

Immunotherapy in Hepatocellular Carcinoma

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*GI-Malignancy Section
Center for Cancer Research*



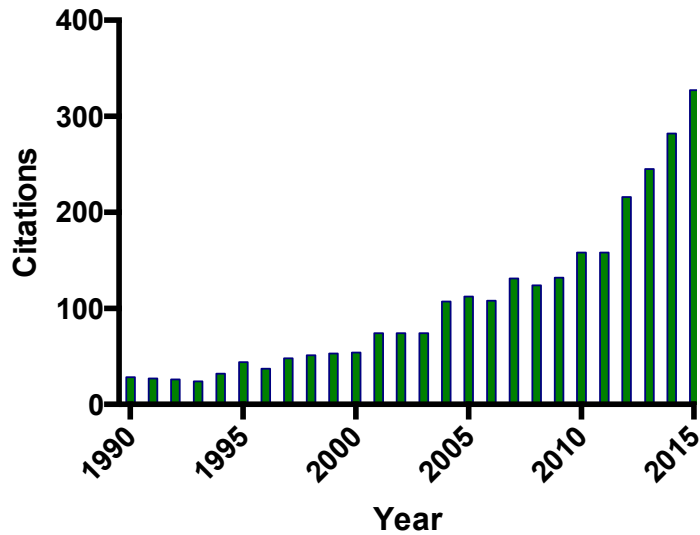
I have no financial relationships to disclose.

**I will discuss the following off investigational use
in my presentation:**

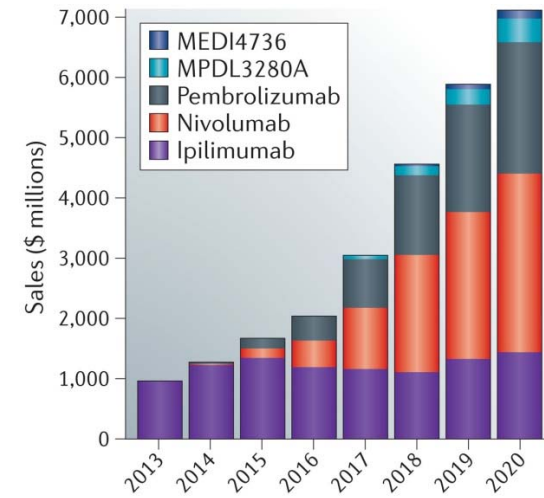
Tremelimumab (anti-CTLA4) for the treatment of
HCC

A growing interest in immunotherapy and HCC

**Pubmed hits for
“hepatocellular AND immune”**



**Estimated market sales for
Immune checkpoint inhibitors**

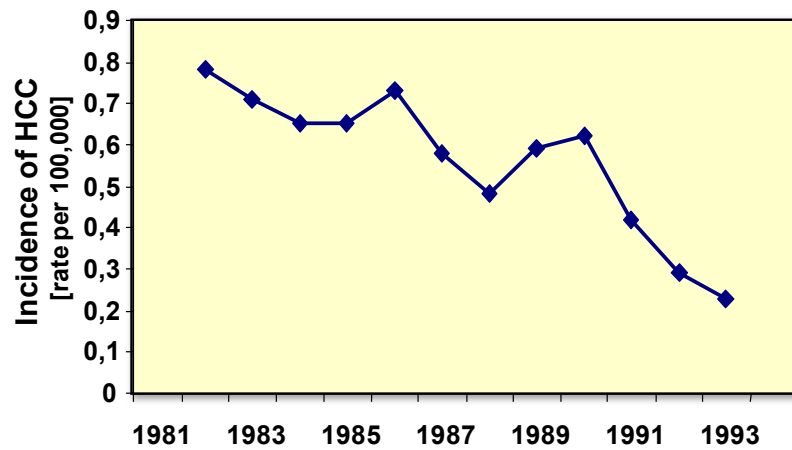


HCC – an inflammation associated cancer?

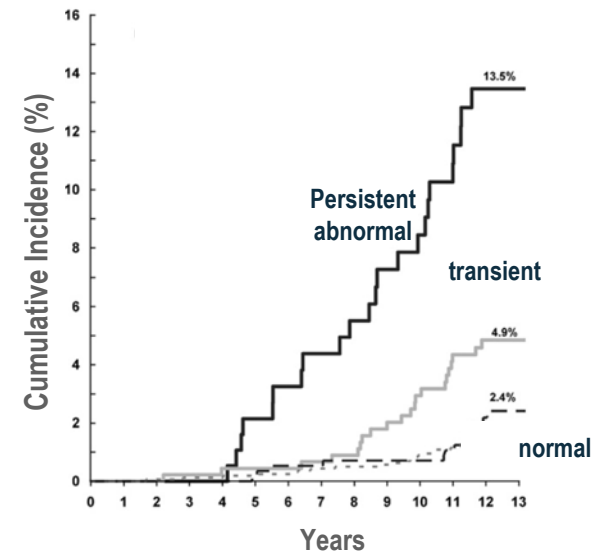
Risk factors

- ▶ HBV
- ▶ HCV

Hepatitis B vaccination prevents HCC



Transaminases and HCC

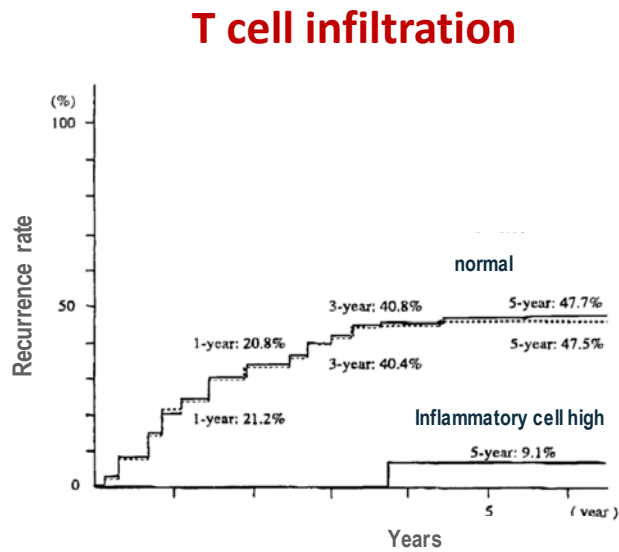


Chang et al, NEJM (1997) 336:1855

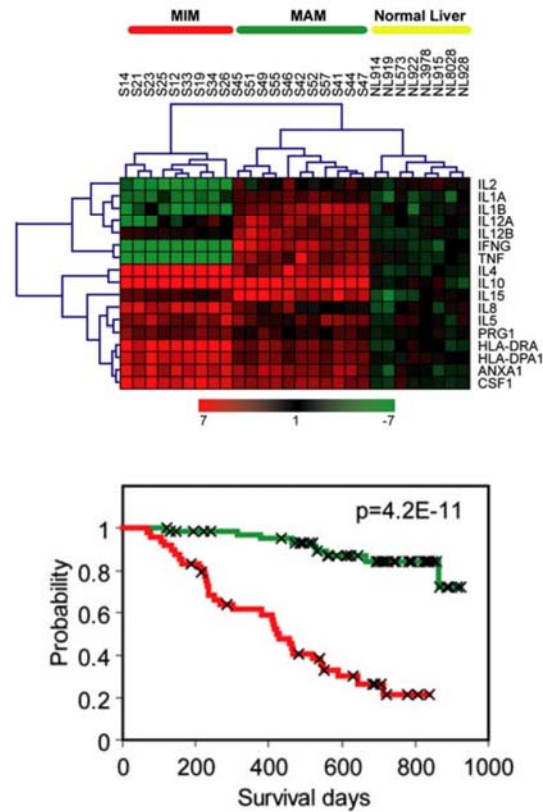
Chen et al. (2011) Gastroenterology 141:1240–1248

Immune correlatives correlate with outcome in HCC

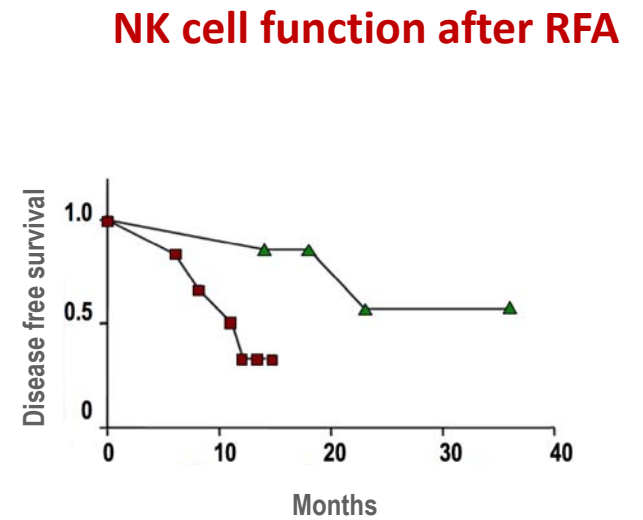
17 gene "immune signature"



Wada et al, Hepatology (1998) 27:407



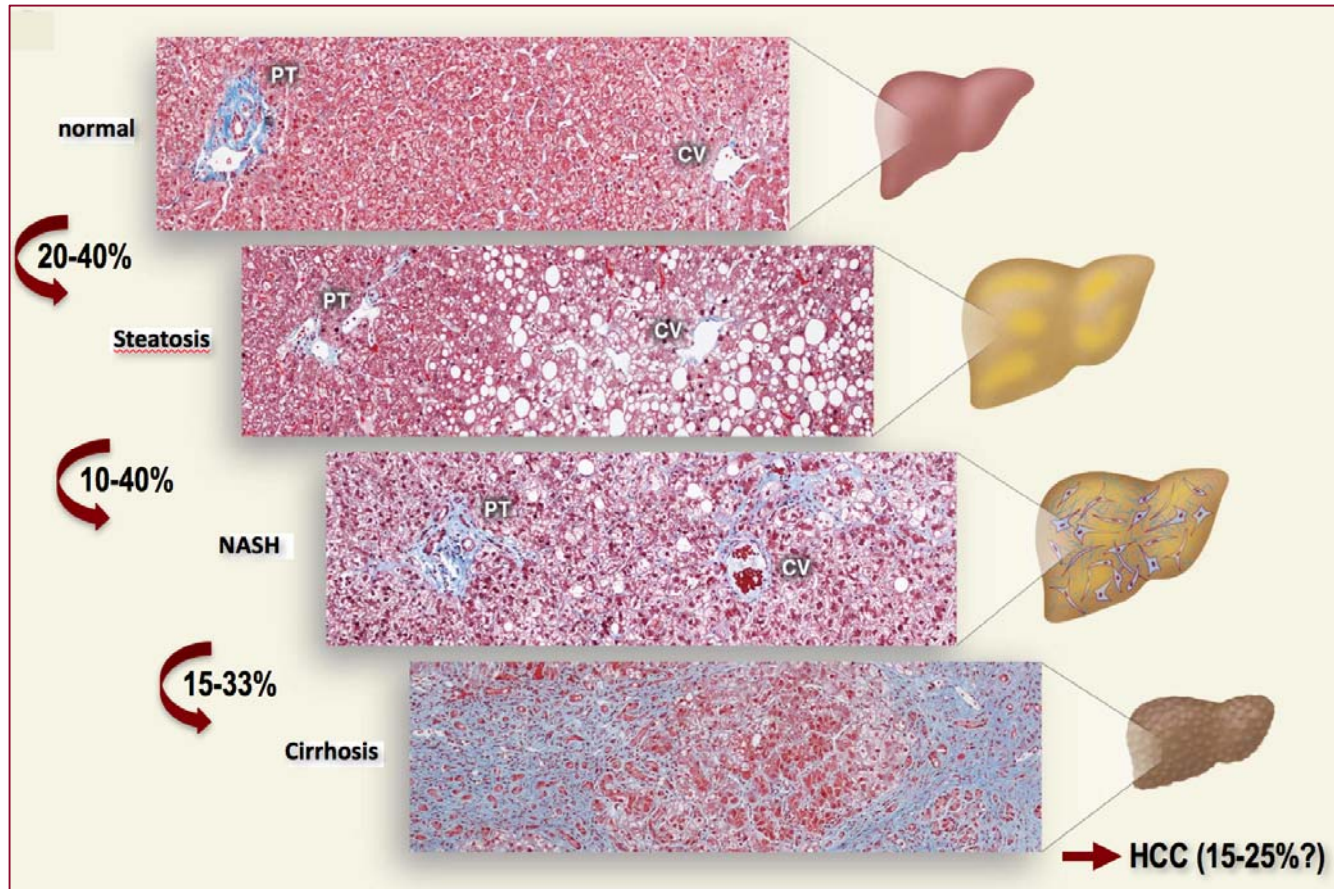
Budhu et al, Cancer Cell (2006) 10:99



Zerbini et al, Gastroenterology (2010) 138:1931

NASH controls adaptive immune responses during hepatocarcinogenesis

NASH - HCC

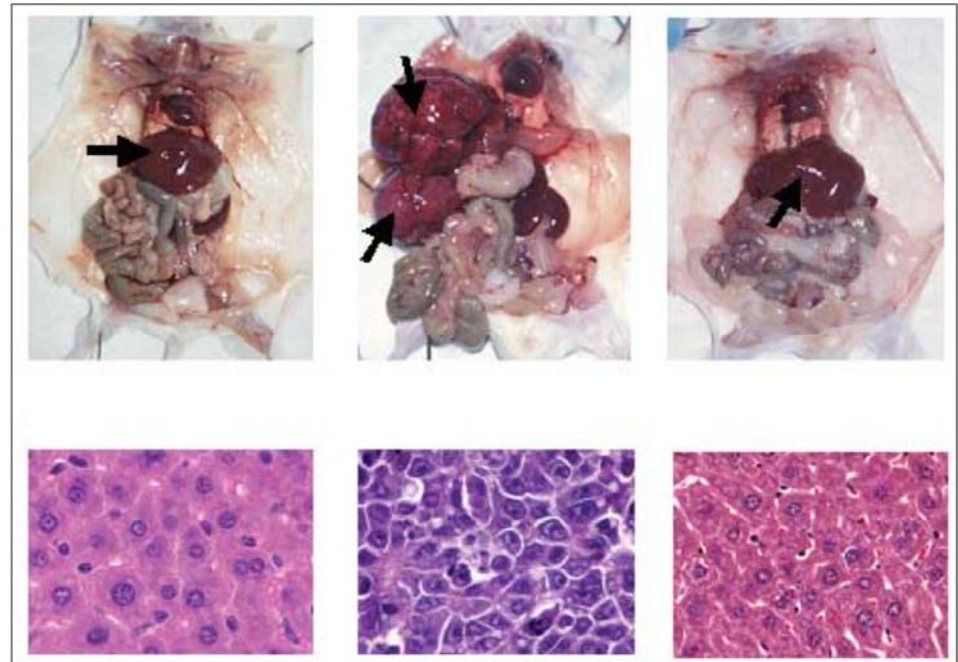
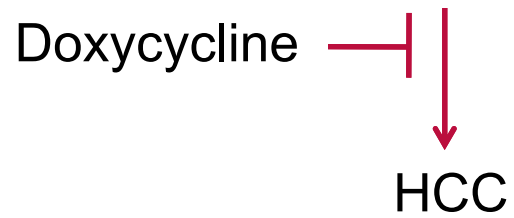


modified from Cohen et al. (2011) Science; 332: 1519-23

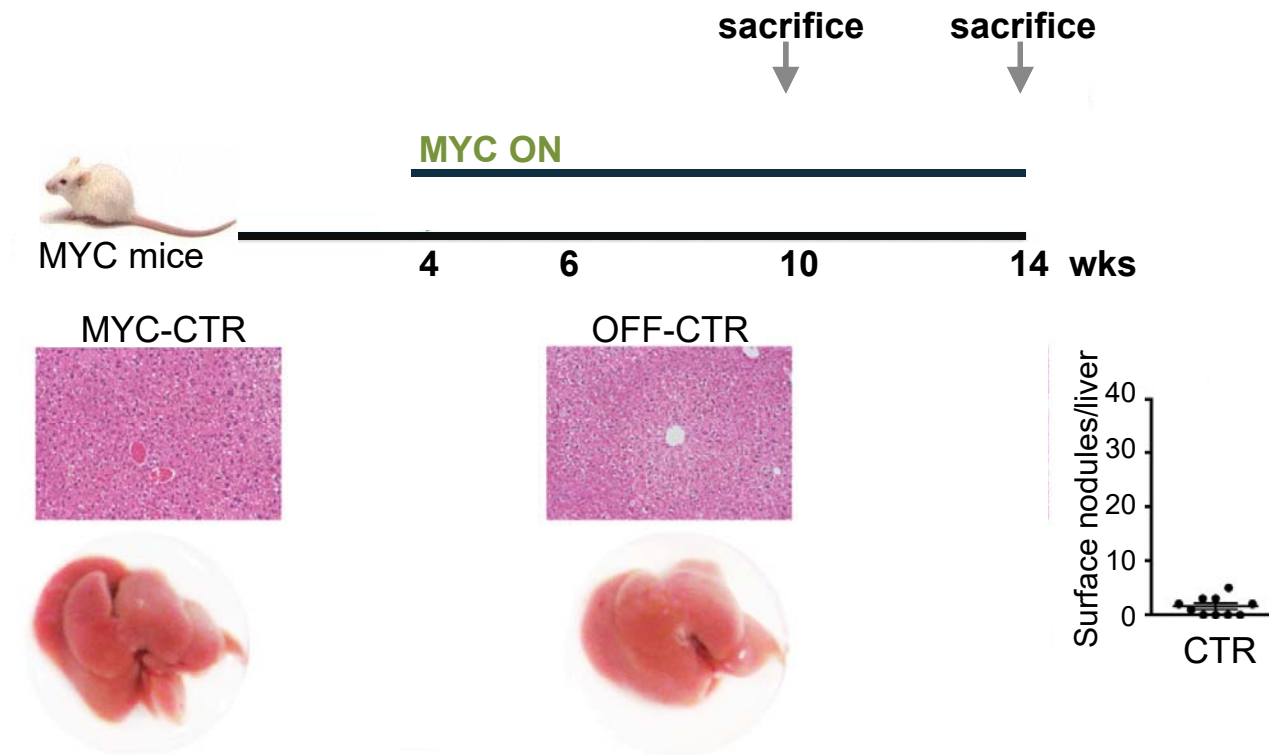
MYC transgenic HCC mouse model



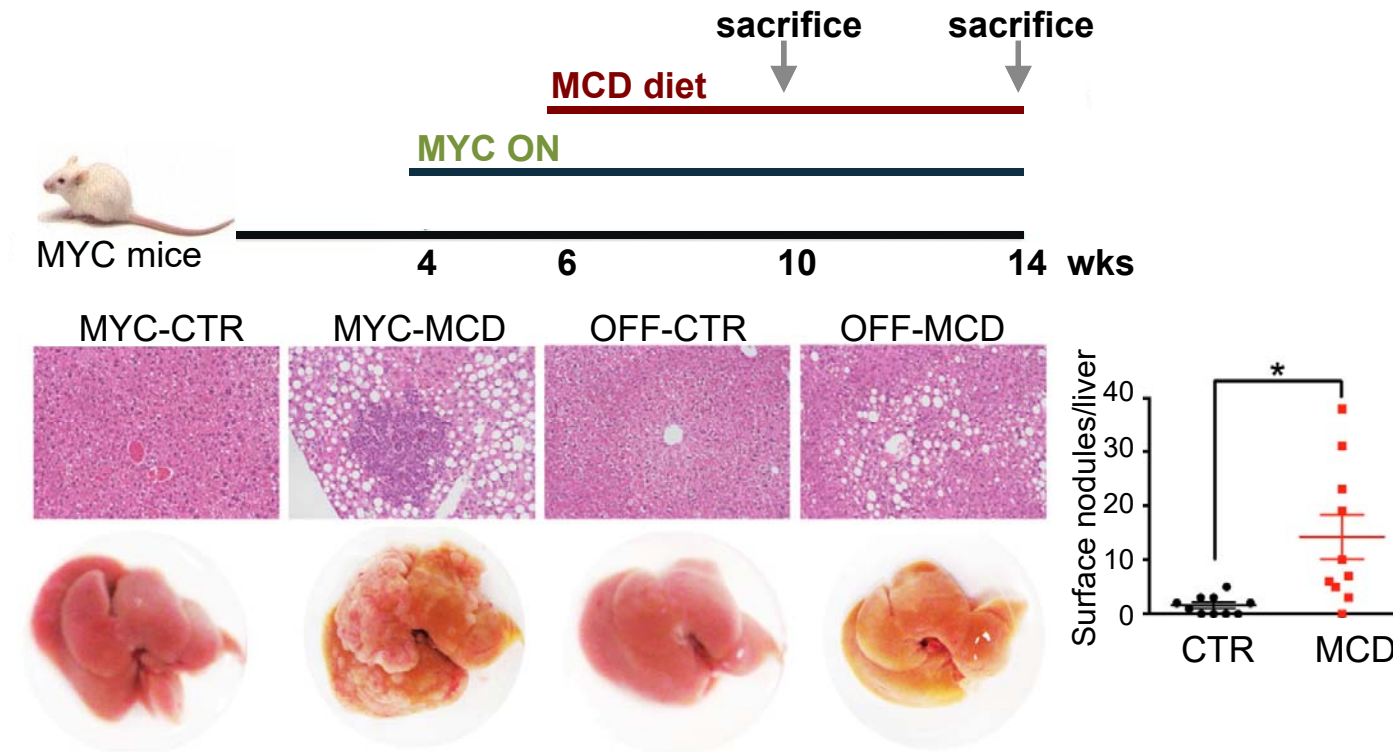
LAP-tTA/TRE-MYC



NASH promotes hepatocarcinogenesis



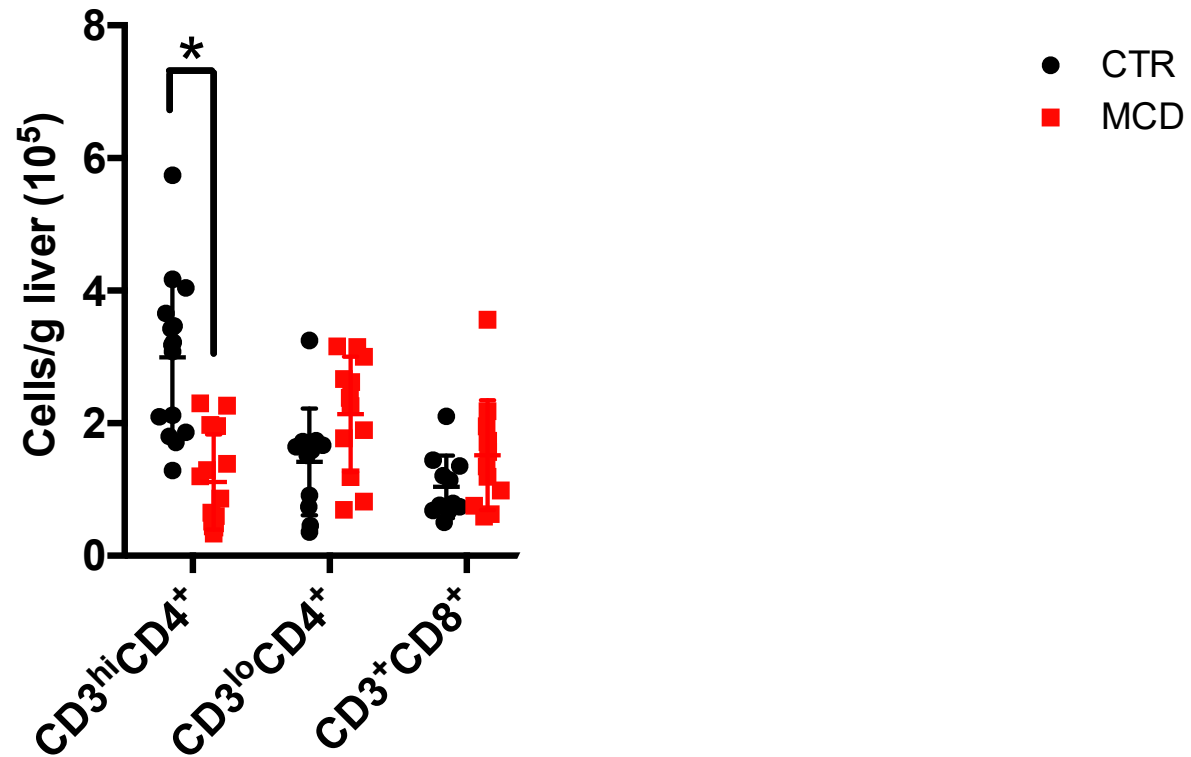
NASH promotes hepatocarcinogenesis



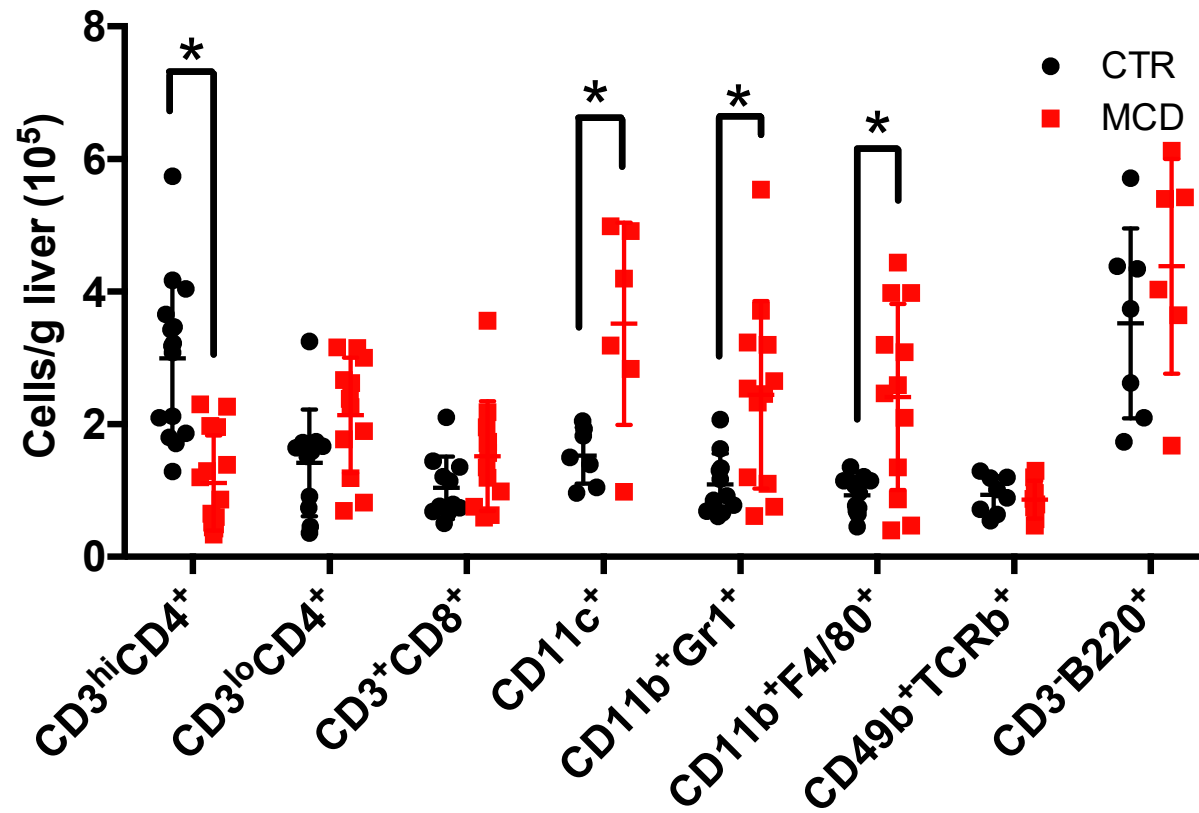
Selective CD4⁺ T cell loss in mice with NASH



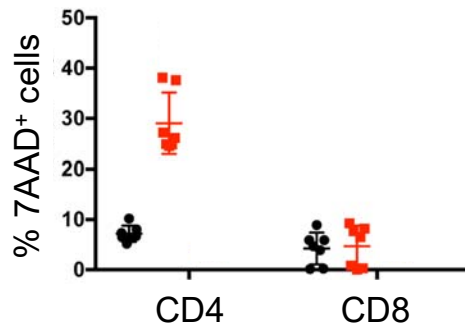
Selective CD4⁺ T cell loss in mice with NASH



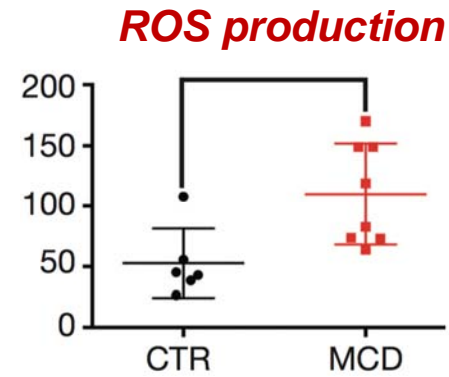
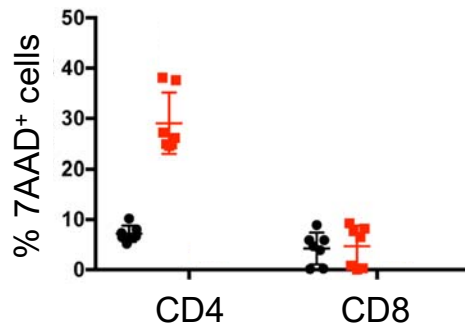
Selective CD4⁺ T cell loss in mice with NASH



CD4⁺ T cells die upon co-culture with hepatocytes from mice with NAFLD

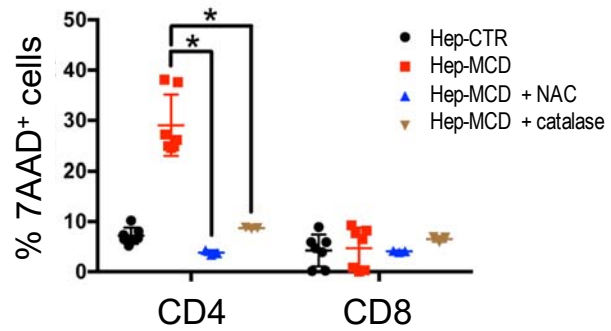


CD4⁺ T cells die upon co-culture with hepatocytes from mice with NAFLD

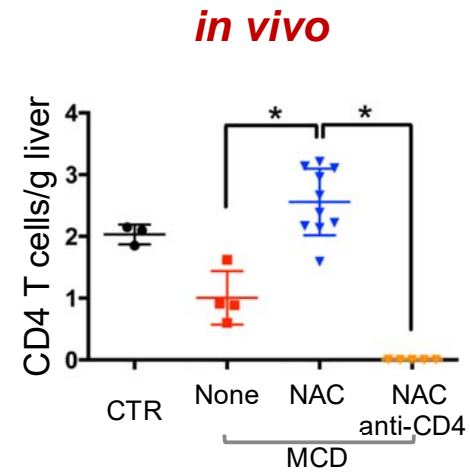
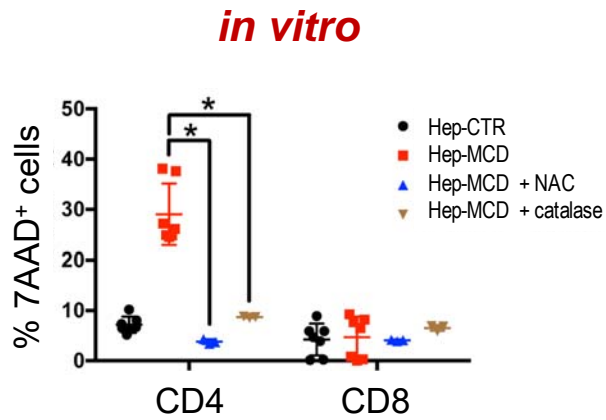


N-acetyl cystein treatment prevents CD4⁺ T cell loss

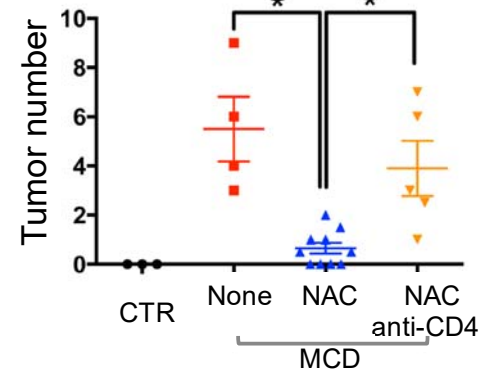
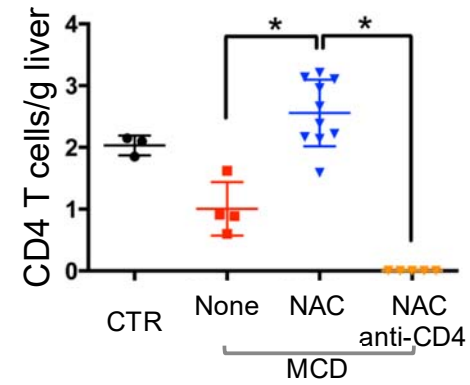
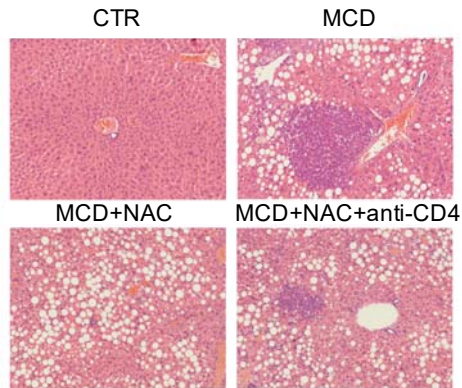
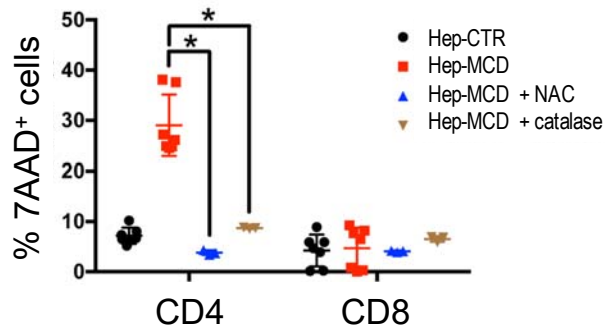
in vitro



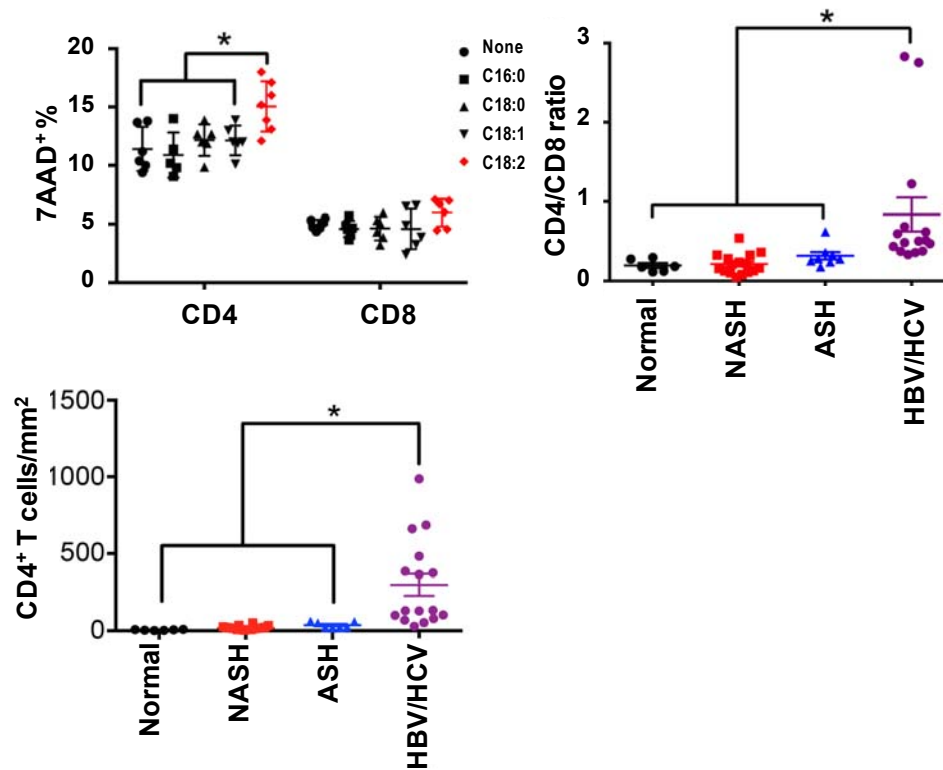
N-acetyl cystein treatment prevents CD4⁺ T cell loss

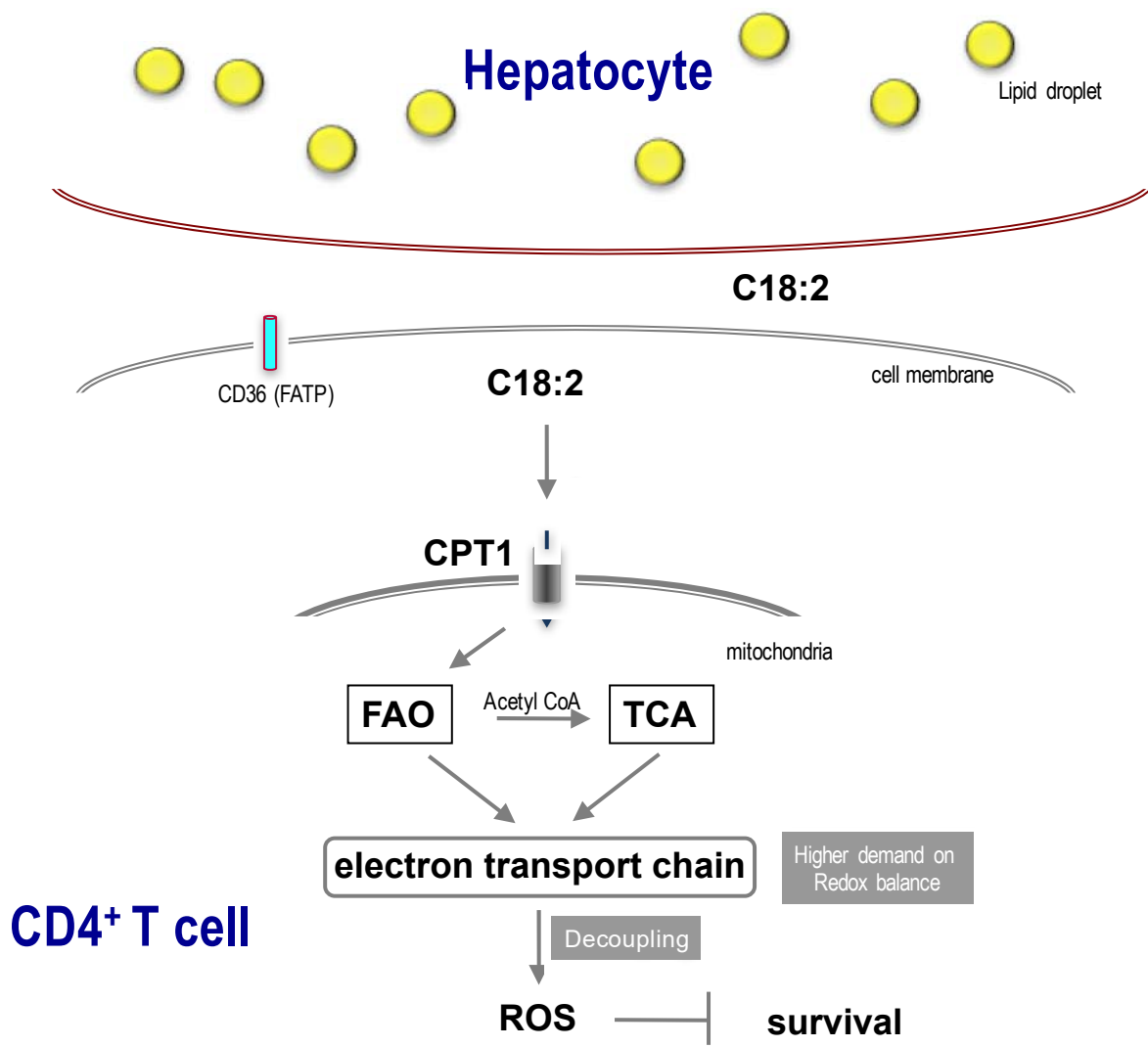


N-acetyl cystein treatment prevents CD4⁺ T cell loss

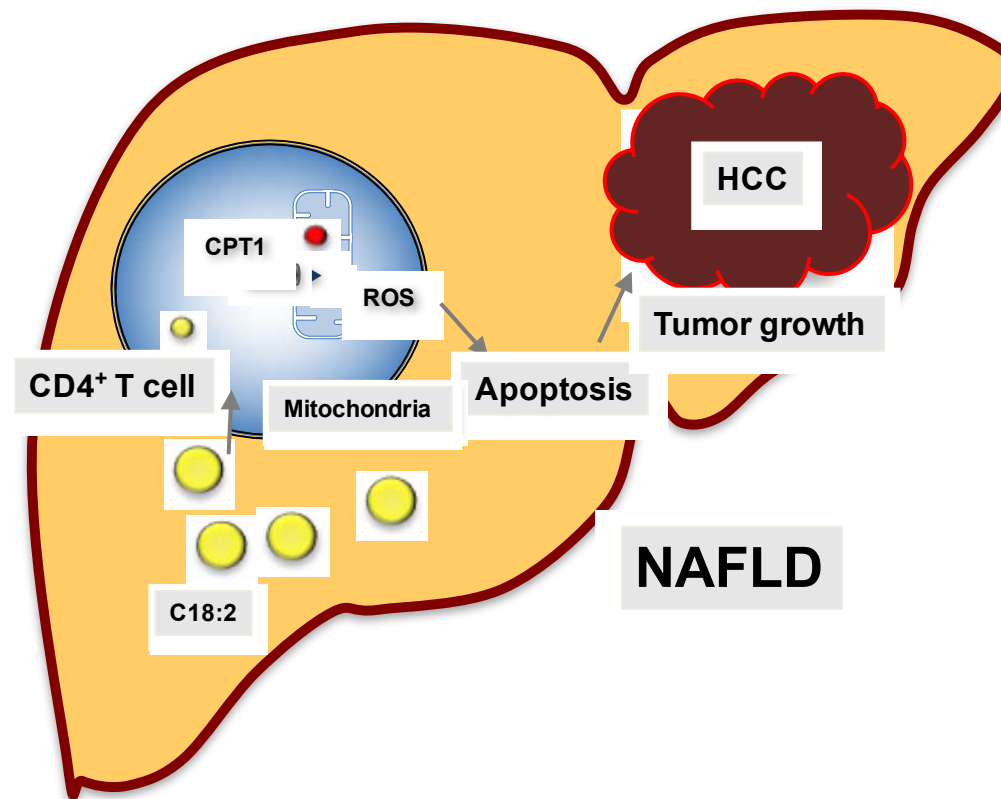


C18:2 kills human CD4⁺ T cells, which are reduced in NASH patients





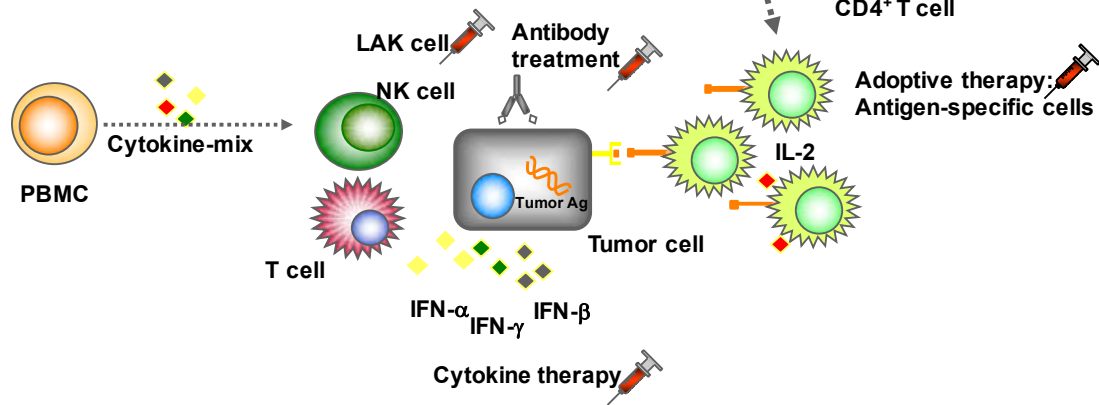
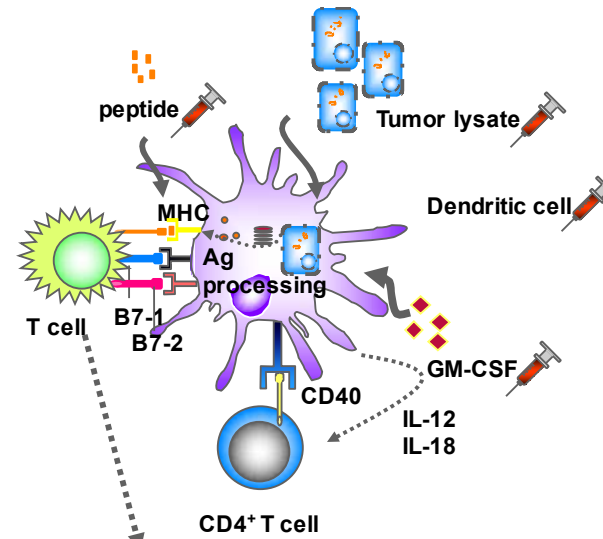
NAFLD causes selective CD4+ T lymphocyte loss and promotes hepatocarcinogenesis



Immunotherapy Trials in HCC

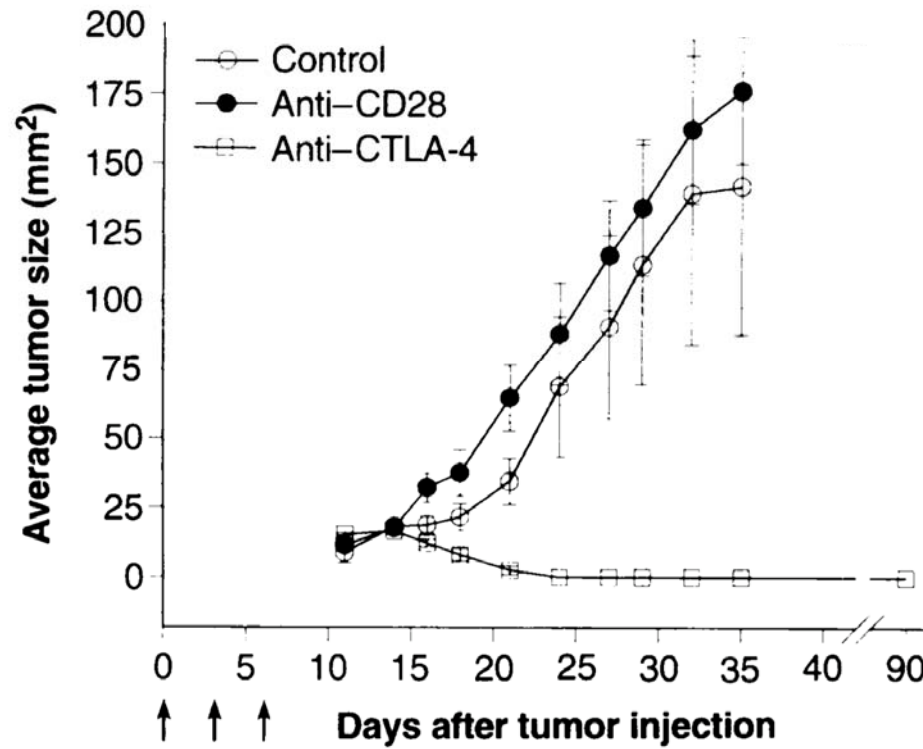
Type of treatment	No. of studies	No. of patients
Cytokine treatment (Ifn and GM-CSF)	32	1496
Cell based treatment	15	340
Antibody treatment	8	316
Vaccines	4	48

Greten et al. (2008) Rev Recent Clin Trials 3:31-9



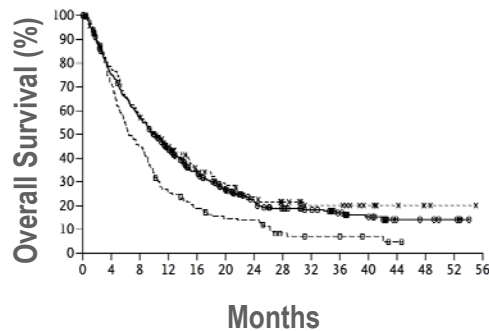
Greten et al. (2006) J.Hepatol. 45:868-78

Enhancement of Anti-Tumor Immunity by CTLA4 Blockade



Treatment of Cancer with Immune Checkpoint Inhibitors

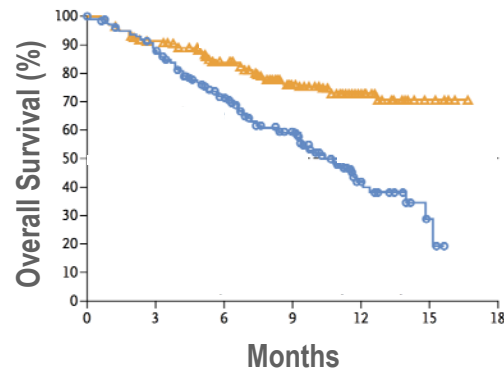
Anti-CTLA4



..... **Melanoma**

Hodi et al. (2010) N Engl J Med **363**:711-23

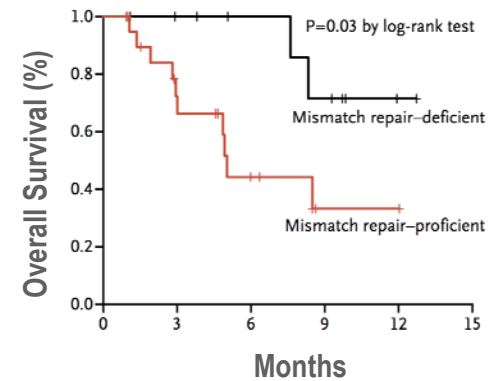
Anti-PD1



..... **Melanoma**

Robert et al. (2015) N Engl J Med **372**:320

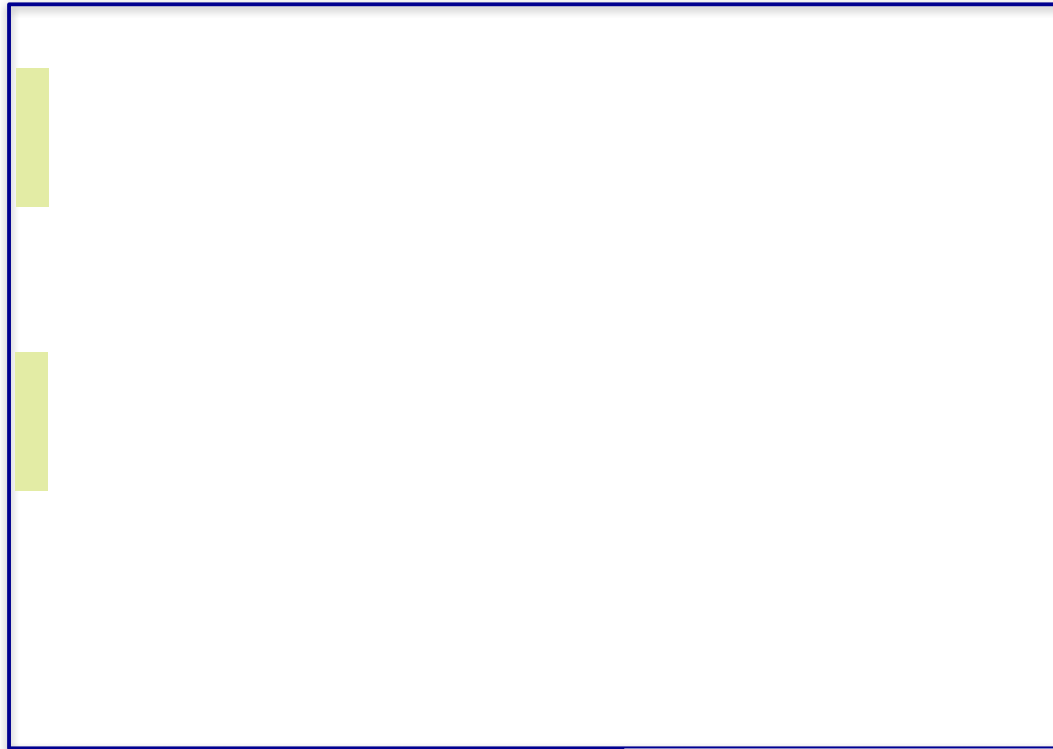
Anti-PD1



MSI^{hi} CRC

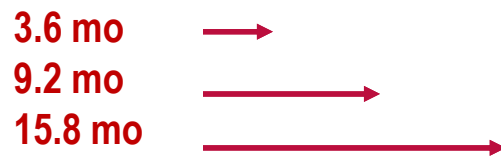
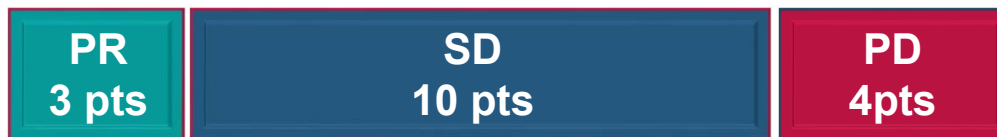
Le et al. (2015) N Engl J Med **372**:2509

Immune checkpoint inhibitors

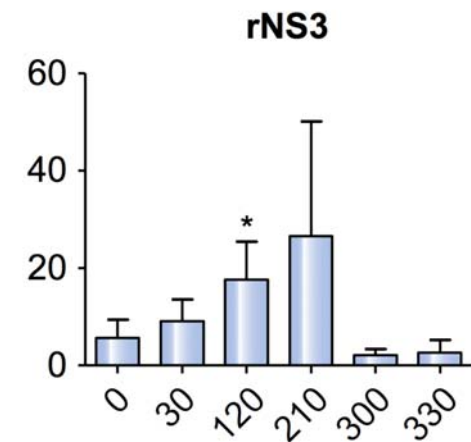


Antiviral and Antitumoral Effects of the Anti-CTLA4 Agent

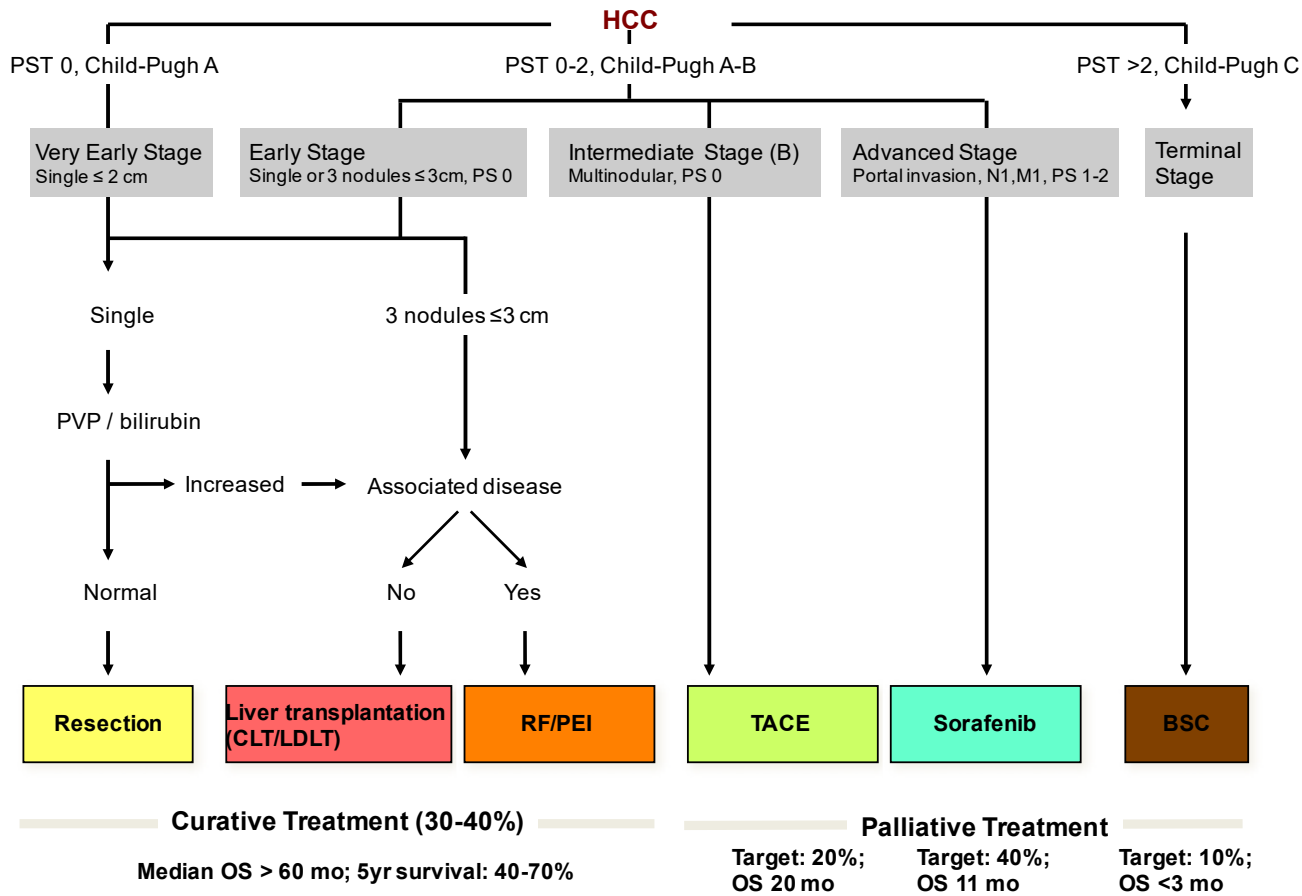
Disease control rate: 76.4%



T cell responses



HCC Management



Curative Treatment (30-40%)

Median OS > 60 mo; 5yr survival: 40-70%

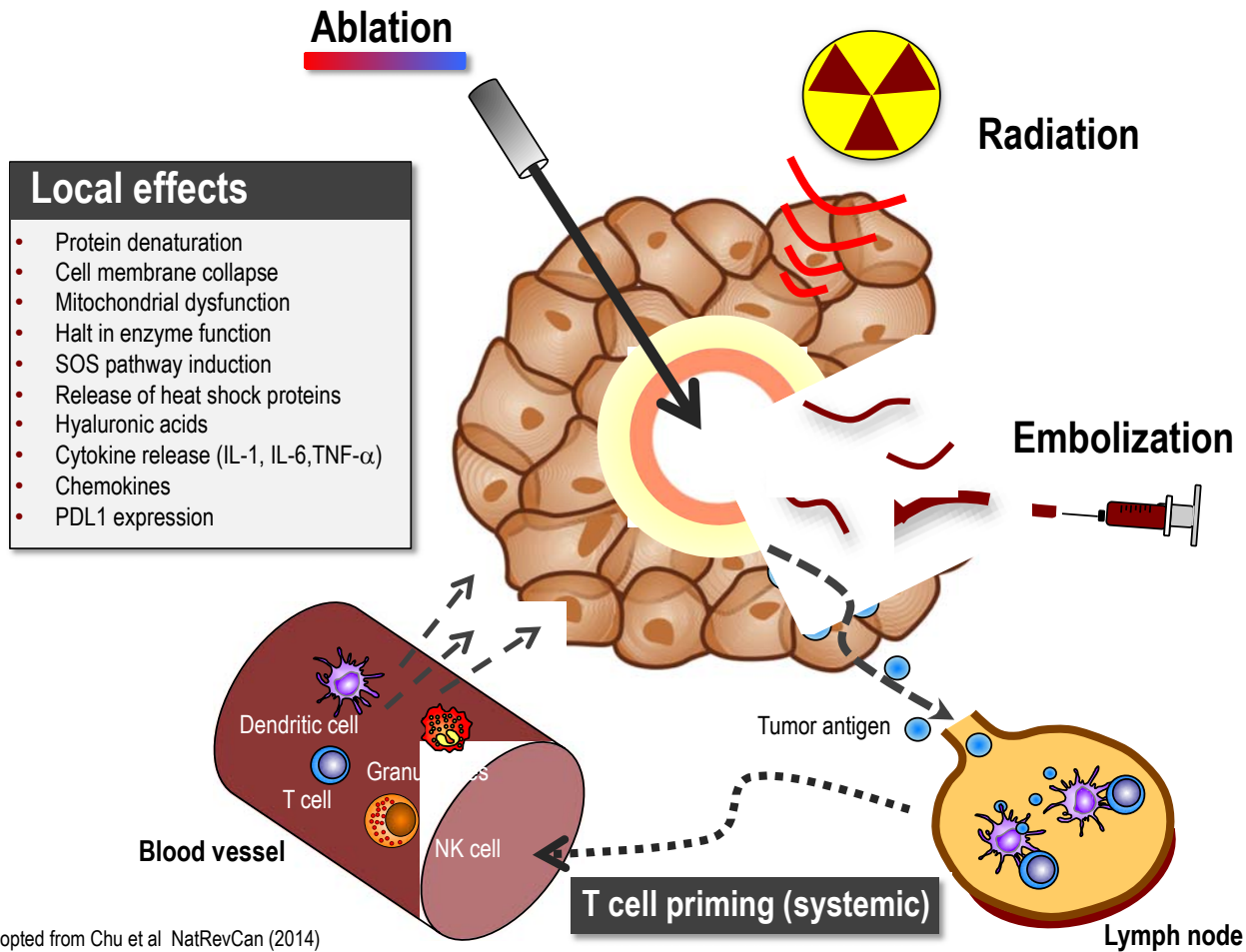
Palliative Treatment

Target: 20%; OS 20 mo

Target: 40%; OS 11 mo

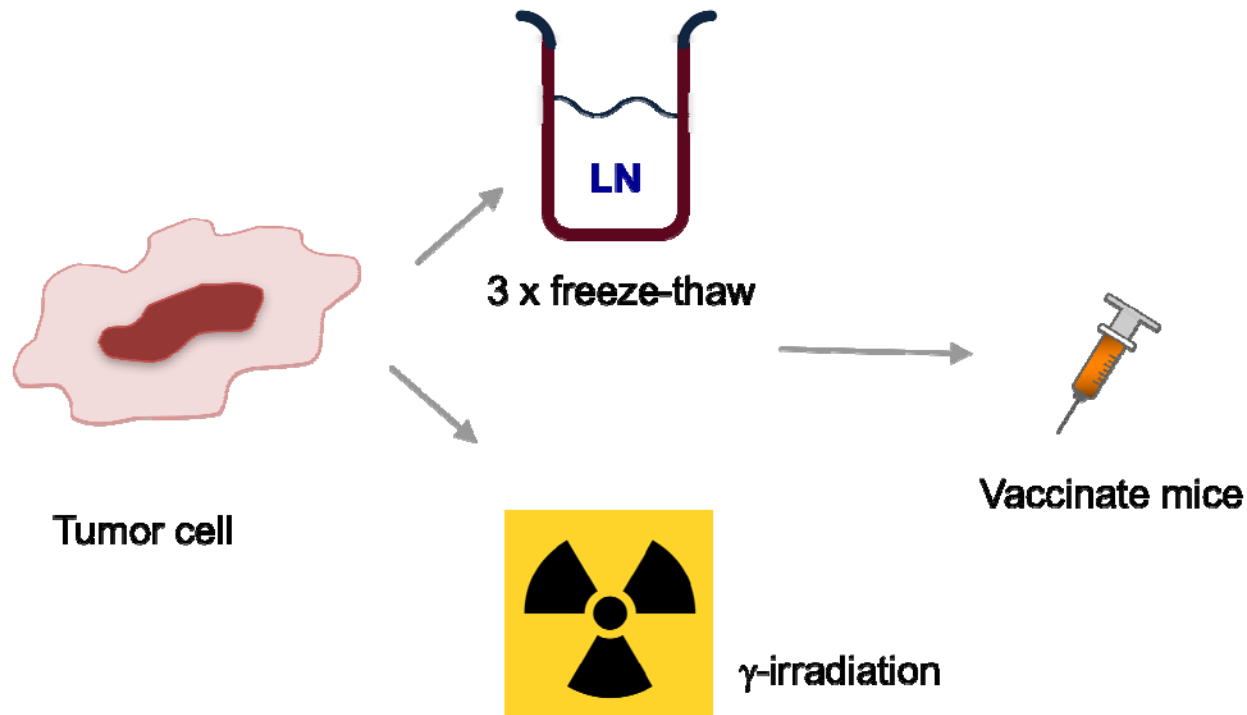
Target: 10%; OS <3 mo

Immunological Effects of Ablative Therapies

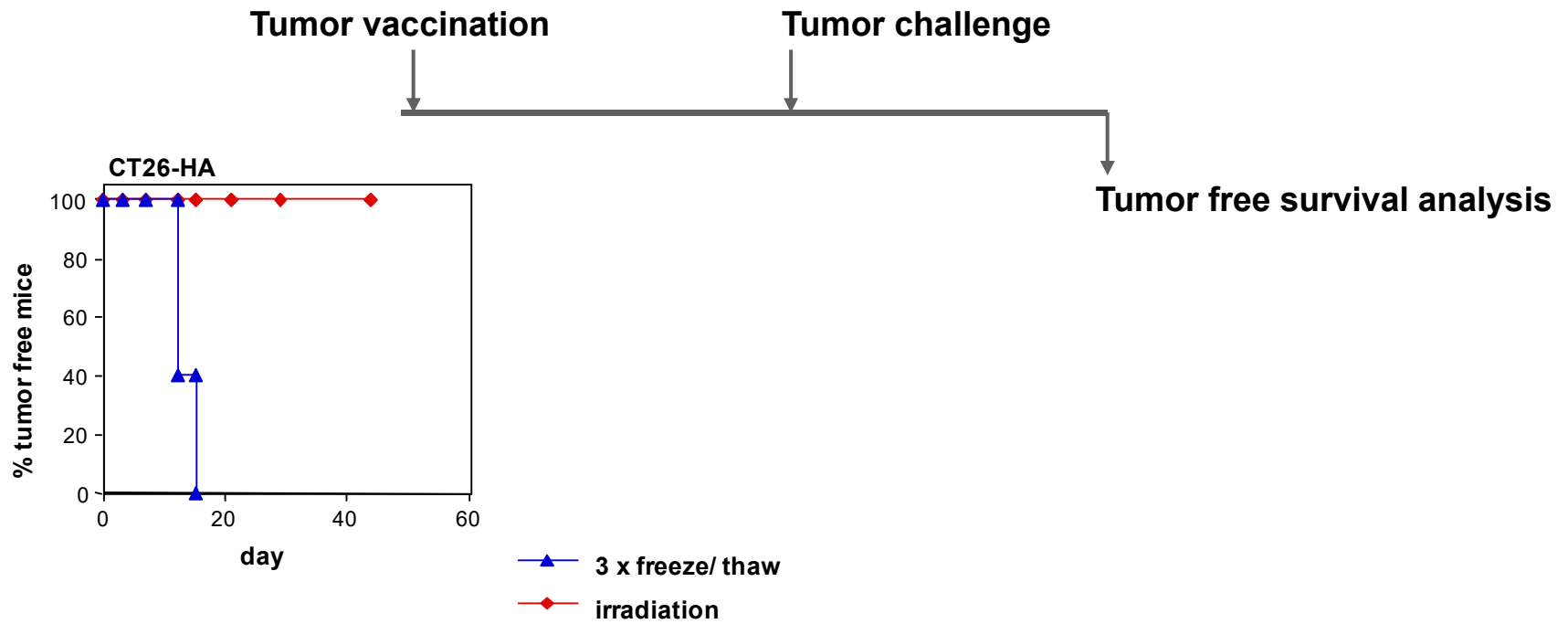


Adopted from Chu et al NatRevCan (2014)

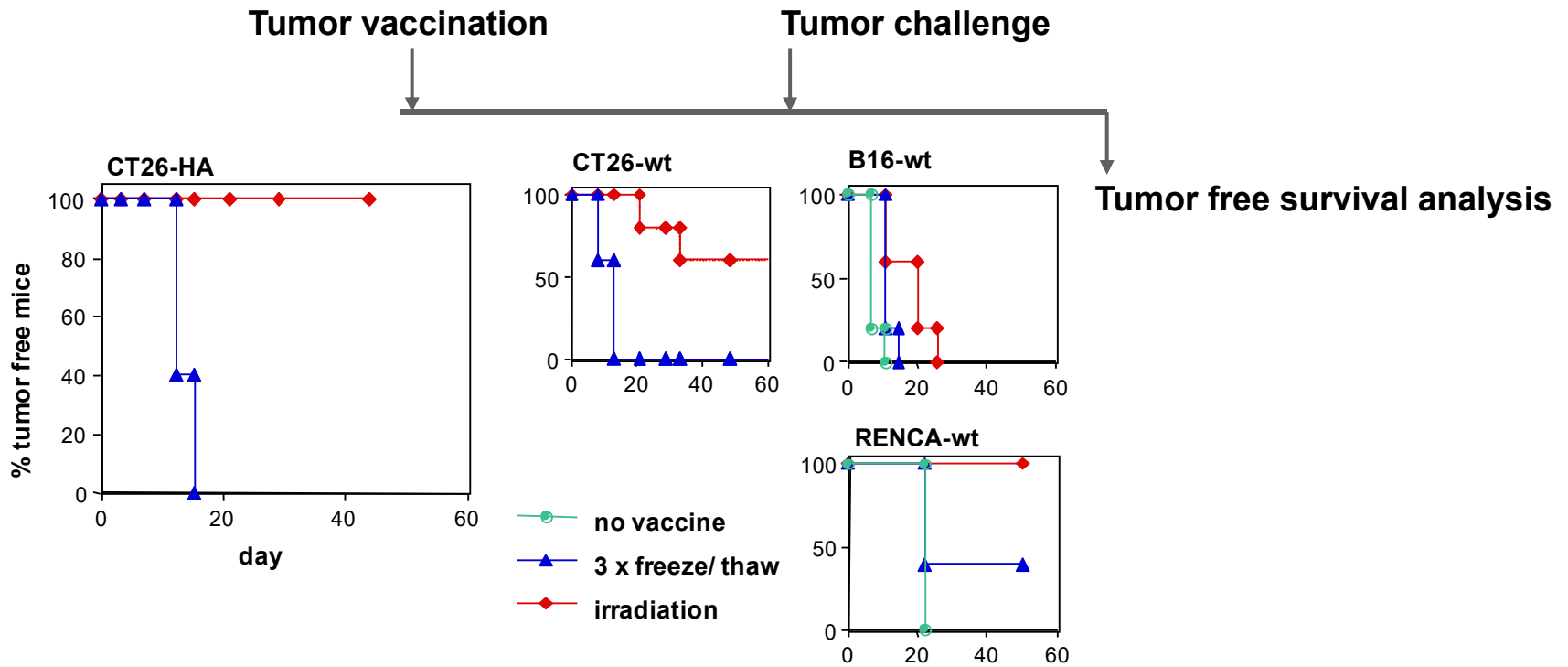
Apoptotic/necrotic tumor cell death



Irradiated vs freeze/thawed tumor cell vaccines

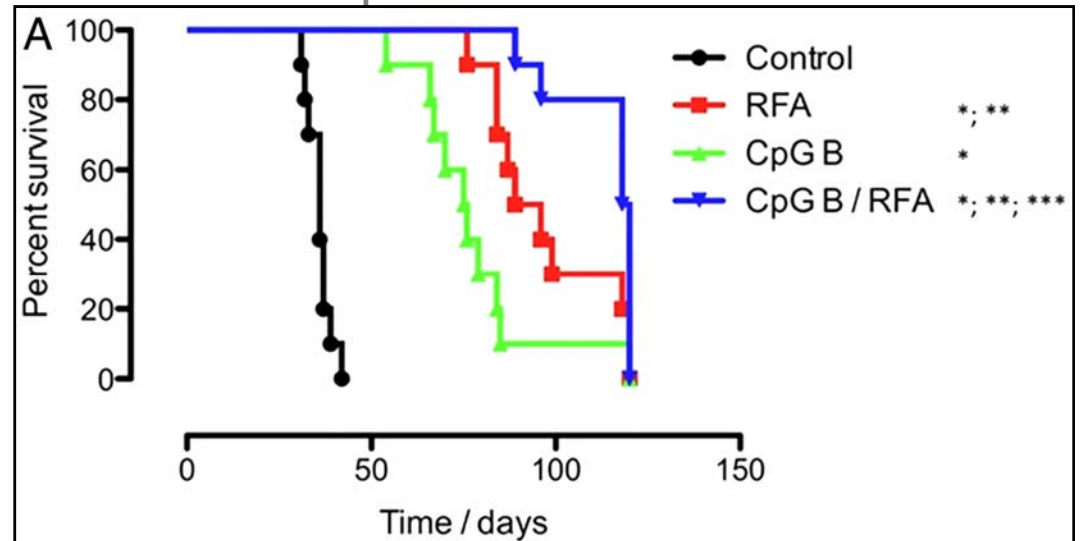
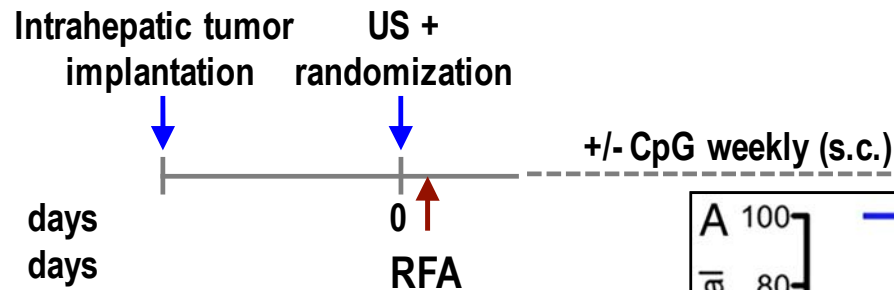


Irradiated vs freeze/thawed tumor cell vaccines

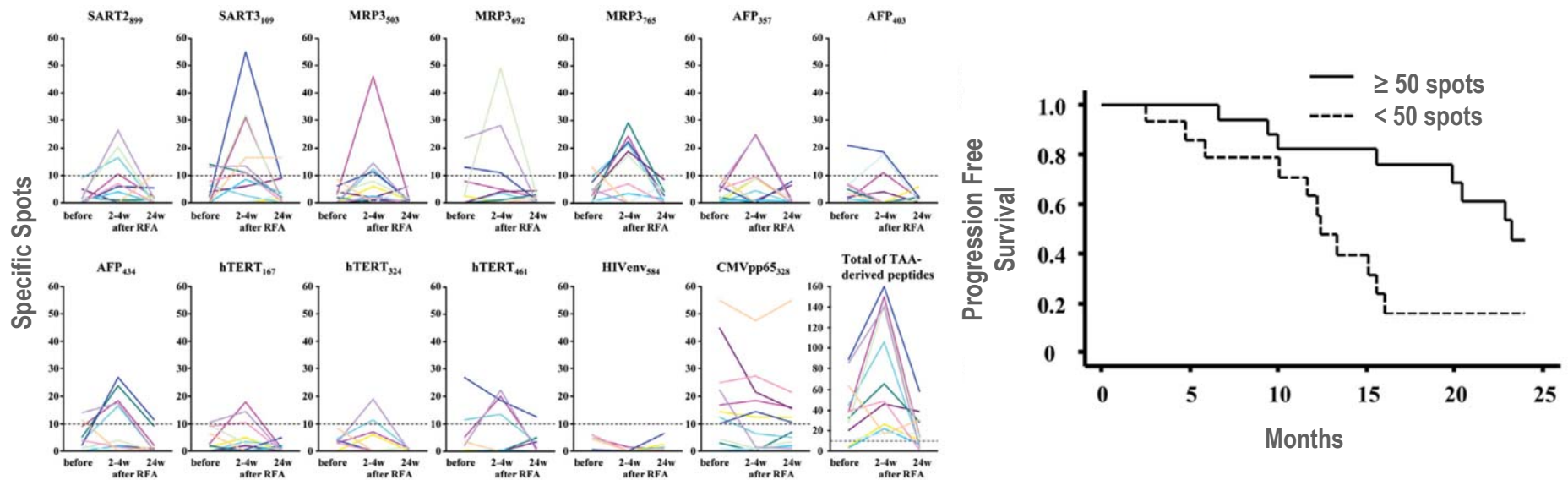


Combined **radiofrequency** ablation and **TLR 9** stimulation in the rabbit VX2 hepatoma model

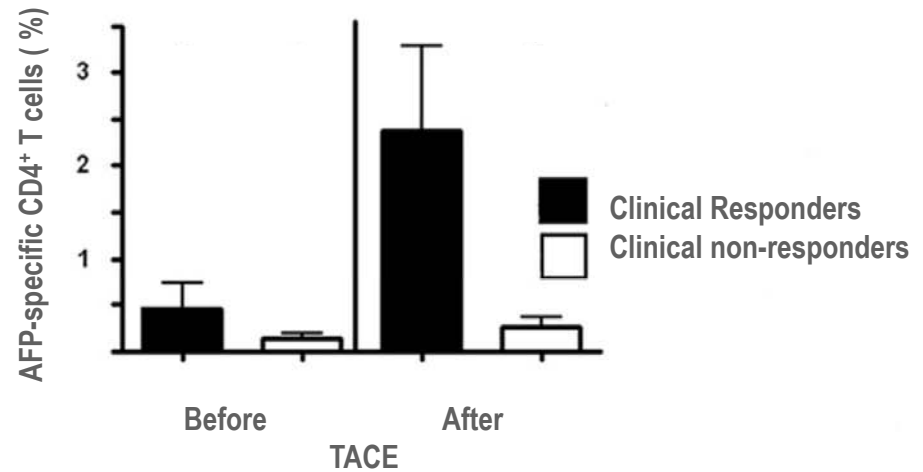
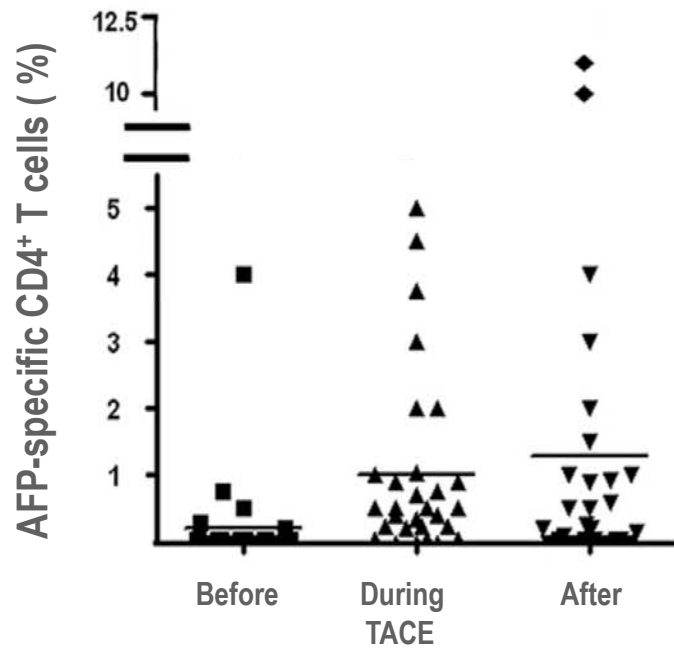
Tumor growth and survival



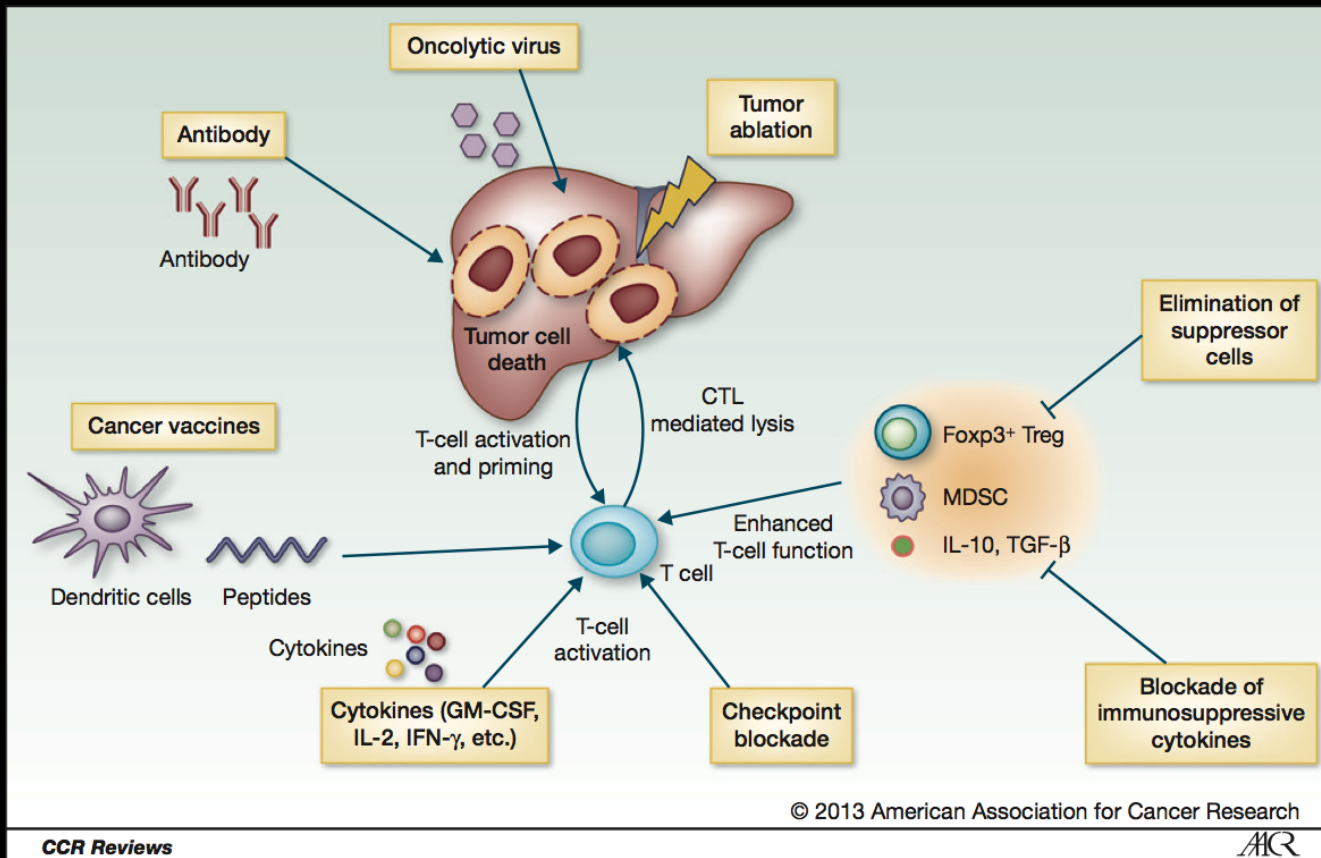
Enhancement of tumor-associated antigen-specific T cell responses by RFA correlates with better survival



TACE induced CD4 T cell responses are associated with better clinical outcome

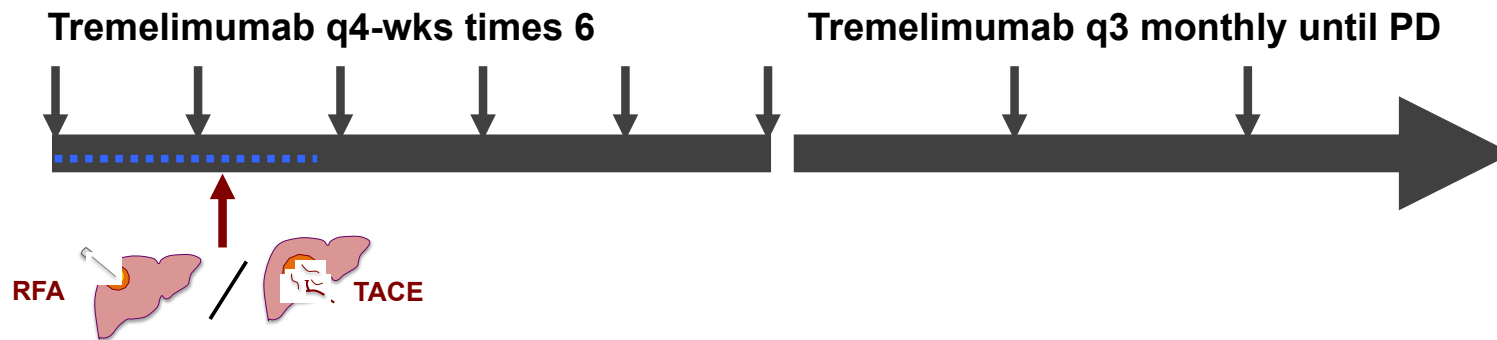


**A phase I/II proof of concept study
evaluating combined locoregional therapy +
anti-CTLA4 (tremelimumab) in HCC**



A Pilot Study of Tremelimumab – A monoclonal antibody against CTLA-4 – in combination with ablation in patients with HCC

Trial design



A Pilot Study of Tremelimumab – A monoclonal antibody against CTLA-4 – in combination with ablation in patients with HCC

Inclusion criteria

- ▶ Biopsy-proven HCC [Childs Pugh A/B7; BCLC Stage (B)/C; ECOG 0/1]
- ▶ Post-sorafenib
- ▶ Tumor biopsies performed at the time of the radiologic procedure.
- ▶ Restaging CT /MRI scan every 8 weeks to evaluate TTP in **non-TACE/RF lesion**.

Patient Characteristics

All(*)	
Number	32 (6/14/12)
Age	
Median (range)	61 (36-76)
Sex	
Male	28 (4/13/11)
Female	4 (2/1/1)
ECOG	
0	8
1	24
Liver Cirrhosis	
Yes	22 (3/11/8)
No	9 (3/2/4)
Cause of Liver disease	
HBV	5 (2/1/2)
HCV	19 (3/11/5)
Baseline Child Pugh Score	
5	14 (2/6/6)
6	5 (1/3/1)
7	3 (-/2/1)

All(*)	
Number of target lesions	
1	5
2	3
3-5	12
>5	8
Extrahepatic disease	
Yes	14 (2/10/2*)
No	17 (4/4/9)
Prior sorafenib	
Yes/no	21/7
D/C'd due to PD/intolerant	18/3
Other systemic therapies	9
Other previous interventions	
TACE	11
Surgery	5
Ablation	5
Reason for discontinuation	
Progressive disease	(5/12/3)
Toxicity	(1/2/1)

Adverse Events

Toxicity	3.5mg/kg (N=6), n		10mg/kg (N=26), n		All patients (N=32), n	
	≥ grade 2	grades 3-4	≥ grade 2	grades 3-4	≥ grade 2	grades 3-4
Hyperbilirubinemia	2	1	5	2	7	3
Aspartate aminotransferase increased	6	4	5	3	11	7
Alanine aminotransferase increased	1	-	5	3	6	3
Pruritus	-	-	3	1	3	1
Rash	3	-	2	-	5	-
Pneumonitis	1	-	-	-	1	-
Colitis	-	-	2	-	2	-
Angioedema	-	-	-	1	-	1
Thyroid dysfunction	-	-	1	1	1	1
Adrenal insufficiency	-	-	-	1	-	1
Discontinued due to toxicity*	1/6		3/25		4 (13%)	

Skin Reaction



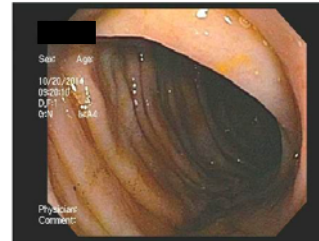
Mild colitis



1 cecum with appendiceal orifice



2 terminal ileum



3 ascending colon



4 transverse colon



5 rectal granular mucosa



6 rectal granular mucosa



7 rectum on retroflexion

Impression:

- Non-thrombosed external hemorrhoids found on perianal exam.
- Granularity in the rectum and in the sigmoid colon. Biopsied.

Mild colitis

10/20/2014 15:18 Surgical Pathology

Surgical Pathology

CASE NUMBER:

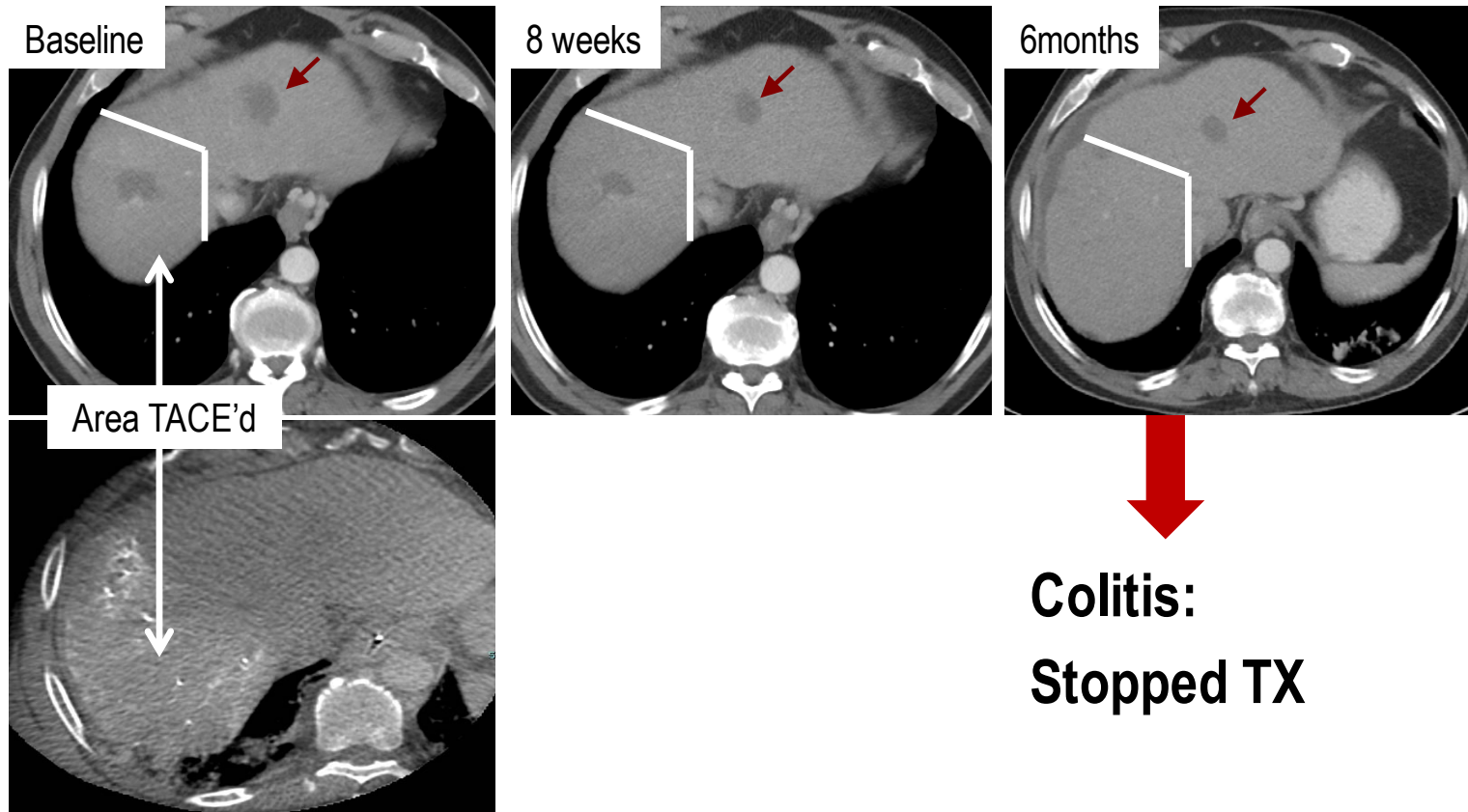
DIAGNOSIS:

1. Ileum, terminal, biopsy: Small bowel mucosa with mild inflammation
2. Colon, ascending, biopsy: Lymphocytic colitis with active colitis. See note.
3. Colon, transverse, biopsy: Lymphocytic colitis with active colitis. See note.
4. Colon, descending, biopsy: Lymphocytic colitis with active colitis. See note.
5. Colon, sigmoid, biopsy: Lymphocytic colitis with active colitis. See note.
6. Rectum, biopsy: Active proctitis.

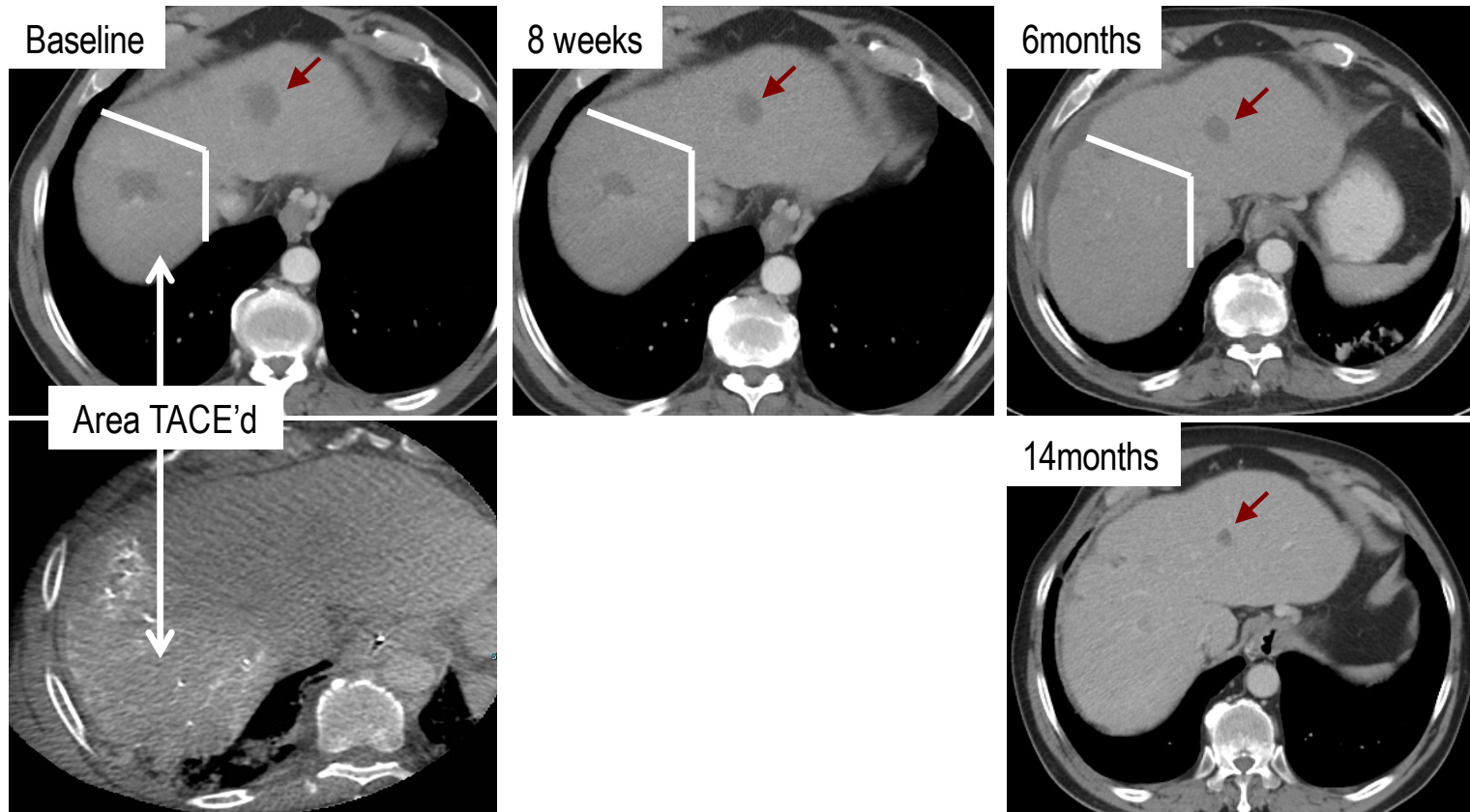
NOTE: Immunohistochemistry stains (CD3 and CD8) are performed on specimens # 2, 3 and 5. CD8 stain highlights an increased number of T cells in the colonic epithelium and the lamina propria. The CD3 stain was not contributory because of poor technical quality.
Case reviewed by Dr. David Kleiner.

Case 1: 60yr old male; HBV; BCLC B; multifocal HCC s/p RFAx2

Case 1: 60yr old male; HBV; BCLC B; multifocal HCC s/p RFAx2

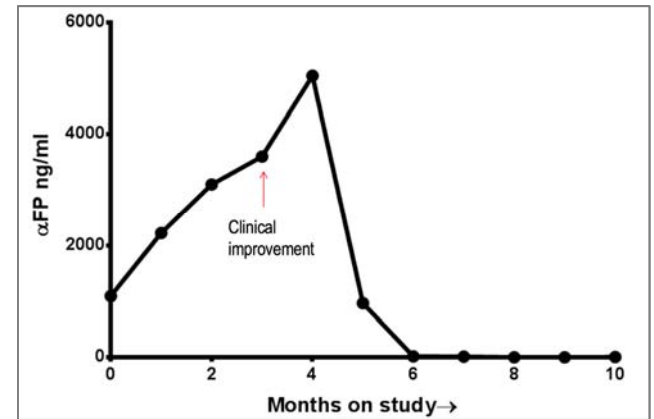
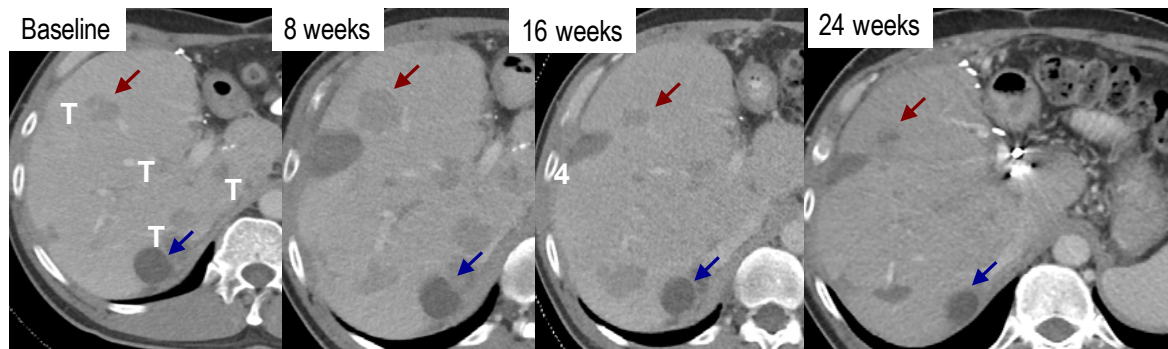
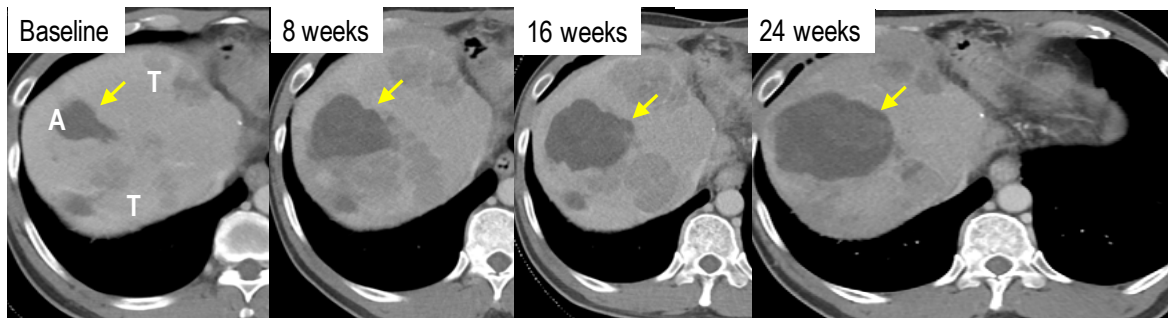


Case 1: 60yr old male; HBV; BCLC B; multifocal HCC s/p RFAx2

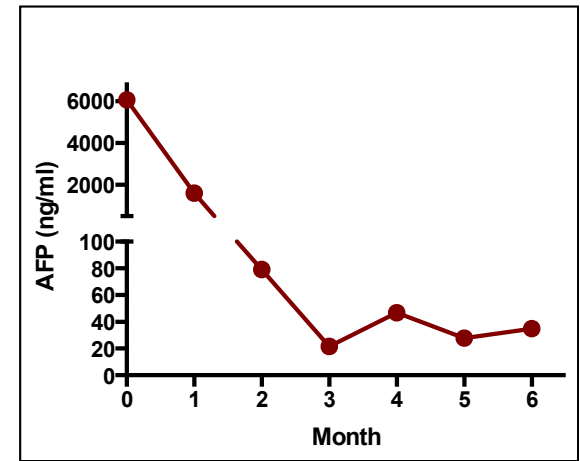
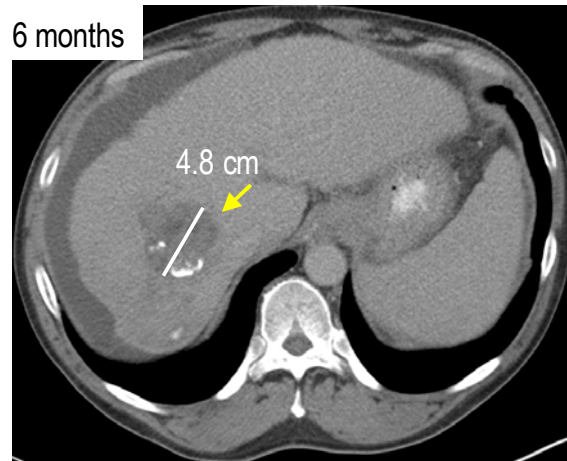
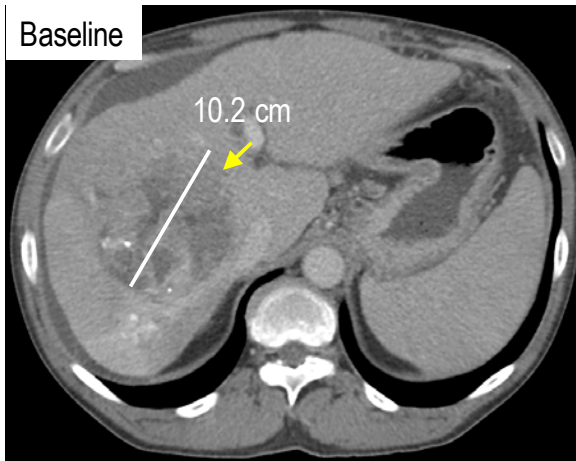


Case 2: 54yr old male; HBV; BCLC C; multifocal HCC s/p 2x part. Hepatectomy, 3x TACE, 1 x Y-90, sorafenib, GemOx, FOLFOX, Avastin+erlotinib

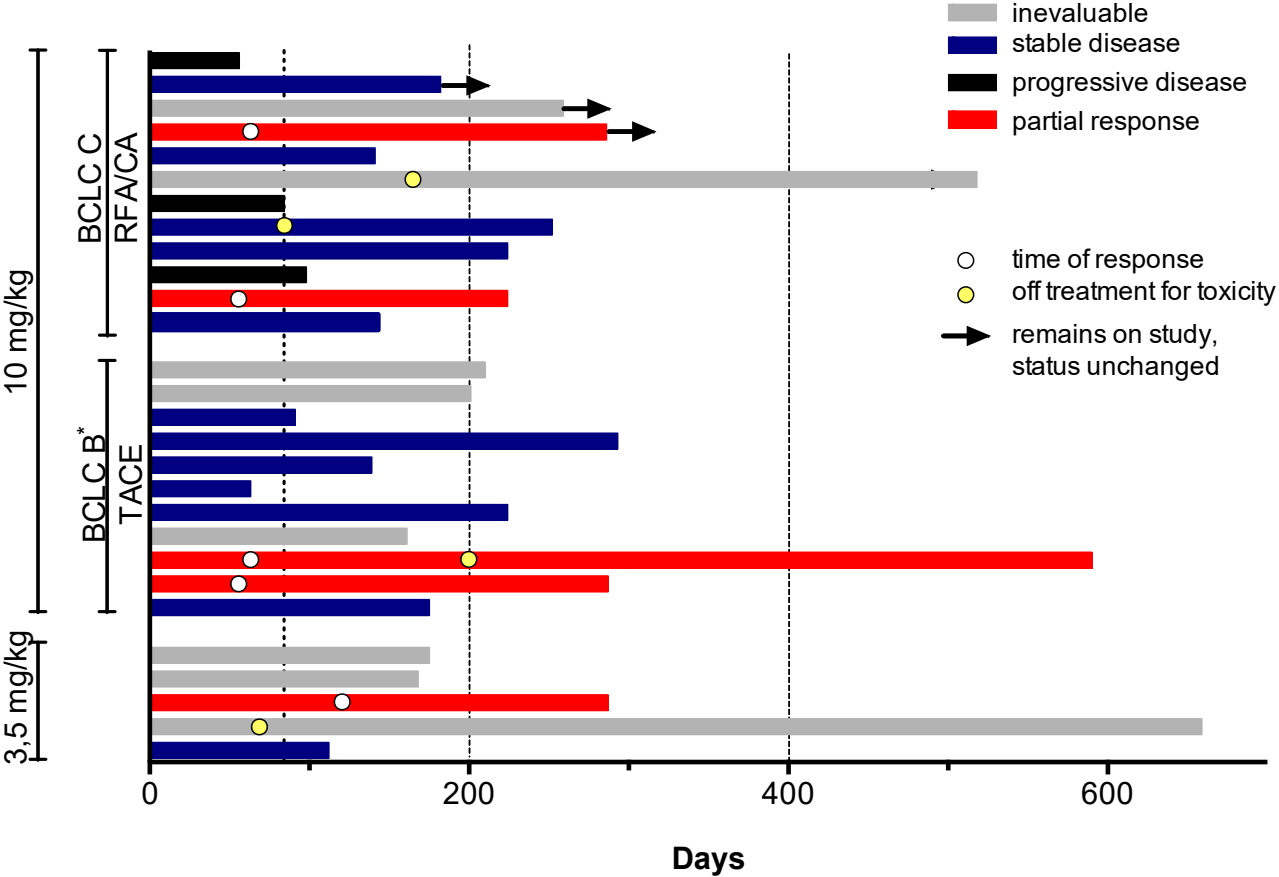
Case 2: 54yr old male; HBV; BCLC C; multifocal HCC s/p 2x part. Hepatectomy, 3x TACE, 1 x Y-90, sorafenib, GemOx, FOLFOX, Avastin+erlotinib



Case 3: 57yr old male; old male; HBV; BCLC B; 3x TACE

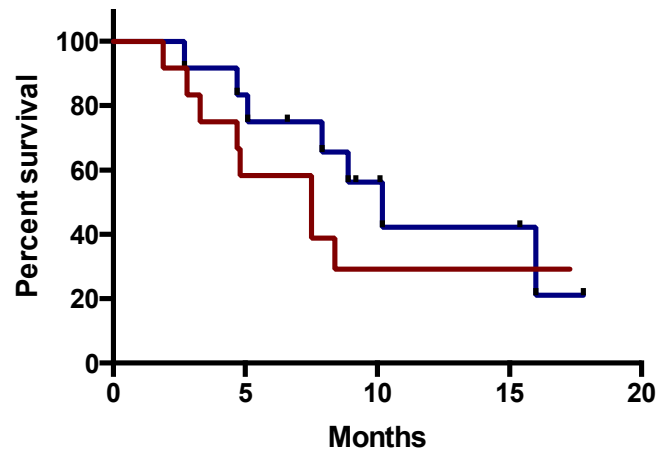


Efficacy



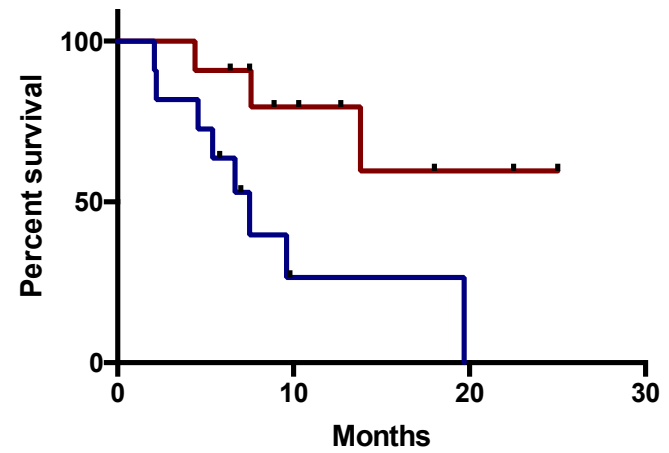
Survival analysis

Ablation



— TTP
— OS

TACE

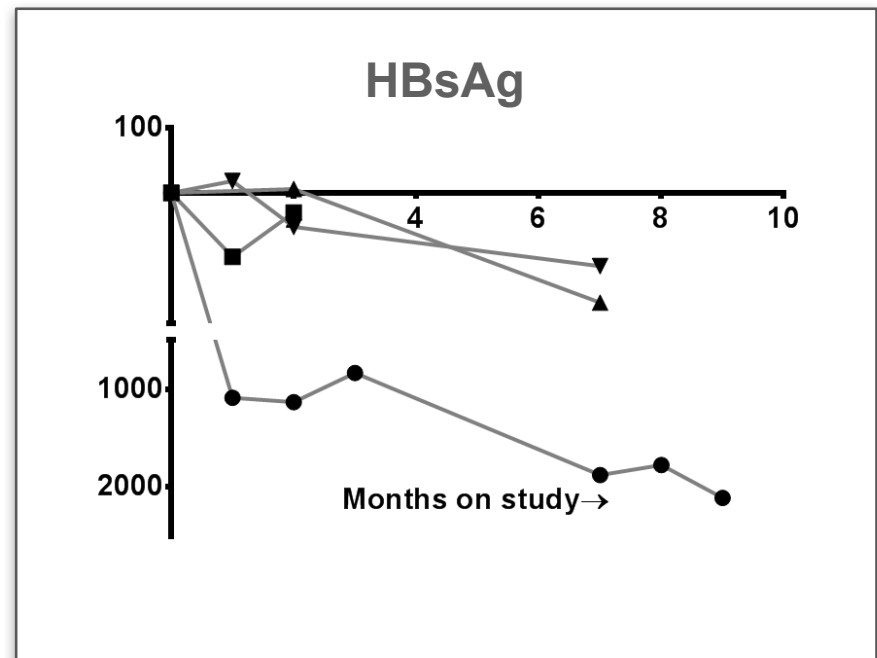
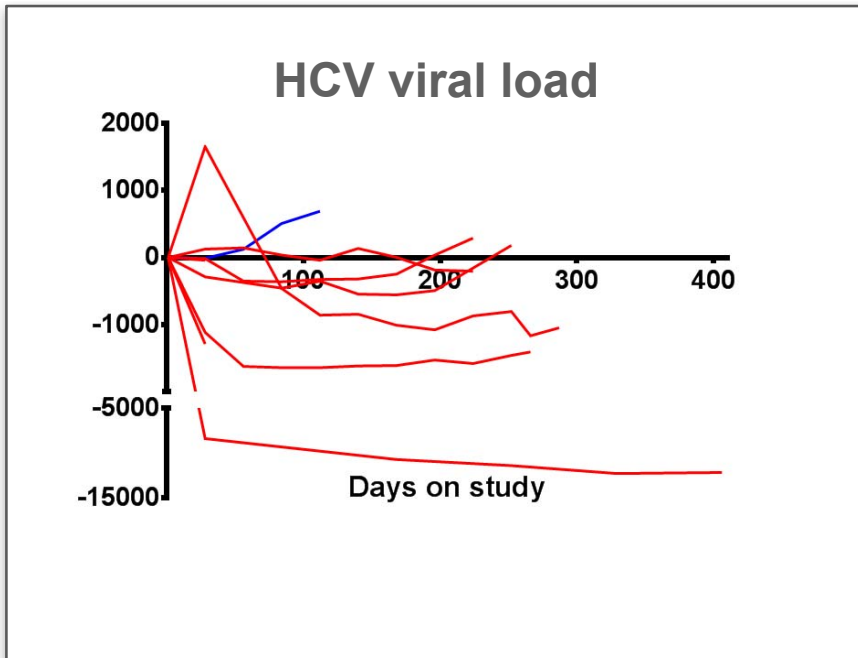


— TTP
— OS

Summary of Efficacy

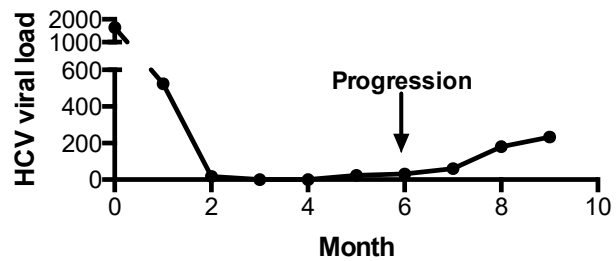
	Median TTP	6-month TTP	12-month TTP	Median OS	6-month survival	12-month survival
Ablation (n= 12)	7.4 months	58.3%	29.2%	10.1 months	75.0%	41.0%
TACE (n= 11)	7.4 months	63.6%	26.5%	NR	81.8%	70.1%
Total population	7.4 months	60.9%	25.1%	13.6 months	78.3%	54.0%

Viral Immunity

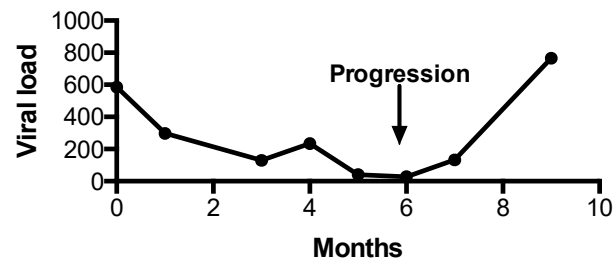


Viral Immunity and Treatment Response

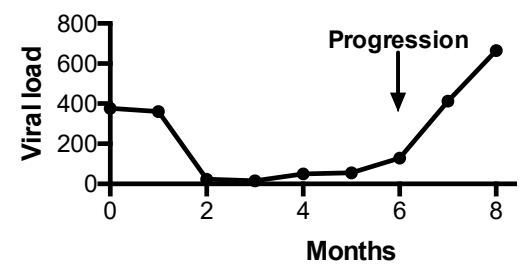
Patient A



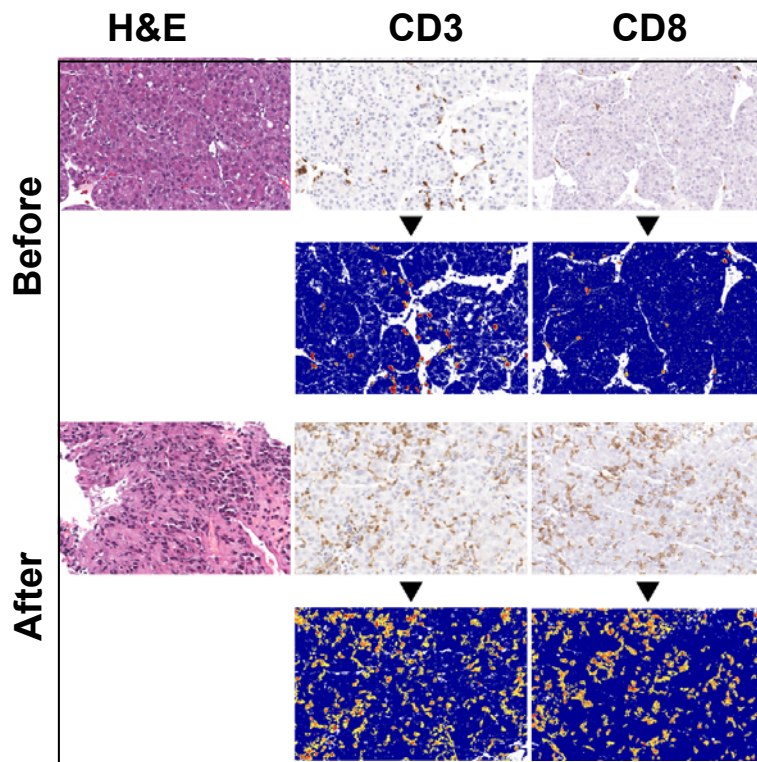
Patient B



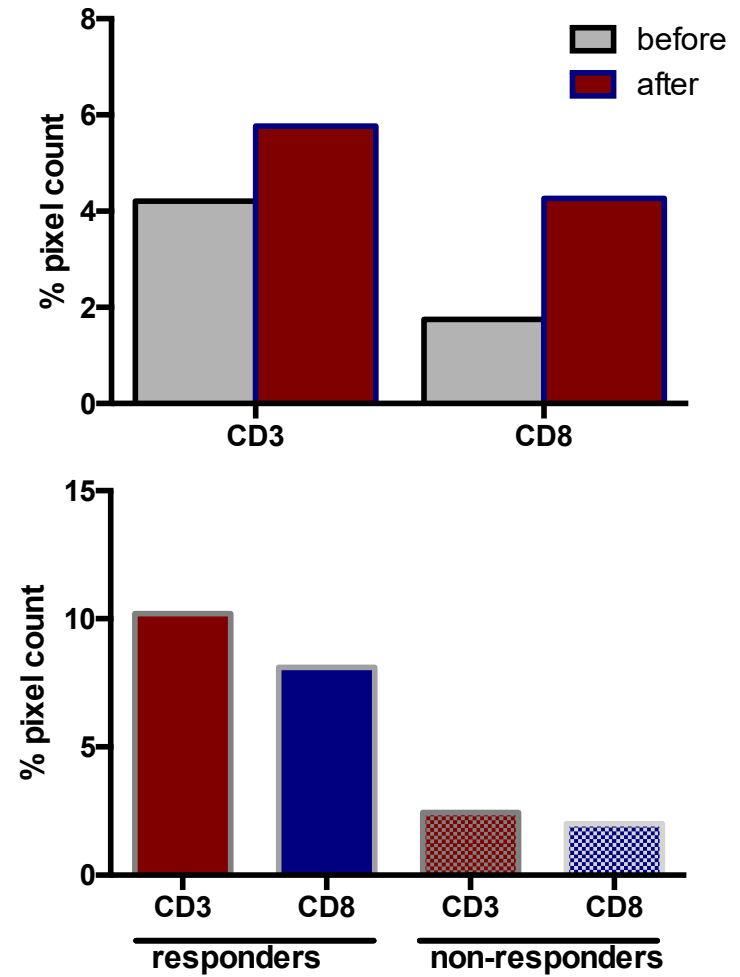
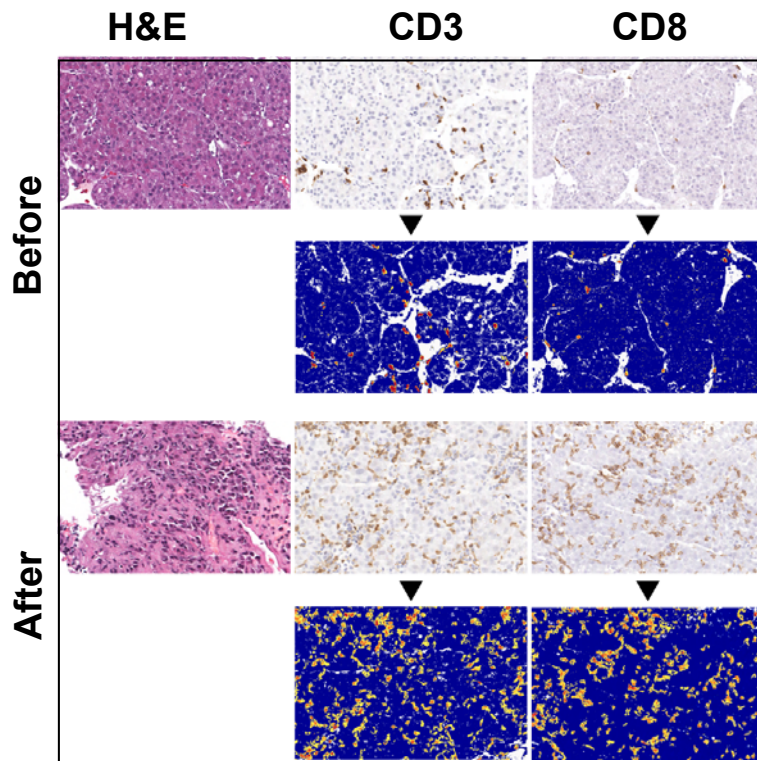
Patient C



Tumor biopsies



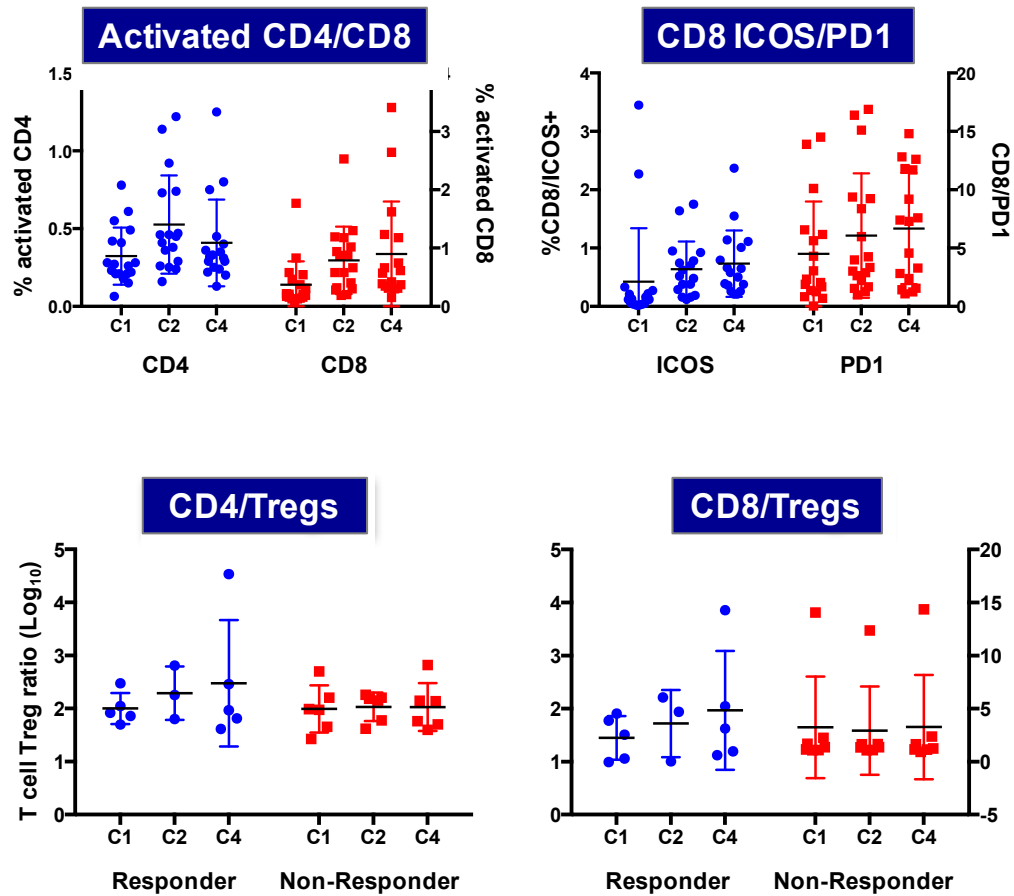
Tumor biopsies



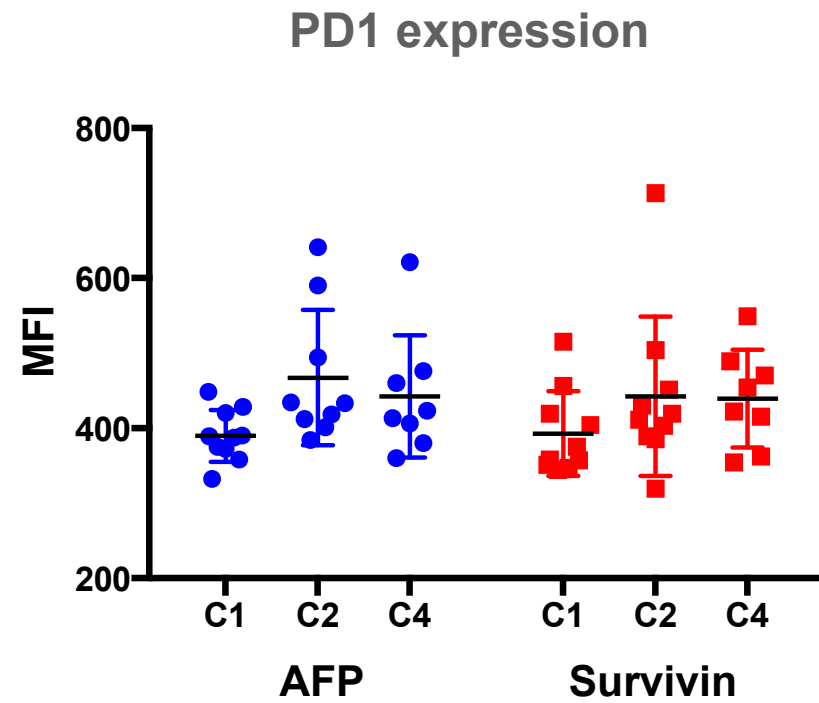
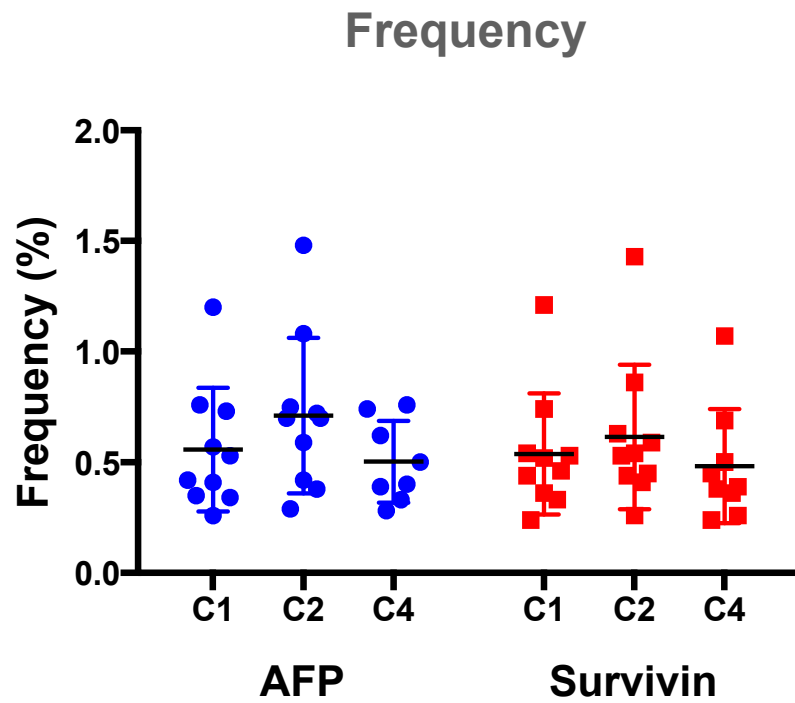
Immune monitoring

Population	Phenotype	Population	Phenotype
Treg CD4	CD3+CD4+CCR4+CD25+CD127low	Central memory CD4+ Tcells	CD4+CCR7+CD45RA-
	CD4+	Naïve CD4+ Tcells	CD4+CCR7+CD45RA+
	CD4+4-1BB+	Effector CD4+ T cells	CD4+CCR7-CD45RA+
	CD4+PD-1+	Effector memory CD4+ Tcells	CD4+CCR7-CD45RA-
	CD4+PD-L1+	Central memory CD8+ Tcells	CD8+CCR7+CD45RA-
	CD4+TIM3+	Naïve CD8+ Tcells	CD8+CCR7+CD45RA+
	CD4+CTLA4+	Effector CD8+ T cells	CD8+CCR7-CD45RA+
	CD4+ICOS+	Effector memory CD8+ Tcells	CD8+CCR7-CD45RA-
	CD4+IL-T2+	Activated CD4+ cells	CD3+CD4+CD38+HLADR+
	CD4+IFNg+	Activated CD8+ cells	CD3+CD8+CD38+HLADR+
	CD4+IL-2+	Th1 cells	CD3+CD4+CXCR3+CCR6-
	CD4+TNFa+	Th2 cells	CD3+CD4+CXCR3-CCR6-
	CD4+Ki67+	Th17 cells	CD3+CD4+CXCR3-CCR6+
CD8	CD8+	B cells	CD19+
	CD8+4-1BB+	Naïve B cells	CD19+CD27-
	CD8+PD-1+	Plasmablast	CD19+CD27+CD20-CD38+
	CD8+PD-L1+	Monocytes	CD19-CD14+
			CD19-CD14-CD20-HLADR +CD123+
	CD8+TIM3+	Plasmacytoid DC	CD19-CD14-CD20-HLADR +CD11c+
	CD8+CTLA4+	Myeloid DC	
	CD8+ICOS+	NK cells	CD19-CD14-CD20-CD56hi/low
	CD8+IL-T2+	MDSC	HLADR-CD14+
	CD8+IFNg+		CD14-CD15+CD33+CD11b+
	CD8+IL-2+	CD8+/Treg	ratio
	CD8+TNFa+	CD8+/MDSC	ratio
	CD8+Ki67+		

Analysis of peripheral T cells



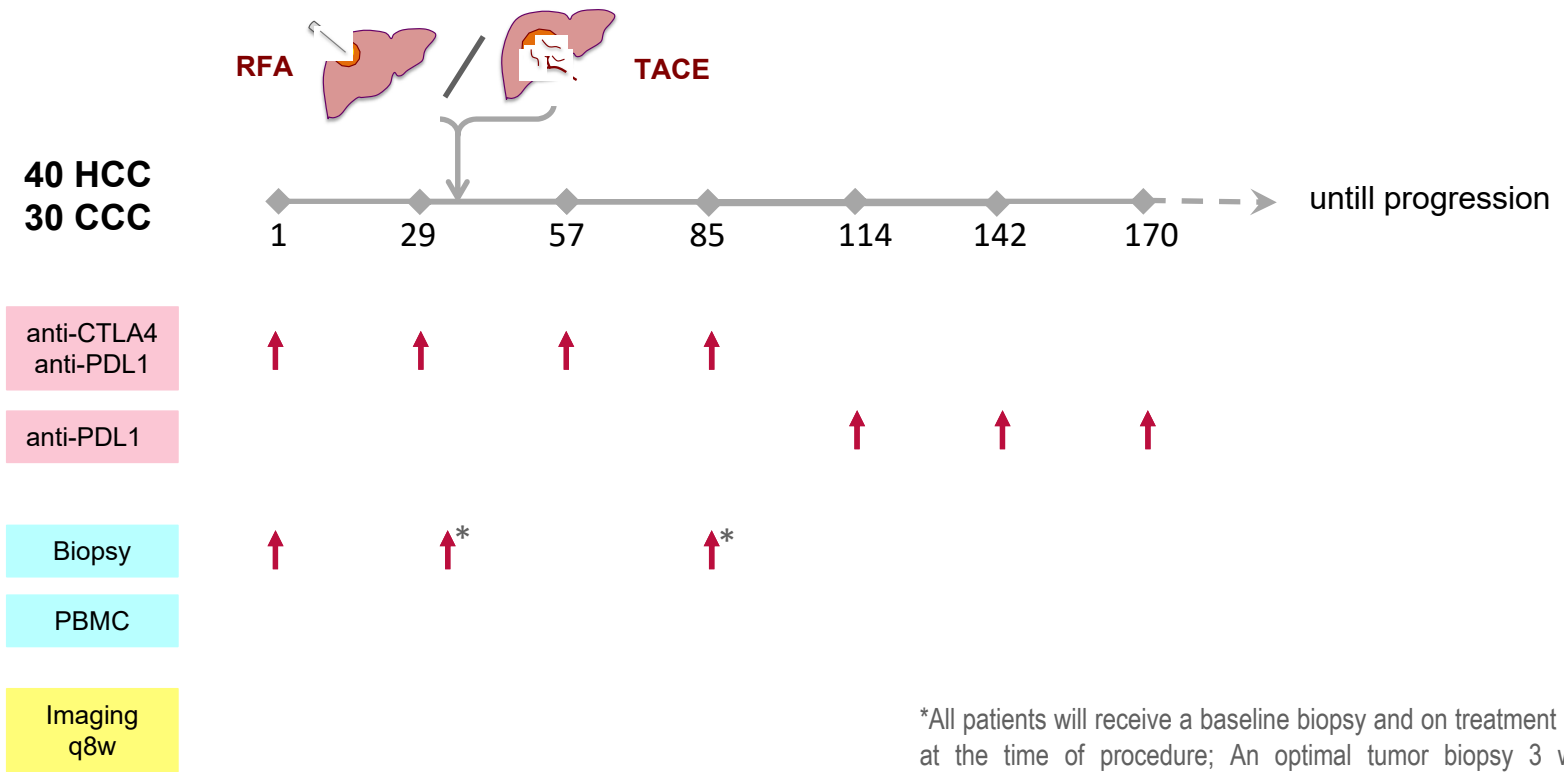
Tumor-specific T cell responses



Summary

- ▶ Combination of tumor ablation and anti-CTLA4 therapy is feasible.
- ▶ The treatment is safe.
- ▶ Immune correlates suggest an activation of tumor virus-specific immune responses.
- ▶ Anti-CTLA4 therapy leads to infiltration of CD8+ T cells in the tumor of responding patients.

A Pilot study of combined anti-CTLA4 + anti-PDL1 in combination with locoregional therapy in subjects with HCC and CCC



*All patients will receive a baseline biopsy and on treatment biopsy at the time of procedure; An optimal tumor biopsy 3 will be requested on day 85.

Immune checkpoint inhibitors in HCC

Treatment	#	BCLC (A/B/C)	Therapy line	Responses	Survival
Tremelimumab 30 mg q 3 months	21	3/6/12	Not amenable to ablative therapies	3/17 PR (3.6, 9.2, 15.8 mo) 76.4% DCR	TTP 6.48 months OS 8.2 months
Tremelimumab 10 mg q 28 days + Ablation	32	-/7/21	BCLC 2 Progressed on sorafenib	5/19 PR 84.2% DCR	TTP 7.4 months OS 12.3 months
Nivolumab 3mg/kg	206	C	Sora naïve/tolerant Progressed on sorafenib	9% ORR 68 of 174 evaluable pts (39%) had a decline in tumor burden	6 mo OS: 69%

Sangro et al. Hepatol. 2013; Duffy et al J.Hepatol. 2016, Sangro et al. ILCA 2016

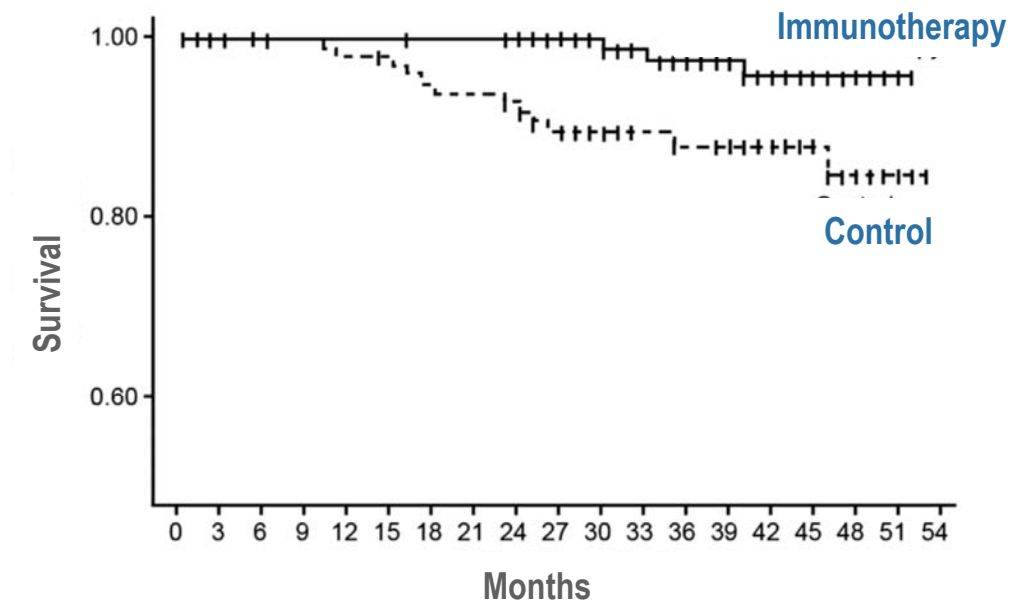
Ongoing and future immunotherapy trials in patients with HCC

Ongoing immunotherapy trials in HCC

- ▶ Immune checkpoint inhibitors
- ▶ Cytokine activated killer cells
- ▶ CAR T cells
- ▶ Antibodies
- ▶ Oncolytic viruses
- ▶ Vaccines

Ongoing immunotherapy trials in HCC

- ▶ Immune checkpoint inhibitors
- ▶ **Cytokine activated killer cells**
- ▶ CAR T cells
- ▶ Antibodies
- ▶ Oncolytic viruses
- ▶ Vaccines



Glypican 3

- ▶ Peptide vaccine
- ▶ Antibody
- ▶ CAR T cells
- ▶ Antibody fusion
- ▶ TCR transduced T cells

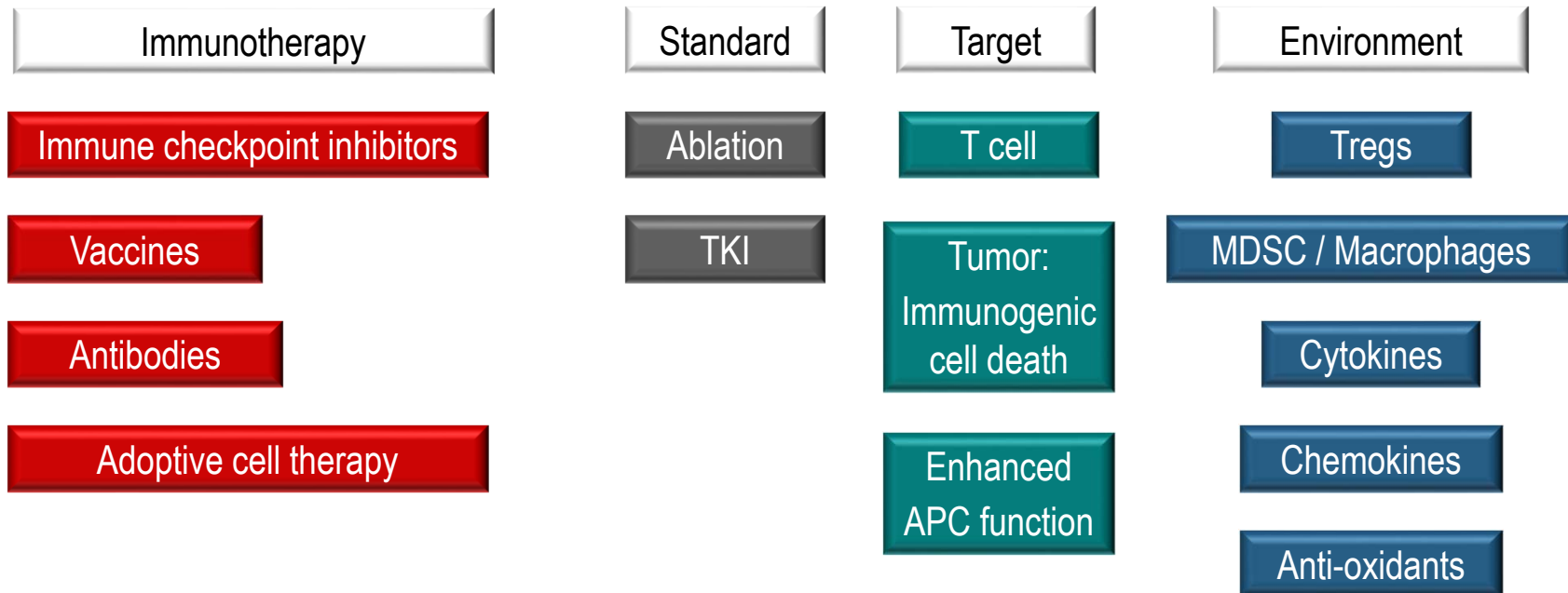
Ongoing clinical trials

Treatment	#	Therapy line	Enrollment start date
Nivolumab (anti PD1) vs sora	726	1 st line	11/2015
PexaVecc + sora vs sora	600	1 st line	10/2015
Pembrolizumab (anti-PD1)	408	2 nd line	5/2016
MEDI4736 (anti-PDL1+tremelimumab)	144	2 nd line	10/2015

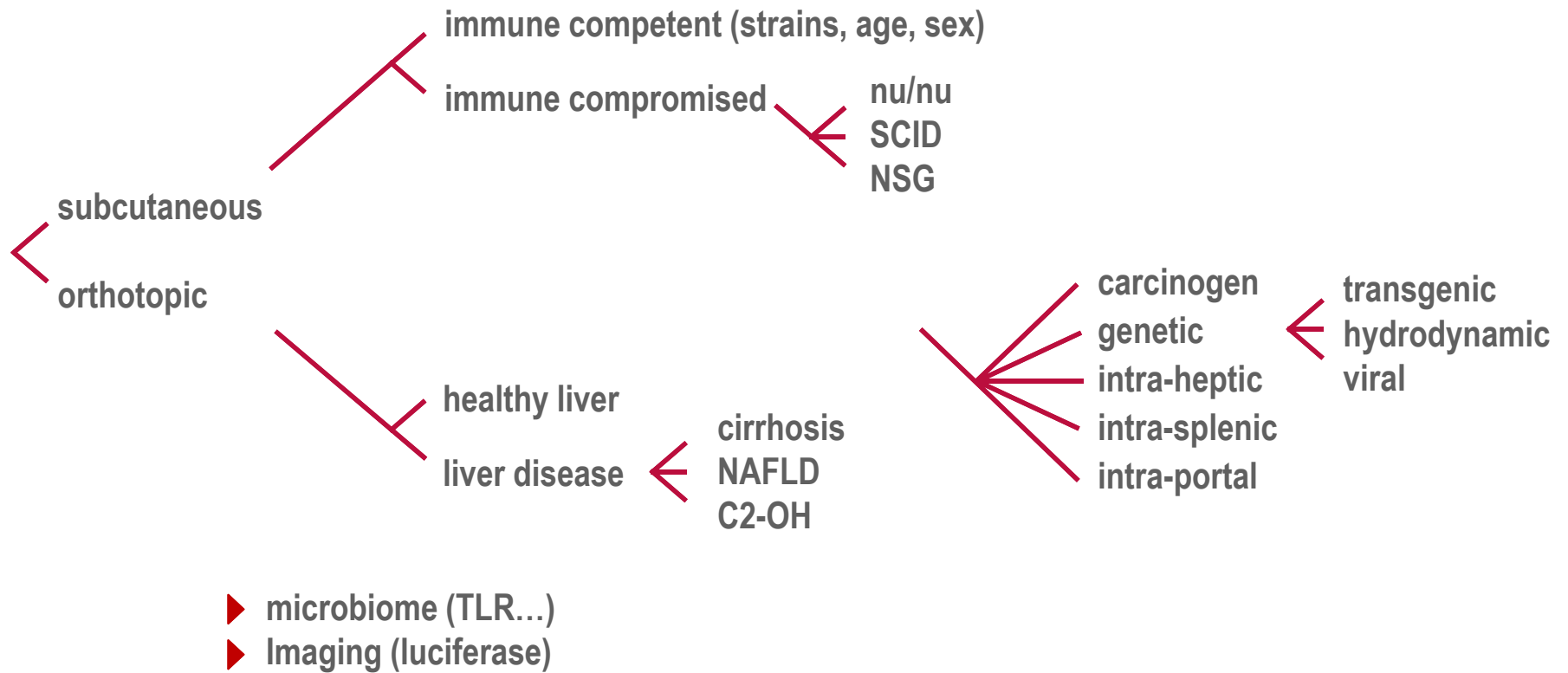
Future immunotherapy trials



Future immunotherapy trials



Which is the best animal model for immunotherapy?



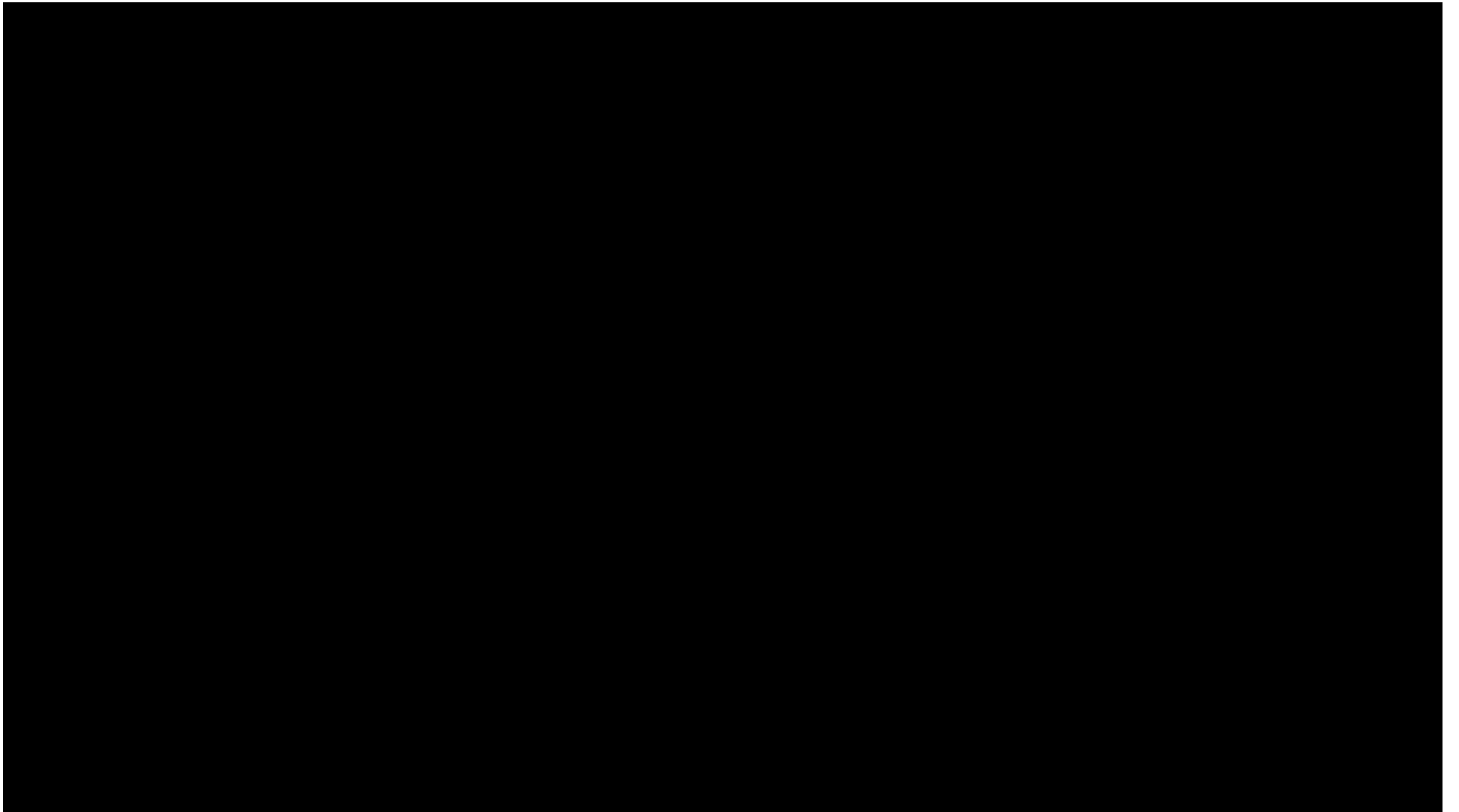
Immune correlatives

Tumor

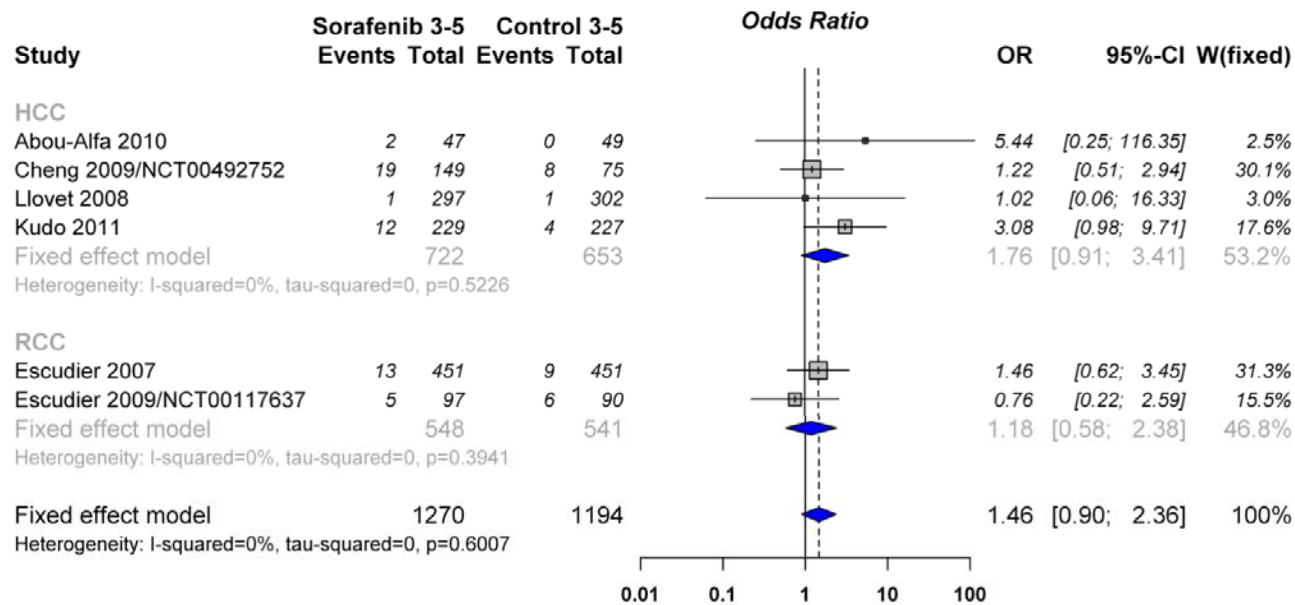
- ▶ Immune infiltrate
- ▶ PDL1 expression
- ▶ Mutational load

Peripheral blood

- ▶ Antigen-specific T cells
- ▶ Activation markers
- ▶ Viral responses
- ▶ Cytokines
- ▶ Suppressor cell populations
- ▶ T cell function



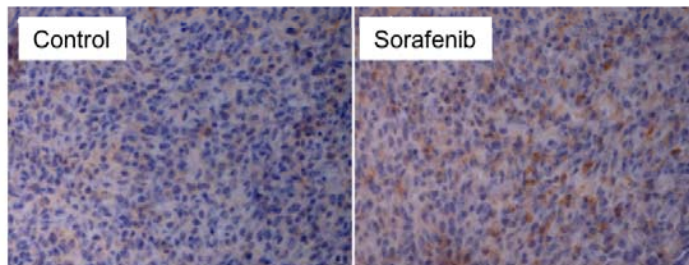
Hemorrhagic Events in Hepatocellular Carcinoma Patients Treated With Antiangiogenic Therapies



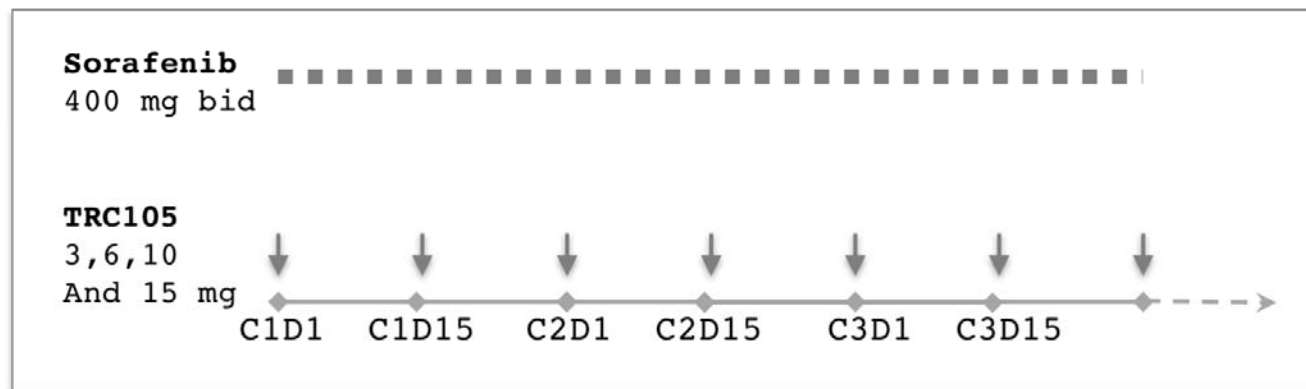
A Phase 1/2 Study Of TRC105 In Combination With Sorafenib In HCC

- ▶ CD105 (endoglin) is expressed in the vascular endothelial cells of HCC tissue ¹
- ▶ VEGF inhibition leads to increased expression of CD105 ²
- ▶ HCC patients with CD105^{hi} tumors have a worse outcome after resection ³

CD105 expression in murine HCC



A Phase 1/2 Study Of TRC105 In Combination With Sorafenib In HCC



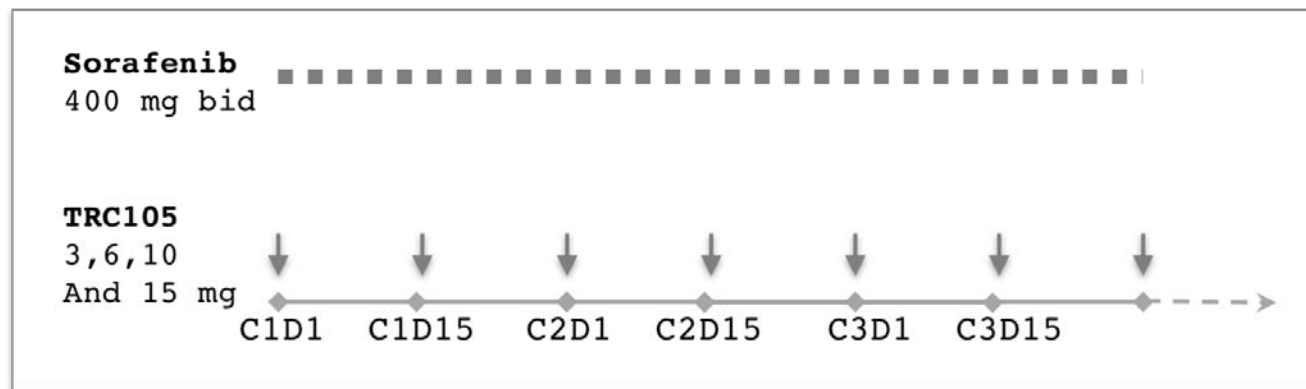
Inclusion Criteria

- ▶ Child Pugh A/B7
- ▶ Platelet >60
- ▶ ALT/AST < x10 ULN
- ▶ Mandatory endoscopy if cirrhosis

Safety and dose

- ▶ Well tolerated
- ▶ no unexpected toxicity
- ▶ Phase II dose 15 mg

A Phase 1/2 Study Of TRC105 In Combination With Sorafenib In HCC



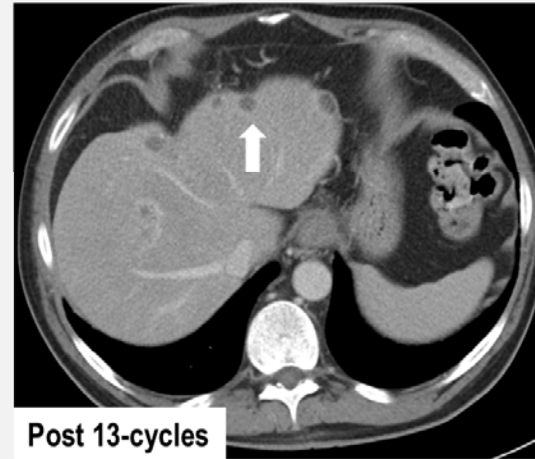
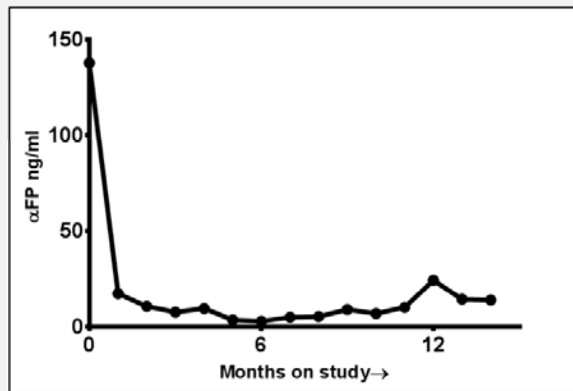
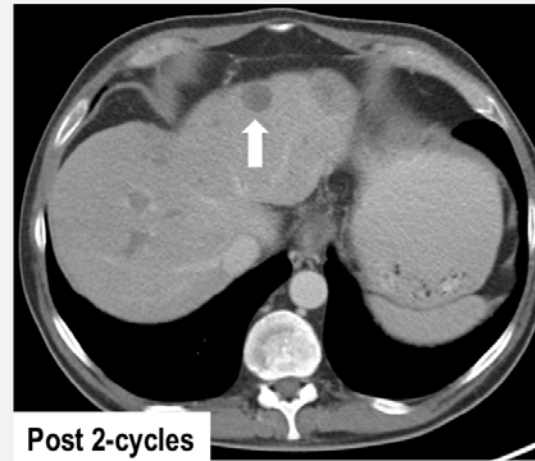
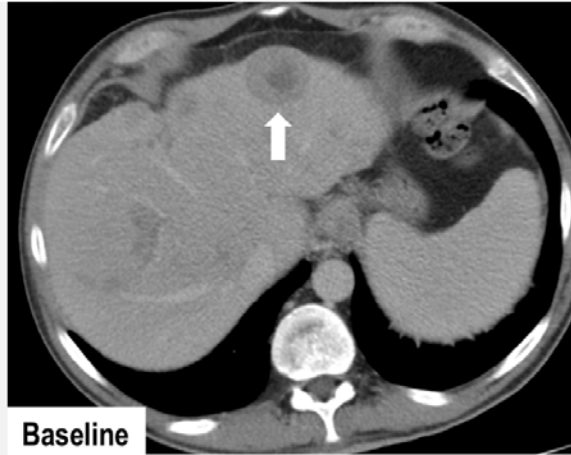
Inclusion Criteria

- ▶ Child Pugh A/B7
- ▶ Platelet >60
- ▶ ALT/AST < x10 ULN
- ▶ Mandatory endoscopy if cirrhosis

Safety and dose

- ▶ Well tolerated
- ▶ no unexpected toxicity
- ▶ Phase II dose 15 mg

Pt #13



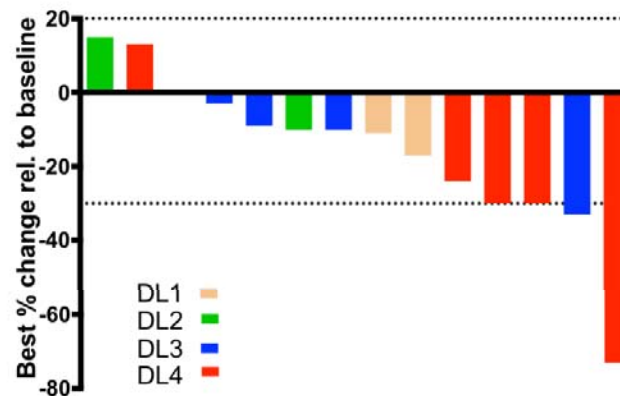
ORIGINAL ARTICLE

Sorafenib in Advanced Hepatocellular Carcinoma

Table 2. Summary of Efficacy Measures.*

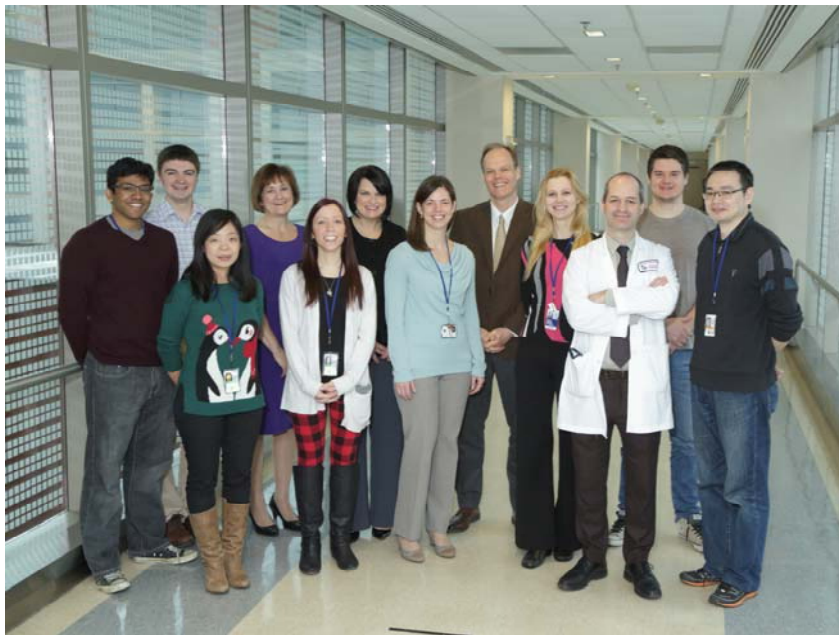
Outcome	Sorafenib (N=299)	Placebo (N=303)	Hazard Ratio (95% CI)	P Value
Overall survival (mo)			0.69 (0.55-0.87)	<0.001
Median	10.7	7.9		
95% CI	9.4-13.3	6.8-9.1		
1-yr survival rate (%)	44	33		0.009
Time to symptomatic progression (mo)†			1.08 (0.88-1.31)	0.77
Median	4.1	4.9		
95% CI	3.5-4.8	4.2-6.3		
Time to radiologic progression (mo)			0.58 (0.45-0.74)	<0.001
Median	5.5	2.8		
95% CI	4.1-6.9	2.7-3.9		
Level of response (%)‡				
Complete	0	0		NA
Partial	2	1		0.05
Stable disease	71	67		0.17
Disease-control rate (%)§	43	32		0.002

TRC105 + Sorafenib



Adverse Events

Toxicity	Phase I			
	3 mg (n=3)	6 mg (n=3)	10 mg (n=6)	15 mg (n=3)
Anemia	-	-	1	-
Hand Foot Syndrome	1	1	4	2
Hypophosphatemia	1	-	-	-
Hyperbilirubinemia	1	1	-	-
AST/ALT elevation	1	-	2	-
Diarrhea	1	-	-	-
Intracranial Hemorrhage	-	1	-	-
Lymphopenia	-	-	2	-
Cardiac Ischemia	-	-	1 (G5)	-



Clinical Team

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Brad Wood, Elliot Levy
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Laboratory of Pathology

David Kleiner, Mark Raffeld, Drew Pratt

Biostatistics and Data Management Section

Seth Steinberg, David Vanzon

Core Facilities

CRC: Nursing Staff and NPs on 3NW
Clinical Pharmacology Program
NIH Tetramer Facility



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