



**UNIVERSITÄTS
KLINIKUM** FREIBURG

HBV immunopathogenesis

Robert Thimme

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University Hospital Freiburg

CD8+ T cells in HBV infection

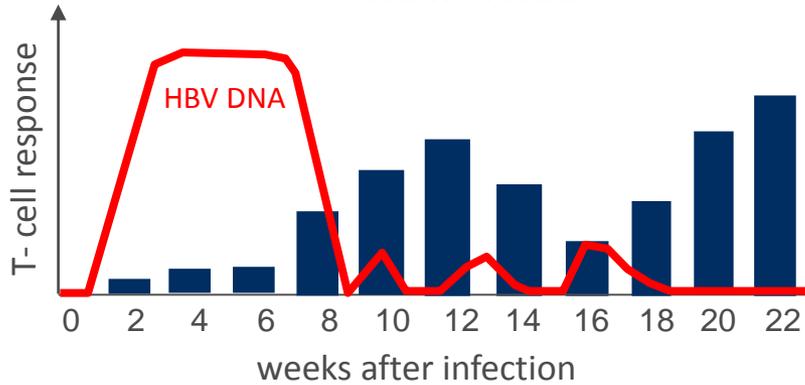
Function of CD8+ T cells

Failure of T cells

- viral escape?
- T cell exhaustion

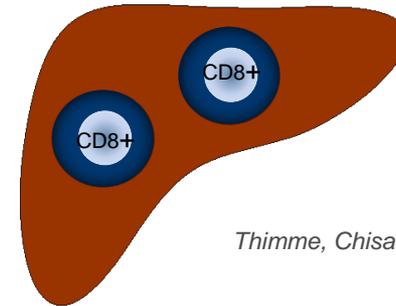
CD8+ T-cell responses in HBV infection

Temporal association of CD8+ T-cell response and viral load



Maini, Bertolotti Gastro 2001
Boettler, Thimme, J Virol 2005

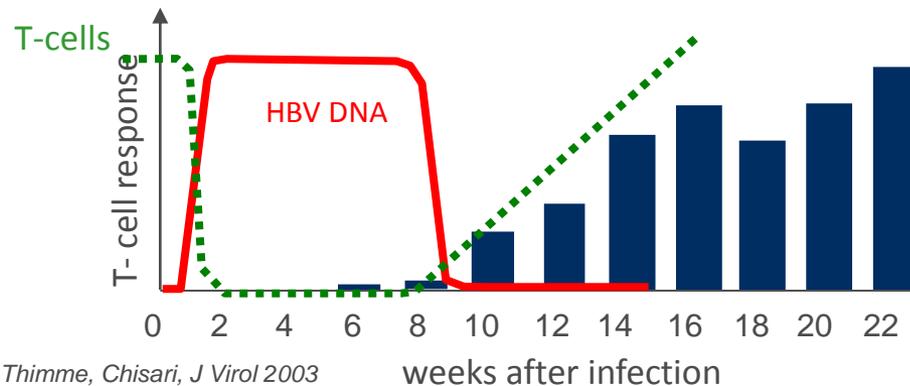
Intrahepatic accumulation of CD8+ T-cell responses during viral clearance



Thimme, Chisari, J Virol 2003



Depletion of CD8+ T cells prolongs viremia

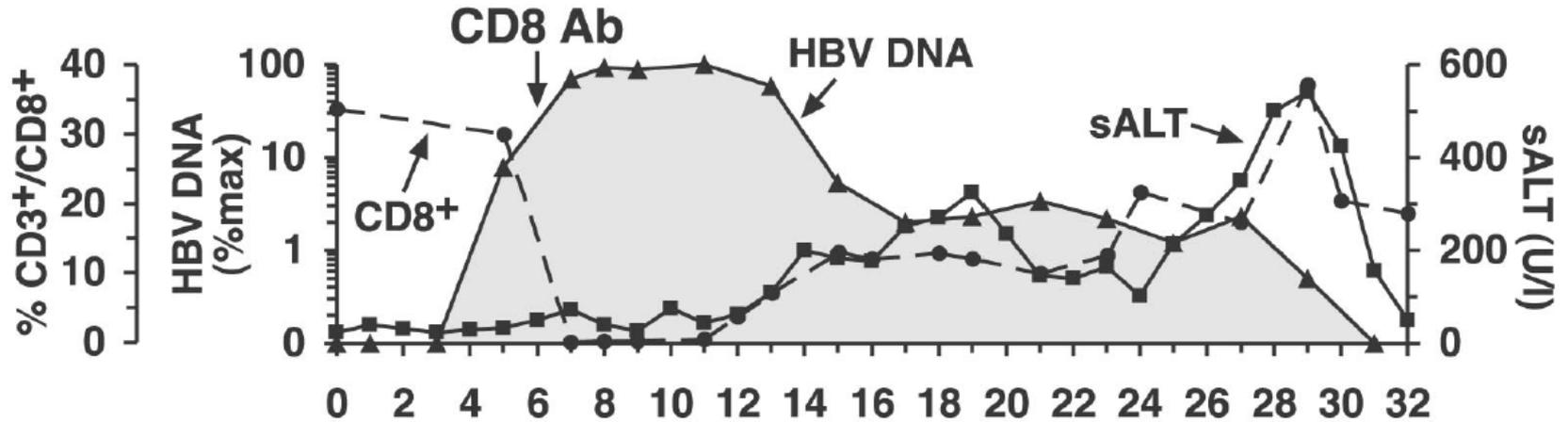


Thimme, Chisari, J Virol 2003

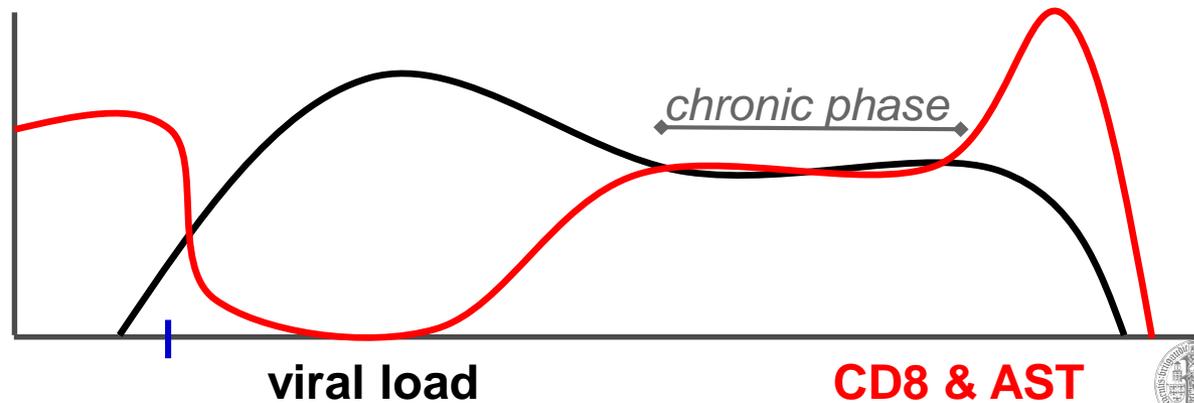
Function of CD8+ T cells in HBV

cytolytic mechanisms

Ch.1620



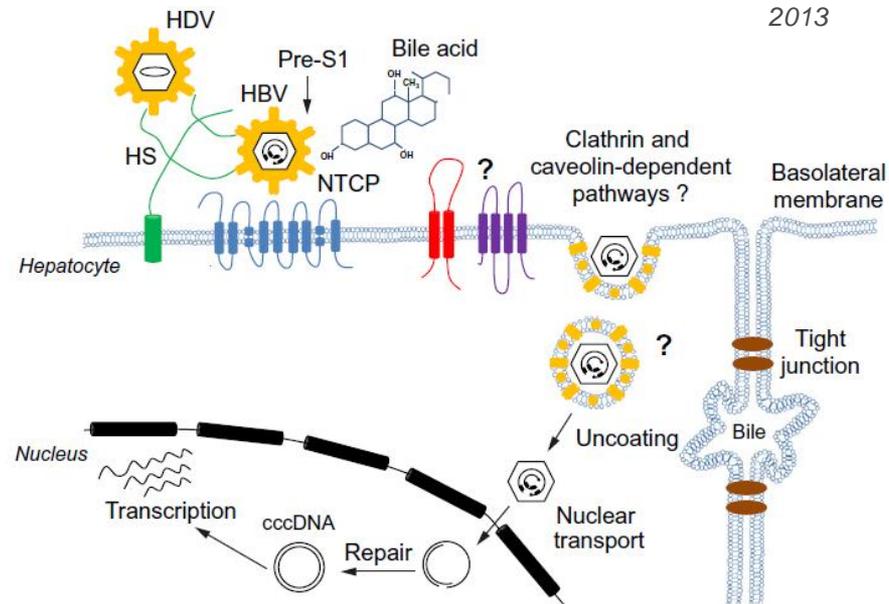
Thimme et al. J. Virol. 2003



Identification of a novel cell culture model



2013



human sodium taurocholate cotransporting polypeptide (hNTCP)

Methods – Experiment set-up

Assay for co-cultures:

- HBV infected HepG2^{hNTCP} cells
- HLA-matched core₁₈₋₂₇-specific CD8⁺ T cells[§]
- duration: 4 d
- Direct and indirect conditions

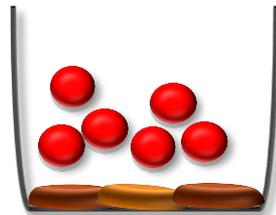
Read-out:

- cytoplasmic viral load (qPCR)
- transaminase level (AST)

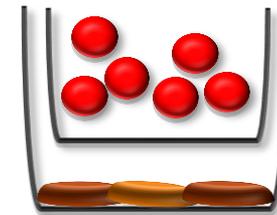
Untreated control



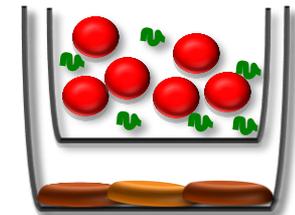
Direct co-culture



Transwell cultures



-



+

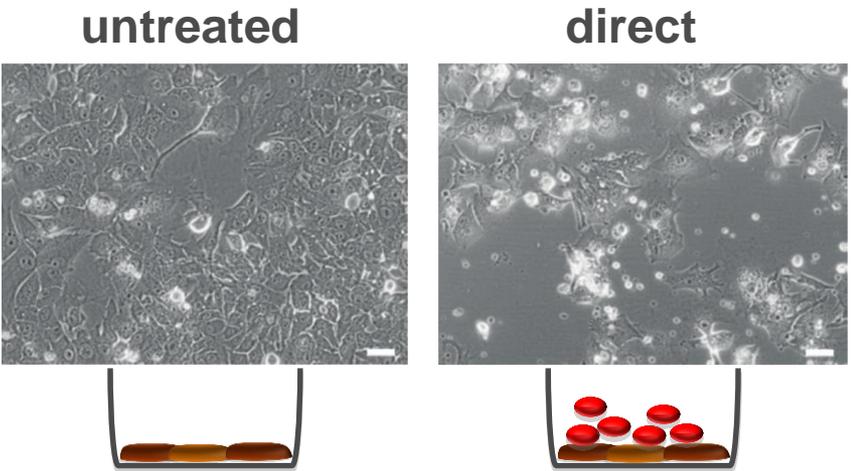
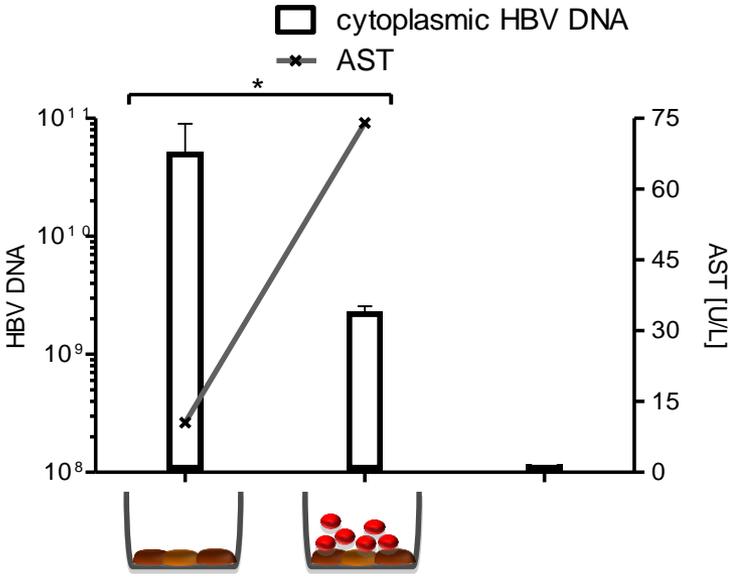
stimulation

 HBV infected hepatoma cell

 CD8⁺ T cell  core₁₈₋₂₇ peptide

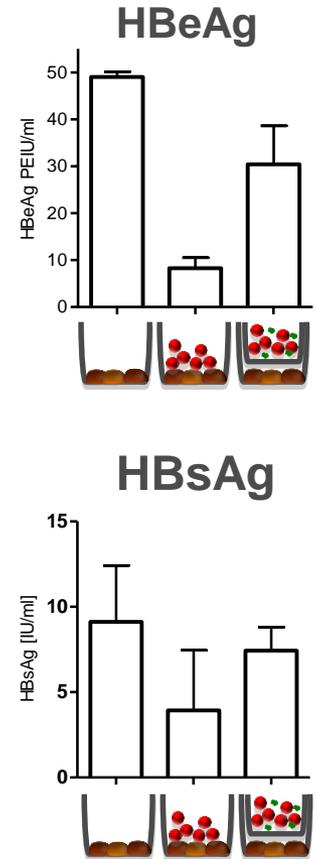
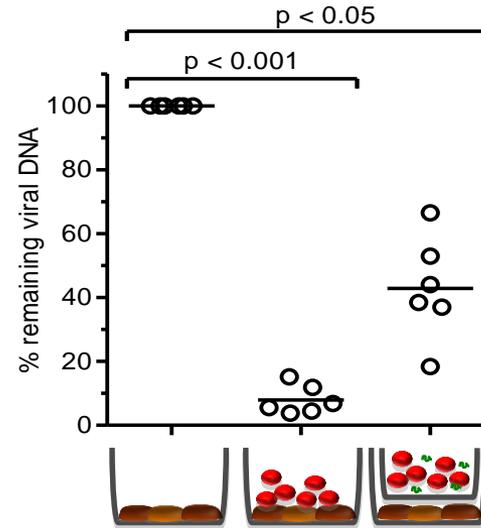
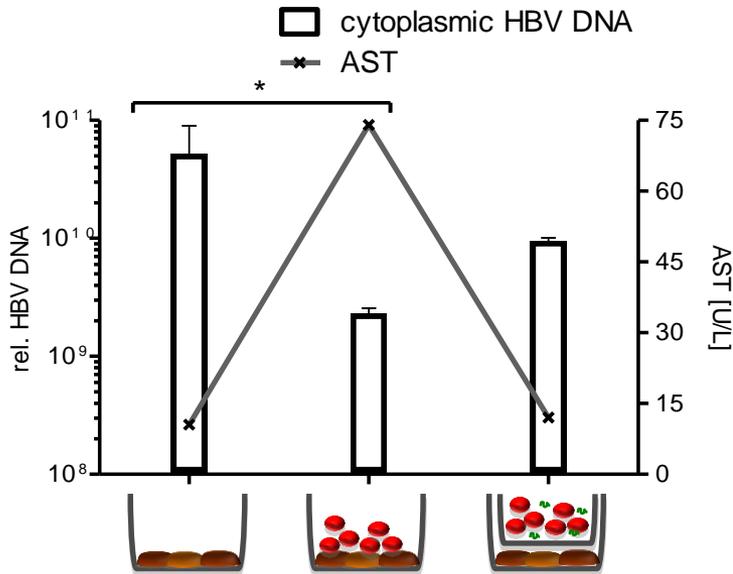
[§] provided by **Antonio Bertoletti**, A*STAR, Singapore

Reduction of viral loads requires cell killing



$\Delta t = 96$ h
E:T = 1:1

Reduction of viral loads requires cell killing



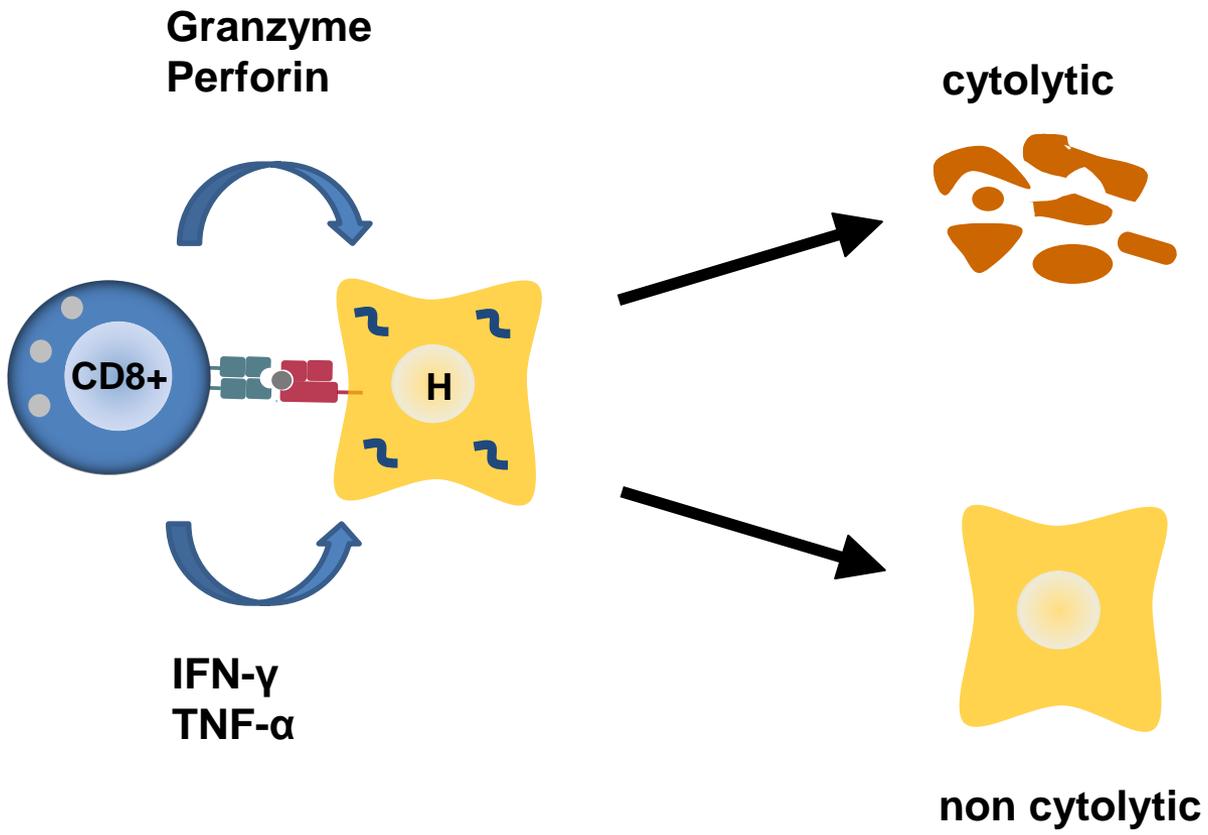
$\Delta t = 96$ h
 E:T = 1:1

Strong reduction (95%) of viral loads requires:

- Direct cell-cell contact
- Cytotoxic effector mechanisms

n = 2

Function of CD8+ T cells in HBV



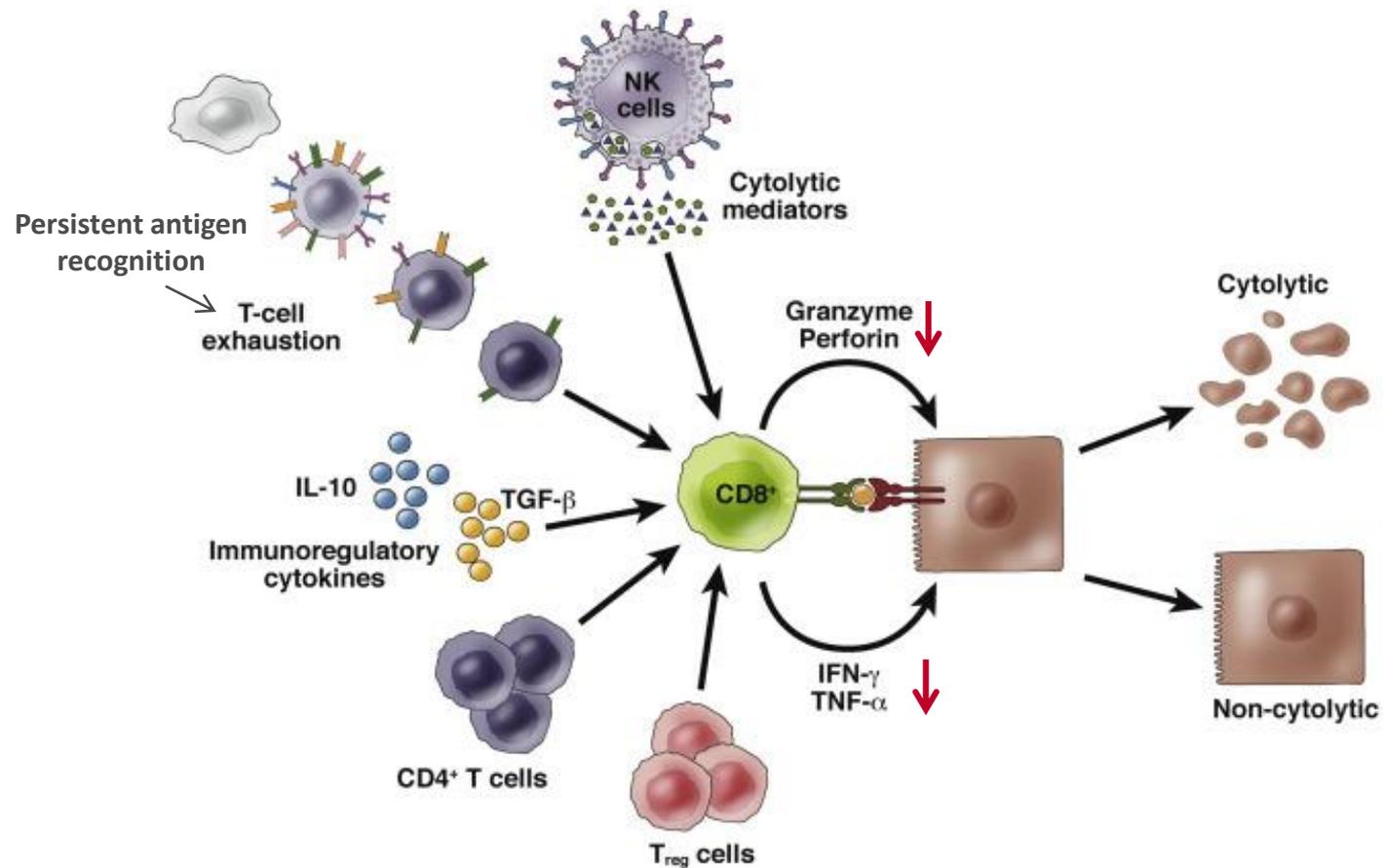
CD8+ T cells in HBV infection

Function of CD8+ T cells

Failure of T cells

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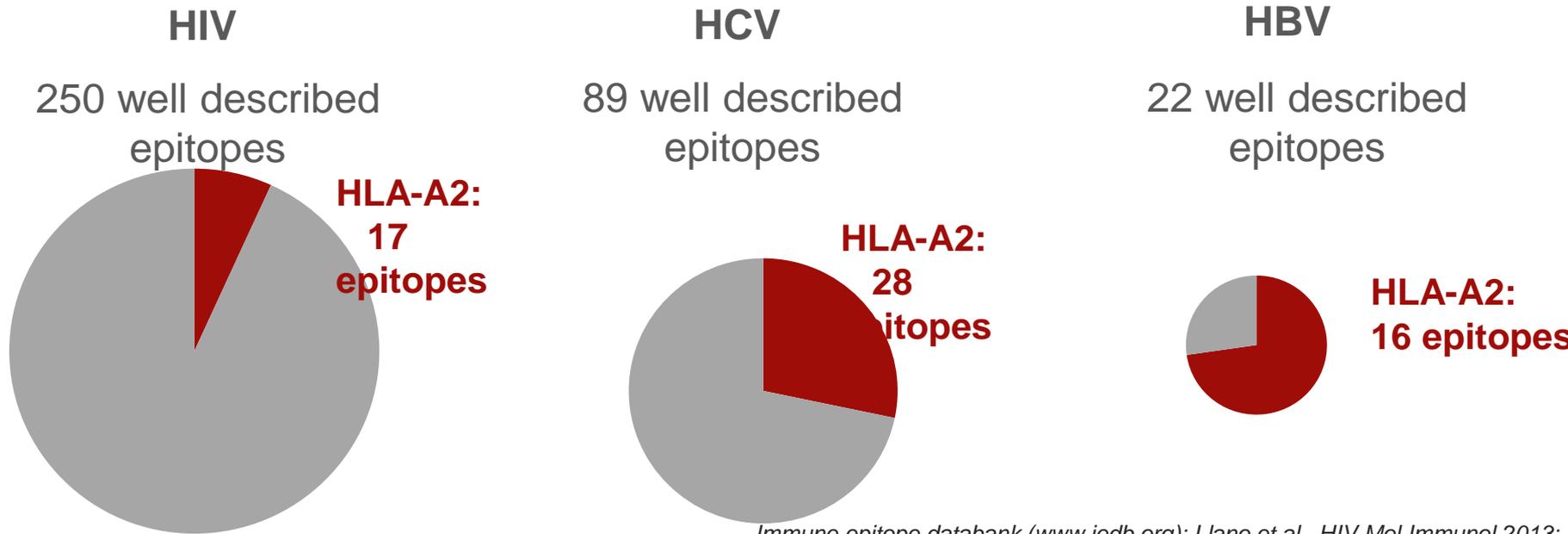
Mechanisms of CD8+ T-cell failure



adapted from Knolle and Thimme, Gastroenterology, 2014

HBV-specific CD8+ T cell response

Repertoire of virus-specific CD8+ T cell epitopes



Mechanisms of virus-specific CD8+ T cell failure

- | HIV | HCV | HBV |
|---|---|---|
| <ul style="list-style-type: none">• T cell dysfunction• Viral escape | <ul style="list-style-type: none">• T cell dysfunction• Viral escape | <ul style="list-style-type: none">• T cell dysfunction• No relevant role of viral escape! |

HLA-B27 is protective in HIV and HCV infection

HIV

- low viral loads
- slow CD4⁺ decline
- late onset of AIDS



HCV

- associated with spontaneous viral clearance



Immunodominant HLA-B27-restricted CD8⁺ epitopes:

gag₂₆₃₋₂₇₂ KRWILGLNK

NS5B₂₈₄₁₋₂₈₄₉ ARMILMTHF



Viral escape



T cell failure

Hypothesis

HLA-B*27 is a dominant driver of viral escape
also in HBV infection

3 experimental approaches:

1. HBV sequence analysis in HLA-B*27 positive and negative patients => „HLA-B*27 footprints“
2. *In silico* HLA-B*27 epitope prediction
3. Overlapping peptides in acute HBV infection

HLA-B*27 footprints

Pol 105-121

	K	R	R	L	Q	L	I	M	P	A	R	F	Y	P	N	V	T	K	Y	L	
B*27+

	.	.	K	K	F

	M	S	L
	G
	K	I
	K
B*27-

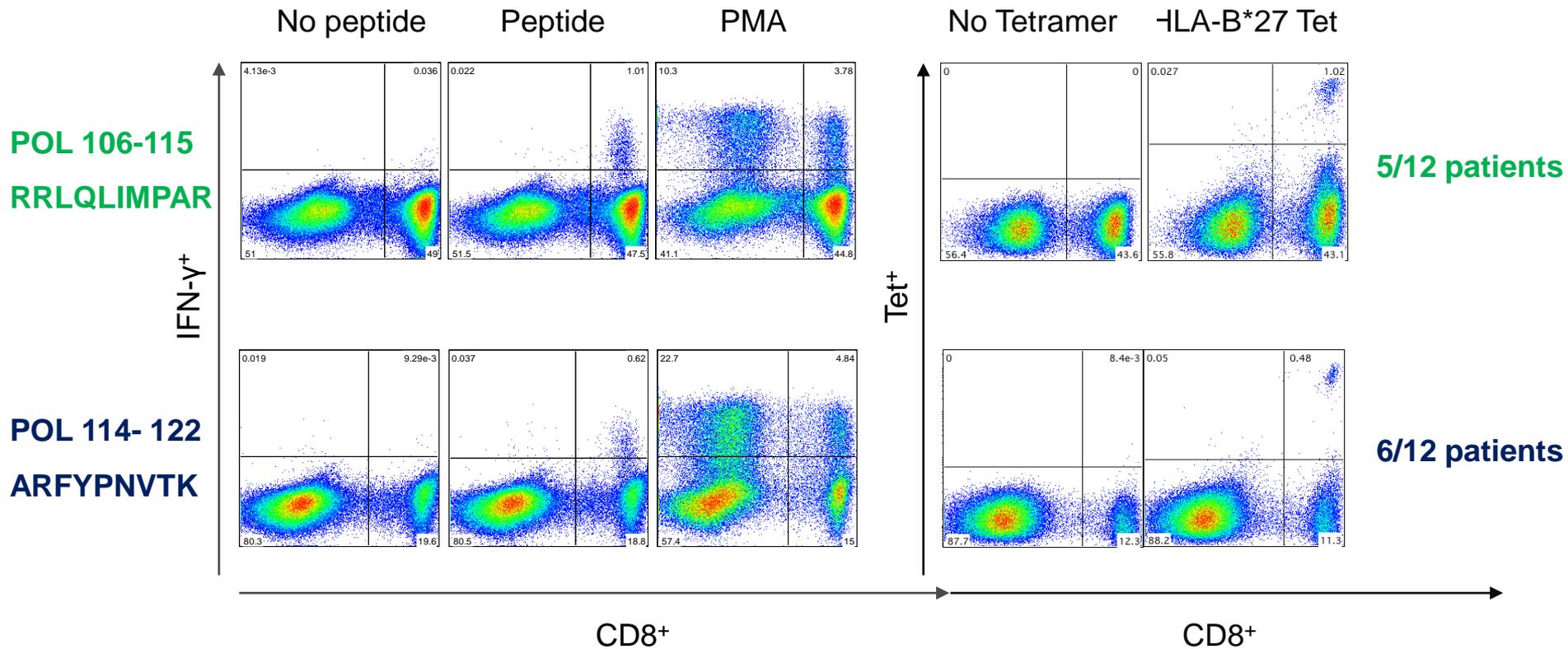
HLA-B*27:05 binding affinity:

IC₅₀: 1.1 nM

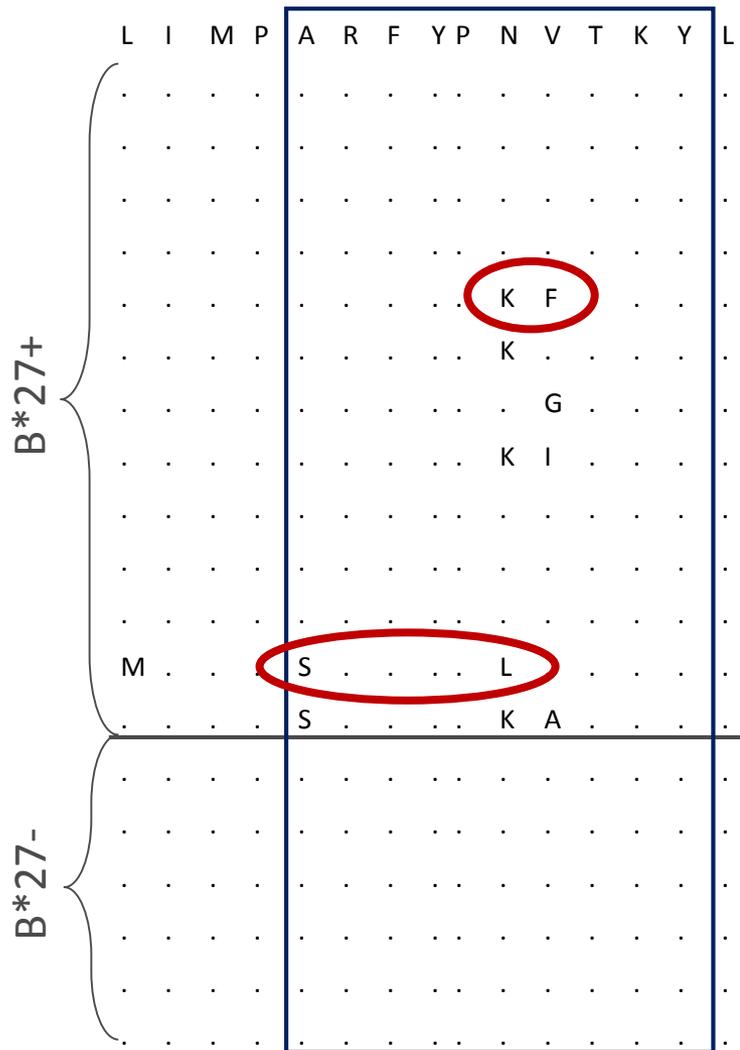
IC₅₀: 1.6 nM

(<50 nM 'optimal binders')

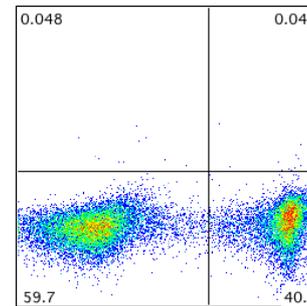
HLA-B*27 restricted CD8+ T cell epitopes



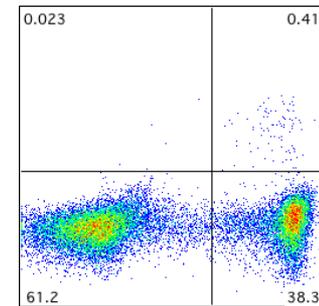
Functional evidence for HLA-B*27 driven viral escape



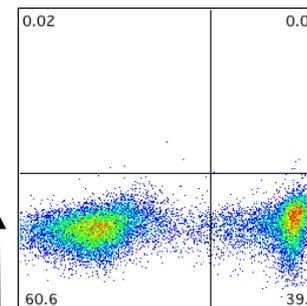
No peptide



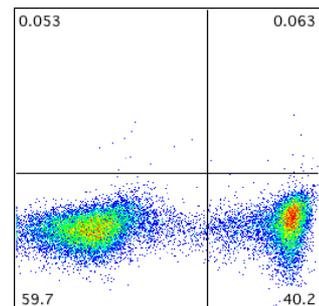
Wild-type
ARFYPNVTK



Variant
.....KF..



Variant
S.....L....

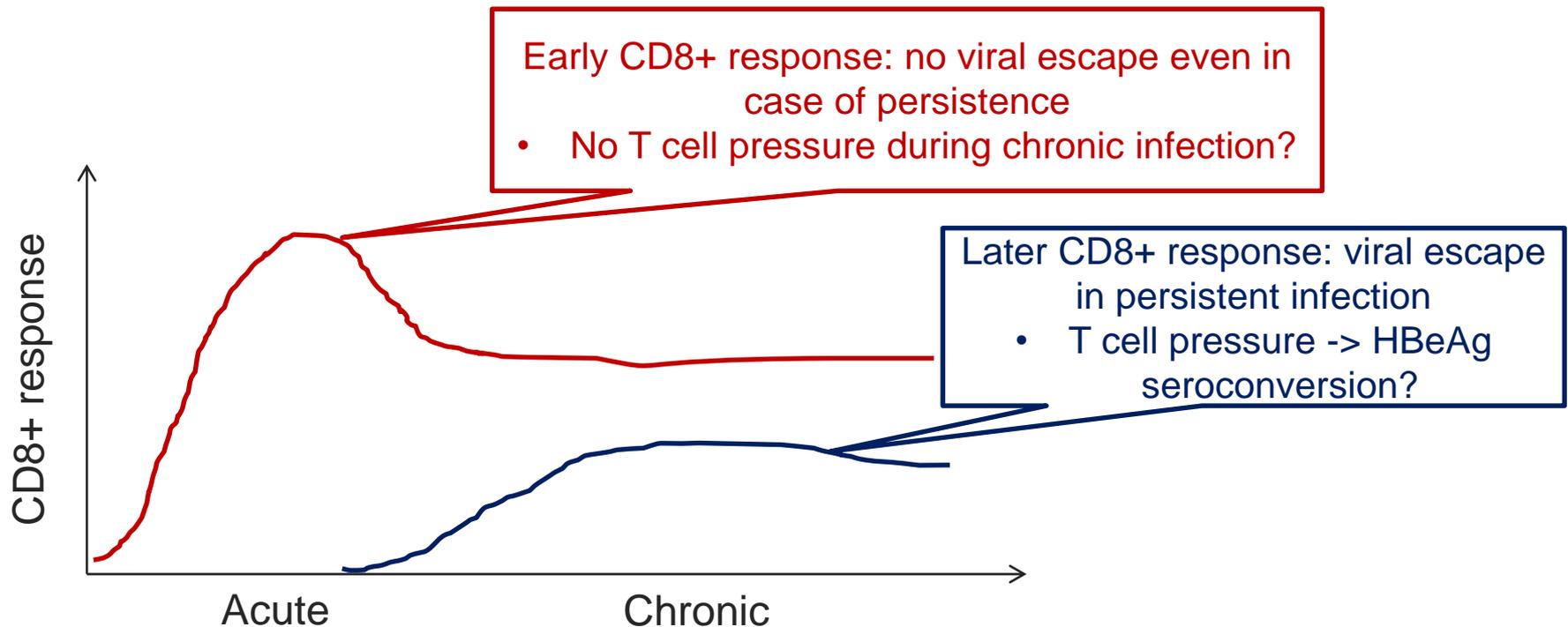


IFN- γ ⁺
CD8⁺

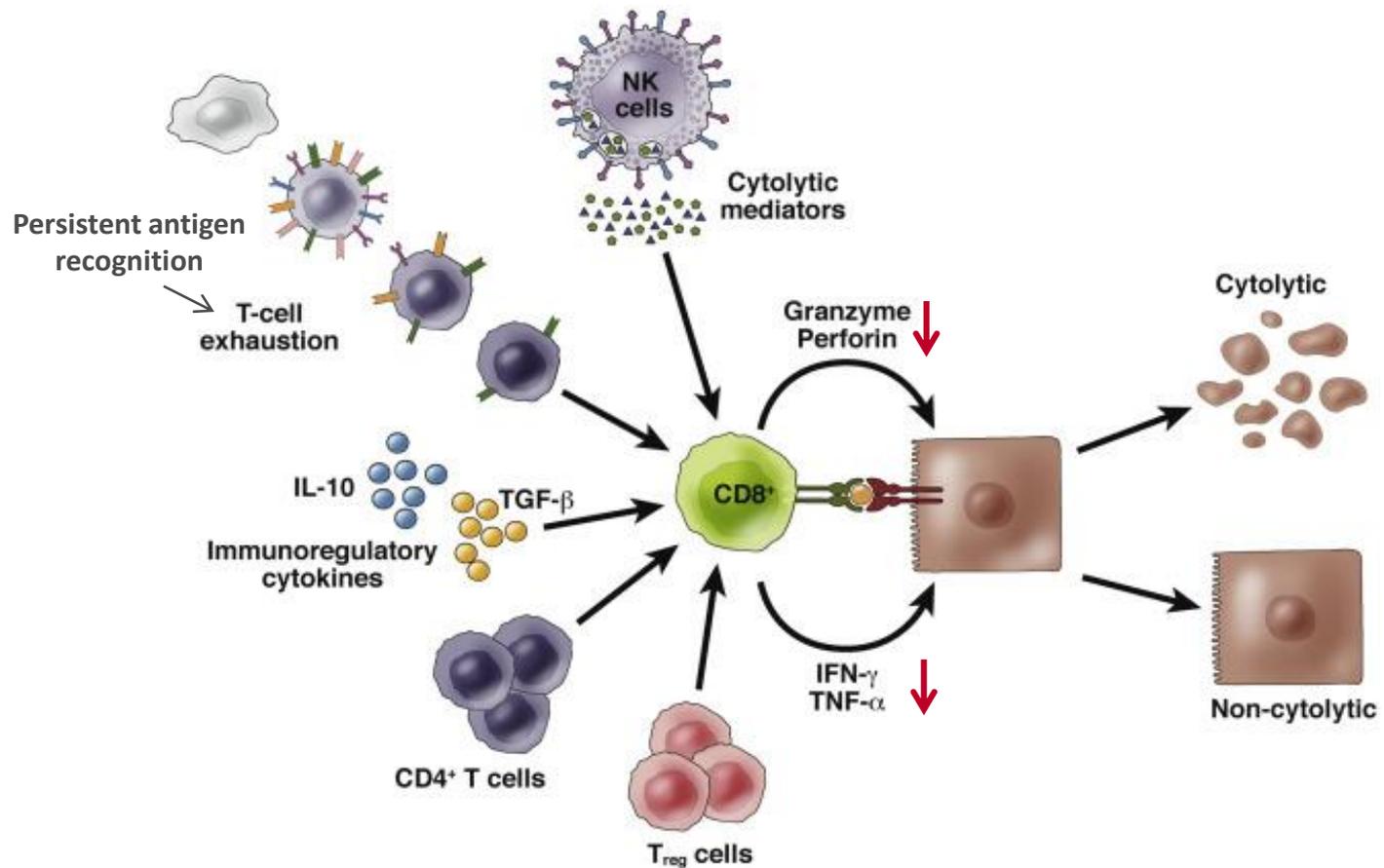
Differential role of HLA-B*27 restricted HBV-specific CD8+ T cells

7 epitopes with evidence of viral escape

5 epitopes without viral evolution

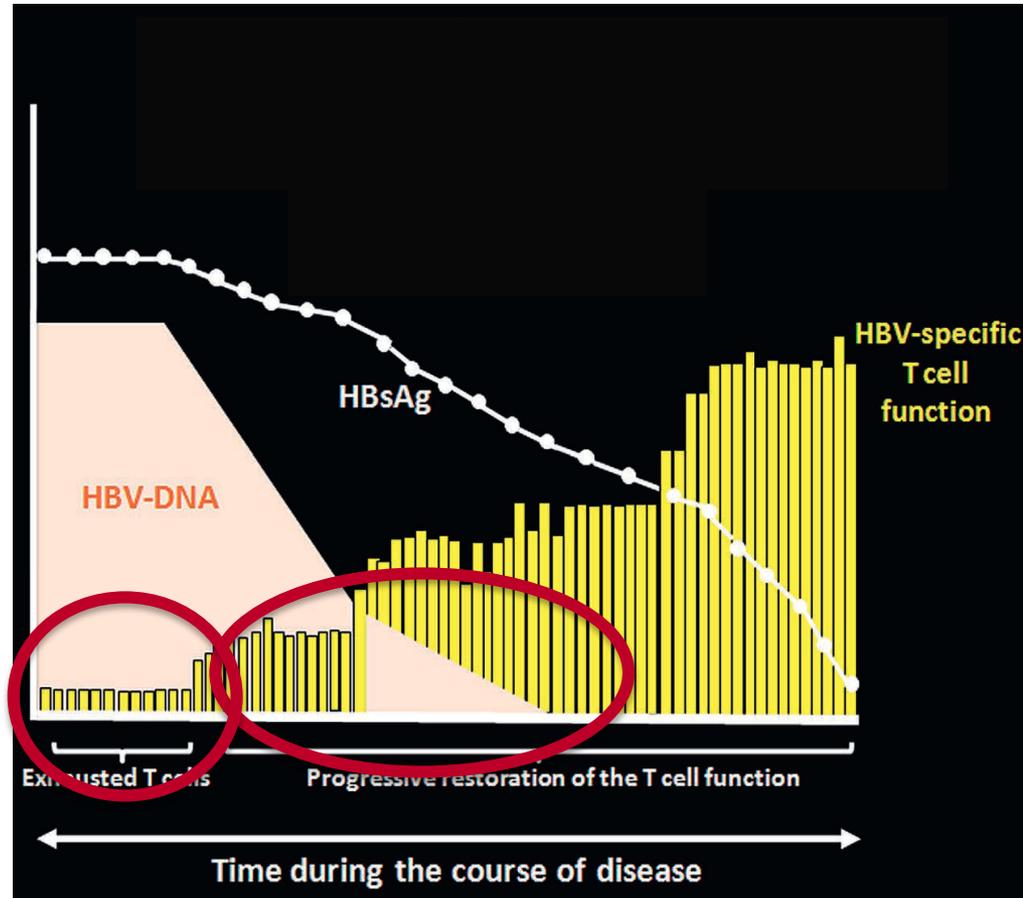


Mechanisms of CD8+ T-cell failure



adapted from Knolle and Thimme, Gastroenterology, 2014

Model: T cells and the course of viral Hepatitis

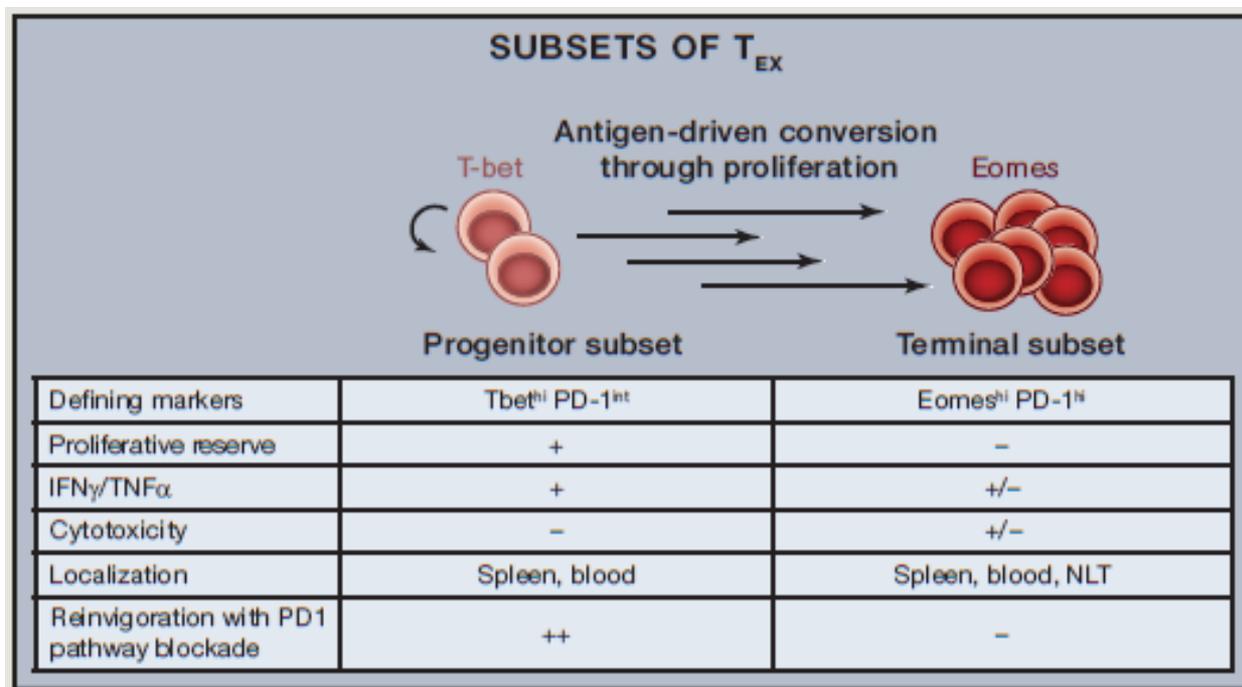


Exhausted CD8+ T cells are heterogeneous

SnapShot: T Cell Exhaustion

Kristen E. Pauken and E. John Wherry

Department of Microbiology and Institute for Immunology, University of Pennsylvania, Philadelphia, PA 19104, USA



Exhaustion of HBV-specific CD8+ T cells

Patient cohorts (cHBV patients)

Phenotypic analyses:

- 15 inactive carriers
- 20 cHBV patients under NUC therapy

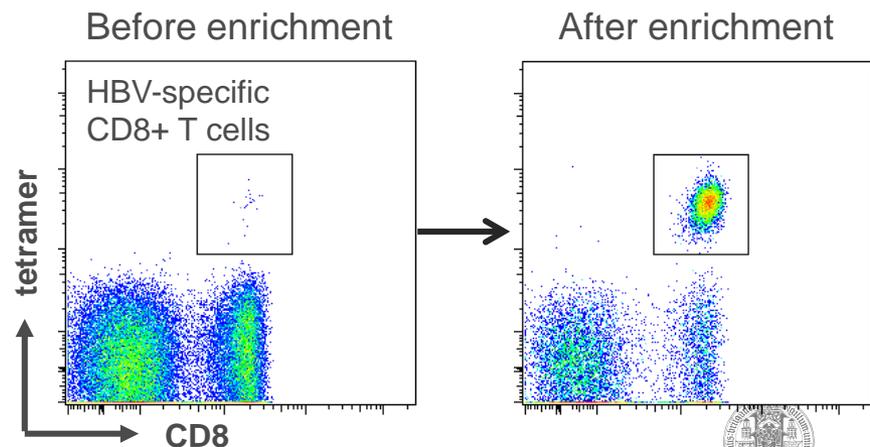
Gene expression profiles:

- 7 inactive carriers
- 2 cHBV patients under NUC therapy

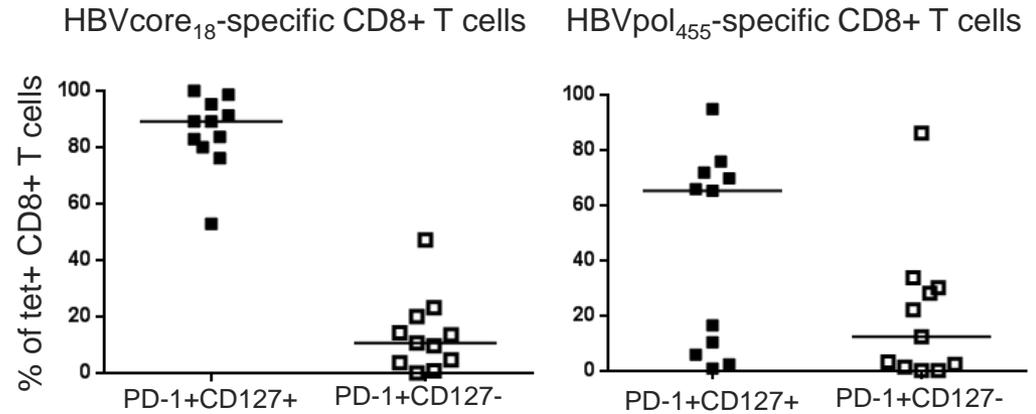
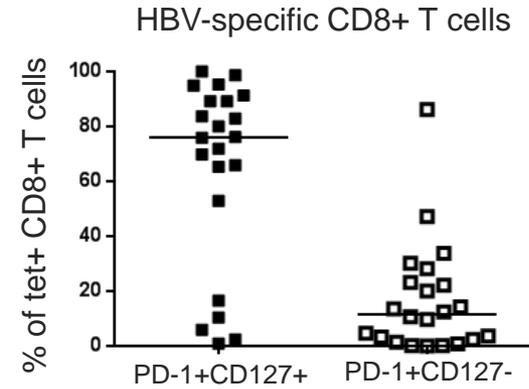
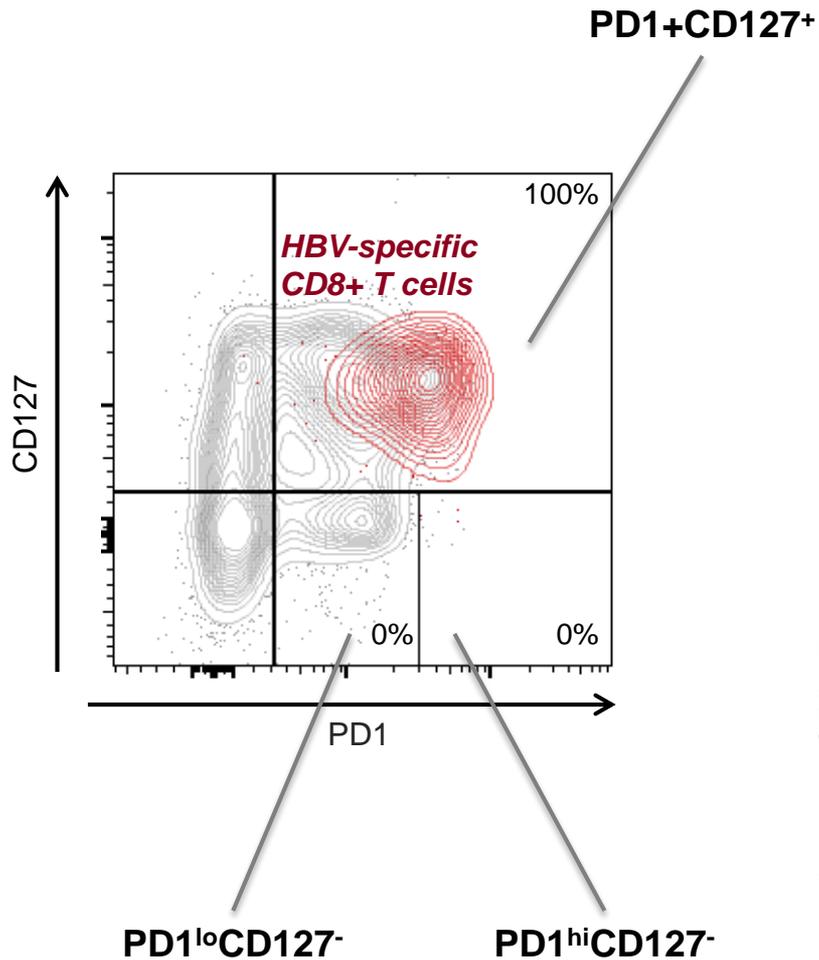
Methods

- Tetramer-based enrichment of HBV-specific CD8+ T cells (*ex vivo*)

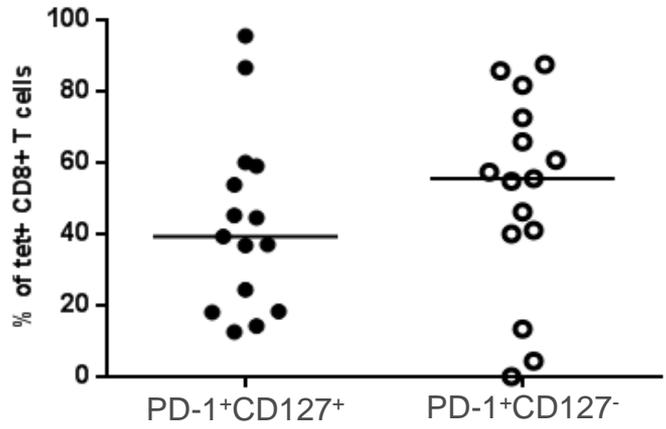
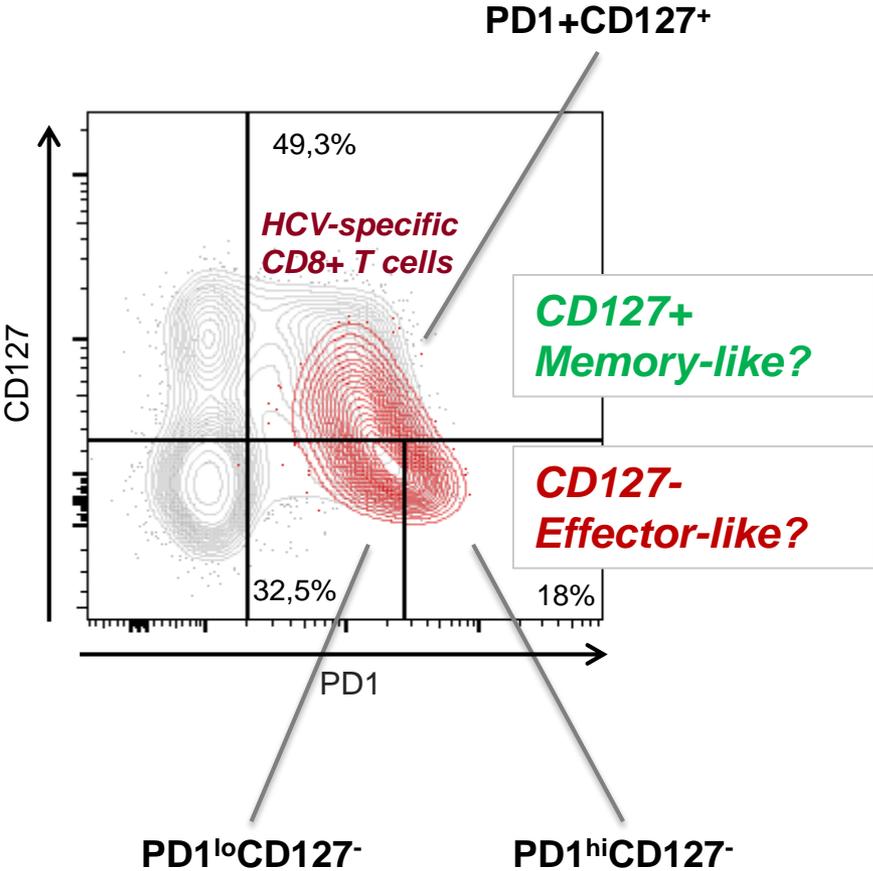
<i>HCV epitopes (HLA-A*0201-Tetramers)</i>	
AA position	Sequence
Core ₁₈₋₂₇	FLPSDFFPSV
Env ₁₈₃₋₁₉₁	FLLTRILTI
Env ₃₃₅₋₃₄₃	WLSLLVPFV
Pol ₄₅₅₋₄₆₃	GLSRYVARL



Exhaustion of HBV-specific CD8+ T cells



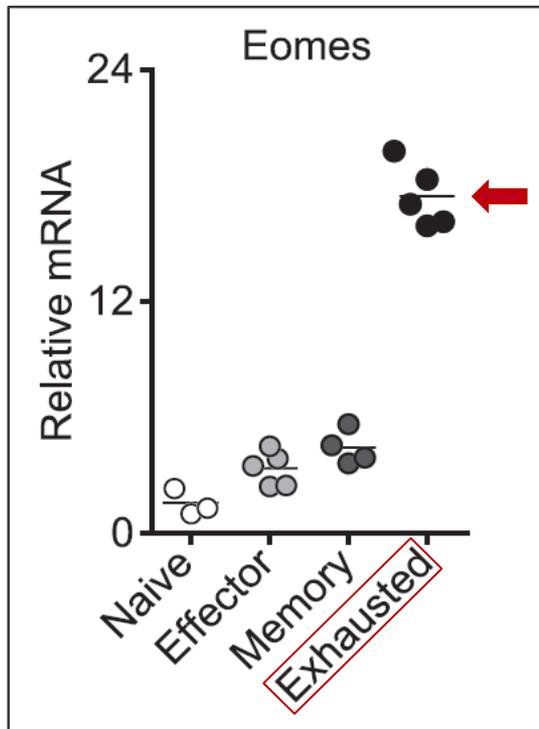
Exhaustion of HCV-specific CD8+ T cells



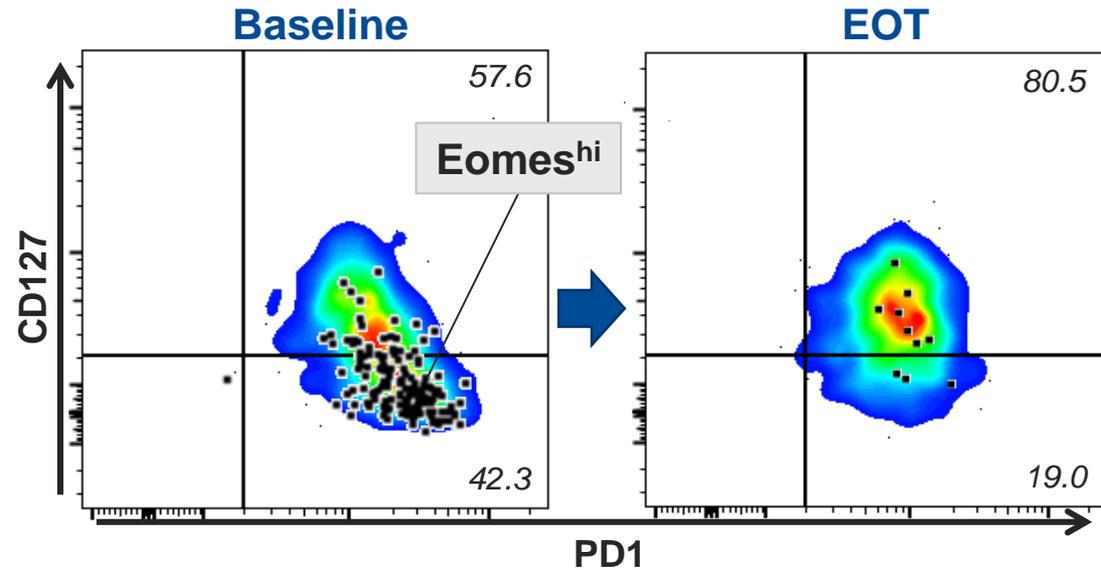
CD8+ T-cell exhaustion on the transcriptional level

Progenitor and Terminal Subsets of CD8⁺ T Cells Cooperate to Contain Chronic Viral Infection

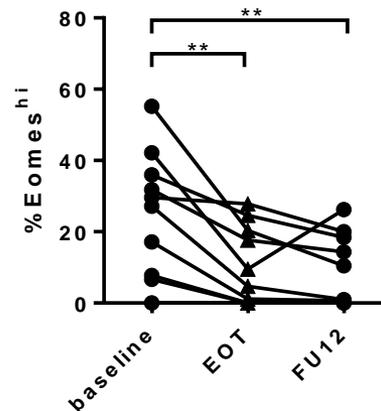
Michael A. Paley,¹ Daniela C. Kroy,² Pamela M. Odorizzi,¹ Jonathan B. Johnnidis,¹ Douglas V. Dolfi,¹ Burton E. Barnett,¹ Elizabeth K. Bikoff,³ Elizabeth J. Robertson,³ Georg M. Lauer,² Steven L. Reiner,^{4*} E. John Wherry^{1†}



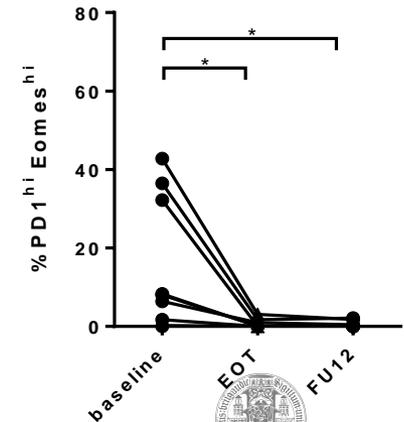
Paley, Wherry, Science 2012



%Eomes^{hi}
of HCV-specific CD8+ T cells



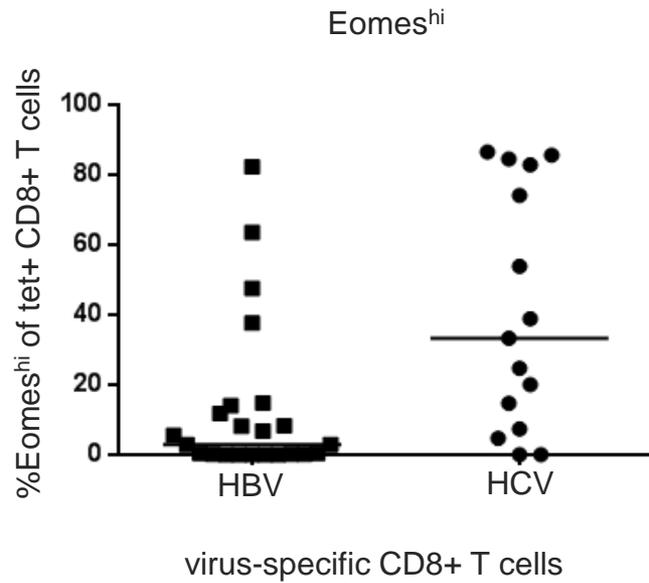
%PD1^{hi} Eomes^{hi}
of HCV-specific CD8+ T cells



Are terminally exhausted subsets absent in HBV-specific CD8+ T-cell populations?

Progenitor and Terminal Subsets of CD8⁺ T Cells Cooperate to Contain Chronic Viral Infection

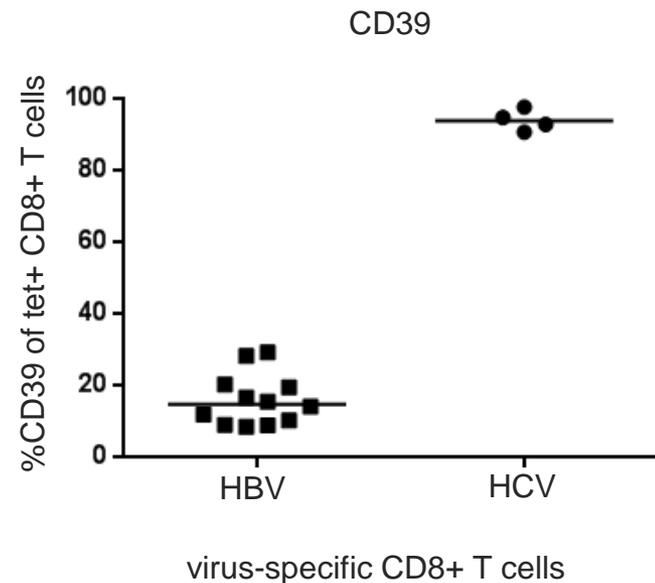
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RESEARCH ARTICLE

CD39 Expression Identifies Terminally Exhausted CD8⁺ T Cells

Prakash K. Gupta^{1,2☉}, Jernej Godec^{1,3☉}, David Wolski^{4☉}, Emily Adland², Kathleen Yates¹, Kristen E. Pauken⁵, Cormac Cosgrove⁶, Carola Ledderose⁷, Wolfgang G. Junger⁷, Simon C. Robson⁸, E. John Wherry⁵, Galit Alter⁶, Philip J. R. Goulder², Paul Klenerman², Arlene H. Sharpe^{3,9}, Georg M. Lauer⁴, W. Nicholas Haining^{1,9,10*}



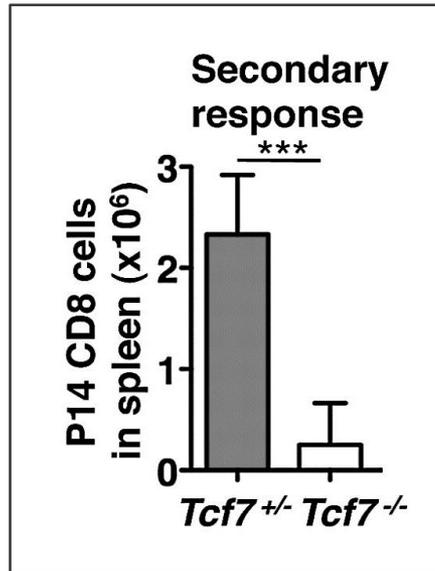
The majority of HBV-specific CD8+ T cells express the memory marker Tcf-1

Essential role of the Wnt pathway effector Tcf-1 for the establishment of functional CD8 T cell memory

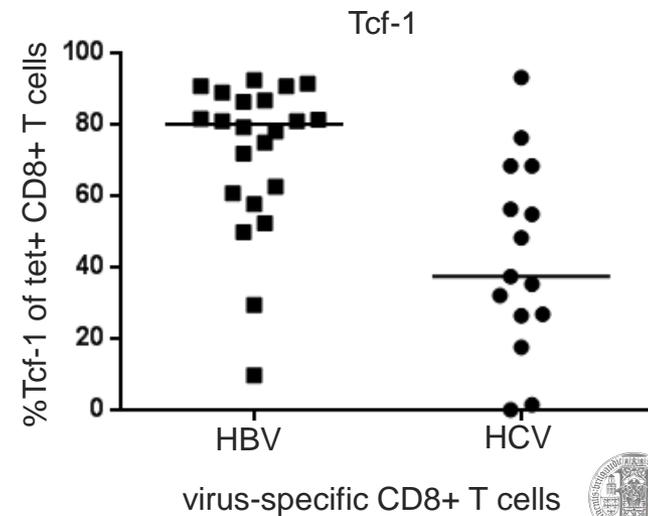
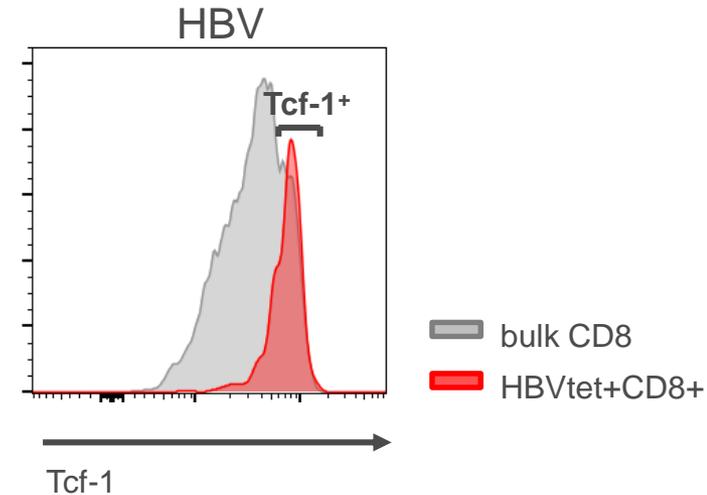
Grégoire Jeannet^{a,1}, Caroline Boudousquié^{a,1}, Noémie Gardiol^a, Joonsoo Kang^b, Joerg Huelsenken^c, and Werner Held^{a,2}

^aLudwig Institute for Cancer Research Ltd., Lausanne Branch and University of Lausanne, 1066 Epalinges, Switzerland; ^bDepartment of Pathology, Graduate Program in Immunology and Virology, University of Massachusetts Medical School, Worcester, MA 01655; and ^cSwiss Cancer Research Institute, Federal University of Technology Lausanne, 1015 Lausanne, Switzerland

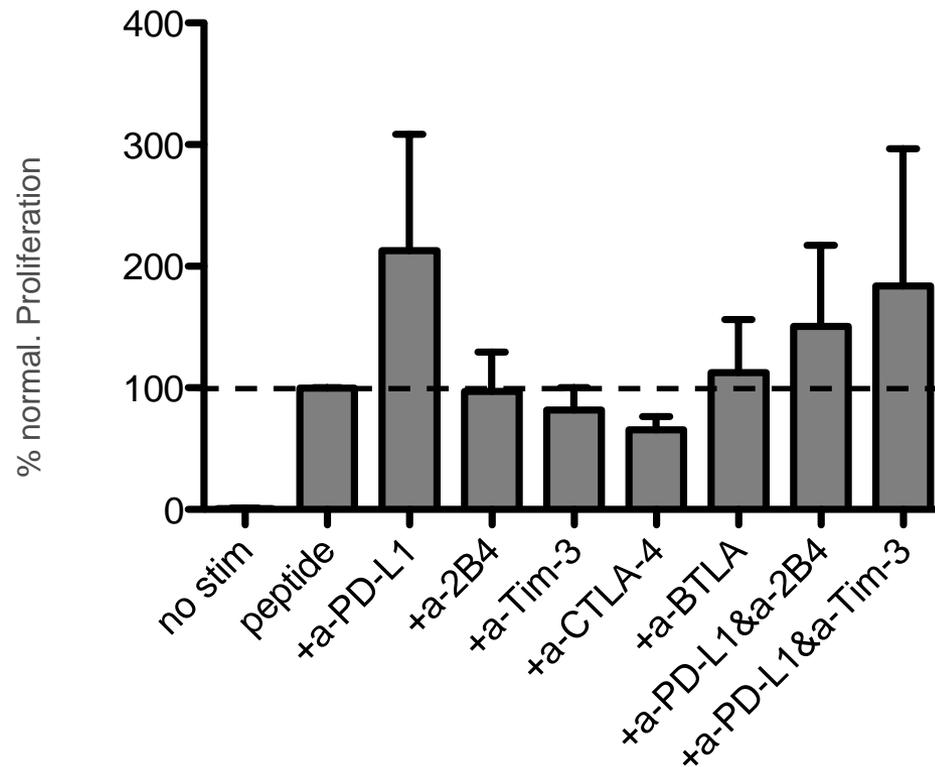
Edited* by Douglas T. Fearon, University of Cambridge School of Clinical Medicine, Cambridge, United Kingdom, and approved April 19, 2010 (received for review December 8, 2009)



Jeannet, Held, PNAS 2010



Variable proliferative response of HBV-specific CD8+ T cells to inhibitory receptor blockade



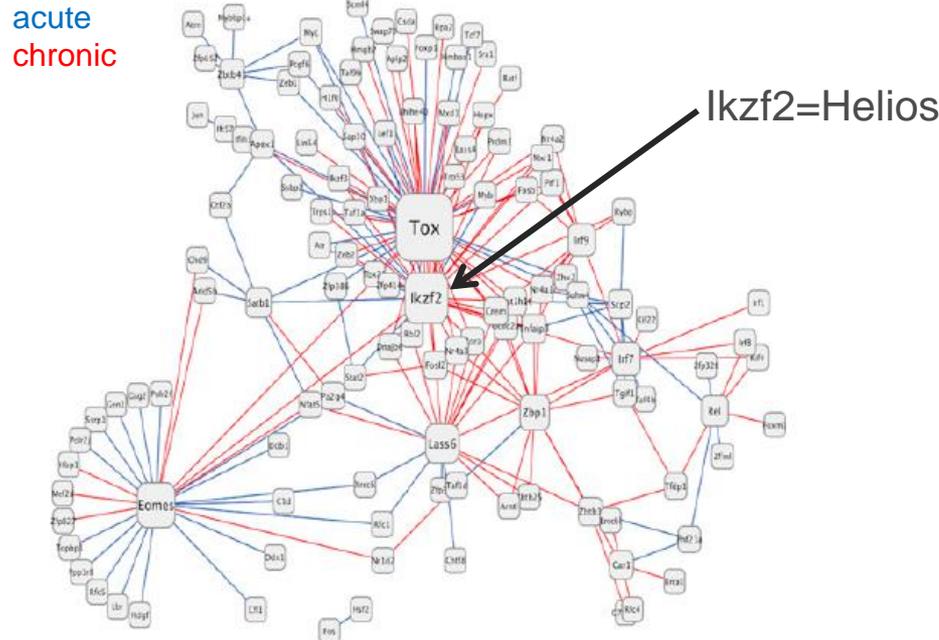
→ PD-L1 blockade has the strongest effect

Is there a signature of exhaustion in HBV-specific CD8+ T cells from asymptomatic carriers?

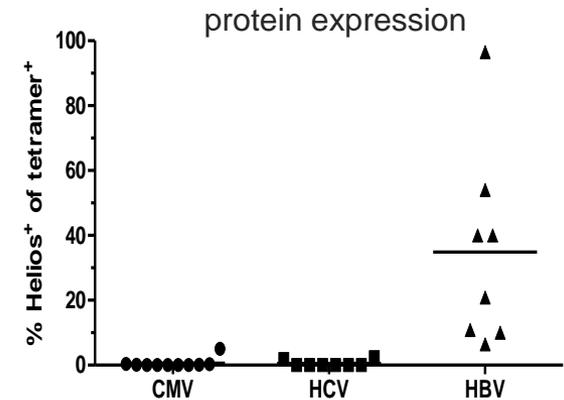
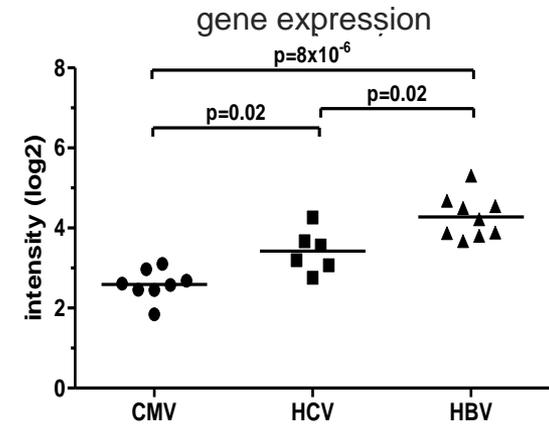
Network Analysis Reveals Centrally Connected Genes and Pathways Involved in CD8+ T Cell Exhaustion versus Memory

Travis A. Doering,^{1,2} Alison Crawford,^{1,3} Jill M. Angelosanto,¹ Michael A. Paley,¹ Carly G. Ziegler,¹ and E. John Wherry^{1,*}
¹Department of Microbiology and Institute for Immunology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA 19104, USA
²Present address: Hofstra North Shore-LIJ School of Medicine, Hempstead, NY 11549, USA
³Present address: Regeneron Pharmaceutical, Tarrytown, NY 10591, USA
 *Correspondence: wherry@mail.med.upenn.edu
<http://dx.doi.org/10.1016/j.jimmuni.2012.08.021>

Transcription Factor Difference Network



Helios (IKZF2)



virus-specific CD8+ T cells

Helios (IKZF2)

- Zinc finger transcription factor
- IKZF proteins found in many hematopoietic cells - Helios in T cells
 - expressed in autoreactive thymocytes undergoing clonal deletion
 - expressed in exhausted CD4+ T cells and a subset of T_{reg} cells
 - expressed in exhausted CD8+ T cells
 - expressed in a model of peripheral CD8+ T-cell tolerance
- Role of Helios in T cells
 - marker of T-cell activation and proliferation
 - induces epigenetic silencing of IL-2 gene expression

Parish et al., Blood 2009
Himmel et al., J Immunol 2013
Daley et al., J Exp Med 2013
Baine et al., J Immunol 2013
Crawford et al., Immunity 2014
Doering et al., Immunity 2014

Summary CD8+ T cells in HBV

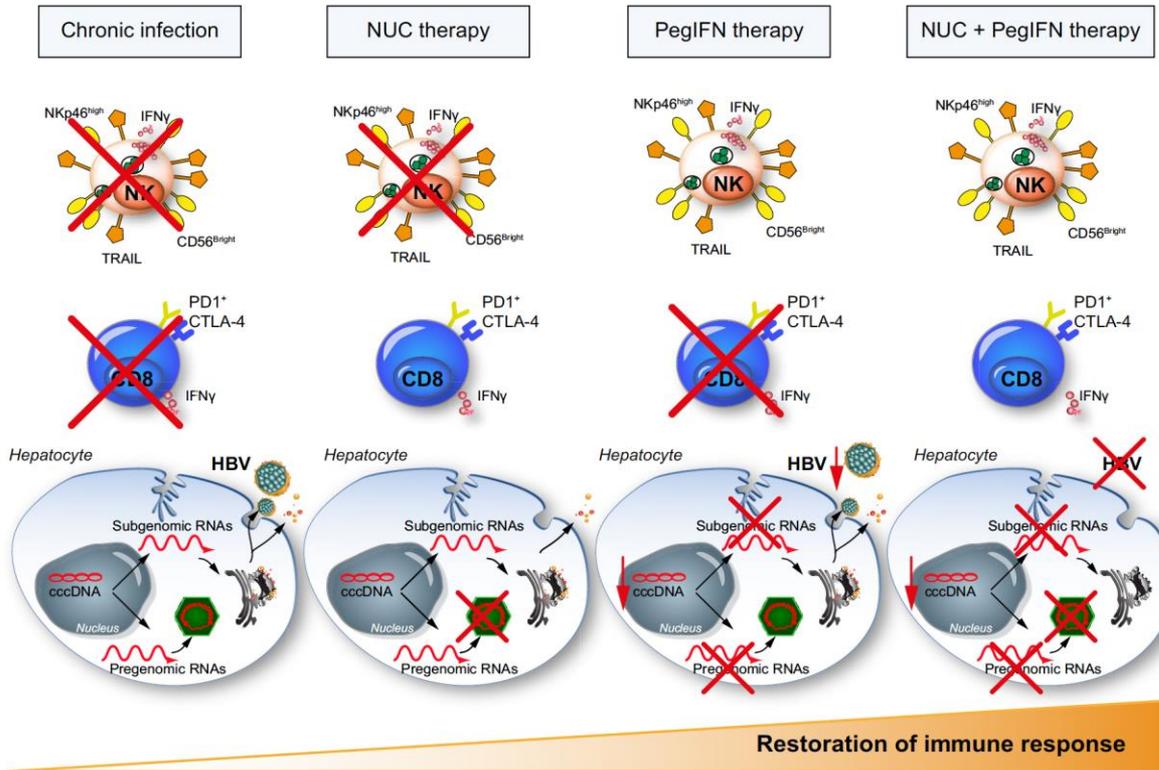
Dominance of cytolytic effector functions

Viral escape is associated with HBV persistence

HBV-specific CD8+ T cells less exhausted than HCV specific CD8+ T cells

Unique expression of Helios of HBV-specific CD8+ T cells

Restoration of HBV-specific CD8+ T cell failure by antiviral therapy



Boni, Ferrari, JCI 1998

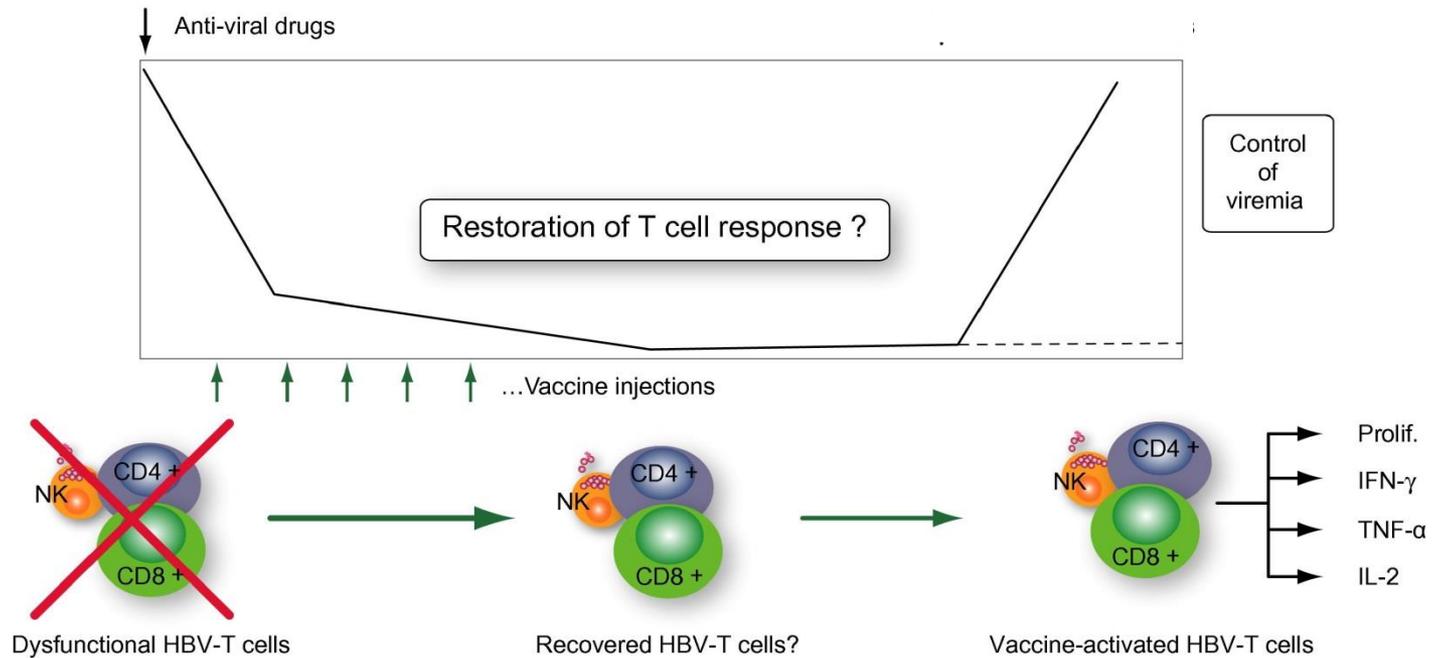
Boni, Ferrari, J Hepatol 2003

Boni, Ferrari, Gastro 2012

Penna, ferrari, J Hepatol 2012

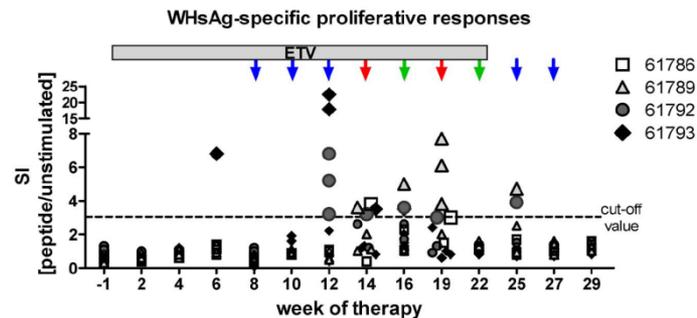
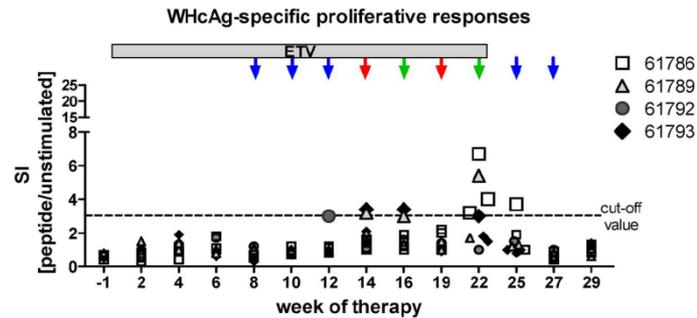
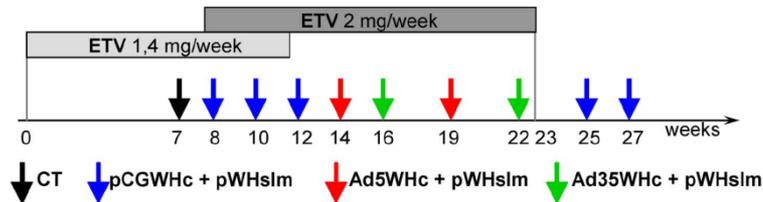
Micco, Maini, J Hepatol 2013

Concepts for immunotherapy



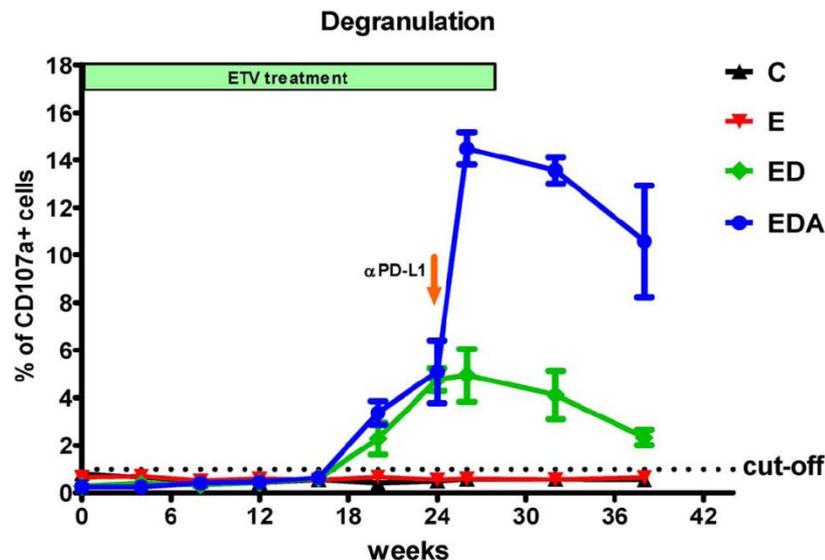
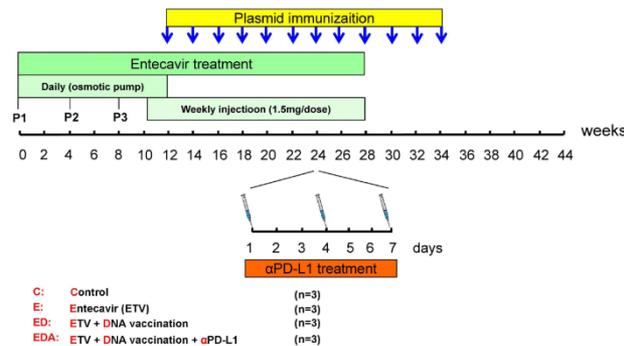
Combination of DNA Prime – Adenovirus Boost Immunization with Entecavir Elicits Sustained Control of Chronic Hepatitis B in the Woodchuck Model

Anna D. Kosinska¹, Ejuan Zhang^{1,2}, Lena Johrden³, Jia Liu¹, Pia L. Seiz⁴, Xiaoyong Zhang^{1,5}, Zhiyong Ma¹, Thekla Kemper¹, Melanie Fiedler¹, Dieter Glebe⁴, Oliver Wildner⁶, Ulf Dittmer¹, Mengji Lu¹, Michael Roggendorf^{1*}



Enhancing Virus-Specific Immunity *In Vivo* by Combining Therapeutic Vaccination and PD-L1 Blockade in Chronic Hepadnaviral Infection

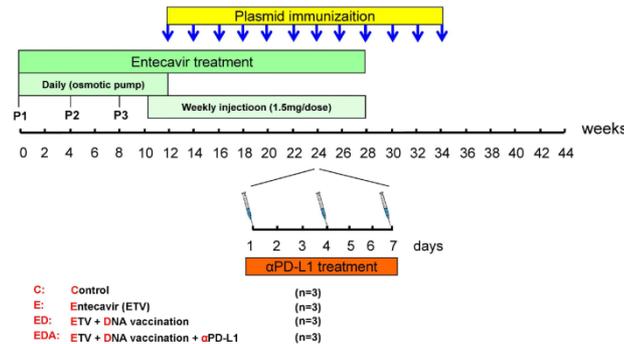
Jia Liu¹, Ejuan Zhang¹, Zhiyong Ma¹, Weimin Wu¹, Anna Kosinska¹, Xiaoyong Zhang^{1,2}, Inga Möller¹, Pia Seiz³, Dieter Glebe³, Baoju Wang⁴, Dongliang Yang⁴, Mengji Lu¹, Michael Roggendorf^{1*}



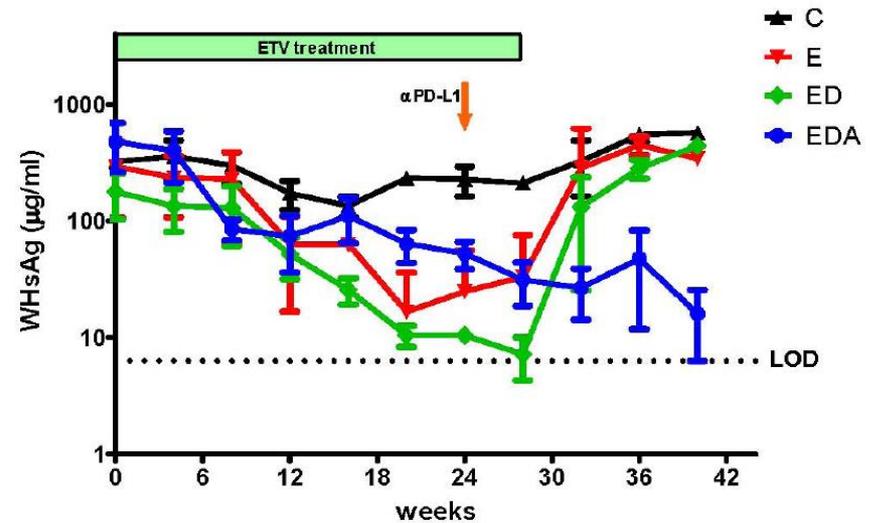
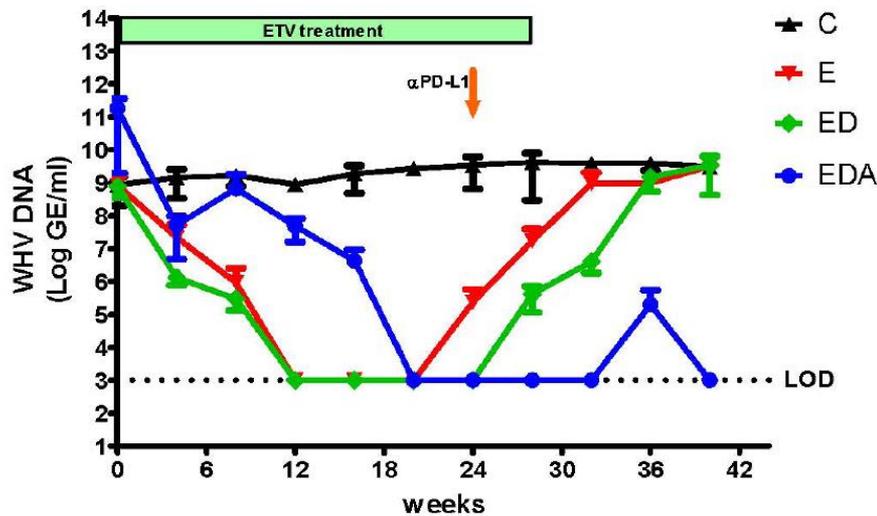
Plos Pathogens 2014

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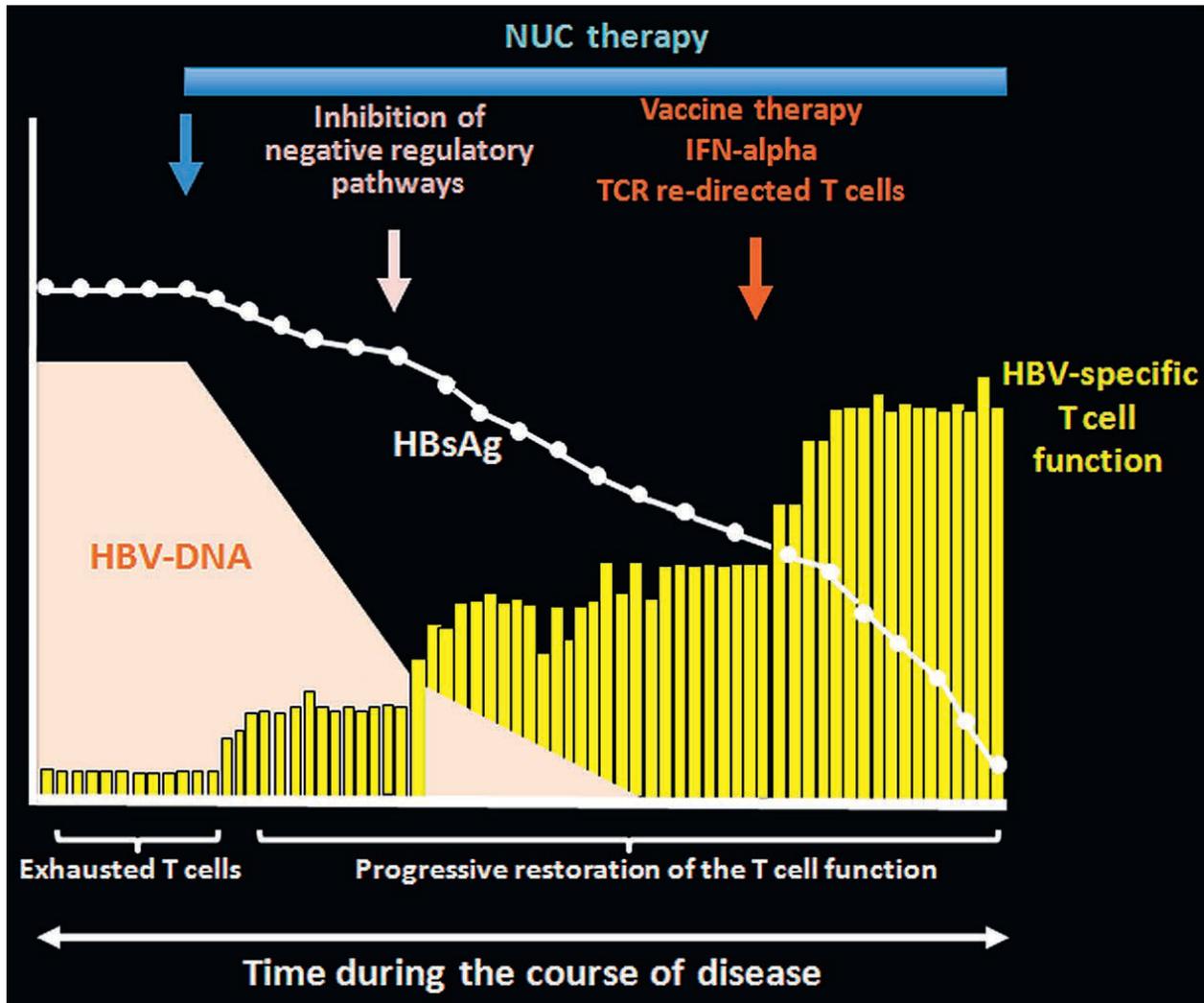
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Plos Pathogens 2014



Outlook



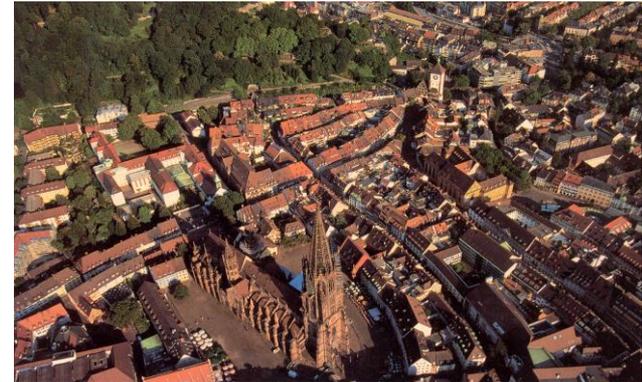
Ferrari, Bertoletti, GUT 2012

THANKS!

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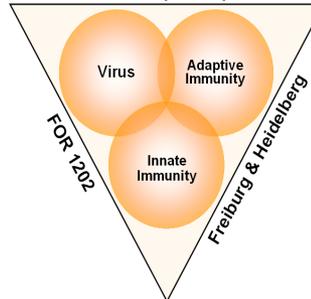
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Persistence of Hepatotropic Viruses



Deutsche Forschungsgemeinschaft

