Vaccines, Tumor Antigens and Immune Responses

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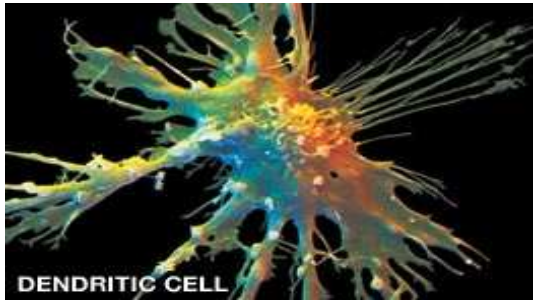
Presenter Disclosure Information

Lisa H. Butterfield, Ph.D.

The following relationships exist, possibly related to this presentation:

Kite Pharma, stock options
Caladrius (formerly NeoStem), Scientific Advisory Board member,
Oxford Immunotec, Affymetrix/eBioscience, Merck, Biodesix, Verastem, Astra Zeneca: consultant/advisory board

DC Vaccines



- 200 DC trials since 1996
- 5 current phase III trials recruiting
- 5 current phase II trials of DC + anti-PD-1

Dendreon Sipuleucel T: >$80,000/patient; UPCI IMCPL: $6,500/pt.

Historically, 5-10% CR+PR in late stage patients in some trials, 0% in other trials.

Recent DC vaccine studies:

1. Schreibelt, De Vries: CaRes 2016: 14 stg. IV melanoma pt., CD1c+ isolated blood DC, 16 hour culture, + gp100 and tyrosinase. 4/14 pt. PFS 12-35 mo.
2. Wilgenhof, Neyns: JCO 2016: 39 “adv. Melanoma” pt., mRNA: gp100, tyrosinase, MAGE-A3, MAGE-C2/DC + ipi. “Encouraging” ORR, 8 CR+7 PR/39.
3. Carreno, Linette: Science 2015: 3 stg. III melanoma pt., DC+neoAg peptides, some + immune responses.

Summary of Completed MART-1-based Clinical Trials

Phase I MART-1_{27-35} pep/DC:

10^5, 10^6, 10^7 DC/injection; routes: i.v. vs. i.d. (18 pt., stg. III-IV)
13/16 immune responses by MHC tetramer; and 13/15 by IFNg ELISPOT
10 pt. w/disease: 2 SD (4, 12 mo.), 1 CR (*w/determinant spreading***)
8 pt. NED: 5/8 remained NED (18+ to 27+ mo.)

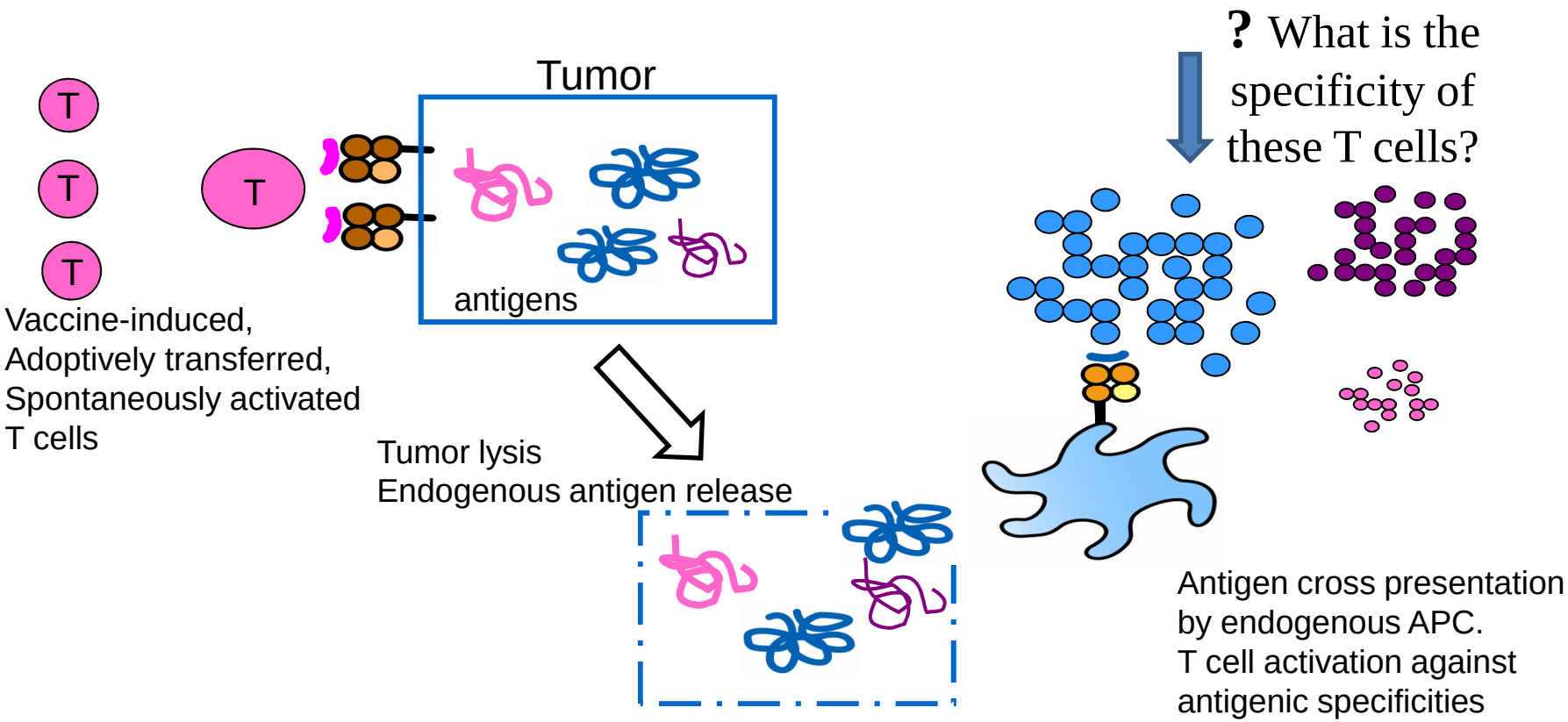
Phase II MART-1_{27-35} pep/DC:

10^7 DC/injection, i.d. (10 pt., stg. II-IV)
9/10 MART-1 immune responses by MHC tetramer and/or IFNg ELISPOT
5 pt. w/disease: 1 MR, 1 SD (6 mo.), 1 CR (*w/determinant spreading***, + ipi).
4/5 NED remained NED (20+ to 27+ mo.)

AdVMART1/DC:

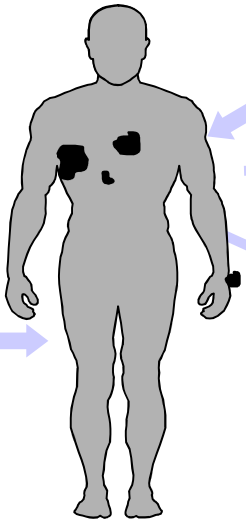
3/02-3/04 (23 enrolled); 14 received all 3 vaccines (all metastatic)
12/13 MART-1 immune responses by IFNg ELISPOT; 9/14 MHC Tetramer+
1 “unevaluable” (54+ mo., *w/determinant spreading***),
4 SD (27, 33, 36**, 42 mo.), 1 became resectable/NED (56+ mo.)**

Determinant Spreading



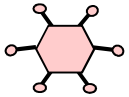
Multi-Antigen-AdV-Transduced DC with IFNα Boost Trial

DIAGRAM



IFNα boost

leukapheresis



3 tumor antigen
Adenovirus

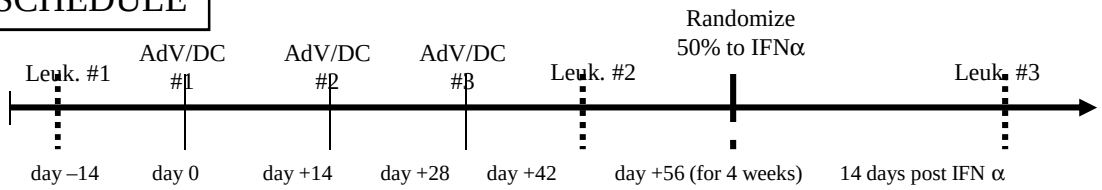
3 vaccines,
intra-dermal,
10^7 DC per
injection,
every other week

Leukapheresis/Biopsy:
CD8+/CD4+ PBMC:
-Multi-cytokine ELISPOT for immunizing antigens
-Determinant Spreading ELISPOT
-Serum Luminex
-Tetramer Assay/phenotyping
-Avidity (A2/DR4)
-NK activation
-TIL analyses
-Tumor antigen analysis

30 Patients Randomized:
1:1 to high dose i.v. IFNα (arm A)

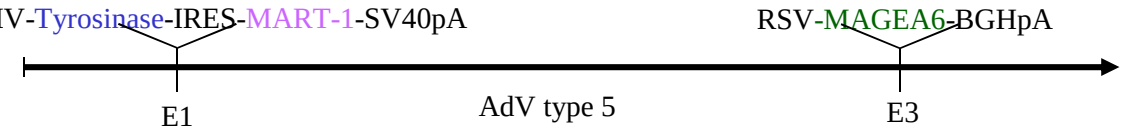
Tumor Biopsy:
Analysis of vaccine antigens
(not inclusion criterion)
Analysis of additional antigens
(for potential determinant spreading assay antigens)

SCHEDULE



MART-1: melanosome structure?
Tyrosinase: oxidase, melanin synthesis
MAGE-A6: ubiquitination?
p53 apoptosis resistance?

VECTOR MAP



Dendritic Cell Vaccines

Successes: >7% clinical response rate in late stage patients

Failures: <6% responses rate that was similar to chemotherapy in a randomized trial; other trials without significant clinical responses

Role for cancer vaccines today:

"inflammed", infiltrated tumors respond better to checkpoint blockade;
need PD-L1+ cells in tumors for PD-1 blockade to be effective; adoptively transferred cells need something to support them (antigen, cytokines); vaccines may be "enough" in a prevention setting?

Current questions:

- **Dose** (10e5 minimum? 10e8 maximum feasible?)
- **Route** (i.d. > i.v.? i.n accuracy? i.lymphatic?)
- **Culture conditions** (can we do better than 6 days in GM-CSF+IL-4?)
- **Maturation conditions** (TLRs, cytokine cocktails…)
- **Antigen loading** (peptides, proteins, lysates, allogeneic cells, autologous tumor)
- **Potency Assay** (IL-12p70? IL-12/10? Phenotype? Transcriptome?)

What testing is performed: safety, purity and identity tests

An example of the specific release tests which are required by the FDA for early phase trials involving autologous, *in vitro* manipulated cellular products: Safety, identity/purity testing, and the candidate potency test being explored are shown.

Viability: The cells are counted by microscopic observation on a hemacytometer, and a differential count (DC vs. lymphocytes) is obtained using trypan blue dye. Minimum 70% viability.

Purity: The DC must express MHC class II and CD86 by flow cytometry in a minimum of 70% of the cells. Additional phenotyping (MHC class I, CD80, CD83, CCR7, etc.) is performed to fully characterize the DC (research).

Sterility: DC are tested by bacterial (aerobic and anaerobic) and fungal cultures. Final results are available in 14 days. Prior to release of the DC for vaccine use, a standard gram stain is performed and must be negative.

Mycoplasma testing of cell suspensions (not supernatants) must be negative for mycoplasma.

Endotoxin testing is performed on the cell culture at the time of harvest and prior to release of the final product. The acceptable endotoxin level is <5 EU/kg of body weight per dose.

Potency: To define a measure of potency for the DC, we determine their ability to produce IL-12p70 and IL-10 by Luminex assay. This test is performed batched, with and without activation by CD40L and/or LPS, and is available several weeks after vaccine injection.

A 0.5 ml sample of the final DC preparation from each vaccination time is cryopreserved for possible ancillary testing in the future.

Patient Enrollment

Aug. 2012-Feb. 2016:

35 pt. enrolled
32/35 completed 3 vaccines and post vaccine blood/pheresis (3 partially vaccinated)
20/35 completed the protocol
(including post vaccine IFN/obser. and d101 blood/pheresis
(5 with d101 peripheral blood instead).
One with no 2nd pheresis (blood only).

Clinical Responses (RECIST):
2 PR: 14 mo., 7 mo. 7 SD (4-7+ mo.)
of the 11 measurable disease completing the protocol.

13/24 "early PD" before protocol completion (@d+101).

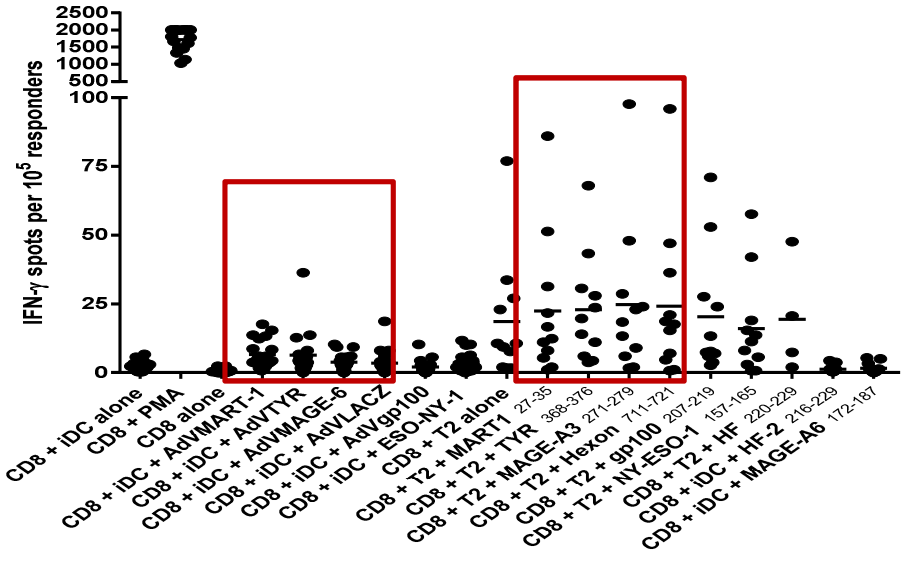
11 high risk NED 2-22+ mo. (6 NED still NED)

(Kirkwood, Tarhini, Tawbi)

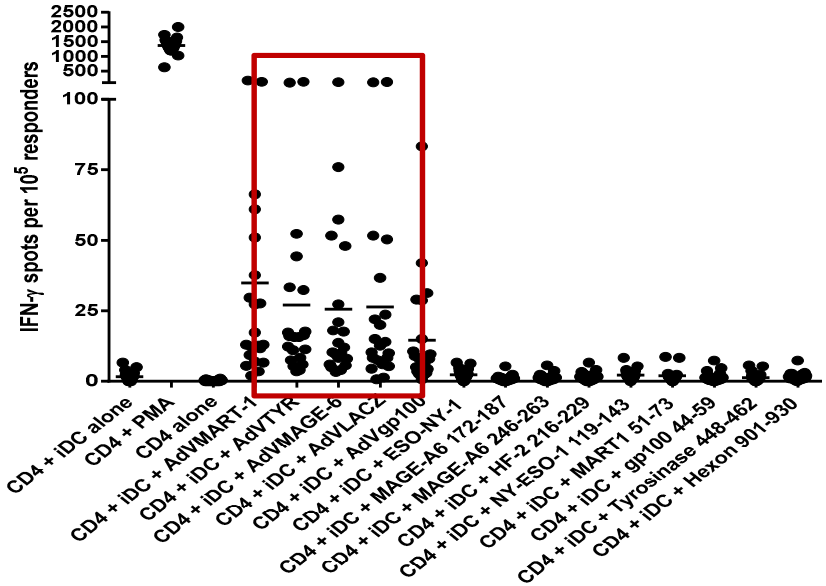
T cell subset ELISPOT analysis: Vaccine-encoded antigens

Did we hit the target/promote vaccine-specific T cell responses?

CD8+

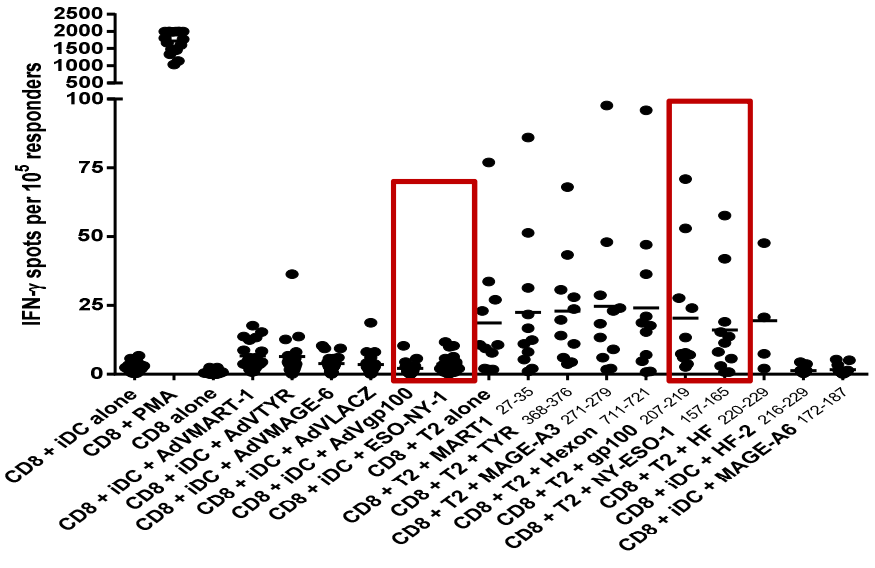


CD4+

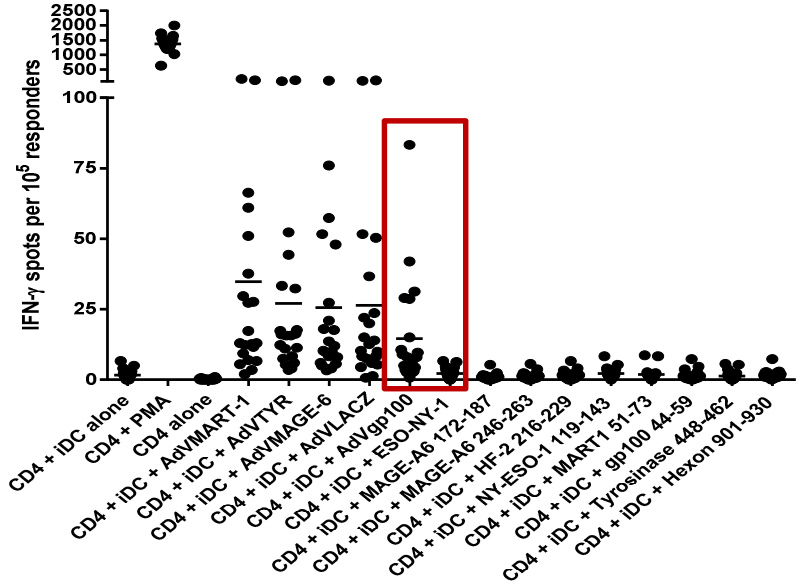


T cell subset ELISPOT analysis: “Determinant spreading” central hypothesis about non-vaccine shared antigens

CD8+



CD4+



		Spontaneous			Induced	
		IL-12p70	**IL-10**		**IL-12p70**	**IL-10**
Spontaneous Range		0 - 4,020	1 - 106	**CD40L**	55 - 7,177	45 - 3,267
	1 (PD)	1237	5		4024	84
	2 (PD)	1640	7		2743	151
	3 (PD)	462	5		1354	45
	4 (PD)	13	5		1205	219
	5 (PD)	4120	38		4108	691
	6 (SD)	7	30		*195*	*844*
	7 (SD)	196	6		730	112
	8 (SD)	54	14		7177	3267
	9 (SD)	***39***	***51***		***55***	***435***
	10 (PR)	**17**	**2**		**348**	**73**
	11 (PD)	69	4		384	279
	12 (SD)	*22*	*23*		*231*	*439*
	13 (NED)	64	12		3009	283
	14 (PD)	433	22		833	203
	15 (NED)	*89*	*106*		*391*	*3169*
	16 (NED)	79	1		250	100
	17 (PD)	13	6		*147*	*350*
	18 (NED)	*1*	*3*		*70*	*188*
	19 (SD)	*4*	*12*		*58*	*317*
	20 (PR)	**30**	**3**		***124***	***197***
	21 (NED)	6	4		*347*	*369*
	Ave.	**409**	**17**		**1323**	**562**

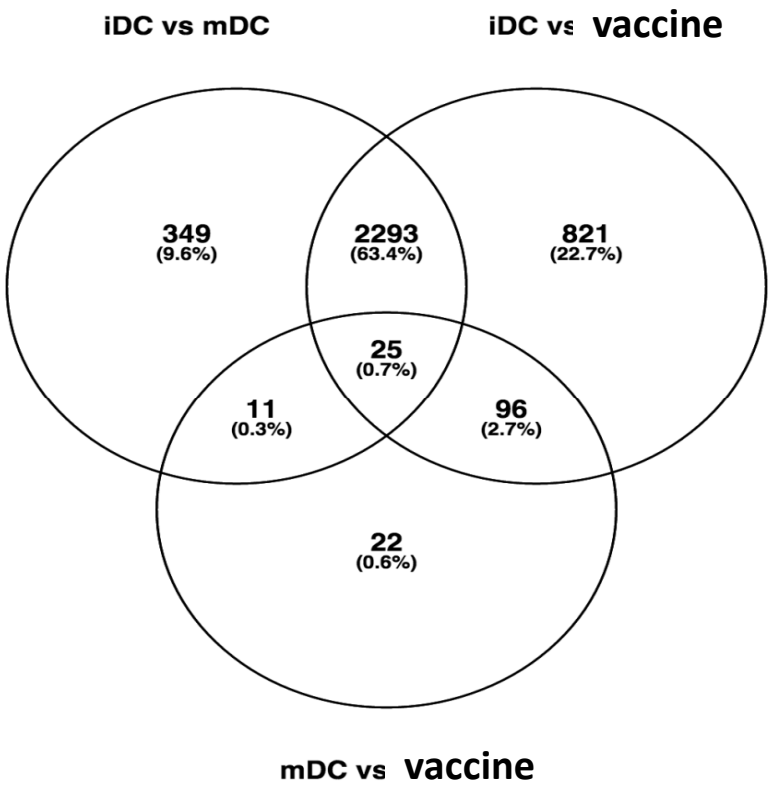
DC Vaccine Potency:
IL-12p70 and IL-10 production (pg/ml)

Most vaccines spontaneously secrete some IL-12 and little IL-10.

CD40 ligation (via J558 cells) triggers increased IL-12 production by vaccines.

Best clinical responses have less "favorable" IL-12p70 secretion and *IL-12/IL-10 ratios*.

Differential Expression Analysis



- Student t-test (unequal variance) is performed in R to test for differential expression.
- P-values are adjusted for multiple comparisons using FDR method.

FDR 5%, FC > 2 fold	# genes
iDC vs mDC	2678
iDC vs vaccine	3235
mDC vs vaccine	154

Stroncek, Chandran

DC vaccine genes associated with:

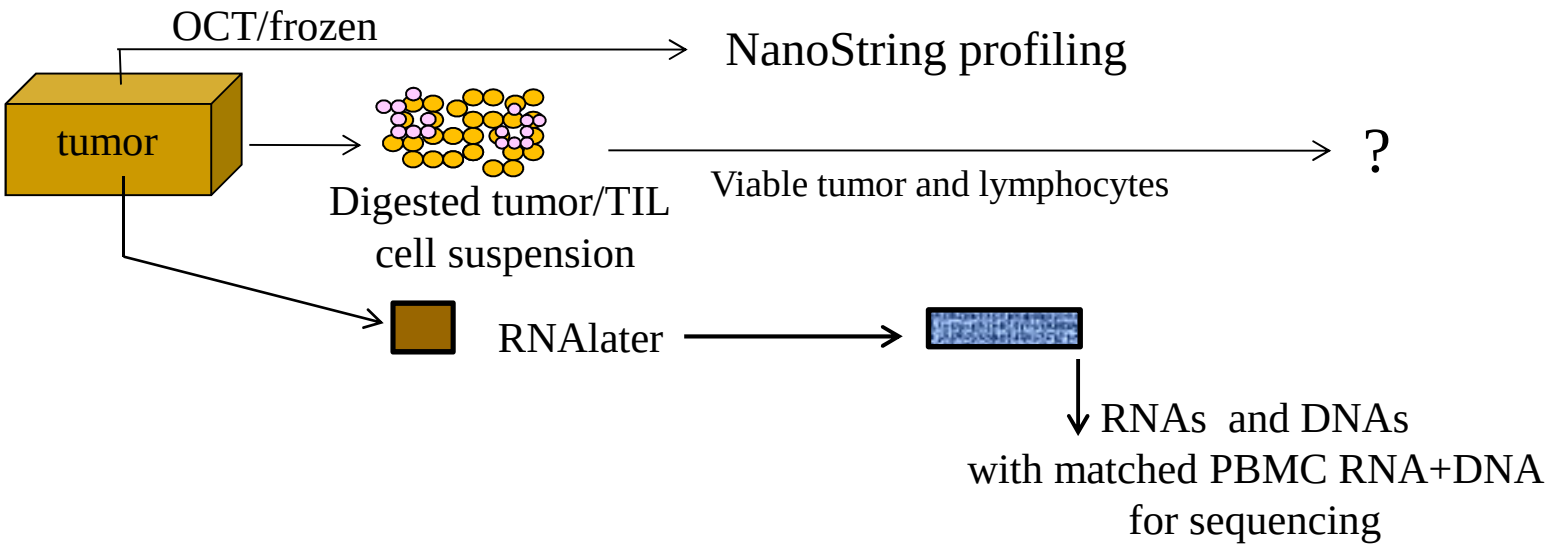
A. IFNγ+ T cell responses to the vaccine-encoded antigens

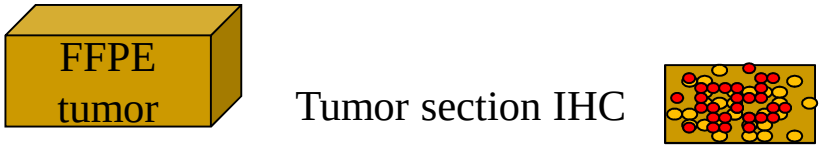
B. IFNγ+ T cell responses to the shared determinant spreading antigens (gp100, NY-ESO-1)

C. clinical outcomes

1. Genes encoding the 3 vaccine antigens: ***no***
2. MHC class I and/or II: ***no***
3. Costimulatory molecules: CD40, CD80, CD86: ***mostly no***
4. Changed transcripts (many, some immune related, to be investigated)

Chandran & Co.





in Pathology banks in Pittsburgh and elsewhere,
received unstained slides for IHC/IF.

Vaccines
Blood
Tumor
Checkpoints

Melanoma DC Vaccine Trial Next Steps:

1. Correlate data:
   a) Clinical outcomes of the patients
   b) DC surface phenotype
   c) DC spontaneous and induced IL-12p70 and IL-10 expression levels
   d) T cell immune responses generated (vaccine antigens)
   e) Determinant spreading (non-vaccine antigens)
   f) Humoral Responses (TAA, AdV)
   g) Gene arrays (DC vaccines, tumors)
   h) Tumor IHC
   i) NK cell responses
   j) Checkpoint molecule expression/modulation in patients (DC, blood)
   k) Improve T and NK cell responses activated by DC with anti-PD-1?

2. Figure out how to make effective vaccines, and to promoting spreading

HCC and Alpha Fetoprotein

Numbers: > 600,000 new HCC cases annually around the globe
Therapy: Last 5 phase III trials failed, Sorafenib adds 2.8 months.

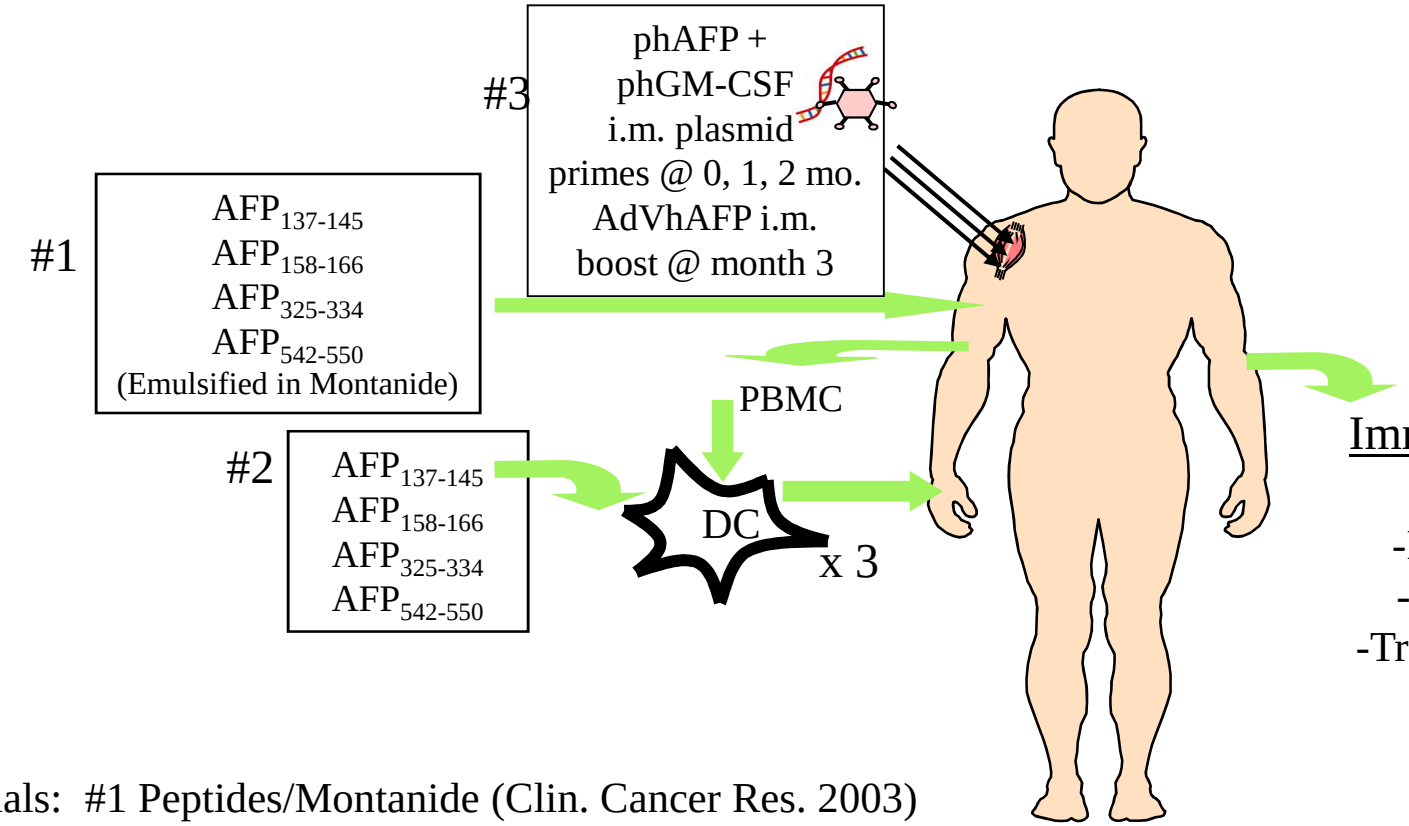
AFP Protein: 609 aa glycoprotein (591 aa secreted size), synthesized in fetal liver and yolk sac, the major serum protein before birth.
AFP Function: Possible roles in serum component transport (fatty acids), binds hormones including estrogen, possible breast cancer prevention role, binds TNFα, **possible immunoregulatory role.**

Serum levels: in fetus: maximum at 10-13 weeks (3 mg/ml), decreases to 30-100 ug/ml at birth, adult levels 1-3 ng/ml.
HCC Biomarker/Expression: 50% to 80% HCC express AFP (serum up to 1 mg/ml).

Immunotherapy: 14 HLA-A2.1-restricted peptides were characterized (4 immuno-dominant, 10 sub-dominant) and the 4 immunodominant were found to be immunogenic *in vivo*, in HCC pt. with high serum AFP.

(Cancer Res. ’99, Molec. Immunol. ’00, J. Immunol. ’01, Clin. Cancer Res. ’03)

AFP Based Immunotherapy Clinical Trials for HCC



Immune Response:
PBMC:
-IFNγ ELISPOT
-MHC Tetramer
-Treg, NK activation

Trials: #1 Peptides/Montanide (Clin. Cancer Res. 2003)
#2 Peptides/DC (Clin. Cancer Res. 2006)
#3 DNA prime/AdV boost i.m. (JTM, 2014)

Summary of Completed AFP-based Clinical Trials

AFP peptides/Montanide:

6 patients, Stage IVa, IVb,
Four AFP peptides in Montanide ISA adjuvant
100 ug, 500 ug each peptide, 3 intradermal injections (skin toxicity only)
6/6 immune responses by MHC tetramer and/or IFNg ELISPOT
No objective clinical responses or AFP decreases, OS = 2-17 months

AFP peptides/DC:

10 patients, stage III-IVb
Four AFP peptides pulsed onto autologous GM-CSF/IL-4 DC
3 injections, intradermal, no toxicities
8/10 immune responses by MHC tetramer and/or IFNg ELISPOT
No objective clinical responses, 2 serum AFP decreases, OS = 2-35 months

AFP DNA prime/AFPAdV boost:

2 patients, stage II
AFP + GM-CSF plasmids x 3, then AdVhAFP x 1; monthly i.m.
Pt. #1 Minimal AFP-specific T cell immunity, low anti-AdV neutralizing antibodies. 9 mo. AFP+ recurrence.
Pt. #2 *Strong* AFP-specific T cell immunity,+anti-AdV neutralizing antibodies. 18 mo. AFP- suspected recurrence.

Antigen Loading of AFP-based DC Vaccines

~~MHC class I-restricted peptides?~~

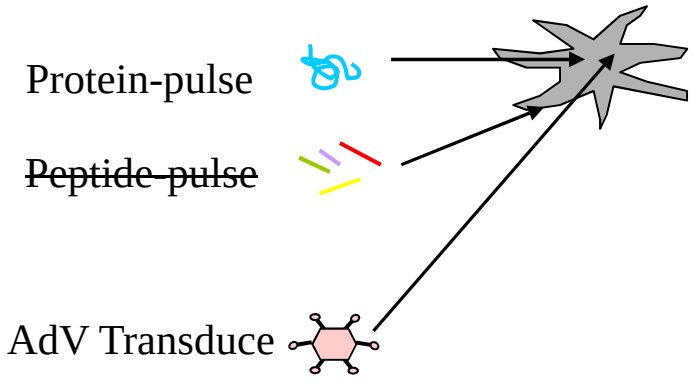
Full-length antigen, non-MHC-restricted, to activate polyclonal CD8+ and CD4+ T cells:

1) Protein (from cord blood, ”normal” nAFP)

2) Protein (from tumor cell lines, ”tumor” tAFP)

3) Tumor lysate (HepG2 cell line)

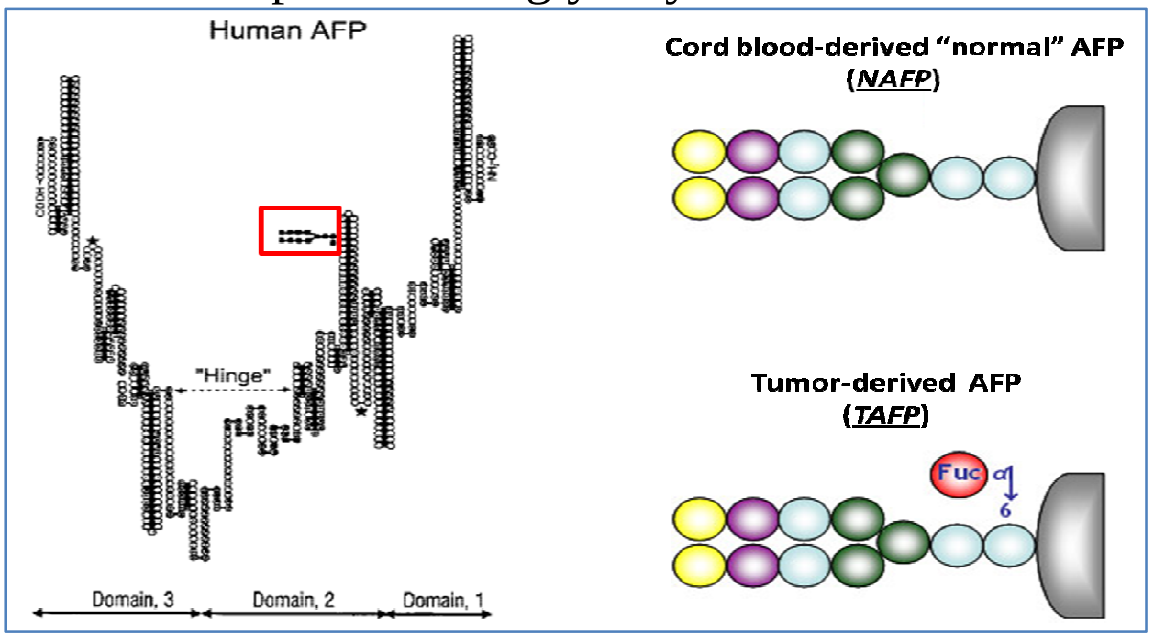
4) Viral Vector (synthetic AFP)



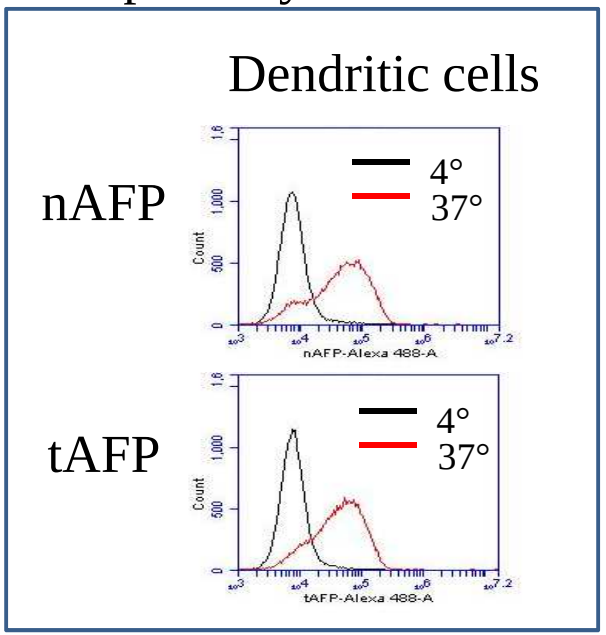
Alpha-fetoprotein

50-80% of HCC express AFP → fucosylated AFP glycoform

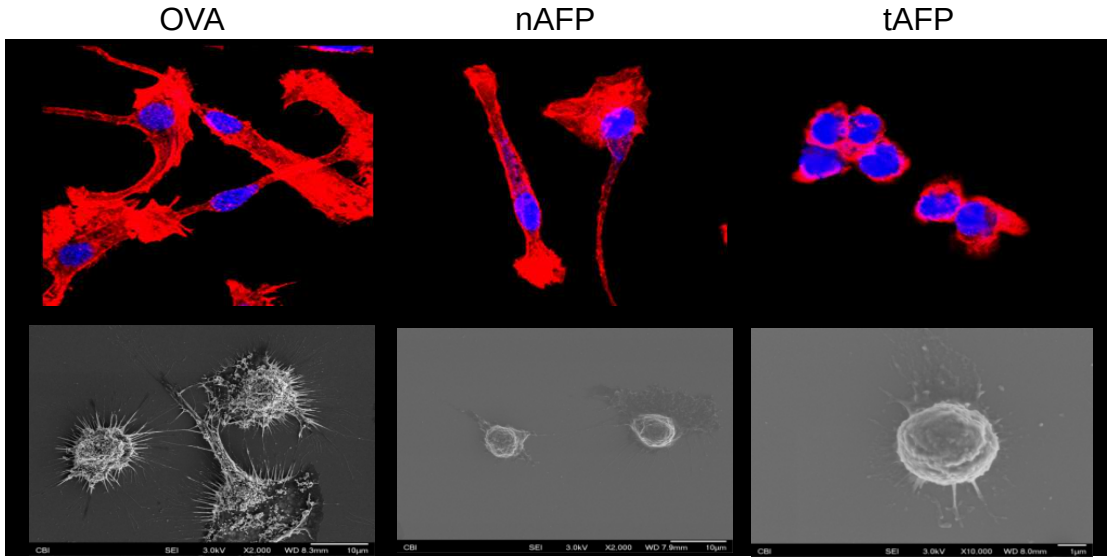
AFP protein and glycosylation

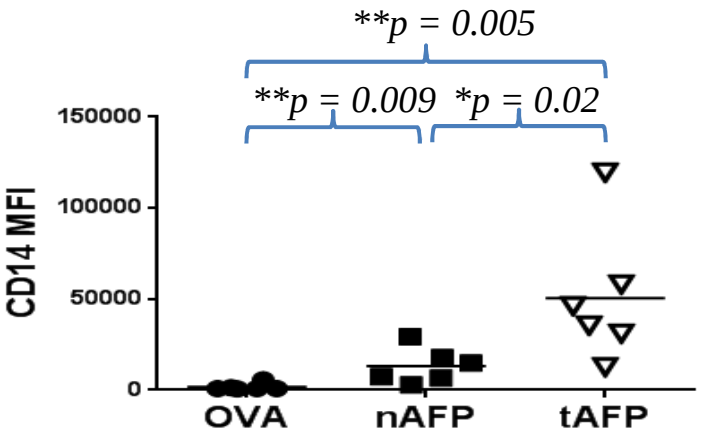


Uptake by human DC



Monocytes treated (10ug/ml AFP protein) during DC culture

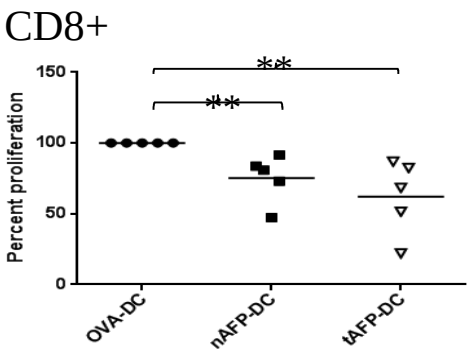


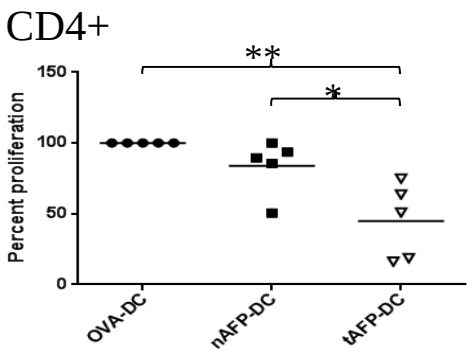


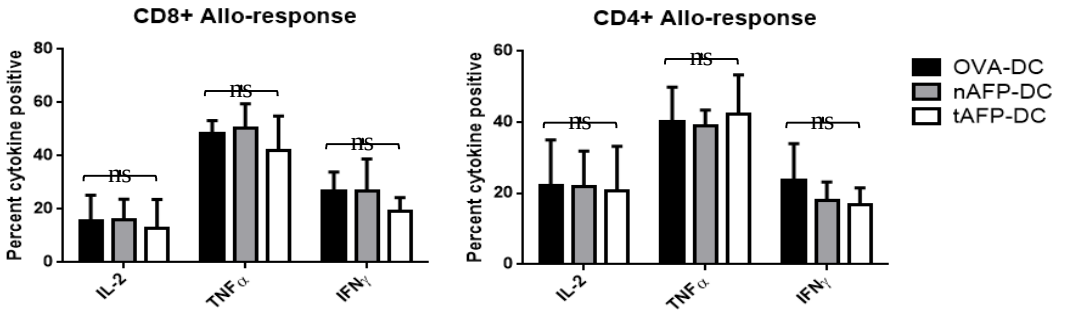
Pardee, J.I. 2014

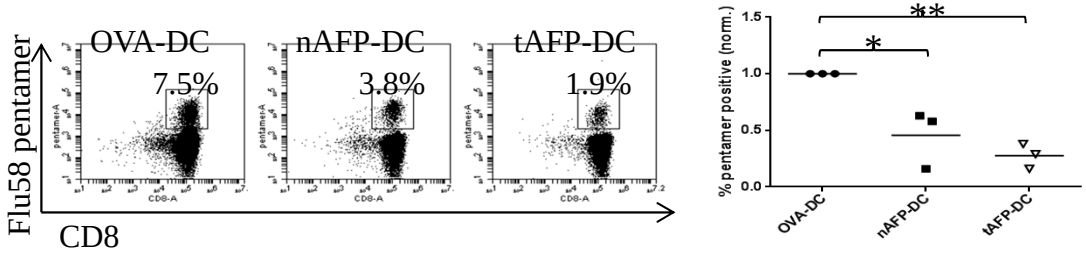
AFP impact on T cell proliferation, cytokine production

Tumor-derived AFP reduces T cell proliferation in the settings of both allo MLR and Flu CTL expansion.
Cytokine production per cell is only minimally affected.









AFP impact on DC metabolism

1. GLUL, Glutamine Biosynthesis. ASNS asparagine synthase *increased*
2. PLA2G5, phospholipase A2 group 5, lipid metabolism
3. ALOX15, arachidonate 15-lipoxygenase
4. ACOX2, acyl-co-A oxidase 2
5. LPL, lipoprotein lipase
6. FASN, fatty acid synthase

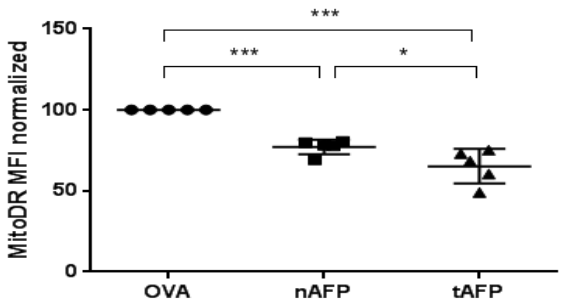
Down with AFP

AFP binds many lipids, and oxidized lipids are known to be taken up directly by DC (via MSR1/scav. rec. A) and can be suppressive (Gabrilovich & Kagan).

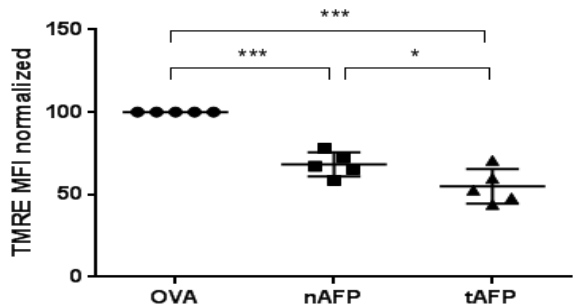
	OVA vs nAFP	OVA vs tAFP
GLUL	2.63x up	1.60x up
ASNS	1.39x up	6.87x up
ALOX 15	1.45x up	4.68x down
ACOX2	2.20x down	8.35x down
MSR1	1.97x down	4.17x down
FASN	1.35x up	2.50x down
LPL	1.83x down	10.92x down
PLA2G5	5.83x down	11.06x down

Reduced mitochondrial mass, active mitochondria and shut down of OxPhos

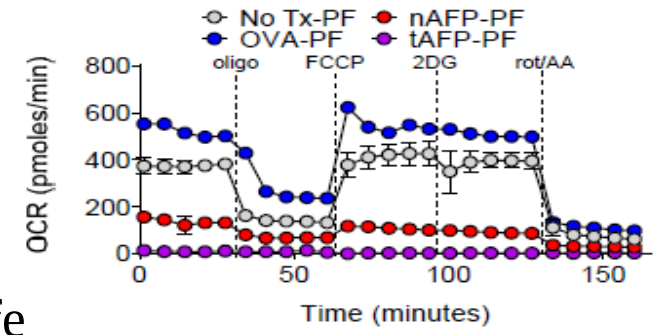
MitoDR
(total mitochondria mass)



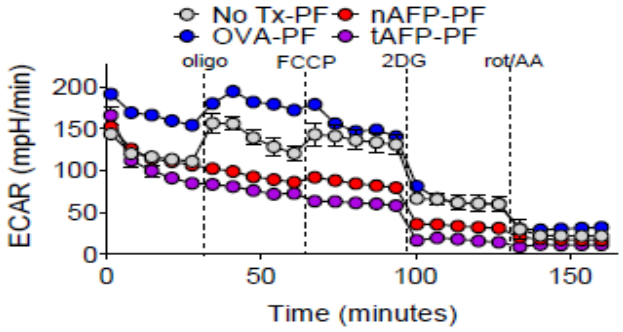
TMRE
(active mitochondria)



OXPHOS

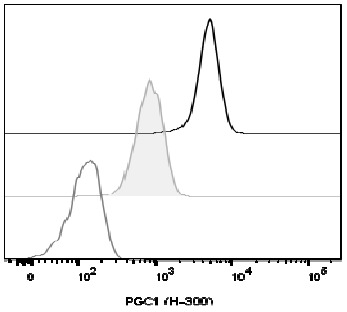


GLYCOLYSIS



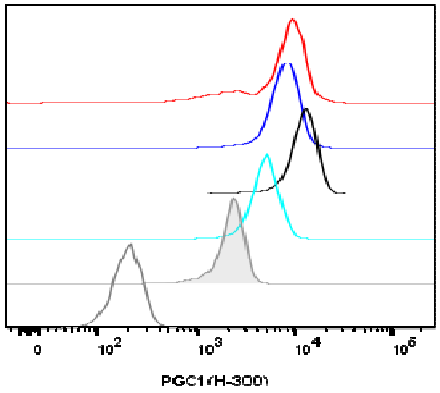
Santos, Delgoffe

PGC1a expression (d5 DC)



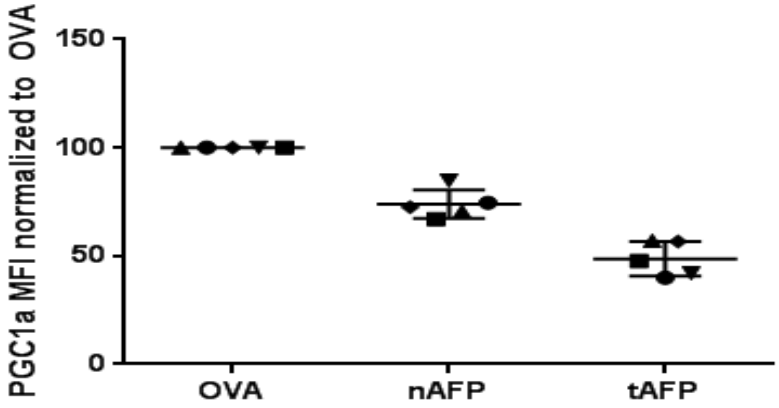
HepG2	4459
No 1°	788
No 2°	121

HepG2 positive control



tAFP	5593
nAFP	7193
OVA	11054
HepG2	4459
No 1°	2018
No 2°	191

PGC1a: Transactivator driving mitochondrial biosynthesis



Conclusions:

1. Not all shared antigens are created equal. Some have immune modulatory activity that must be considered in design and conduct of trials.
2. Cancer Vaccines may be critical for driving immunity in those patients with minimal, skewed immunity and non-infiltrated/inflamed tumors, as well as in early stage/prevention settings.
3. A combination of well standardized assays (ELISPOT, multimer, Luminex) with high throughput profiling (arrays, sequencing) and newer advanced technologies (ncounter, TCR sequencing, multiplexed immunofluorescence) will yield improved biomarker data and mechanistic analysis.
4. Can targeting shared antigens promote determinant spreading to neoantigens?

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