In vitro and in vivo immunogenicity of a dendritic cell-based vaccine targeting HBV epitopes

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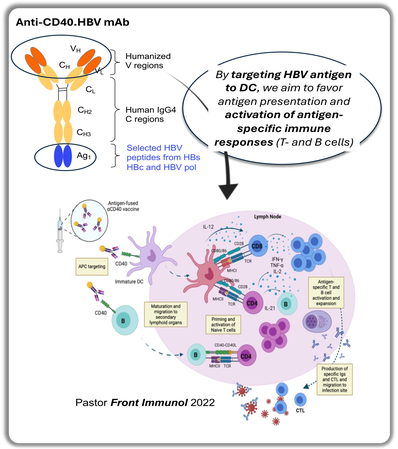
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BACKGROUND

- Persistent chronic HBV infection (CHB) is associated with impaired functional T-cell specific responses and a lack of memory T-cells against core (C), polymerase (P), and envelope proteins (S) [1].
- Therapeutic vaccination is aimed to relieve exhausted T-cells and to stimulate recall memory T-cells. We have developed an antigen-presenting cell (APC)-targeting approach allowing the delivery of specific antigens through a mAb targeting the CD40 receptor.

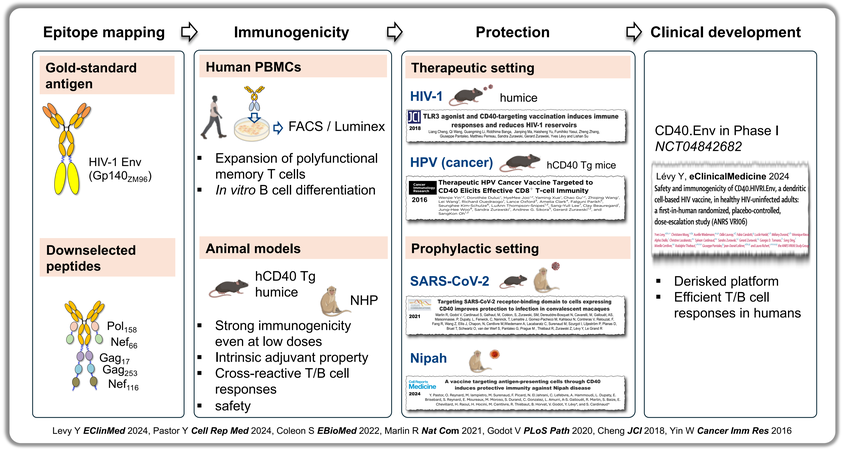
[1] Rossi M ***Gut*** 2023, Dusséaux ***Gastroenterology*** 2017, Thimme R, ***J Virol*** 2001, Boni C ***J Virol*** 2007, Guidotti ***Science***, 1999

HYPOTHESIS



Targeting selected immunogenic HBV peptides to DC demonstrated immunogenicity *in vivo* and efficiently recalled *in vitro* memory T-cells of NUC-treated CHB patients, even at low doses

PROOF-OF-CONCEPTS OF THE CD40-TARGETING VACCINE PLATFORM



Levy Y ***EClinMed*** 2024, Pastor Y ***Cell Rep Med*** 2024, Coleon S ***EBioMed*** 2022, Marlin R ***Nat Com*** 2021, Godot V ***PLoS Path*** 2020, Cheng ***JCI*** 2018, Yin W ***Cancer Imm Res*** 2016

METHODS

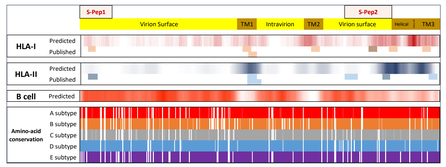
1. HBV C, P, and S proteins (genotype Dayw) were screened *in silico* to identify immunogenic and conserved epitopes. Six selected peptides were fused to a humanized anti-CD40 IgG4 mAb.
2. PBMCs from NUC-treated chronically infected donors (n=19) were stimulated *in vitro* with 1nM to 10nM of the vaccine, and the expansion of functional CD4+ and CD8+ memory T-cells was analyzed by flow cytometry.
3. The immunogenicity of the resulting CD40.HBV vaccines was evaluated in hCD40 transgenic mice in a prime/boost regimen (13 µg/mouse with 50 µg of Poly-ICLC). IgG binding to S and C was measured via Luminex assay, while T-cell immunity was assessed using IFN-γ ELISpot.

Statistical analysis. (i) human PBMCs: Wilcoxon matched-pairs *t*-test (ii) mouse studies: ordinary two-way ANOVA; **p*<0.05, ***p*<0.01; ****p*<0.01, *****p*<0.0001

RESULTS

1. *In silico* down-selection of HBV immunogenic peptides

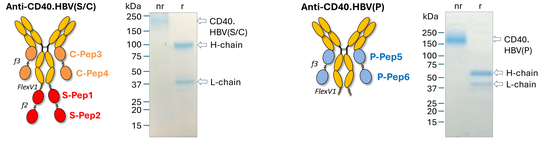
● T- and B-cell epitope mapping of HBV S, C and P



●● Down-selection of 6 HBV immunogenic peptides

	Antigens	Lenght	predicted T cell epitopes coverage HLA-I	HLA-II	described epitopes coverage HLA-I	HLA-II	described epitopes HLA-I	HLA-II	B cell epitopes Nb	among HBV genotypes AA percentage
HBs	S-Pep1	35	19	12	44%	11%	1	1	32	85%
	S-Pep2	53	61	80	77%	56%	1	2	22	91%
HBc	C-Pep3	42	34	145	81%	67%	3	2	40	96%
	C-Pep4	59	60	145	93%	74%	2	0	38	91%
Pol	P-Pep5	78	101	253	92%	92%	3	1	30	96%
	P-Pep6	73	83	190	96%	78%	1	0	154	96%

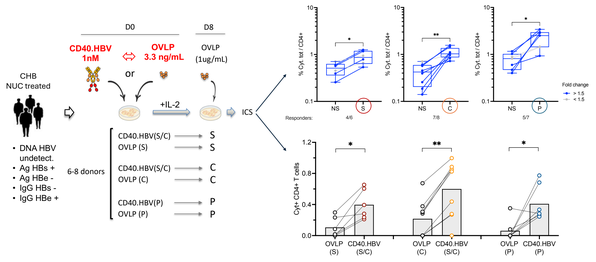
●●● Production of 2 vaccine candidates in CHO cells



Two peptides from S and 2 from C were selected and successfully conjugated with the CD40 mAb (CD40.HBV(S/C)), or the non-targeting IgG4 control mAb (IgG4.HBV(S/C)) while 2 peptides from P were associated with a second vaccine (CD40.HBV(P))

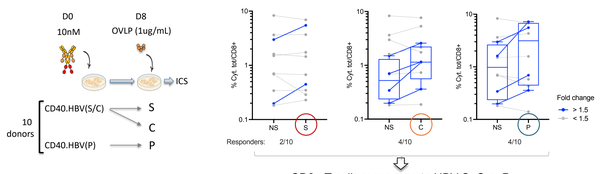
2. *In vitro* expansion of human memory T-cells by CD40.HBV

● Vaccine-stimulated HBV-specific CD4+ T-cells (1nM)

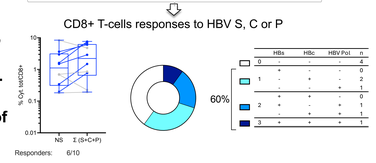


Both vaccines activated human polyfunctional (IFN-γ±IL-2±TNF) CD4+ T-cells specific to S, C, and P *in vitro*, with **significantly enhanced responses compared to equivalent amounts of non-targeted antigens (3 ng/mL of peptide pools).**

●● Vaccine-stimulated HBV-specific CD8+ T-cells (10nM)

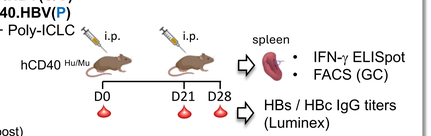


60% of patients showed *in vitro* CD8+ T-cell responses to CD40.HBV vaccines, with **HBc- and HBV Pol-specific responses amplified in 40% of NUC-treated CHB patients.**

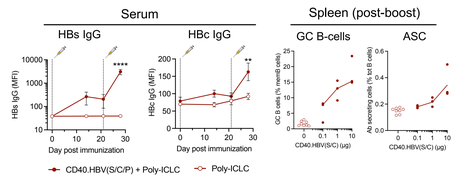


3. Cellular and humoral responses in hCD40 tg mice

● Study plan

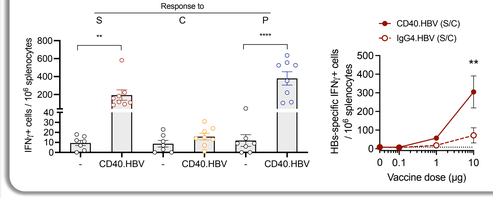


●● HBs and HBc humoral responses



The co-injection of CD40.HBV(S/C) and CD40.HBV(P) induced **significant serum HBs and HBc IgG responses** (P<0.0001 and P<0.01, respectively)

●●● HBV-specific T-cells responses



- Vaccine candidates induced *in vivo* **HBs and HBV P-specific IFN-γ ELISpot responses** (p<0.01, p<0.0001, respectively) compared to mock injection.
- HBV C-Pep4 was not predicted to be presented by the mouse MHC I-A^b.
- We confirmed *in vivo* **the advantage of the CD40 targeting in eliciting specific T-cell responses** by comparing the CD40.HBV(S/C) with IgG4.HBV(S/C).

CONCLUSION

Two CD40-targeting vaccine candidates, incorporating 6 highly immunogenic HBV peptides, were produced with high quality and demonstrated immunogenicity *in vivo*, and were able to recall memory T-cell responses from PBMCs of NUC-treated CHB patients. **These vaccines are promising candidates for further testing in humanized mice and for assessing their therapeutic potential.**

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