15 maggio 2015

Prospettive di immunoterapia per l'epatite cronica B

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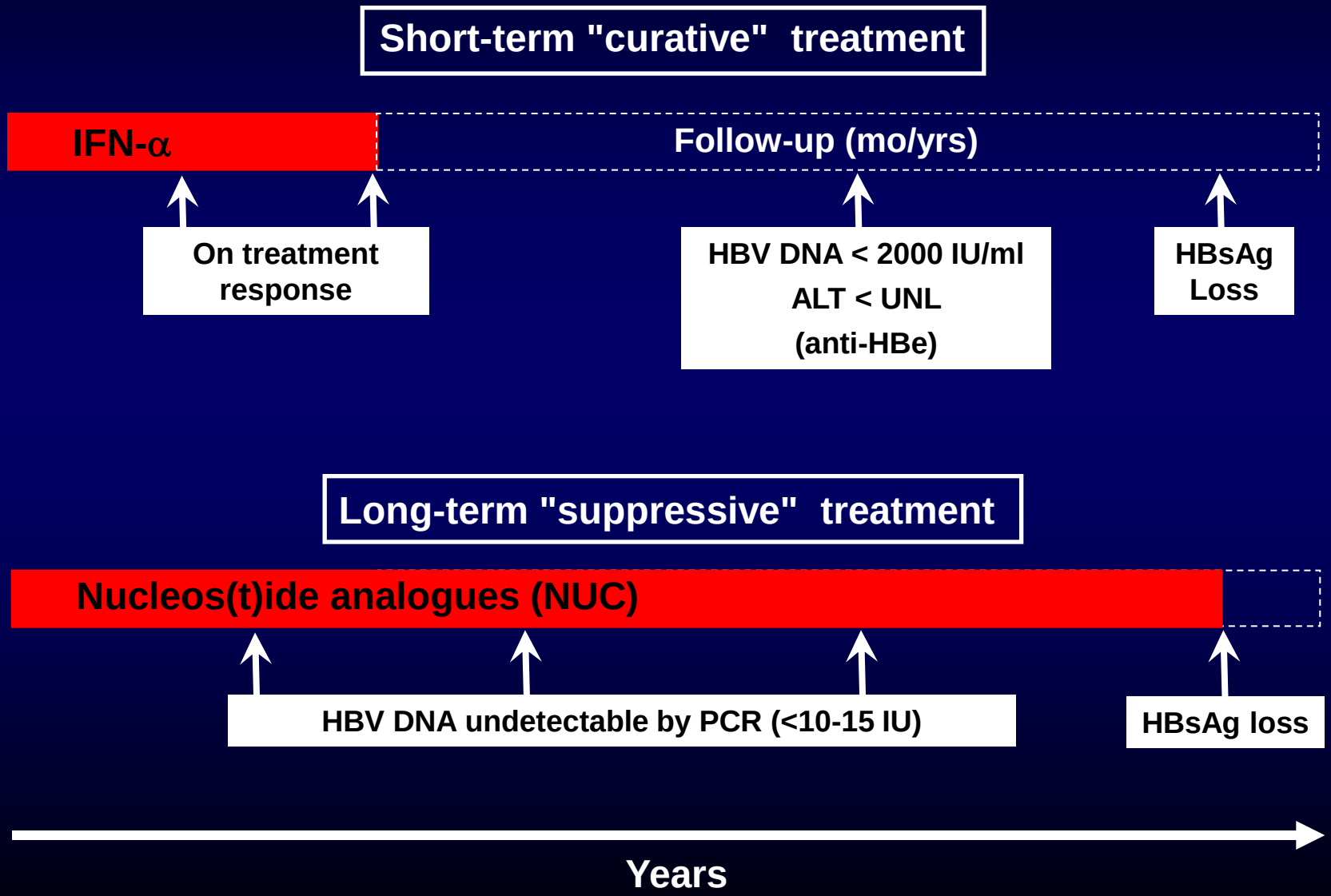
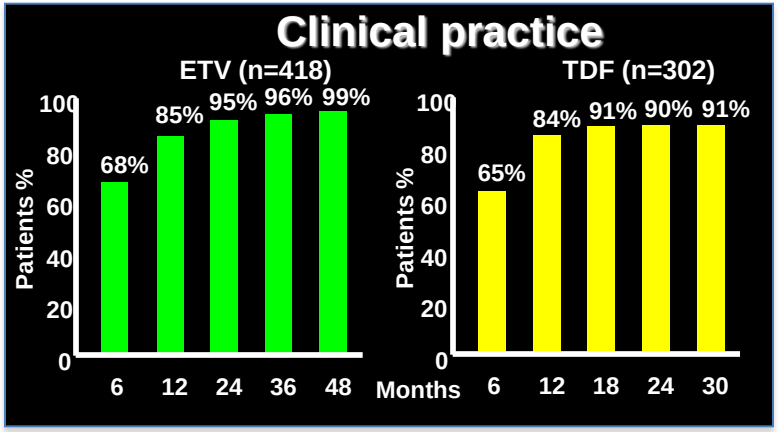
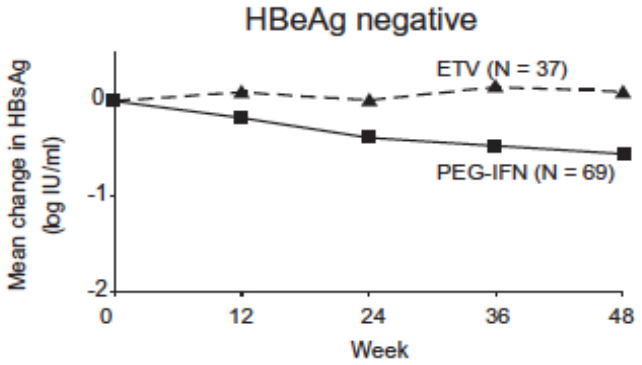
Therapeutic strategies for HBeAg negative chronic hepatitis B



Efficient control of HBV replication by NUC therapy

Slow HBsAg decline during NUC therapy: need of life-long NUC administration





Reijnders JGP et al J-Hepatol. 2011

Clinical needs in HBV therapy for CH-B:

to shorten NUC therapy by accelerating

HBsAg clearance

Clinical needs in HBV therapy for genotype D HBeAg negative CH-B

- How to shorten NUC therapy?
  - Combination therapies to accelerate HBsAg clearance
  - Predictors of efficient immune control

- How to consolidate and optimize IFN-α effect?

Clinical needs in HBV therapy for genotype D HBeAg negative CH-B

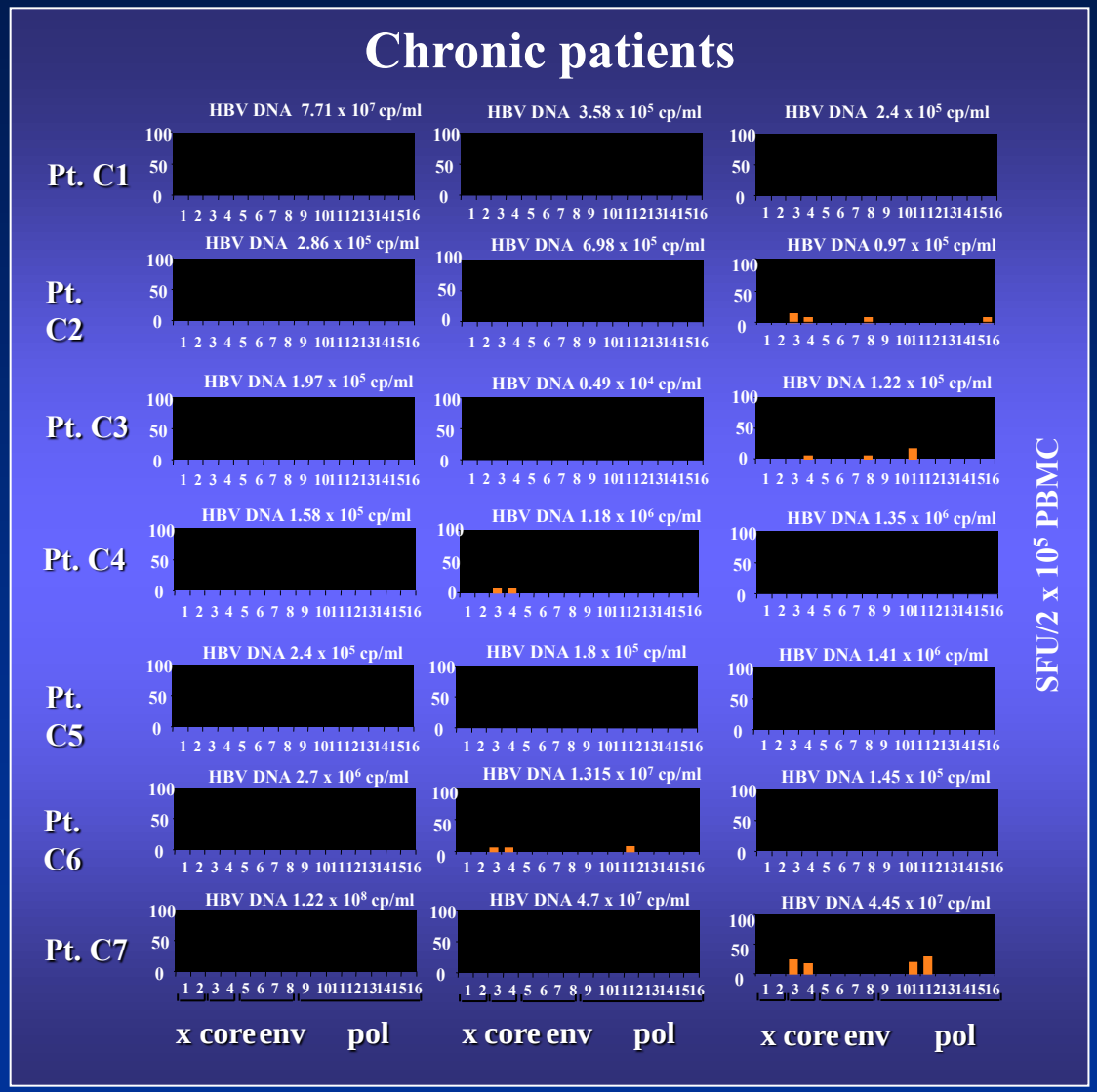
- How to shorten NUC therapy?
  - Combination therapies to accelerate HBsAg clearance

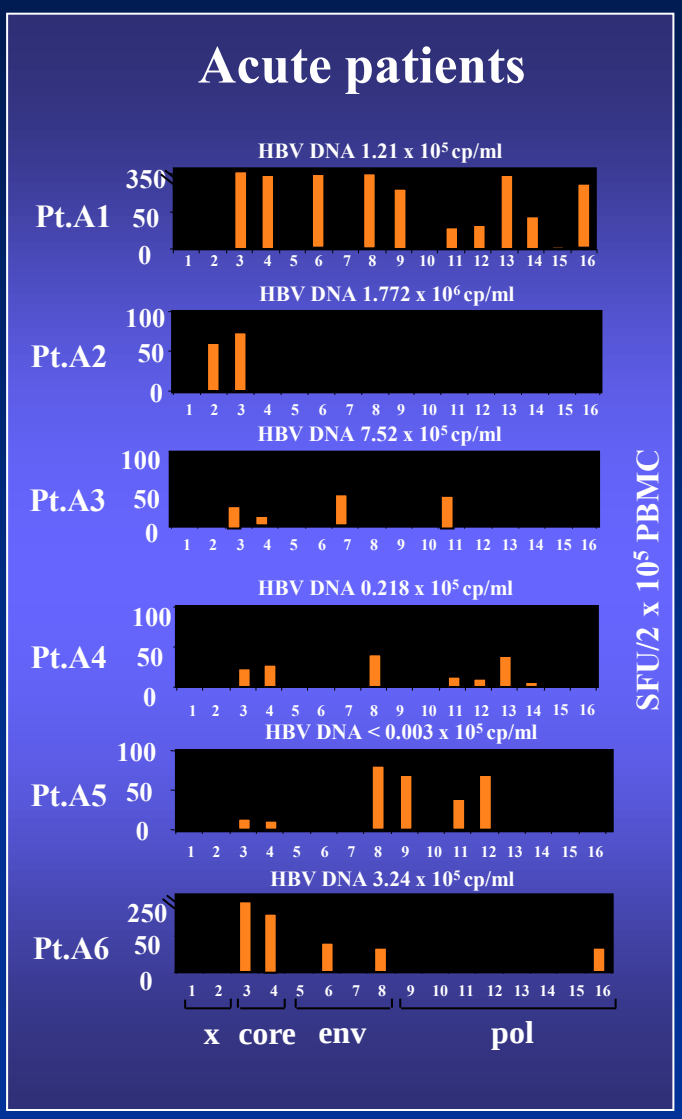
↓

Stimulation of anti-viral immunity:
de-novo or *sequential* combination therapies?

The HBV-specific T cell function is deeply impaired in anti-HBe+ chronic HBV infection

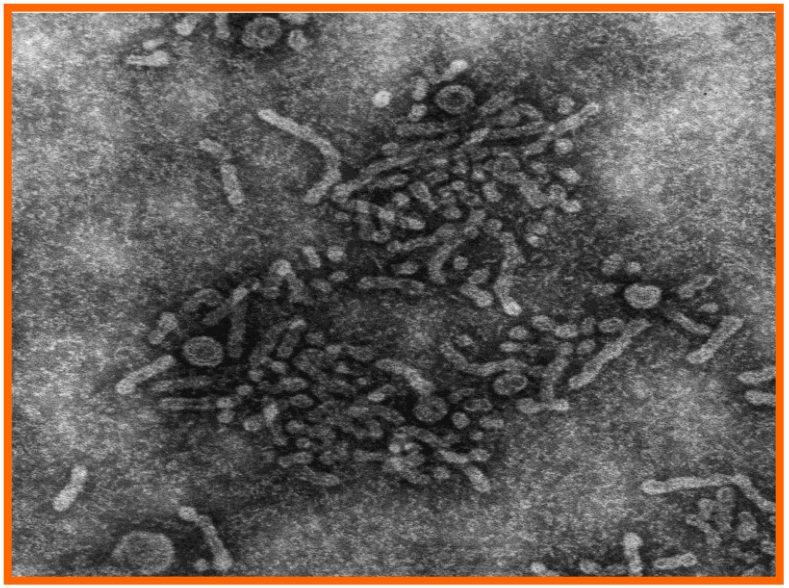
(Boni C, Fisicaro P. et al. J.Virol.2007)



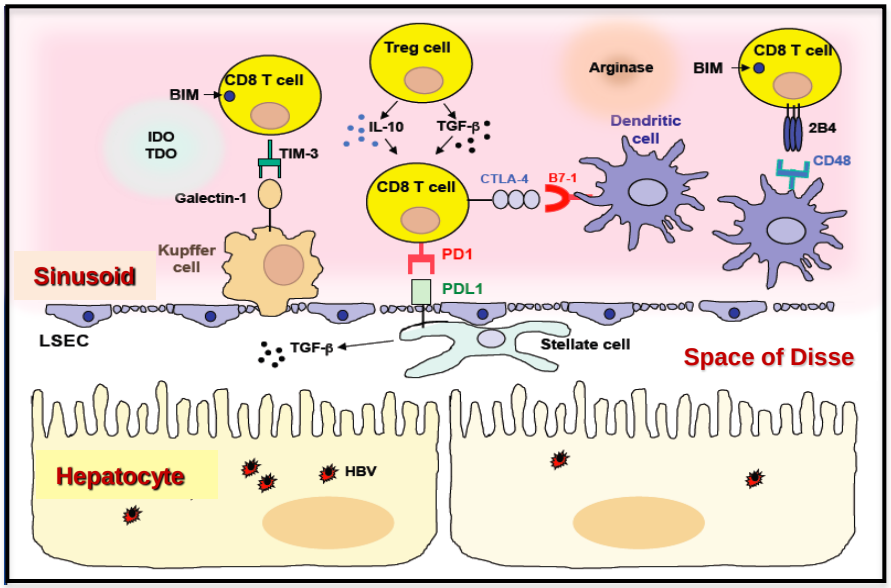


PUTATIVE MECHANISMS OF T CELL EXHAUSTION IN HBV INFECTION

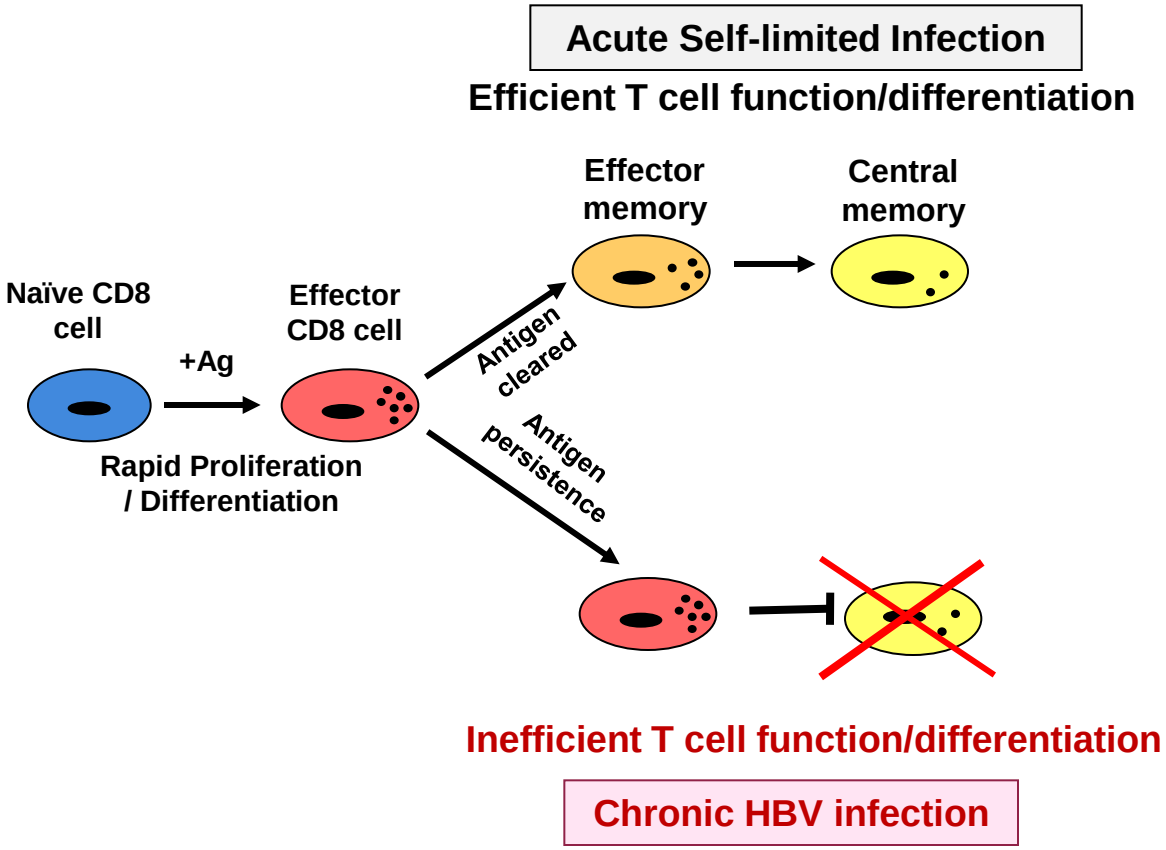
High viral load with massive production of secretory proteins (HBsAg, HBeAg)



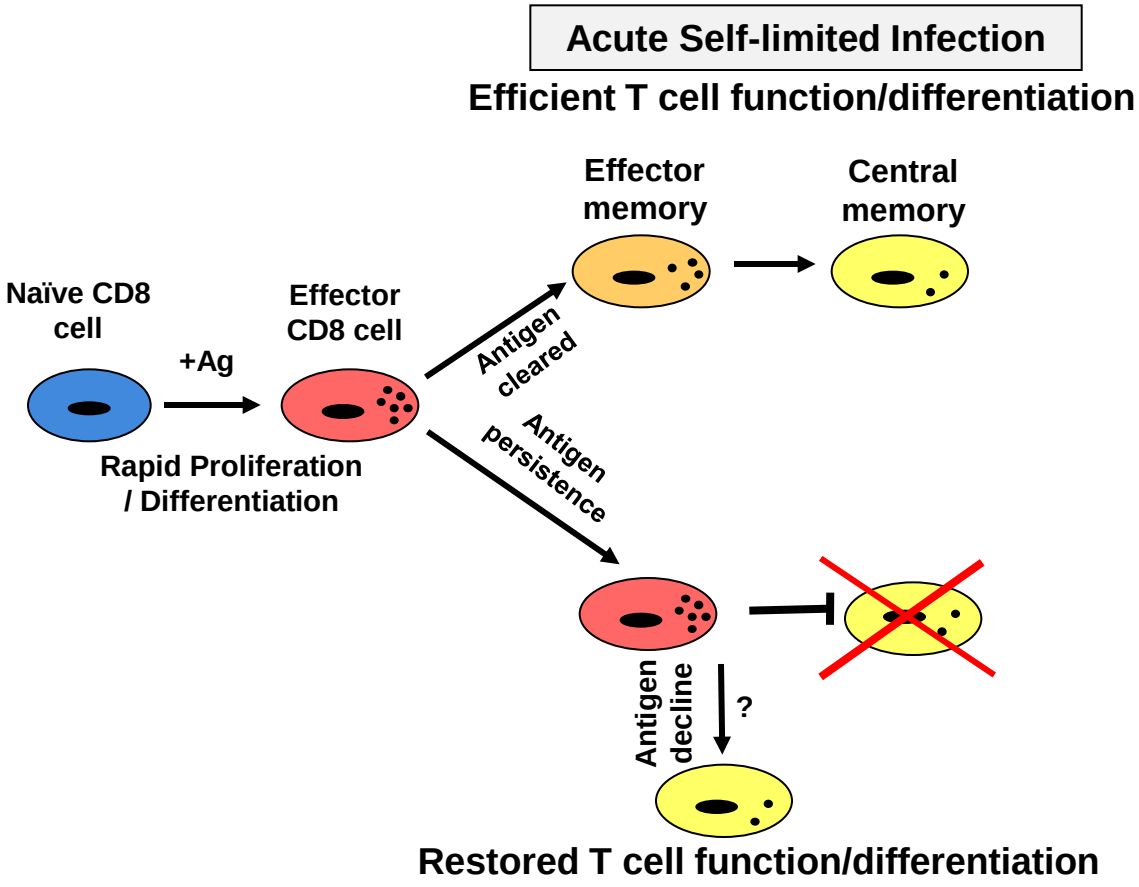
Tolerizing liver environment



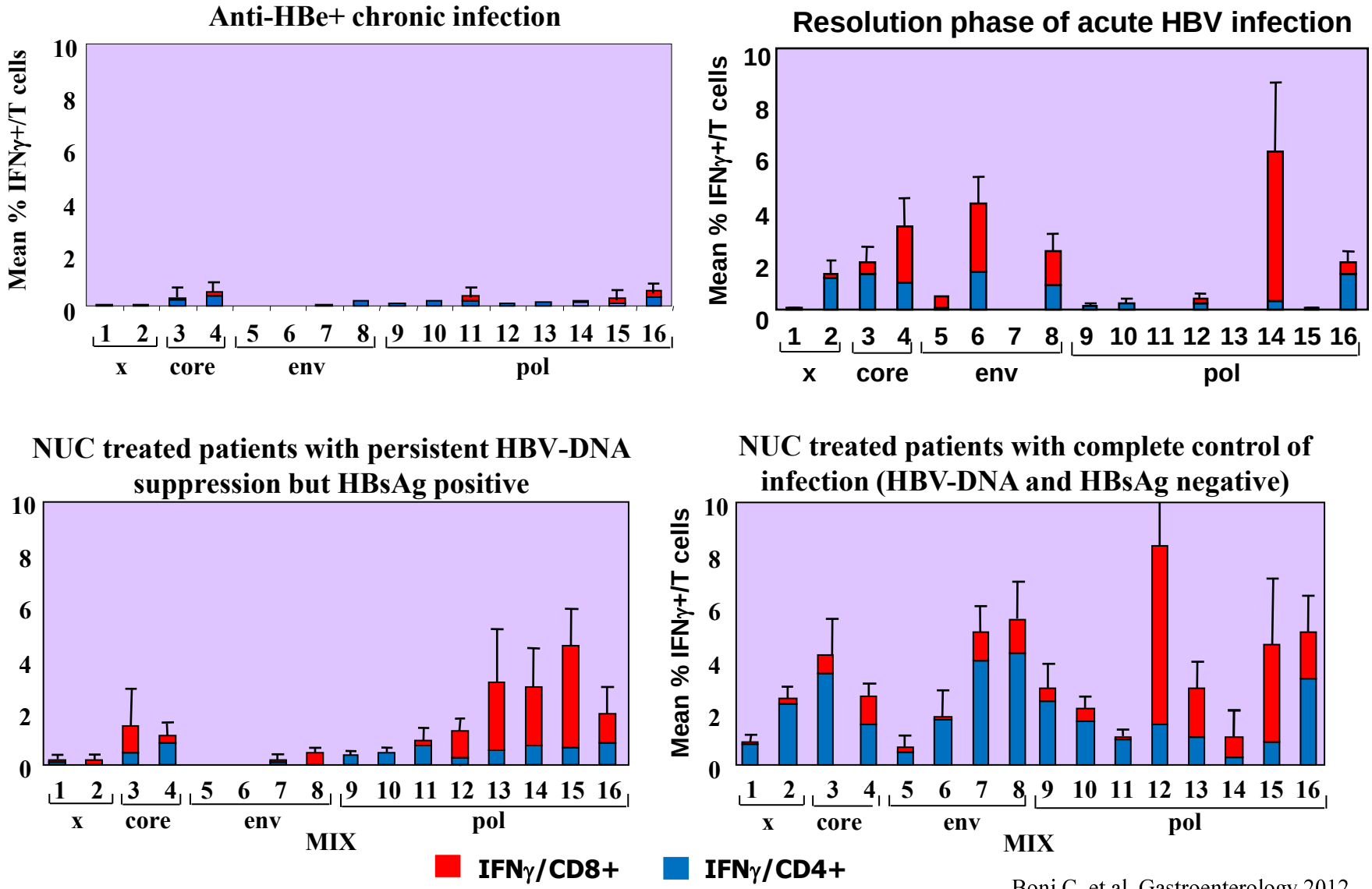
T CELL FUNCTIONAL IMPAIRMENT IN CHRONIC HBV INFECTION



CAN THE HBV-SPECIFIC T CELL FUNCTION BE RESTORED IN CHRONIC HBV PATIENTS?



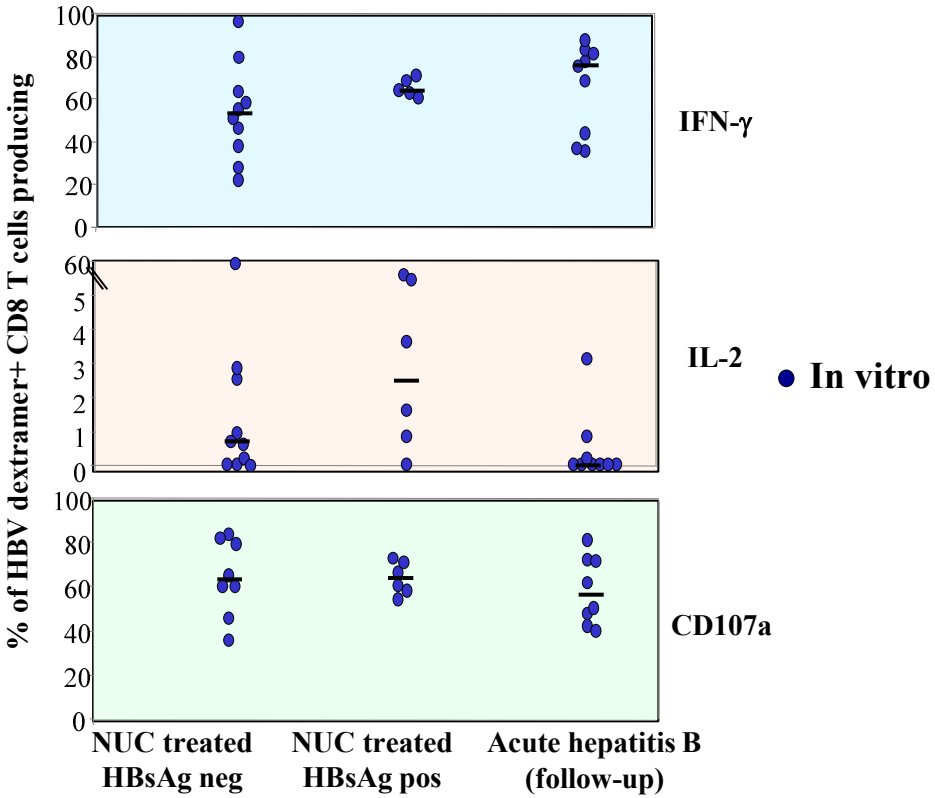
Functional T cell restoration after long-lasting suppression of HBV replication in NUC treated patients

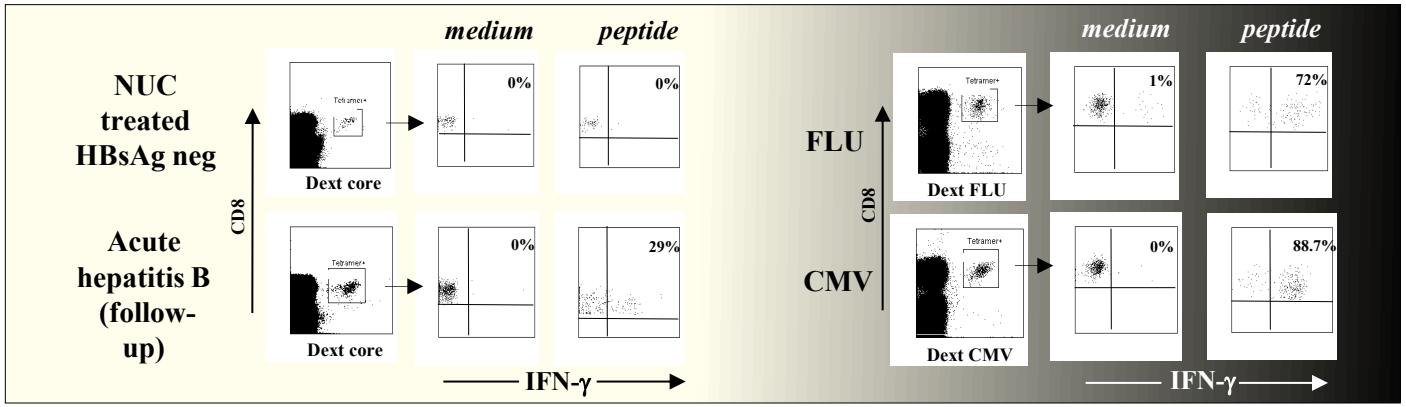


Boni C. et al. Gastroenterology 2012

CD8 cell restoration is efficient in vitro in NUC treated patients

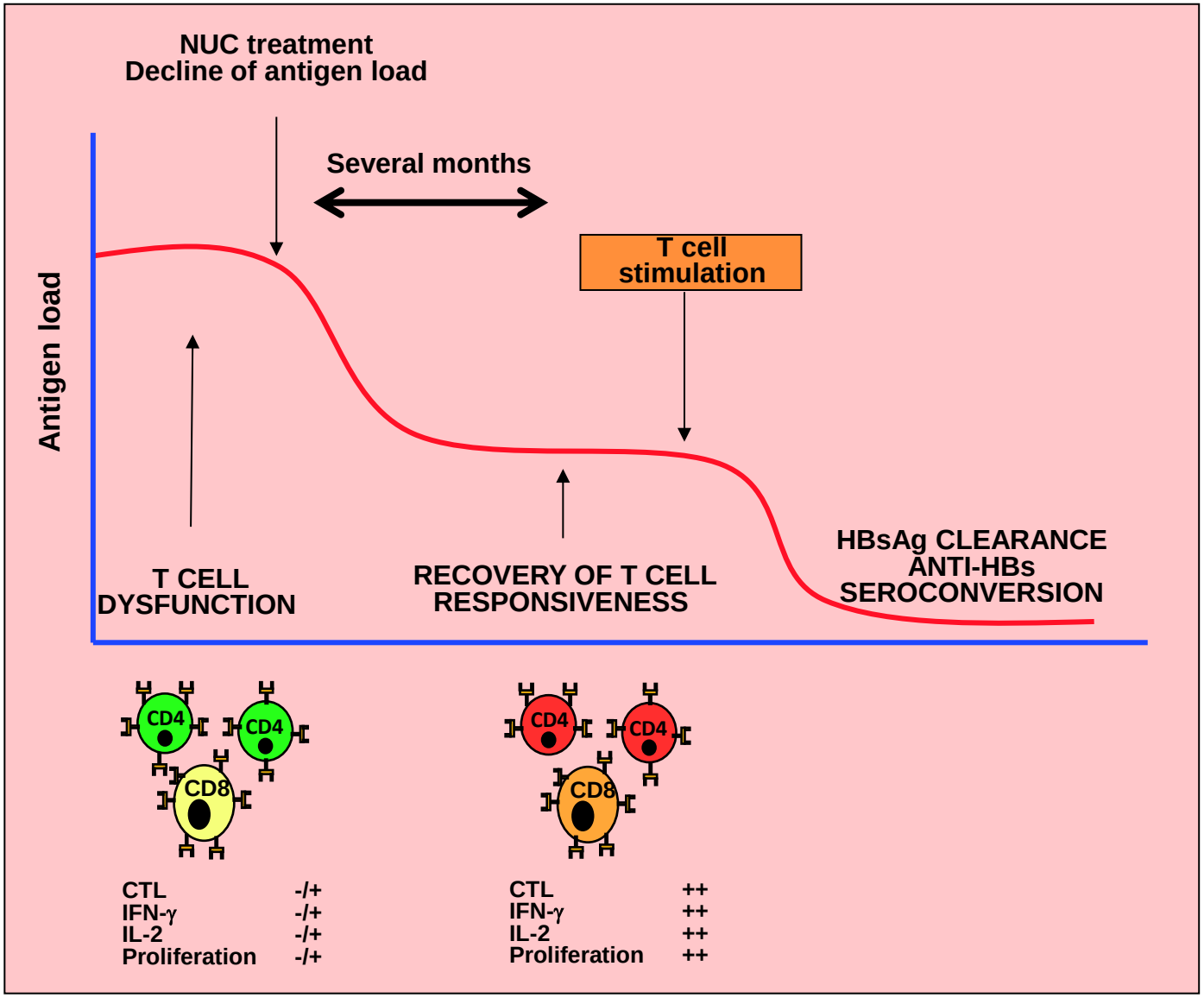
Boni C. et al. Gastroenterology 2012





SEQUENCIAL NUC/IFN-α THERAPY

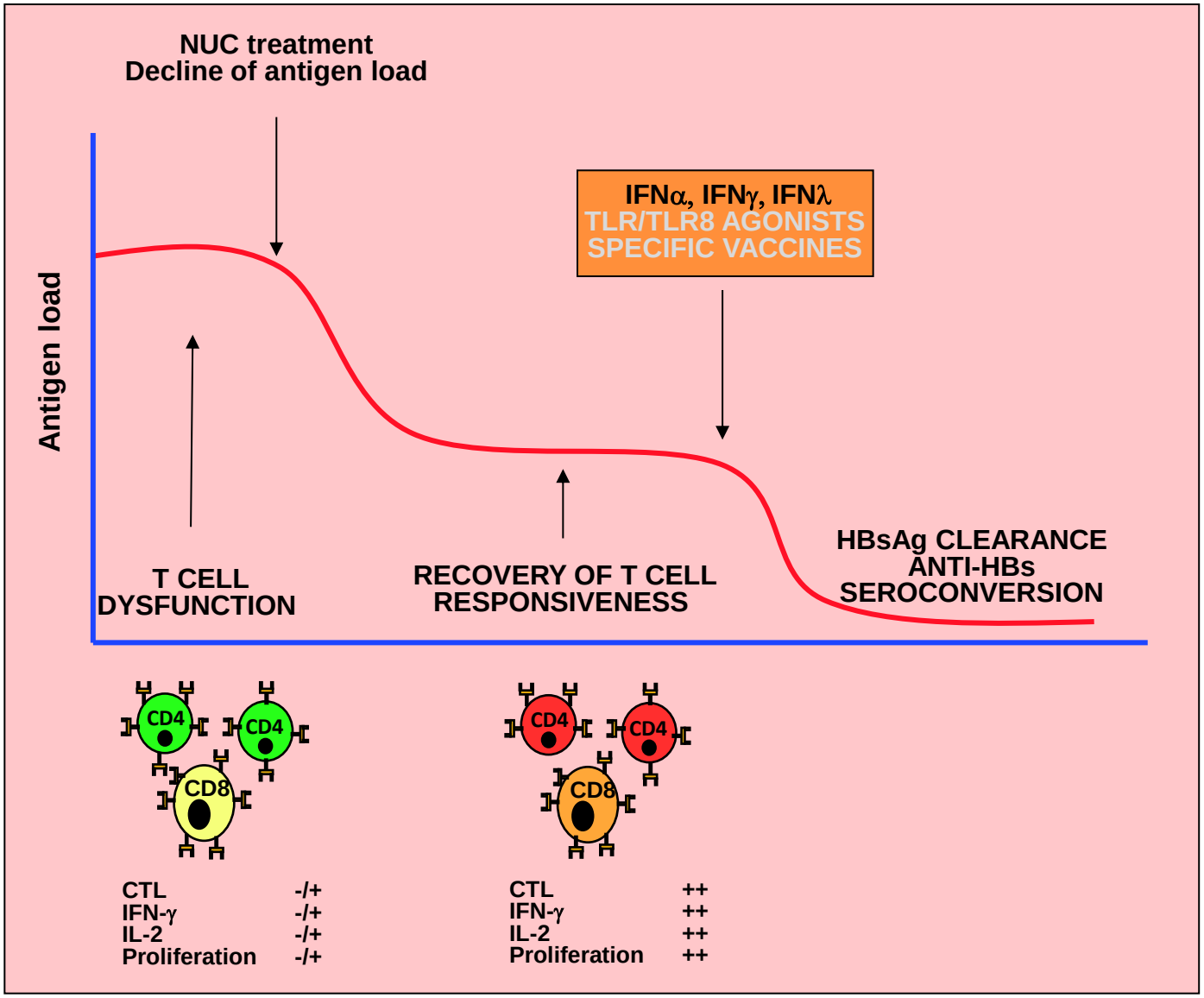
Potential strategy to shorten NUC therapies



Modified from: Ferrari C. Gastroenterology 2008

SEQUENCIAL NUC/IFN-α THERAPY

Potential strategy to optimize IFN-α efficacy and to shorten NUC therapies



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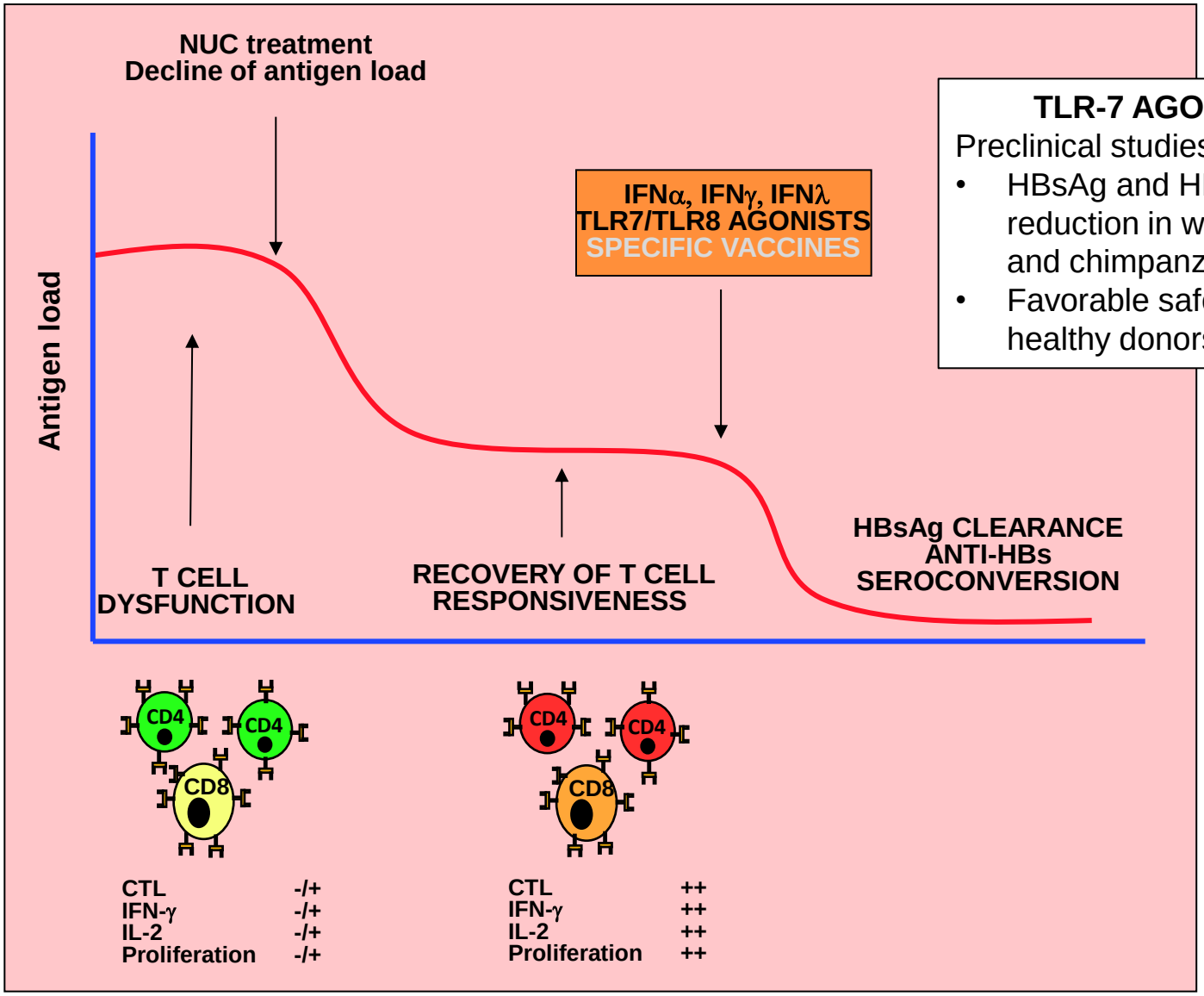


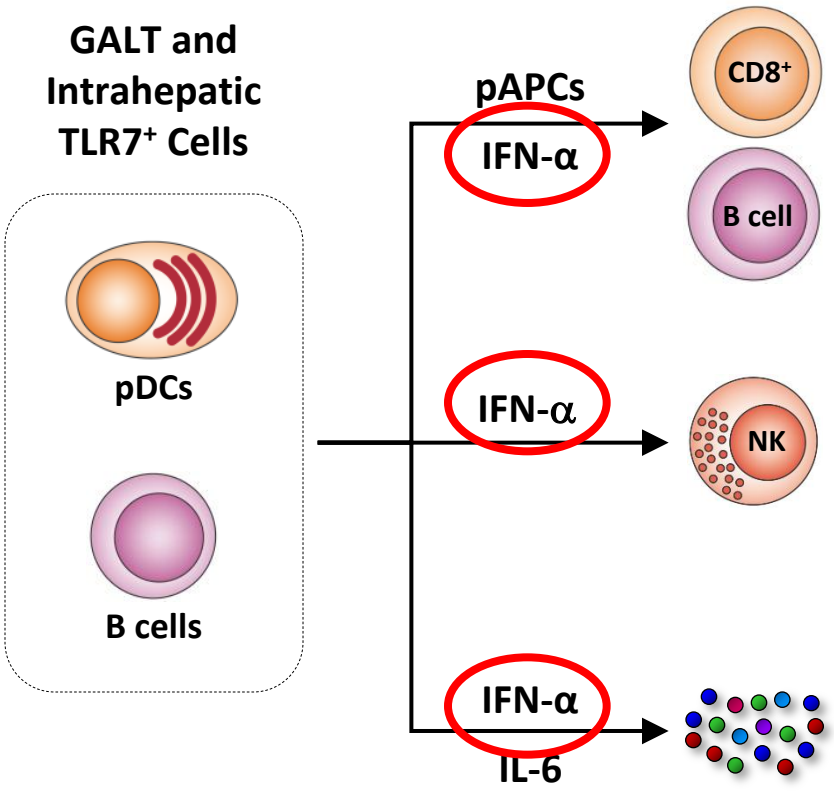
TLR-7 AGONISTS

Preclinical studies:

- HBsAg and HBV-DNA reduction in woodchucks and chimpanzees
- Favorable safety profile in healthy donors

Modified from: Ferrari C. Gastroenterology 2008

Rationale for TLR7 Targeting



❶ Adaptive immunity

-Kill infected cells

-Antiviral cytokines (e.g. IFN-γ)

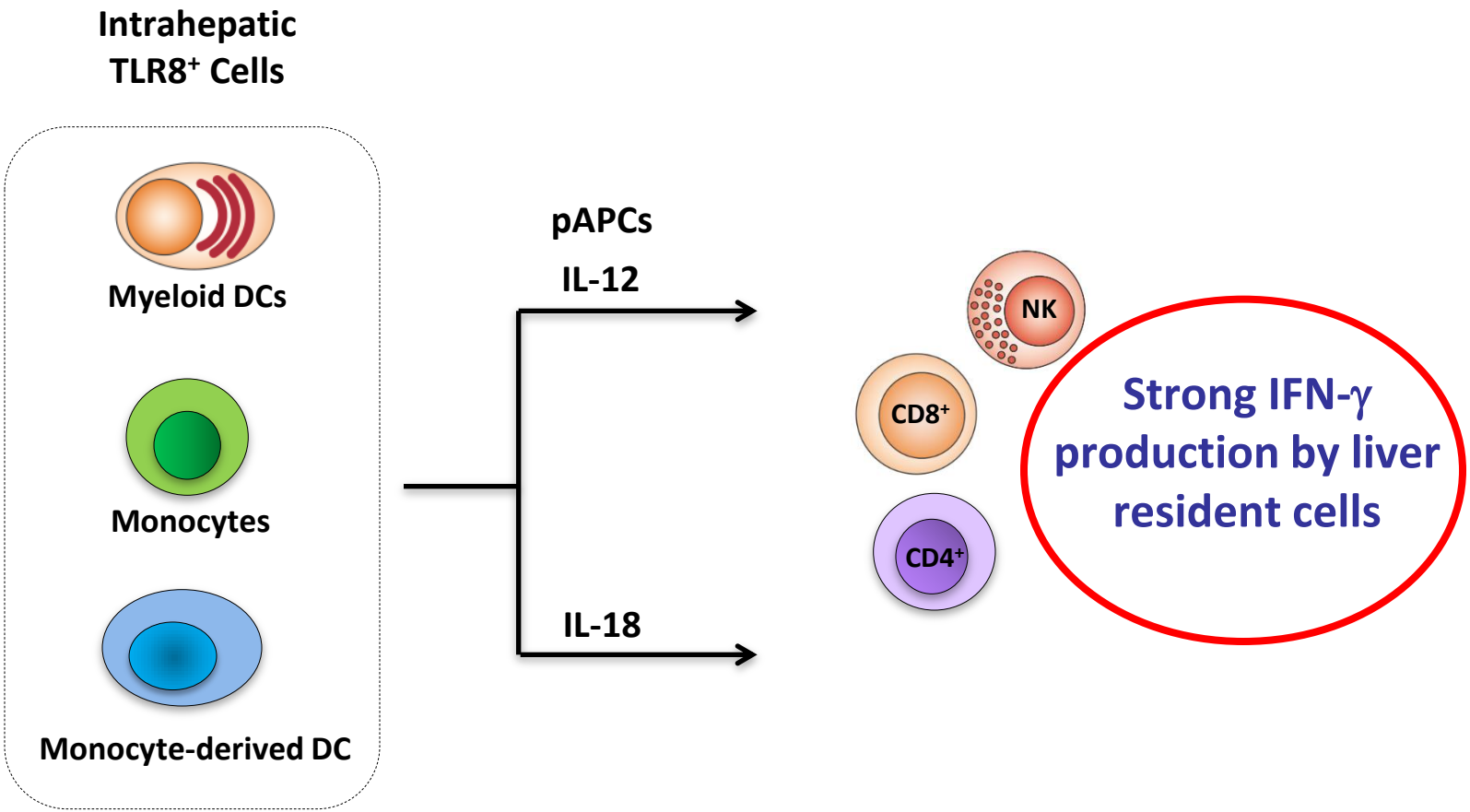
-Neutralizing antibodies

❷ Innate Immunity

- Kill infected cells
- Antiviral cytokines (e.g. IFN-γ)

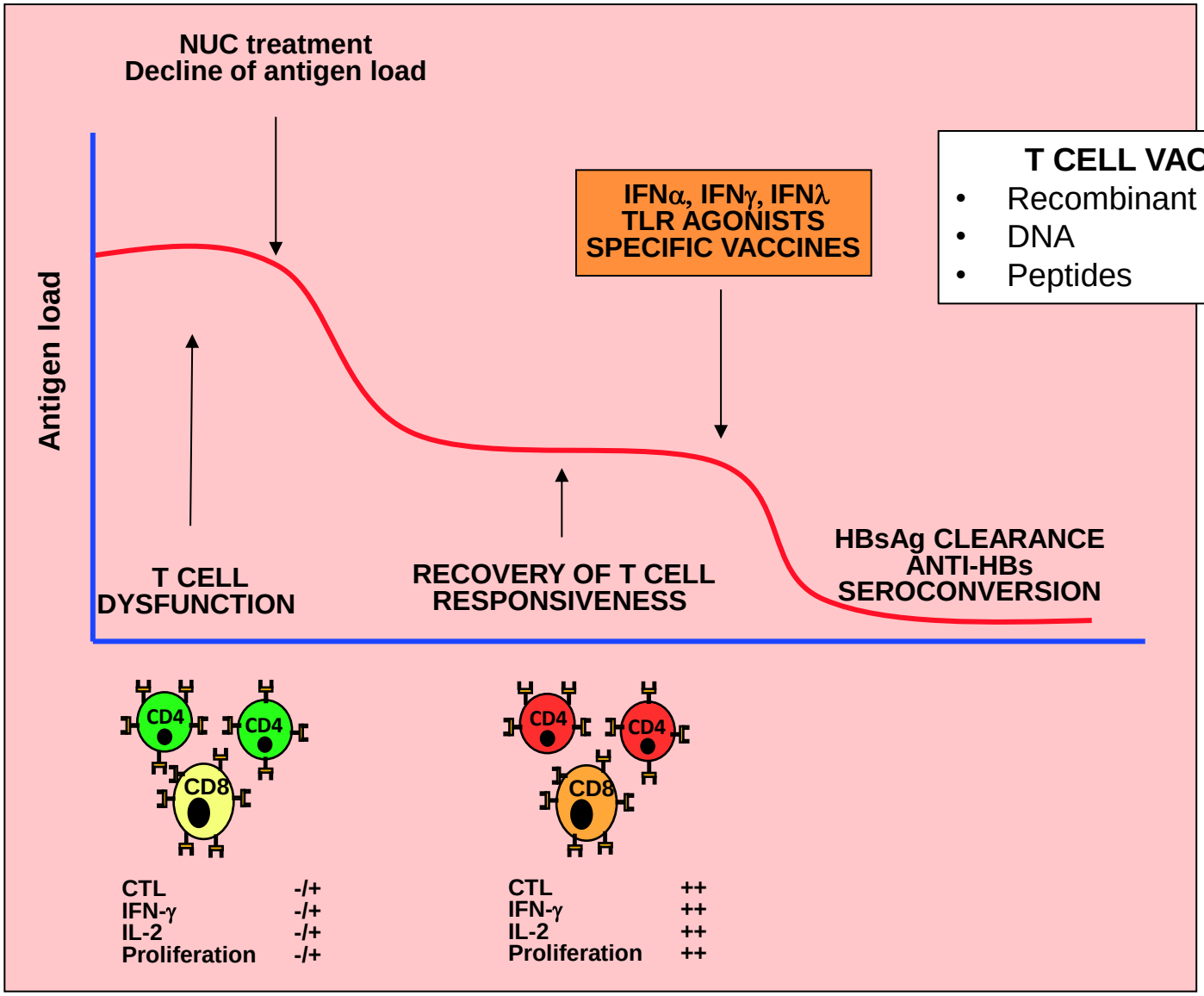
❸ Antiviral cytokines

Rationale for TLR8 Targeting



SEQUENCIAL NUC/IFN-α THERAPY

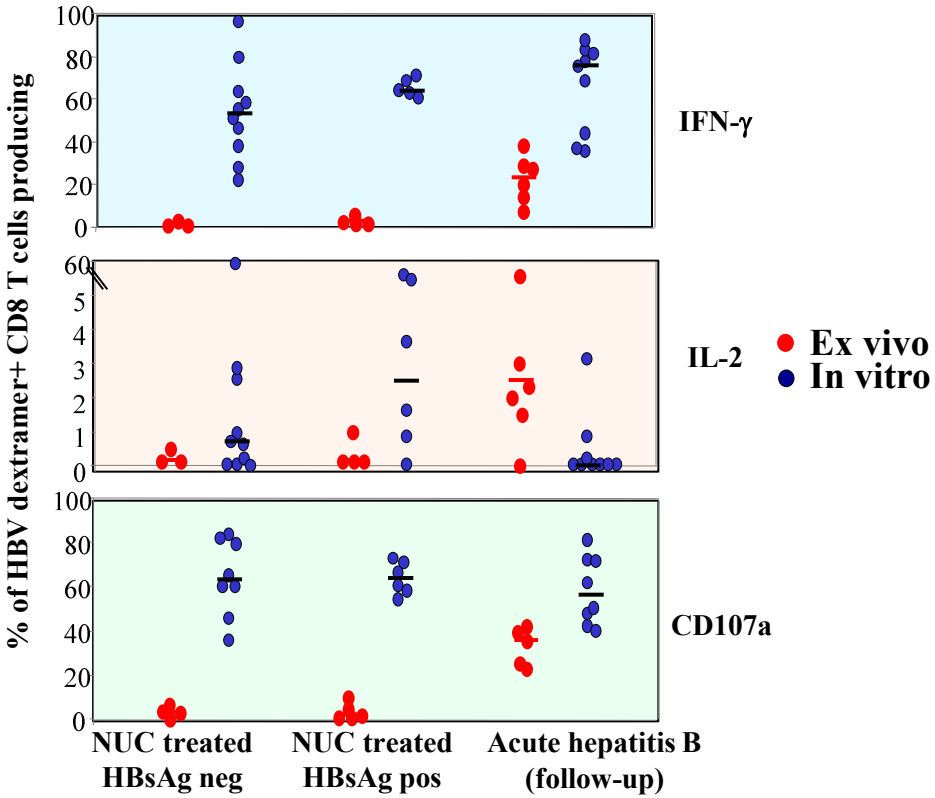
Potential strategy to optimize IFN-α efficacy and to shorten NUC therapies

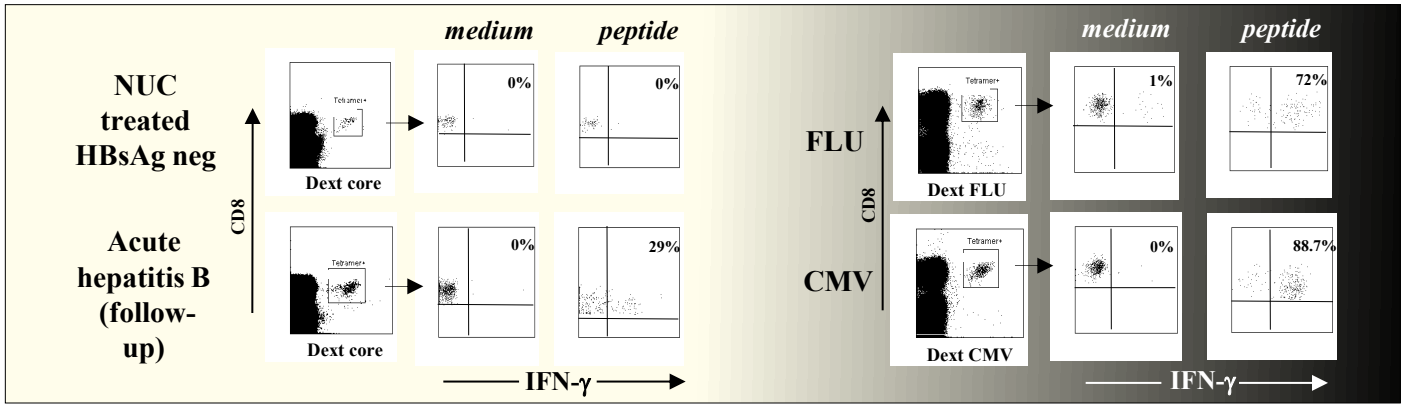


Modified from: Ferrari C. Gastroenterology 2008

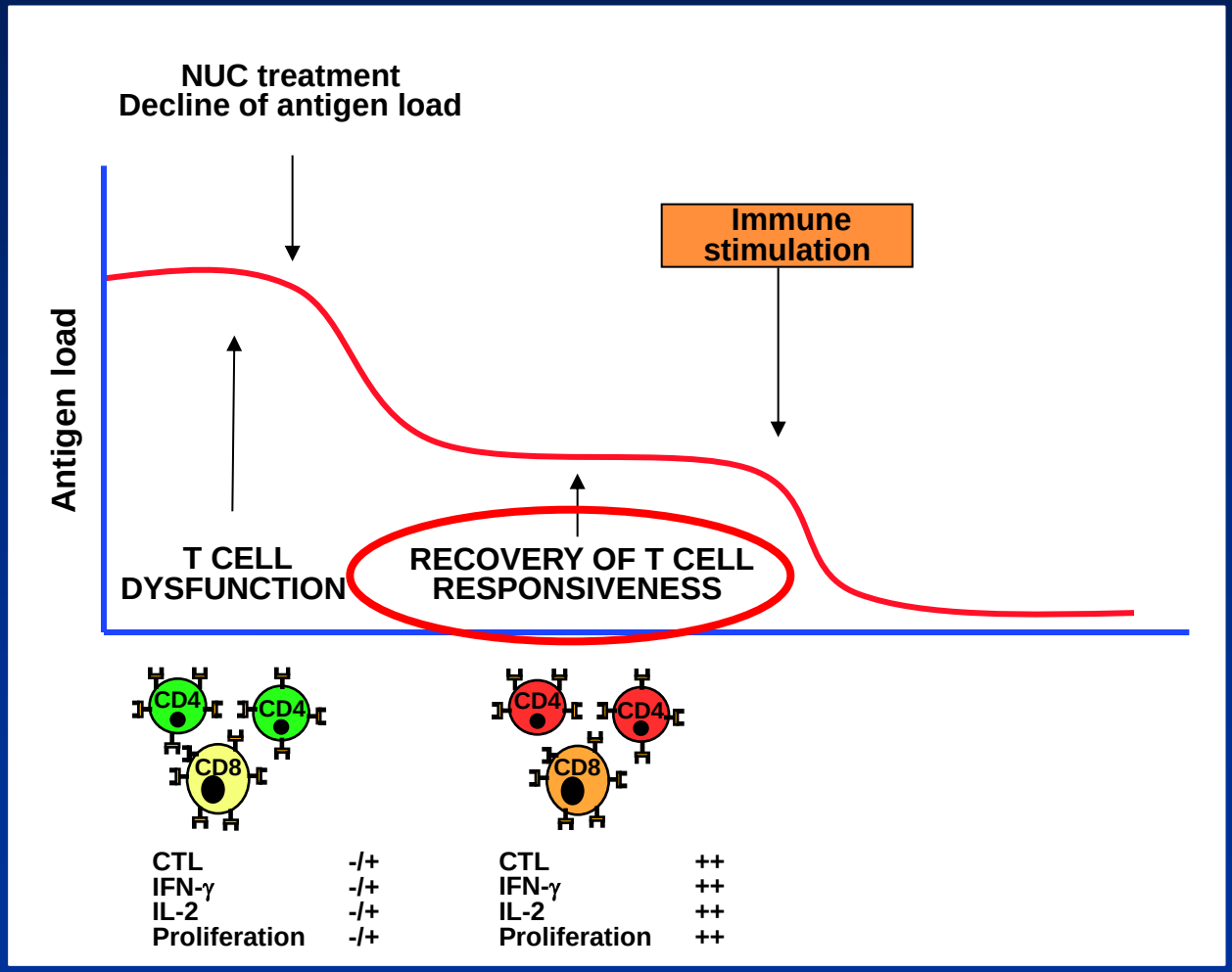
CD8 cell restoration is less efficient ex vivo upon NUC treatment

Boni C. et al. Gastroenterology 2012



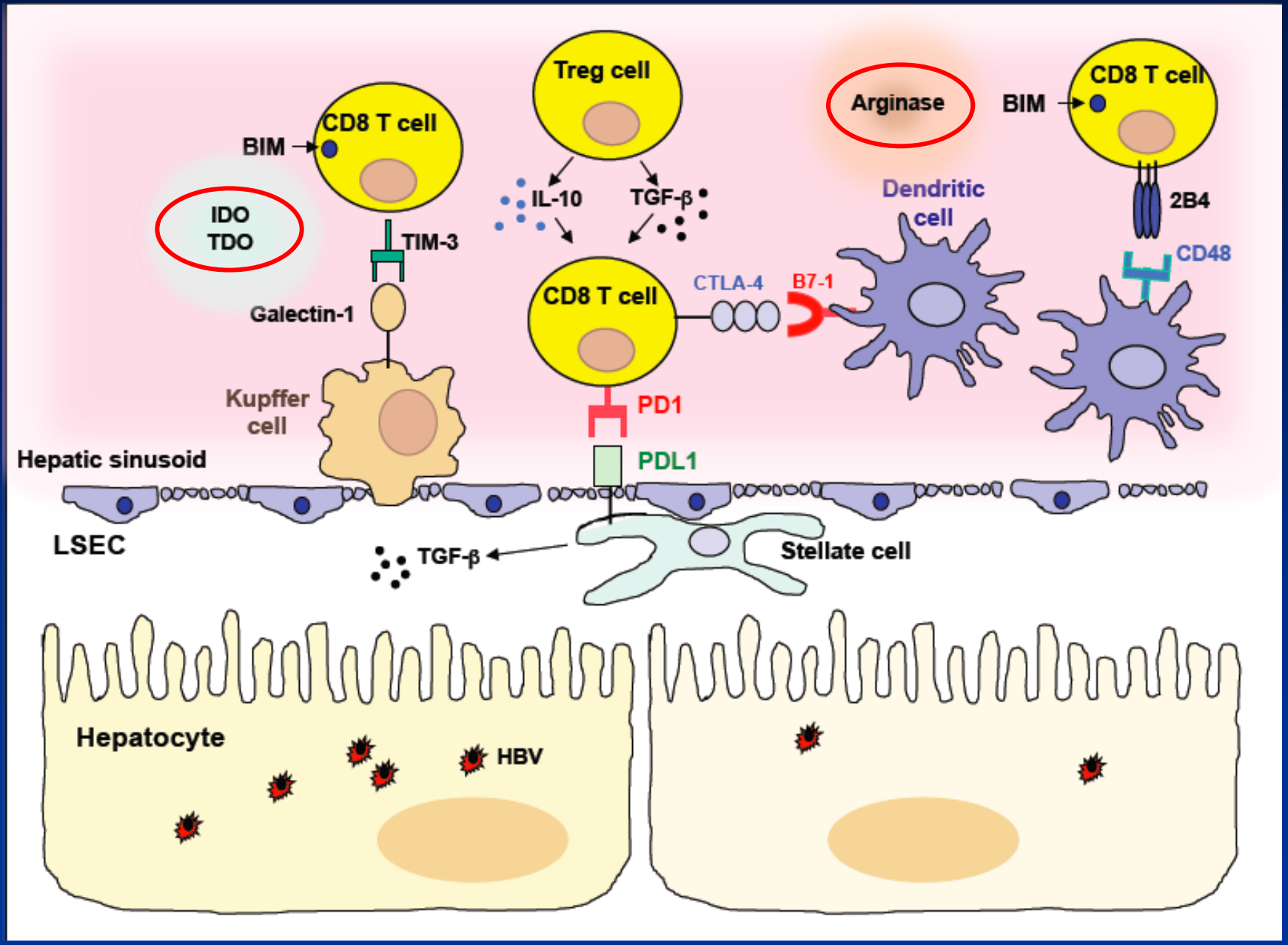


Are there other possible strategies to improve the functional T cell restoration induced by antigen decline?



INTRAHEPATIC INHIBITORY MECHANISMS

(*modified from* U. Protzer et al. Nature Reviews in Immunology 2012)



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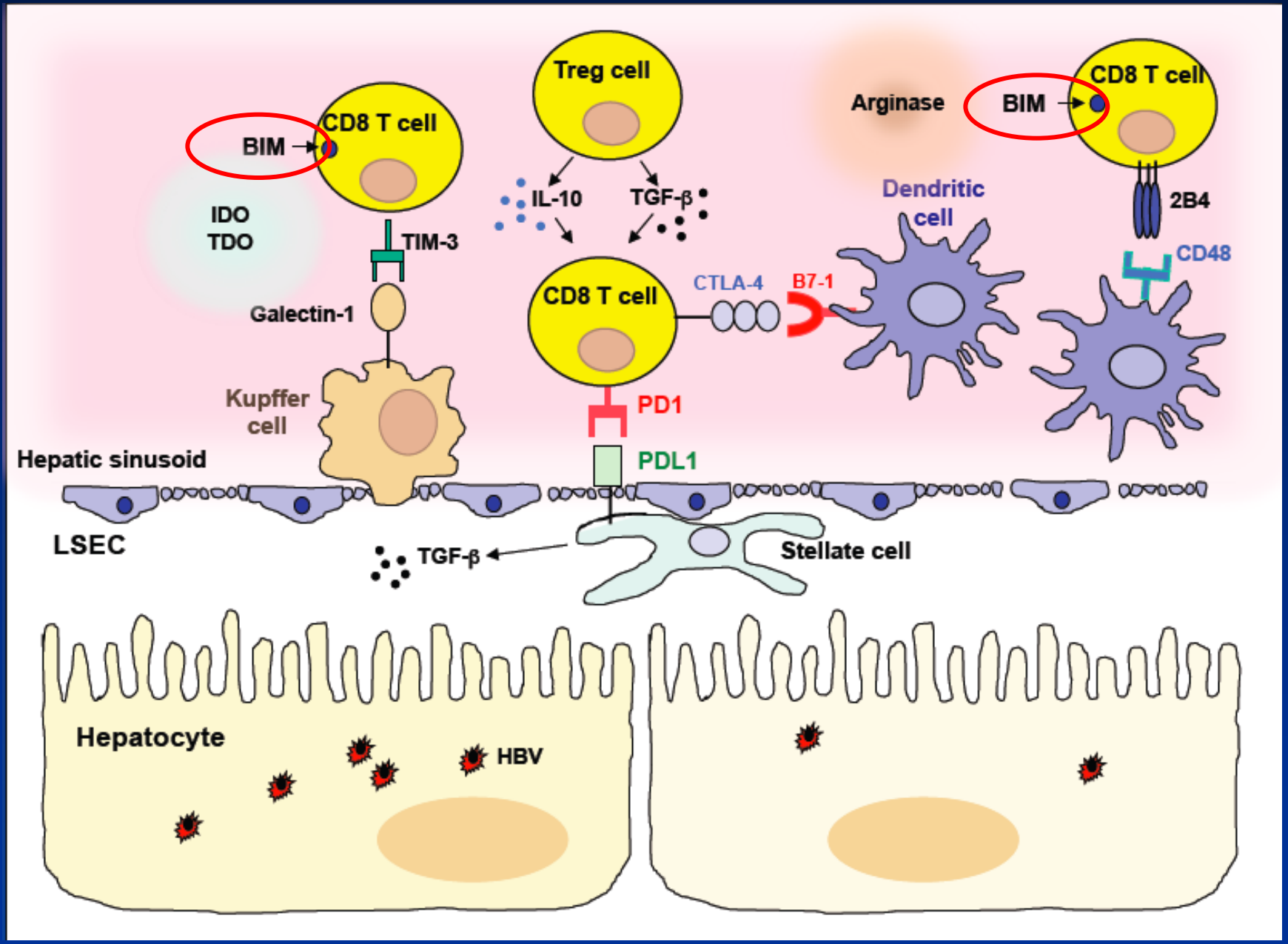

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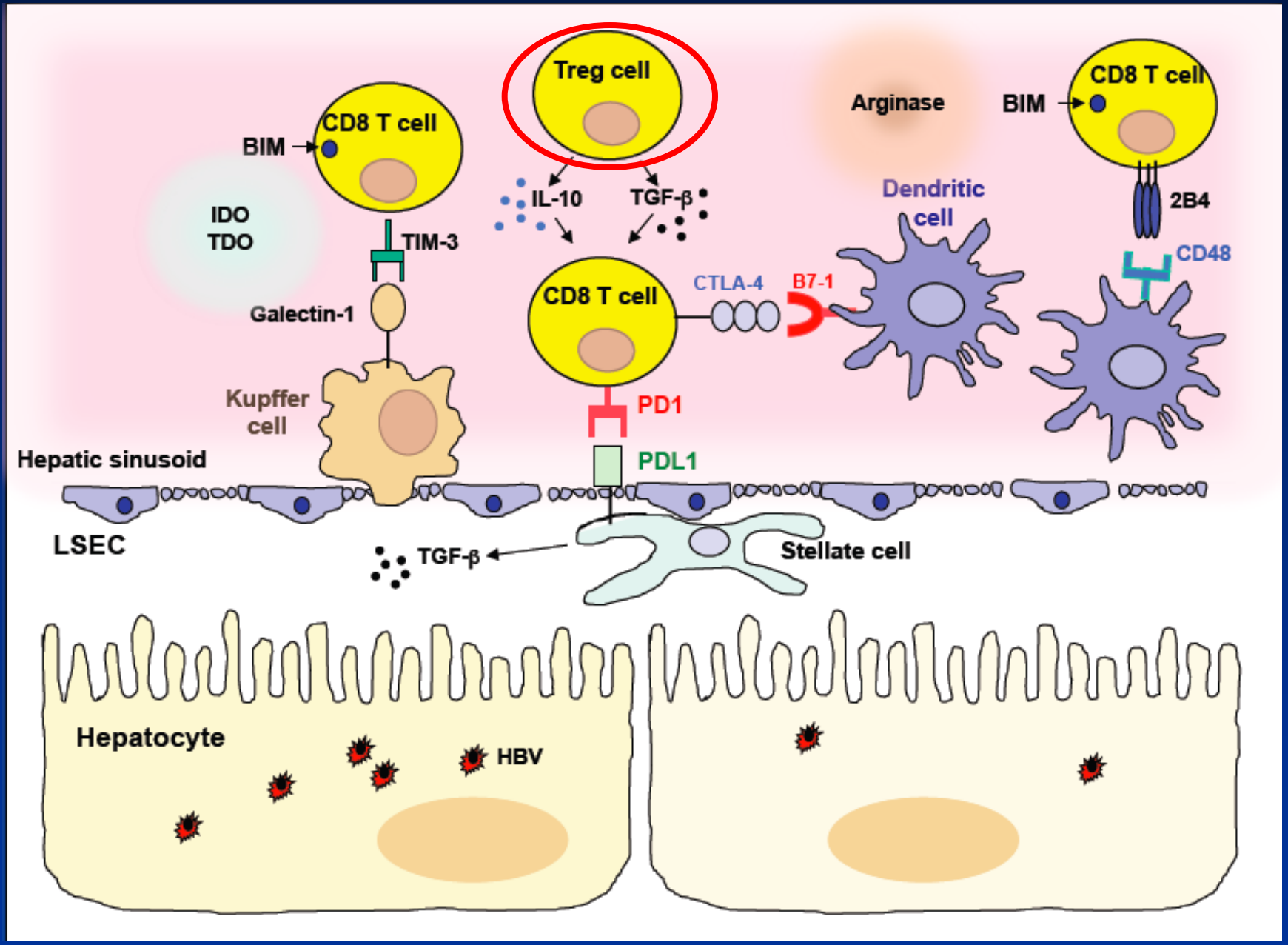

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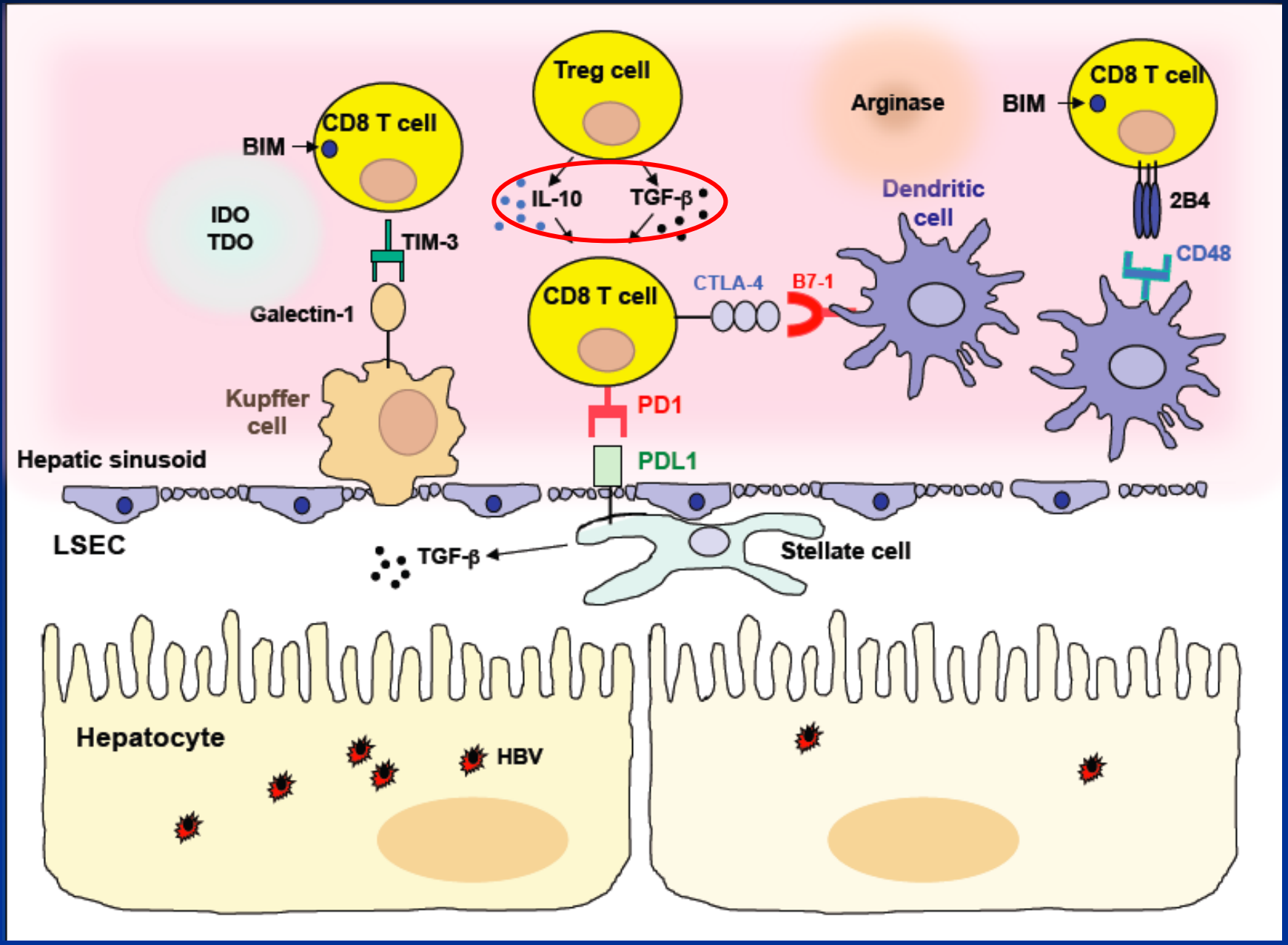

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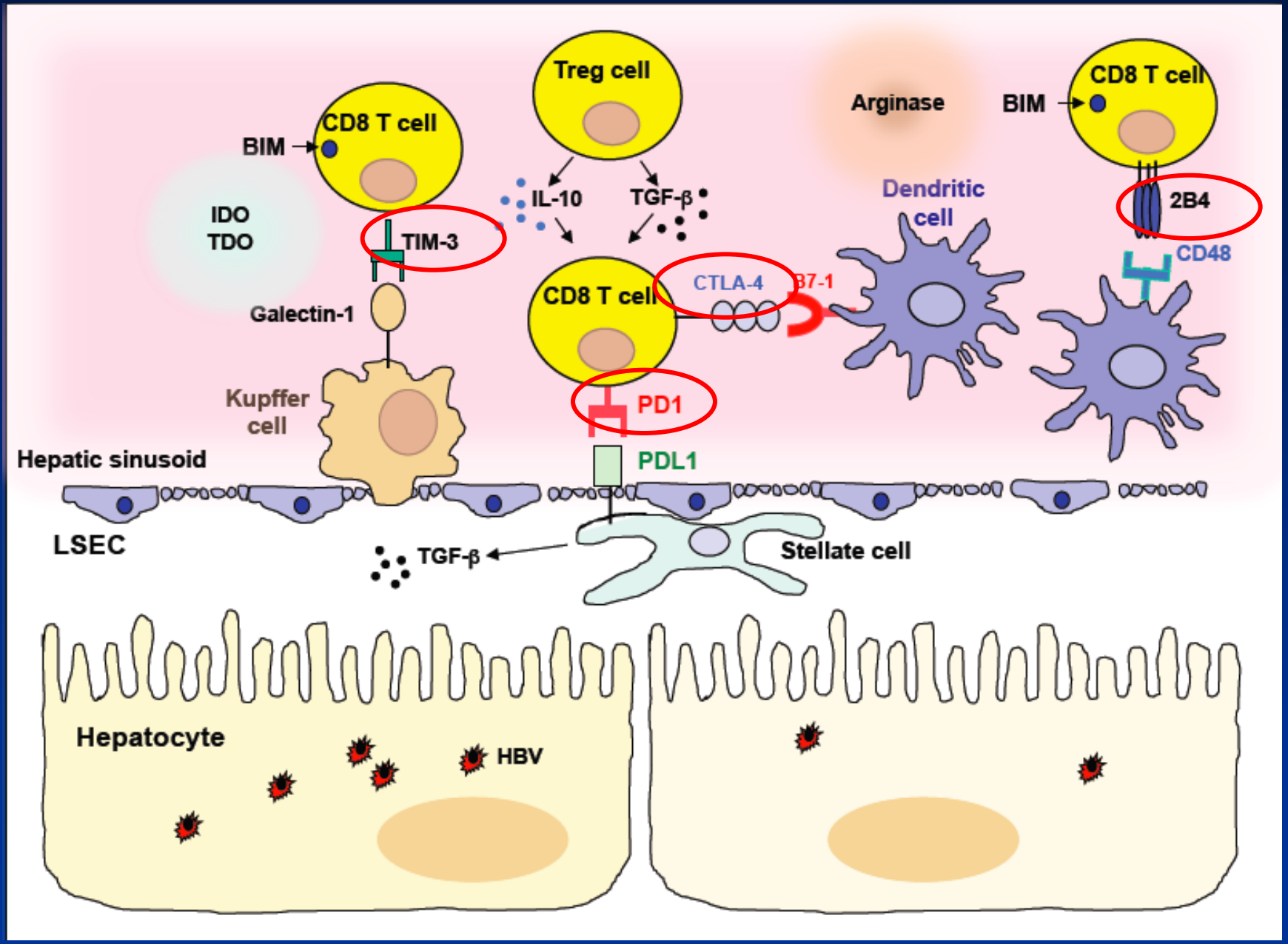
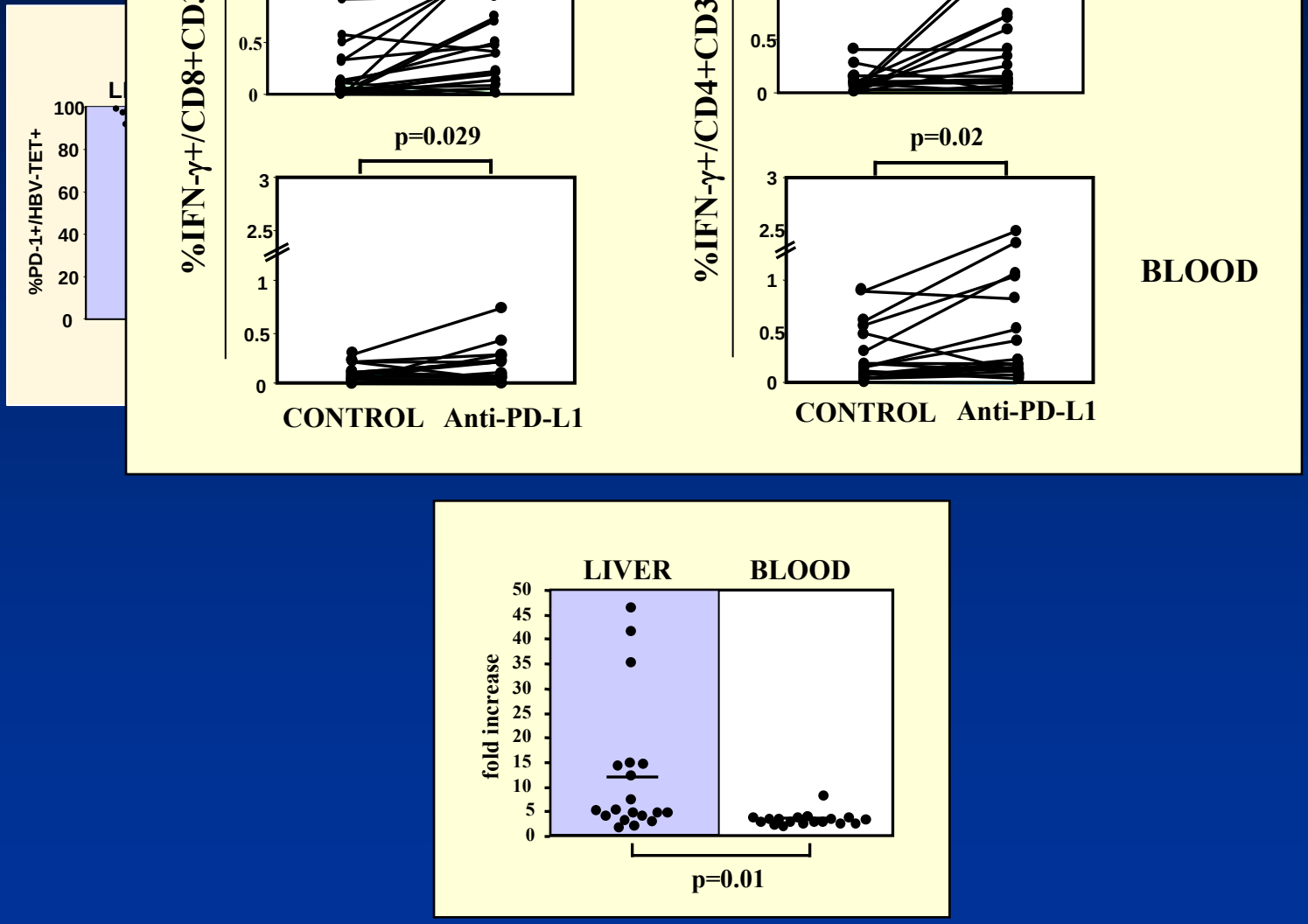
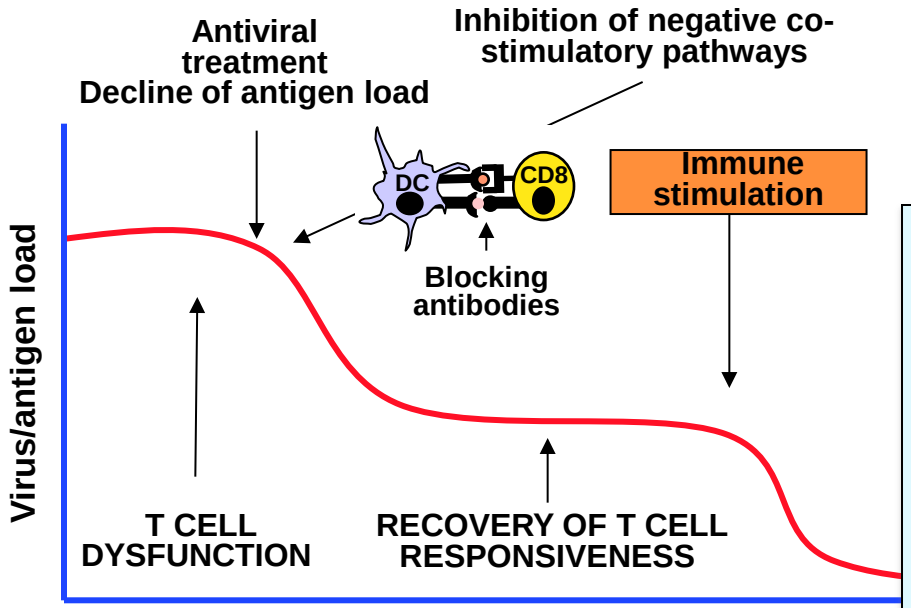


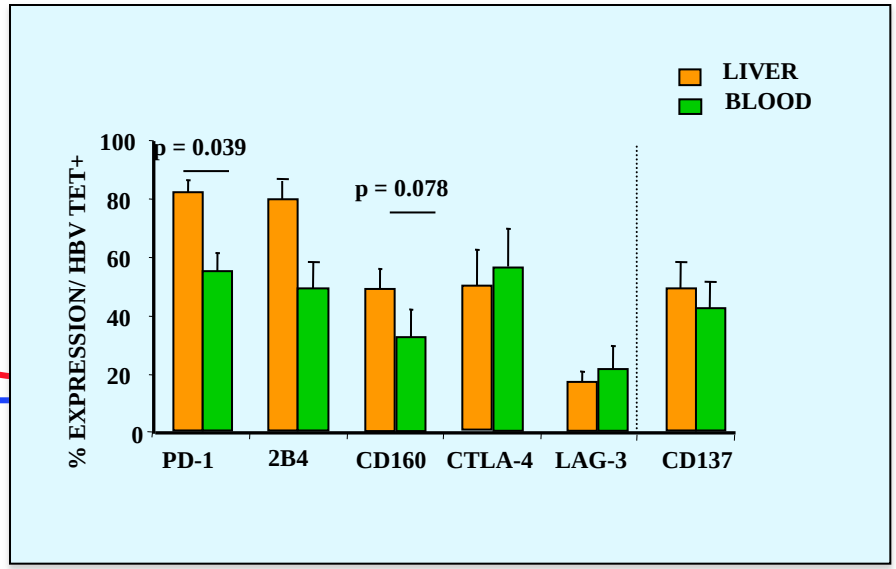
Expression of PD-1 and CD127 on circulating and intrahepatic virus-sp…on and

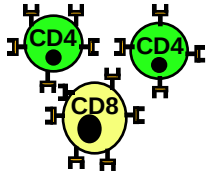


Fisicaro P. et al. Gastroenterology 2010, 2012

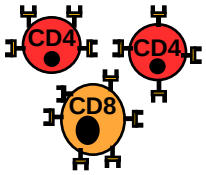
Future perspectives of anti-HBV immune therapy





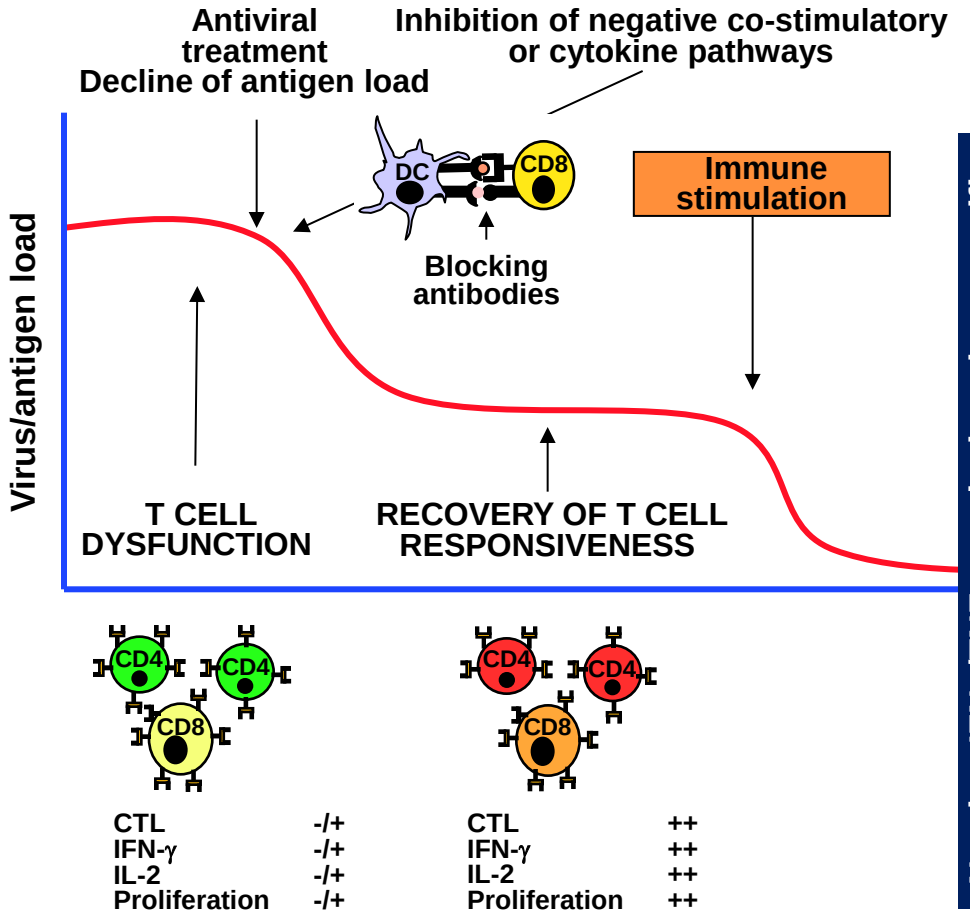


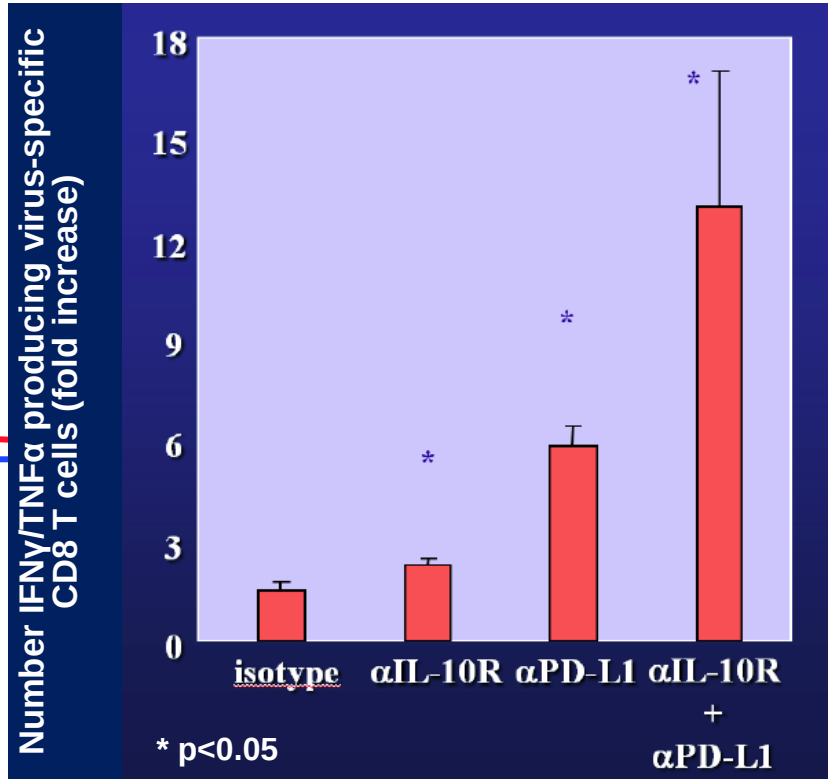
CTL	-/+
IFN-γ	-/+
IL-2	-/+
Proliferation	-/+



CTL	++
IFN-γ	++
IL-2	++
Proliferation	++

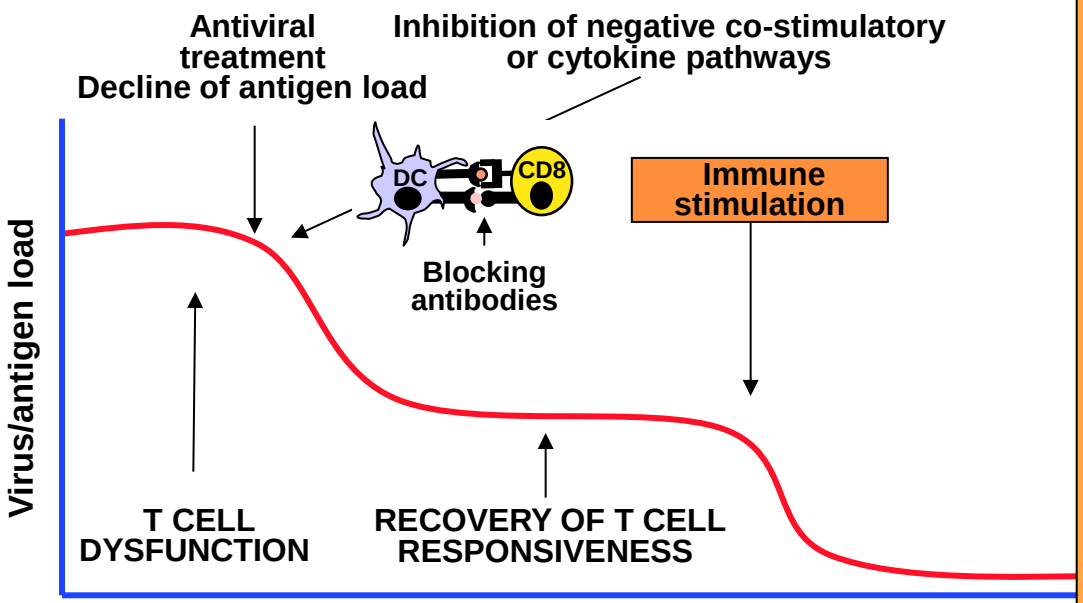
Future perspectives of anti-HBV immune therapy

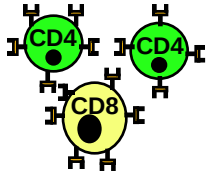




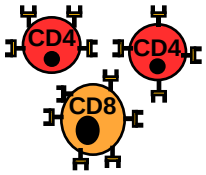
Adapted from: Ferrari C. *Gastroenterology* 2008;**134**:1601-1614. 2. Brooks DG, et al. *PNAS* 2008;**105**:20428-20433.

Future perspectives of anti-HBV immune therapy



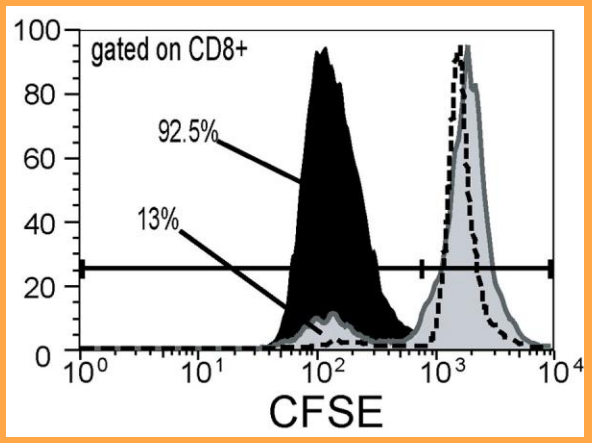


CTL	-/+
IFN-γ	-/+
IL-2	-/+
Proliferation	-/+



CTL	++
IFN-γ	++
IL-2	++
Proliferation	++

Arginase and HBV infection: role for arginase inhibitors



medium+L-arginine

medium-L-arginine

Das A et al. J. Exp. Med. 2008

Adapted from: Ferrari C. *Gastroenterology* 2008;**134**:1601-1614. 2. Brooks DG, et al. *PNAS* 2008;**105**:20428-20433.

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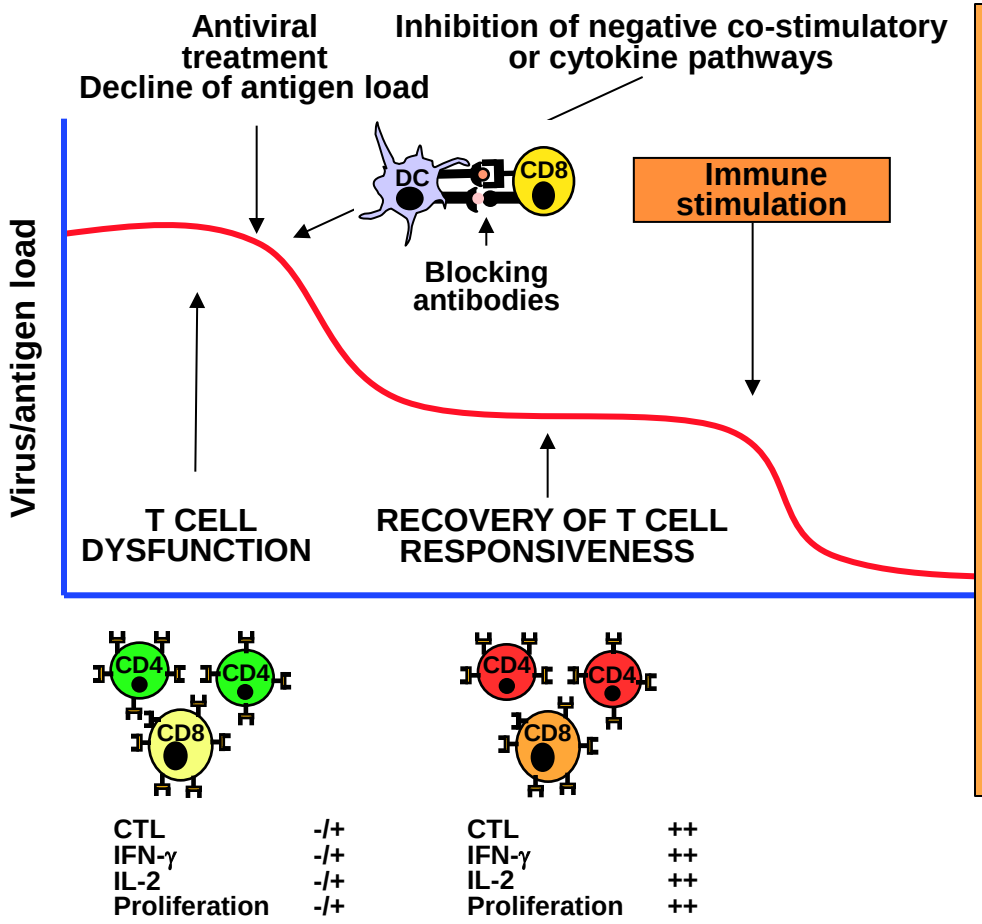
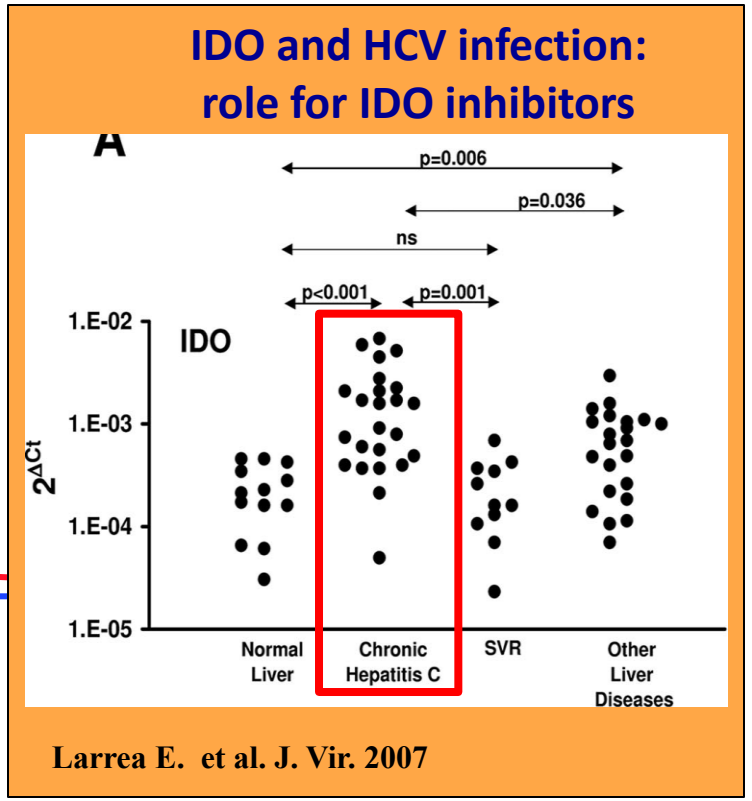




Adapted from: Ferrari C. *Gastroenterology* 2008;**134**:1601-1614. 2. Brooks DG, et al. *PNAS* 2008;**105**:20428-20433.

HBV-specific T cell dysfunction

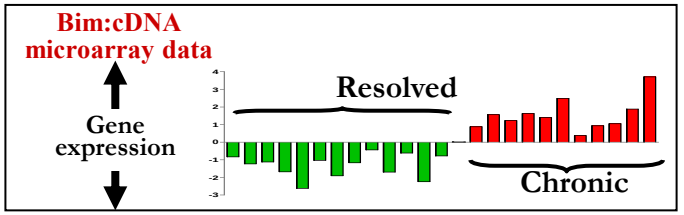
Possible strategies to overcome it

Persistent exposure to high Ag doses
Persistent inflammation

Amplification of negative costimulatory pathways

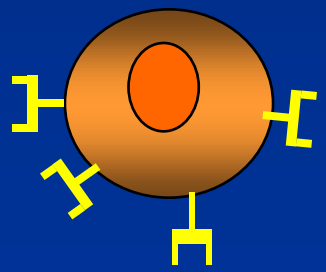
Blocking antibodies

Up-regulation of Bim in HBV-specific CD8 from chronic infection



Lopes AR et al. J. Clin. Invest, 2008

Anti-apoptotic drugs



Restoration of HBV-specific T cell functions

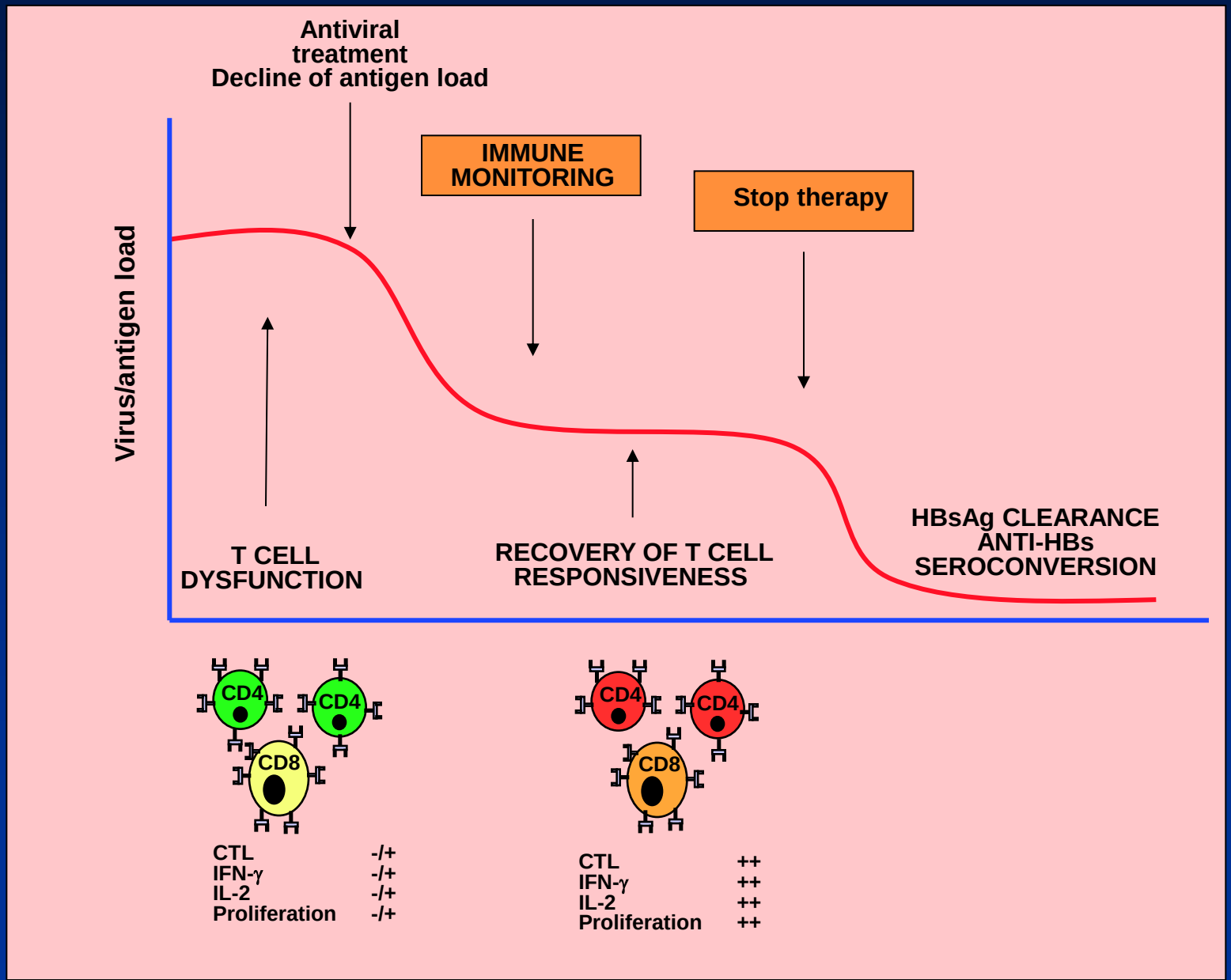
Clinical need in HBV therapy for HBeAg negative CH-B

- To shorten NUC therapy
  - Combination therapies to accelerate HBsAg clearance
  - Reliable predictors of efficient immune control

SEQUENCIAL THERAPY

Strategies to accelerate HBsAg clearance and shorten NUC therapies

(*Modified from:* Ferrari C. Gastroenterology 2008)

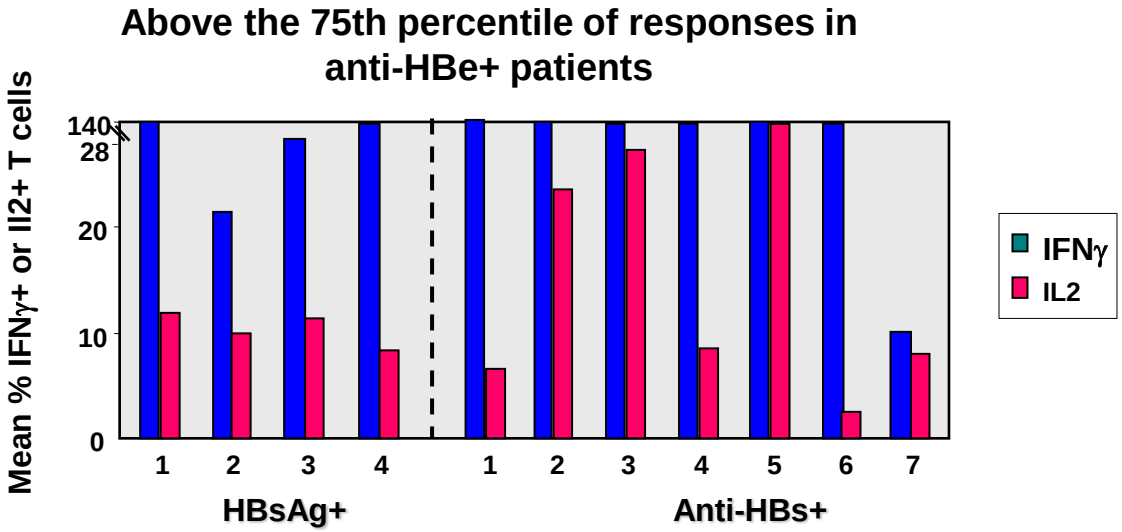


IMMUNOMONITORING

Strategies to shorten NUC therapies

(Boni C et al. Gastroenterology 2012)

A subgroup of NUC treated HBsAg positive patients show levels of IL2 and IFN-γ production comparable to those of NUC treated anti-HBs positive patients with an optimal restoration of T cell functions (around 20%)



IMMUNOMONITORING

Immune control for a safe NUC withdrawal

HBV-specific T cell function

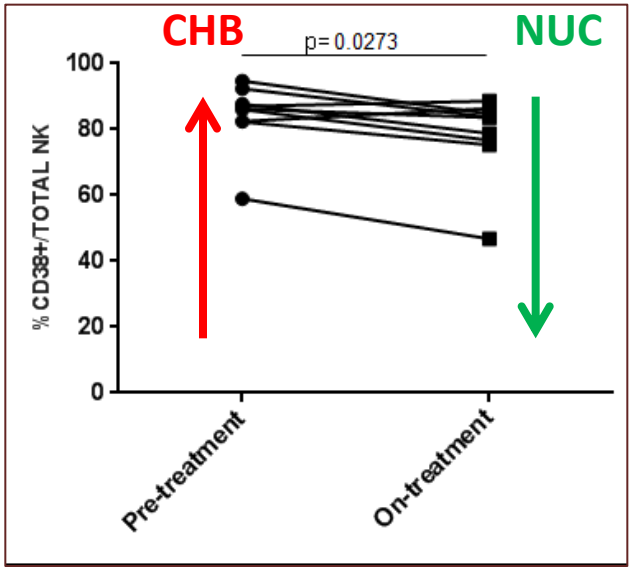
Daily clinical practice: too complex

NK cell phenotype and/or function

Daily clinical practice: can they be used?

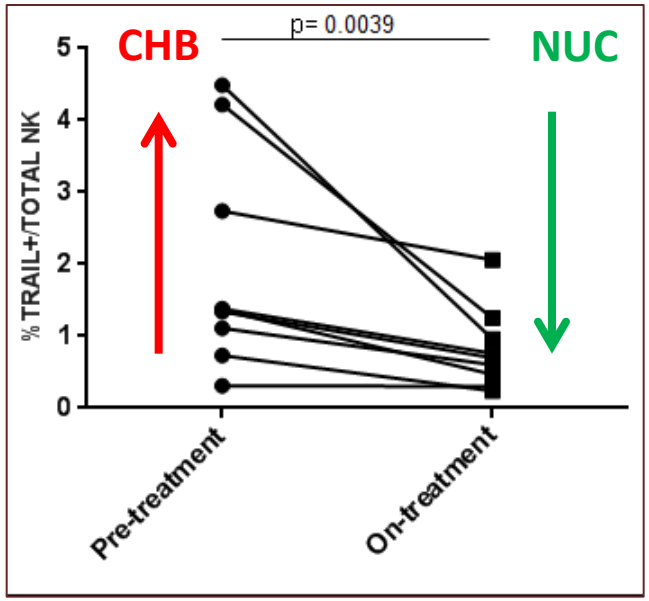
NK CELLS

CD38

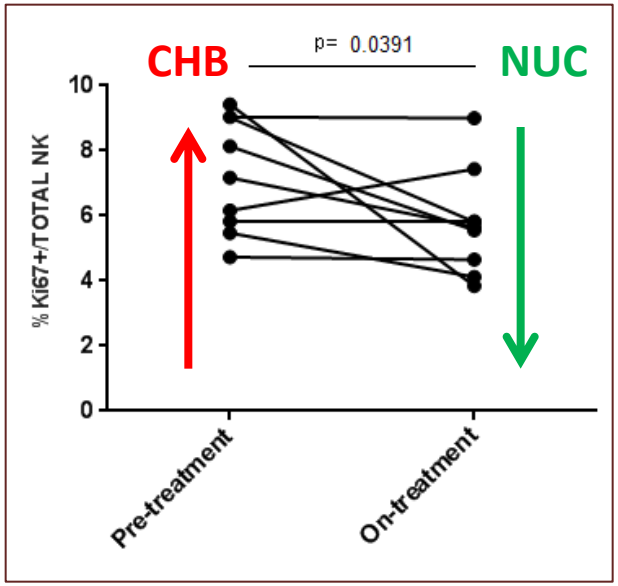


NORMALIZATION OF INFLAMMATORY STATUS INDUCED BY NUC THERAPY

TRAIL



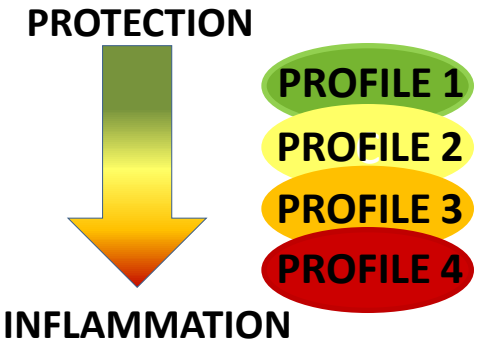
Ki67



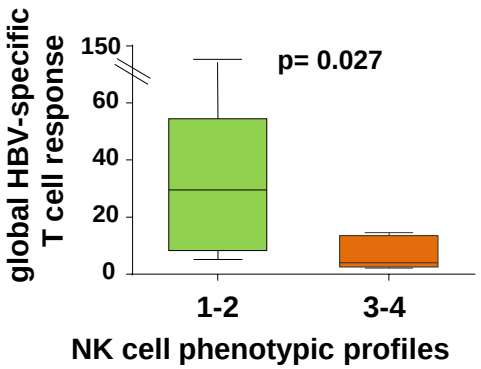
SIGNIFICANT DECREASE OF CD38, TRAIL, Ki67 EXPRESSION ON TREATMENT

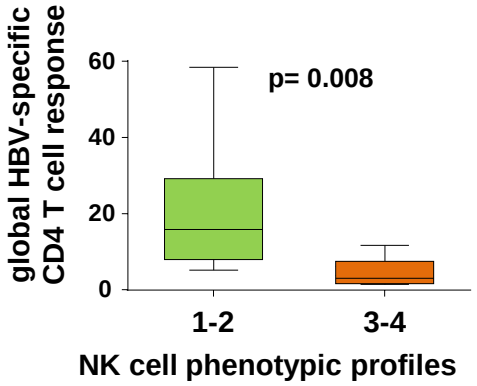
Boni C. et al. 2015 submitted

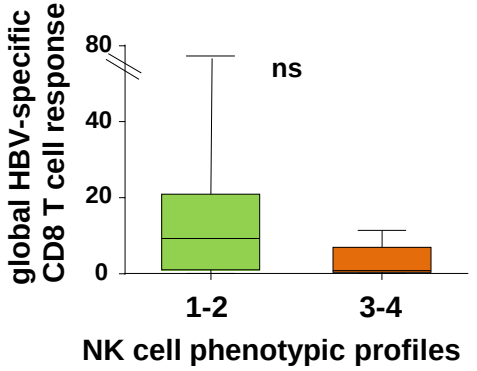
Association between NK cell phenotypic profiles and T cell responses

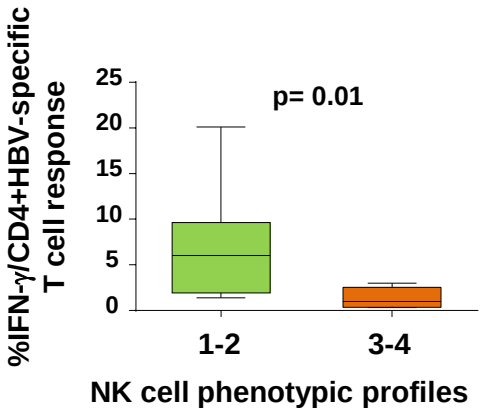


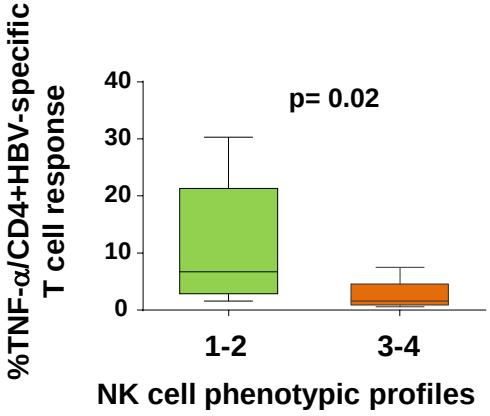
NUC-treated

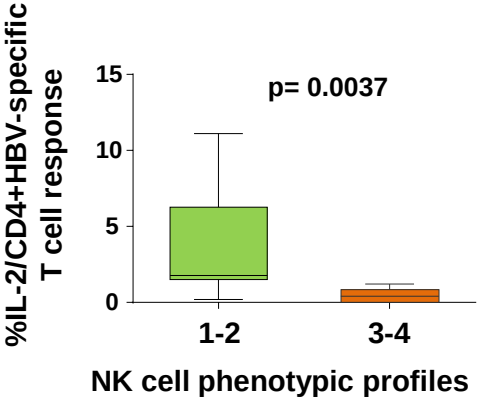












Boni C. et al. 2015 submitted

IMMUNOMONITORING

Strategies to shorten NUC therapies

NK cell phenotype analysis may predict control of infection

because maturation of a resting phenotype is associated

with restoration of a better anti-viral T cell function

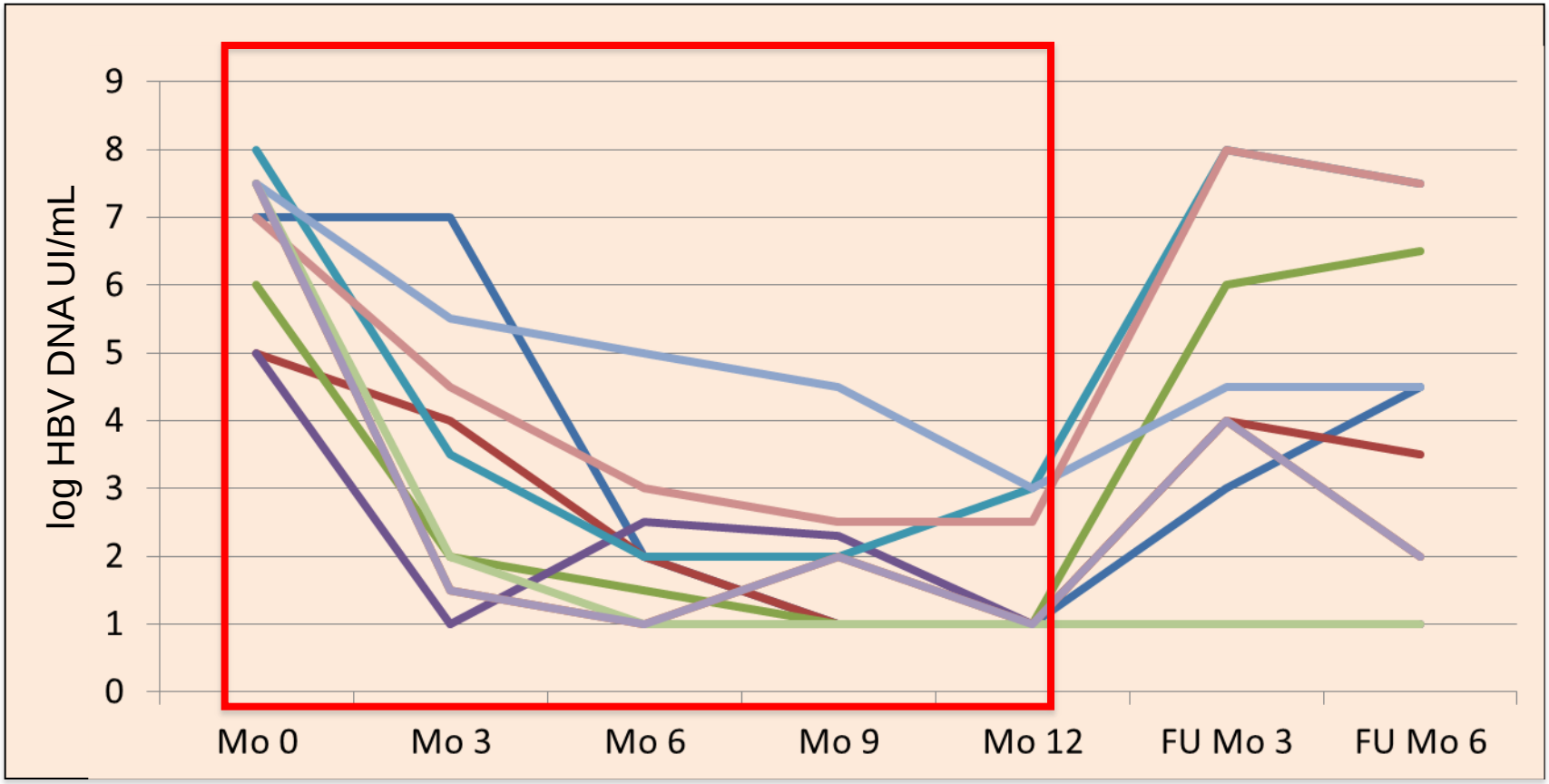
↓

BIO-MARKERS FOR AN EARLY THERAPY WITHDRAWAL ?

Clinical needs in HBV therapy for genotype D HBeAg negative CH-B

- How to shorten NUC therapy?
  - Combination therapies to accelerate HBsAg clearance
  - Predictors of efficient immune control
- How to consolidate and optimize IFN-α effect?

On-treatment effect of PEG-IFN therapy on HBV viremia in genotype D HBeAg negative CH-B

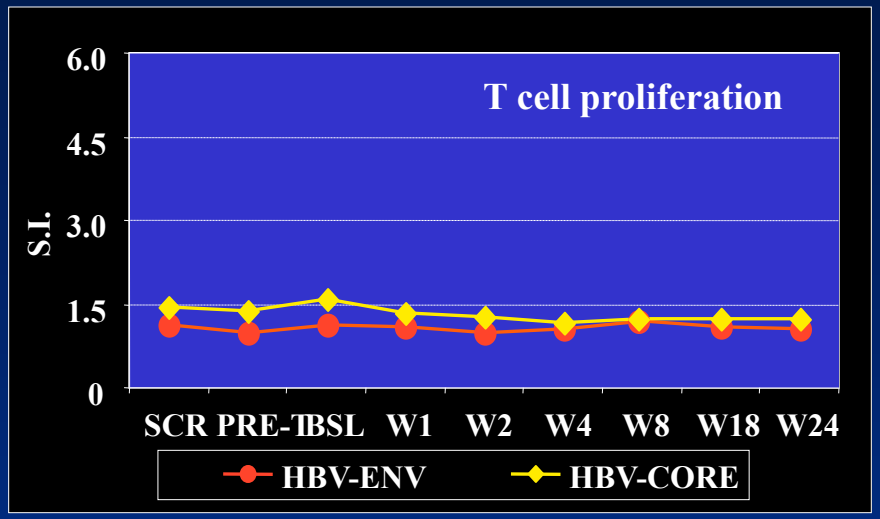


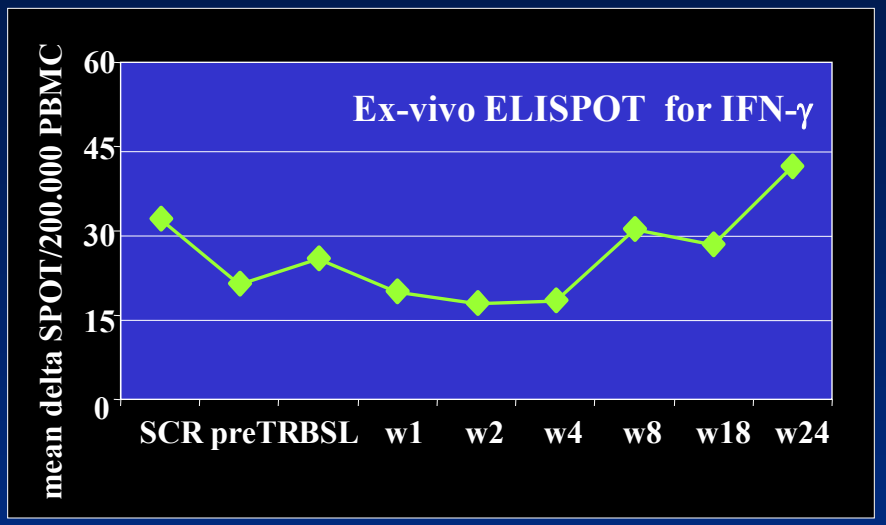
How can we improve PEG-IFN efficacy ?

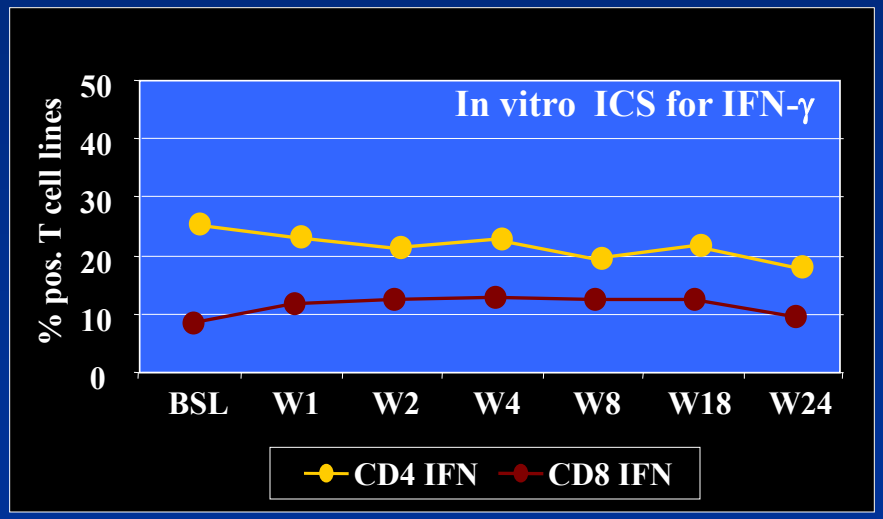
- Duration of therapy
- Pre-treatment predictors of response
- On-treatment predictors of response
- *De-novo* or *sequential* combination therapies

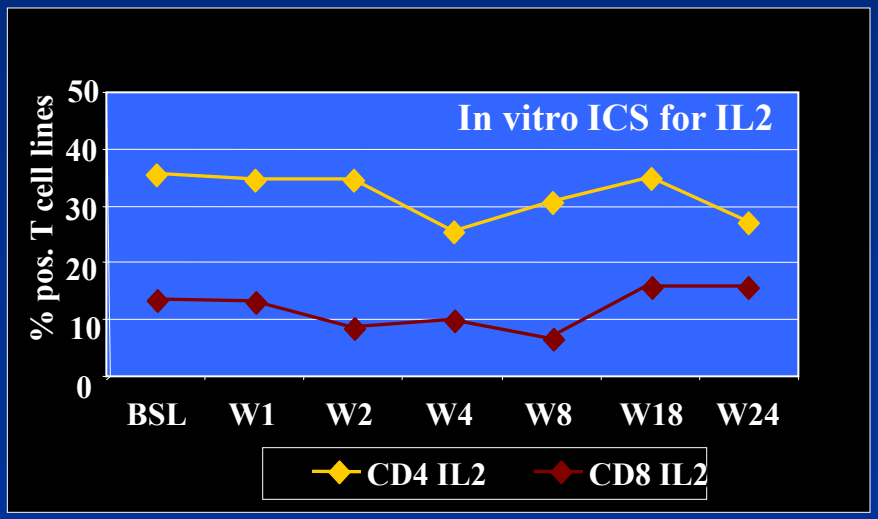
PBMC proliferation, ex-vivo ELISPOT, in vitro ICS before and during PEG-IFN therapy

15 anti-HBe+ patients



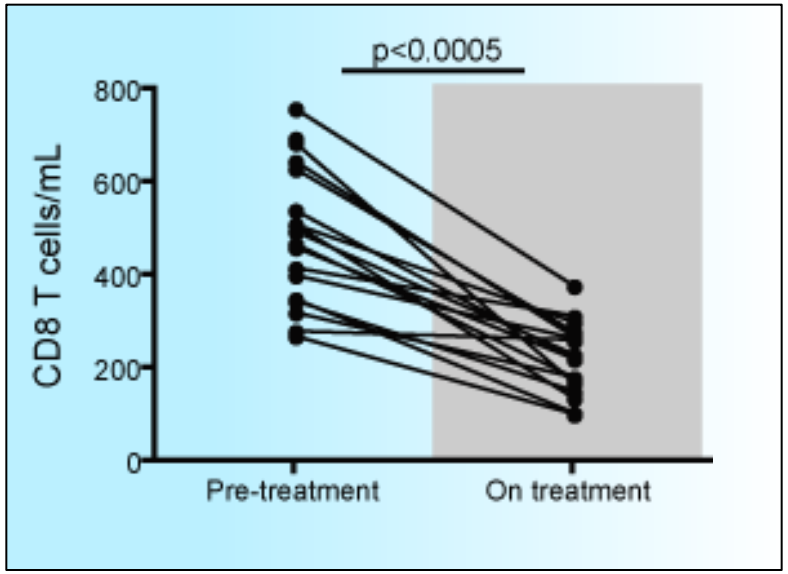


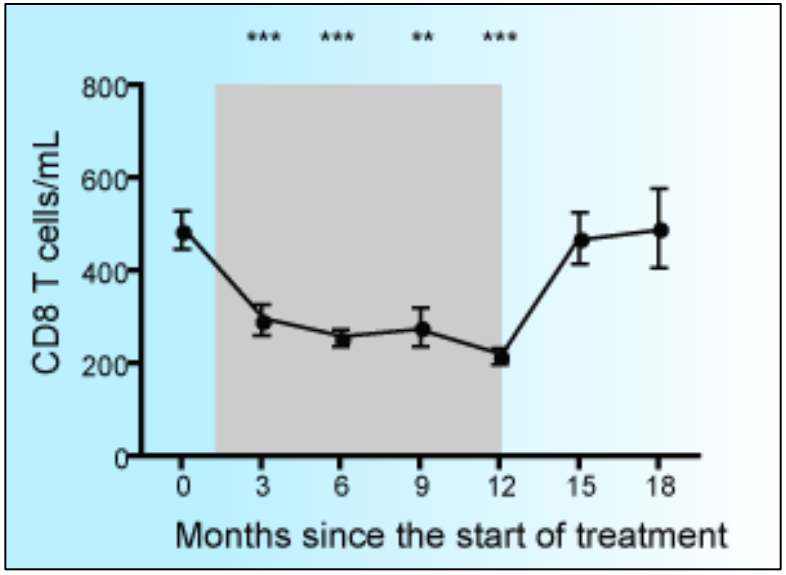




A. Penna et al J.Hepatol. 2012

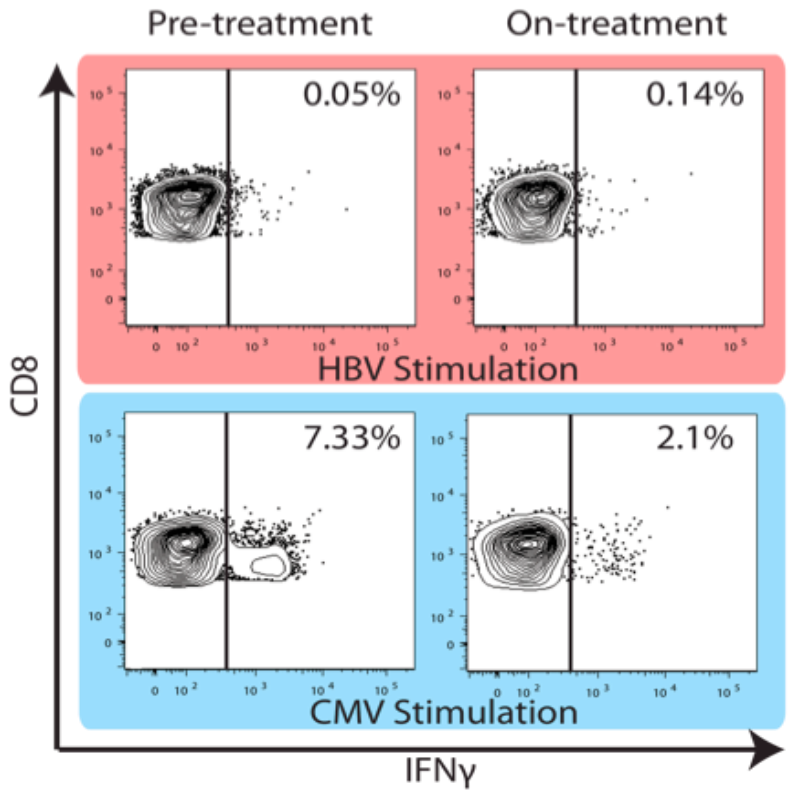
CD8 T cells are markedly reduced during Peg-IFNα treatment

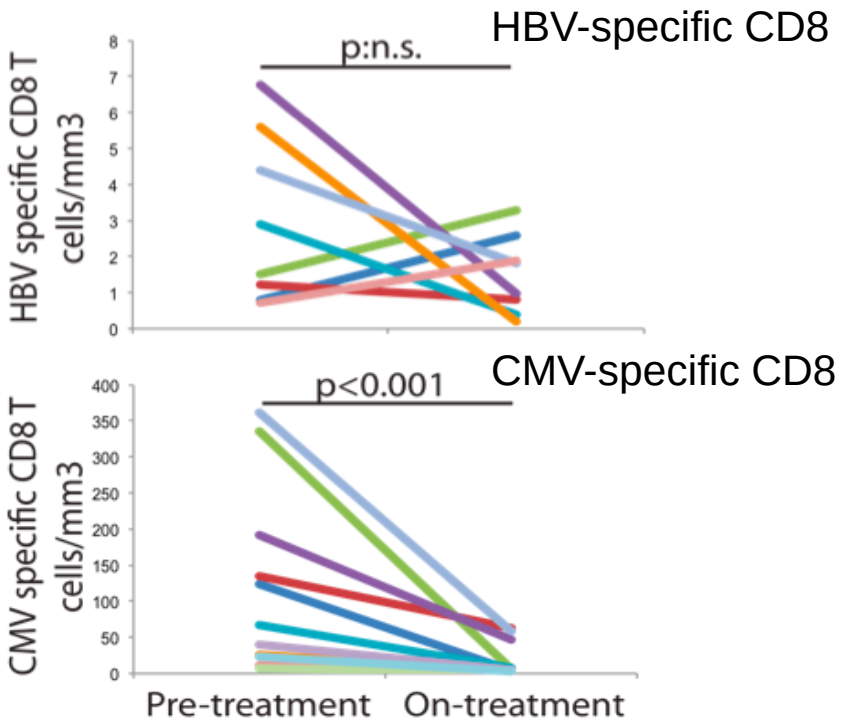




Micco L et al J. Hepatol. 2012

Pegylated interferon-alpha therapy does not reconstitute virus-specific T cell responses





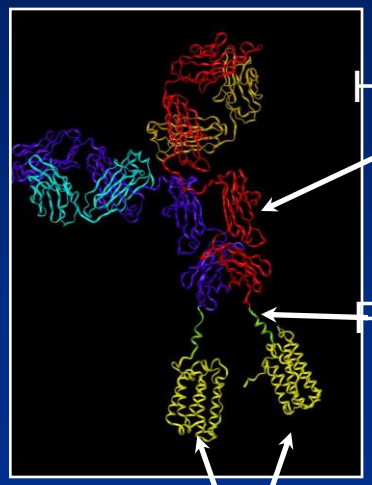
Micco L et al J. Hepatol. 2012

Possible immune therapeutic strategies to improve PEG-IFNa effect

- To target antiviral cytokines to the liver
- To generate in vitro functional autologous T cells to reinject in chronic patients

Therapeutic potential: cytokine delivery to HBV-infected cells

Changhua Ji, et al Hepatology 2012

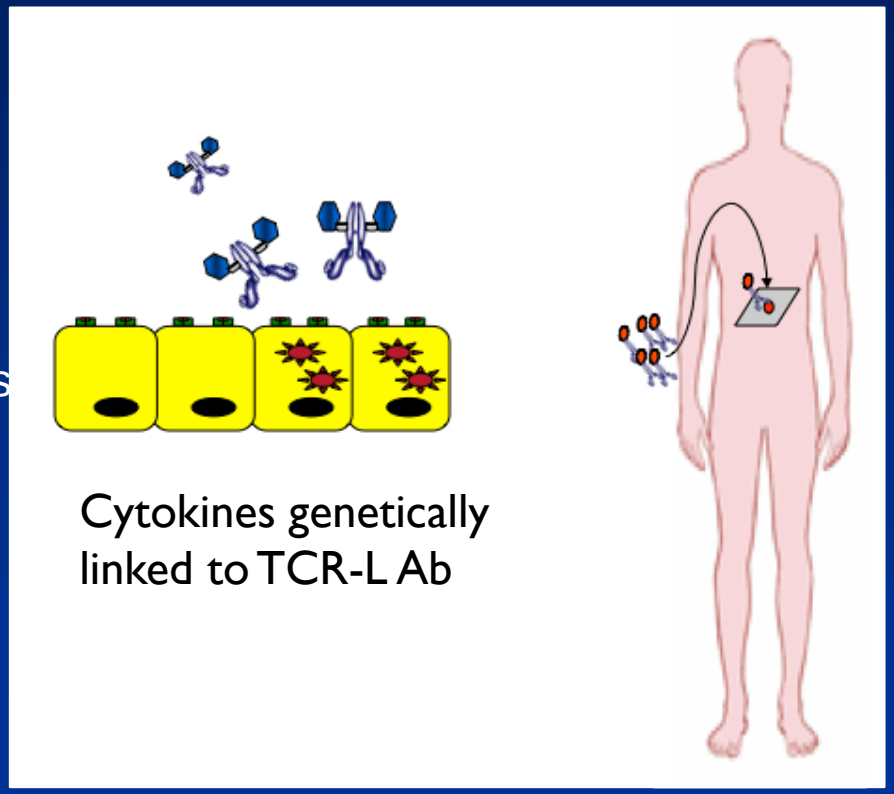


Humanized TCR-like Ab

Flexible (GLY4Ser)2 linkers

2 IFN-alpha 2a molecules

IFN-gamma
TNF-alpha

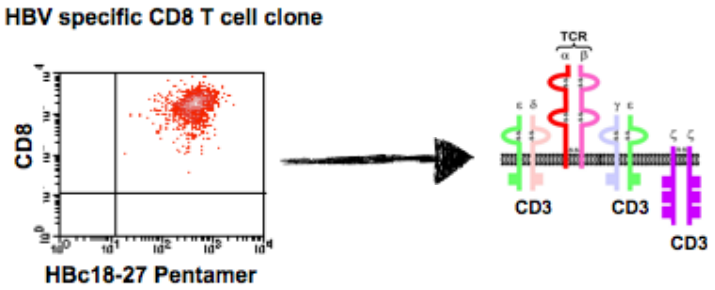


Additional immune therapeutic strategies

- To target antiviral cytokines to the liver
- To generate in vitro functional autologous HBV-specific T cells to reinject in chronic patients

T Cell Receptor (TCR) Gene Therapy in HBV

Library of TCRs



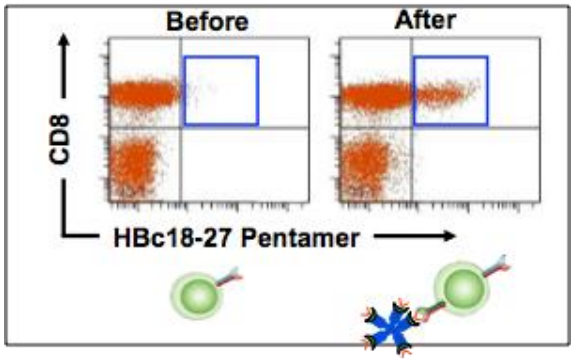
Clone DNA of TCR

Insertion on viral vectors

Production of viral vectors

Transduction of T cells Healthy and CHB patients

TCR-Modified T cells



Gehring, AJ et al. **J HEPATOL** 2011

Qasim W, Brunetto M et al. **J HEPATOL** 2014

CONCLUSIONS

- Available therapies for HBeAg negative chronic HBV infection are highly effective but they require strategies to further optimize their anti-viral effect

- NUC have potent anti-viral effect and association with immune stimulatory strategies may allow to shorten the duration of therapy

- IFN-α has a good but transient anti-viral effect and a poor modulatory activity on HBV-specific T cells; restoration of the T cell function may represent a strategy to consolidate the anti-viral effect of IFN-α and to decrease the relapse rate.

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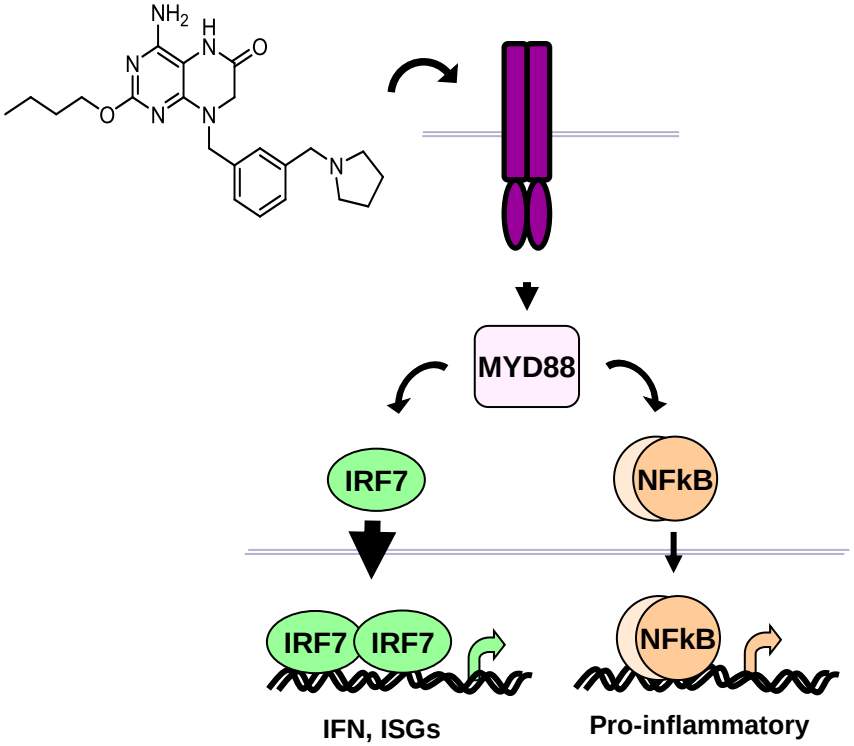
RER HBV/HCV Network

TLR-7

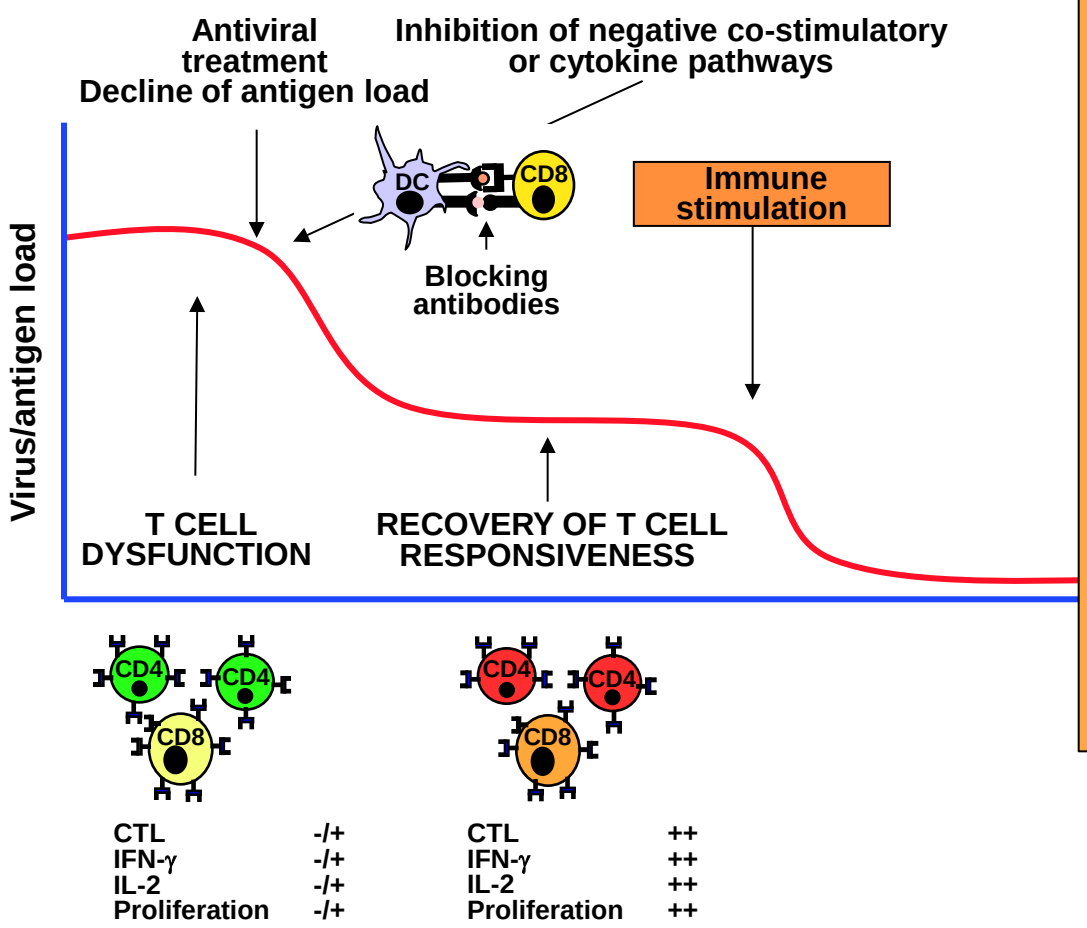


Toll-like receptor 7 (TLR-7) is a pattern-recognition receptor located in the endolysosomal compartment of plasmacytoid dendritic cells (pDC) and B cells

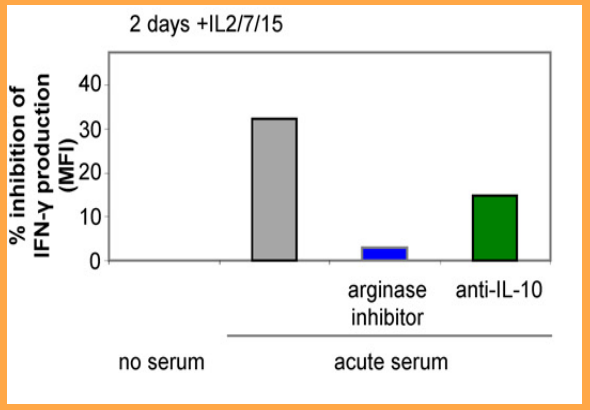
TLR-7 activation results in innate and adaptive immune stimulation through:

- Secretion of type I interferon (IFN) by pDC1
- Increased expression on pDC of molecules associated with antigen (Ag) presentation and T-cell costimulation
- B Lymphocytes differentiation in immunoglobulin producing plasma cells

Future perspectives of anti-HBV immune therapy



Arginase and HBV infection: role for arginase inhibitors



arginase inhibitors

anti-IL-10

Sandalova E. et al. Gastroenterology 2012

Adapted from: Ferrari C. *Gastroenterology* 2008;**134**:1601-1614. 2. Brooks DG, et al. *PNAS* 2008;**105**:20428-20433.