Other Topics in Biomarkers Discussion

- Moderator: Adrian Bot, MD, PhD – Kite Pharma, Inc.
- Blood-Based Markers: Michael D. Kalos, PhD – Eli Lilly and Company
- Tissue Markers: Naiyer Rizvi, MD – Columbia University Medical Center

Blood-based Biomarkers in Immuno-oncology

Michael Kalos, PhD
Chief Scientific Officer, Cancer Immunobiology
Eli Lilly and Company

SITC annual meeting
Biomarker debate
November 8, 2015, Bethesda, MD

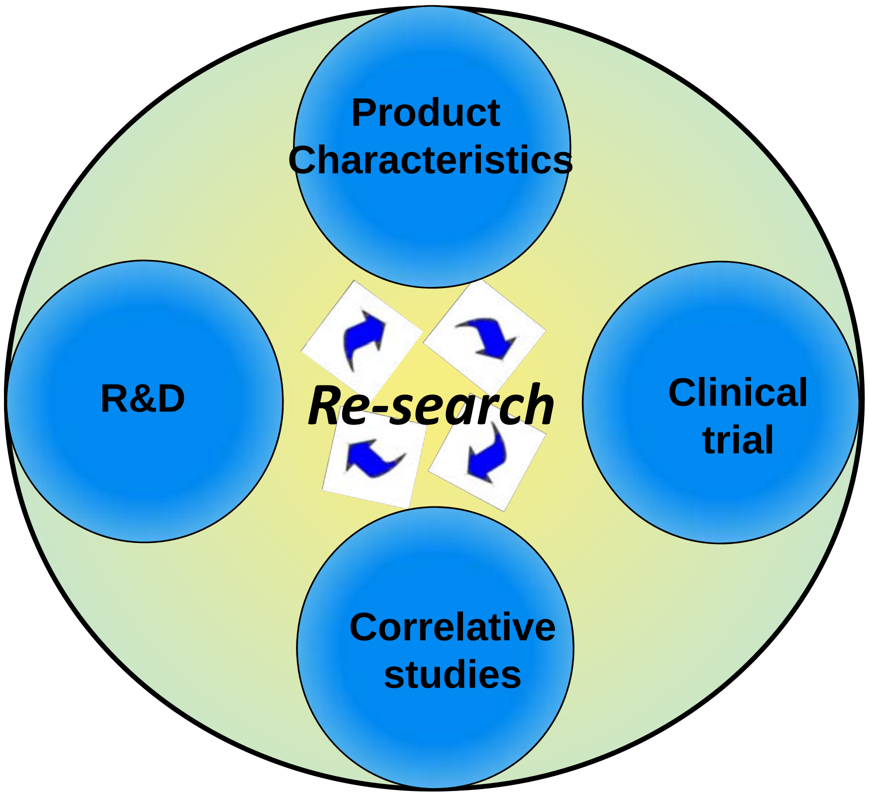
COI Disclosure Information

I have the following relevant financial relationships to disclose:

- Patents and potential royalties from Novartis Pharmaceuticals-CAR technology
- Scientific Advisory Board member (stock grants) for Adaptive Biotechnologies (ex)
- Eli Lilly and Company- Employment (current)

Biomarkers Drive The Translational and Clinical Research Engine

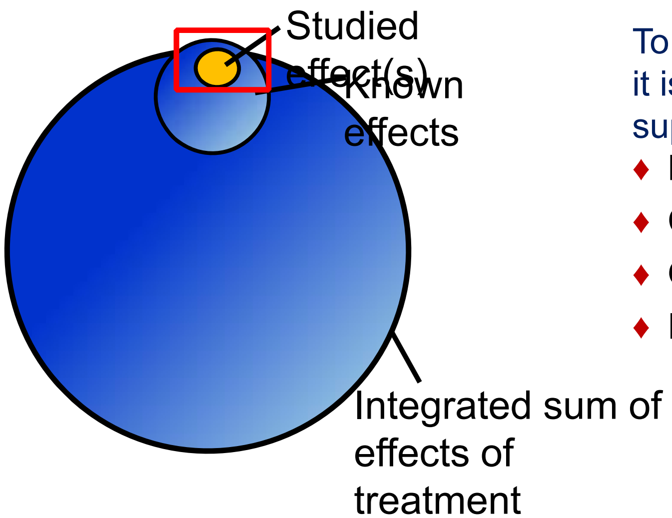
- Biological effects from in -vitro and in-vivo models
- Pharmacodynamic and biophysical characteristics of molecule
- Patient genetics and biology, clinical measurements
- Clinical correlative studies



- ➢ Understand tailoring
- ➢ Understand pharmacodynamics, activity, and toxicity
- ➢ Understand MOA and efficacy
- ➢ Develop surrogate endpoints

Critical issues in Biomarker research

Our ability to define and implement appropriate hypothesis focused biomarker and tailoring strategies is compromised by our lack of a comprehensive understanding of the biological effects of the therapeutic agents on the immune system and the tumor milieu. Accordingly, ***hypothesis testing alone is inadequate as a strategy***



To develop appropriate and relevant tailoring strategies it is imperative to build infrastructure and commit to support:

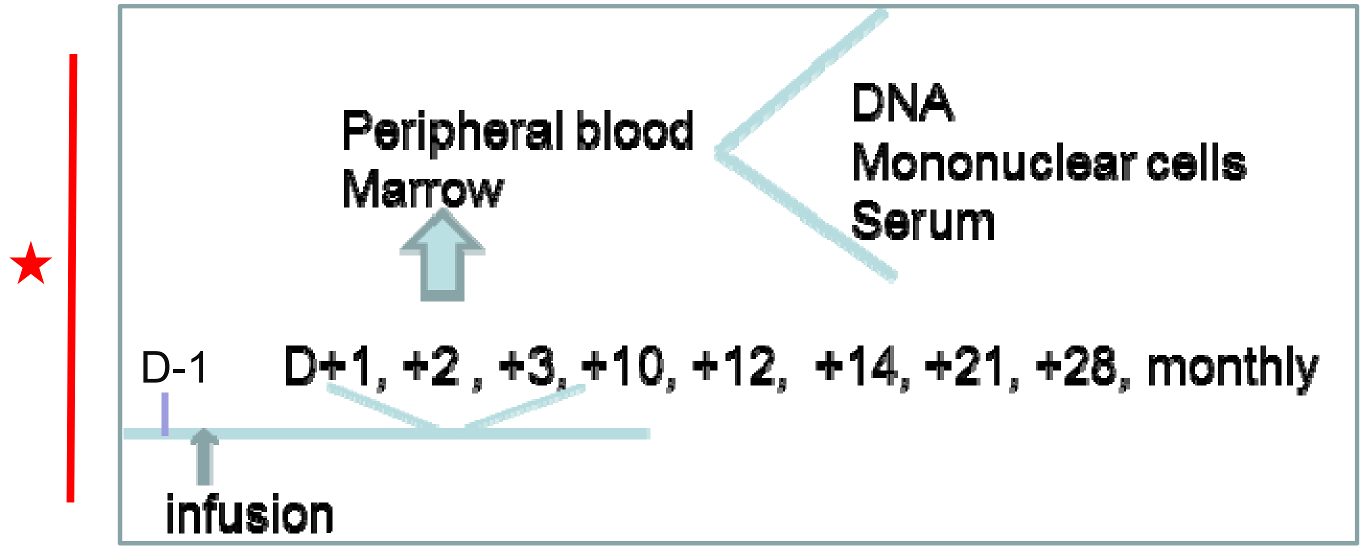
- Robust sampling strategies, including biopsy tissues
- Comprehensiveness- hypothesis generating science
- Objective Quality
- Integrated and systematic meta-analysis

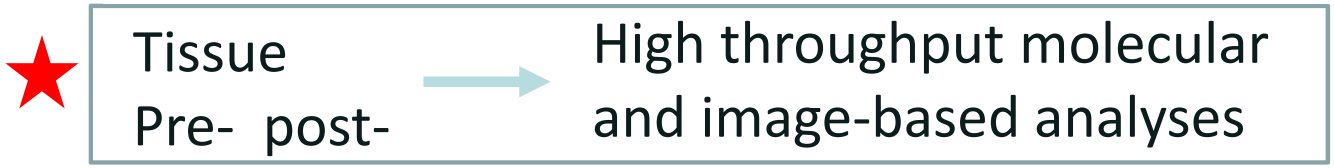
Kalos, M. J. Trans Med 2010, 2011

Biomarker strategies in the 21st century

Comprehensive biomarker strategy required to enable mechanistic insights to guide rational clinical development

- Temporal kinetics of activity must be captured





What is the value for blood-based biomarkers in Immuno-oncology?

Reasonably well-established that a principle site for biomarker interrogation is the tumor: "Where the action is"

Beyond studying blood-borne cancers, what can blood-based analyses tell us in immunooncology?

Blood-based testing can inform about:

- Systemic consequences of activity at tumor site
- Temporal modulation of subsets of relevant cells
- Pharmacodynamic measures of drug half-life and activity

High-throughput and comprehensive platforms to evaluate blood-based biomarkers

Potential samples and available platforms

- **Whole blood**:
  - Flow cytometry, biochemical and biophysical platforms
- **Isolated cell subsets**:
  - Flow cytometry
- **Plasma/serum**:
  - Luminex, mesoscale, protoarray
- **Nucleic acid**:
  - Nanostring, quantigene, whole-exome sequencing, TCR sequencing
- **Circulating tumor cells**:
  - Flow cytometry, nucleic acid-based
- **Subcellular particles (exosomes, etc.)**:
  - Molecular characterization

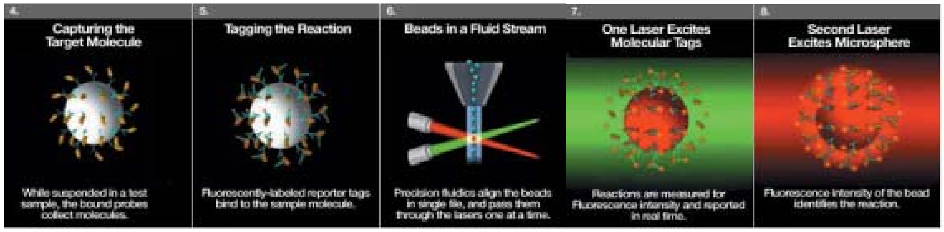
Categories and attributes of T cell biomarkers

Category`	Platforms	Assay	Advantages	Disadvantages
Presence	Flow cytometry	Surface marker detection	Individual cells detected	Sample intensive Low sensitivity Specific detection reagent
	PCR	Transgene-specific amplification	High sensitivity	Bulk analysis
	Deep sequencing	Detection of specific TcR clonotypes	Extremely high sensitivity	Technology intensive

Kalos, M. J. Transl. Med. 2011

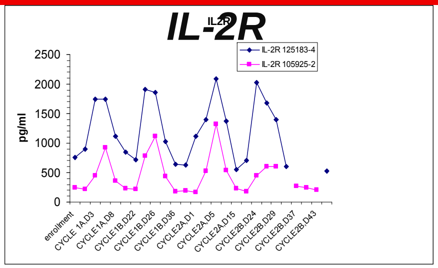
Lilly

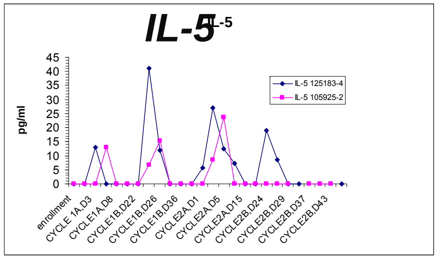
Multiparameter bead array (Luminex)

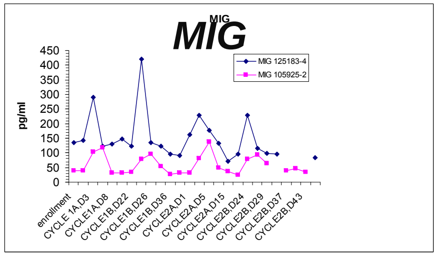


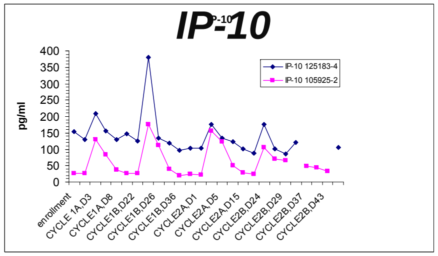
- Advantages:
  - ✓ High on the comprehensiveness scale
  - ✓Minimal sample volumes required
  - ✓ Robust and quantitative platform
- Applications
  - ➢ Soluble factors
  - ➢ Phosphoproteins
  - ➢ Nucleic acid

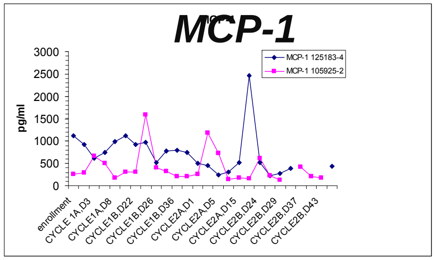
Multiplex cytokine analysis reveals unpredicted patterns of systemic cytokine modulation in models systems

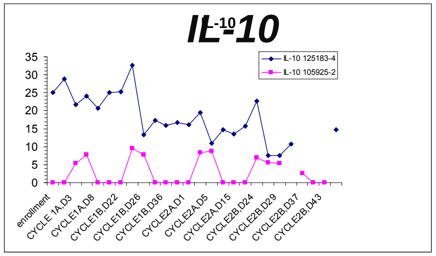


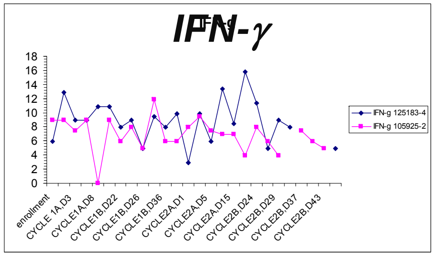








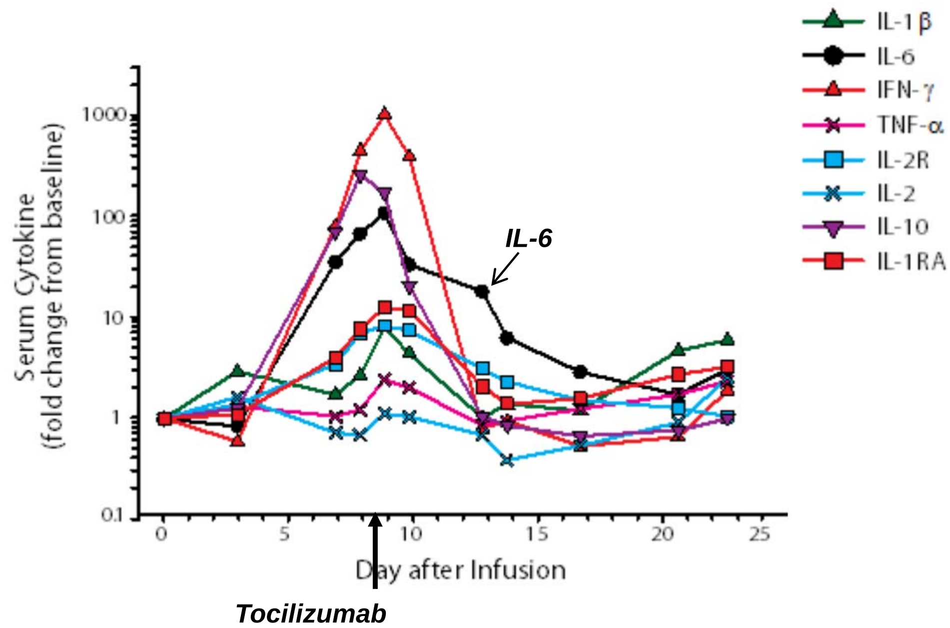




Lilly

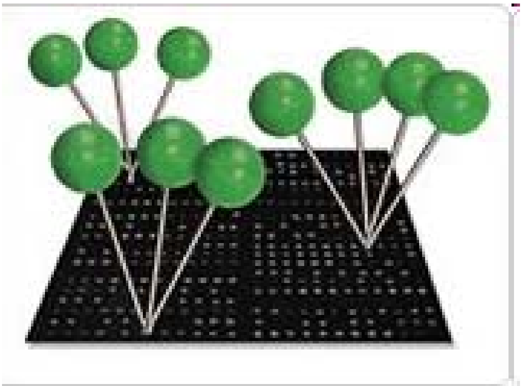
Agnostic cytokine analysis reveals unexpected elevations in IL-6 and drives development of a new treatment paradigm

On-Target Delayed Cytokine Release Syndrome following CART-19 therapy is mitigated by anti IL-6 therapy



Grupp S. , Kalos, M. et al NEJM 2013

Seromics- Invitrogen Protoarray



- Over 10,000 full-length human proteins displayed on array chip
- Proteins expressed by baculovirus expression system as GST fusions
- Proteins are purified under non-denaturing conditions and printed to preserve native protein structure
- Arrays probed with sera from patients to identify autoantibodies that develop during treatment

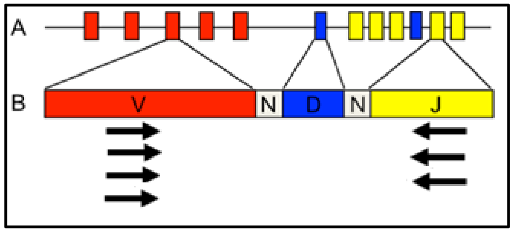
Protoarray analysis provides evidence for epitope spreading following CART therapy

Seromics: Protoarray analysis of serum samples from pancreatic cancer patient against 10,000 human proteins

			Pre-	day+44	
Database ID	**Ultimate ORF ID**	**Description**	**Intensity**	**Intensity**	**Ratio post/pre**
BC003548.1	IOH4864	polymerase (DNA directed), lambda (POLL)	564	62,437	110.70
NM_015129.3	IOH27517	septin 6 (SEPT6), transcript variant II	431	28,447	66.08
NM_003677.3	IOH56971	Density-regulated protein	431	18,521	43.02
NM_145802.1	IOH14040	septin 6 (SEPT6), transcript variant V	431	12,692	29.48
NM_033003.1	IOH5665	general transcription factor II, i (GTF2I), transcript variant 4	654	18,769	28.70
NM_053031.2	IOH59941	Myosin light chain kinase, smooth muscle	430	10,117	23.50
NM_015927.2	IOH3924	transforming growth factor beta 1 induced transcript 1 (TGFB1I1), transcript variant 2	687	15,098	21.98
NM_000431.1	IOH10122	Mevalonate kinase	2,517	49,352	19.61
NM_003315.1	IOH14566	DnaJ (Hsp40) homolog, subfamily C, member 7 (DNAJC7)	430	7,733	17.96
NM_006759.3	IOH26550	UDP-glucose pyrophosphorylase 2 (UGP2), transcript variant 1	697	12,385	17.78
XM_376764.2	IOH40703	paraneoplastic antigen MA2 (PNMA2)	1,759	27,277	15.51
NM_016954.2	IOH46151	T-box 22 (TBX22), transcript variant 2	430	6,653	15.45
BC012899.1	IOH11155	sialidase 4 (NEU4)	636	9,721	15.28
BC036846.1	IOH28739	protease, serine, 33 (PRSS33)	899	13,007	14.48
BC007637.1	IOH6973	chromosome 1 open reading frame 94 (C1orf94)	950	10,953	11.54
NM_024825.2	IOH29237	podocan-like 1, mRNA (cDNA clone MGC:71618 IMAGE:30347370), complete cds	430	4,865	11.30
BC000525.1	IOH3627	glutamic-oxaloacetic transaminase 2, mitochondrial (aspartate aminotransferase 2) (GOT2)	6,105	62,995	10.47
BC007560.1	IOH6825	LIM and SH3 protein 1 (LASP1)	431	4,295	10.00

Beatty G. Haas A. et al. Cancer Immunol. Res. 2014

Immune cell diversity profiling-Deep sequencing



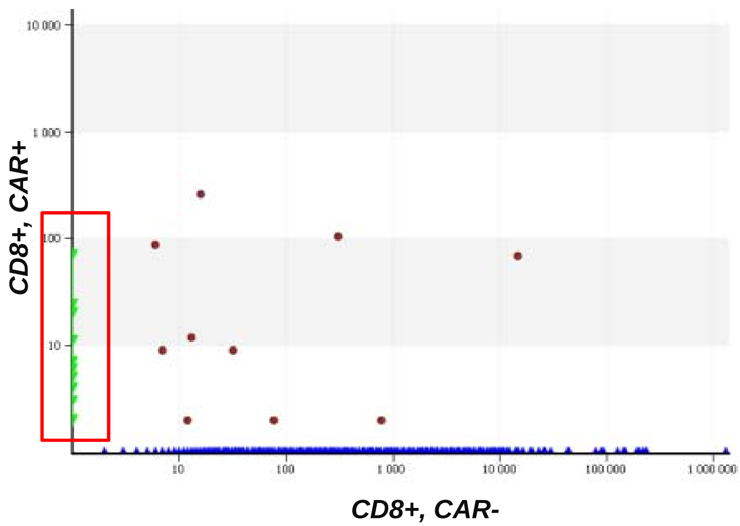
Adaptive Biotechnologies, Inc

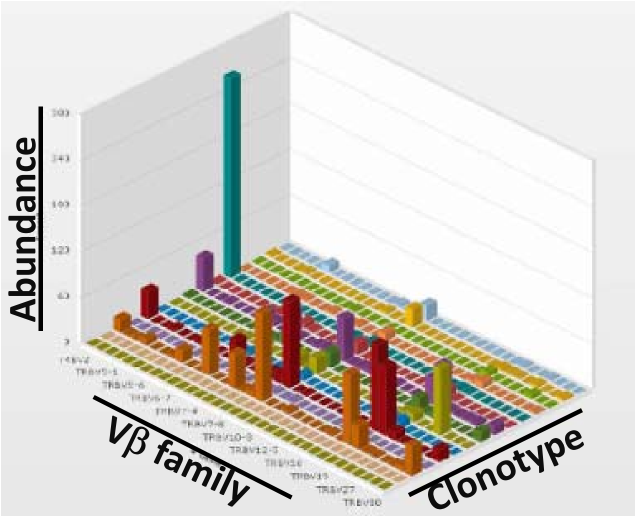
- Illumina HySEQ compatibe sequencing using multiplex PCR with primers to all known V and J segments
- Amplification of rearranged TcRβ CDR3 and IgH sequences
- Custom software to verify, align, catalogue, and quantify individual sequences
- Compatible with genomic DNA
- Provides integrated and quantitative snapshot of T and B cell diversity and abundance

Deep sequencing reveals a diverse population of persisting gene-modified T cells post immunotherapy

6 month post infusion sample, sorted on CAR19+ and negative CD8+ cells

CD8+ CART19 cells





Adaptive Biotechnologies, Seattle, WA

9 clonotypes >1%
c.a. 20 clonotypes >0.1%

Complete responses are associated with deep molecular remissions: IGH deep seqencing

Patient	Tissue	timepoint	Cell equivalents	total productive reads	Total unique sequences	Total tumor reads	tumor clone frequency
UPN01	Blood	-1	158, 730	408,579	48	407,592	99.8
		28	158, 730	0	0	0	0
		176	79,365	285,305	7362	0	0
	Marrow	28	158,730	0	0	0	0
		176	158,730	202,535	4451	0	0
		720	279,924	261	13	0	0
UPN02	Blood	-1	61,270	1,385,340	4,534	1,231,018	88.9
		31	158, 730	0	0	0	0
		176	317,460	0	0	0	0
	Marrow	31	277,778	0	0	0	0
		176	158730	0	0	0	0
		741	222,019	707	29	0	0
CHP959-100	Blood	-1	111,340	189	6	185	97.88
		23	218,210	0	0	0	0
		87	288,152	0	0	0	0
		180	420,571	6	2	0	0
	Marrow	-1	317,460	59,791	318	59,774	99.97
		23	362,819	37	2	33	89.19
		87	645,333	10	1	10	100
		180	952,381	45	7	0	0
CHP959-101	Blood	-1	152,584	38,170	52	30,425	79.71
		23	417,371	92	5	18	19.6
	Marrow	-1	158,730	68,368	65	50,887	74.43
		23	305,067	1,414	11	946	66.9
		60	916,571	530,833	206	363,736	68.9

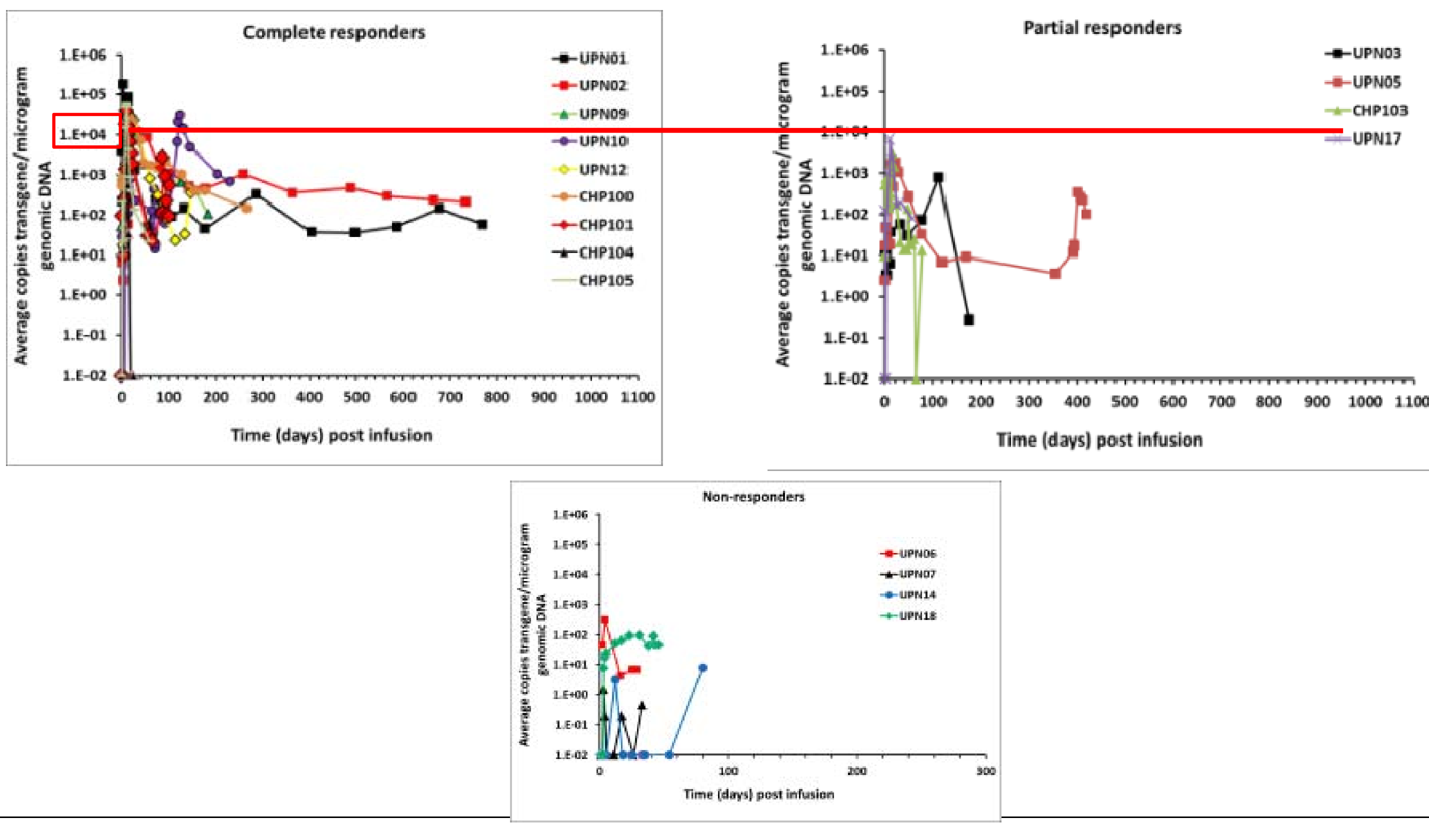
Potential solid tumor applications?

Kalos et al. Sci TM 2011
Grupp, Kalos, et al NEJM 2013

Potential companion diagnostic- Higher levels of peripheral CTL019 cells detected in complete responders

Q-PCR analysis

CTL019 cells/microgram genomic DNA



Porter et al. Sci TM, 2015

Blood-based biomarker studies can provide important insights for immunotherapy-based studies

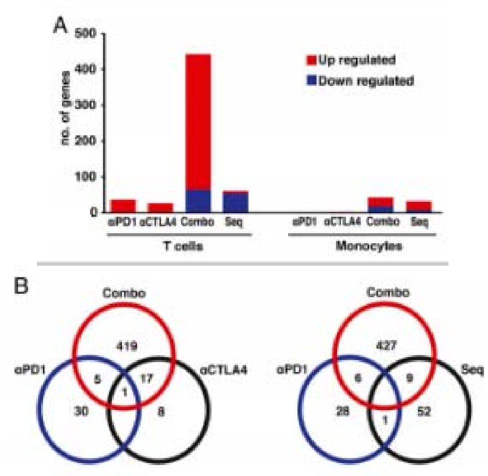
MOA insights: Treatment and combination-unique signatures detected in peripheral blood by exome sequencing

Combination Therapy with Anti–CTLA-4 and Anti–PD-1 Leads to Distinct Immunologic Changes In Vivo

Rituparna Das,[*,†] Rakesh Verma,[*,†] Mario Sznol,[*,†] Chandra Sekhar Boddupalli,[*,†] Scott N. Gettinger,[*,†] Harriet Kluger,[*,†] Margaret Callahan,[‡] Jedd D. Wolchok,[‡] Ruth Halaban,[§] Madhav V. Dhodapkar,[*,†] and Kavita M. Dhodapkar[*,†,¶]

Journal of Immunology, 2015

Affymetrix GeneChip Human Transcriptome 2.0 exon array



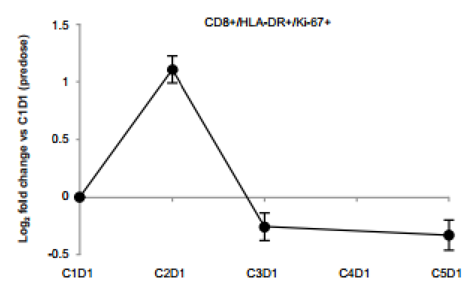
PD measures: Transient proliferative response observed in CD8 cells observedpost anti-PDL1 therapy

LETTER

doi:10.1038/nature14011

Predictive correlates of response to the anti-PD-L1 antibody MPDL3280A in cancer patients

Roy S. Herbst[1], Jean-Charles Soria[2], Marcin Kowanetz[3], Gregg D. Fine[3], Omid Hamid[4], Michael S. Gordon[5], Jeffery A. Sosman[6], David F. McDermott[7], John D. Powderly[8], Scott N. Gettinger[1], Holbrook E. K. Kohrt[9], Leora Horn[10], Donald P. Lawrence[11], Sandra Rost[3], Maya Leabman[3], Yuanyuan Xiao[3], Ahmad Mokatrin[3], Hartmut Koeppen[3], Priti S. Hegde[3], Ira Mellman[3], Daniel S. Chen[3] & F. Stephen Hodi[12]



Summary/Conclusions

- **Successful development and implementation of biomarker studies requires:**
  - **Robust sampling schemes to capture temporal kinetics of modulation**
  - **High throughput and broad assays that enable hypothesis generating insights**
  - **Quality-supporting infrastructure**
  - **Ability to support integrated meta-analysis of data**
- **Rationally designed blood-based biomarkers play an important role in the clinical development of immuno-oncology programs**