



mRNA-based T cell engineering for virus-related cancers (HBV-HCC) and viral infections

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Disclosure

Antonio Bertoletti acts as a consultant and serves on the advisory boards of Gilead Sciences, Janssen-Cilag, MolecularTherapies, Roche, Bri-Bio, Vir and Abivax.

Cofounder of Lion TCR Pte Ltd, a biotech company developing T cell receptors for treatment of virus-related cancers

Summary



Overview of immunotherapy
potential for HBV and HBV-HCC



mRNA HBV-specific TCR-redirected T
cells. Pros and Cons -no genetic
modification- transient effect

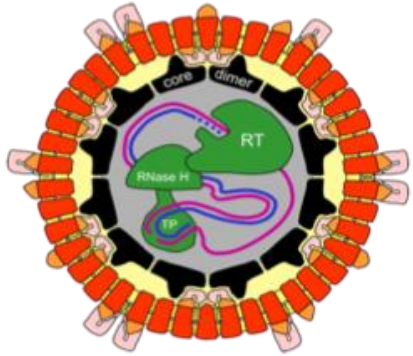


What do we want to achieve and
theoretical mechanisms of clinical
efficacy

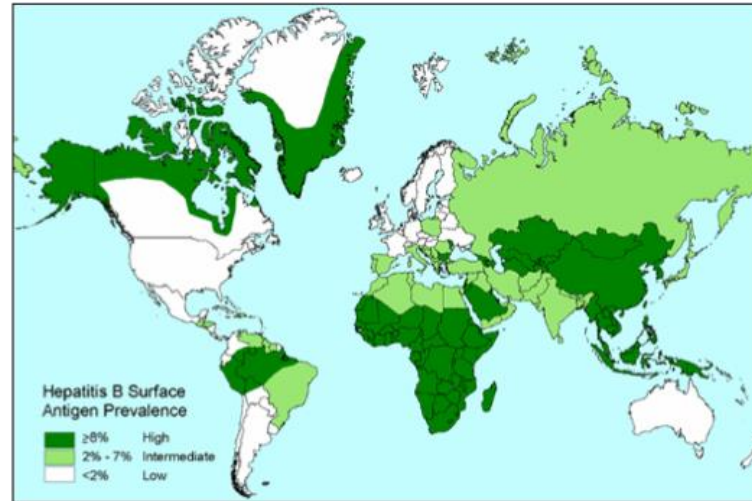
The extend of the problem...

Epidemiology/pathology

HBV



DNA-Virus Hepatotropic
Non-cytopathic



250-300 Million People infected

75% of them in Asia

Therapy blocks HBV replication/but no clearance

Chronic viral hepatitis



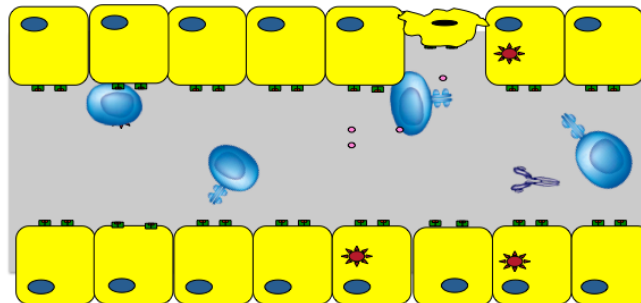
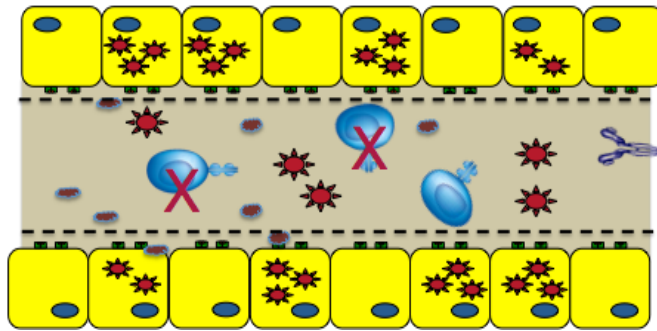
Cirrhosis



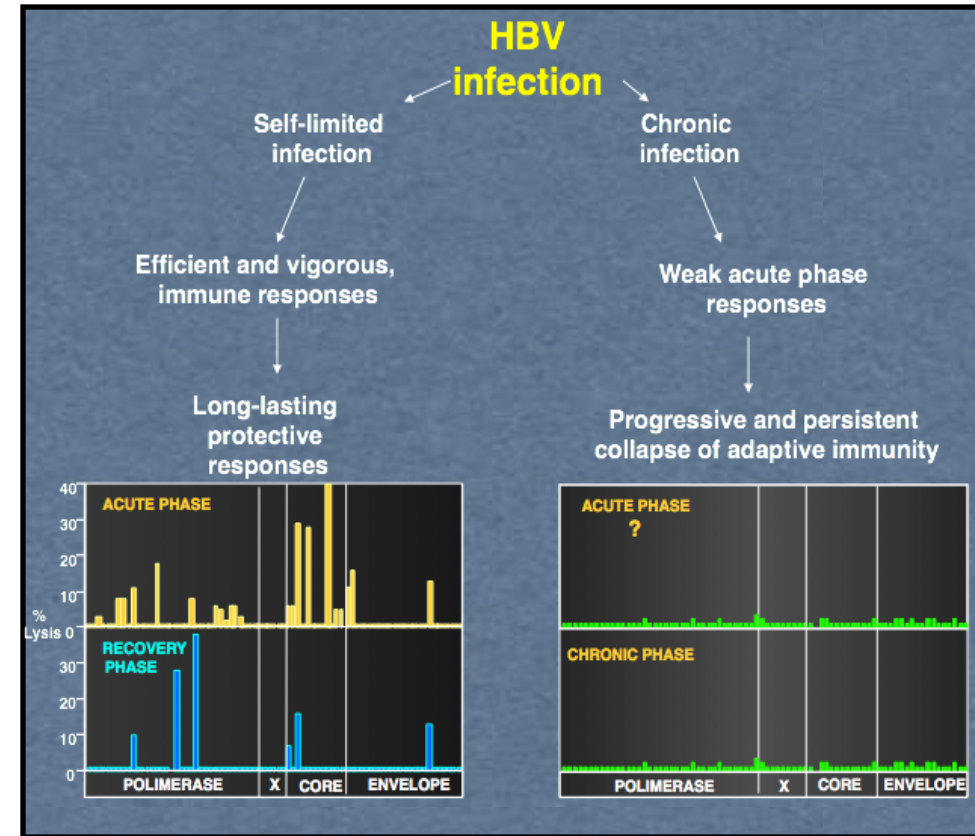
HCC

Reconstitution of HBV-specific T cell immunity is a potential cure for HBV chronic infection .

Chronic HBV Infection



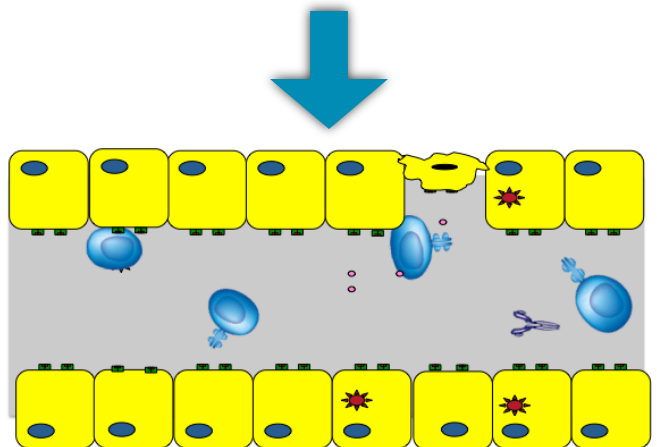
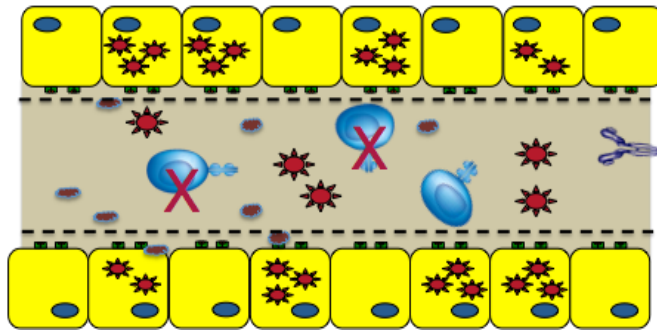
Resolved HBV
"Functional Cure"



Functional Dichotomy of Adaptive Immunity
in chronic versus resolved patients

Reconstitution of HBV-specific T cell immunity is a potential cure.

Chronic HBV Infection



Resolved HBV
"Functional Cure"

**Transplantation of HBV
primed bone marrow causes
HBV functional control in
CHB patients.**

Lau GK et al (1977) H antigen after bone marrow
transplantation: role of adoptive immunity transfer.
Hepatology 25:1497–1501. doi: 10.1002/hep.510250631.

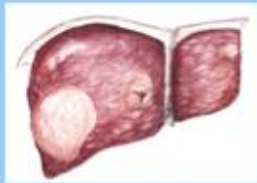
Ilan Y, et al. (1993) Ablation of persistent hepatitis B by bone
marrow transplantation from a hepatitis B-immune donor.
Gastroenterology 104:1818–1821.

- HBV-HCC the extend of the problem .

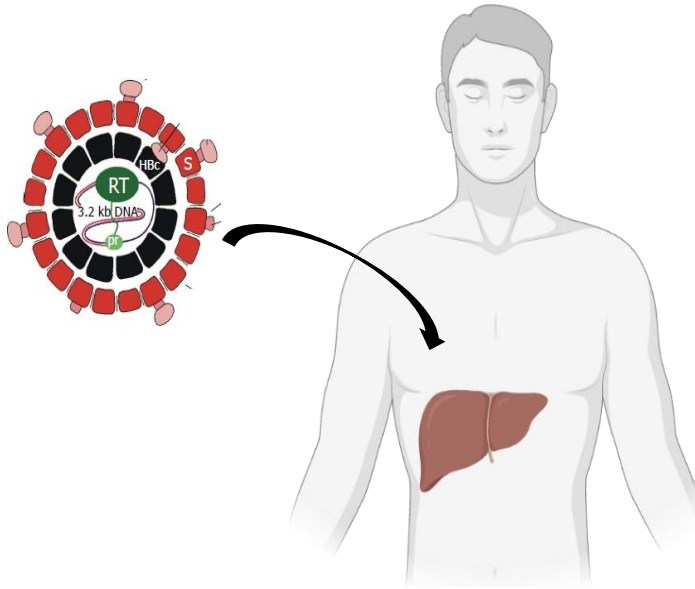
Chronic viral hepatitis



Cirrhosis



HCC



HCC approximately 600.000 new cases at year(55% in China).
~ 80% are HBV related

Median Survival Time (**advanced HCC**): 3-6 months (untreated)
15 months survival (TACE + Sorafenil or checkpoint inhibitors)

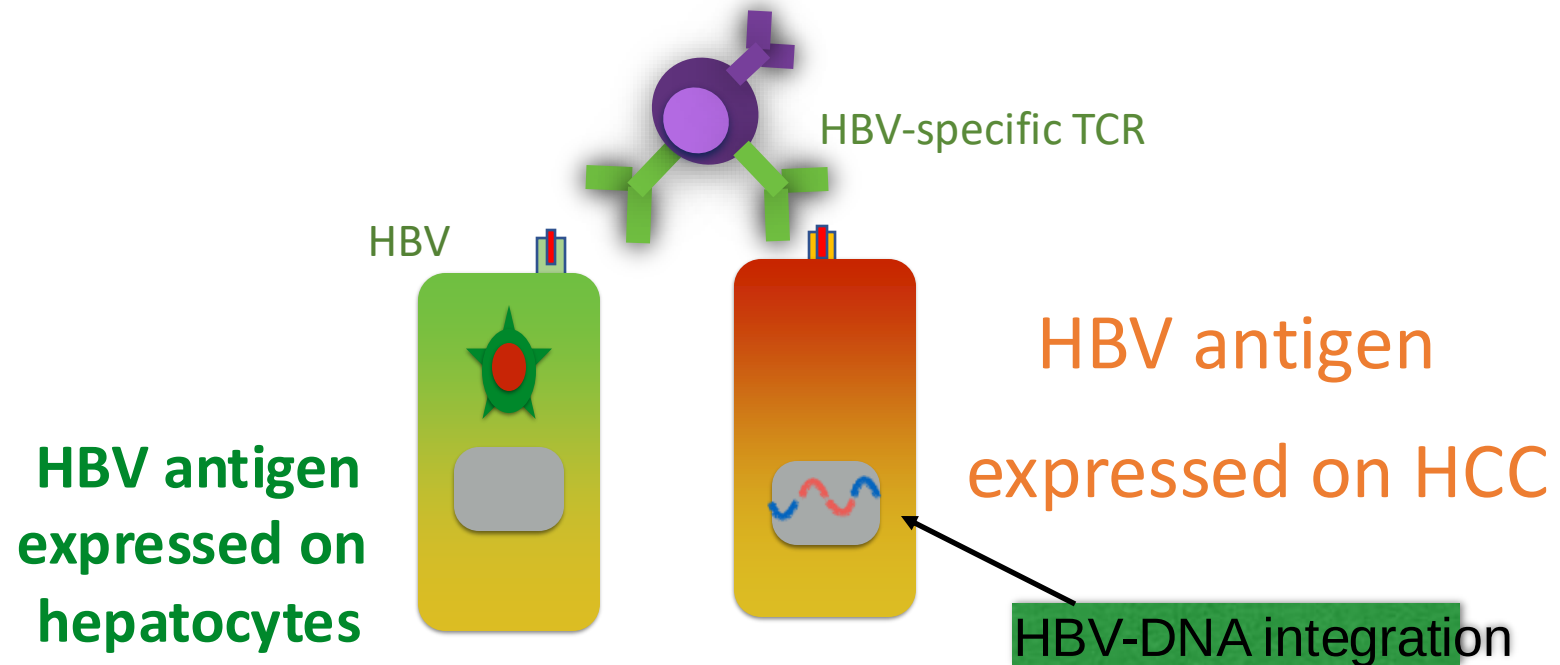
HBV can Integrate

HBV-DNA integration in about 80/90% HCC

HBV-specific CD8 T cells deleted in HBV-HCC patients.

Can we use HBV-specific T cells to treat HBV-HCC?

Rational -Viral antigen as Tumor antigen



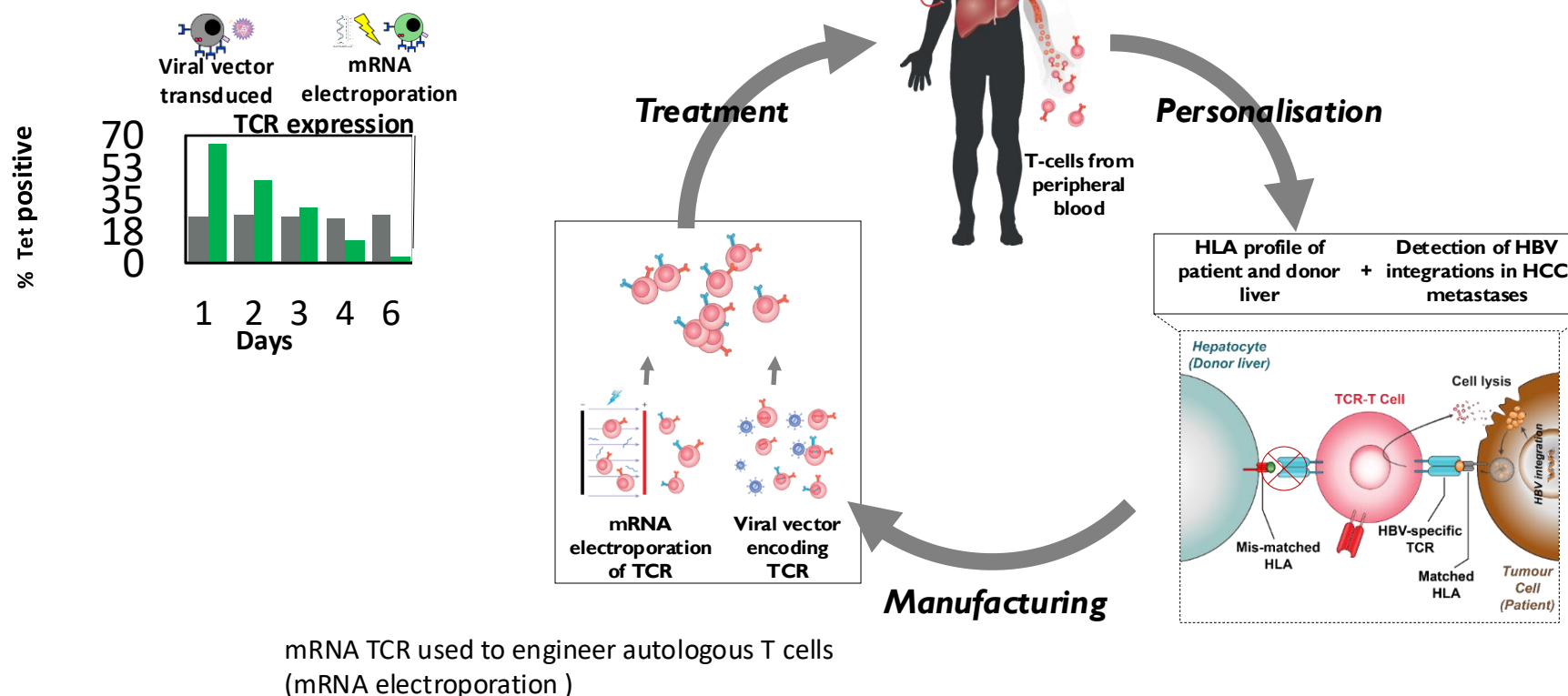
Engineering Lymphocytes for treatment of HBV-HCC

PRIMARY AND SECONDARY

Transient TCR expression: Toxicity control
Escalating doses TCR-T cells (up to 107 x kg)

We are targeting HBV epitopes produced from
HBV-DNA integration present in HCC.

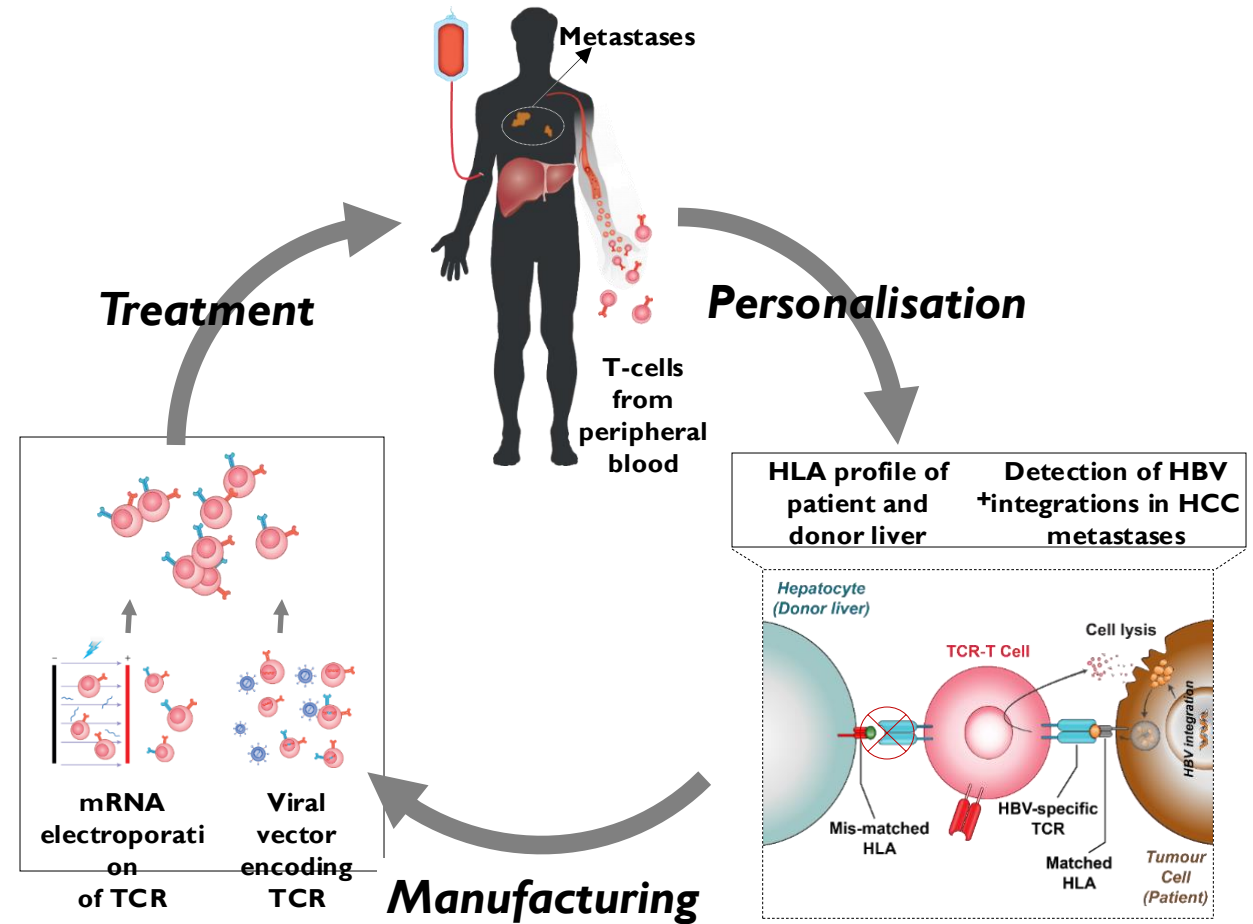
HBV-TCR library recognising different epitopes



Questions

Why TCR and not CAR?

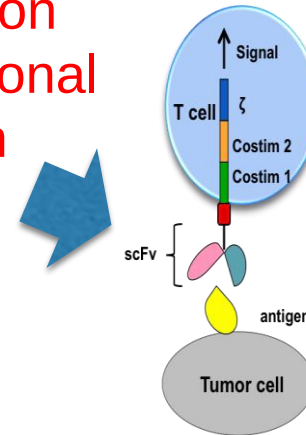
How do we produce HBV-TCR?



Why TCR and not CAR -T cells?

CAR Chimeric antigen receptor

Recognition
Conformational
Antigen

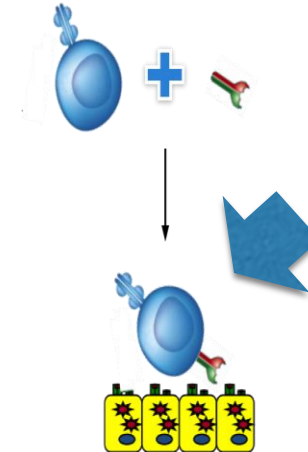


- Target cells expressing whole proteins in correct conformation and expressed on cell surface

NON HLA-restricted

TCR T cell receptor

Recognition
HLA/peptide
complex



- Target cells produce antigenic fragments that are processed and presented

HLA-restricted

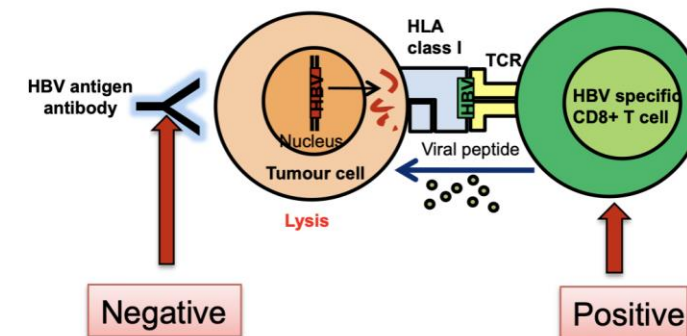
Why TCR and not CAR -T cells?

1- HBV-TCR not inhibited by circulating viral antigens (HBsAg)



(HBsAgI -50 ug/ml in sera)

2- HBV-DNA integration often not complete in HBV-HCC:
not production of whole antigens but short chimeric protein



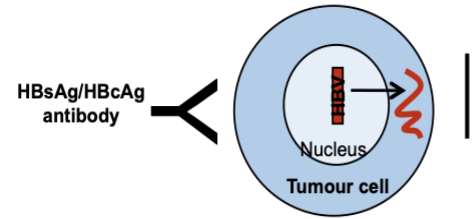
Gastroenterology. 2019 Jan 31. pii: S0016-5085(19)30348-8. doi: 10.1053/j.gastro.2019.01.251. [Epub ahead of print]

Use of Expression Profiles of HBV DNA Integrated Into Genomes of Hepatocellular Carcinoma Cells to Select T Cells for Immunotherapy.

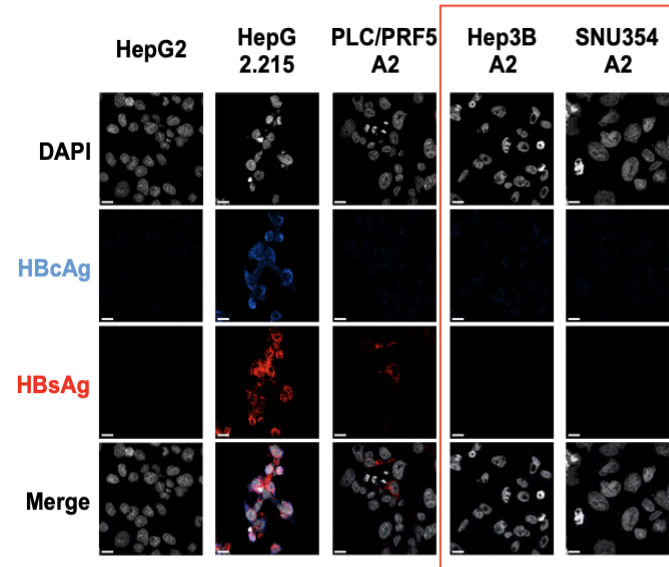
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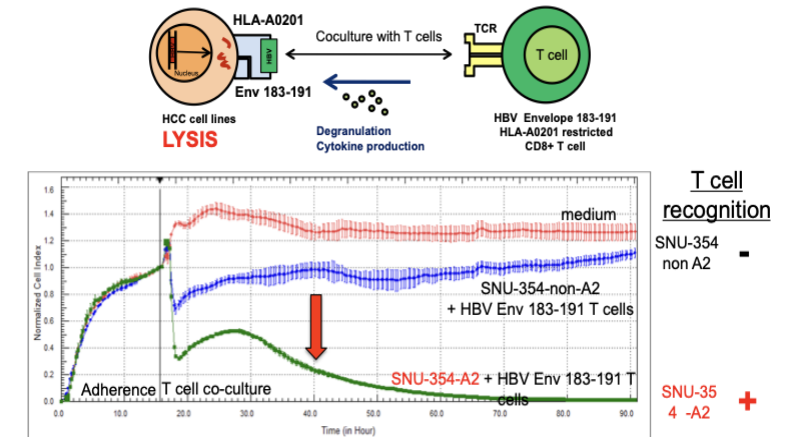
HBV Antigen expression



HBV-TCR T cells recognize HBsAg negative HCC with HBV-DNA integration



- Direct cytotoxicity measurement through impedance

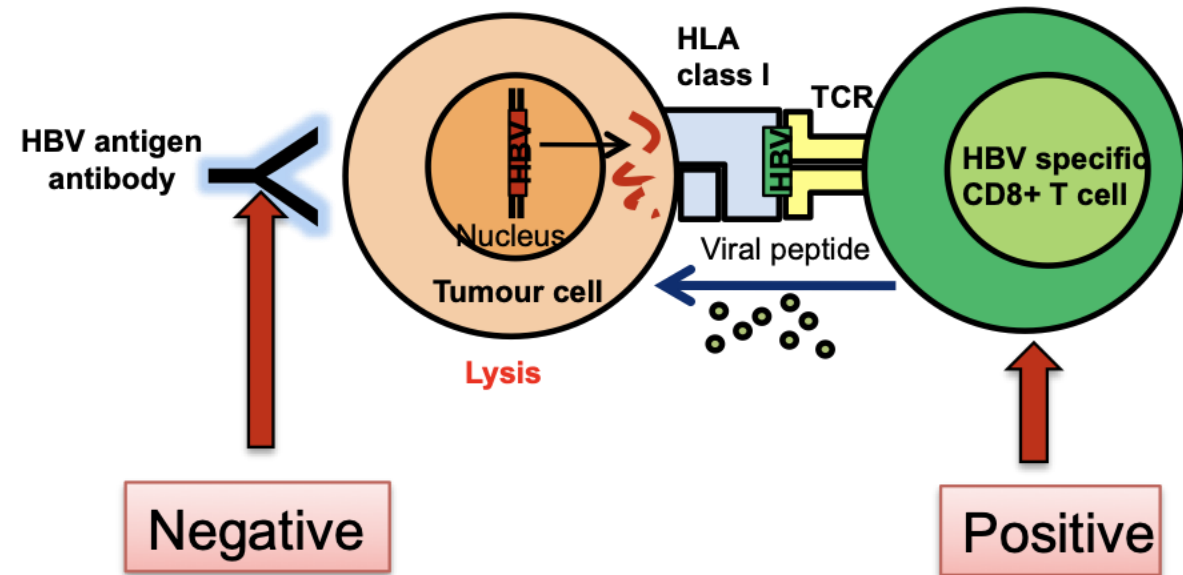


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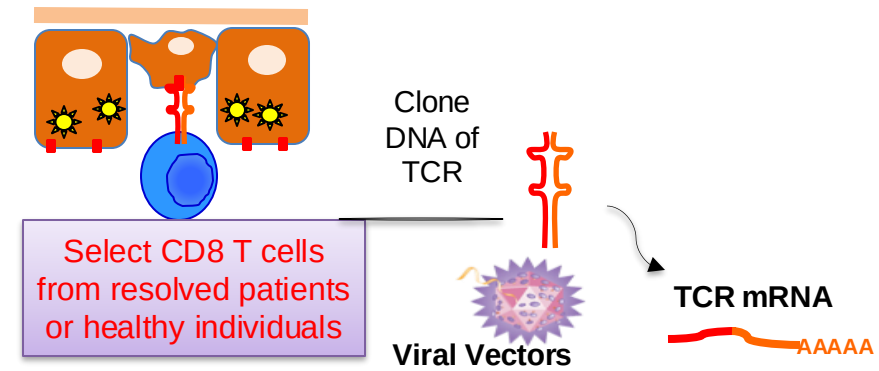
Why TCR and not CAR -T cells?

HBV-DNA fragments present in HBsAg negative HCC produce antigens that are processed and presented to TCR of CD8+ T cell

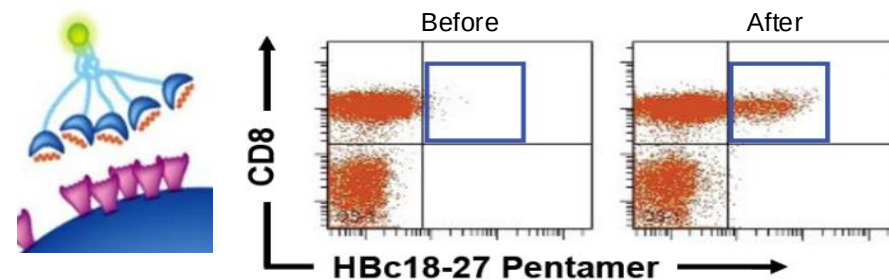


HBV-HCC recognised by T cells but not antibodies

Production of HBV- specific TCR T cells

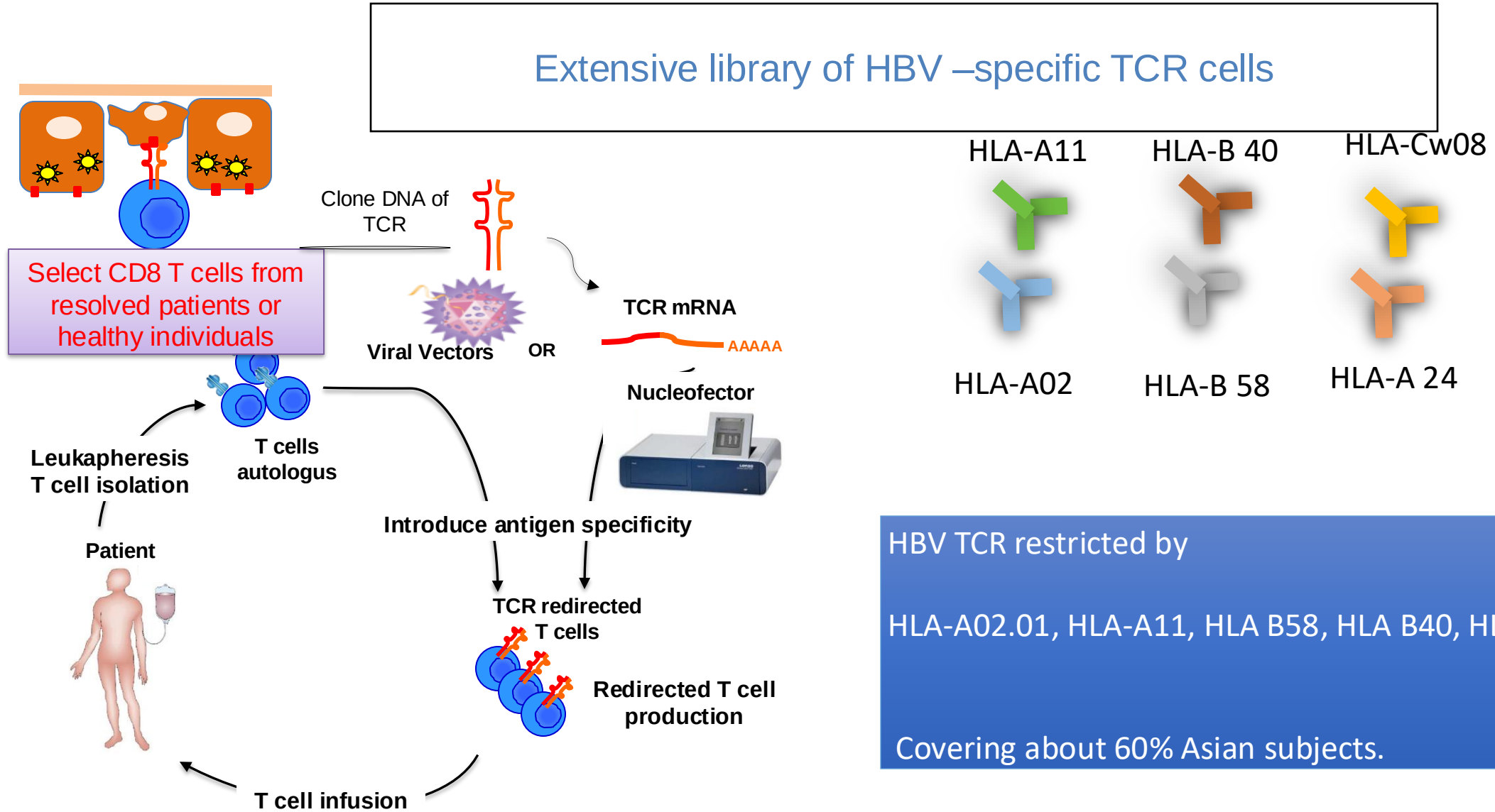


TCR-gene modified T cells



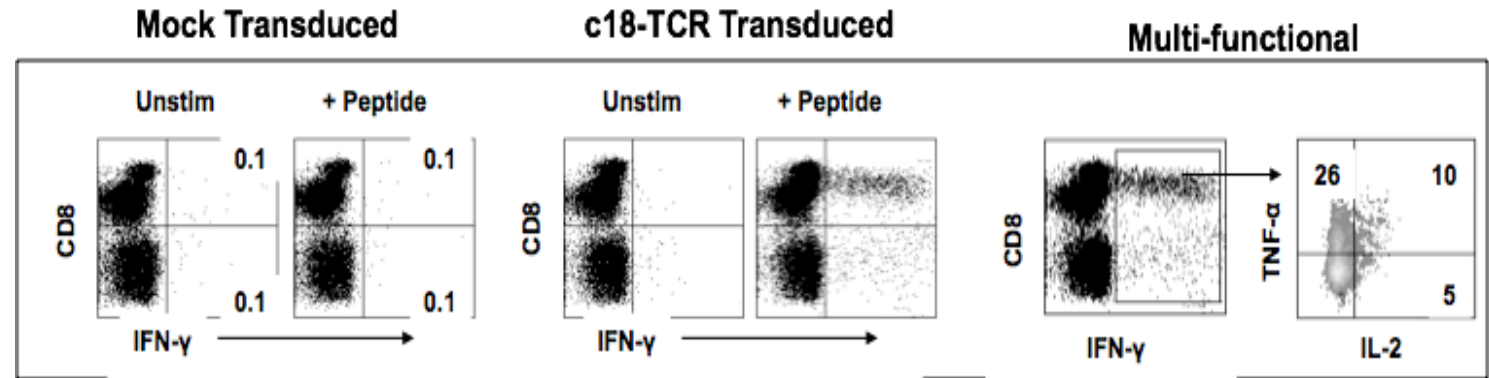
TCR-gene modified T cells are functional – produce IFN- γ and TNF- α in antigen-specific manner

Production of HBV-specific TCR-redirected T cells

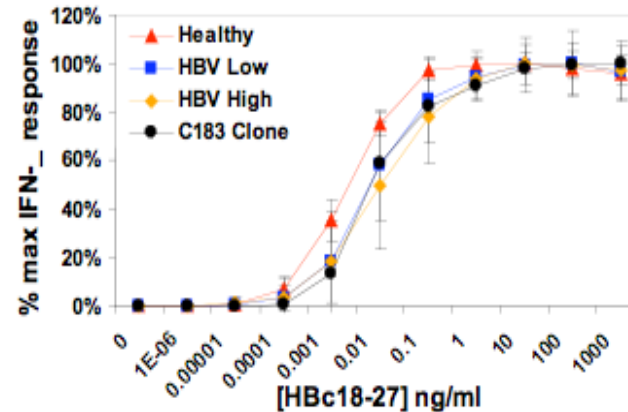


We can engineer HBV-specific T cells with HBV-TCR

Function identical to HBV-T cells of patients who resolved HBV

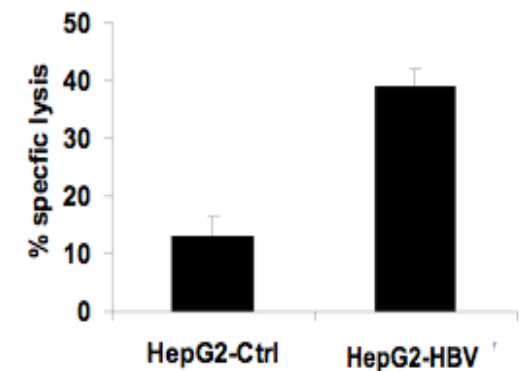


Sensitivity of T cell activation



1. A2+ T2 cells pulsed with decreasing peptide
2. Compare to original T cell clone from resolved patient
3. 5 patients/group; Mean +/- Std Dev

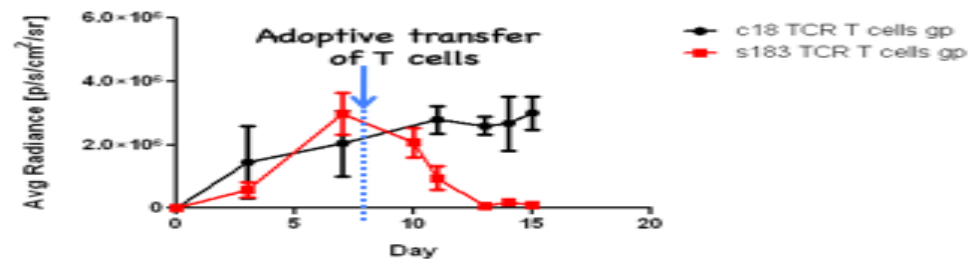
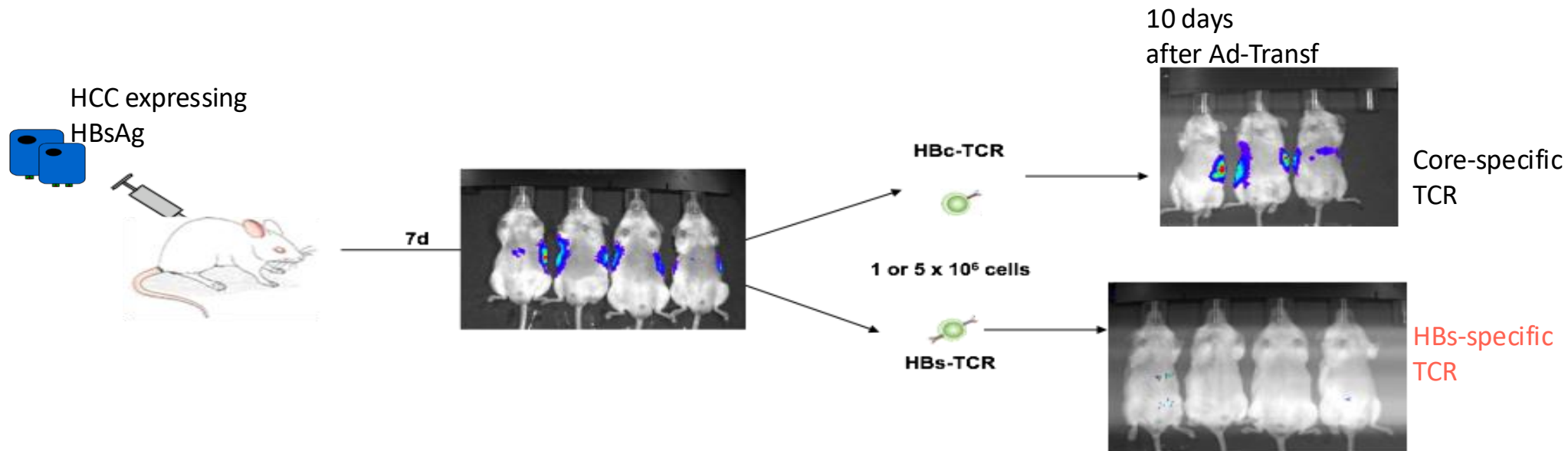
Cytotoxicity



1. HepG2 expressing HBV virus
2. 6 h assay, E:T = 1:1 (effectors CD8+IFN-γ+)

HBV-specific TCR T cell ability to lyse HBV/HCC \ (Animal Model)

Function of TCR-gene modified T cells (in vivo) against HBV-HCC (xenograft model)

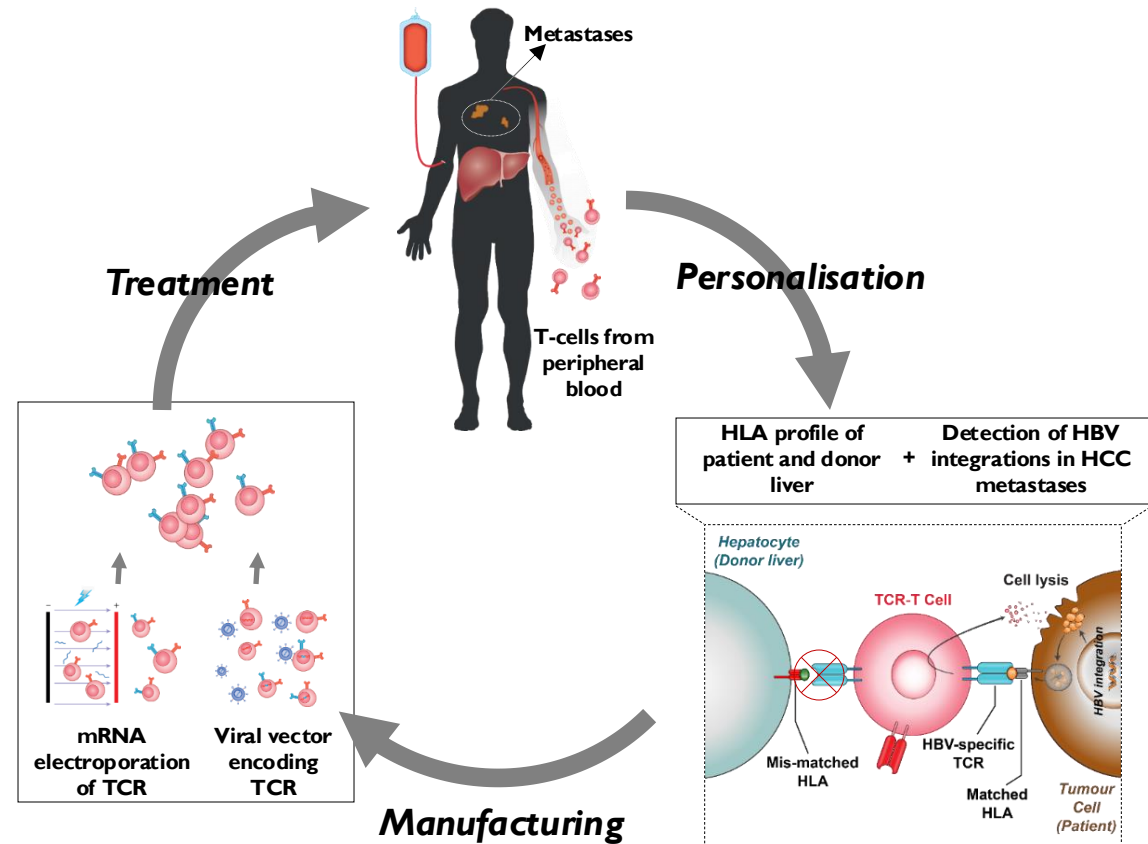


Question

Evidence in HBV-HCC patients?

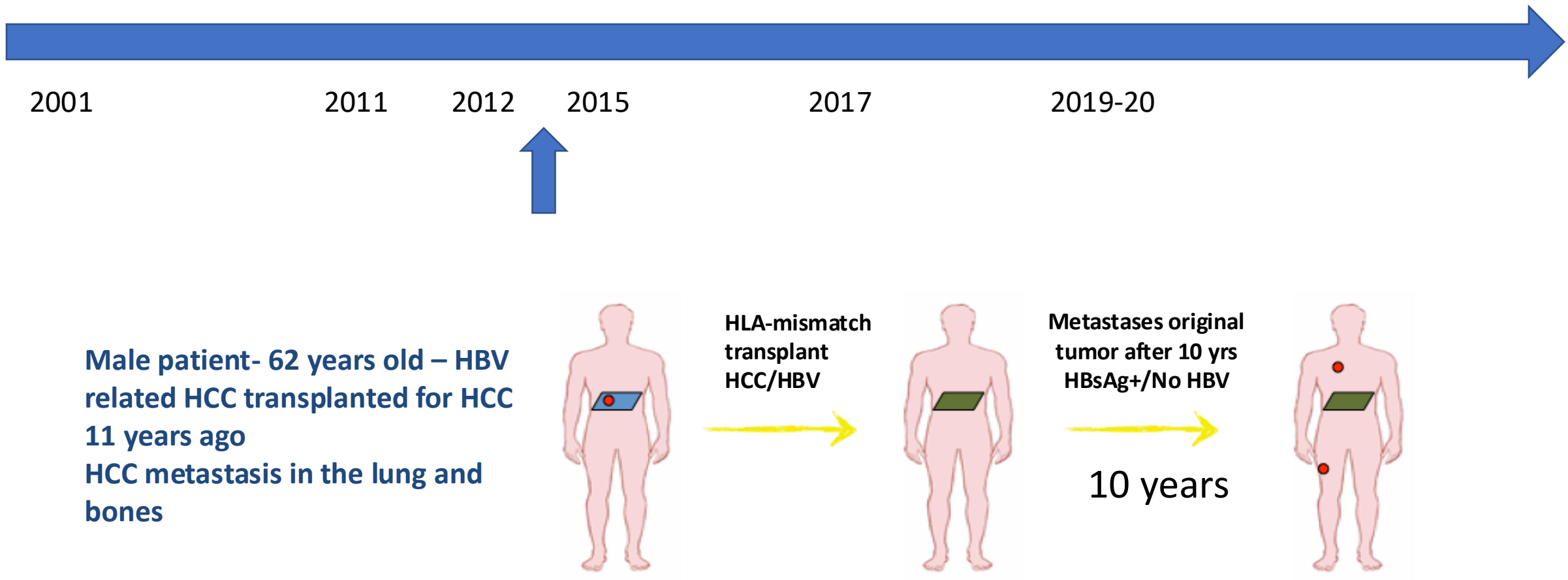
Engineering Lymphocytes for HBV-HCC treatment

PRIMARY AND SECONDARY



FROM DISCOVERY OF HBV-SPECIFIC TCRs INTO THE CLINICS

Proof of principle



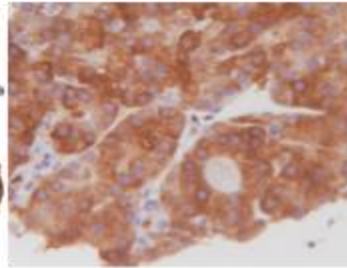
FROM DISCOVERY OF HBV-SPECIFIC TCRS INTO THE CLINICS

Proof of principle

HCC metastasis



HBsAg +



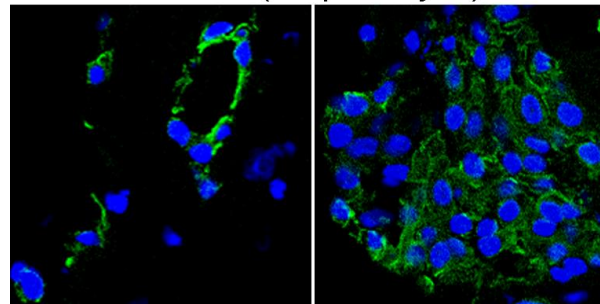
HBV sequence

FLLTRILTI
HBV Gen D

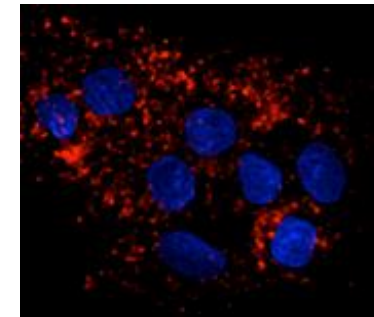
Cytokeratin 18

TCR-like Ab
Specific for the
HLA-A2/Env183-91

Characterization HBV antigen
on HCC relapses



Are cells of hepatic origin?



Do HCC cells present
A2/HBs complexes?

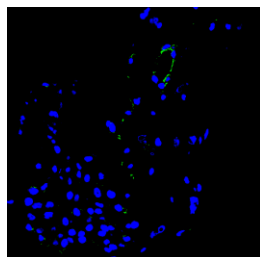
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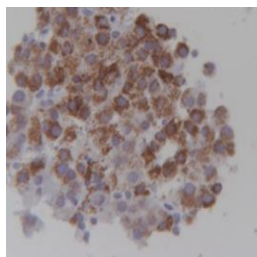
Transplanted liver:
HBsAg- HBcAg- HBV DNA-

Metastasis:

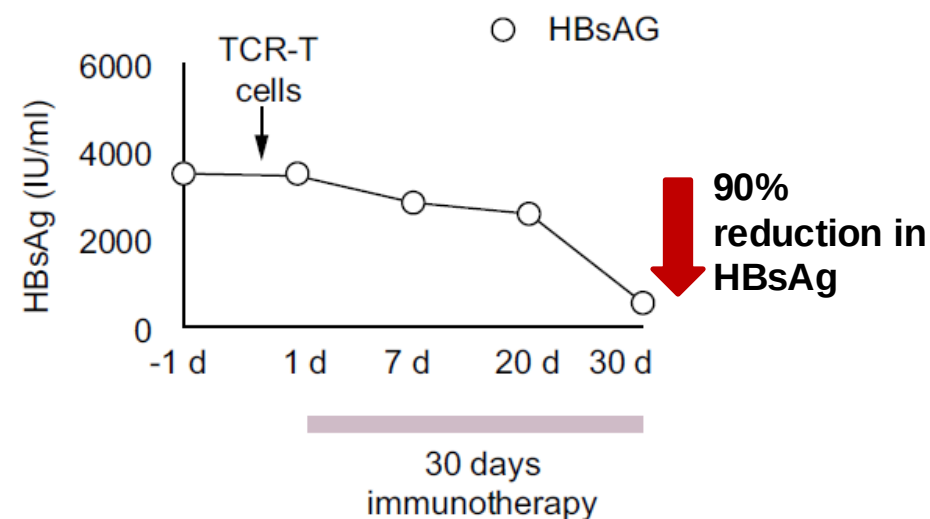
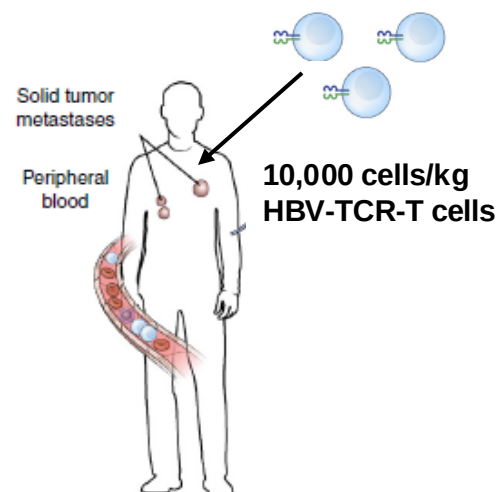
Biopsy of
metastases



CK18+
(Hepatocytes)



HBsAg+

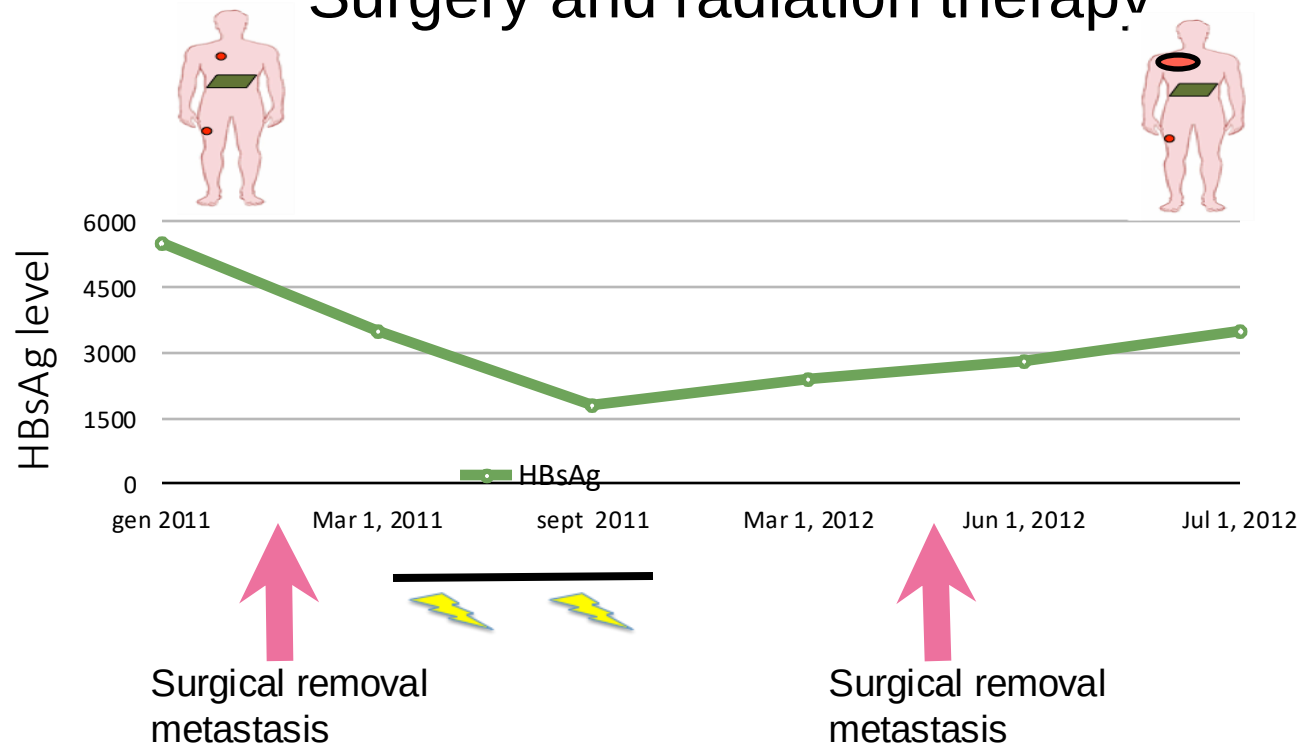


Qasim et al, 2015 J. Hepatology

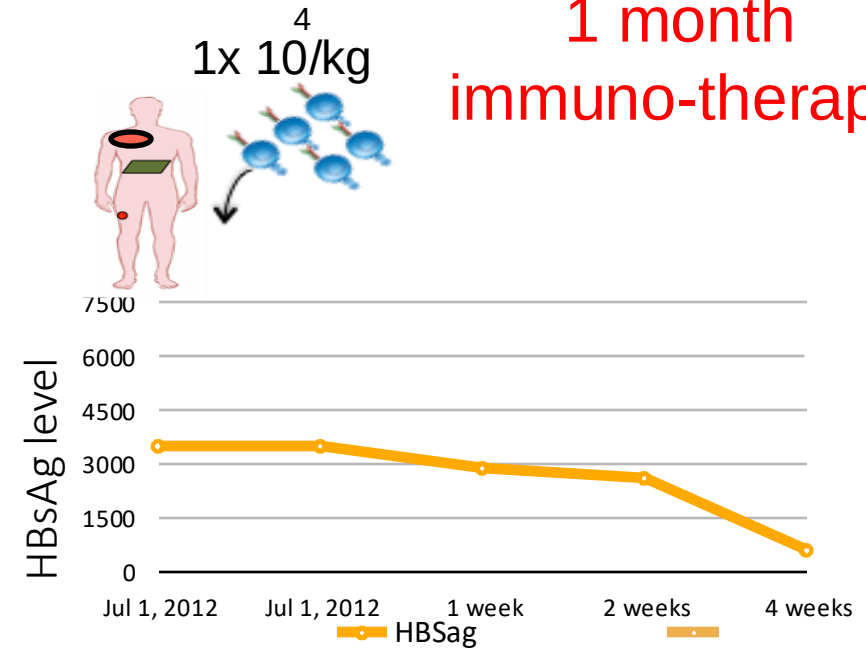
Efficacy of TCR-redirected T cells in controlling HBsAg production.

Proof of principle

18 months
Surgery and radiation therapy

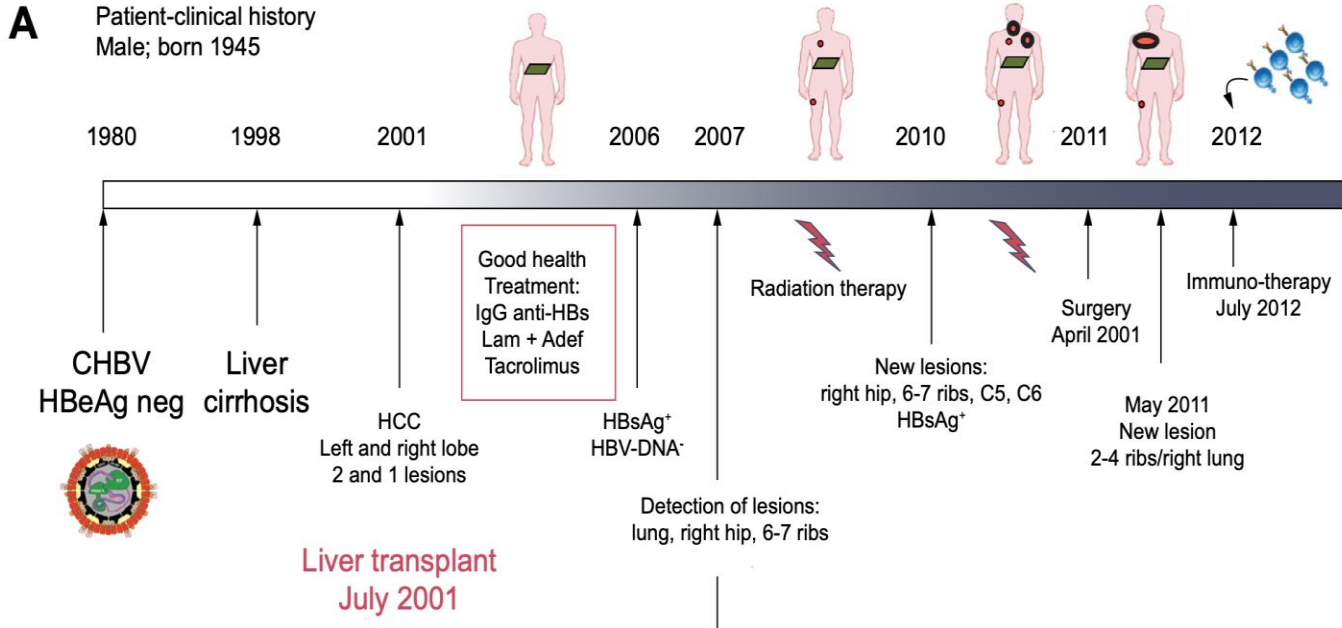


1 month
immuno-therapy



FROM DISCOVERY OF HBV-SPECIFIC TCRS INTO THE CLINICS

Proof of principle

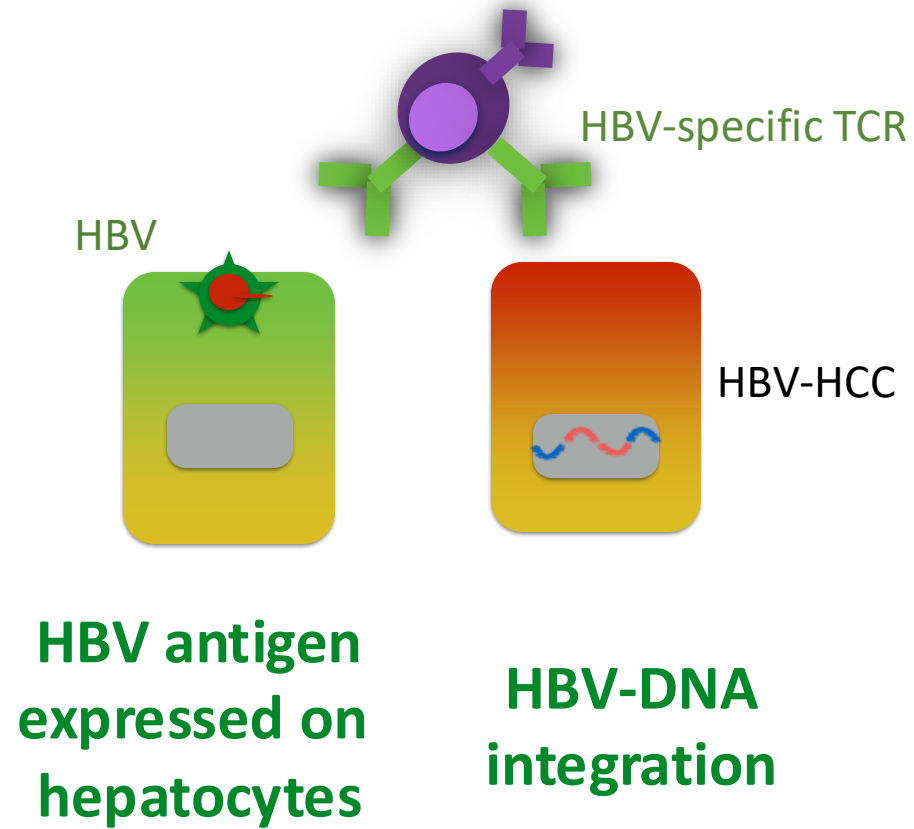


Very peculiar patient

- a) HBsAg produced only by integration
- b) Liver transplant not HBV re-infected

HBV-TCR do not
differentiate
between infected
or tumor
hepatocytes

Safety issue in HBV-HCC



HBV-TCR do not differentiate between
HBV infected hepatocytes and HBV-HCC !!!!

Summary



Overview of immunotherapy potential for HBV and HBV-HCC



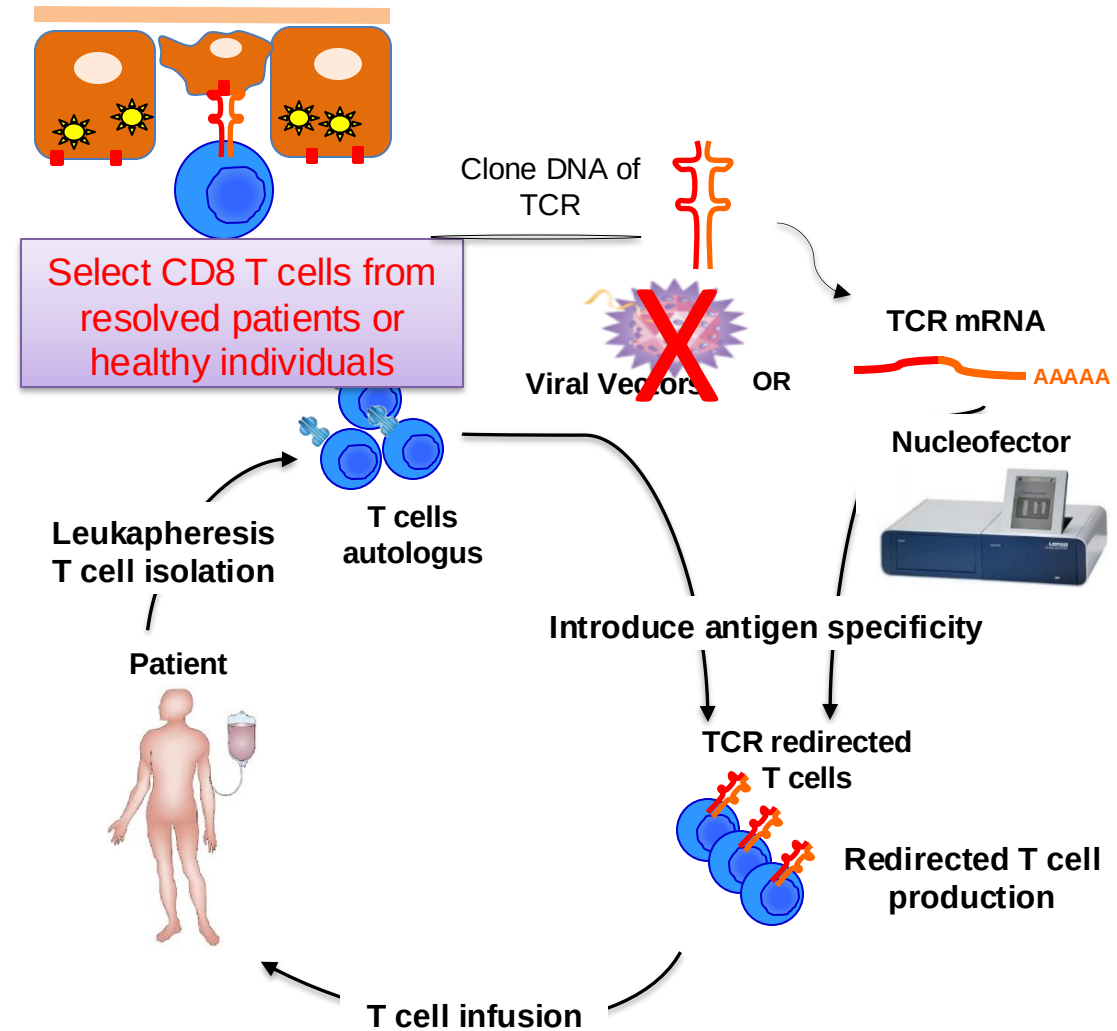
mRNA HBV-specific TCR-redirected T cells. Pros and Cons -no genetic modification- transient effect



What do we want to achieve and theoretical mechanisms of clinical efficacy

mRNA TCR and not
viral vector TCR-T cells

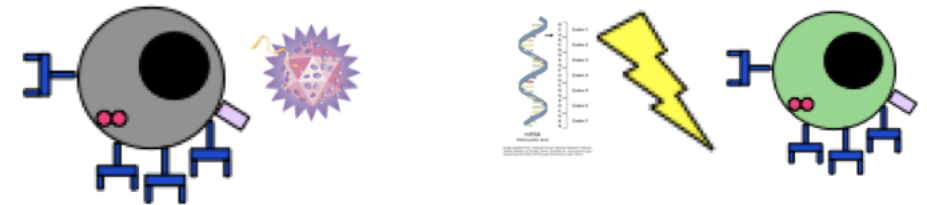
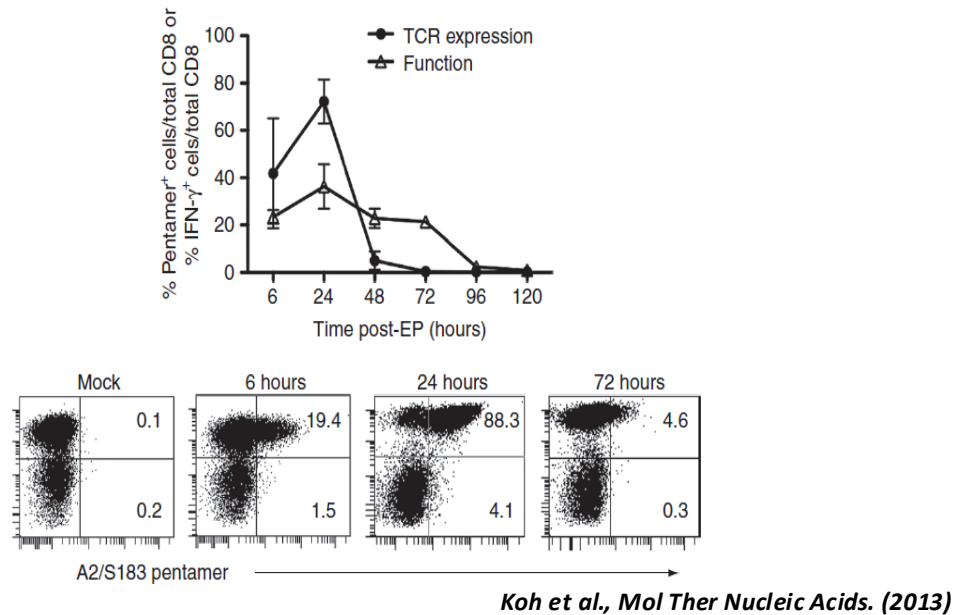
Safety..



Features of mRNA HBV-TCR T cells

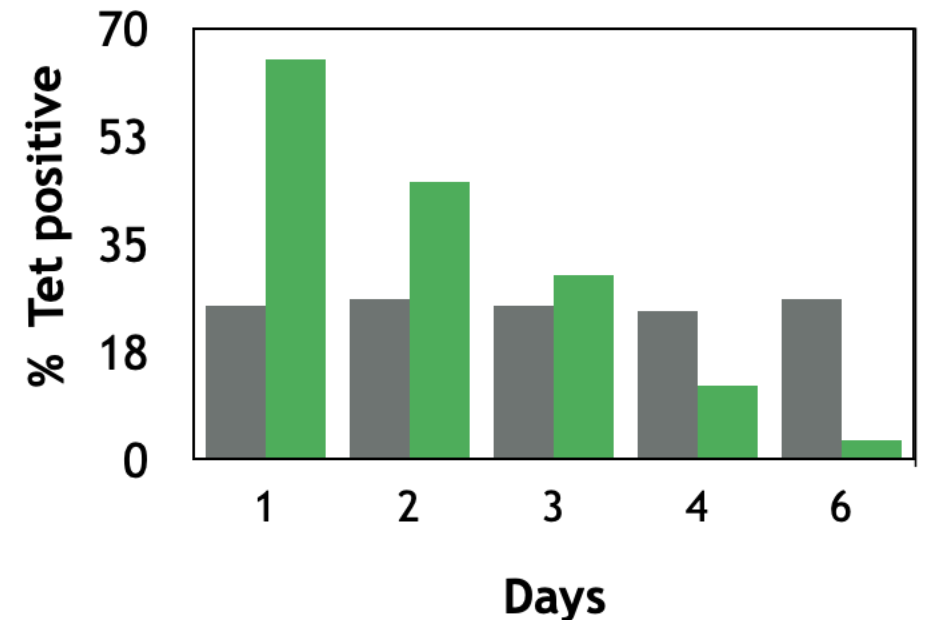
NO GENETIC MANIPULATION

- Temporally limited HBV-specific function due to the gradual decay of introduced HBV-TCR mRNA



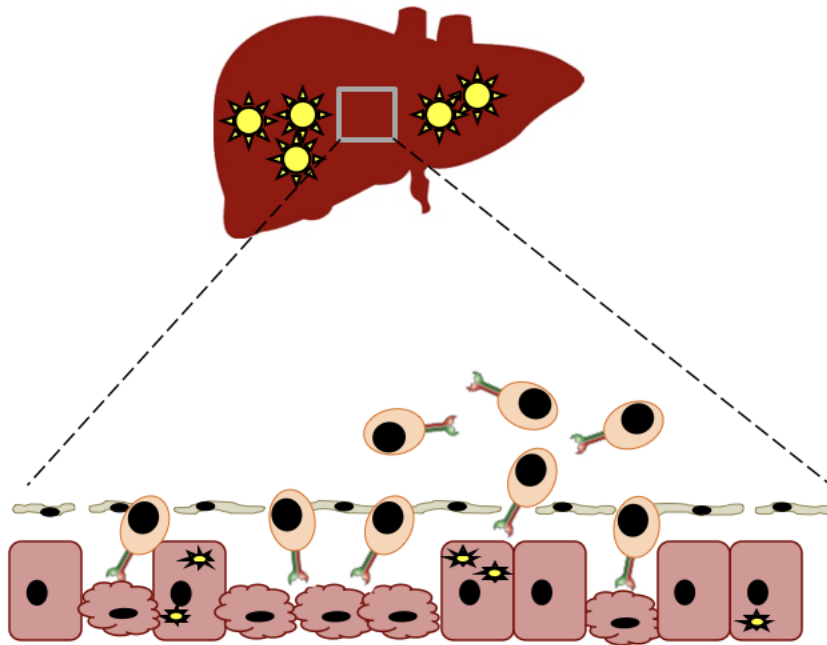
RetroV-TCR mRNA-TCR

TCR expression



Features of mRNA HBV-TCR T cells

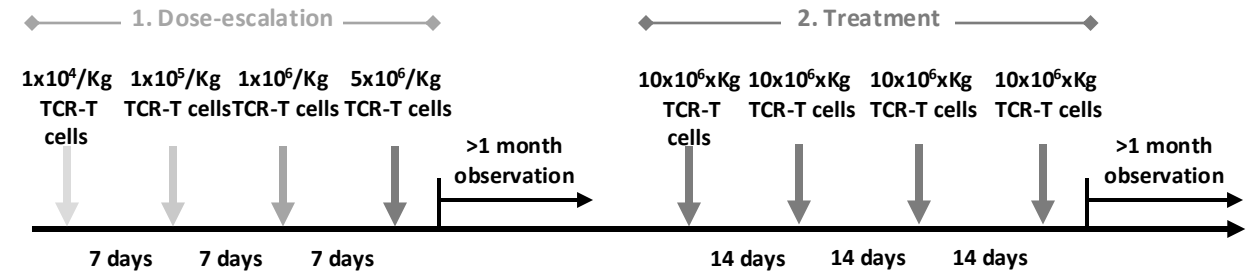
Time controlled TCR-expression



Less damage

TCR- T cells can be used in escalating doses and multiple times.

- Possible to deploy a dose-escalation infusion strategy



mRNA HBV-TCR T cells



Anti-viral and safety
In mouse model

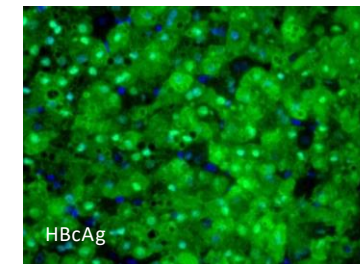
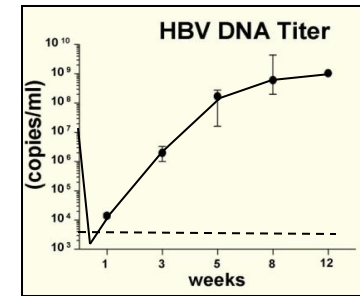
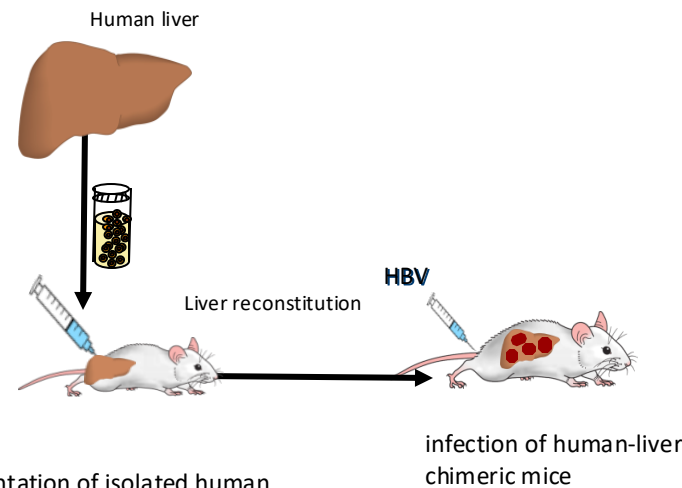
Antiviral activity of electroporated human effector T cells in vivo using HBV infected human liver chimeric uPA/SCID/IL2 γ (USG) mice



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HAMBURG



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The Journal of Clinical Investigation

RESEARCH ARTICLE

Lymphocytes transiently expressing virus-specific T cell receptors reduce hepatitis B virus infection

Janine Kah,¹ Sarene Koh,^{2,3} Tassilo Volz,¹ Erica Ceccarello,^{2,4} Lena Allweiss,¹ Marc Lütgehetmann,⁵ Antonio Bertoletti,^{2,4} and Maura Dandri^{1,6}

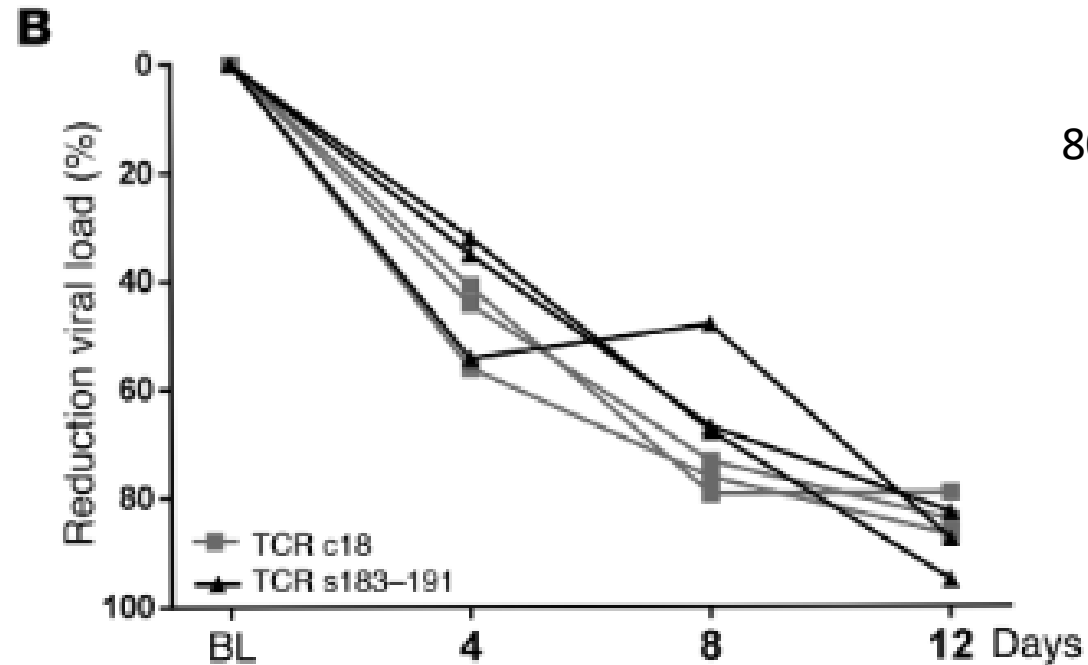
¹Medical Department, Center for Internal Medicine, University Medical Center Hamburg-Eppendorf, Hamburg, Germany; ²Singapore Institute for Clinical Sciences, Agency for Science Technology and Research, Singapore; ³Lion TCR Pte. Ltd., Singapore; ⁴Emerging Infectious Diseases Programme, Duke-NUS Medical School, Singapore; ⁵Institute of Microbiology, Virology and Hygiene, University Medical Center Hamburg-Eppendorf, Hamburg, Germany; ⁶German Center for Infection Research, Hamburg-Lübeck-Borstel Partner Site, Hamburg, Germany.

mRNA HBV-TCR T cells



Anti-viral and safety
In mouse model

12 days- 3 injections- 80% reduction



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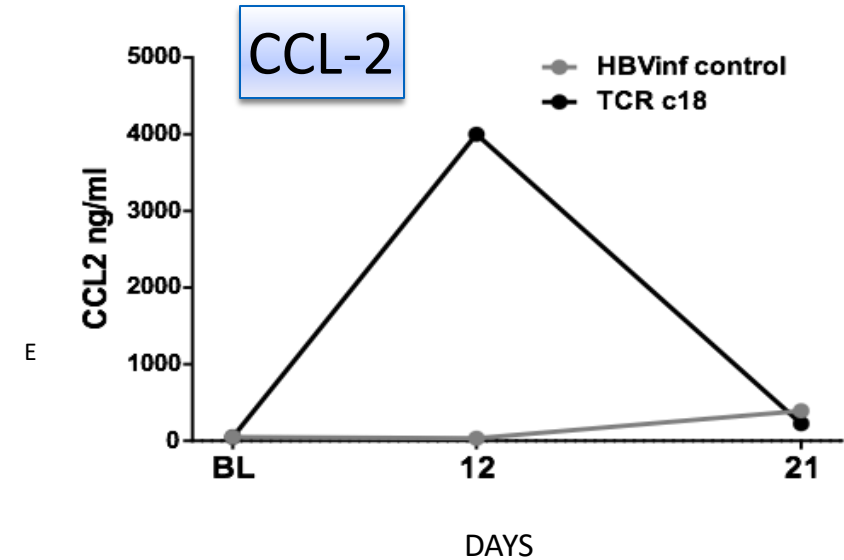
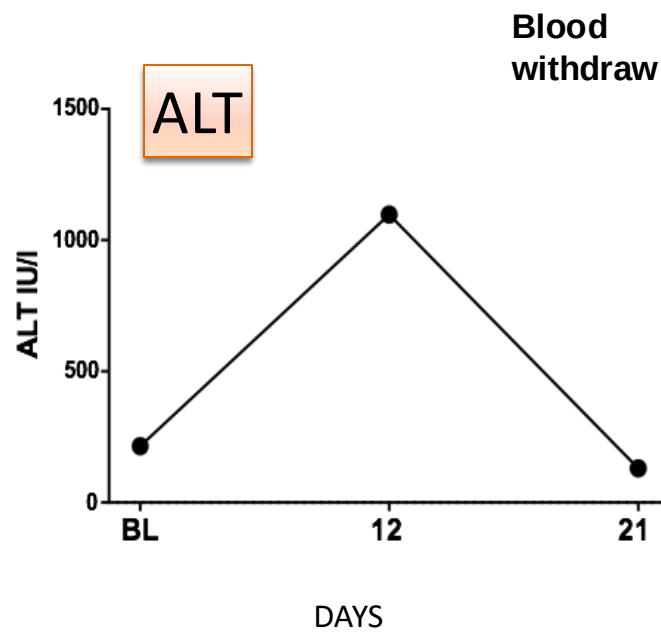
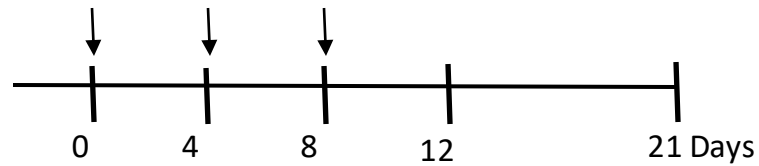
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mRNA HBV-TCR T cells Inflammation is time-limited



Anti-viral and safety
In mouse model

Multiple adoptive transfer of 0.5 million
effector T cells transduced with *TCRc18*



Transient Inflammation



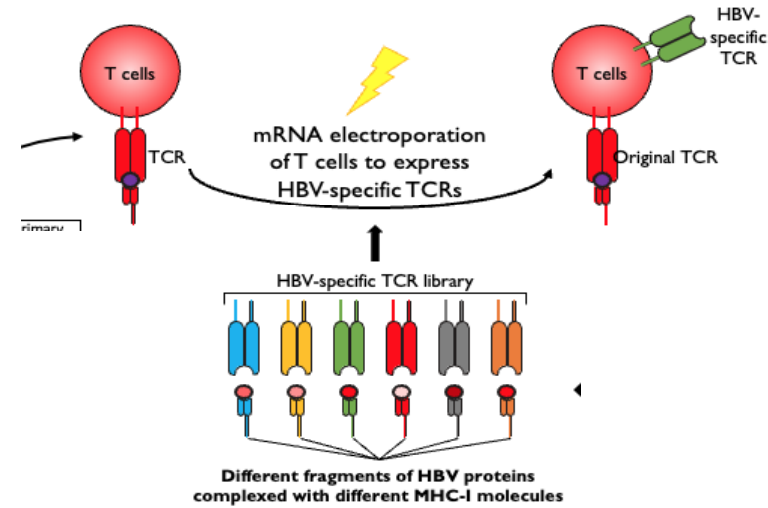
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Why mRNA TCR and not viral Vectors TCR ?

Advantages



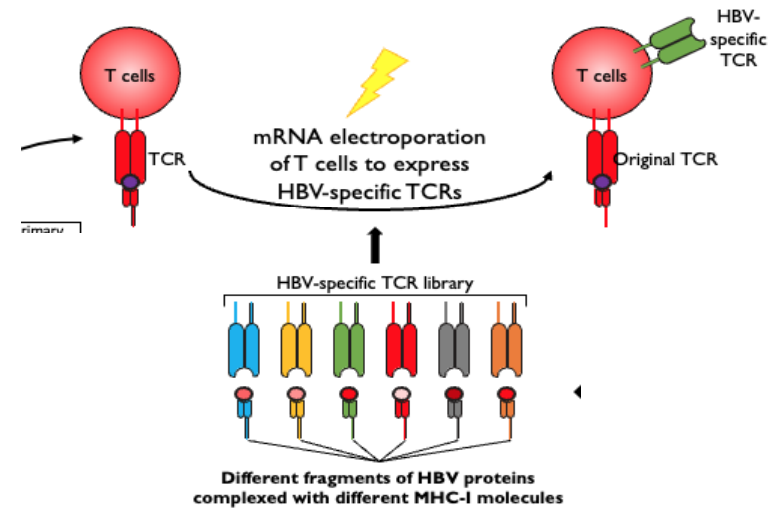
Safety
Possibility of dose escalation

NO GENETIC MANIPULATION

Bypass T cell exhaustion
But temporally limited
efficacy...

Why mRNA TCR and not viral Vectors TCR ?

Disadvantages and
Limitations?

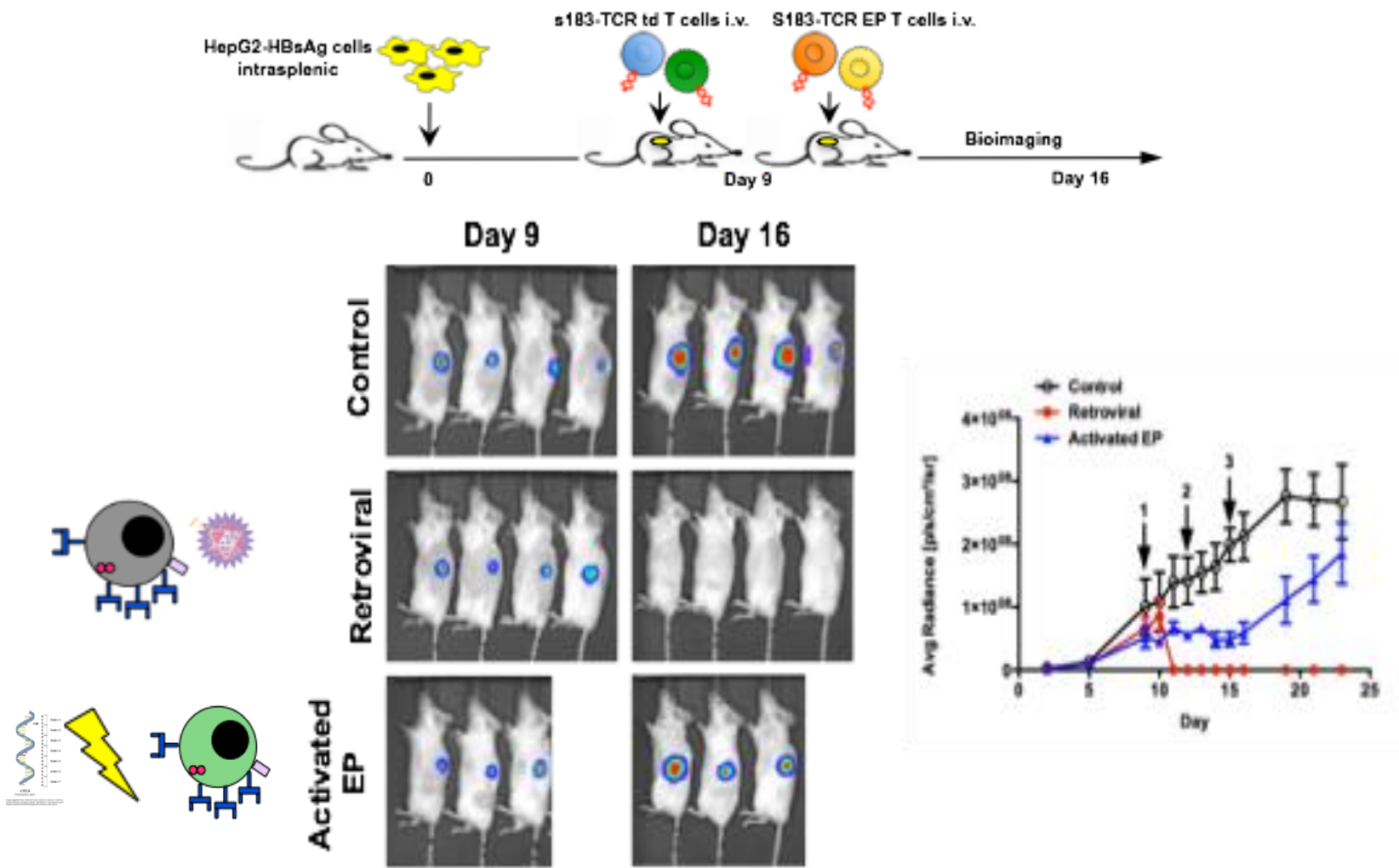


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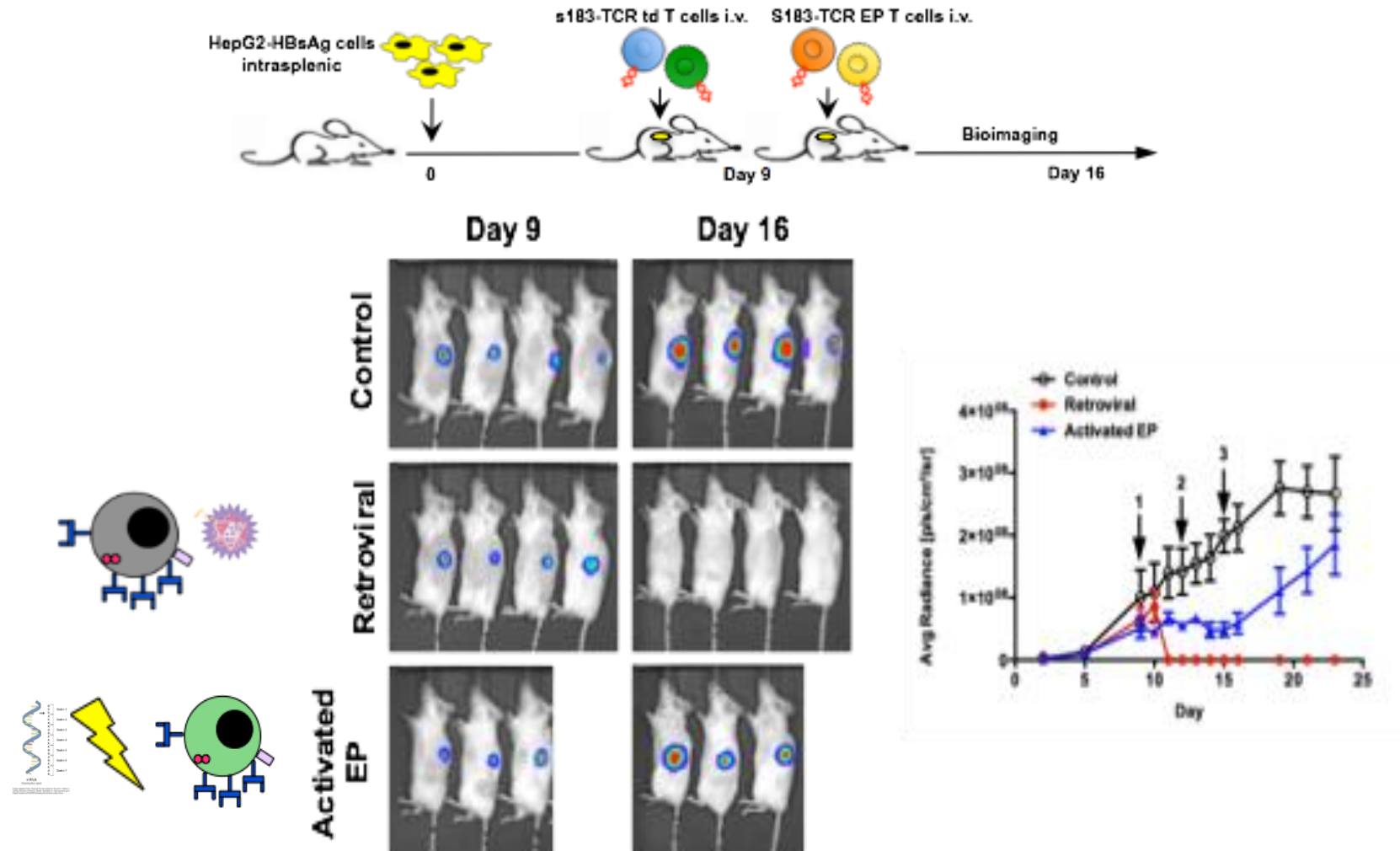
Bypass T cell exhaustion
But temporally limited
efficacy...



Is this occurring in patients?

EXPERIMENTS Immunodeficient Mice

Bypass T cell exhaustion
But temporally limited
efficacy...



Summary



Overview of immunotherapy
potential for HBV and HBV-HCC

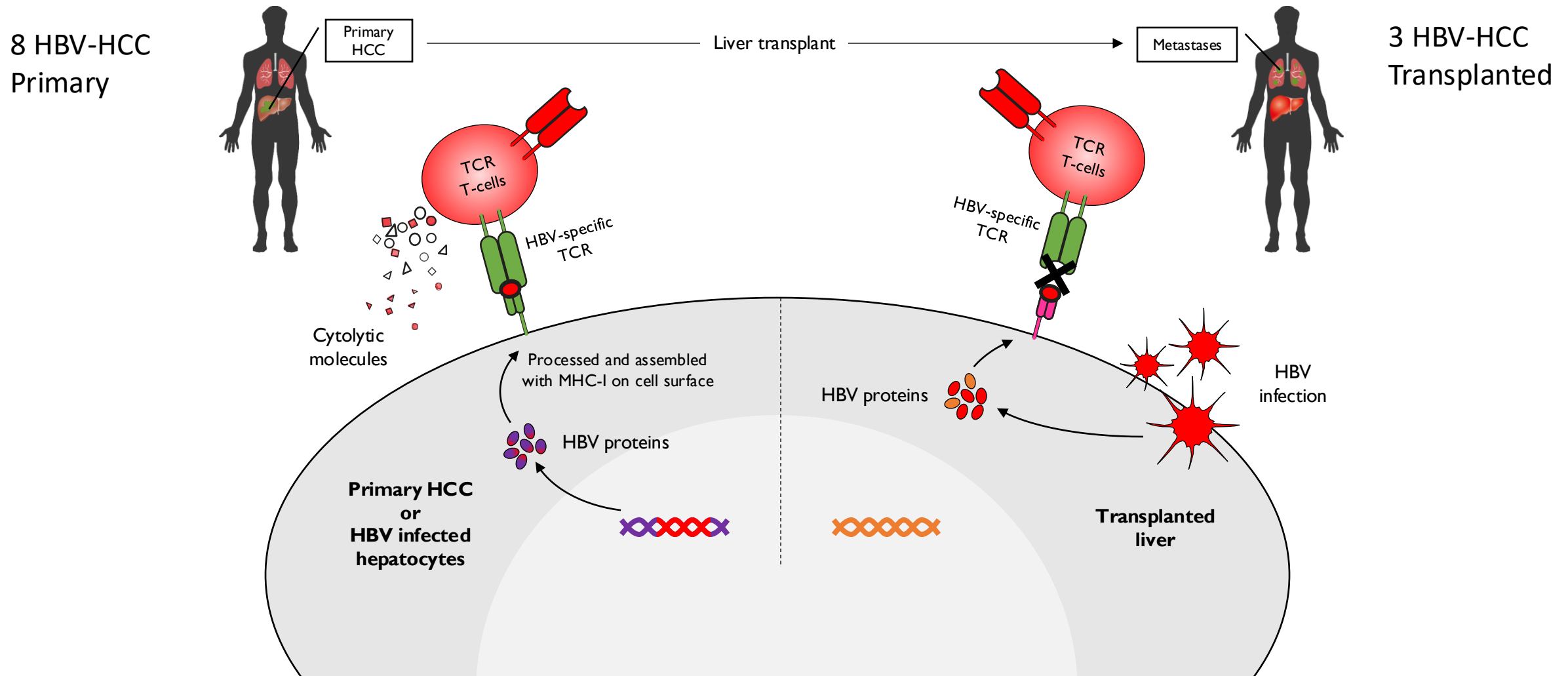


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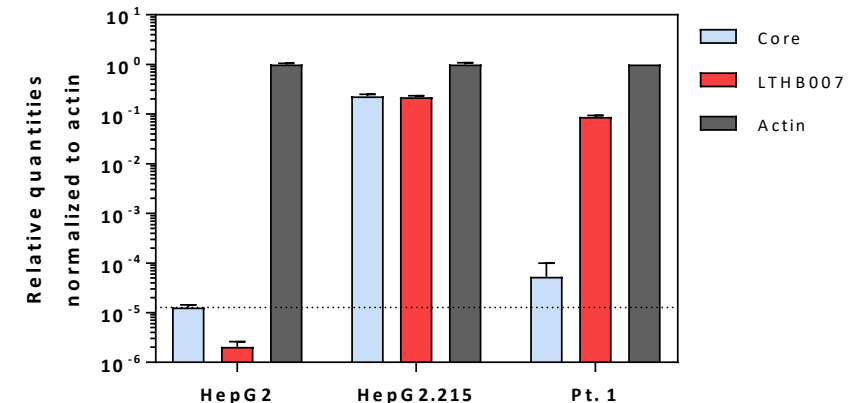
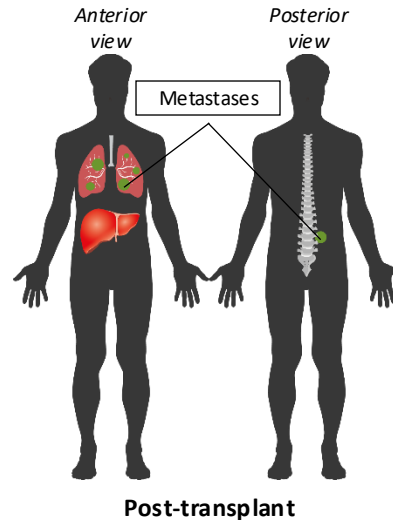
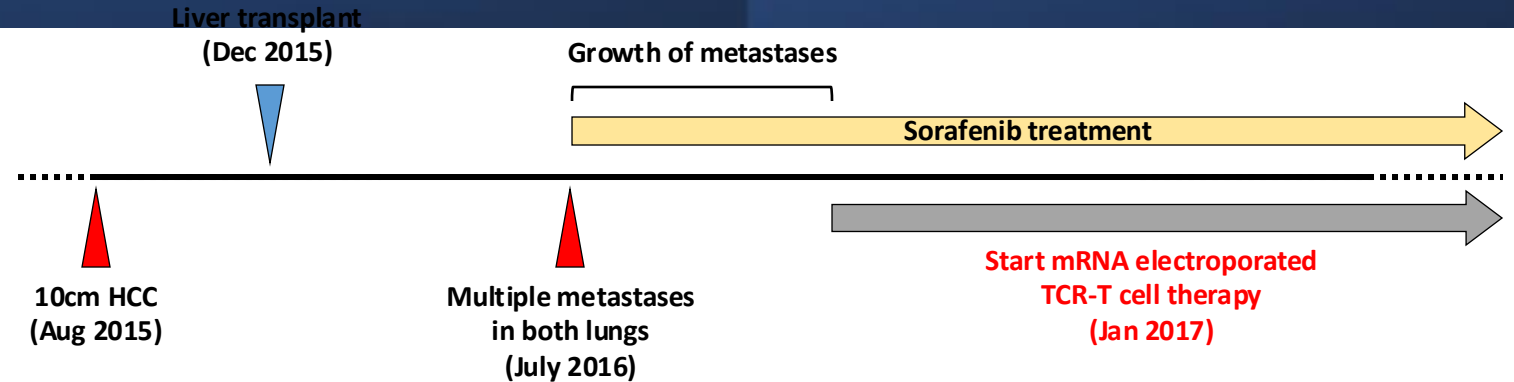
HBV-HCC therapy using mRNA HBV-TCR T cells



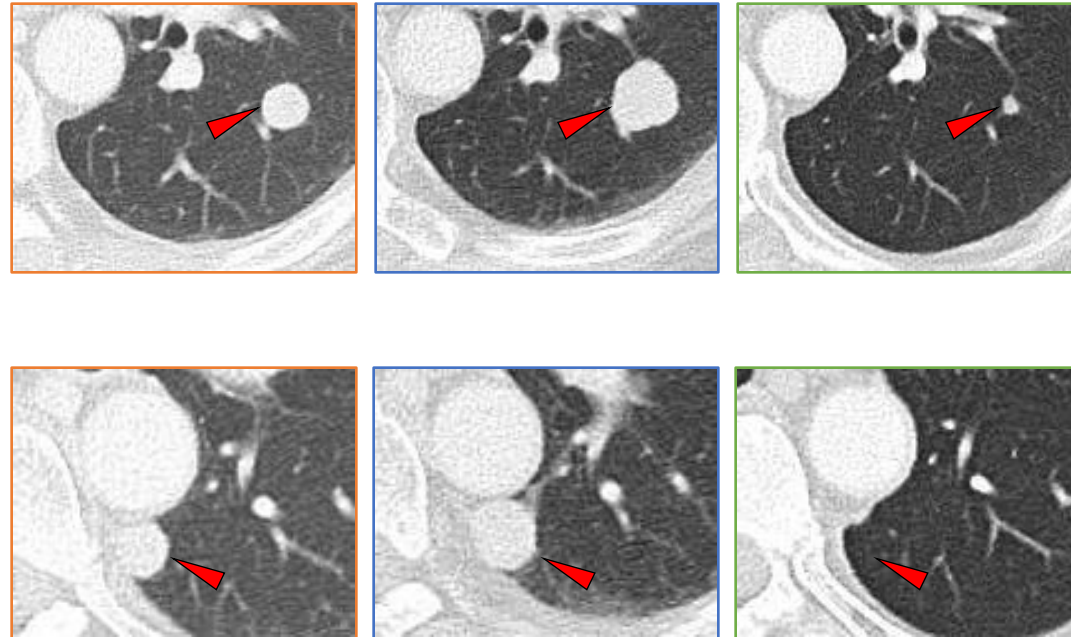
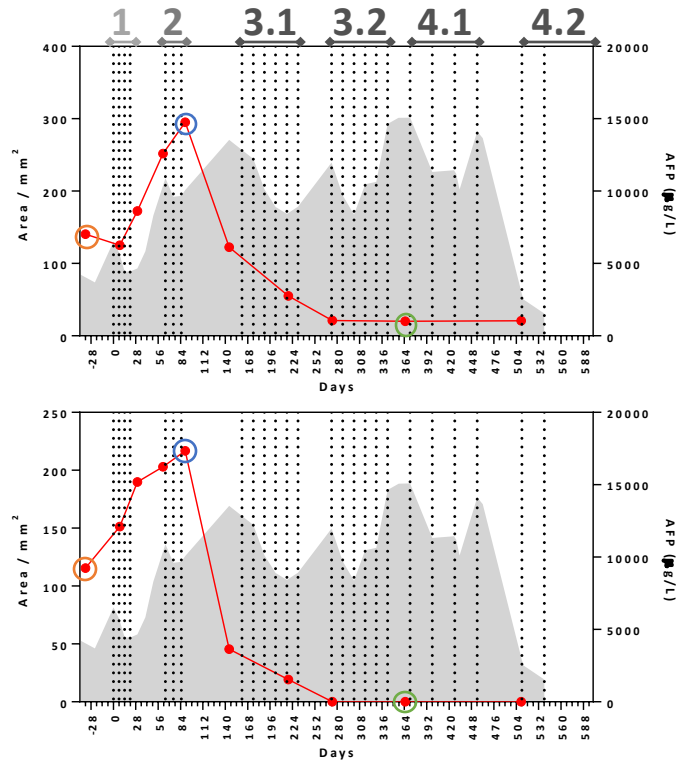
HBV-HCC therapy using mRNA HBV-TCR T cells

- 56 yr old Chinese male chronically infected with HBV, treated with long-term entecavir.
- HCC confirmed by CT in Aug 2015.
- Living donor liver transplant was performed in Dec 2015.
- In ~7 months, multiple metastatic nodules were detected in both lungs by CT.

Patient treated with HLA-B58 restricted HBV Env-Specific T cells (LTHB007)



HBV-HCC therapy using mRNA HBV-TCR T cells

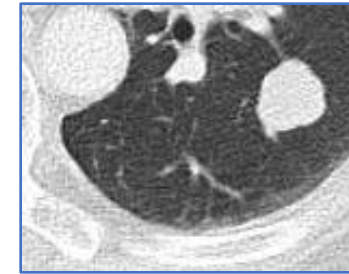
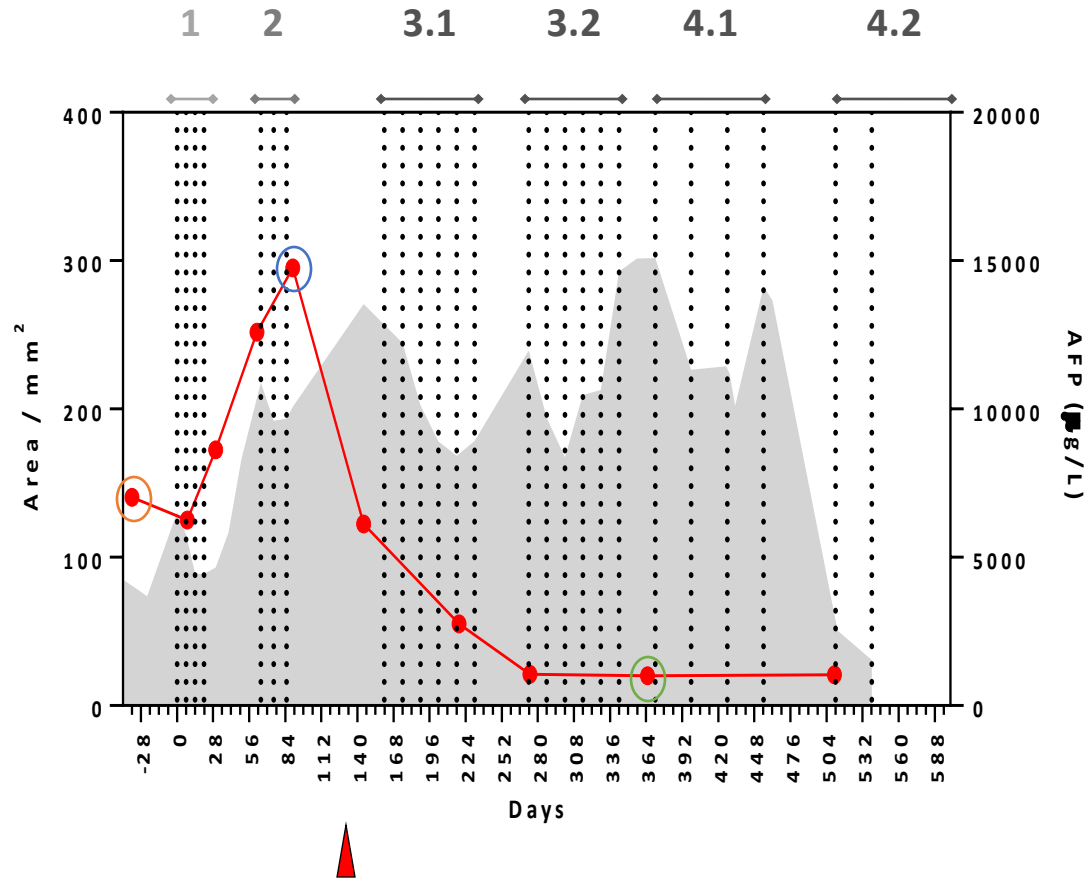


- CT scans revealed an anti-tumour response with a reduction in the size of 6/7 lung lesions.
- 2 representative lung lesions are shown.

Gastroenterology, 2019 Jan 31. pii: S0016-5085(19)30348-8. doi: 10.1053/j.gastro.2019.01.251. [Epub ahead of print]

Use of Expression Profiles of HBV DNA Integrated Into Genomes of Hepatocellular Carcinoma Cells to Select T Cells for Immunotherapy.

HBV-HCC therapy using mRNA HBV-TCR T cells

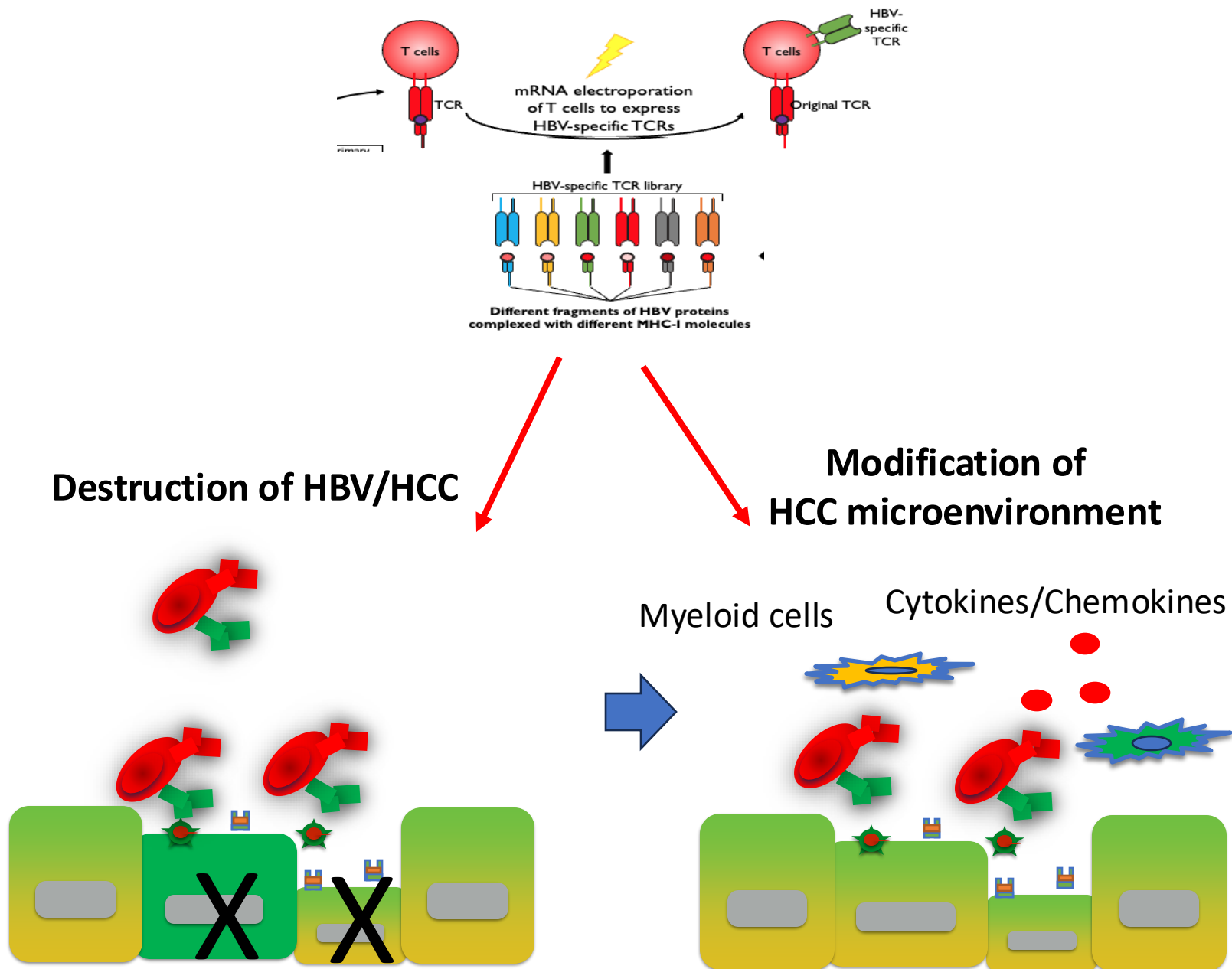


During this period....no infusion

Volume reduction not proportional to mRNA HBV-TCR T cell infusion

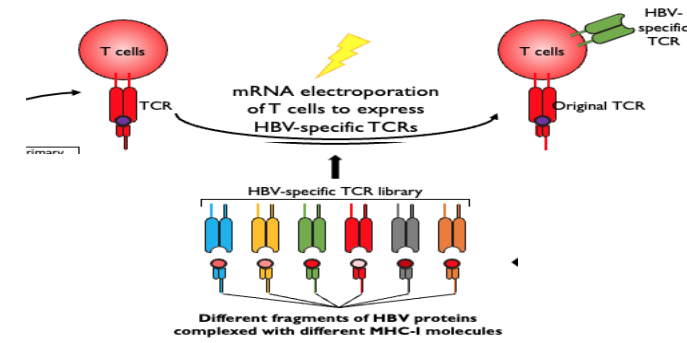
Hypothesis

Can mRNA-TCR T cells modify the HCC micro-environment



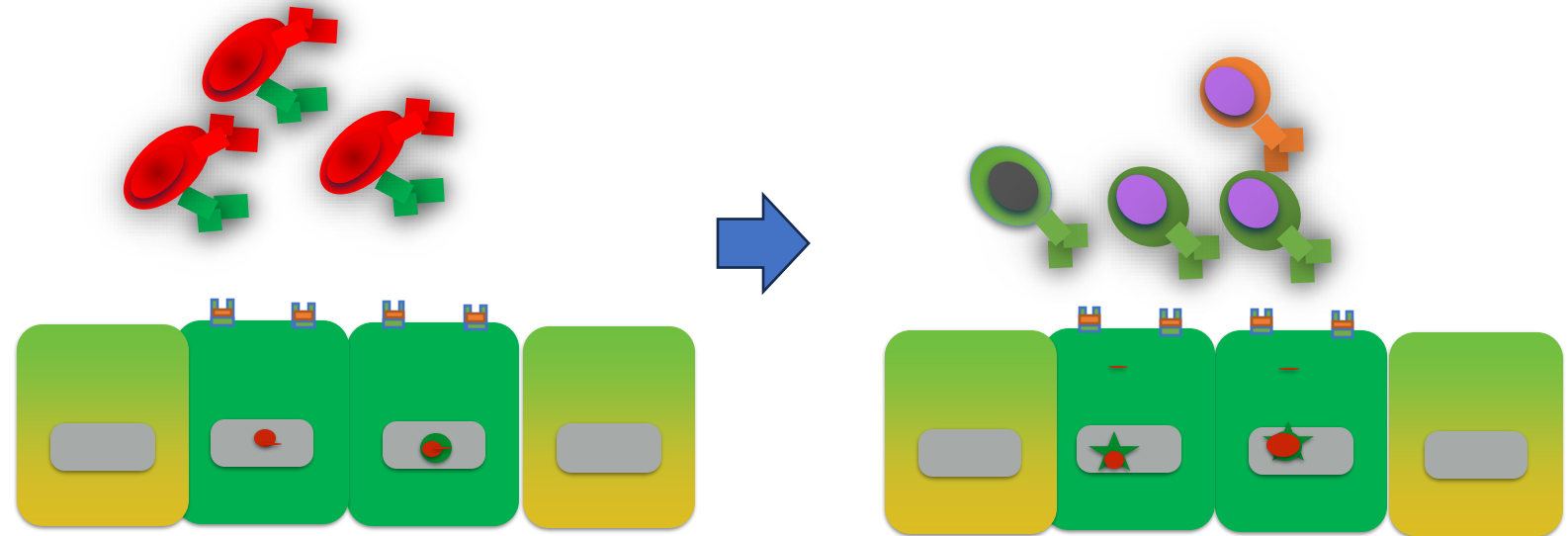
Hypothesis

Can induce new HCC-T cells?



Engineered HBV-specific T cells

Autologous HCC-specific T cells



Transient

Stable

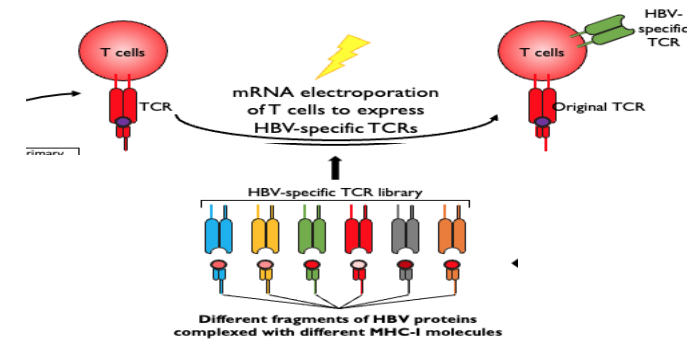
Engineered

Natural

Hypothesis

mRNA-TCR T cells modify
the HCC micro-environment

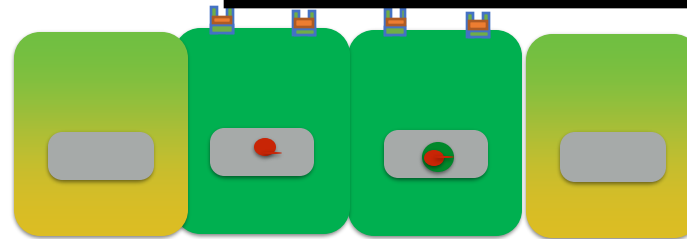
Can induce new HCC-T cells?



Engineered HBV-specific T cells

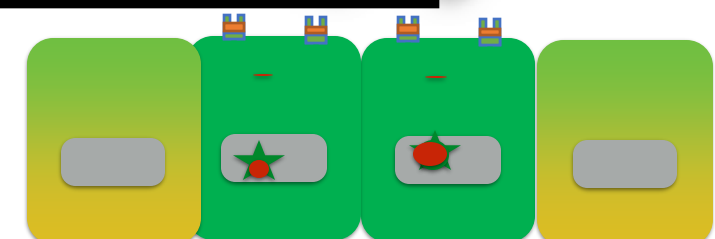
Autologous HCC-specific T cells

We have preliminary data
supporting this hypothesis



Transient

Engineered

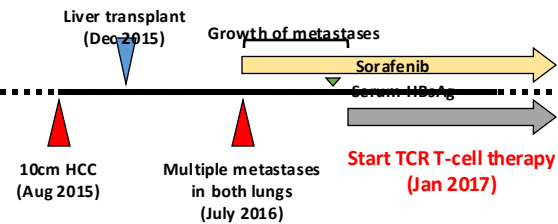
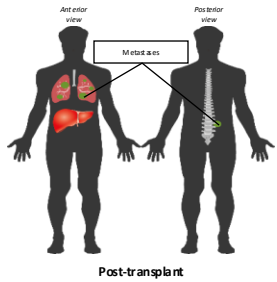


Stable

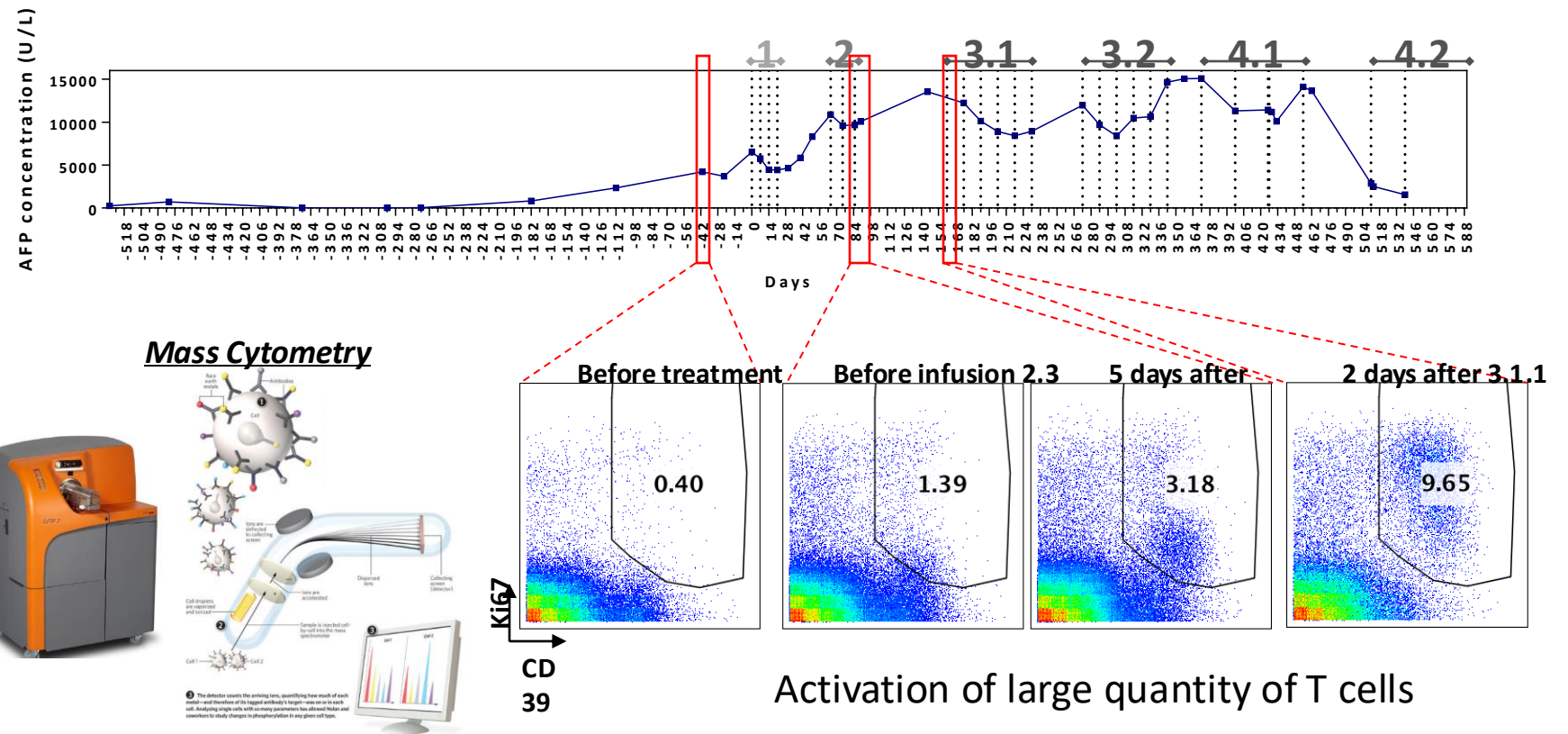
Natural

mRNA HBV-TCR therapy in HBV-HCC patients

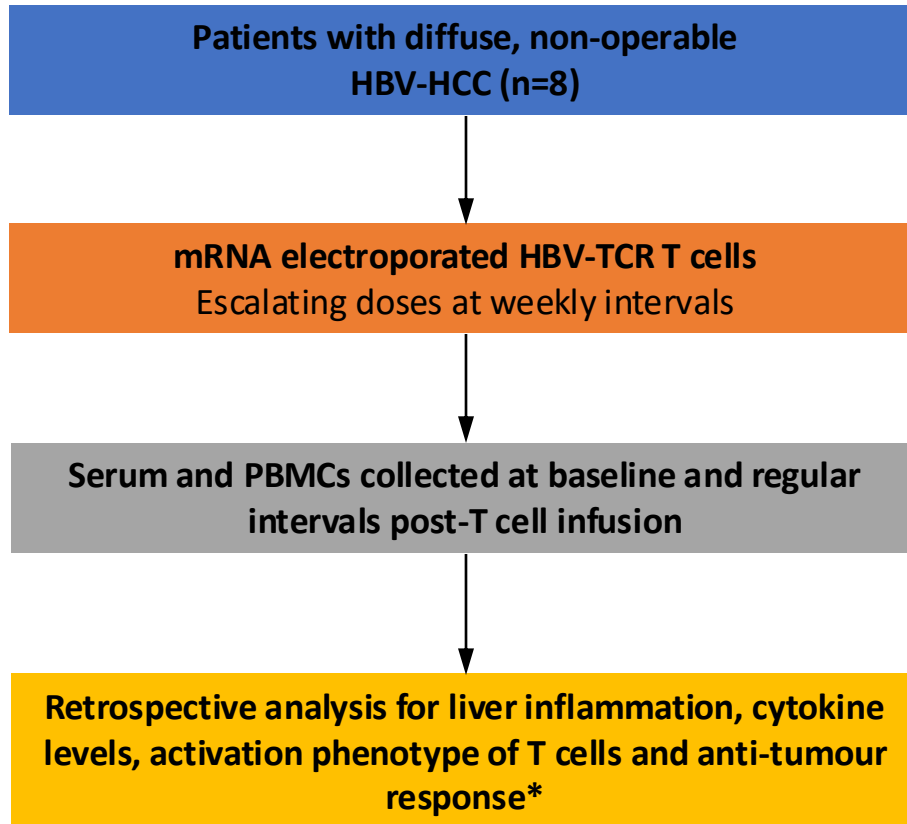
Peripheral blood T cell activation and CXCL-10/CXCL-9 increase



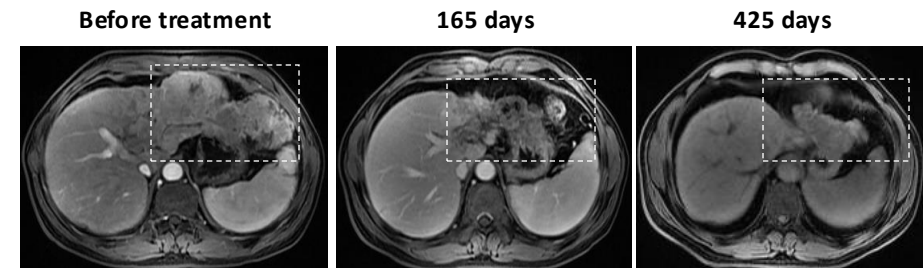
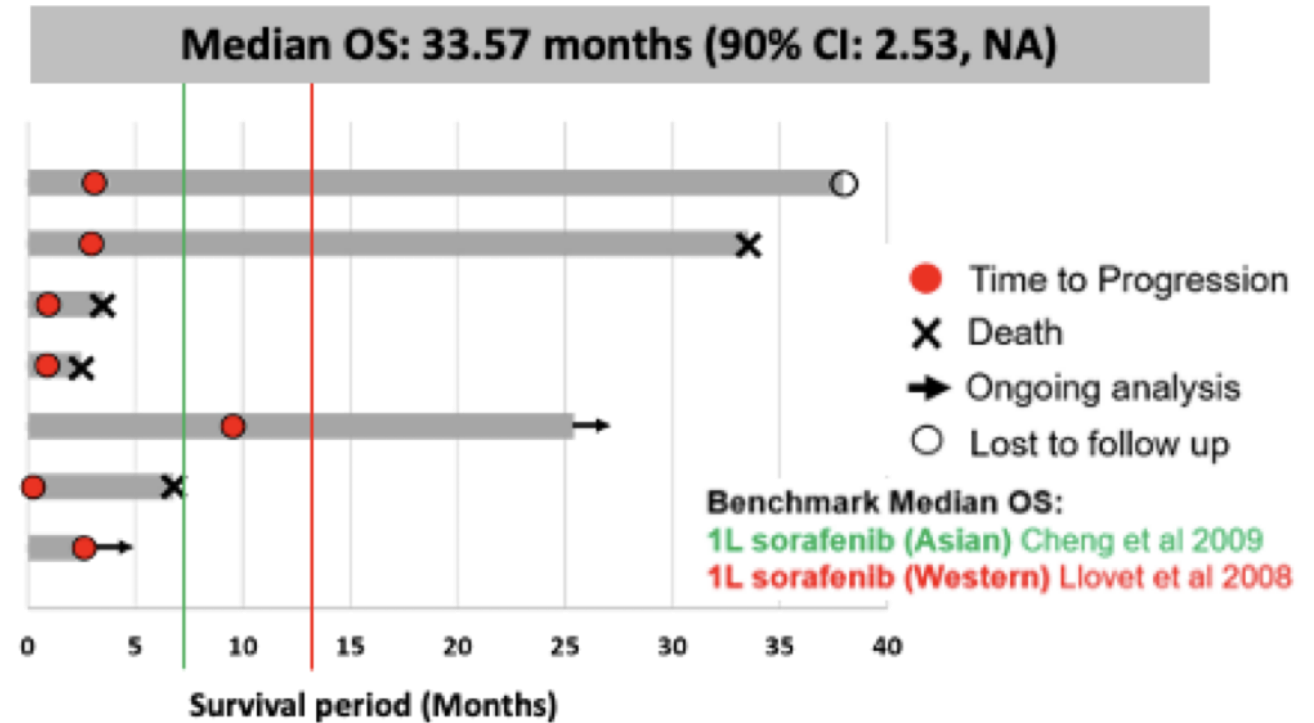
Adoptive transfer ~ 200×10^6 TCR-T cells x infusion



Trial design HBV-HCC Primary



*Evaluated primarily by radiological imaging

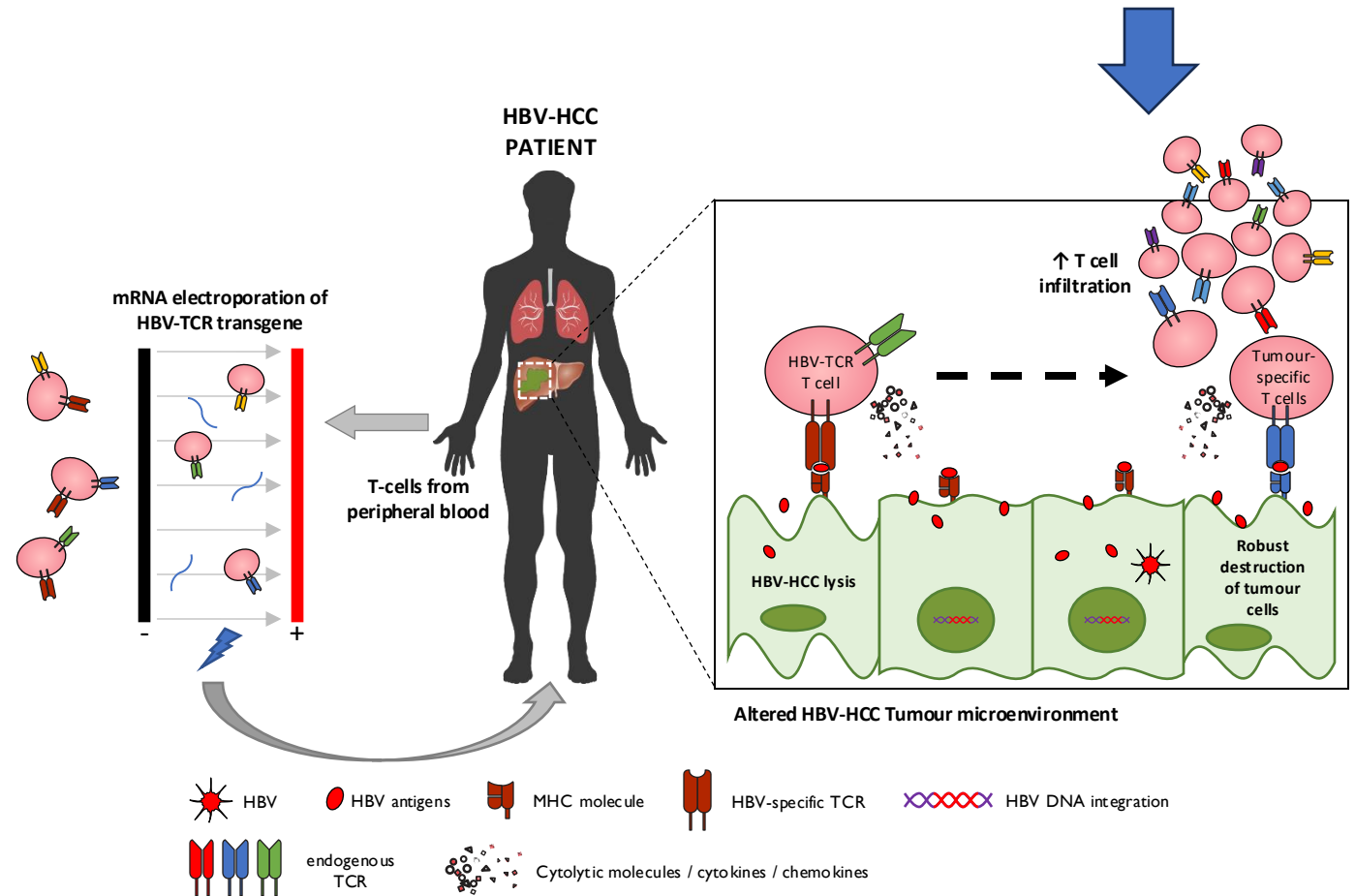


Data in Collaboration with Prof Fu-Sheng Wang;
Tan, A. T. *et al.* Immunological alterations after immunotherapy with short lived HBV-TCR T cells associates with long-term treatment response in HBV-HCC.
Hepatology Commun (2021) doi:10.1002/hep4.1857

Hypothesis

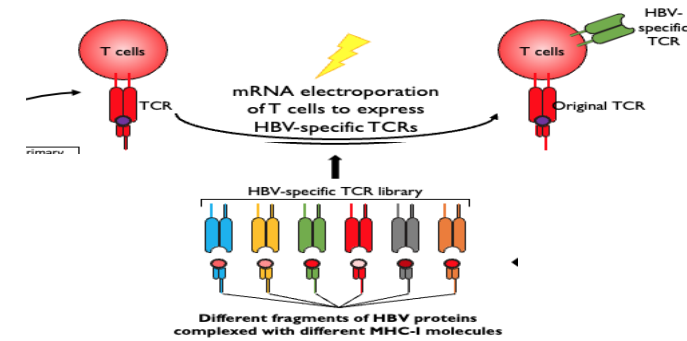
Can induce new HCC-T cells?

Let's try to measure Antigen-specific T cells



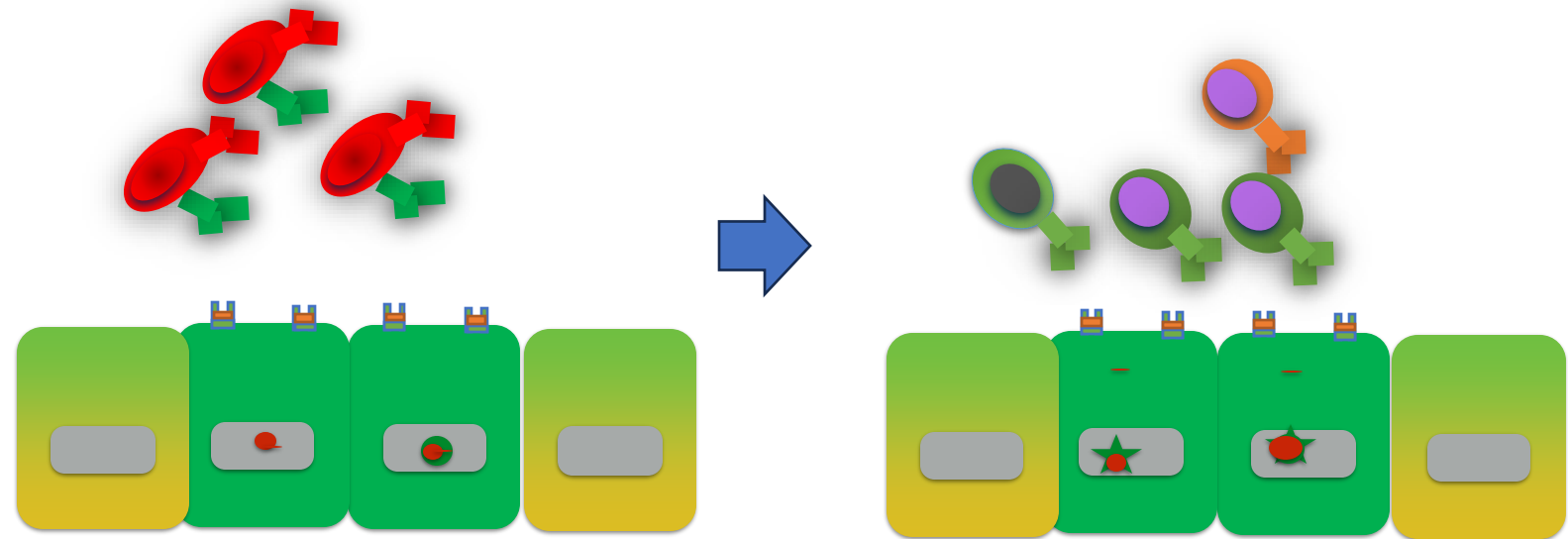
Hypothesis

Can induce new HCC-T cells?



Engineered HBV-specific T cells

Autologous HCC-specific T cells



Transient

Stable

Engineered

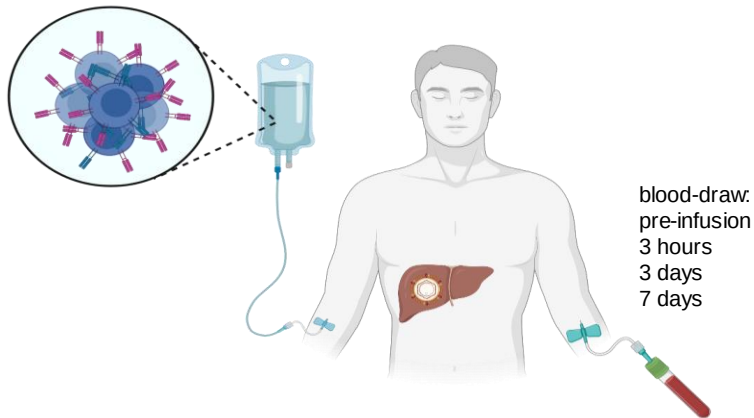
Natural

Quantification of New HBV -T cells functionality in vivo

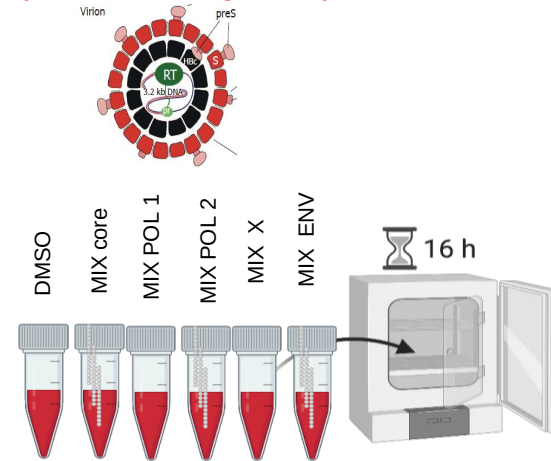


Patient HBV-HCC
Primary

Infusion of
HBV-S183 TCR+ T cells

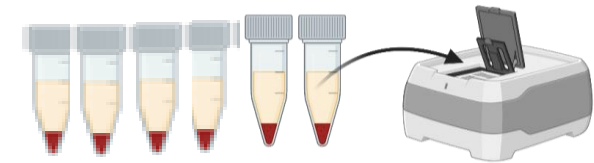


Stimulation of whole blood with
Mix peptides covering HBV proteins



Plasma collection

Cytokine
quantification



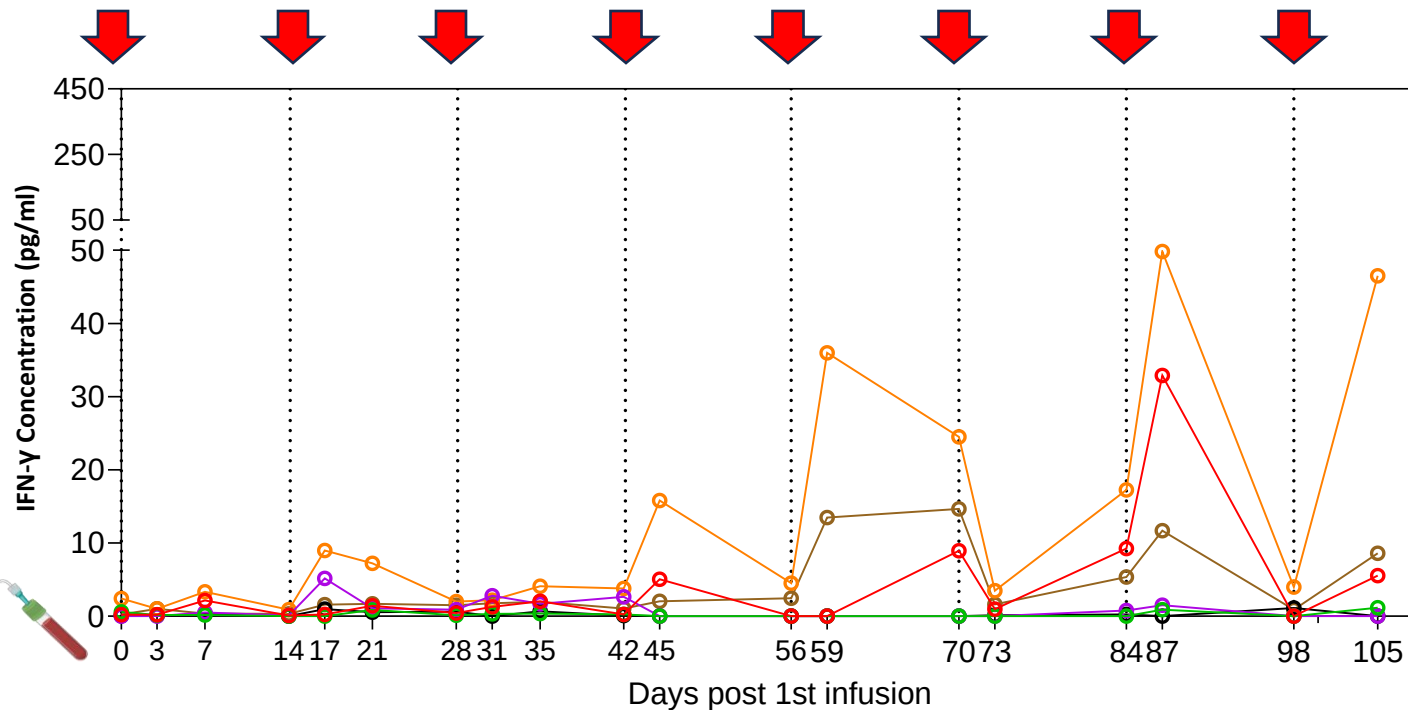
Quantification of New HBV -T cells functionality in vivo



Patient HBV-HCC
Primary

Analysis
HBV-specific T cells

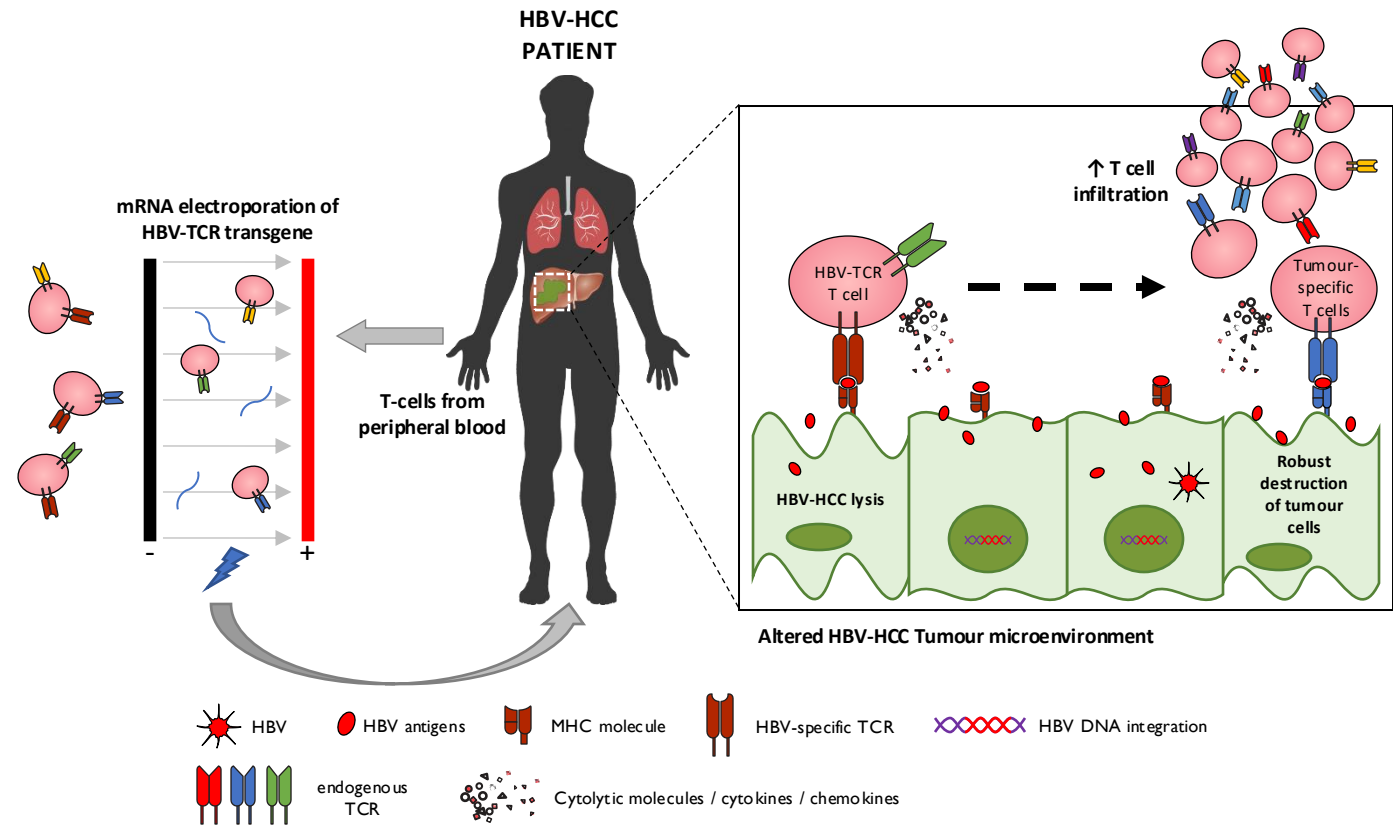
IFN γ pg/ml



Adoptive transfer of
mRNA HBV-specific TCR T cell
 $5-10 \times 10^6 \times \text{kg}$

Epitope spreading?

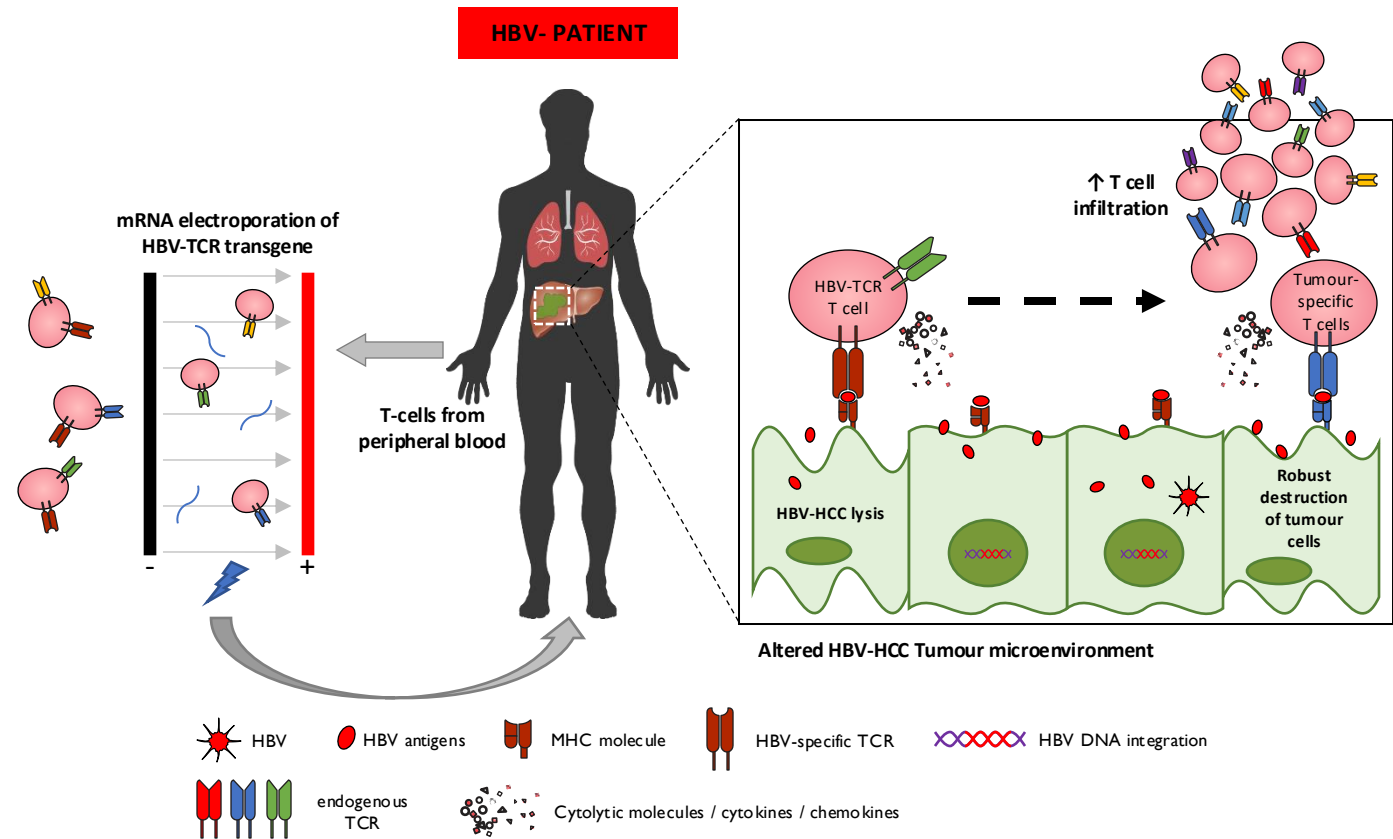
Conclusions



Direct HBV-HCC lysis induces robust destruction of HBV-HCC cells through the recruitment of endogenous novel or pre-existing tumour-specific T cells.

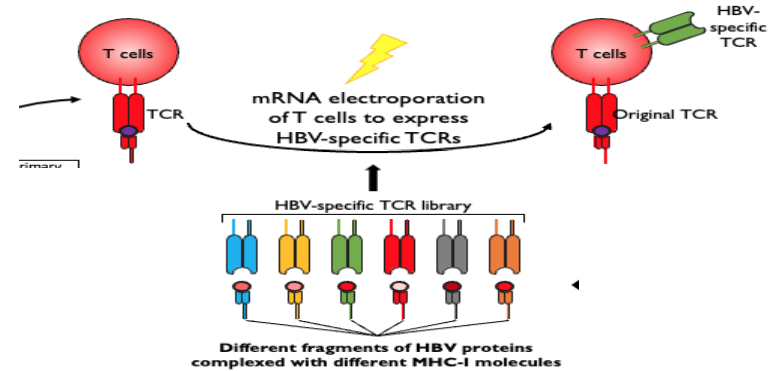
Conclusions

Concept of Epitope spreading can be applied also to Chronic HBV infection



Direct HBV- lysis can induce novel HBV-specific T cells and reconstitute the HBV-specific T cell repertoire

mRNA-based TCR T cell



We can produce HBV-specific T cells utilizing mRNA coding for HBV-TCR

These transient HBV-TCR T cells are able to recognize HBV infected and HBV/HCC

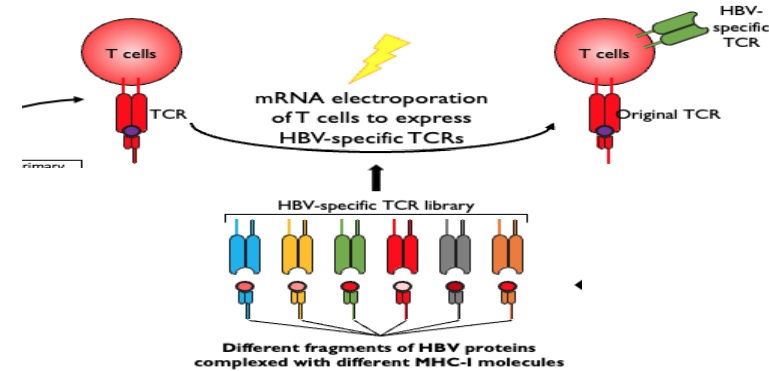
Advantages

NO GENETIC MANIPULATION

Reduce cost

Safety
Possibility of dose escalation

mRNA-based TCR T cell



We can produce HBV-specific T cells utilizing mRNA coding for HBV-TCR

These transient HBV-TCR T cells are able to recognize HBV infected and HBV/HCC

LIMITATIONS

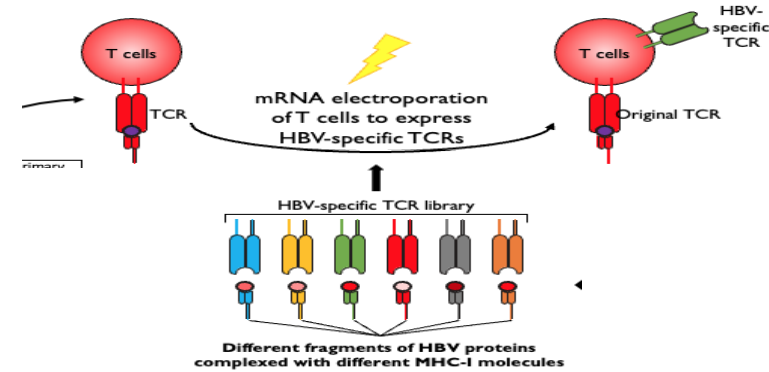
Transient expression

Need of multiple infusion

Compensate by the ability to induce modification
of HBV/HCC microenvironment

mRNA-based TCR T cell

Other Advantages?

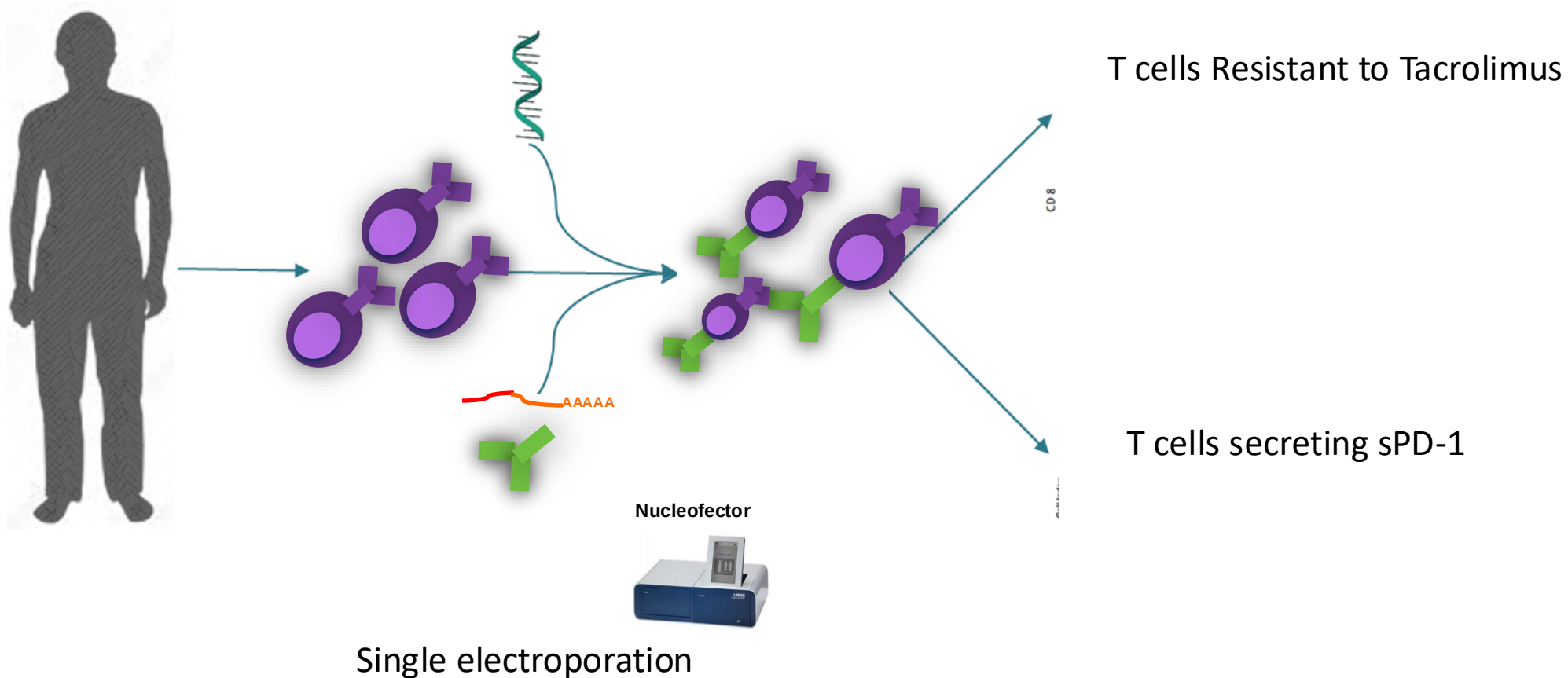


Flexibility of usage

Modification of T cell specificity (TCR) and
T cell function in a single electroporation

Modification of T cell specificity (TCR) and T cell function in a single electroporation

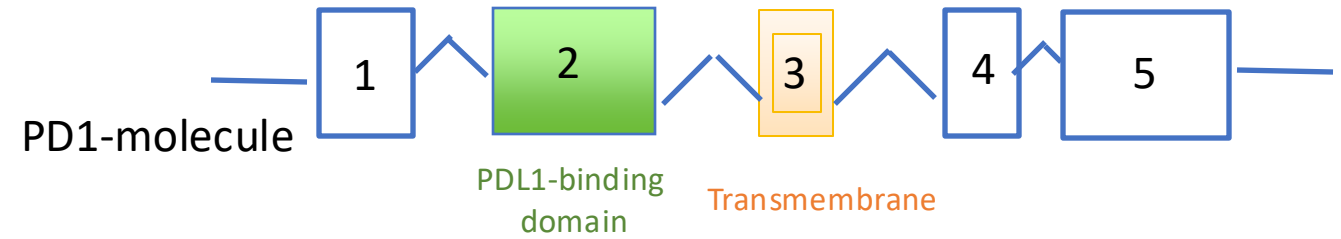
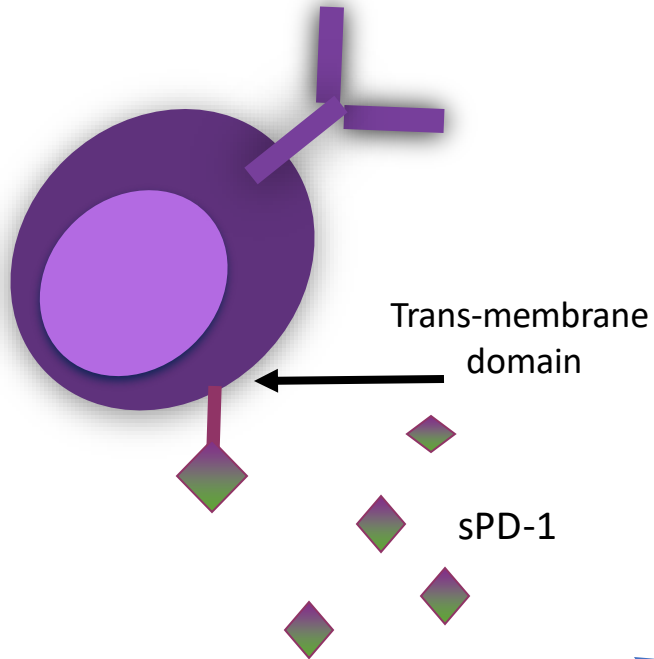
Anti-sense Nucleotides or mRNA coding for Altered CalcB



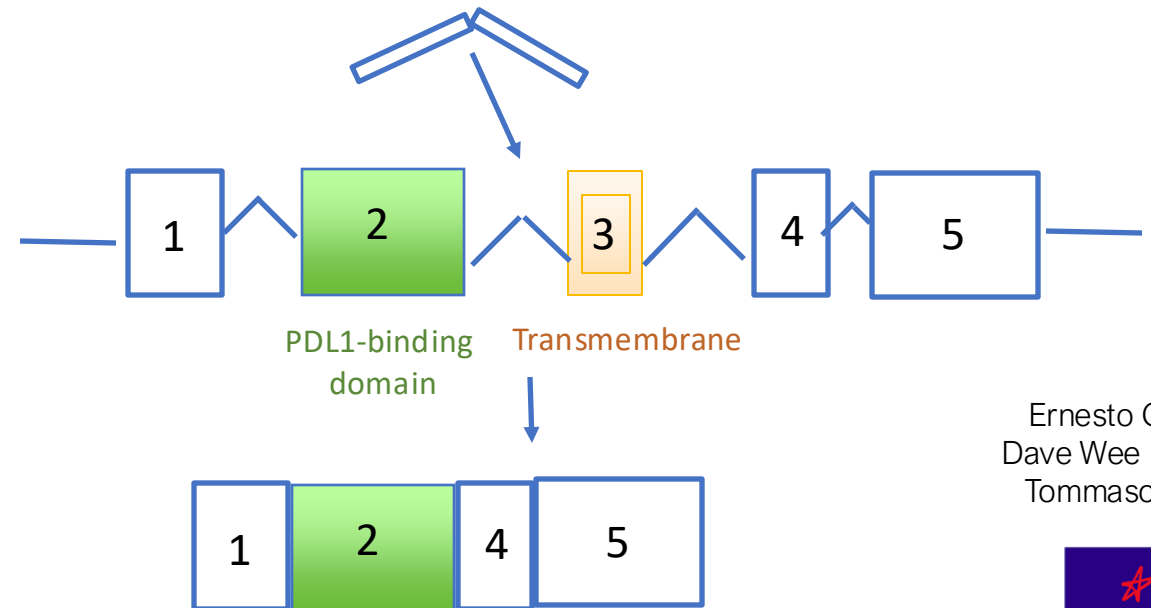
Ernesto Guccione
Dave Wee Keng Boon
Tommaso Tabaglio

We designed ASO altering mRNA PD-1

sPD-1 ASO



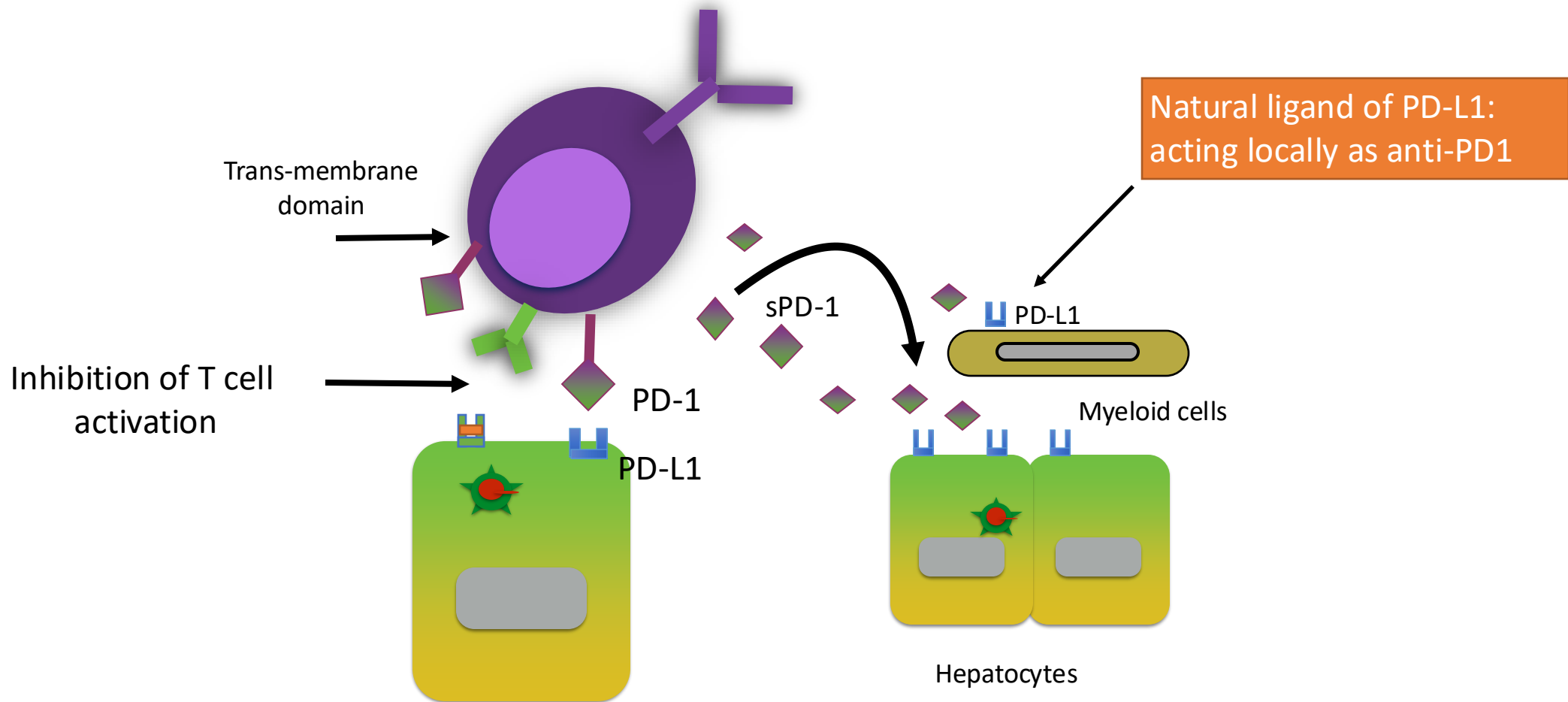
AON altering the splicing of pre mRNA of PD-1



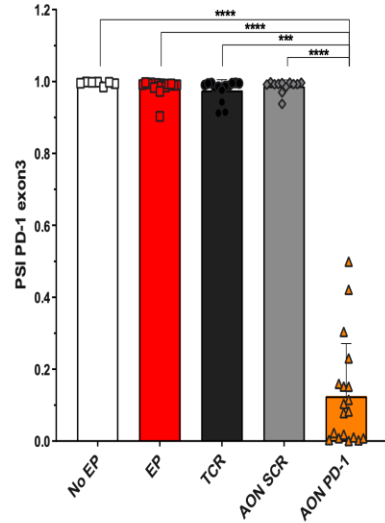
PD1-molecule without transmembrane domain

Ernesto Guccione
Dave Wee Keng Boon
Tommaso Tabaglio

PD-1 can be expressed on the membrane of T cells but also can be secreted as soluble PD-1

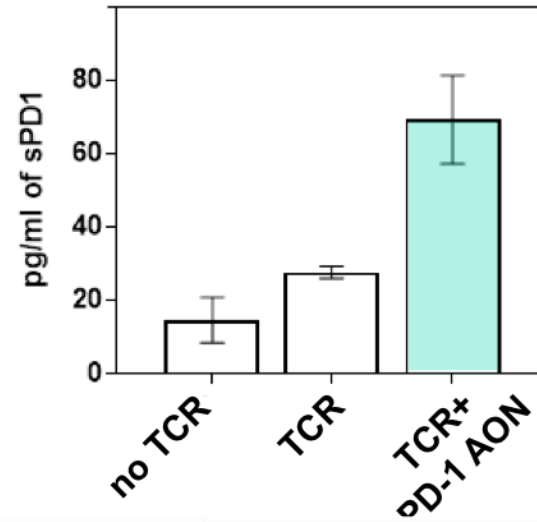


T Lymphocytes modified with TCR and sPD-1 AON secrete PD-1 and are more efficient in vivo (HBV-HCC humanised models)

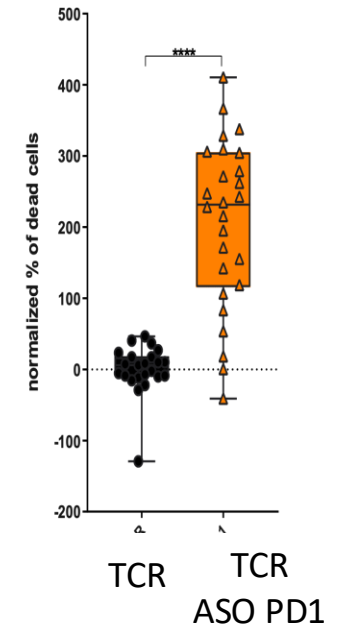


The AONs are promoting an efficient exclusion of PD-1 exon 3

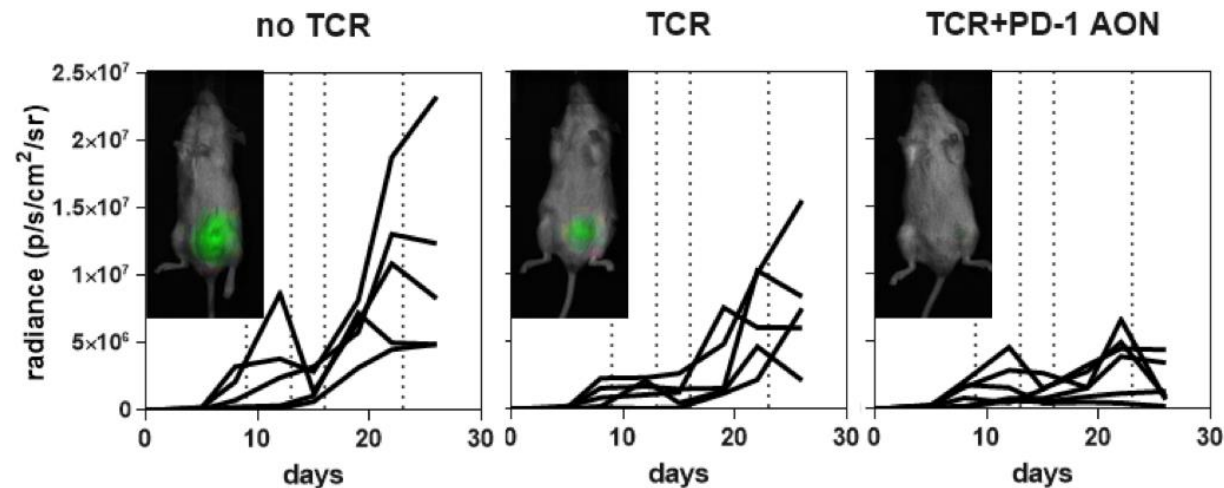
More soluble PD-1



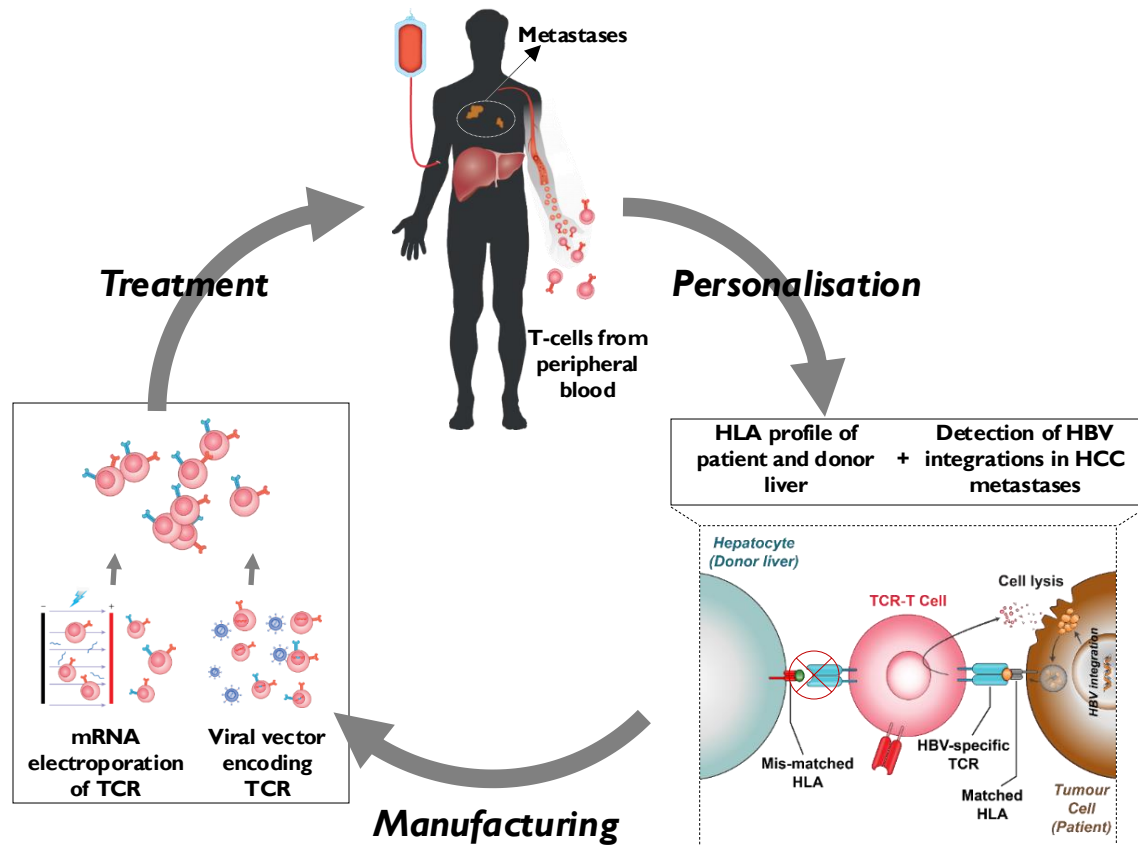
Increased T cell cytotoxicity 3D



In Vivo



Liver Transplant Patients are under Immunosuppressive therapy

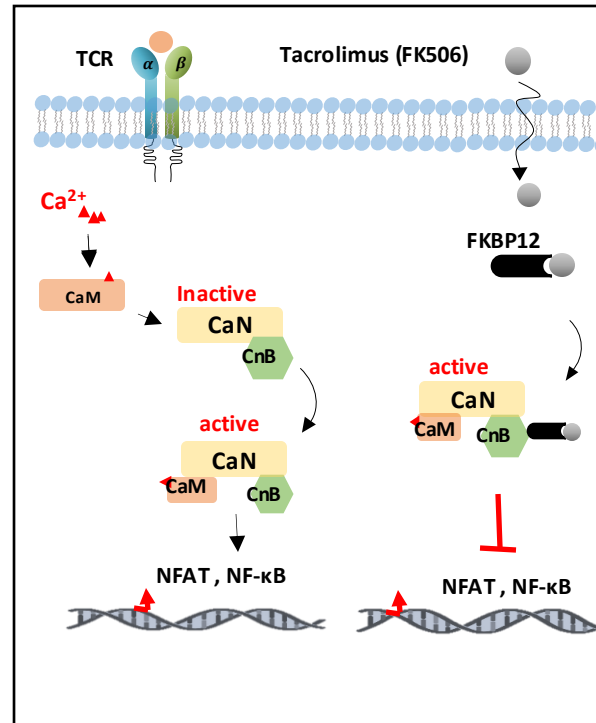


Problem: These drugs can reduce T cell efficacy which can reduce our immunotherapy outcome

Aims: Developing transient drug-resistant T cell for immunosuppressive and kinase inhibitors (maybe steroids)

HOW DOES TACROLIMUS BLOCK T CELL ACTIVATION?

Mechanism of TAC –inhibition

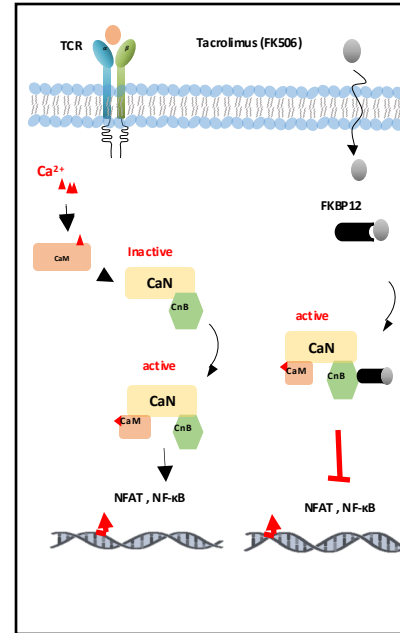


CaM: Calmodulin
CaN: Calcineurin
CnB: Calcineurin subunit B

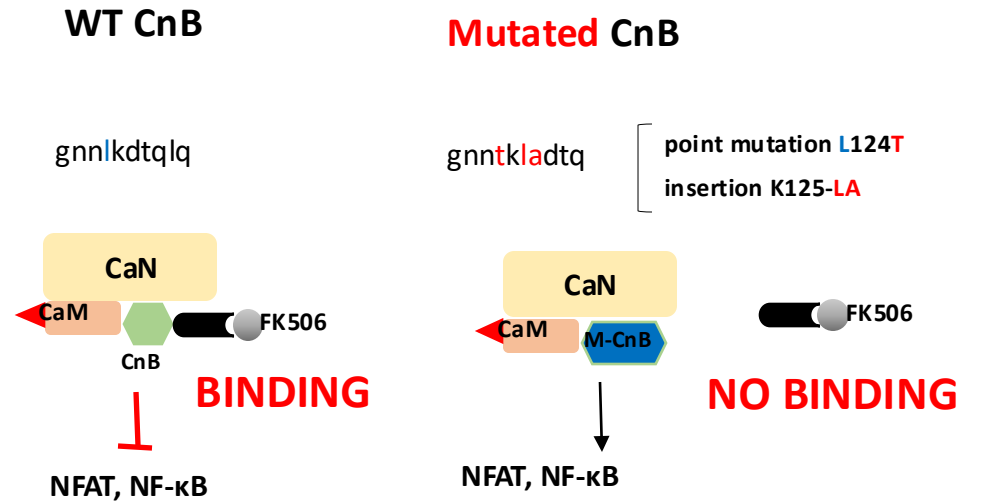
TACROLIMUS BLOCK ACTIVATION OF
CALCINEURIN (Binding to Calc B)

HOW DOES TACROLIMUS BLOCK T CELL ACTIVATION?

Mechanism of TAC –inhibition

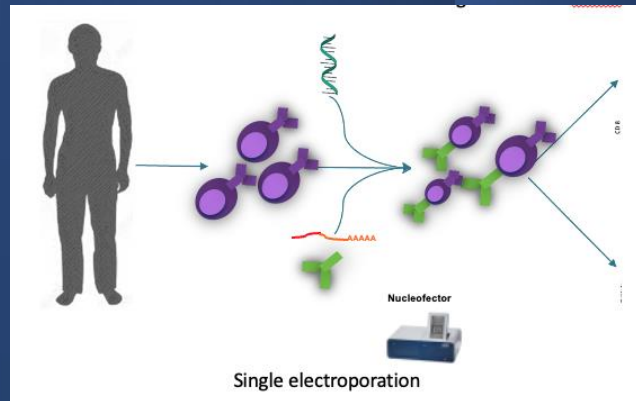


CaM: Calmodulin
CaN: Calineurin
CnB: Calcineurin subunit B



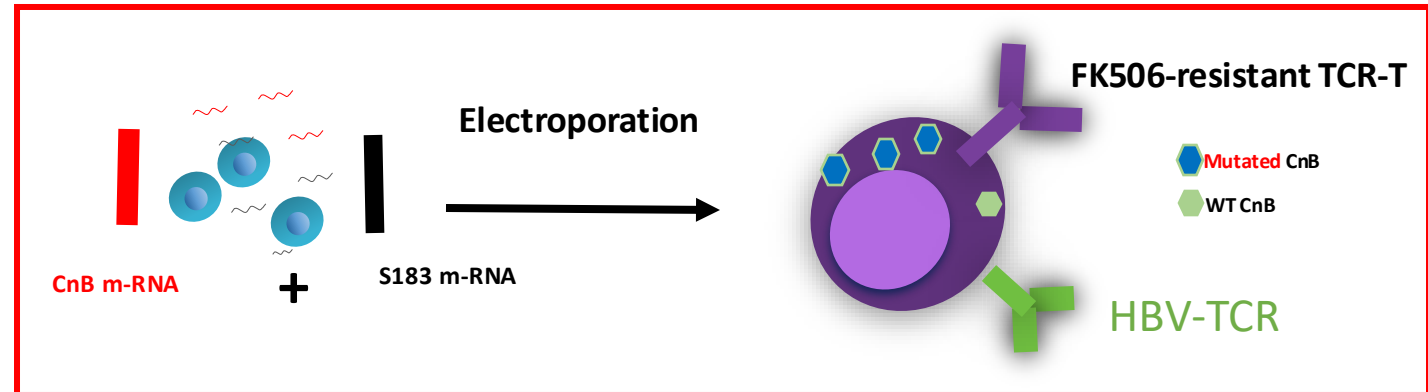
A MUTATED FORM OF CNB DOES NOT BIND TAC (FK506)

EXPRESSION OF MUTATED CnB with mRNA

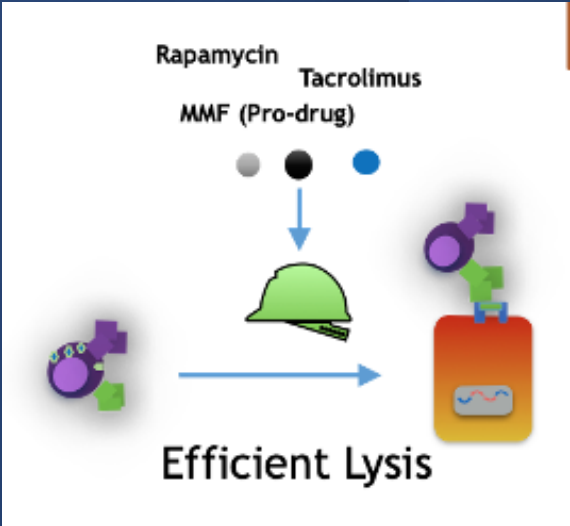


Hafezi M, Lin M, et al. Immunosuppressive Drug Resistant Armored TCR T cells for immune-therapy of HCC in liver transplant patients. Hepatology 2020. doi:10.1002/hep.31662.

Engineering TCR-T cells expressing a new TCR and a mutated form of CnB

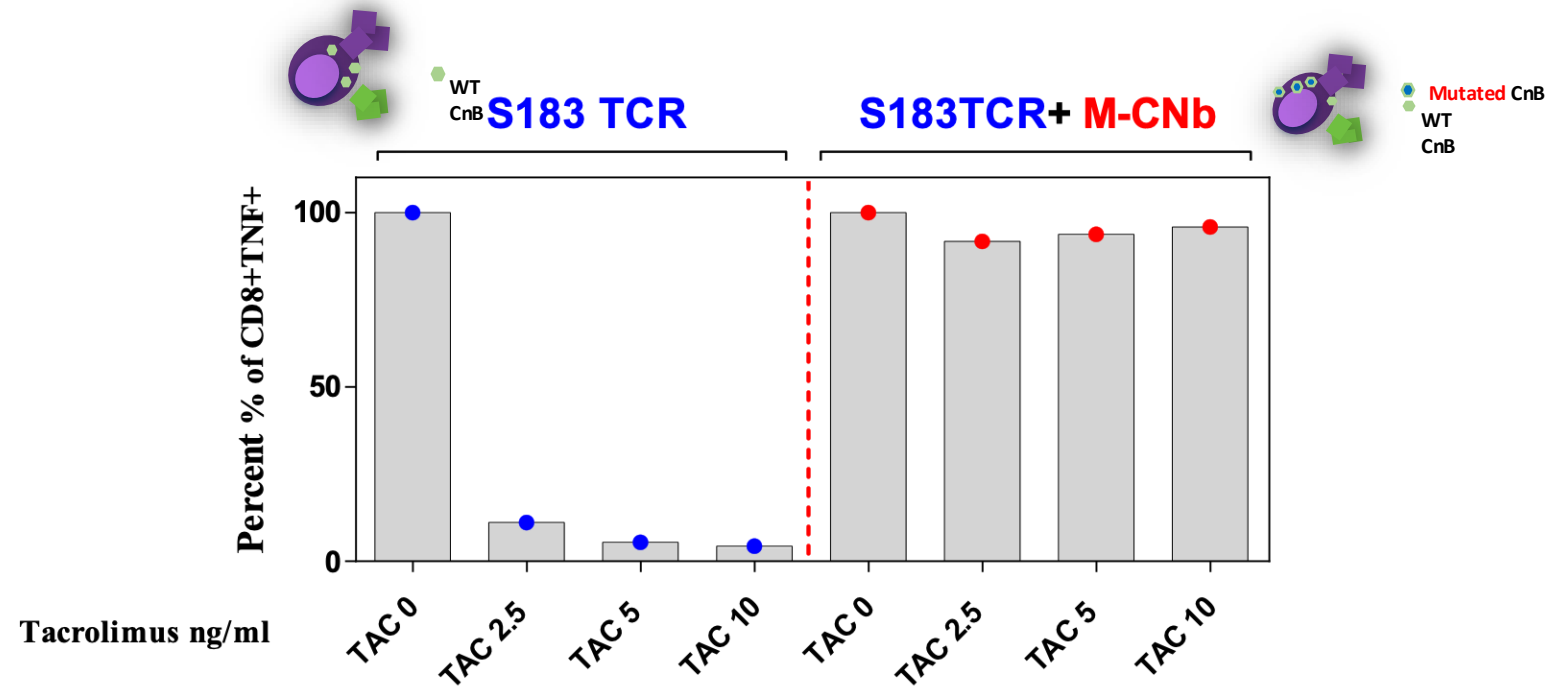
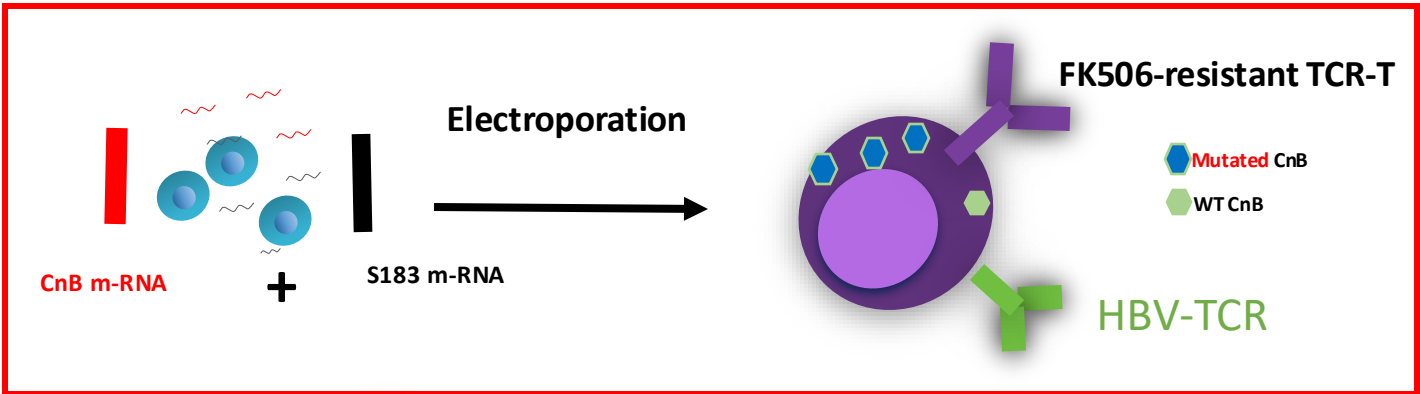


Immunosuppressive Drug Resistant Armored T cells (IDRA)



Hafezi M, Lin M, et al. Immunosuppressive Drug Resistant Armored TCR T cells for immune-therapy of HCC in liver transplant patients. Hepatology 2020. doi:10.1002/hep.31662.

Engineering TCR-T cells expressing a new TCR and a mutated form of CnB



Take home messages

We can engineer Virus-specific (HBV)-TCR T cells with stable (Viral Vector) or transient (mRNA) TCR expression



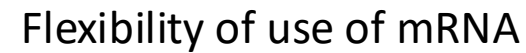
mRNA TCR electroporation: safe, no genetic manipulation, multiple injections



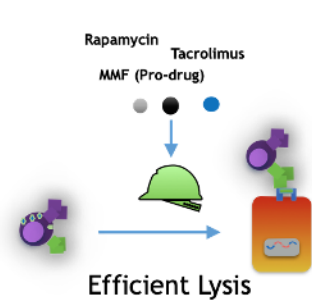
mRNA TCR T cells in HBV-HCC: efficacy not proportional number of TCR-T cells

TCR-T cells act as a modifier of Tumor microenvironment ?

Induction of new T cells, epitope spreading



We can simultaneously modified the specificity and function of T cells trough introduction of TCR-mRNA and ASO or mRNA for CalcB (IDRA T cells)



Acknowledgements



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Ho Zi Zong
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Tina Wang



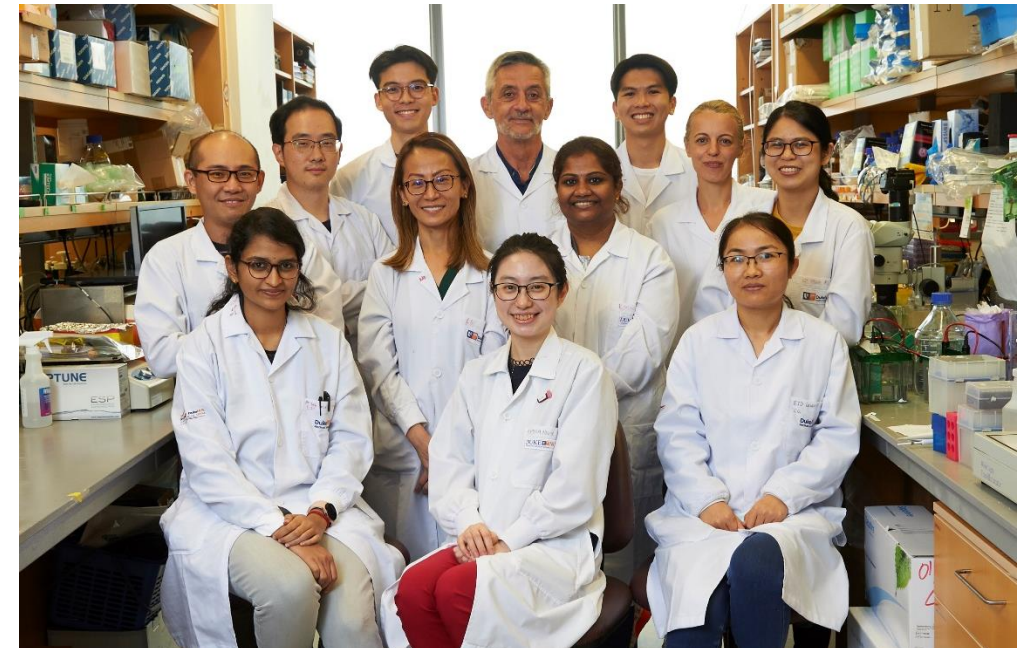
Chow Wan Cheng
Thinesh Krishnamoorthy
William Hwang
Tan Hiang Keat
Rajneesh Kumar



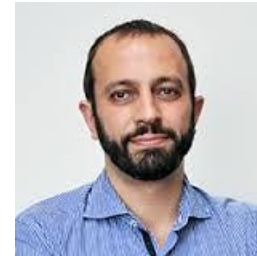
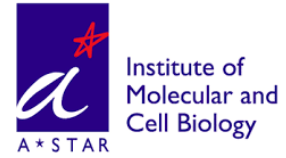
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Meng Fanping



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Andrea Pavesi



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Wasesem Qasim
Hans Stauss



Prof. Mala Maini



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