

Comprehensive Biomarkers on Immunotherapy Clinical Trials: A Longitudinal Approach

***SITC Immuno-Oncology Biomarkers: State-of-the-Art
San Francisco, CA
May 16th, 2018***



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Disclosures

- **Advisory Board/Speaker:** Genentech/Roche, Bristol-Myers Squibb, Bayer, Boehringer Ingelheim, Medscape, Astra Zeneca/Medimmune, Pfizer, Ariad, HTG Molecular, Asuragen, Merck.
- **Research support:** Genentech, Oncoplex, HTG Molecular, DepArray, Merck, NCCN/Bristol-Myers Squibb, GlaxoSmithKlein, Medimmune, Adaptive, Adaptimmune, EMD Serono, Pfizer, Takeda, Amgen, Karus.

Immune Profiling and Monitoring Clinical Trials Settings

- **Advanced Tumors:**
 - Target analysis
 - Biological mechanism
 - Biomarkers of response and toxicity
- **Surgically Resectable Tumors:**
 - Neo-adjuvant and adjuvant approaches
- **Premalignant Lesions:**
 - Immune-chemoprevention on high-risk lesions

Developing Markers for Immunotherapy

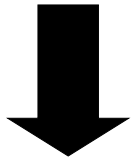
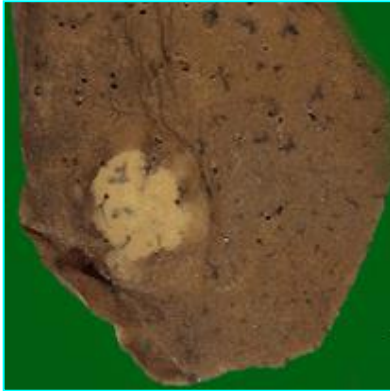
Category	Assay	Specimen
Immune cells characterization	Immunohistochemistry*/** (image analysis)	Tissue
	Immunofluorescence (multiplex; image analysis)**	Tissue
	Flow cytometry** (panels)	Tissue and blood
	MIBI and CyTOF (panels)	Tissue and blood
Functional assessments	Flow cytometry detection of T cell activation**	Tissue and blood
	ELISPOT	Blood
	Neo-antigen prediction (from WES and RNA-seq)	Tissue
	TCR** and BCR** sequencing	Tissue and blood
Host factors	Cytokine analyses (MSD**, Luminex**, ELISA**)	Blood
	Microbiome (16S deep sequencing)	Stool (others)
Tumor and malignant cells genomics	Next generation sequencing (WES, RNA-seq, targeted*/**)	Tissue
	Low-input gene expression signatures (Nanostring**, HTG-Edge Seq**, Affymetrix arrays)	Tissue (FFPE)
	Liquid biopsy (cfDNA*/**, exosomes, CTC)	Blood

*Assays/platforms available at CLIA-certified laboratories

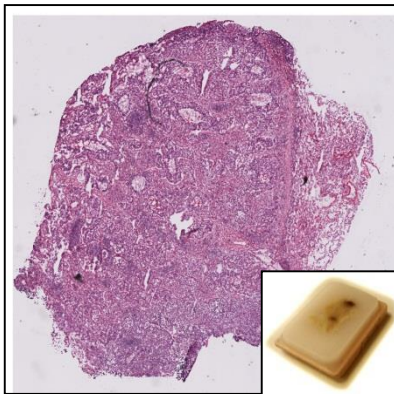
** Analytical validation (non-CLIA).

Types of Tumor Specimens In Lung Cancer

Surgical Resection



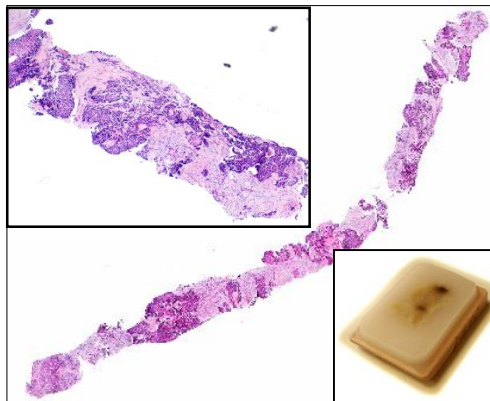
Histology



Advanced Tumor

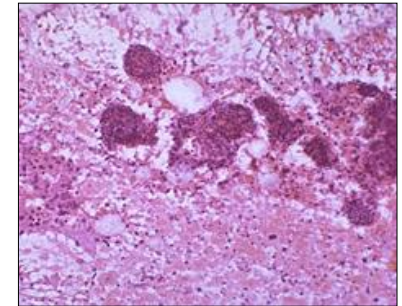


Core Needle Biopsy (CNB)



Formalin-fixed and
Paraffin-embedded (FFPE)

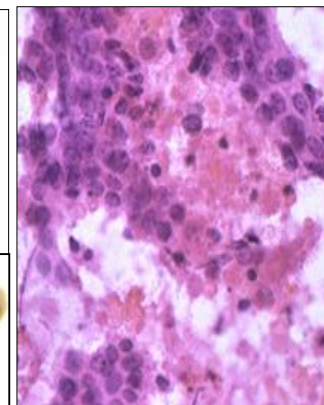
Endobronchial Ultrasound (EBUS) or Pleural Fluid



Alcohol-fixed



Fine Needle Aspiration (FNA)



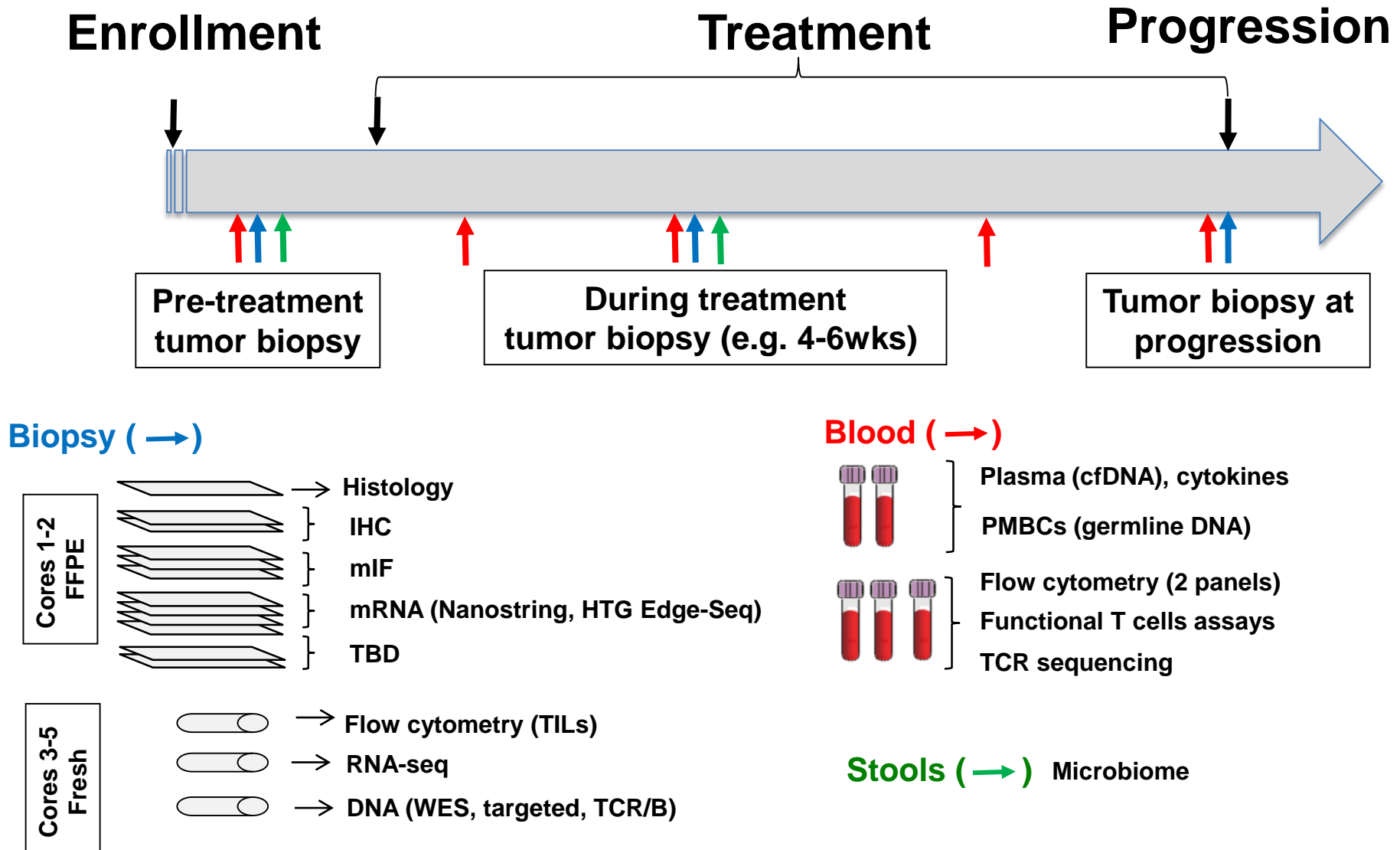
Alcohol-fixed



Alcohol-fixed -
Cell Block

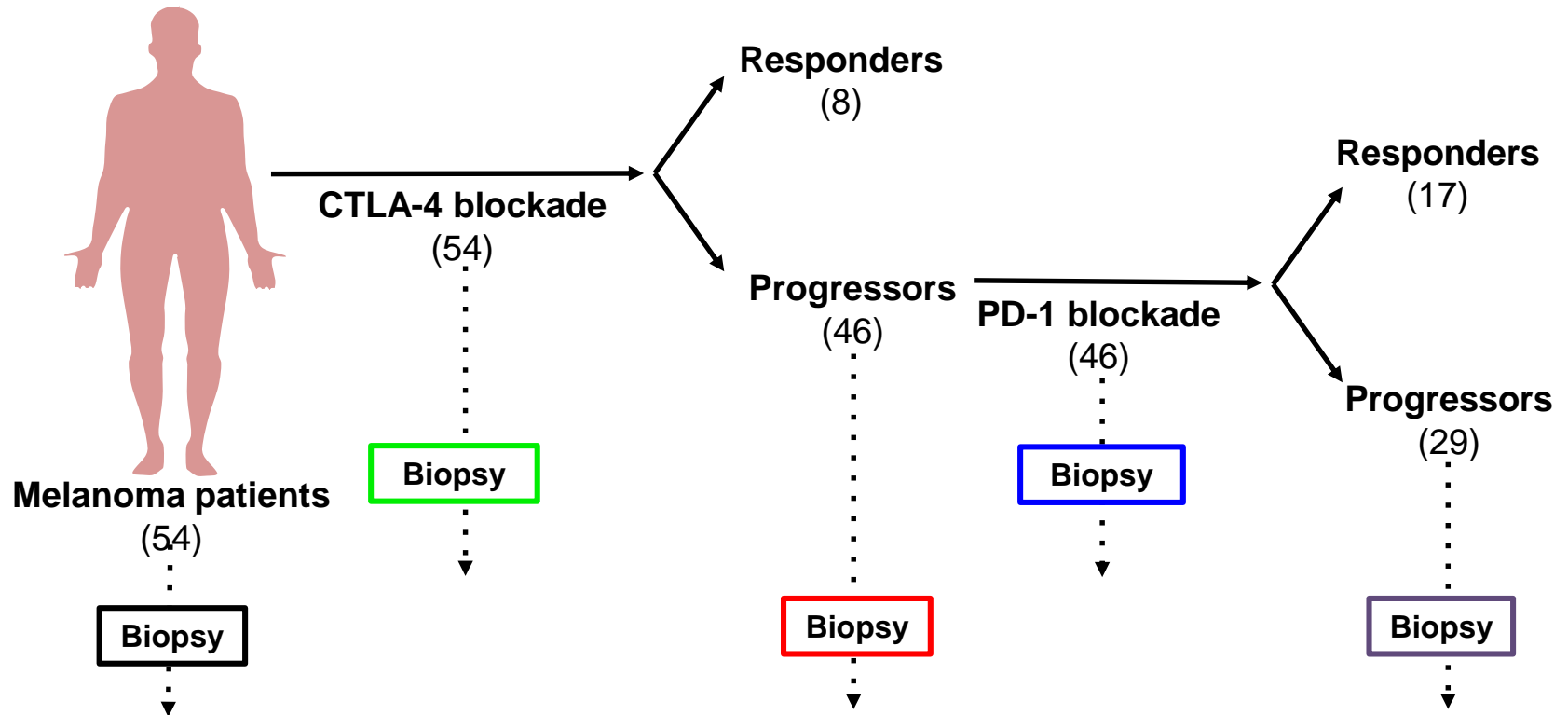
Biomarker Discovery Strategy

Immunotherapy Trials/APOLLO Platform MD Anderson Cancer Center



MD Anderson APOLLO Platform - Melanoma

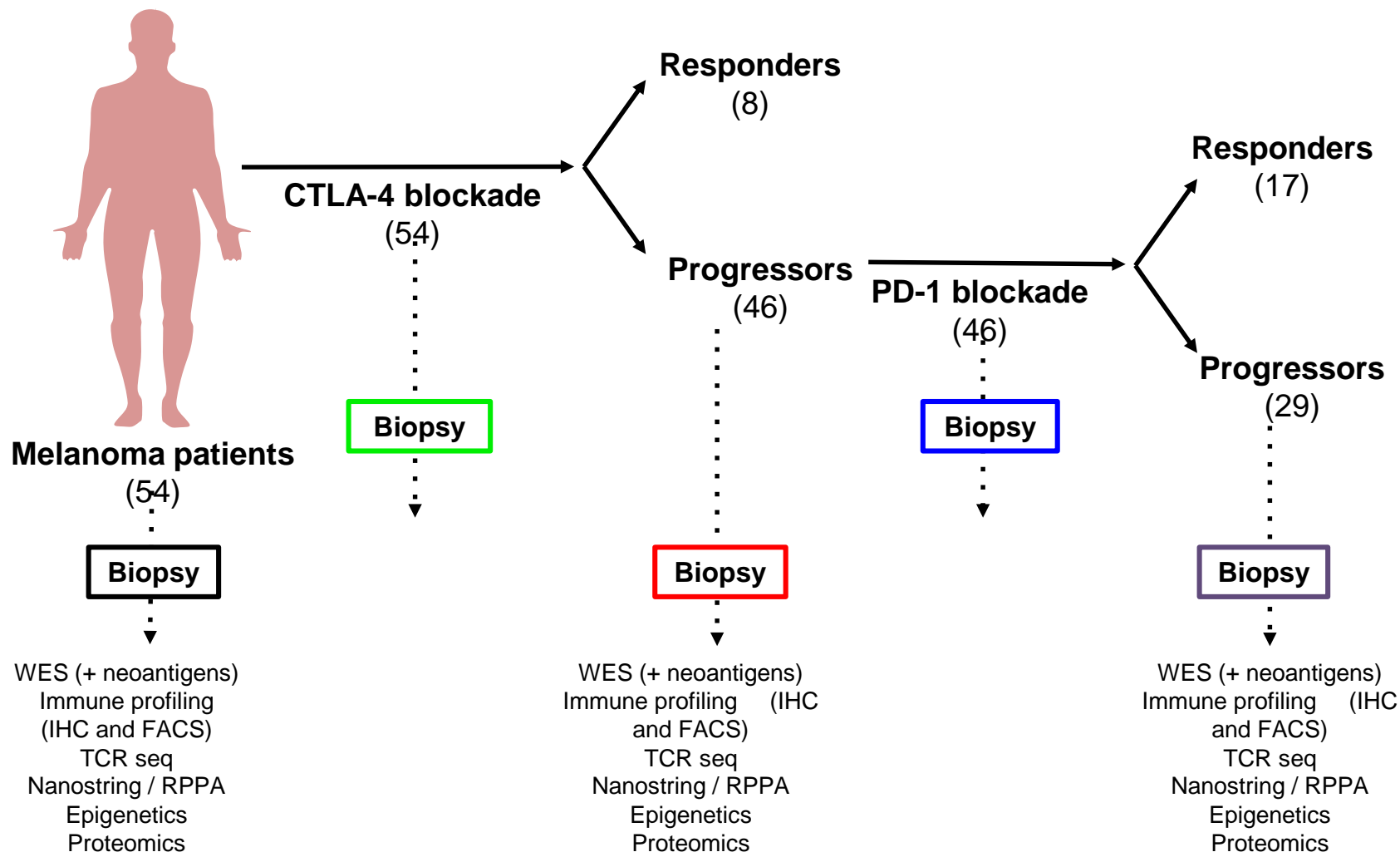
Understanding Evolving Responses to Therapy



Rather than a snapshot view with endpoint data, we can interrogate the system in molecular detail as it evolves

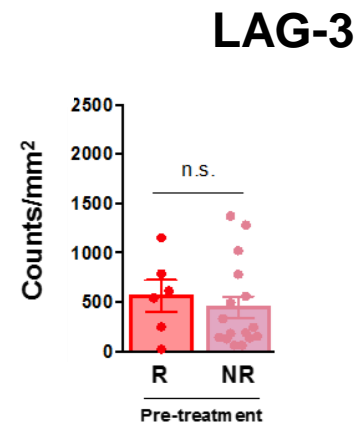
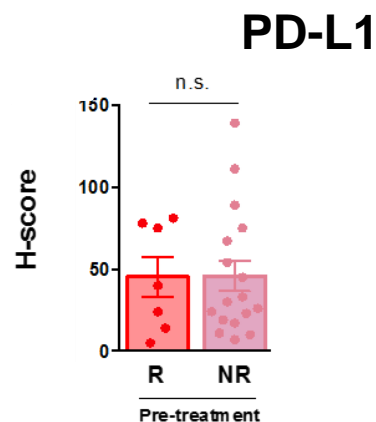
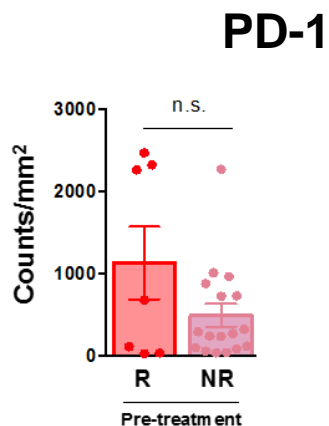
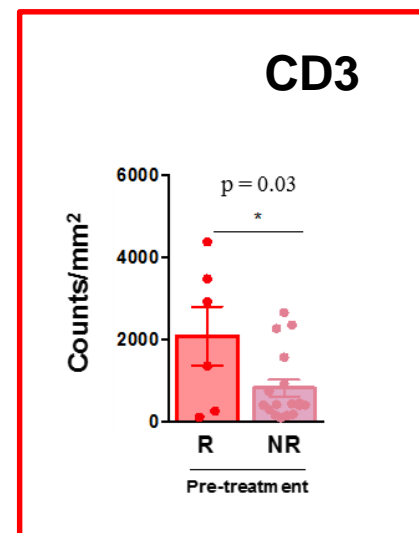
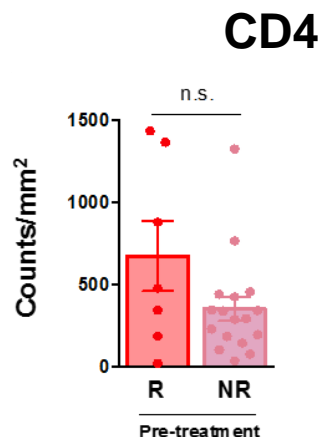
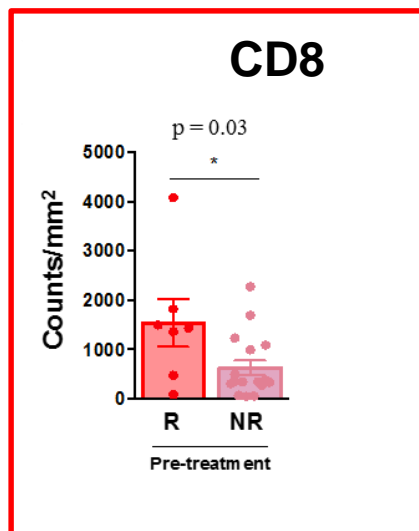
MD Anderson APOLLO Platform - Melanoma

Understanding Evolving Responses to Therapy



Immune Signatures in Pre-Treatment Tumor Biopsies Largely Fail to Predict Response to PD-1 Blockade

But Signatures in On-treatment Biopsies Were Highly Predictive



APOLLO Platform at MD Anderson Cancer Center

Longitudinal Collection of Tumor Tissue in Immunotherapy Trials*

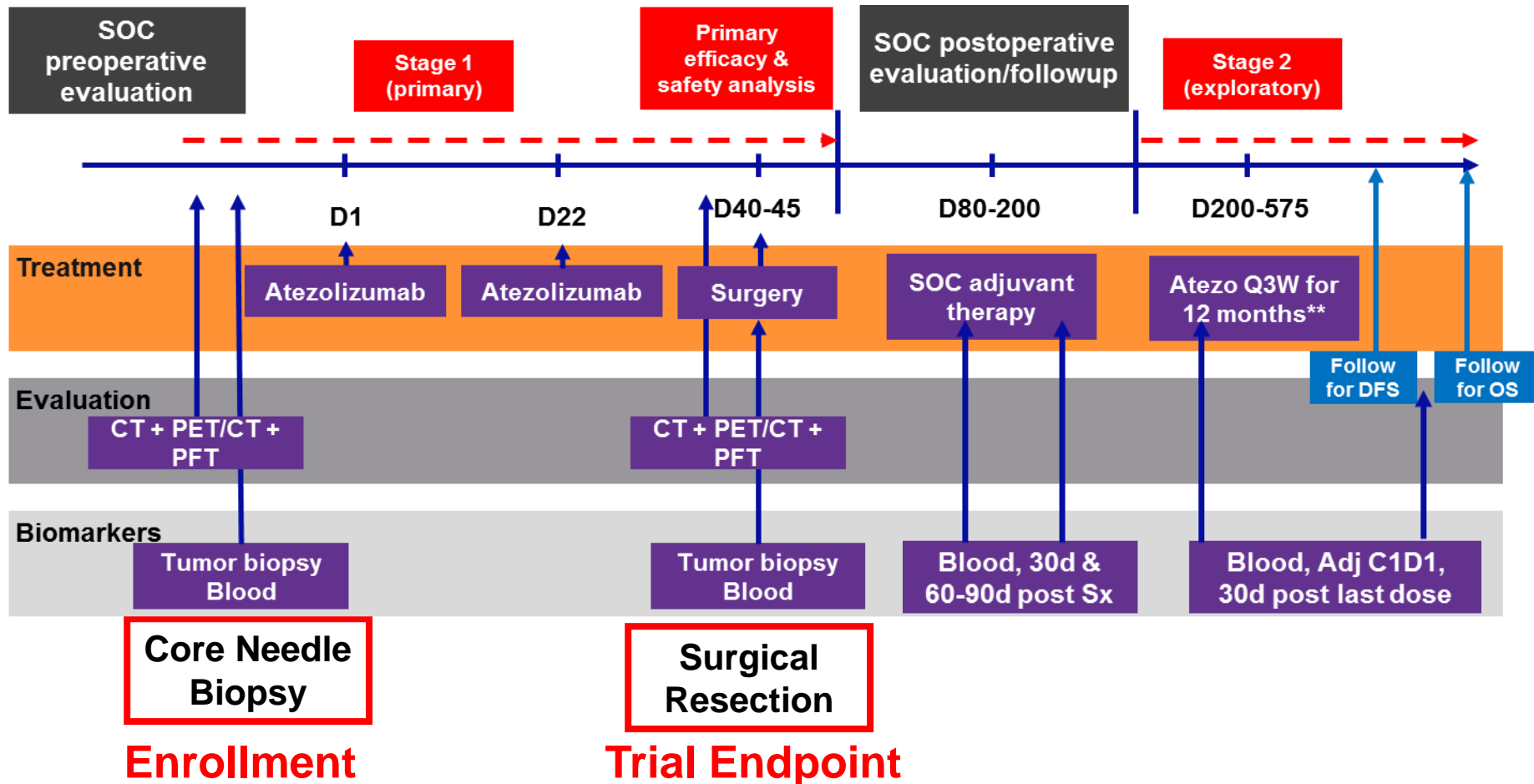
Total Biopsies (05/2018) = 448 (383 QC'ed)

Time-points	Collection of 5 Tissue Cores	Biopsies with $\geq 30\%$ Tumor w/Viable Malignant Cells	
		FFPE	Fresh Frozen
Pre-Treatment	221 (92%)	207 (80%)	198 (76%)
Post-Treatment	162 (94%)	149 (78%)	148 (78%)

* 20 open phase I/II clinical trials

Overall Goal: 2,700 patients, >5,000 biopsies

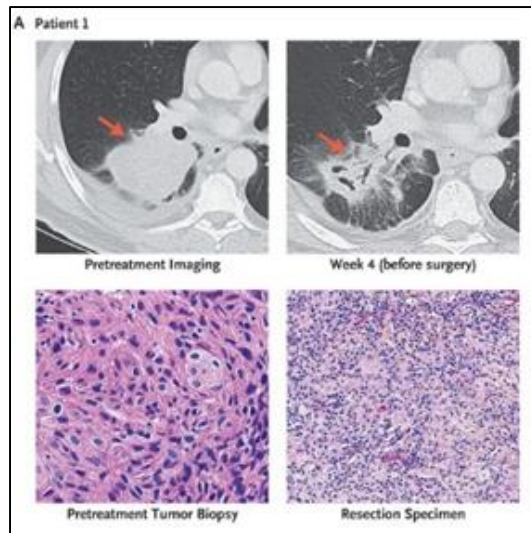
NSCLC Neoadjuvant Anti-PD-L1 LCMC-3 Trial Scheme



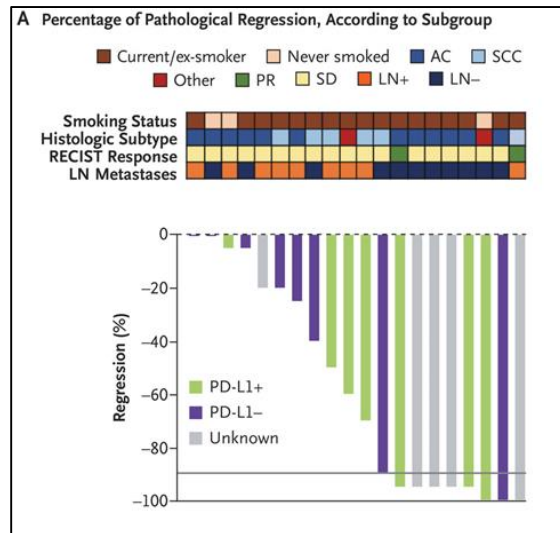
- Phase II, open-Label, multicenter, single-arm study to investigate the efficacy and safety of atezolizumab as neoadjuvant and adjuvant therapy in patients with stage IB, II, IIIA resectable and untreated NSCLC - Primary endpoint, pathological response.

Neoadjuvant PD-1 Blockade in Resectable NSCLC (n=21 Cases)

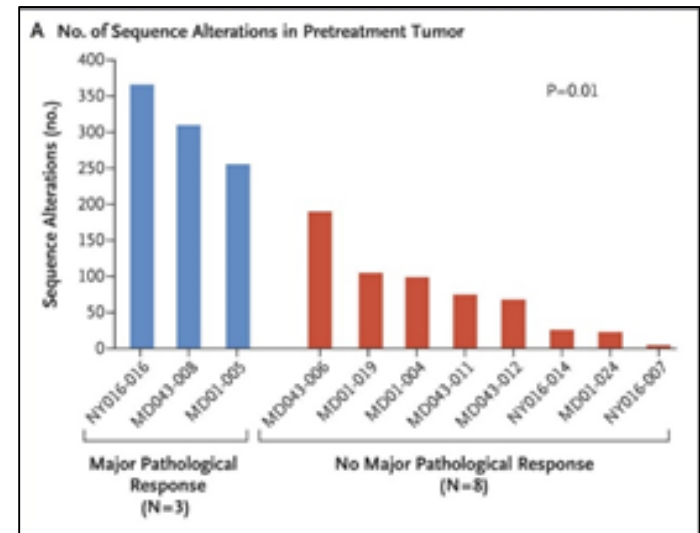
- MPR (≤ 10 malignant cells) in 9/20 (45%)
- No associated to PD-L1 IHC expression
- WES-based on 11 cases, MPR associated to tumor mutational burden
- TC receptor sequencing showed T-cell clones expansion in tumor and blood in 8/9 patients



MPR - Histology



PD-L1 IHC Expression



Tumor Mutational Burden

Biomarker Discovery Strategy

Immunotherapy Trials/APOLLO Platform MD Anderson Cancer Center

Enrollment

Treatment

Progression

Toxicity

Pre-tox

Post-tox

Tox-resolution

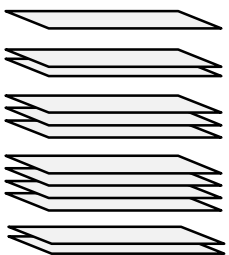
Pre-treatment
tumor biopsy

During treatment
tumor biopsy (e.g. 4-6wks)

Tumor biopsy at
progression

Biopsy (→)

Cores 1-2
FFPE



Histology

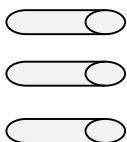
IHC

mIF

mRNA (Nanostring, HTG Edge-Seq)

TBD

Cores 3-5
Fresh



Flow cytometry (TILs)

RNA-seq

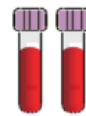
DNA (WES, targeted, TCR/B)

Blood (→)



Plasma (cfDNA), cytokines

PMBCs (germline DNA)



Flow cytometry (2 panels)

Functional T cells assays

TCR sequencing

Stools (→) Microbiome

(→) No tumor biopsy (*skin, colon, etc.*)
Blood
Fluids (BAL)
Skin microbiome



Thank You