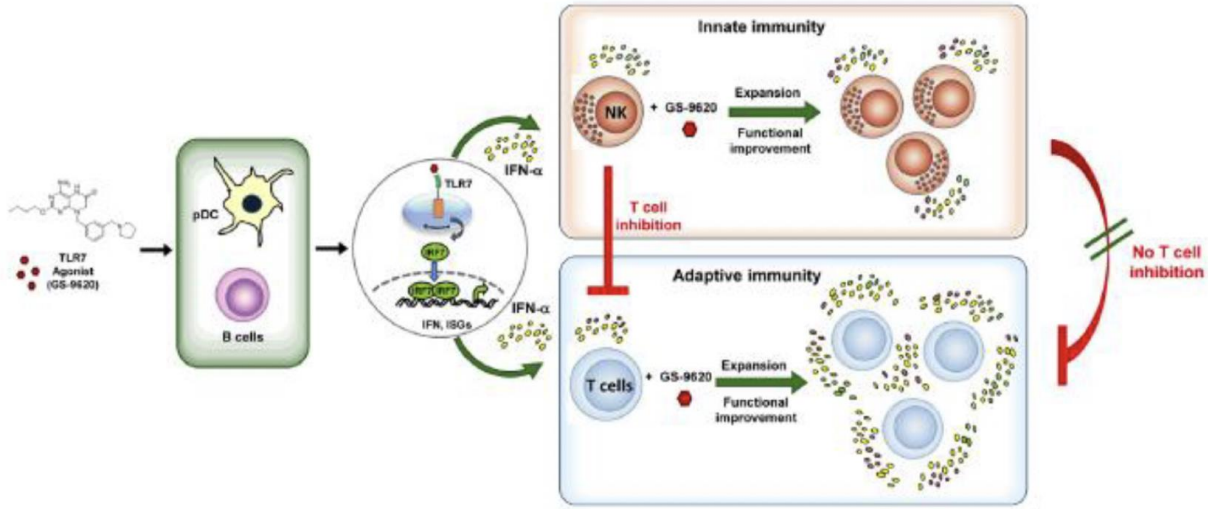
- ***induces an ISG response in patients in vivo but no durable HBsAg loss***

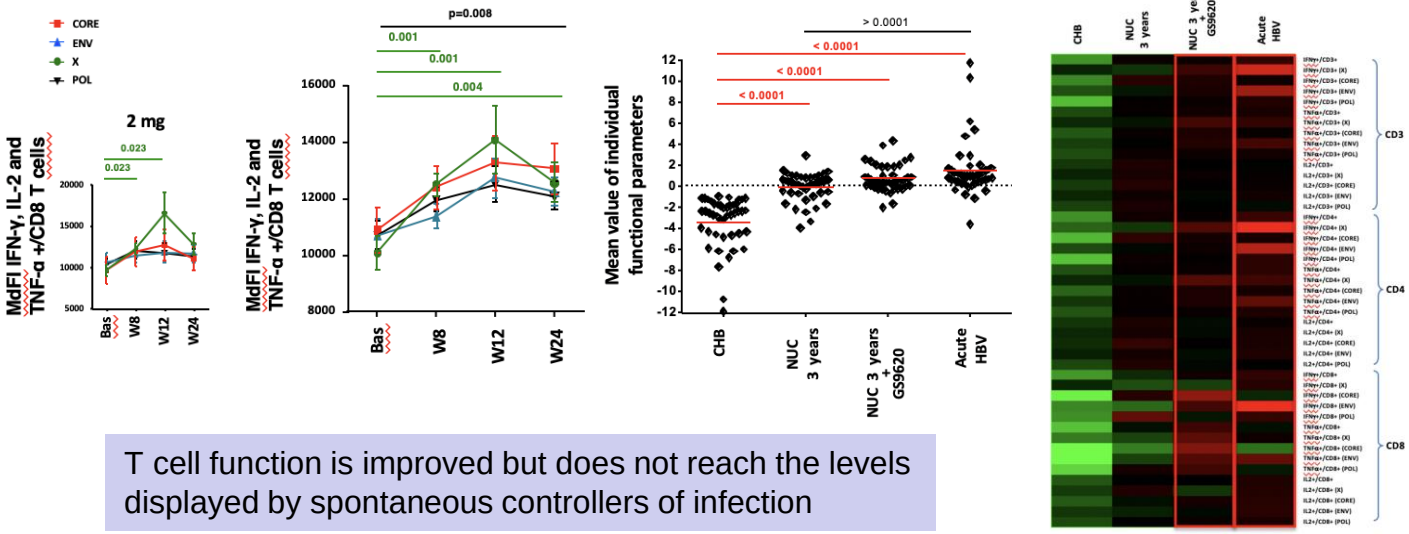
Gane et al, J Hepatol 2015
Janssen et al, J Hepatol, 2017

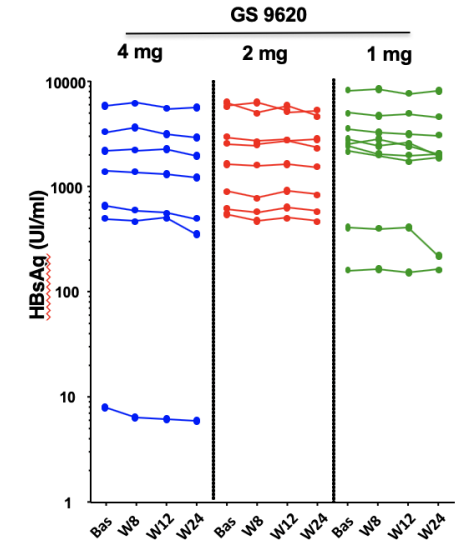


J.Jo et al. PLOS Pathogens 2014; Menne S et al. J Hepatol 2015; Lanford RE et al. Gastroenterology 2013; Gane EJ et al. J Hepatol 63:320–328; 2015; Huang LR et al. Nat Immunol 2013; Maini and Gerhing J. Hepatol. 2016

TLR-7 agonist increases responses of HBV-specific T cells and natural killer cells in CHB patients treated with NUCs





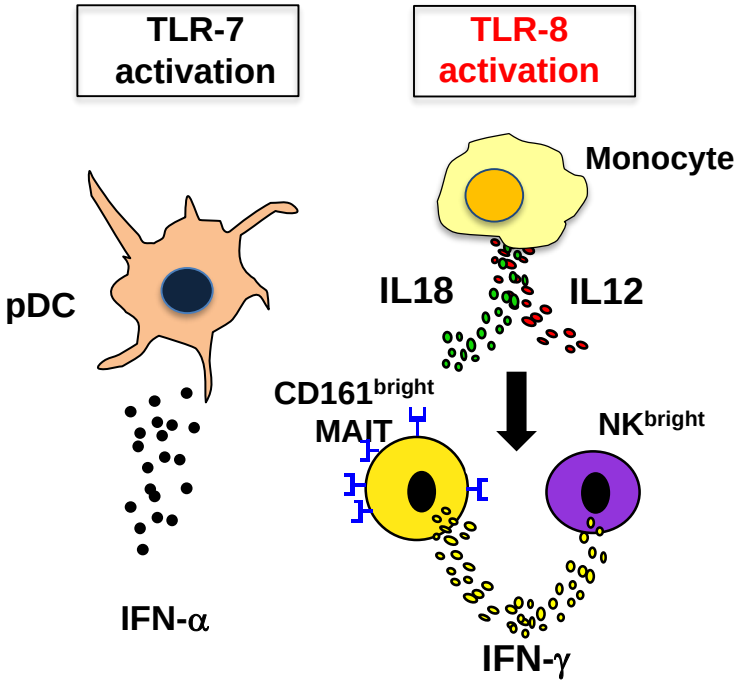


T cell function is improved but does not reach the levels displayed by spontaneous controllers of infection

no patients with >0.5-log10 declines in HBsAg at week 24;
no patients had HBsAg loss at week 24

Boni et al, Gastroenterology 2018

Manipulation strategies for innate immunity

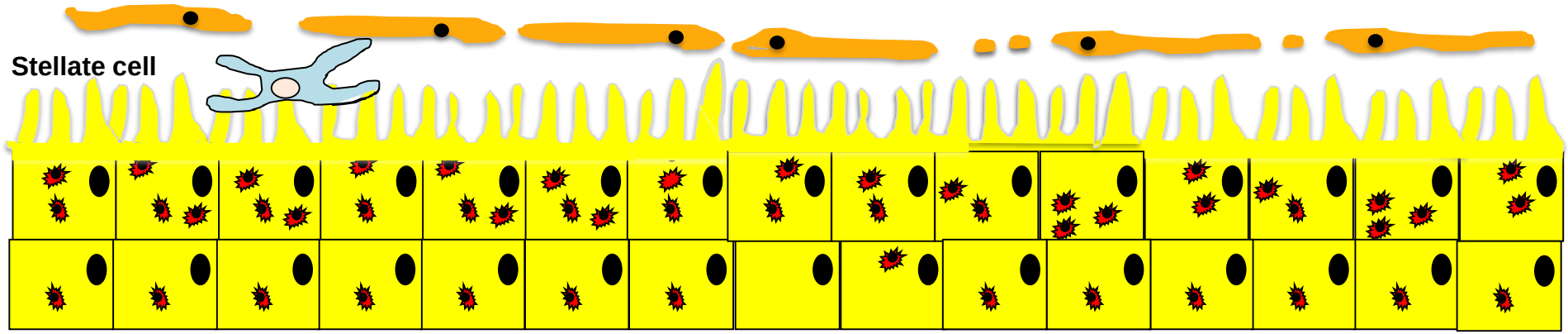


***TLR8 agonist GS-9688* : sustained efficacy and surface antigen seroconversion in the woodchuck model of CHB**

Daffis et al J Hep 2017, 66:S692-S693

PHASE Ib clinical trial of TLR8 agonist GS-9688

- *Blood samples from HBV patients treated withGS-9688 for 2 to 7 days and analyzed for cytokine response*
- *GS-9688 induced cytokines and also reduced the frequency of conventional regulatory Tcells (Tregs)*
- *GS-9688 triggered dose-dependent activation of NK cells*
- *GS-9688 triggered the production of IL12, IFNg & TNFa: potential for stimulation of innate immunity, adaptive immunity, and direct antiviral effect*



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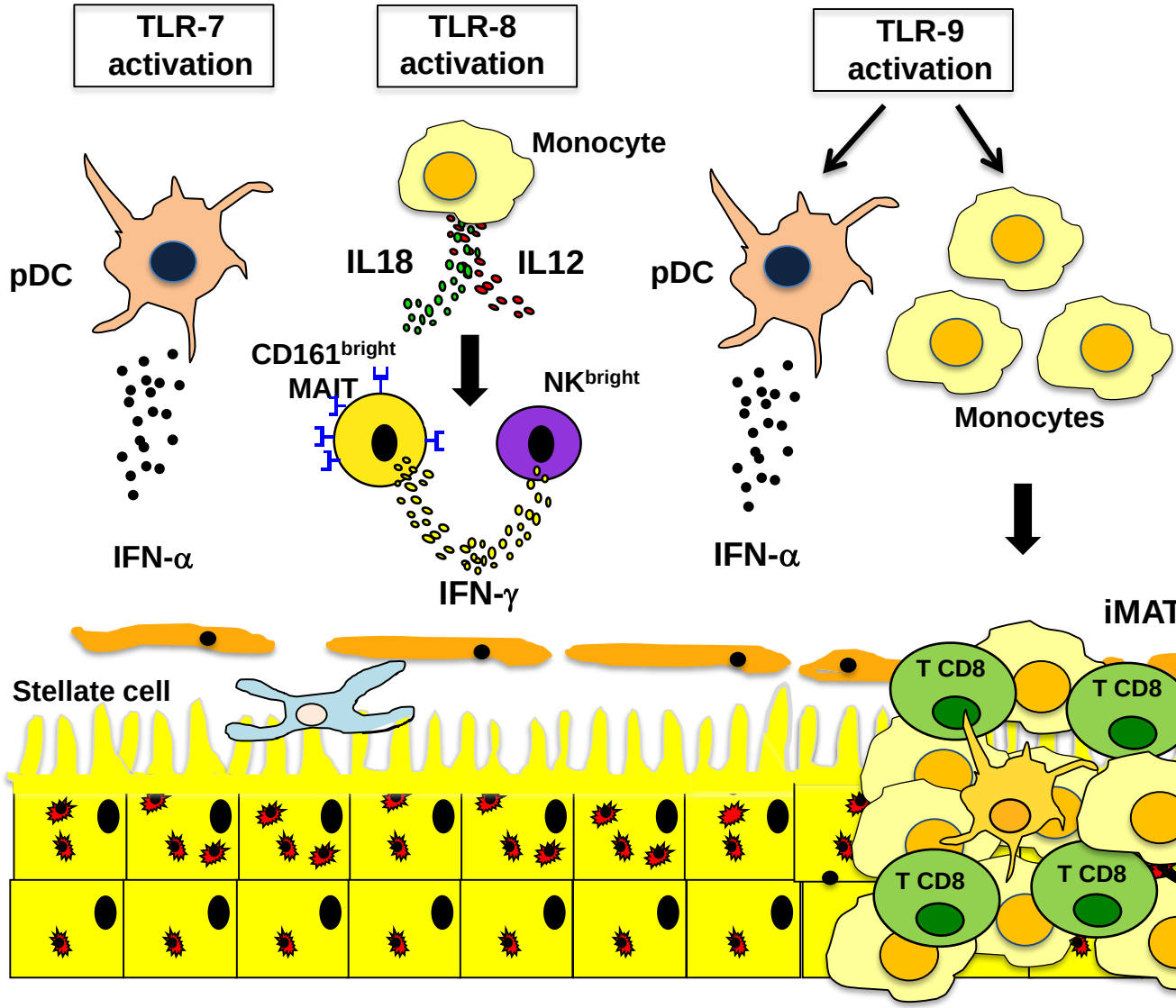
Manipulation strategies for innate immunity

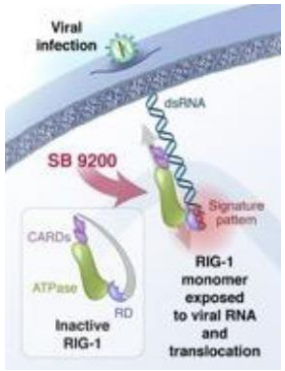


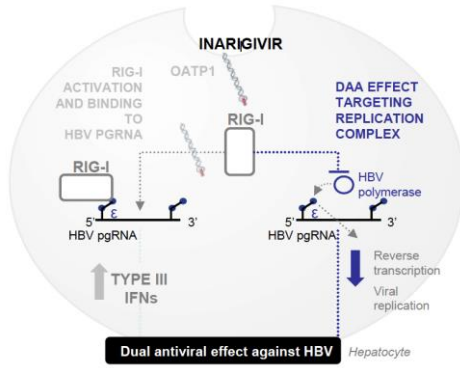
Subcutaneous TLR9 agonist induces IFN-α and intrahepatic T cell aggregates and shows antiviral efficacy in the woodchuck model of CHB during treatment in combination with entecavir

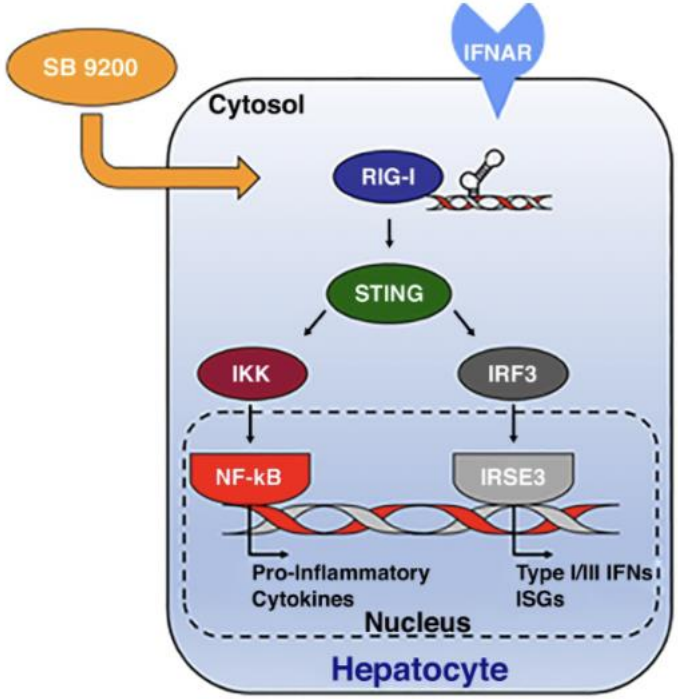
Meng et al Antiviral Res 2016
Huang L-R Nature Immunol. 2013

J.Jo et al. PLOS Pathogens 2014; Menne S et al. J Hepatol 2015; Lanford RE et al. Gastroenterology 2013; Gane EJ et al. J Hepatol 63:320–328; 2015; Huang LR et al. Nat Immunol 2013; Maini and Gerhing J. Hepatol. 2016

RIG-I agonist (SB 9200): *restoration of endogenous IFN production & interference on Polymerase/pgRNA interaction*

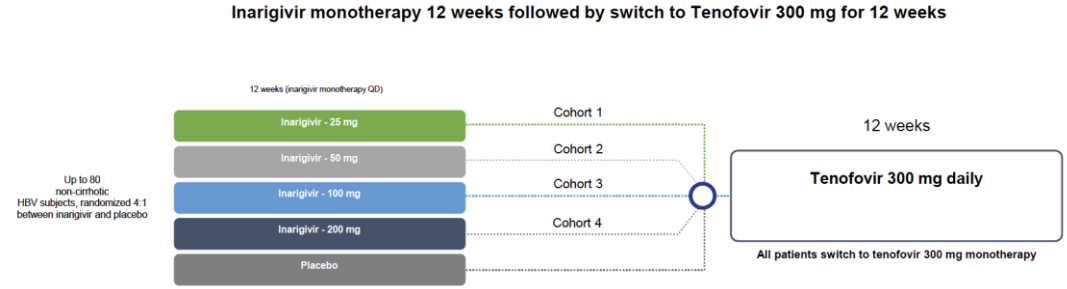






***RIG-I agonist SB 9200*: antiviral efficacy in the woodchuck model of CHB during sequential treatment with SB 9200 and entecavir**
Suresh et al Plos One 2017

PHASE II ACHIEVE trial of oral RIG-I agonist Inarigivir and Tenofovir
Yuen et al Hepatology 2017,66:22A



Targeting Adaptative Immunity

- **Antigen load reduction ?**
- **Therapeutic vaccines :** stimulation of HBV-specific CD4 and CD8 T cells
- **Check-point inhibitors** : rescue exhausted HBV specific CD4 and CD8 T cells
- **Targeting mytochondrial disfunction :** restoration of the antiviral activity of exhausted HBV-specific CD8 T cells
- **T cells engineering :** redirecting T cells to infected hepatocytes

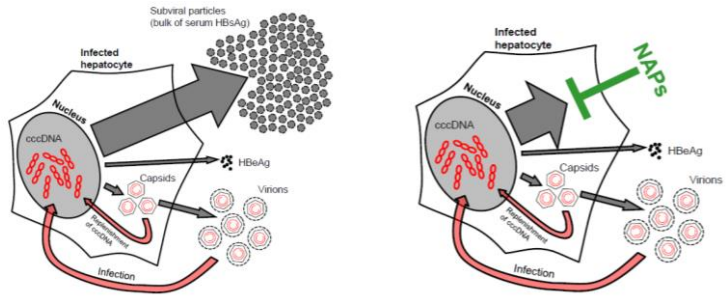
Maini et al, J Hepatol 2016;
Bertoletti et al, J Hepatol 2016;
Fisicaro et al, Nature Medicine 2017

Reduce antigen load *to restore T cell responses*

- HBsAg clearance is a a therapeutic end-point
- Reduction of HBsAg should translate in a revival of HBV-specific *exausted* T cells
- Multiple strategies are evaluated:
  - Interfering RNAs (siRNA): «genetic silencing»
  - Nucleic Acid Polymers (NAPs): HBsAg secrétion
  - Anti-HBs antibodies

Nucleic Acid Polymers (NAPs)

REP 2139
REP 2165

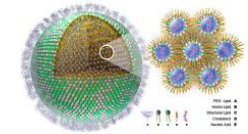


MoA unknown

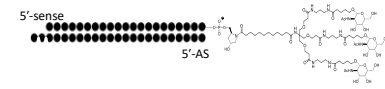
Vaillant, 2016. Antiviral Res. 133: 32-40
Real et al.,2016 J. Hepatol. 64: S395
Noordeen et al., 2015 PLOS One 10: e0140909
Noordeen et al., 2013 AAC 57: 5299-5306
Nooreen et al., 2013 AAC 57: 5291-5298

siRNAs ASO

arrowhead pharmaceuticals
janssen PHARMACEUTICAL COMPANIES OF Johnson & Johnson
Arbutus BIOPHARMA Aspiring to Cure Chronic Hepatitis B
Alnylam PHARMACEUTICALS
VIR
gsk
IONIS



Lipid Nanoparticles for IV infusion



GalNAc-Conjugate for sc administration

An ideal therapeutic vaccination approach

Vaccine:

- *incorporating core, pol and surface antigens*
- *inducing multispecific broadly cross-reactive T cells*
- *inducing functional B cells and neutralizing antibodies*
- *accompanied by immunomodulation to overcome HBV-specific immune exhaustion*

Therapeutic vaccine trials in chronic hepatitis B

Homologous vaccines

-	HepT cell	peptide + adjuvant	Phase I
-	INO-1800	DNA-vaccine	Phase I
-	CVI-HBV-002	DNA-vaccine	Phase I/II
-	HB-110/100	DNA-vaccine	Phase I
-	ppdpSC18	DNA-vaccine	Phase I/II
-	HBO2-VAC-ADN	DNA-vaccine	Phase I/II
-	Theravax	protein + adjuvant	Phase Ib
-	GS-4774	protein + adjuvant	Phase II
-	ePA-44	peptide + adjuvant	Phase II
-	ABX 203	protein	Phase II/III
-	TG1050	adeno vector vaccine	Phase II

failed

Heterologous prime – boost vaccines

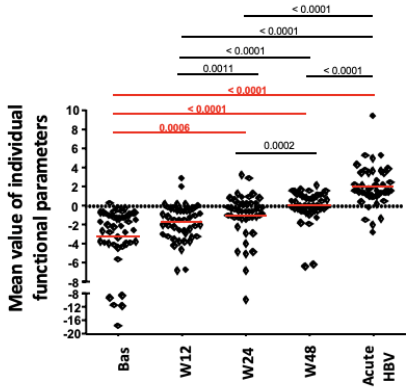
-	pSG2.HBs/MVAHBs	DNA-vaccine + MVA	Phase Ib/II (S only, no Ab) failed
-	***TherVac B***	**protein + MVA (broad)**	**preclinical PoC**

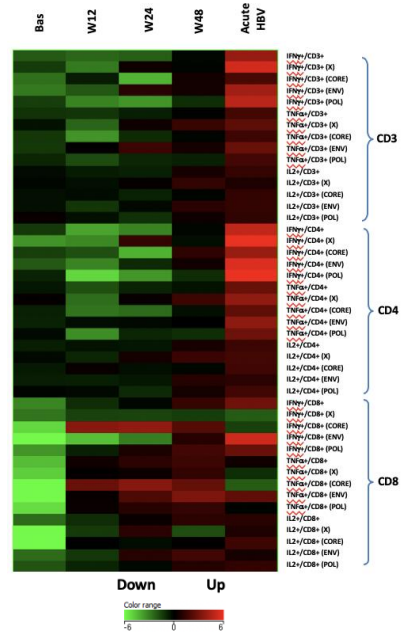
Therapeutic vaccines for chronic HBV infection

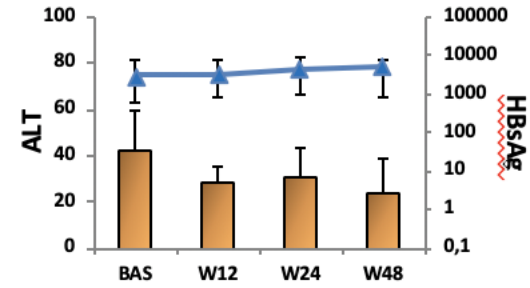
GS-4774 (Tarmogen)

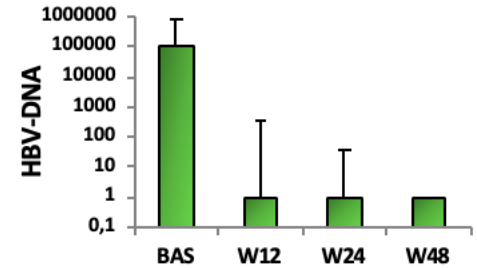
Recombinant yeast is efficiently taken up by professional antigen-presenting cells (APCs)

Processed viral antigens are then presented to T cells via MHC I and II.







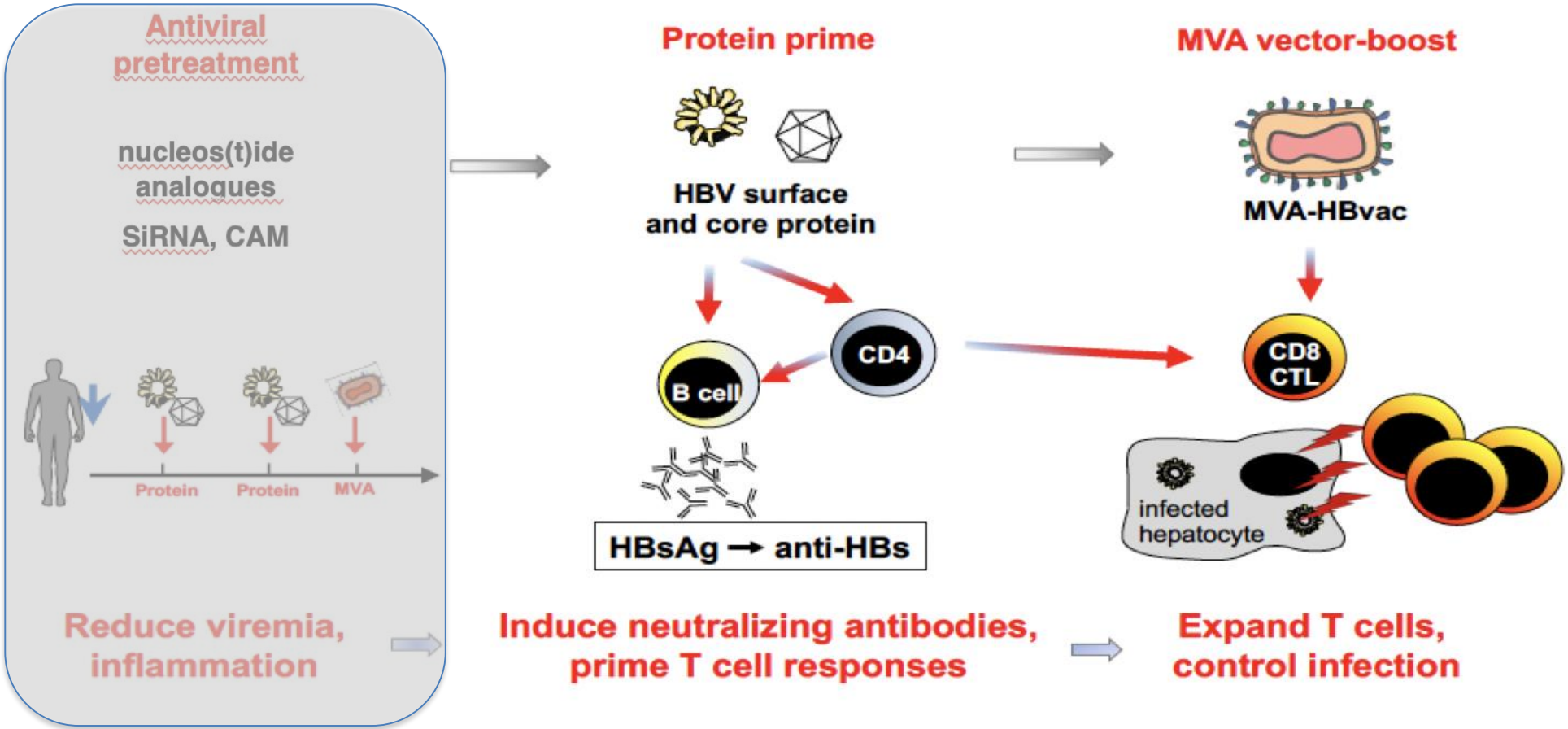


T cell responses are improved by vaccination but still remain much lower than after spontaneous resolution of infection

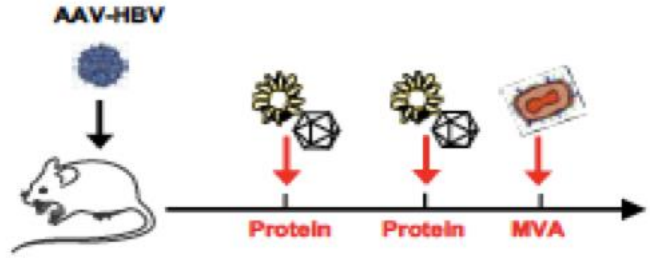
- All patients normalized ALTs, most became HBV-DNA neg
- No patients had HBsAg loss at week 48

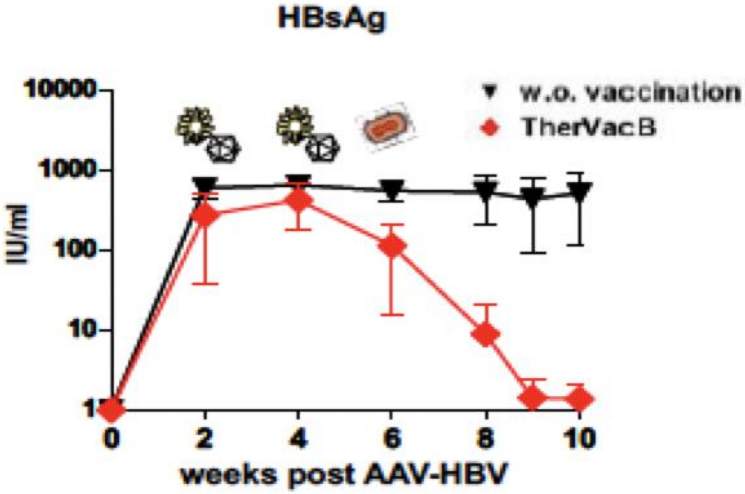
Boni C. et al. Gastroenterology 2018

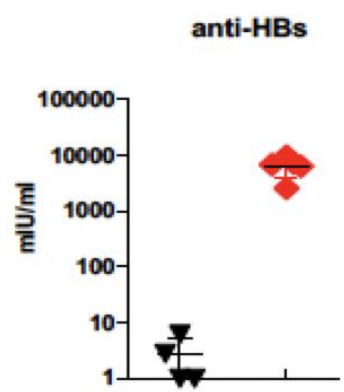
Therapeutic vaccination with prime boost

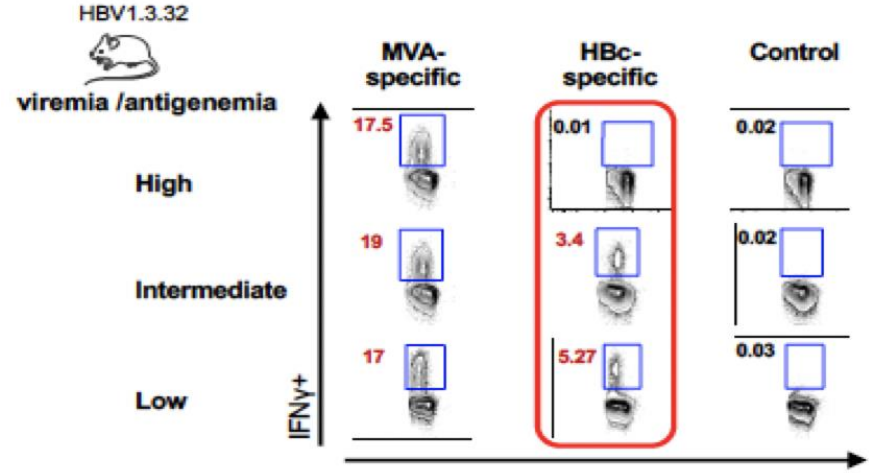


Therapeutic vaccination (TherVac) with prime boost





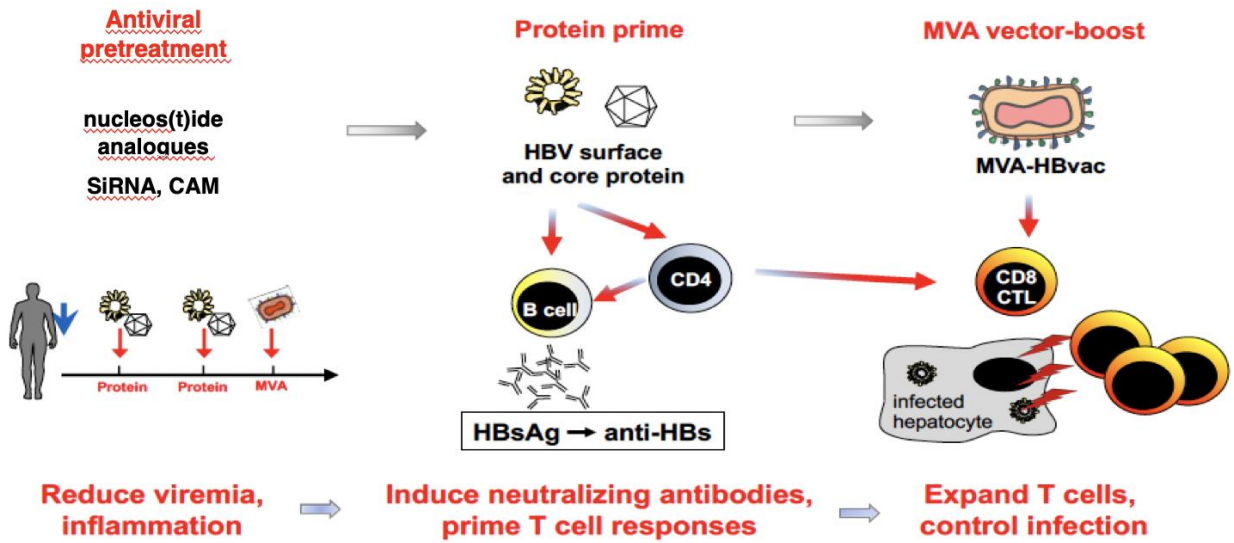


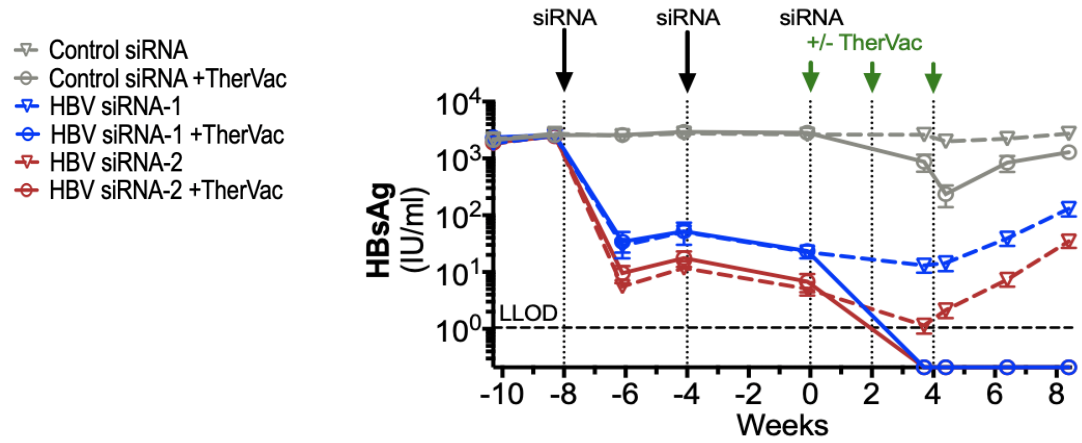


High antigen levels limit the immunological response to therapeutic vaccination in HBV-Tg mice

Bakes et al. Vaccine 2016;34:923-932

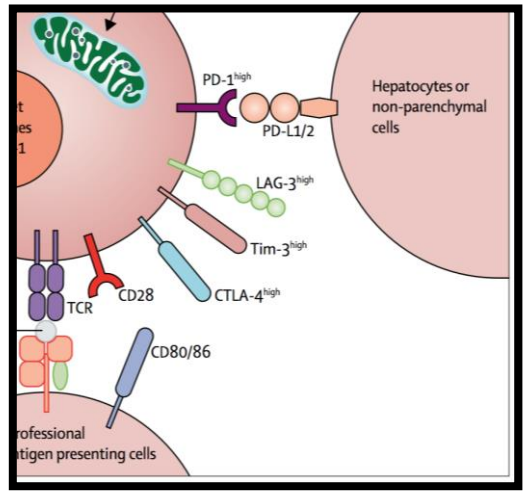
Response to *TherVac* is increased by prior decline of antigen

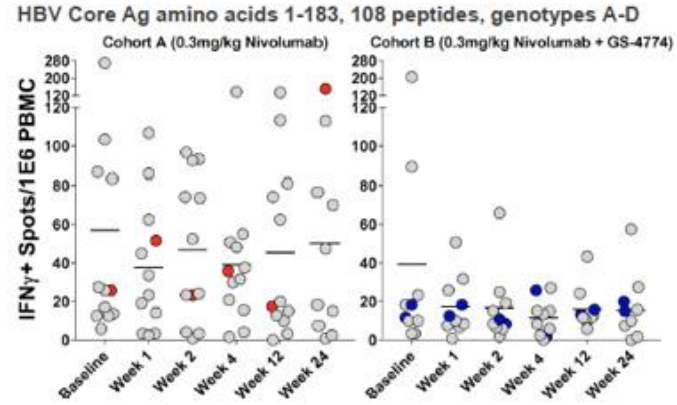




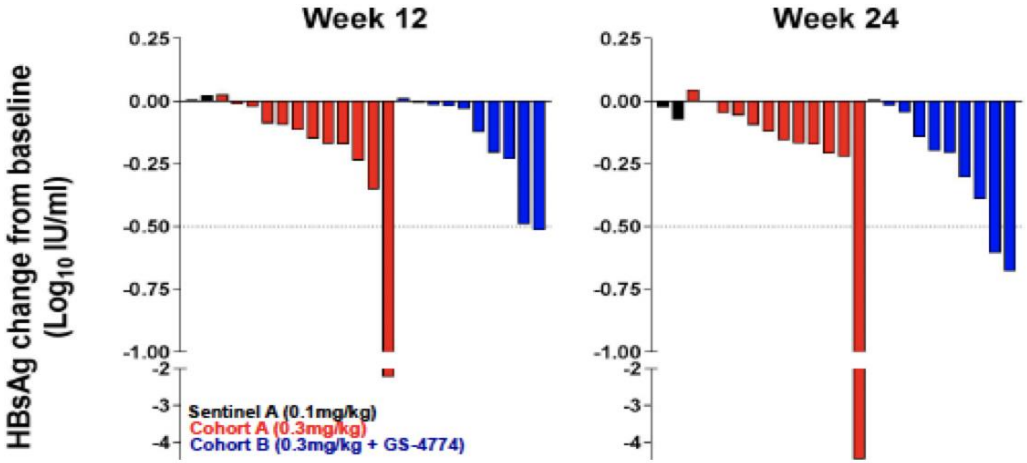
- siRNA pre-treatment and ***TherVacB*** allow to "cure" HBV

HBV-specific T cell responses in patients with chronic HBV infection treated with anti-PD-1 alone or in combination with the GS-4774 vaccine

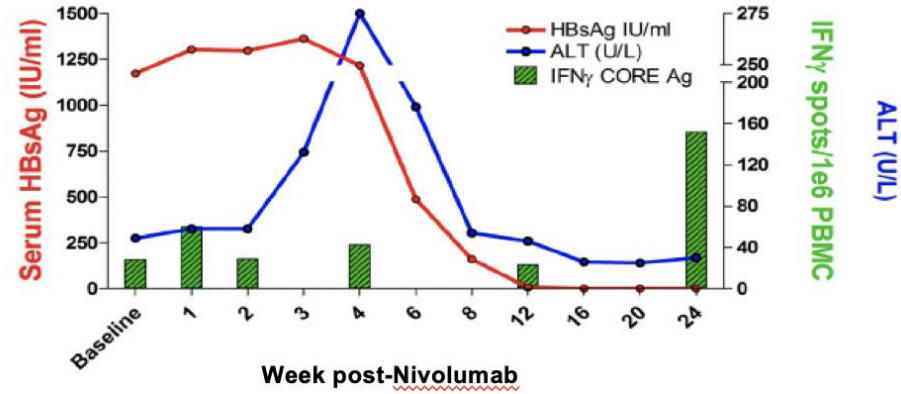




- HBc- and HBs-specific T cells detected ex vivo in 18/24 pts
- No increase with Nivolumab



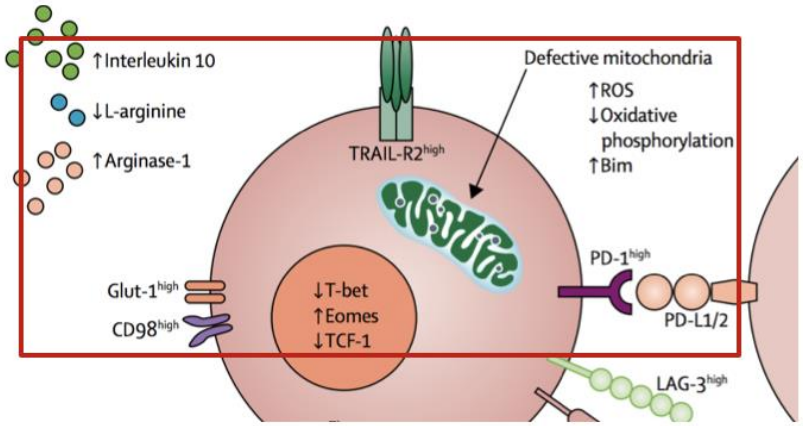
- 2/22 (9%) at wk 12 and 3/22 (14%) at wk 24 with > 0.5 log_{10} reduction in HBsAg
- 19/22 pts treated with 0.3 mg/Kg showed some decline in HBsAg by wk 24



- 1 patient off treatment for > 9 mo with sustained S loss and anti-HBs seroconversion

Gane et al, 2018

Targeting underlying HBV-specific T cell mitochondrial/metabolic dysfunction

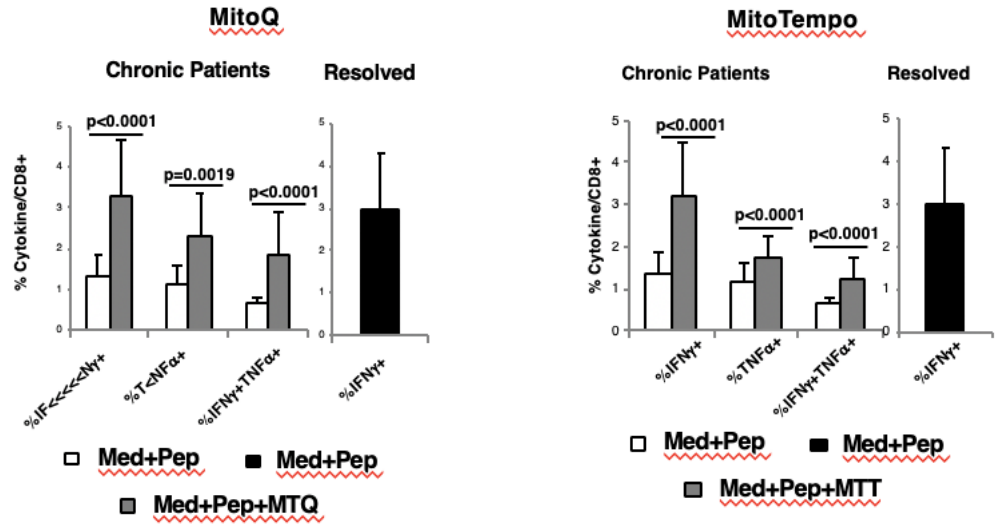


Schurich et al Cell Reports 2016
Fisicaro et al Nat Med 2017

Exhausted CD8 T cells are poorly able to use oxphos to meet their energy demands

MitoQ: Ubiquinone moiety CONJUGATED TO A TPP CATION

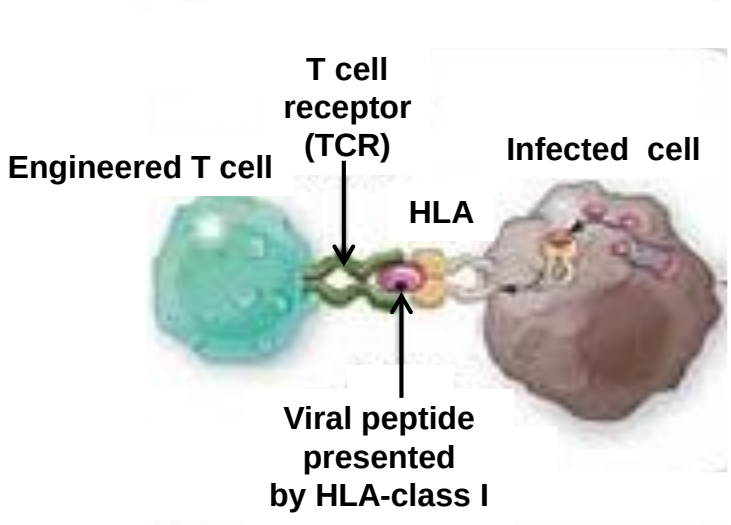
MitoTEMPO: Superoxide dismutase mimetic action
Catalase-like action
CONJUGATED TO A TPP CATION



Functional restoration of antiviral effector CD8 responses by mitochondrial targeted anti-oxidant compounds

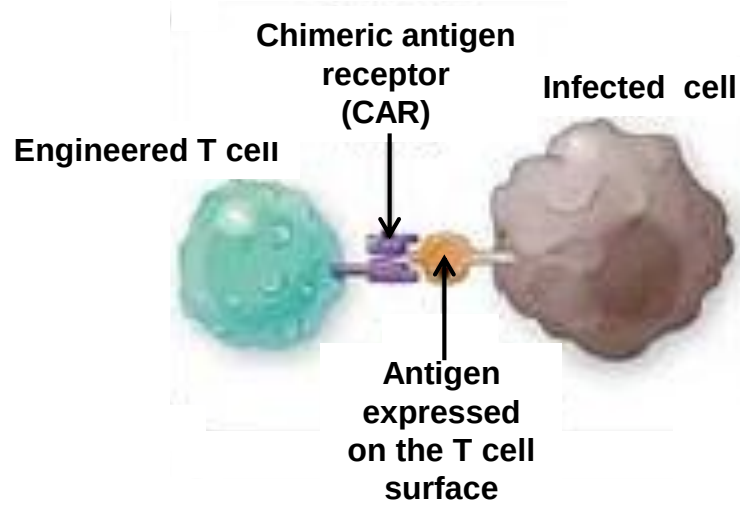
Adapted from Maini and Pallett, Lancet Gastroenterol Hepatol 2018

Adoptive cell therapy through T cell engineering



T cells are HLA-class I restricted

Gehring et al, J Hepatol 2011,Qasim et al, J Hepatol 2015
Kah et al, JCI 2017, Koh et al, Gastro 2018



T cells recognize conformational antigen

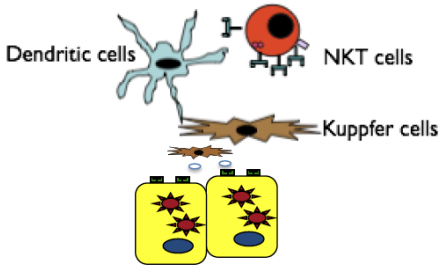
Bohne et al, Gastro 2008, Krebs et al, Gastro 2013

Why Immune therapies didn't (so far) work?

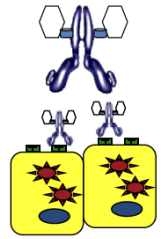
IFN-alpha is the only therapy that increases seroconversion

Activation of Intrahepatic Innate Immunity

TLR 7/8 RIG-I Agonists

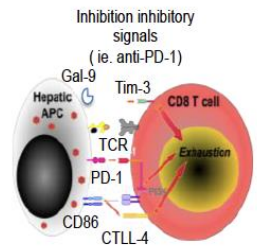


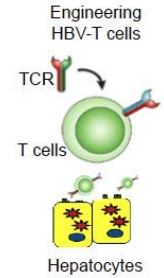
Cytokines/ direct/ antibody delivery..



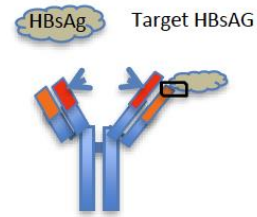
Restoration of HBV-specific Immunity

T cell boosting

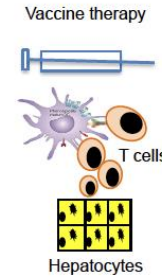




Antibodies



Vaccines

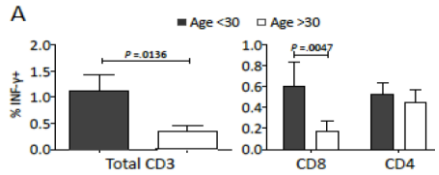


Adapted from: Bertoletti A SHC 2019

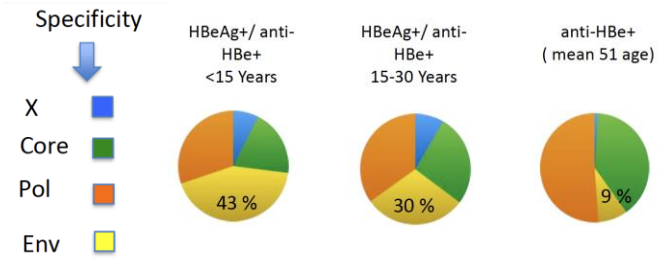
Because we are targeting the wrong patient population ?

- Increased frequency of HBV specific T cells in "Young Immunotolerant" pts

  Kennedy et al Gastroenerology 2012

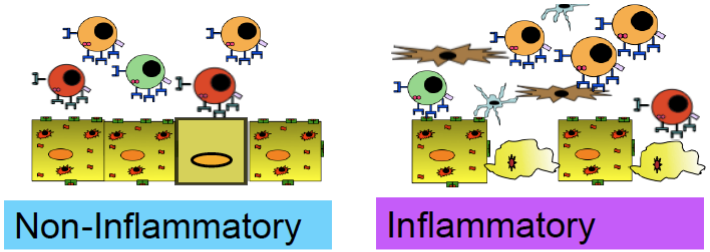


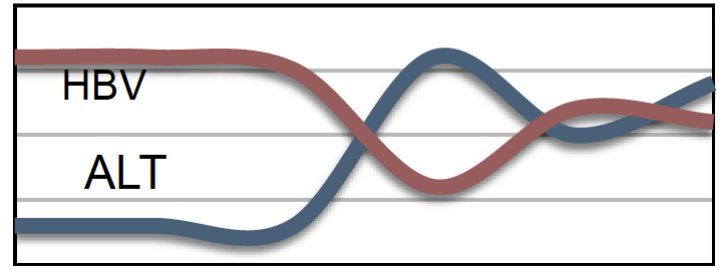
- More complete T cell repertoire



Some HBV T cells (tolerant / anergic) Tolerogenic environment

Few HBV T cells (exhausted) Suppressive environment





CHB patients, mainly anti-HBe, under NA therapy

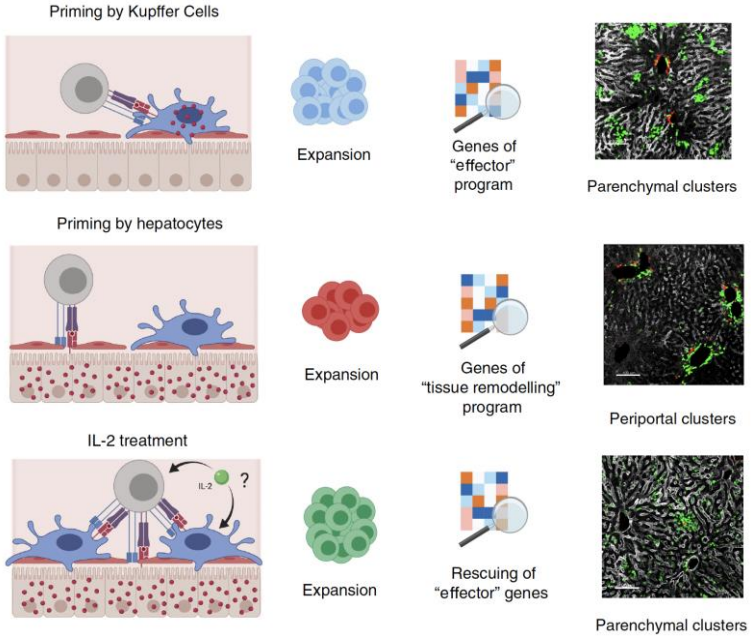
Adapted from: Bertoletti A SHC 2019

Consequences of HBV hepatic priming

NATURE | VOL 574 | 10 OCTOBER 2019

Dynamics and genomic landscape of CD8+ T cells undergoing hepatic priming

Alexandre P. Bénéchet[1,11], Giorgia De Simone[1,2,11], Pietro Di Lucia[1], Francesco Cilenti[2,3], Giulia Barbiera[3], Nina Le Bert[4], Valeria Fumagalli[1,2], Eleonora Lusito[3], Federica Moalli[1], Valentina Bianchessi[2,3], Francesco Andreata[1], Paola Zordan[1], Elisa Bono[1], Leonardo Giustini[1], Weldy V. Bonilla[5], Camille Bleriot[6], Kamini Kunasegaran[4], Gloria Gonzalez-Aseguinolaza[7], Daniel D. Pinschewer[5], Patrick T. F. Kennedy[8], Luigi Naldini[2,3], Mirela Kuka[1,2], Florent Ginhoux[6,9], Alessio Cantore[2,3], Antonio Bertoletti[4,6], Renato Ostuni[2,3,12], Luca G. Guidotti[1,2,12] & Matteo Iannacone[1,2,10,12]✉



- exhausted T cells
- tolerant / anergic
- not sensitive to anti-PD1
- IL2 dependent

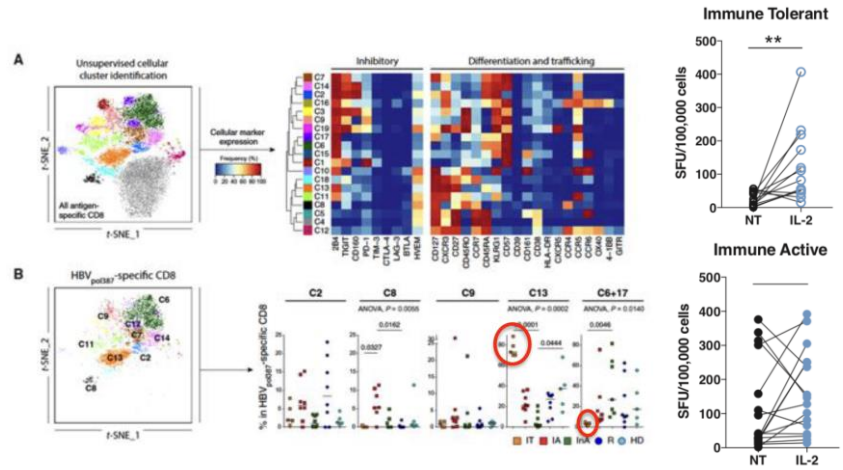
SCIENCE IMMUNOLOGY | RESEARCH RESOURCE

HEPATITIS

Cheng *et al.*, *Sci. Immunol.* **4**, eaau6905 (2019)

Multifactorial heterogeneity of virus-specific T cells and association with the progression of human chronic hepatitis B infection

Yang Cheng[1], Yuan O. Zhu[2], Etienne Becht[1], Pauline Aw[2], Jinmiao Chen[1], Michael Poidinger[1], Paola Flórez de Sessions[2], Martin Lloyd Hibberd[2,3], Antonio Bertoletti[1,4], Seng Gee Lim[5], Evan W. Newell[1,6]✉



- *Different HBV specific CD8 T cells in (young) IT*
- *and (adult) CHB patients*
- *Phenotypically they show that they form a different cluster (cluster 13, CD127++ (IL7R), CXCR3++, CD27++)*

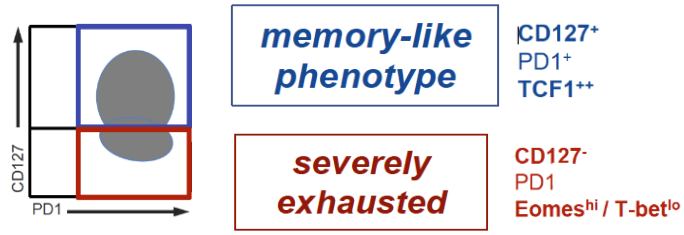
HBV specific CD8 T cells in IT (HBe pos CI) or IA (HBe neg CH) are different

Young / IT : Vac therapy ? IL-2 ? TLR-7/8 ?

Older CHB : Reconstitution of T cells ? PDL1 ?

Heterogeneity of HBV specific CD8+ T cells

- **Distinct subsets**



- **Different target epitopes**

Phenotypic and functional differences of HBV core-specific versus HBV polymerase-specific CD8+ T cells in chronically HBV-infected patients with low viral load

Anita Schuch,[1,2,3] Elahe Salimi Alizei,[1,2,4] Kathrin Heim,[1,2,3] Dominik Wieland,[1,2] Michael Muthamia Kiraithe,[1,2] Janine Kemming,[1,2,3] Sian Llewellyn-Lacey,[5] Özlem Sogukpinar,[1,2] Yi Ni,[6] Stephan Urban,[6,7] Peter Zimmermann,[1,2,3] Michael Nassal,[1,2] Florian Emmerich,[8] David A Price,[5] Bertram Bengsch,[1,2] Hendrik Luxenburger,[1,2] Christoph Neumann-Haefelin,[1,2] Maike Hofmann,[1,2] Robert Thimme[1,2]

Phenotype and function of HBV-specific T cells is determined by the targeted epitope in addition to the stage of infection

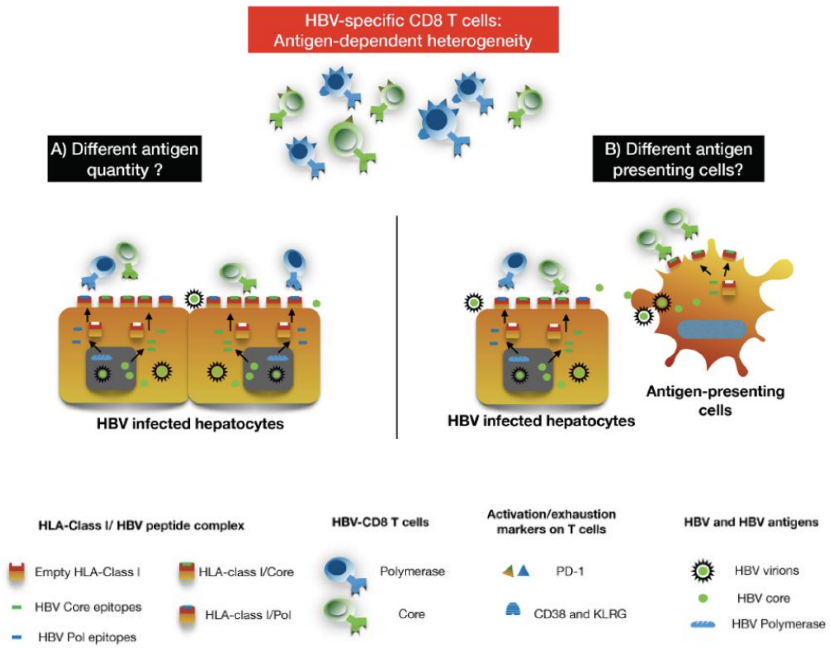
Ruben C Hoogeveen,[1,2] Maxwell P Robidoux,[1] Tatjana Schwarz,[3] Laura Heydmann,[4] James A Cheney,[1] Daniel Kvistad,[1] Jasneet Aneja,[1] Juliana G Melgaço,[5] Carlos A Fernandes,[6] Raymond T Chung,[1] Andre Boonstra,[2] Arthur Y Kim,[7] Thomas F Baumert,[4] Jörg Timm,[3] Lia L Lewis-Ximenez,[5] Pierre Tonnerre,[1] Georg M Lauer[1]

Gut, 2019

Conclusions

HBV antiviral immunity: not all CD8 T cells are born equal

Antonio Bertoletti,[1,2] Patrick T F Kennedy[3]



- Immune therapy could be an important asset for HBV cure but patients selection is crucial
- Immune therapies need to be personalized in relation to the immune profile of disease and not only to virological parameters

Thanks to Antonio Bertoletti, Rober Thimmeand Carlo Ferrari for providing me slides and Carlo Ferrari for the continuing collaboration that keeps me in the immunology loop

INSERM U1052
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Lab of Gene Expression


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INSERM U1052
Equipe 15

Fabien Zoulim
Barbara Testoni
and her team