What Relevant Immune Monitoring and Correlative Study Criteria Do We Use?

Lisa H. Butterfield, Ph.D.

Associate Professor of Medicine, Surgery and Immunology
University of Pittsburgh Cancer Institute
Director, UPCI Immunologic Monitoring and Cellular Products Laboratory

Disclosure

The following relationships exist related to this presentation:
None

The following relationships exist:
Advisory Board participant: EMD Serono, Novartis, Ziopharm
Special Advisor: Kite Pharma

Correlations between Immune Monitoring and Clinical Outcome

Vaccination of vulvar intraepithelial neoplasia patients with long peptides from HPV16 {Welters '10}. Investigators tested lymphocyte proliferation and cytokine production to immunizing antigens as well as circulating regulatory T cells (Treg). Their study showed that the patients with the **strongest proliferation, positive IFNγ and IL-5 production, and lowest Treg were those with complete responses (CR)** to therapy.

A trial testing WT-1 antigen in AML patients indentified significant changes in **WT-1 specific $CD8^+$ T cell frequencies,** and more dramatic **activation of circulating NK cells in patients with CRs** {Van Tendeloo '10}.

A recent study testing a multi-peptide vaccine in renal cancer patients demonstrated that **inhibition of Treg** strengthened antitumor immunity, **frequencies of specific subsets of MDSC at baseline were critical,** and that improved clinical outcome correlated with **a larger number of peptides** responded to {Walter '12 }. This last observation has been made in previous multi-peptide vaccine trials {Banchereau, '01}.

In patients treated with ipilimumab, a number of groups have examined humoral and cellular responses from peripheral blood. Recently, **integrated humoral and $CD8^+$ T cell response to the shared, immunogenic cancer/testes antigen NY-ESO-1** was shown to be a significant immunologic measure correlating with clinical outcomes {Yuan '11}. In a related publication, circulating **T cells specific to NY-ESO-1 and MART-1** antigens (but not MAGE-A3 or survivin antigens) were correlated with melanoma patient survival {Weide '12}.

A final example of an immune measure that several independent groups have correlated to clinical outcome is "epitope spreading" or "**determinant spreading**". This acquisition of T cell and antibody reactivity to shared antigens or epitopes other than those used in a vaccine, has been observed in melanoma {Ranieri '00; Butterfield, '03; Ribas '04; Butterfield '08}, renal cancer {Wierecky '06 } and breast cancer {Disis '02 }.

Correlations between Immune Monitoring and Clinical Outcome

Vaccination of vulvar intraepithelial neoplasia patients with long peptides from HPV16 {Welters '10}. Investigators tested lymphocyte proliferation and cytokine production to immunizing antigens as well as circulating regulatory T cells (Treg). Their study showed that the patients with the **strongest proliferation, positive IFNγ and IL-5 production, and lowest Treg were those with complete responses (CR)** to therapy.

A trial testing WT-1 antigen in AML patients indentified significant changes in **WT-1 specific $CD8^+$ T cell frequencies,** and more dramatic **activation of circulating NK cells in patients with CRs** {Van Tendeloo '10}.

A recent study testing a multi-peptide vaccine in renal cancer patients demonstrated that **inhibition of Treg** strengthened antitumor immunity, **frequencies of specific subsets of MDSC at baseline were critical,** and that improved clinical outcome correlated with **a larger number of peptides** responded to {Walter '12 }. This last observation has been made in previous multi-peptide vaccine trials {Banchereau, '01}.

In patients treated with ipilimumab, a number of groups have examined humoral and cellular responses from peripheral blood. Recently, **integrated humoral and $CD8^+$ T cell response to the shared, immunogenic cancer/testes antigen NY-ESO-1** was shown to be a significant immunologic measure correlating with clinical outcomes {Yuan '11}. In a related publication, circulating **T cells specific to NY-ESO-1 and MART-1** antigens (but not MAGE-A3 or survivin antigens) were correlated with melanoma patient survival {Weide '12}.

A final example of an immune measure that several independent groups have correlated to clinical outcome is "epitope spreading" or "**determinant spreading**". This acquisition of T cell and antibody reactivity to shared antigens or epitopes other than those used in a vaccine, has been observed in melanoma {Ranieri '00; Butterfield, '03; Ribas '04; Butterfield '08}, renal cancer {Wierecky '06 } and breast cancer {Disis '02 }.

Correlations between Immune Monitoring and Clinical Outcome

Vaccination of vulvar intraepithelial neoplasia patients with long peptides from HPV16 {Welters '10}. Investigators tested lymphocyte proliferation and cytokine production to immunizing antigens as well as circulating regulatory T cells (Treg). Their study showed that the patients with the **strongest proliferation, positive IFNγ and IL-5 production, and lowest Treg were those with complete responses (CR)** to therapy.

A trial testing WT-1 antigen in AML patients indentified significant changes in **WT-1 specific $CD8^+$ T cell frequencies,** and more dramatic **activation of circulating NK cells in patients with CRs** {Van Tendeloo '10}.

A recent study testing a multi-peptide vaccine in renal cancer patients demonstrated that **inhibition of Treg** strengthened antitumor immunity, **frequencies of specific subsets of MDSC at baseline were critical,** and that improved clinical outcome correlated with **a larger number of peptides** responded to {Walter '12 }. This last observation has been made in previous multi-peptide vaccine trials {Banchereau, '01}.

In patients treated with ipilimumab, a number of groups have examined humoral and cellular responses from peripheral blood. Recently, **integrated humoral and $CD8^+$ T cell response to the shared, immunogenic cancer/testes antigen NY-ESO-1** was shown to be a significant immunologic measure correlating with clinical outcomes {Yuan '11}. In a related publication, circulating **T cells specific to NY-ESO-1 and MART-1** antigens (but not MAGE-A3 or survivin antigens) were correlated with melanoma patient survival {Weide '12}.

A final example of an immune measure that several independent groups have correlated to clinical outcome is "epitope spreading" or "**determinant spreading**". This acquisition of T cell and antibody reactivity to shared antigens or epitopes other than those used in a vaccine, has been observed in melanoma {Ranieri '00; Butterfield, '03; Ribas '04; Butterfield '08}, renal cancer {Wierecky '06 } and breast cancer {Disis '02 }.

Correlations between Immune Monitoring and Clinical Outcome

Vaccination of vulvar intraepithelial neoplasia patients with long peptides from HPV16 {Welters '10}. Investigators tested lymphocyte proliferation and cytokine production to immunizing antigens as well as circulating regulatory T cells (Treg). Their study showed that the patients with the **strongest proliferation, positive IFNγ and IL-5 production, and lowest Treg were those with complete responses (CR)** to therapy.

A trial testing WT-1 antigen in AML patients indentified significant changes in **WT-1 specific $CD8^+$ T cell frequencies,** and more dramatic **activation of circulating NK cells in patients with CRs** {Van Tendeloo '10}.

A recent study testing a multi-peptide vaccine in renal cancer patients demonstrated that **inhibition of Treg** strengthened antitumor immunity, **frequencies of specific subsets of MDSC at baseline were critical,** and that improved clinical outcome correlated with **a larger number of peptides** responded to {Walter '12 }. This last observation has been made in previous multi-peptide vaccine trials {Banchereau, '01}.

In patients treated with ipilimumab, a number of groups have examined humoral and cellular responses from peripheral blood. Recently, **integrated humoral and $CD8^+$ T cell response to the shared, immunogenic cancer/testes antigen NY-ESO-1** was shown to be a significant immunologic measure correlating with clinical outcomes {Yuan '11}. In a related publication, circulating **T cells specific to NY-ESO-1 and MART-1** antigens (but not MAGE-A3 or survivin antigens) were correlated with melanoma patient survival {Weide '12}.

A final example of an immune measure that several independent groups have correlated to clinical outcome is "epitope spreading" or "**determinant spreading**". This acquisition of T cell and antibody reactivity to shared antigens or epitopes other than those used in a vaccine, has been observed in melanoma {Ranieri '00; Butterfield, '03; Ribas '04; Butterfield '08}, renal cancer {Wierecky '06 } and breast cancer {Disis '02 }.

Immunologic Biomarkers in Cancer

Organizers:

Lisa H. Butterfield, PhD
University of Pittsburgh

Mary (Nora) L. Disis, MD
University of Washington

Samir Khleif, MD
National Cancer Institute

Francesco Marincola, MD
National Institutes of Health

Magdalena Thurin, MD
National Institutes of Health

Participating Organizations:

- Association for Immunotherapy of Cancer (CIMT)
- Biotherapy Development Association (BDA)
- Cancer Vaccine Consortium (CVC) of the Cancer Research Institute (CRI)
- Food and Drug Administration (FDA)
- Italian Network for Tumor Biotherapy (NIBIT)
- Japanese Society of Cancer Immunology (JSCI)
- National Cancer Institute (NCI)
- National Institutes of Health (NIH)
- Nordic Center for Development of Antitumour Vaccines (NCV-network)

Special Report

Clinical Cancer Research

May 2011

Recommendations from the iSBTc-SITC/FDA/NCI Workshop on Immunotherapy Biomarkers

Lisa H. Butterfield[1], A. Karolina Palucka[4,5], Cedrik M. Britten[23,24], Madhav V. Dhodapkar[6], Leif Håkansson[28], Sylvia Janetzki[7], Yutaka Kawakami[29], Thomas-Oliver Kleen[9], Peter P. Lee[10], Cristina Maccalli[31], Holden T. Maecker[11], Vernon C. Maino[12], Michele Maio[33], Anatoli Malyguine[13], Giuseppe Masucci[27], Graham Pawelec[25], Douglas M. Potter[2], Licia Rivoltini[32], Lupe G. Salazar[19], Dolores J. Schendel[26], Craig L. Slingluff, Jr.[20], Wenru Song[21], David F. Stroncek[15], Hideaki Tahara[30], Magdalena Thurin[18], Giorgio Trinchieri[14], Sjoerd H. van Der Burg[34], Theresa L. Whiteside[3], Jon M. Wigginton[8], Francesco Marincola[16], Samir Khleif[17], Bernard A. Fox[22], and Mary L. Disis[19]

Variability:	**Recommendations:**
1. Patient	1. Save DNA/RNA/cells/tumor; include healthy donor control
2. Blood draw	2. Standardized procedures
3. Processing/cryo/thaw	3. Standardized procedures
4. Cellular product	4. Functional assays to characterize/develop potency
5. Assay choice	5. Standardized, functional
6. Assay conduct	6. SOP
7. Assay analysis	7. Appropriate biostatistical methods
8. Data reporting	8. Full details, controls, QA
9. Next cool new assay	9. Sufficient blood/tissue to interrogate the samples *now*, as well as *later*, to generate new hypotheses.

ImmunoAssay Proficiency Panel Program

The program's objectives are:
1)to offer an external validation program, and
2)to enhance assay harmonization.

The Proficiency Panel Program seeks to identify:

- **issues and deficiencies of current assay practices;**
- **sources for assay variability within and among institutions;**
- **protocol details that optimize assay performance.**

Define criteria for and support harmonization & validation of the assay for individual member laboratories.
Determine the value of resulting criteria in the cancer immunotherapy community.
Offer training programs to the cancer immunotherapy community to enhance assay performance and comparability between laboratories.
Establish immune assays as standard monitoring tools, if feasible.

ELISPOT -- Dr. Sylvia Janetzki, ZellNet Consulting
ICS -- Dr. Lisa McNeil, Wyeth
Multimer (Tetramer/Pentamer) Staining -- Dr. Pedro Romero, Ludwig Institute for Cancer Research, Lausanne, Switzerland, and Dr. Cedrik Britten, University of Leiden, The Netherlands
Luminex – Dr**.** Michael Kalos, Univ. Pennsylvania

Central laboratory services, cells, and shipping are provided by the **Immunology Quality Assurance Center** (IQAC) of the Duke Human Vaccine Institute under the leadership of Dr. Thomas Denny.

Immunity 37, July 27, 2012

T Cell Assays and MIATA: The Essential Minimum for Maximum Impact

C.M. Britten,* S. Janetzki, L.H. Butterfield, G. Ferrari, C. Gouttefangeas, C. Huber, M. Kalos, H.I. Levitsky, H.T. Maecker, C.J.M. Melief, J. O'Donnell-Tormey, K. Odunsi, L.J. Old, T.H.M. Ottenhoff, C. Ottensmeier, G. Pawelec, M. Roederer, B.O. Roep, P. Romero, S.H. van der Burg, S. Walter, A. Hoos, and M.M. Davis

Lack of conformity on reporting data hampers the ability to objectively evaluate data.

Minimal Information About T cell Assays ("MIATA"): we propose to generate recommendations on the minimum information required to allow an objective and thorough interpretation of published results from immunological T cell assays.
Any recommendation in the proposed Modules is strictly based on supporting published data.

Long-term goal of MIATA: adapt the reporting framework for annotations of immune monitoring data sets from human studies for a central database, possibly in the context of a Human Immunity Project. This may include structured database vocabulary.

MIATA Reporting Framework

CIP CIMT Immunoguiding Program

CIC Cancer Immunotherapy Consortium

Special Report

Clinical Cancer Research

May 2011

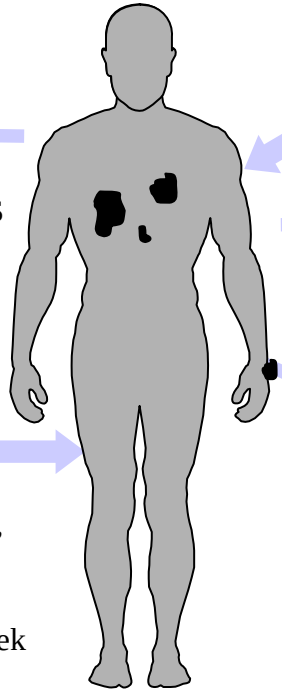
Recommendations from the iSBTc-SITC/FDA/NCI Workshop on Immunotherapy Biomarkers

Lisa H. Butterfield[1], A. Karolina Palucka[4,5], Cedrik M. Britten[23,24], Madhav V. Dhodapkar[6], Leif Håkansson[28], Sylvia Janetzki[7], Yutaka Kawakami[29], Thomas-Oliver Kleen[9], Peter P. Lee[10], Cristina Maccalli[31], Holden T. Maecker[11], Vernon C. Maino[12], Michele Maio[33], Anatoli Malyguine[13], Giuseppe Masucci[27], Graham Pawelec[25], Douglas M. Potter[2], Licia Rivoltini[32], Lupe G. Salazar[19], Dolores J. Schendel[26], Craig L. Slingluff, Jr.[20], Wenru Song[21], David F. Stroncek[15], Hideaki Tahara[30], Magdalena Thurin[18], Giorgio Trinchieri[14], Sjoerd H. van Der Burg[34], Theresa L. Whiteside[3], Jon M. Wigginton[8], Francesco Marincola[16], Samir Khleif[17], Bernard A. Fox[22], and Mary L. Disis[19]

Putting the recommendations into practice:

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

DIAGRAM



leukapheresis

IFNα boost

3 tumor antigen Adenovirus

DC

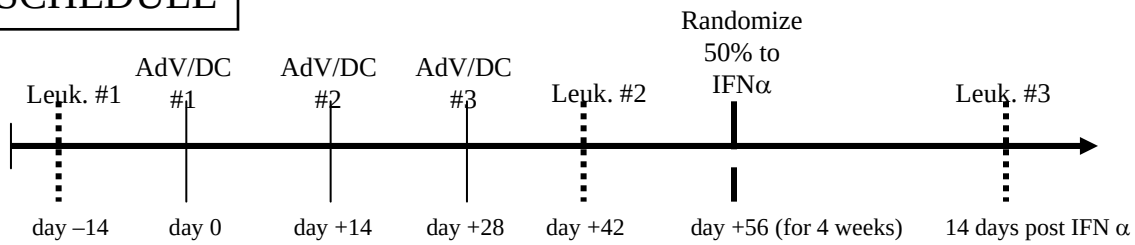
3 vaccines, intra-dermal, 10^7 DC per injection, every other week

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

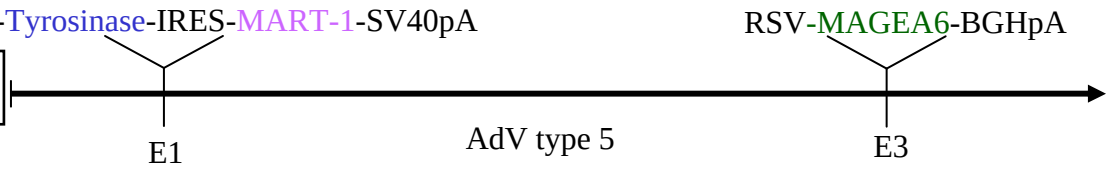
30 Patients Randomized:
1:1 to high dose i.v. IFN α

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

SCHEDULE



VECTOR MAP



Immune Response, Clinical Response and Determinant Spreading

Immune response to the vaccine-encoded antigens can correlate with clinical response, but the magnitude of CD8+ T cells to a specific immunizing peptide rarely defines the clinical responders.

Total *immune score*, *breadth* of immunity and *determinant spreading* may be more critical.

How to strengthen and broaden immunity:

1. Multiple CD8/class I epitopes (dominant and subdominant)
2. Include cognate CD4 helper stimulation
3. Further improve the DC (subtype, maturation)
4. Promote innate immunity (NK cells)
5. Immunize with multiple antigens
6. Boost the immune response (cytokines)

In Vivo Cross-Presentation

A recent study testing a multi-peptide vaccine in renal cancer patients demonstrated improved clinical outcome correlated with **a larger number of peptides** responded to {Walter '12 }. This last observation has been made in previous multi-peptide vaccine trials {Banchereau, '01}.

Recently, **integrated humoral and $CD8^+$ T cell response to the shared, immunogenic cancer/testes antigen NY-ESO-1** was shown to be a significant immunologic measure correlating with clinical outcomes {Yuan '11}.

Several independent groups have correlated to clinical outcome is "epitope spreading" or "**determinant spreading**". This acquisition of T cell and antibody reactivity to shared antigens or epitopes other than those used in a vaccine, has been observed in melanoma {Ranieri '00; Butterfield, '03; Ribas '04; Butterfield '08}, renal cancer {Wierecky '06 } and breast cancer {Disis '02 }.

Immunologic biomarkers as correlates of clinical response to cancer immunotherapy.
There is a need to identify immune biomarkers capable of predicting clinical response……Biomarkers that measure the magnitude of the Type I immune response generated with immune therapy, epitope spreading, and autoimmunity are readily detected in the peripheral blood and, in clinical trials of cancer immunotherapy, have been associated with response to treatment. {Disis, CII, '11}.

In Vivo Cross-Presentation

Immunologic biomarkers as correlates of clinical response to cancer immunotherapy. There is a need to identify immune biomarkers capable of predicting clinical response……Biomarkers that measure the magnitude of the Type I immune response generated with immune therapy, epitope spreading, and autoimmunity are readily detected in the peripheral blood and, in clinical trials of cancer immunotherapy, have been associated with response to treatment. {Disis, CII, '11}.

How should this be assessed?

T cells infiltrating the **tumor** (antigen source)?

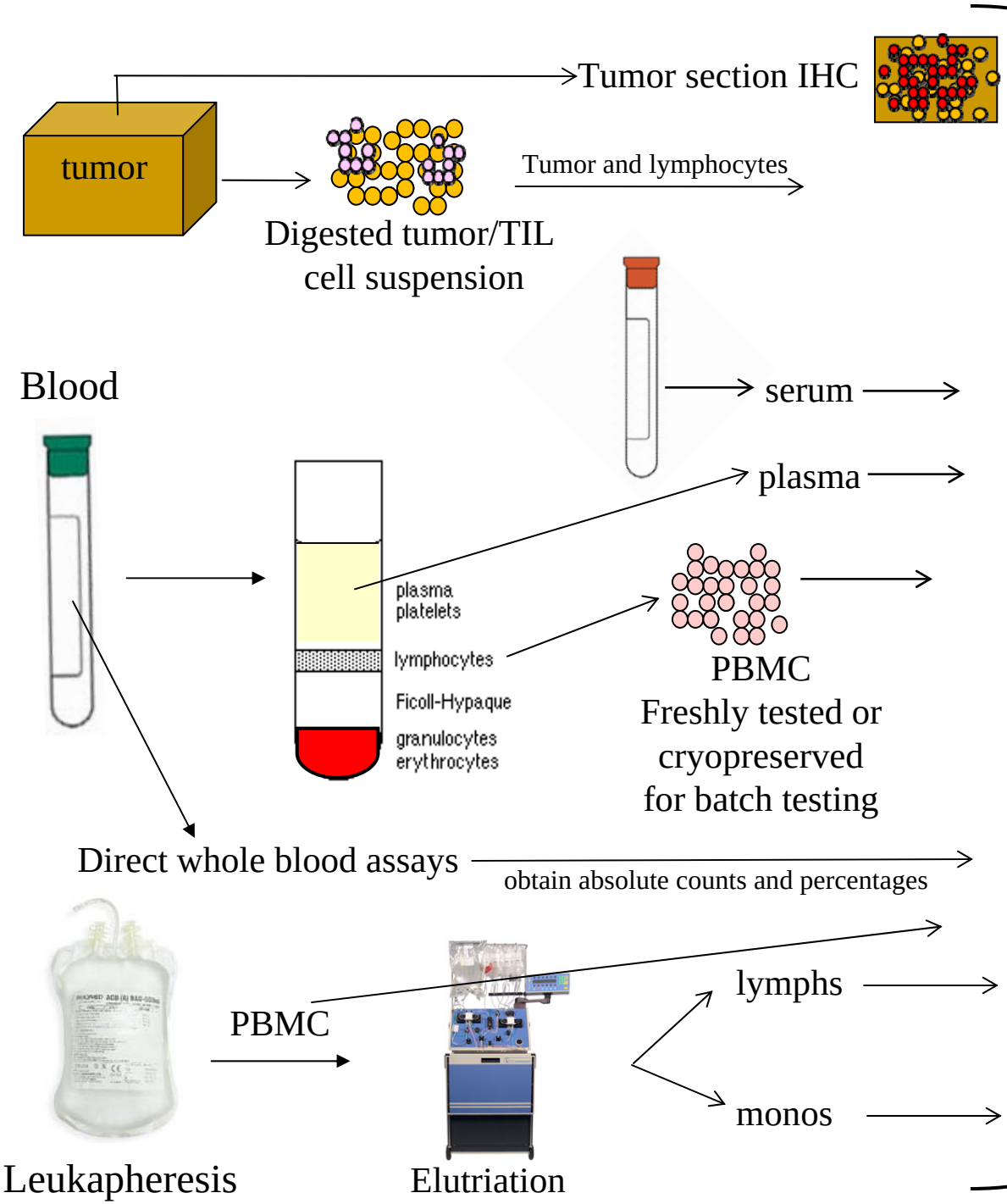
"Inflammed" **tumor** (mRNA/protein)?

Broad antigen recognition (many CD8+ T cell clones activated)?

Functional cellular + humoral immunity induced?

Reduced immune suppression?

Tumor private/mutated antigen responses?



TESTING:

Total lymphocyte subsets

Antigen-specific T cells
(CD4+, CD8+)

Antigen-specific antibodies

NK cells

Myeloid DC
Plasmacytoid DC

Cytokine/chemokines/
growth factors

Treg

MDSC

Frequency, phenotype,
function, activation,
suppression,
expression of key molecules
genetic polymorphisms
RNA expression

AdVTMM2/DC +/- IFNα clinical trial (30 pt.)

a. Clinical outcomes in stage IV patients

b. Immunologic outcomes (blood and TIL; post-vaccine and post-IFNα)
- i. CD8+ T cells specific for vaccine antigens
- ii.CD4+ T cells specific for vaccine antigens
- iii. NK cell activation (flow), infiltration (TIL flow, IHC)
- iv. T cell response to “spreading” antigens: gp100, NY-ESO-1, etc.
- v. T and NK cell responses to autologous tumor (blood and TIL)
- vi. Antibody responses to melanoma antigens
- vii. Treg and MDSC regulatory status

c. AdV responses
- i. CD4+ hexon T cells (frequency, skewing)
- ii. CD4+ and CD8+ T cells to the total viral proteins
- iii. Anti-AdV total antibodies (ELISA)
- iv. Anti-AdV neutralizing antibodies (flow)

AdVTMM2/DC +/- IFNα clinical trial

a. Clinical outcomes in stage IV patients

b. Immunologic outcomes (blood and TIL; post-vaccine and post-IFNα)

primary immune endpoint

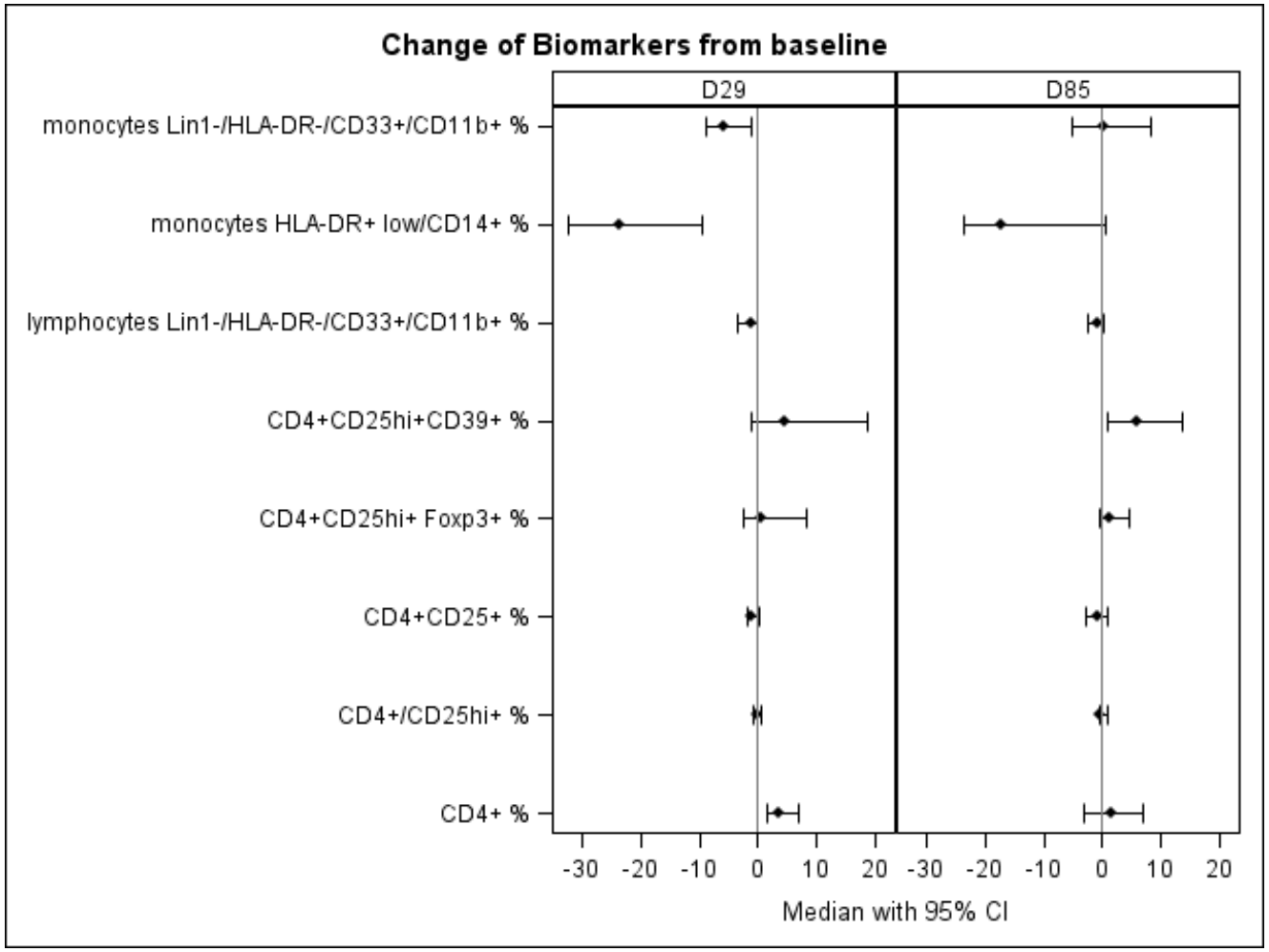
- **i. CD8+ T cells specific for vaccine antigens**
- **ii.CD4+ T cells specific for vaccine antigens**
- iii. NK cell activation (flow), infiltration (TIL flow, IHC)
- **iv. T cell response to "spreading" antigens: gp100, NY-ESO-1, etc**
- v. T and NK cell responses to autologous tumor (blood and TIL)
- vi. Antibody responses to melanoma antigens
- vii. Treg and MDSC regulatory status

"exploratory"
"hypothesis-generating"

c. AdV responses

- i. CD4+ hexon T cells (frequency, skewing)
- ii. CD4+ and CD8+ T cells to the total viral proteins
- iii. Anti-AdV total antibodies (ELISA)
- iv. Anti-AdV neutralizing antibodies (flow)

Exploratory can be important



MDSC

Reduced MDSC correlates with better outcome

Treg

Treg *increasing* in the more effective immunotherapy trial

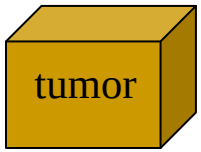
Forest plot of the multicolor flow cytometry data comparing T-reg and MDSC on PBMC at baseline and following treatment (day 29 and day 85) in patients treated with tremelimumab and interferon-α. Tarhini AA, J Immunother., 2012.

Mechanism of Response and Biomarkers of Response

from the AdVTMM2/DC +/- IFNα clinical trial

d. Vaccine Transcriptional Profiling (+/- AdVTMM2)

Compare to standard DC vaccine pheno/cytokine/Ag expression



e. Tumor Transcriptional Profiling (pre, post DC, post IFN)

How are tumors modulated by DC and IFNa therapy?

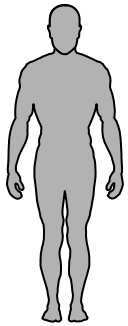
Baseline tumor profile?

f. Peripheral blood signaling

g. Serum profiling (autoimmunity antibodies, LDH, CRP)

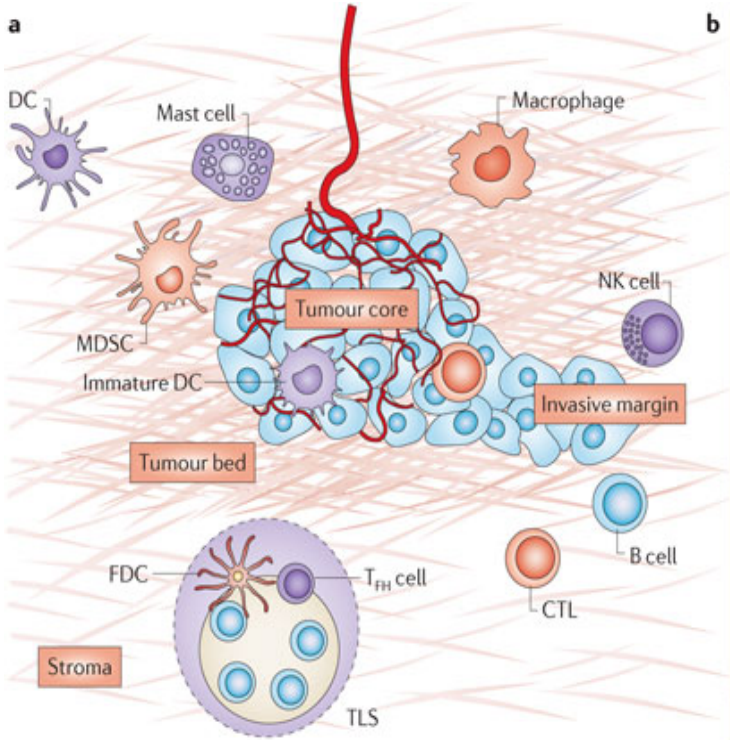
Support previously identified serum biomarkers/autoimmunity?

h. Molecular mimicry with mycoplasma



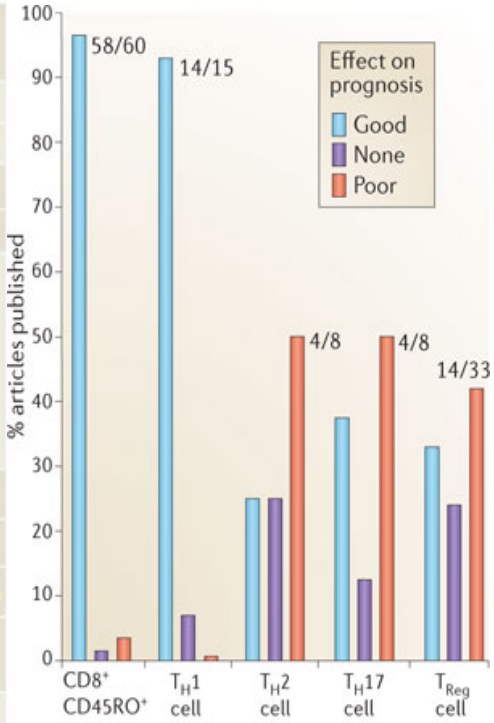
i. Germline DNA SNP analysis

Immune Score



b

Immune contexture	Parameters: positive association with survival
Type	CTLs ($CD3^+CD8^+$)
	Memory T cells ($CD45RO^+$)
Location	Core of the tumour
	Invasive margin
Density	Number of cells per mm^2: 1, 10, 100, 1,000, 10,000 — $CD3^+_{CT}$, $CD3^+_{IM}$, $CD8^+_{CT}$, $CD8^+_{IM}$, $CD45RO^+_{CT}$, $CD45RO^+_{IM}$
Functional orientation	T_H1 cell-associated factors (IFNγ, IL-12, T-bet and IRF1)
	Cytotoxic factors (granzymes, perforin and granulysin)
	Chemokines (CX3CL1, CXCL9, CXCL10, CCL5 and CCL2)
	T_H17 cells, T_{Reg} cells and T_H2 cells have a variable effect on survival, depending on tumour type
TLS	Presence and quality



Nature Reviews | Cancer

Nature Reviews | Cancer

Tumor anatomy showing the features of the immune contexture *J. Galon, W. Fridman; Science, 2006.*

The Immune Score as a New Possible Approach for the Classification of Cancer

Jérôme Galon[1,2,3,4,5*], Franck Pagès[1,2,3,4], Francesco M Marincola[5,6], Magdalena Thurin[7], Giorgio Trinchieri[8], Bernard A Fox[5,9,10], Thomas F Gajewski[5,11] and Paolo A Ascierto[12,13] **Journal of Translational Medicine 2012**

Immunologic Monitoring and Cellular Products Laboratory

- The Cellular Products Laboratory (CPL) is dedicated to preparation of products for tumor vaccines and for cellular and gene therapy of cancer.
  - vaccine preparation
  - sterility and safety evaluations of generated products
  - assists in the preparation of INDs
  - operates according to FDA criteria for current good manufacturing practice (cGMP).
- The Immunologic Monitoring Laboratory (IML) is responsible for serial monitoring of immunologic functions in patients with cancer.
  - state-of-the-art immunologic assays
  - rigorous quality control program
  - development of new assays
  - advice on test selection and result interpretation
  - The Tissue Procurement Facility (TPF) provides tissue and blood banking support under current Good Tissue Practice (cGTP) criteria.

CLIA certified; inspected by CAP and the state of PA; registered with FDA (3004571535)

FDA Master file: BB-MF-12244; FACT accredited

UPMC LIFE CHANGING MEDICINE

Services

- Specimen intake, processing, banking
- Cell bank preparation
- Culture of peptide-specific T cells
- Culture of tumor infiltrating lymphocytes (TIL)
- DC preparation (peripheral blood, stem cells)
- Gene transfer: any human cells
- Expansion of stem cells
- Vaccine production for intra-tumor,-nodal, -lymphatic or intra-venous administration
- Peptide lyophilization, testing
- Adenovirus production (not fully GMP, with Vector Core)

- Assay standardization, normal control ranges
- Mixed lymphocyte-tumor cultures
- Cytotoxicity (^{51}Cr-release, FLOCA, CD107a).
- Proliferation (^{3}H-thymidine and CFSE)
- Multiparameter flow cytometry (effectors, regulatory (Treg, MDSC) cells)
- Single-cell T-cell assays (ELISPOT, CFC, and tetramer)
- Signaling molecules
- Frequency of apoptotic T cells
- Anti-tumor antibodies
- Cytokine/chemokine analysis (Luminex, ELISA)
- Data entry and analysis

UPMC LIFE CHANGING MEDICINE

Standard Operating Procedures (SOPs)

UNIVERSITY OF PITTSBURGH CANCER INSTITUTE
IMMUNOLOGIC MONITORING AND CELLULAR PRODUCTS LABORATORY

PROCEDURE FOR VIRUS INFECTIVITY NEUTRALIZATION ASSAY

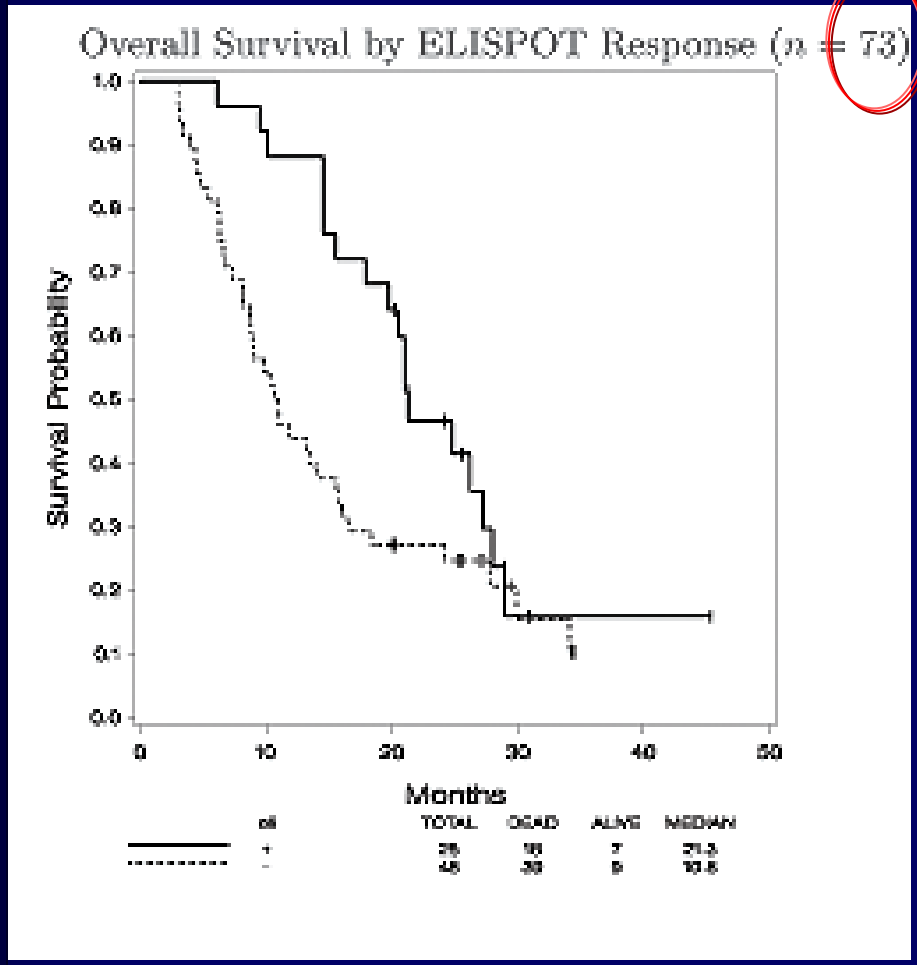
Purpose and Scope

The purpose of this procedure is to test serum for the presence of antibodies to adenovirus which are capable of neutralizing viral infectivity. The assay tests decreasing dilutions of serum for the ability to reduce the efficiency by which an easily transduced cell line (A549) can be transduced with adenovirus encoding eGFP (AdVeGFP). The virus makes A549 cells GFP-positive, which is detected by flow cytometry. Serum samples with high levels of neutralizing antibodies will be able to reduce AdveGFP transduction of A549 cells, even after many serial dilutions.

This procedure is designed to quantitatively measure the inhibition of adenoviral transduction by serotype-specific neutralizing antibodies that are present in the patient serum. The serum dilutions for this assay are 1:4, 1:8, 1:16, 1:32, 1:64, 1:128, 1:256 and 1:512 in addition to a negative control (A549 cells + medium) and a positive control (A549 cells + virus + medium).

Immune Response Correlates with Overall Survival

Multiple melanoma antigen peptide vaccine ± GM-CSF ± IFNα2b

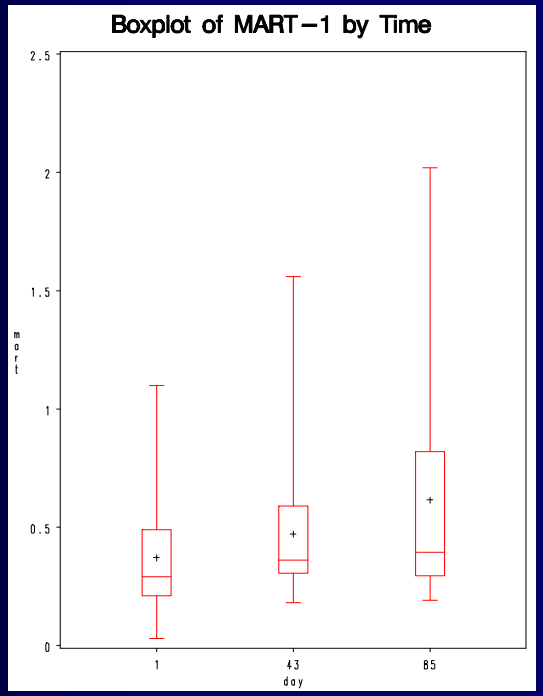


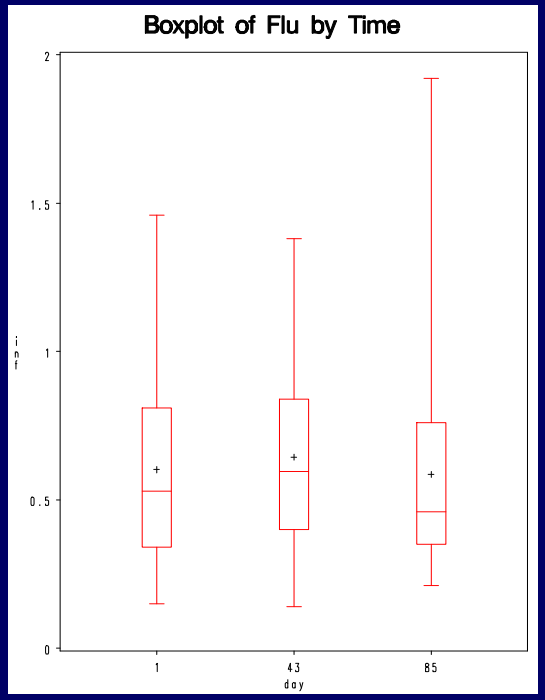
The Kaplan-Meier plot for OS by immune response status is shown for E1696 (Phase II).

There was a significant difference in OS by immune response status. Immune responders lived longer than the non-immune responders (median OS 21.3 versus 10.8 months, *p*=0.033).

(Kirkwood, J.M., Clin. Cancer Res. 2009)

Immune Response: E1696
Melanoma antigen peptide-specific CD8+ T cells





%MART-1 CD8+ cells:	.29%	.36%	.39%	.53%	.53%	.43%
%effector cells:	10%	16%	18%	17%	17%	16%
		(p=0.048)			(p = ns)	

MHC Tetramer Analysis:

The frequency of vaccine peptide-specific CD8+ T cells was measured by MHC tetramers, showing *significant increases for all 3 melanoma antigen peptides* (not Flu).

The MART-1 and gp100-specific cells *differentiated towards effector cells* with vaccination.

Schaefer, Int. J. Cancer, 2011

AdVTMM2/DC +/- IFNα trial pt. #1 DC Vaccine

•Vaccine Phenotype:

CPL#	HLA-DR+	CD86+	CD80+	CD83+	CCR7+	CD40+	CD11c+
CPL-12-19	98%	95%	93%	88%	28%	95%	96%

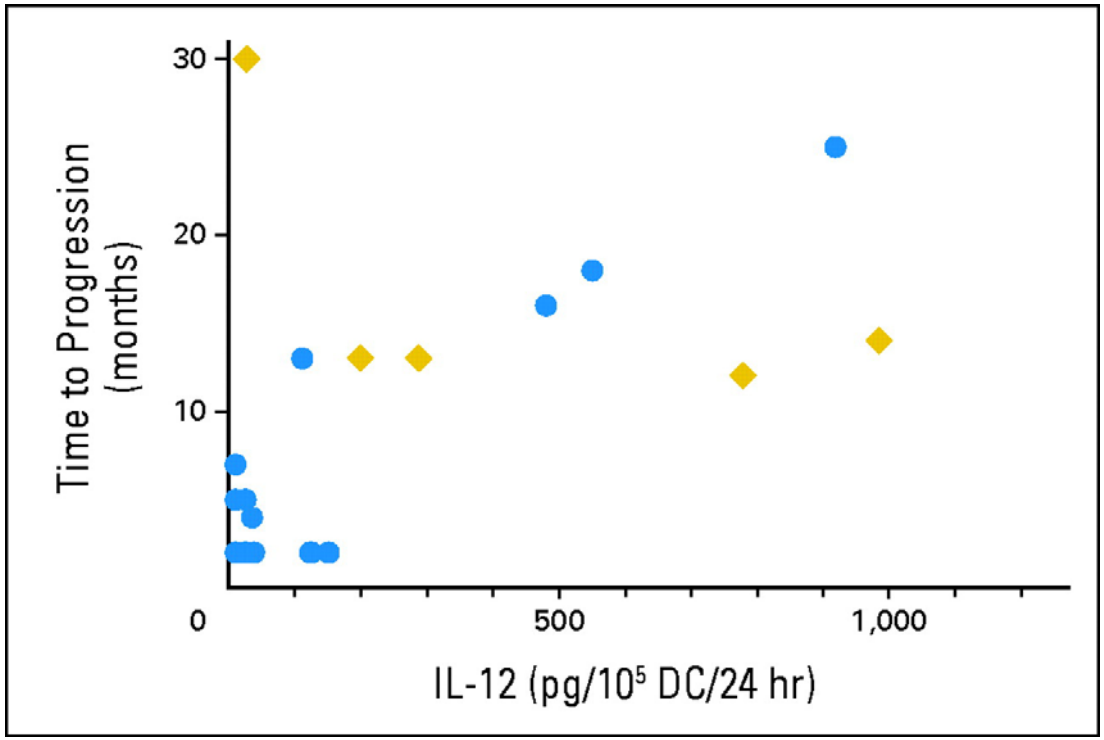
CPL#	SAMPLE	Intracellular: MART-1	TYROSINASE	MAGE-A6
CPL-12-19	mDC (control)	0	2	4
	prematAdVTMM2/DC (vaccine)	6%+	18%+	10%+

•IL-12p70/IL-10 production by vaccine:

IL-12p70	HD DC	Pt. mDC	Pt. AdV/DC vaccine		IL-10	HD DC	Pt. mDC	Pt. AdV/DC vaccine
DC only	0	2430	**1237**			0	8	**0**
DC+CD40L	323	5551	**4024**			505	130	**84**
DC+LPS	99	2840	**1344**			285	23	**19**
DC+CD40L+LPS	877	6102	**3838**			1043	411	**268**

•Immature DC, matDC, premat/AdV/DC (vaccine): *frozen for mRNA per SOP*

DC Vaccine IL-12p70 Production and Clinical Outcome



IL-12p70 production levels positively correlated with time to progression. *P* .0255 is based on Cox regression followed by likelihood-ratio test. Circles indicate patients who have already experienced disease progression; diamonds represent patients who have not experienced recurrence to date (Okada, H. JCO 2010). Assay standardization (Butterfield, JIT 2008).

Anti-CTLA-4, Anti-PD-1/PD-L1

- Absolute lymphocyte count (ALC) >1,000, increased ALC
- Serum LDH, CRP, IL-17
- Treg
- MDSC
- NY-ESO-1 cellular and humoral response
- Tumor IDO
- Tumor TIL
- Tumor PD-L1
- Ki67/EOMES in CD4+/CD8+ T cells
- Exhausted T cells (PD-1/TIM3/LAG-3)

Ascierto, Kalos, Schaer, Callahan & Wolchok CCR 2013

In Vivo Cross-Presentation

How should this be assessed?

T cells infiltrating the **tumor** (antigen source)?

"Inflammed" **tumor** (mRNA/protein)?

tumor

Broad antigen recognition (many CD8+ T cell clones activated)?

Functional cellular + humoral immunity induced?

Tumor private/mutated antigen responses?

effectors

Reduced immune suppression?

suppression

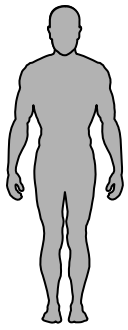
Recommendations:

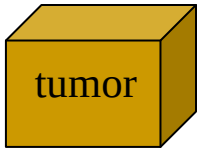
Variability:

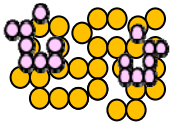
1. Patient
2. Blood draw
3. Processing/cryo/thaw
4. Cellular product
5. Assay choice
6. Assay conduct
7. Assay analysis
8. Data reporting
9. Next cool new assay

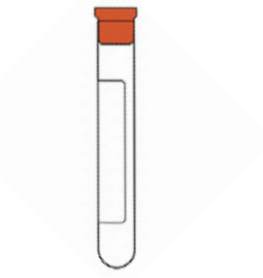
Recommendations:

1. Save DNA/RNA/cells/tumor; include healthy donor control
2. Standardized procedures
3. Standardized procedures
4. Functional assays to characterize/develop potency
5. Standardized, functional
6. SOP
7. Appropriate biostatistical methods
8. Full details, controls, QA
9. Sufficient blood/tissue to interrogate the samples *now*, as well as *later*, to generate new hypotheses.









Acknowledgements

SITC Immunotherapy Biomarkers Taskforce

UPCI/Butterfield Lab

Lazar Vujanovic, Ph.D. , Angela Pardee, Ph.D.

Jian Shi, M.D., Hadas Prag Naveh, M.D.

Univ. Pittsburgh Center for Biological Imaging (S. Watkins),Vector Core (A. Gambotto), Immunologic Monitoring and Cellular Products Lab

University of Pittsburgh Cancer Institute

NIH RO1 CA104524, NIH RO1 CA 138635

NIH 1P50 CA121973

(Kirkwood, J.M., PI, Skin SPORE)

The Pittsburgh Foundation

The Hillman Foundation