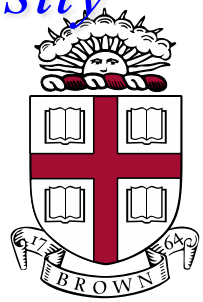
Immunotherapy for HCC

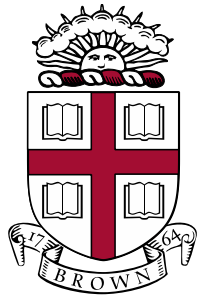
Jack R. Wands, MD

Jeffrey and Kimberly Greenberg - Artemis and Martha Joukowsky, Professor in Gastroenterology and Professor of Medical Sciences, Director, Division of Gastroenterology and Liver Research Center, Brown University

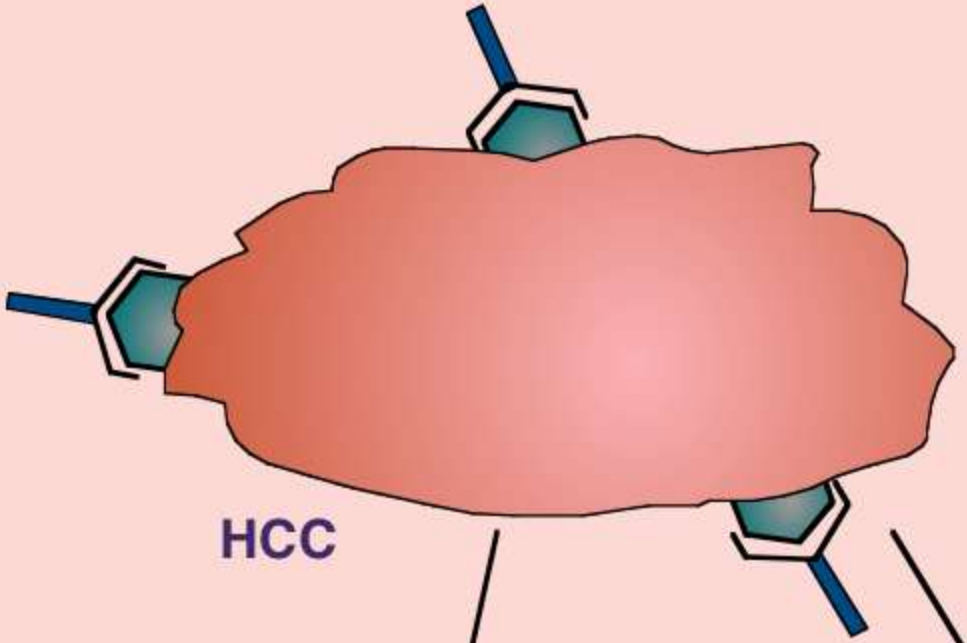


The Target Antigen

How was ASPH discovered?

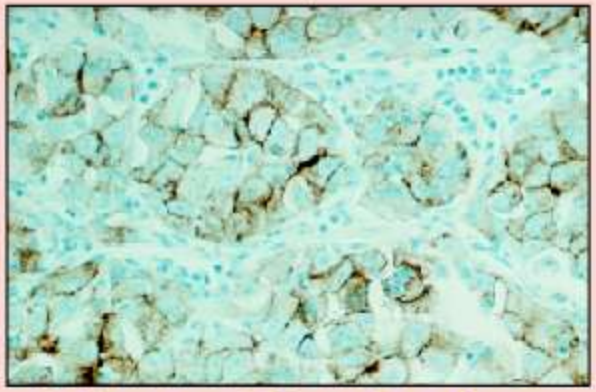


Immunologic Approach to Hepatocellular Carcinoma

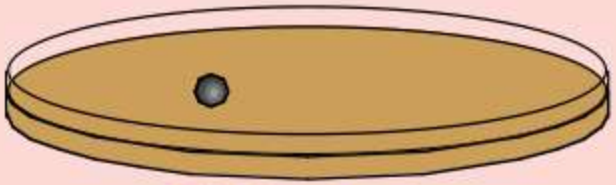


HCC

Biochemical Analysis



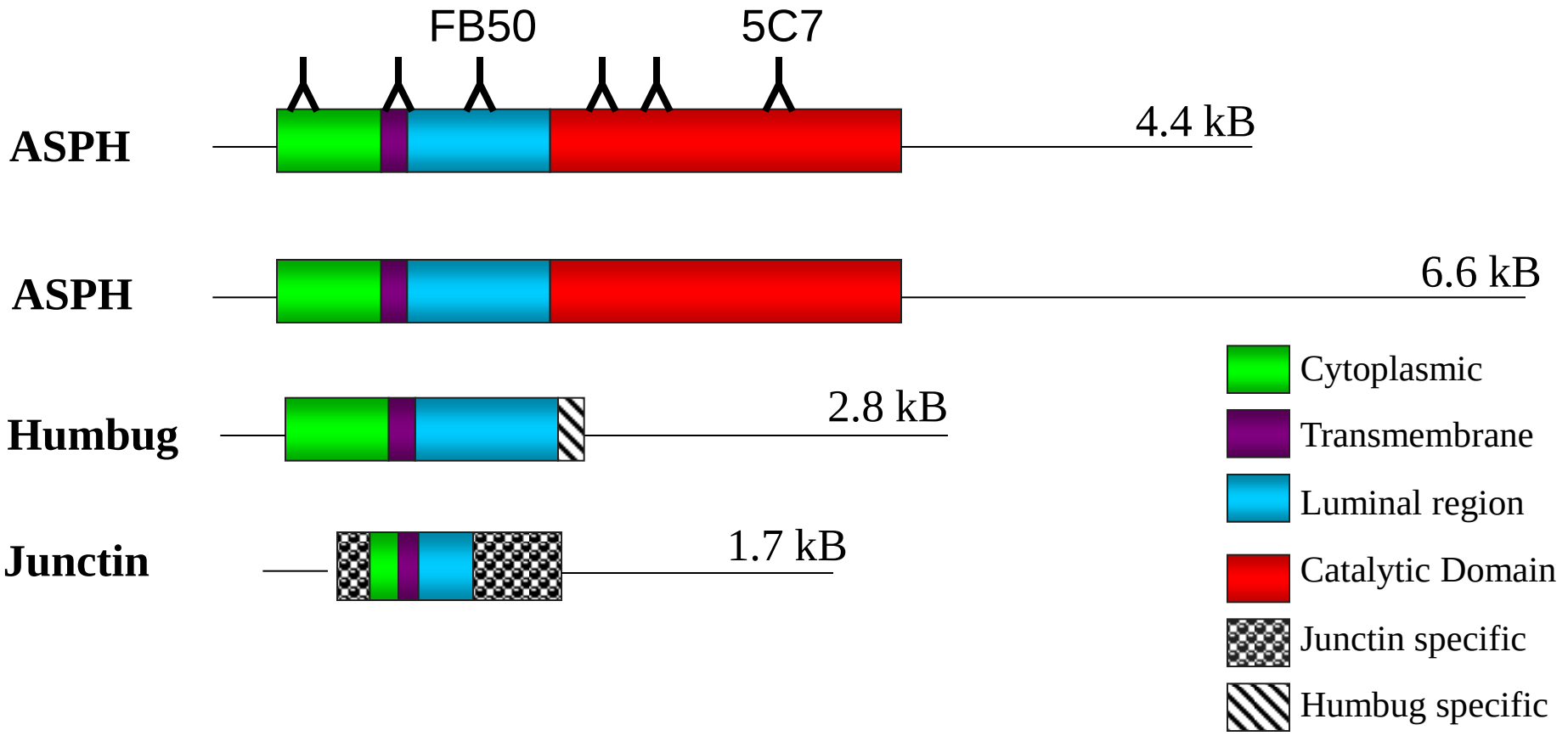
Tissue Distribution



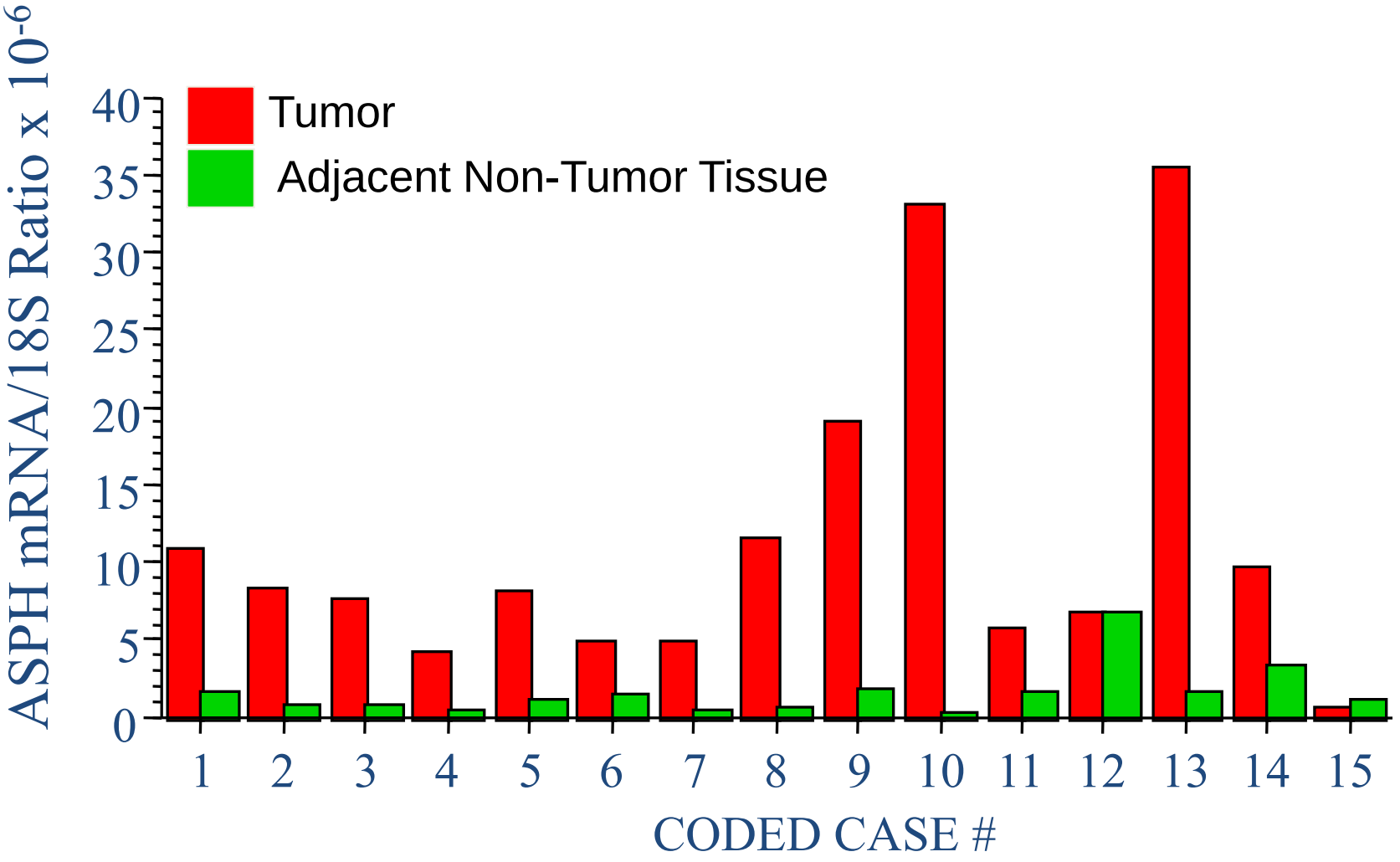
cDNA cloning

Structure of ASPH and Splice Variants

- Human Aspartyl (asparaginyl)-β hydroxylase (ASPH); α-ketogluterate dependent dioxygenase; Mr ~86 kD
- ASPH gene encodes 3 proteins: ASPH, Humbug, Junctin

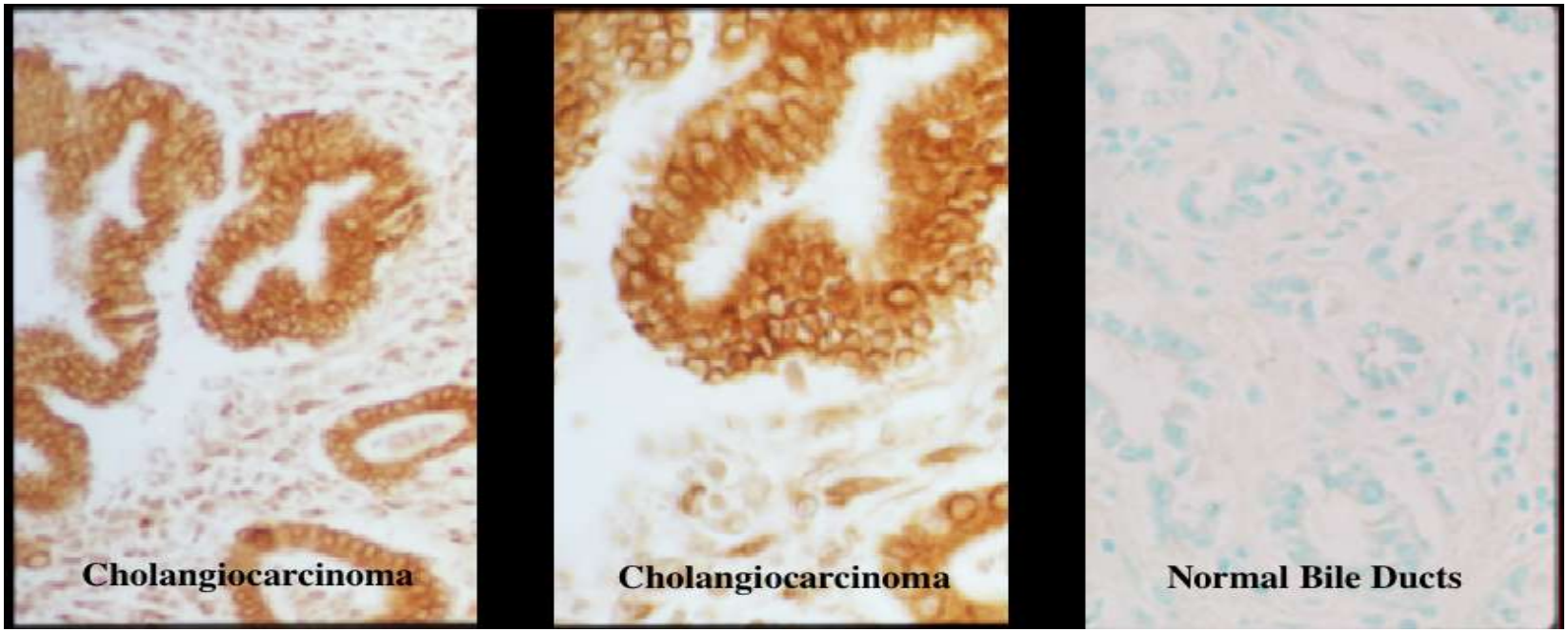


Expression of ASPH (full length) Gene in HBV and HCV Related HCC



ASPH expression in Cancer of the Bile Ducts

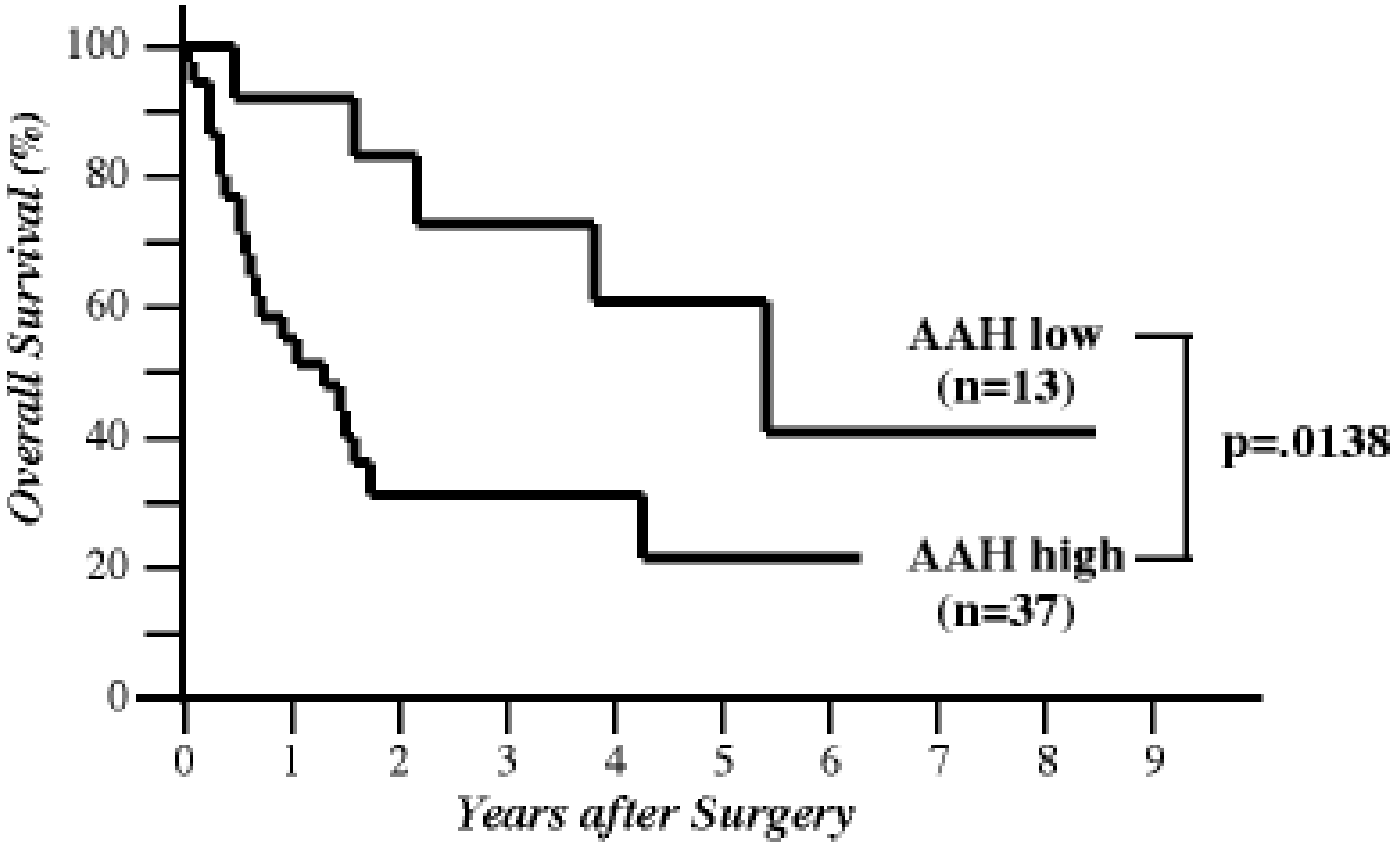
A



B

Disease	No. Pos/No. Studied	%Positive
Cholangiocarcinoma	20/20	100
Sclerosing Cholangitis	0/20	0

Patient Survival after Surgical Resection of Cholangiocarcinoma



Properties of ASPH

1. Overexpressed in >90% of HCC.
2. Translocates from the ER to the cell surface during hepatic oncogenesis.
3. Excellent molecular target for immunotherapy.

Properties of ASPH cont'd…

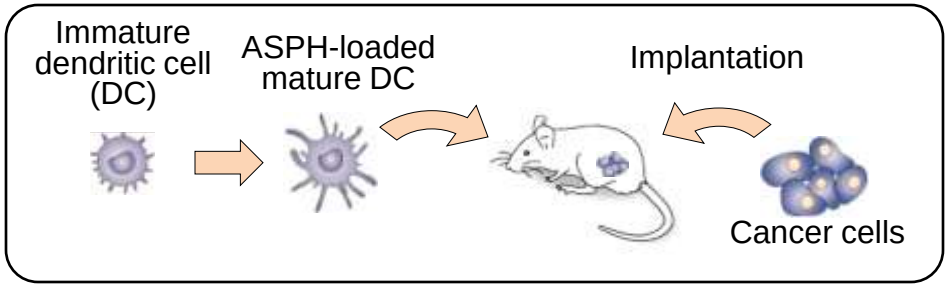
4. Biologic function to promote tumor cell migration and invasion.
5. Transcriptional regulation by IN/IGF-1/IRS-1, Wnt/β-catenin signaling.
6. Exerts biologic function by downstream Notch activation.

Immunotherapy of HCC

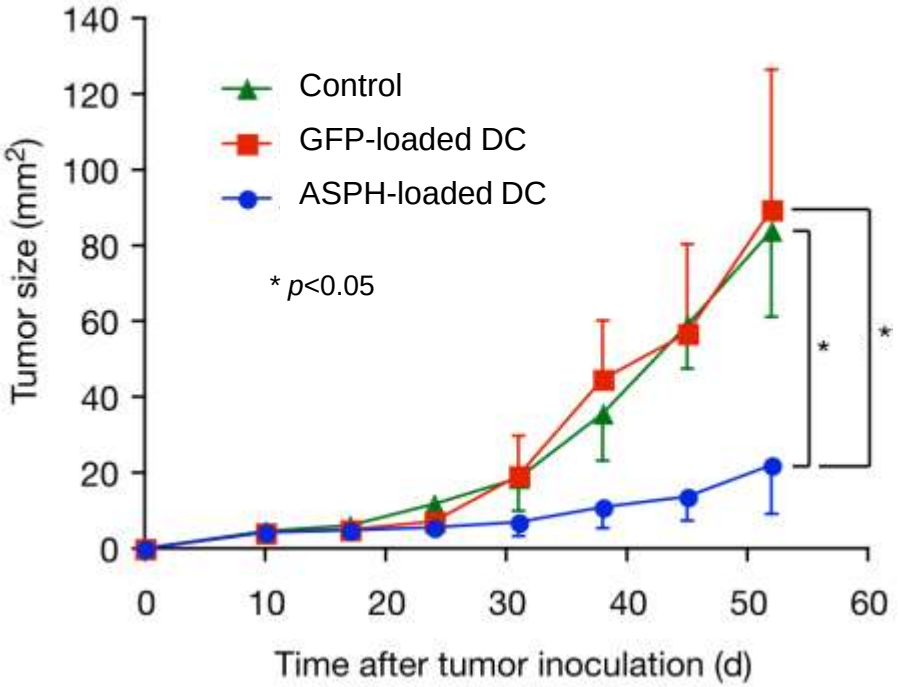
1. Identification and characterization of the target protein.

2. Immunotherapeutic approach.

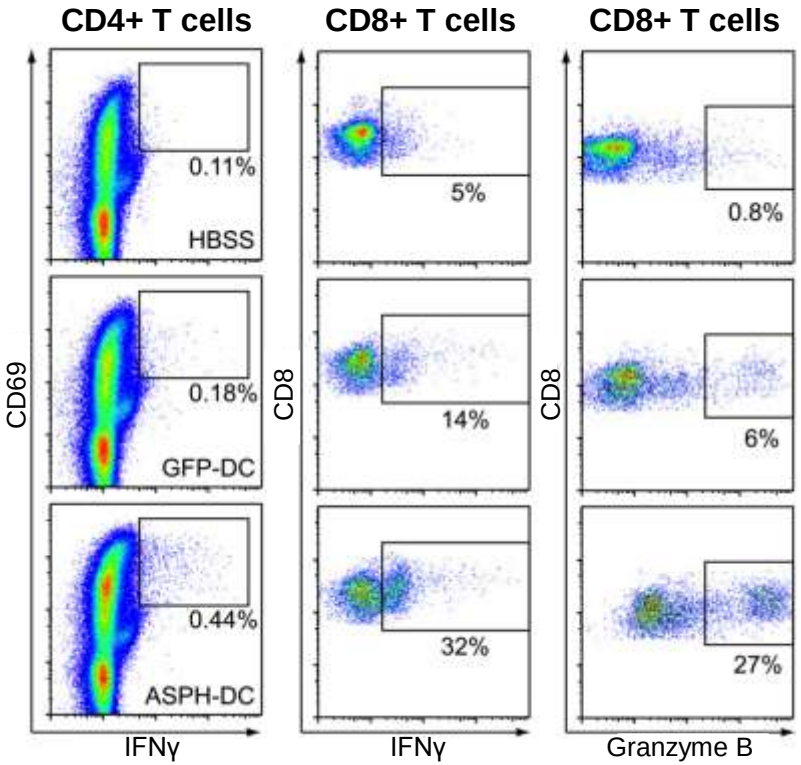
Anti-tumor effect of ASPH protein-loaded DC immunization in mouse HCC model



Tumor growth curve

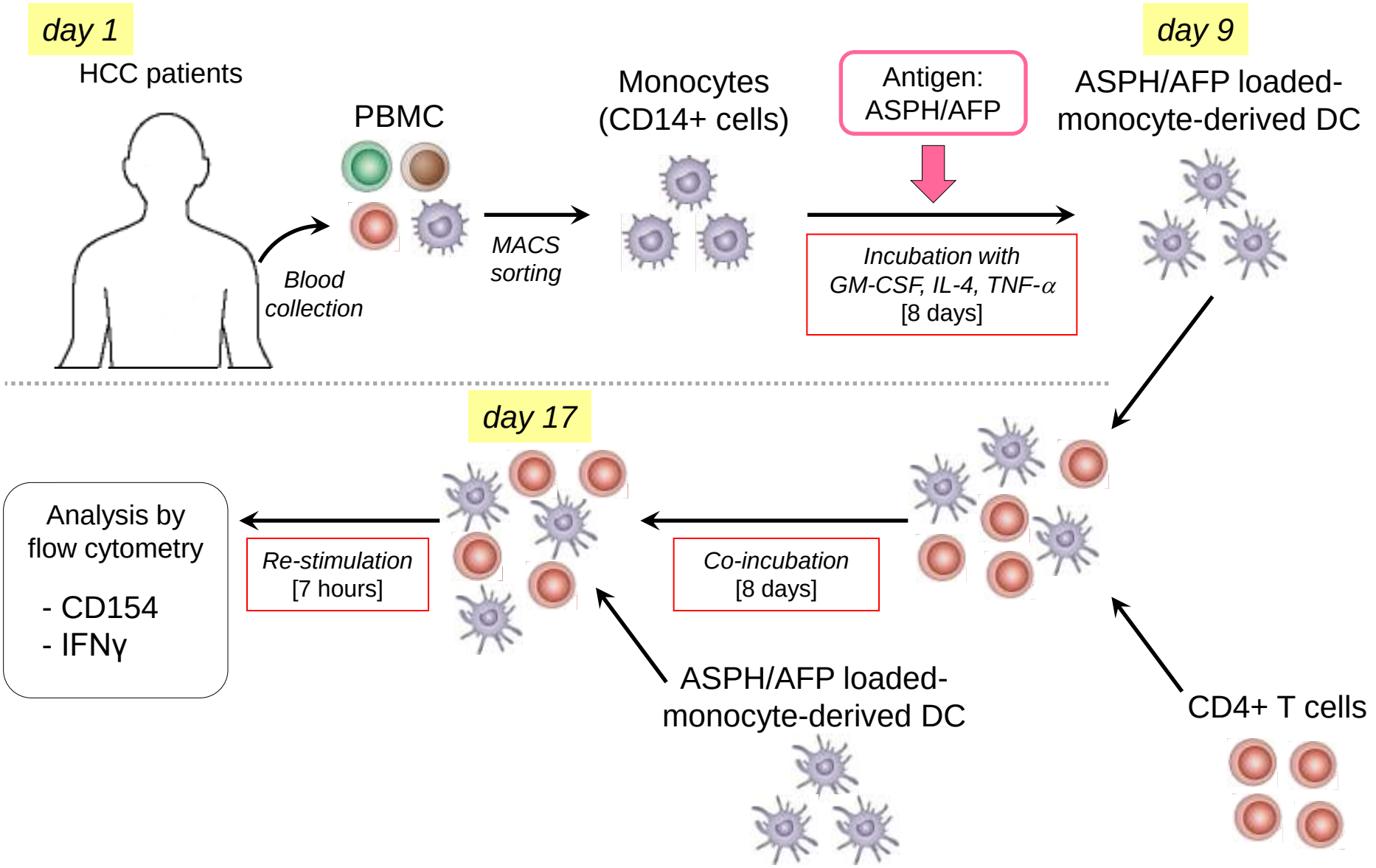


T cell activation



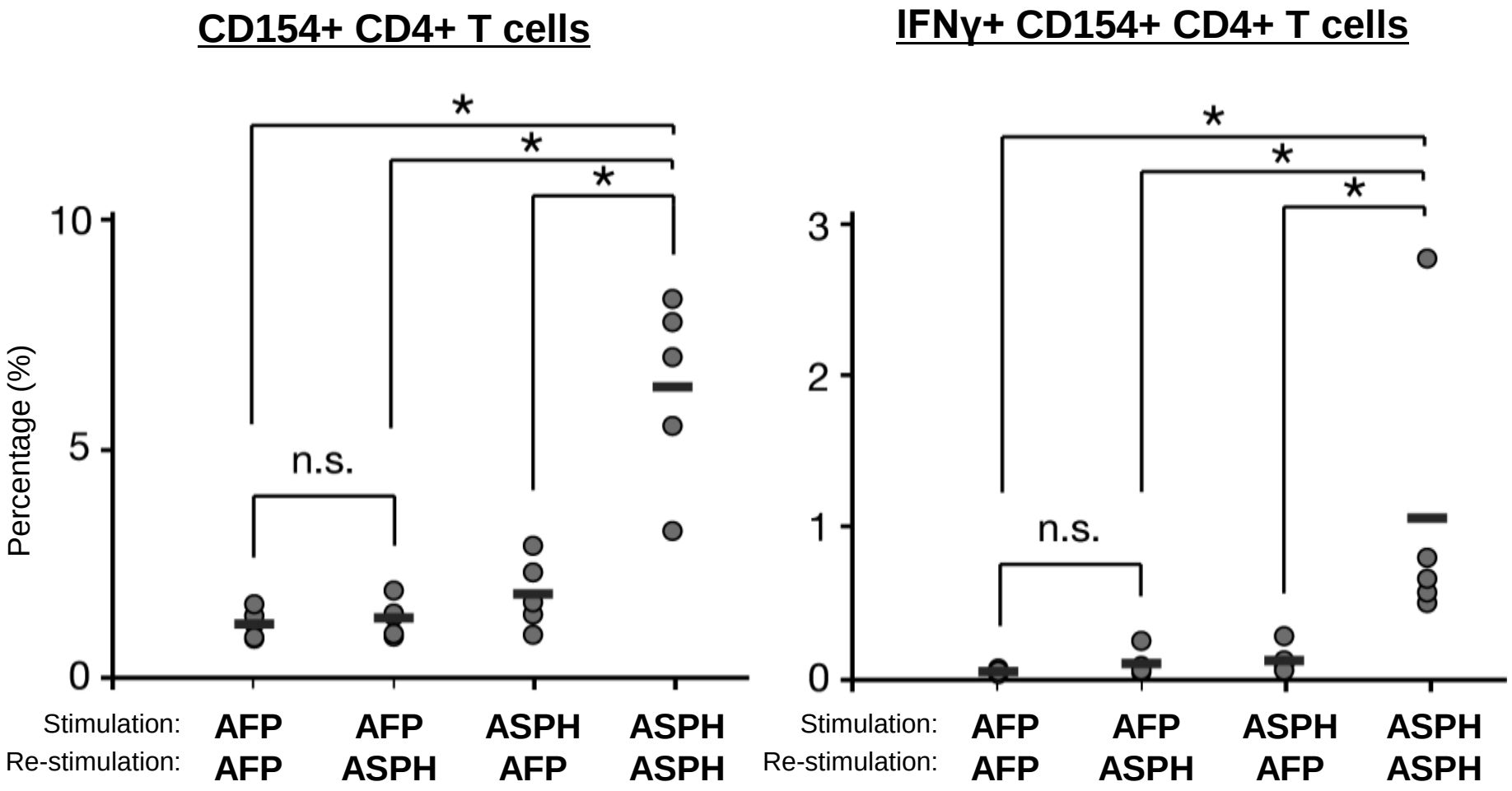
Shimoda M, *et al*. J Hepatol. 2012

Induction of antigen-specific CD4+ T cell response



Shimoda M, *et al*. J Hepatol. 2012

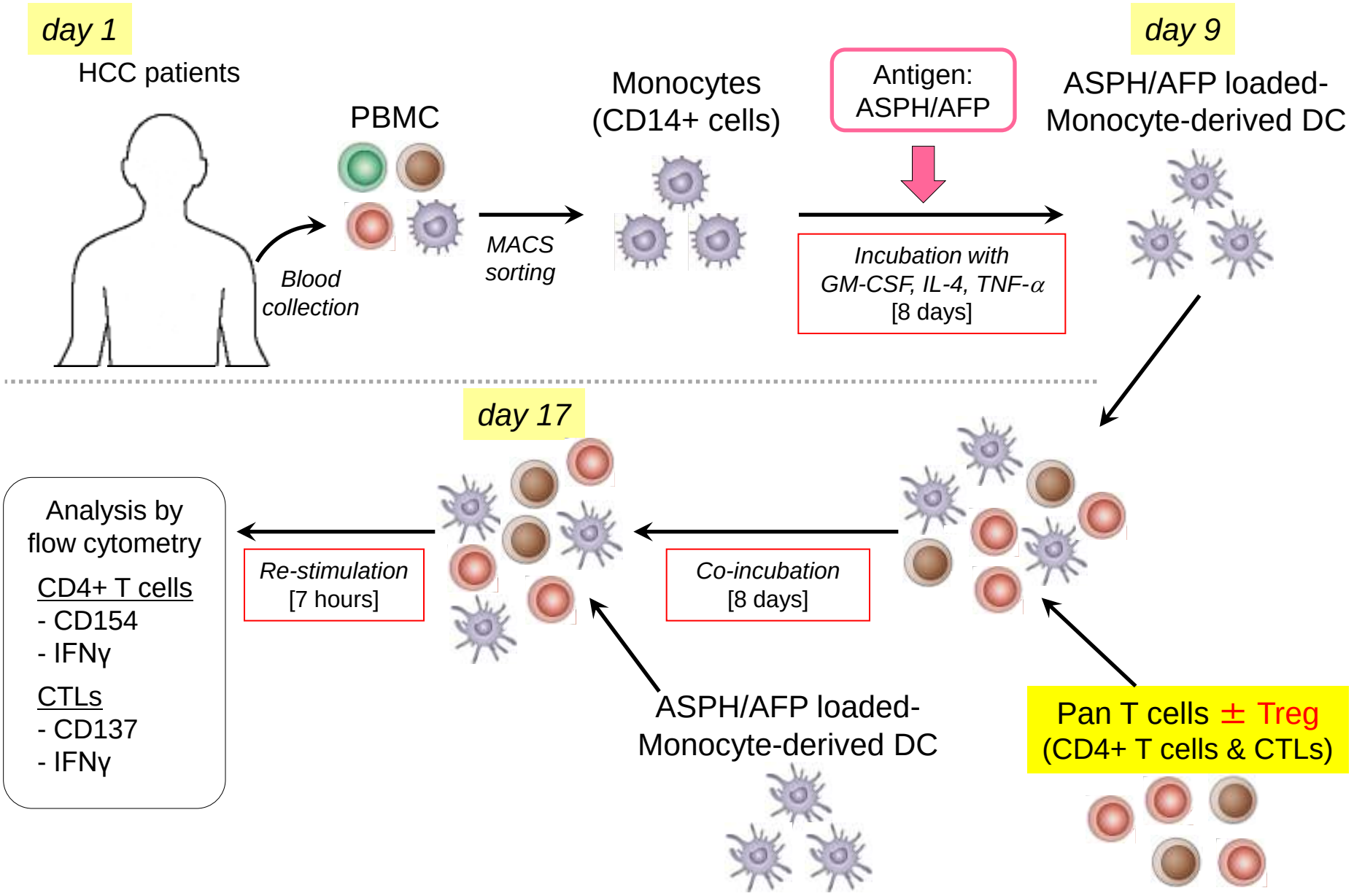
CD4+ T cell activation in HCC patients



* $p<0.05$

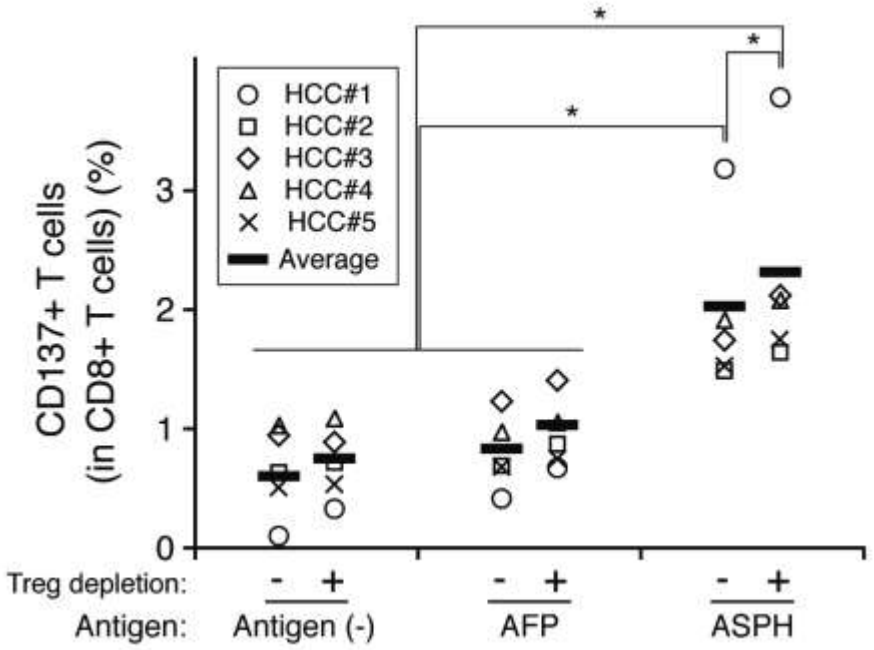
Shimoda M, *et al*. J Hepatol. 2012

Induction of antigen-specific CTL response

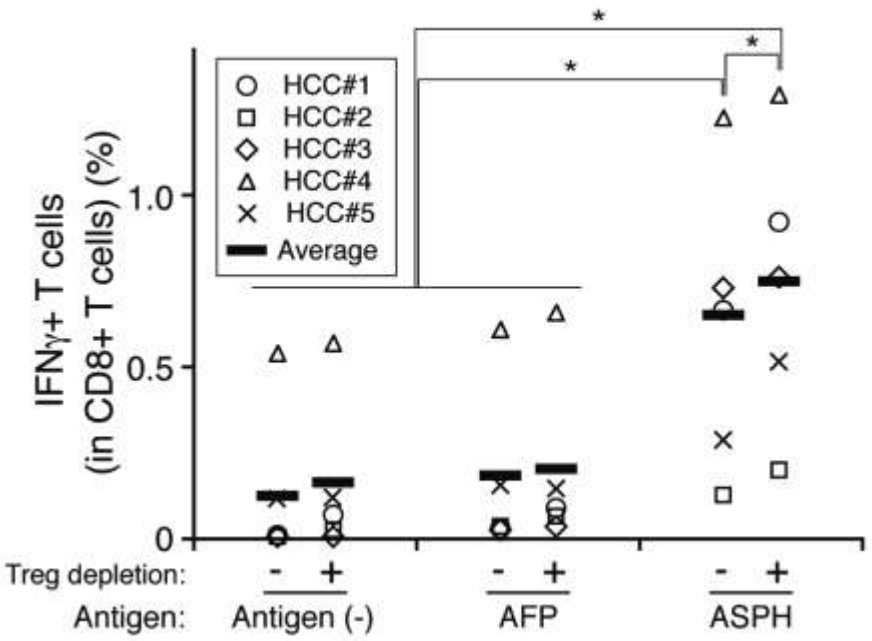


CTL activation in HCC patients

CD137+ CD8+ T cells



IFNγ+ CD8+ T cells



* $p<0.05$

Generation of HLA class II-restricted ASPH peptides

- Epitope prediction was performed using EpiMatrix System in EpiVax, Inc..
- Based on the prediction, 15 HLA class II-restricted peptides with >95% binding probability to MHC diversity in the human population were designed.

ASPH sequence (758 amino acids)

MAQRKNAKSSGNSSSSGSGSGSTSAGSSSPGARRETKHGGHKNGRKGGLSGTSFFTWFMVIALLGVWTSVAVVWFDLVDYEEVLGKLGIYDADGDGDFDV

DDAKVLLGLKERSTSEPAVPPEEAEPHTEPEEQVPVEAEPQNIEDEAKEQIQSLLHEMVHAEHVEGEDLQQEDGPTGEPQQEDDEFLMATDVDDRFETLE

PEVSHEETEHSYHVEETVSQDCNQDMEEMMSEQENPDSSEPVVEDERLHHDTDDVTYQVYEEQAVYEPLENEGIEITEVTAPPEDNPVEDSQVIVEEVSI

FPVEEQQEVPPETNRKTDDPEQKAKVKKKKPKLLNKFDKTIKAELDAAEKLRKRGKIEEAVNAFKELVRKYPQSPRARYGKAQCEDDLAEKRRSNEVLRG

AIETYQEVASLPDVPADLLKLSLKRRSDRQQFLGHMRGSLLTLQRLVQLFPNDTSLKNDLGVGYLLIGDNDNAKKVYEEVLSVTPNDGFAKVHYGFILKA

QNKIAESIPYLKEGIESGDPGTDDGRFYFHLGDAMQRVGNKEAYKWYELGHKRGHFASVWQRSLYNVNGLKAQPWWTPKETGYTELVKSLERNWKLIRDE

GLAVMDKAKGLFLPEDENLREKGDWSQFTLWQQGRRNENACKGAPKTCTLLEKFPETTGCRRGQIKYSIMHPGTHVWPHTGPTNCRLRMHLGLVIPKEGC

KIRCANETRTWEEGKVLIFDDSFEHEVWQDASSFRLIFIVDVWHPELTPQQRRSLPAI

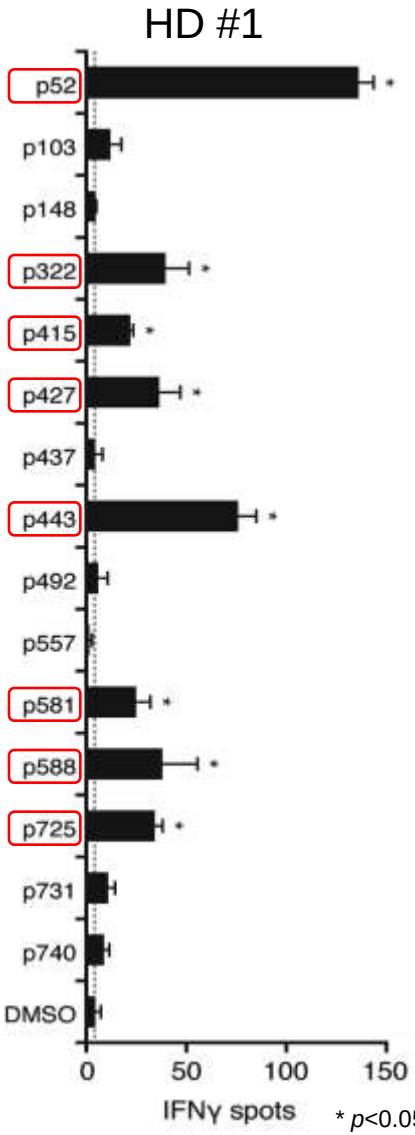
HLA binding assay

ID	EpiMatrix hits	EpiMatrix cluster score	IC_{50} (μg/ml) to HLA-DRB1			
			*0101	*0301	*0701	*1501
p52	14	23.72	294.66	---	6.26	225.24
p103	6	9.47	150.78	11.71	---	33.59
p148	12	16.58	14.62	24.92	16.53	20.35
p322	8	16.05	---	---	---	---
p415	14	27.86	---	---	---	---
p427	8	13.76	< 3.13	---	14.41	7.14
p437	7	12.94	17.47	---	---	< 3.13
p443	8	13.77	< 3.13	37.89	---	< 3.13
p492	16	32.14	< 3.13	---	---	< 3.13
p557	12	18.44	4.94	---	62.29	3.26
p581	8	12.54	126.53	158.00	164.52	13.06
p588	11	17.18	< 3.13	< 3.13	< 3.13	< 3.13
p725	4	7.15	3.63	< 3.13	< 3.13	< 3.13
p731	4	5.36	< 3.13	13.24	< 3.13	< 3.13
p740	7	10.40	---	194.12	---	---

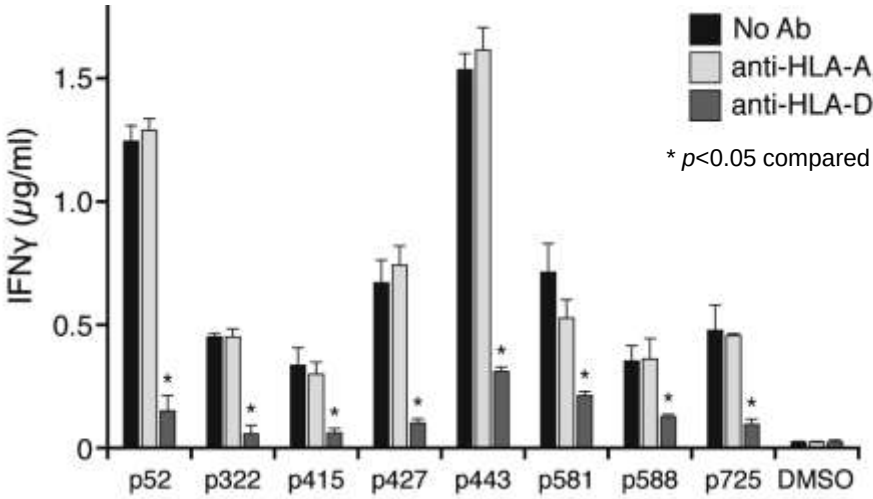
Strong binding affinity
Moderate binding affinity
Weak binding affinity

Characterization of the immune response (2)

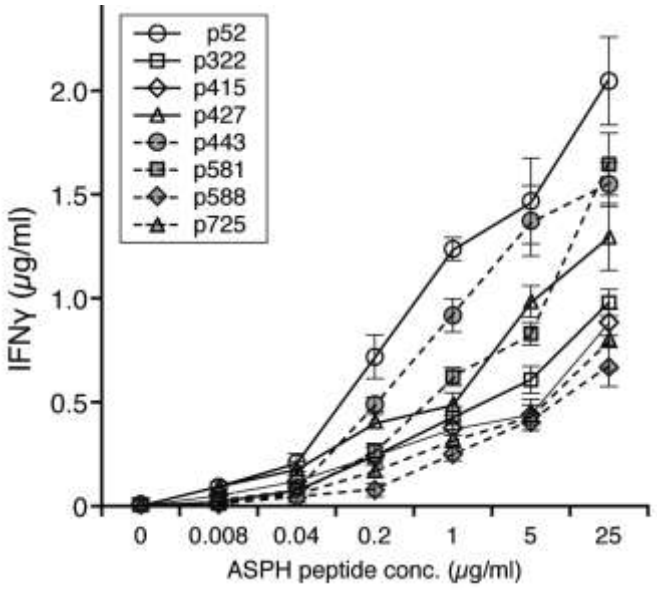
The response is HLA-DR- and peptide dose-dependent



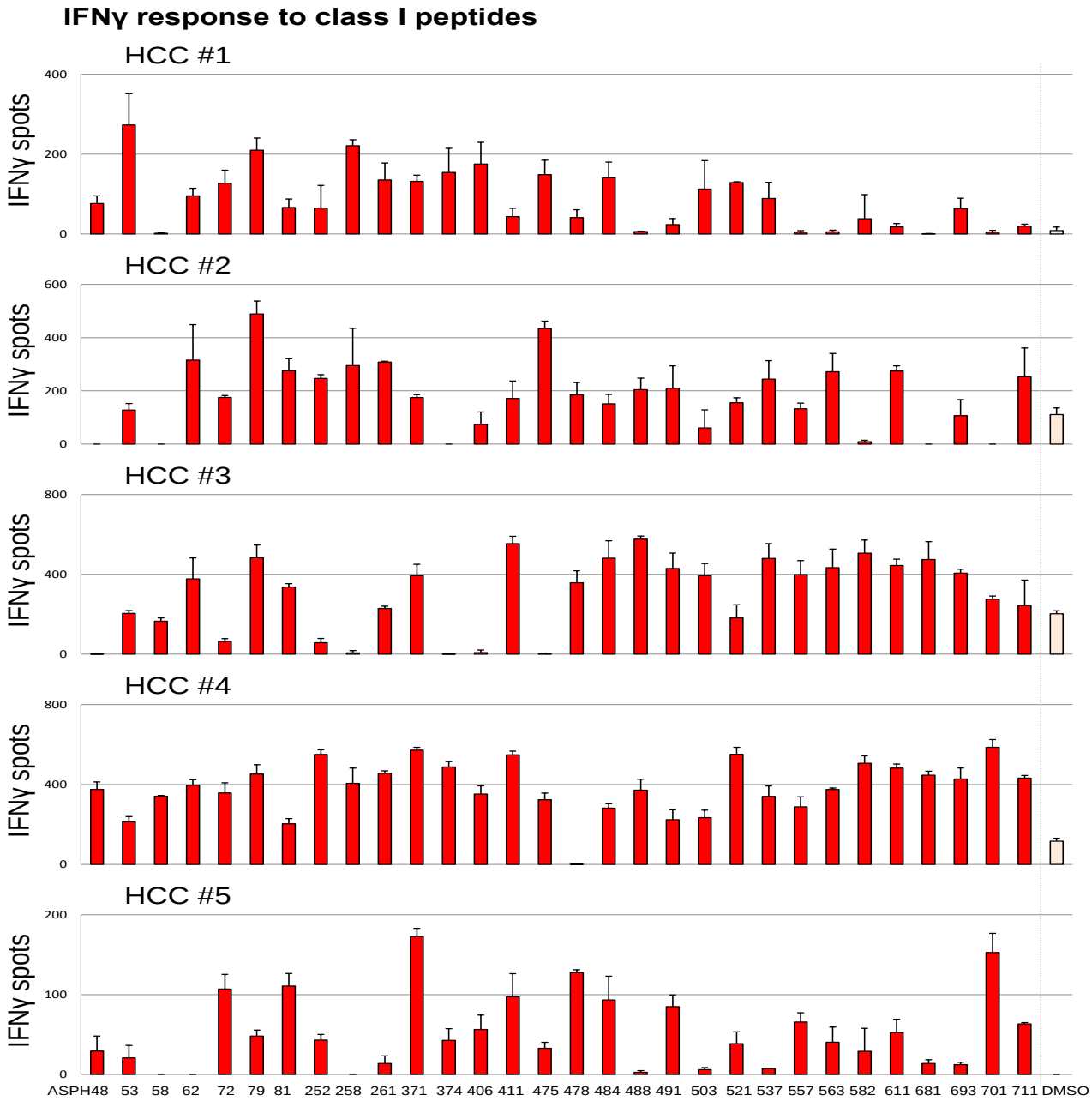
* p<0.05 compared to DMSO



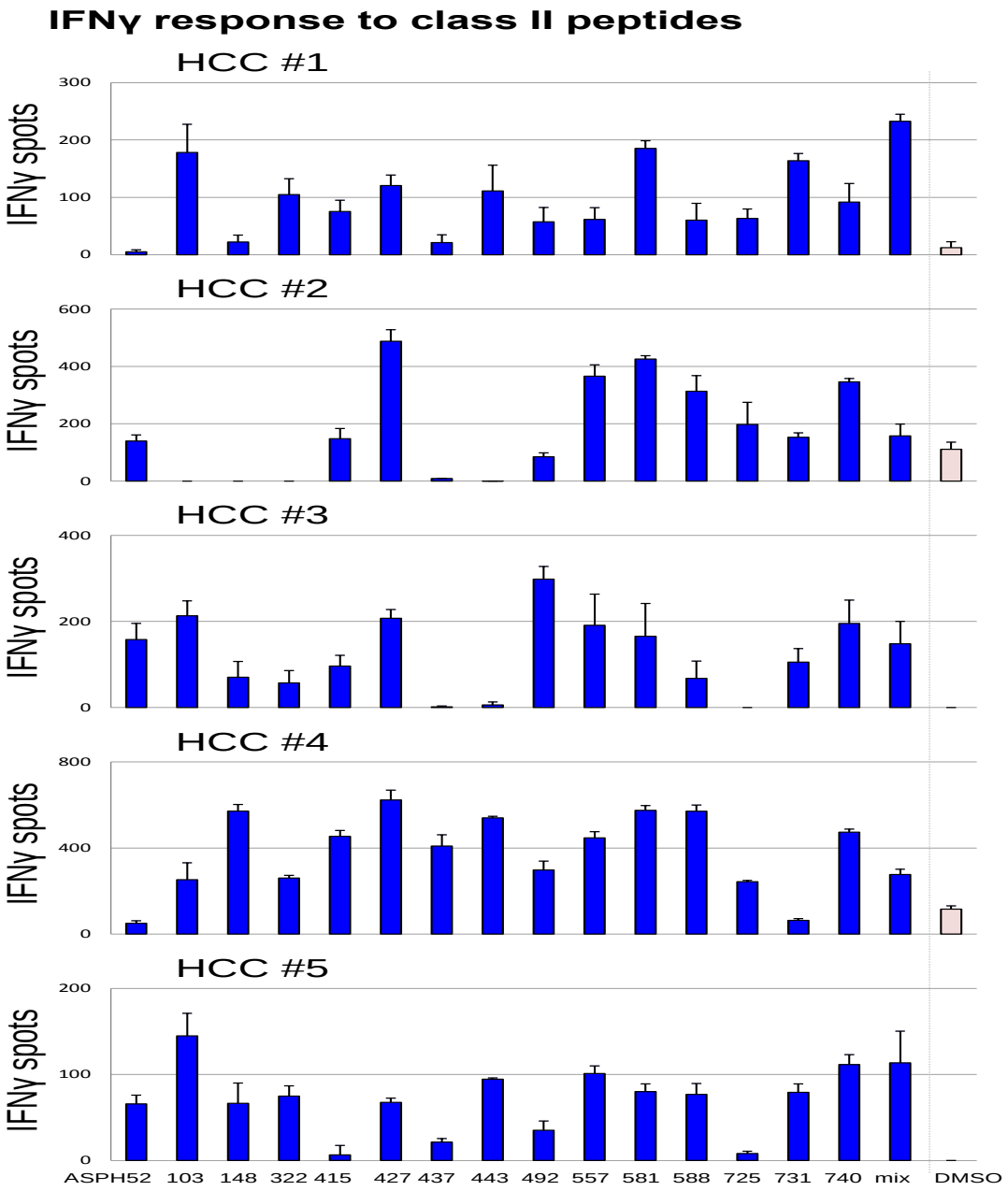
* p<0.05 compared to No Ab



Immune Response to ASPH Peptides in HCC patients

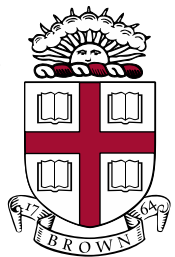


Immune Response to ASPH Peptides in HCC patients



Conclusions

- ASPH protein-loaded DC immunization induced anti-tumor effect through T cell activation in mouse HCC model.
- ASPH protein-loaded DC triggered antigen-specific immune response of CD4+ T cell and CTL in HCC patients.
- Treg depletion enhanced ASPH protein-inducible T cell activation.
- ASPH-derived HLA class I and II-restricted peptides induced immune responses in healthy donors and patients with HCC.



Future plans

Evaluation of immunogenicity of the ASPH peptides in more HCC patients.

Clinical application of the ASPH peptides as cancer vaccines for immunotherapy against HCC and cholangiocarcinoma.

Additional studies…

- Define vaccine adjuvants
- Vaccine design without Treg-stimulating epitopes
- Application to other ASPH expressing cancers

Acknowledgements

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