

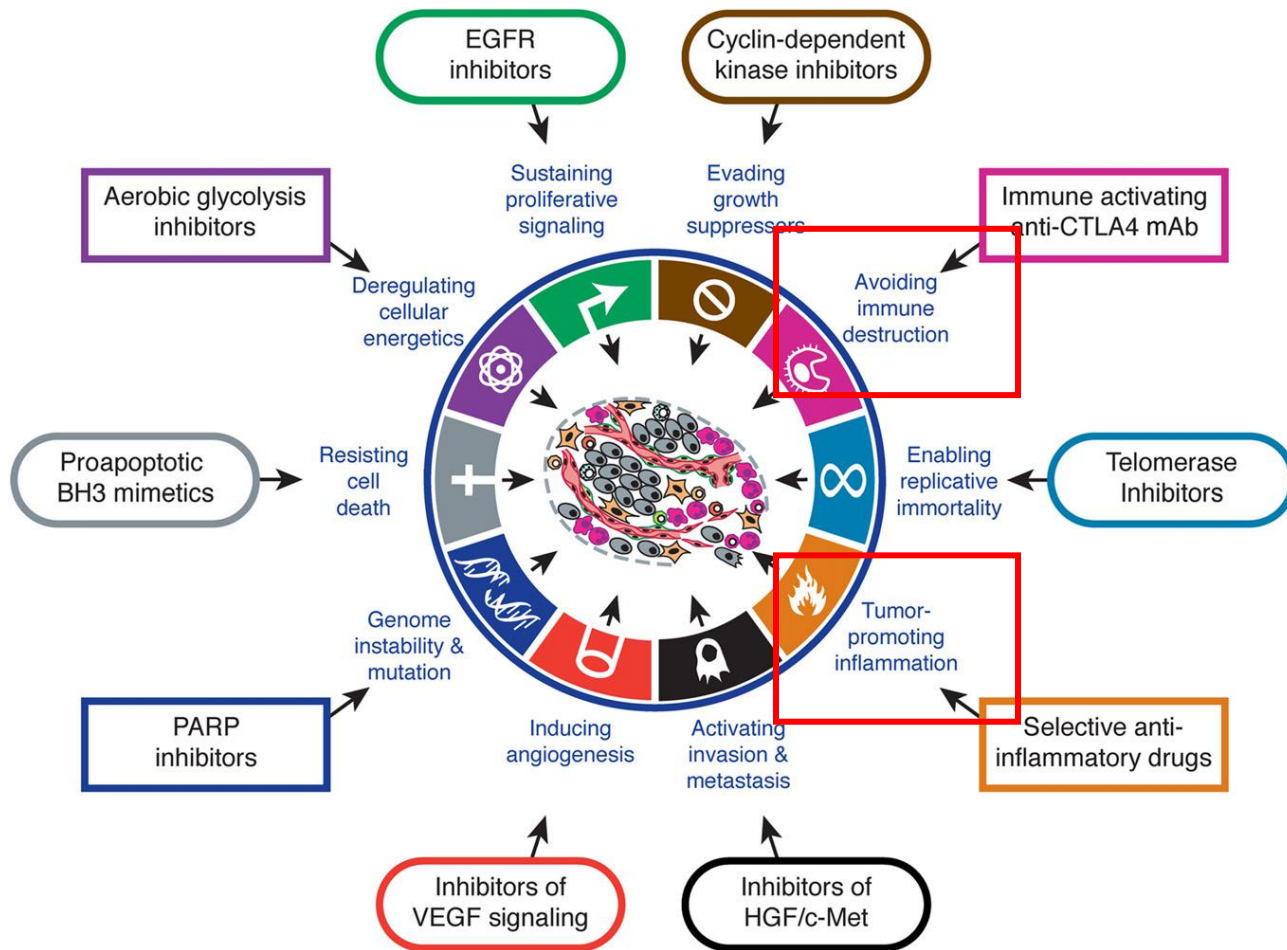
RESEARCH BRIEF

Analysis of Immune Signatures in Longitudinal Tumor Samples Yields Insight into Biomarkers of Response and Mechanisms of Resistance to Immune Checkpoint Blockade

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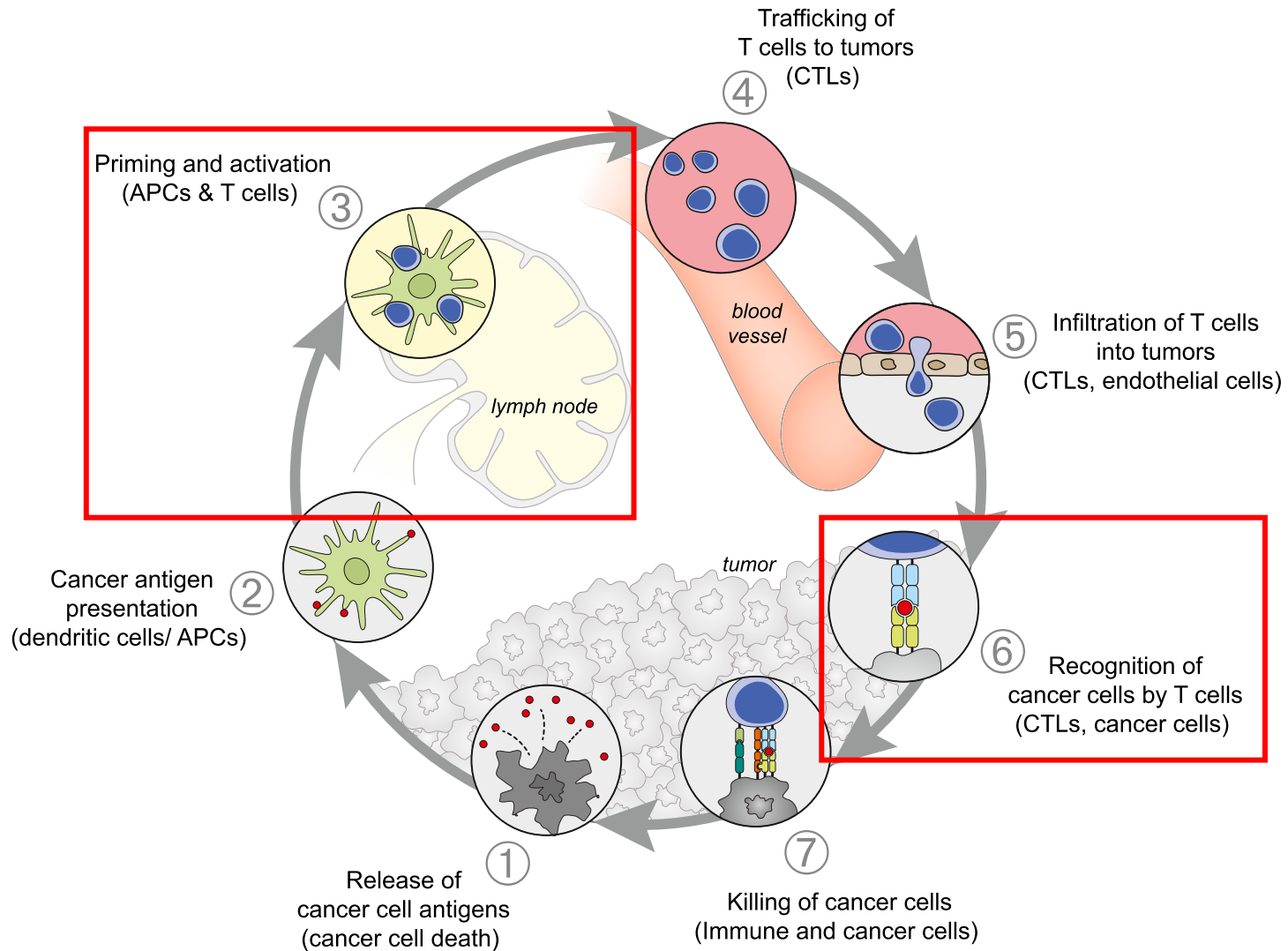
Cancer Discovery - Research Letter 08/2016

Hallmarks of cancer



Modified from Hanahan et al., Cell 2011

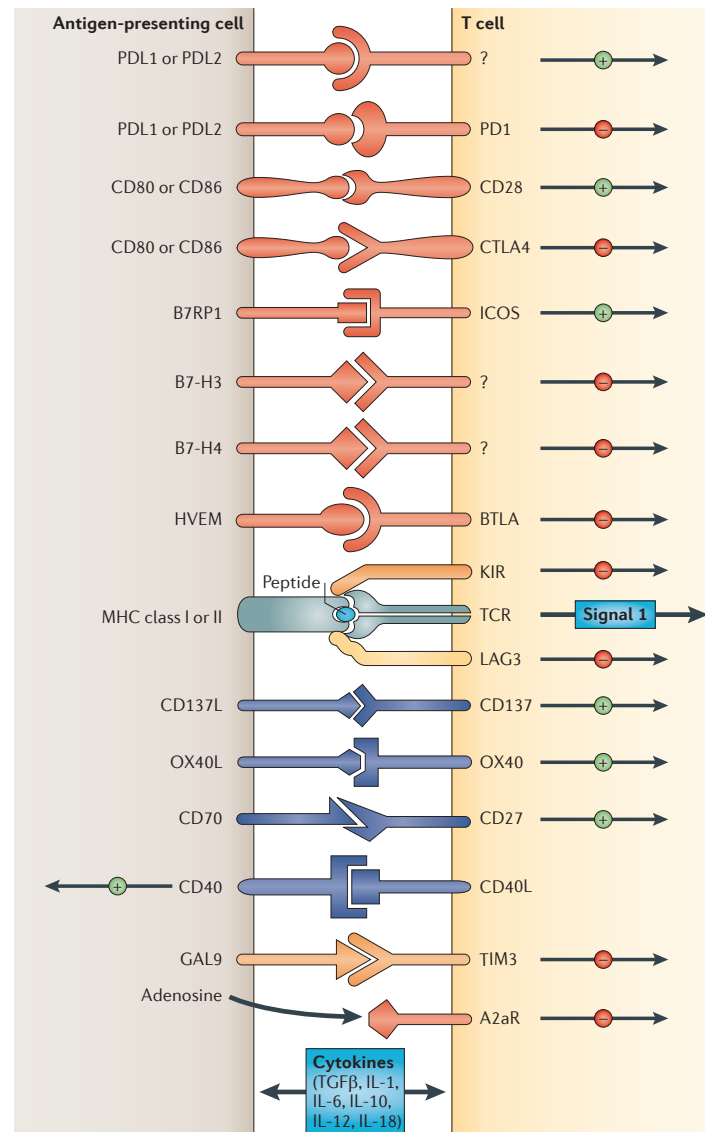
The cancer-immunity cycle



Chen et al., *Immunity* 2013

T cell response regulation

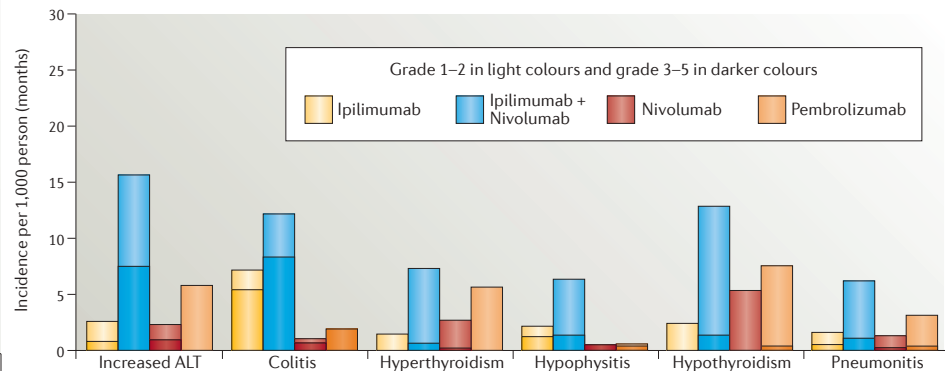
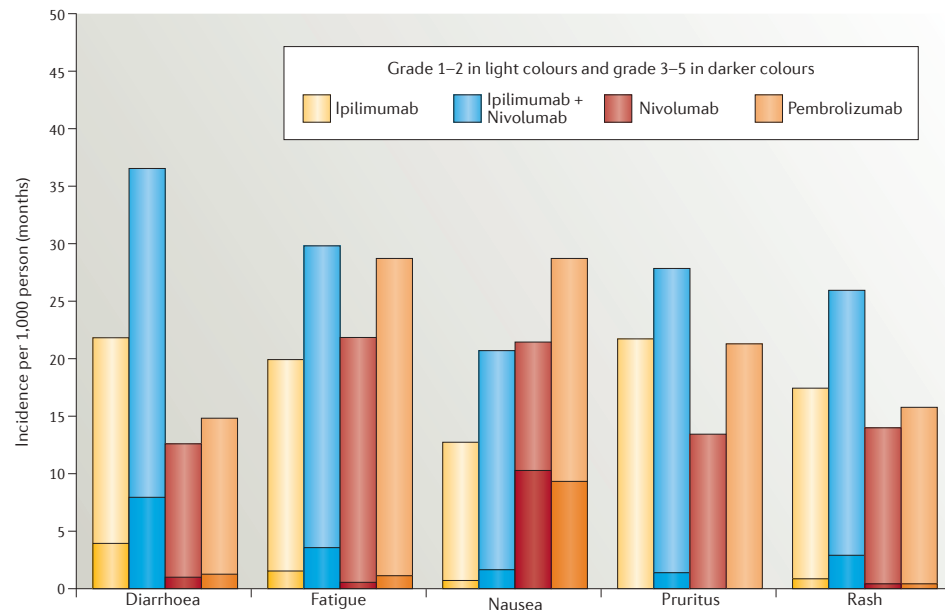
- Multiple co-stimulatory and inhibitory interactions for regulation
- “Checkpoint-Inhibitors”
- In lymph nodes or in peripheral tissue
- Bidirectional communication between APC/cancer cells and T cells



Pardoll et al., Nature Reviews 2012

Limitations

- Response rates 8-44%
- Those that do not response: severe AEs
- Costs: \$300.000 / patient (Ipi + Nivo Combo)



Andrews et al., Health Economics 2015
 Boutros et al., Nature Reviews 2016
 Schadendorf et al., J Clin Oncol 2015

Topalian et al., N Engl J Med 2012
 Topalian et al., J Clin Oncol 2014
 Wolchok et al., Ann Oncol 2013

Aim of the study

- Lack of predictive biomarkers
- Genomic, immune predictors investigated in pretreatment biopsies ¹⁻⁷
- CD8, CD4, PD1, PD-L1 densities in pretreatment biopsies ^{8,9}
- Mutational load, neoantigen structure ^{10, 11}
- Different effects of CIs on transcriptional profiles of TILs, JAK STAT ¹²

→ no robust results

→ Need for comprehensive analyses of longitudinal tumor samples

¹Topalian et al., *N Engl J Med* 2012

²Andtbacka et al., *J Clin Oncol* 2015

³Larkin et al., *N Engl J Med* 2015

⁴Postow et al., *N Engl J Med* 2015

⁵Wolchok et al., *N Engl J Med* 2013

⁶Topalian et al., *J Clin Oncol* 2014

⁷Rizvi et al., *Science* 2015

⁸Tumeh et al., *Nature* 2014

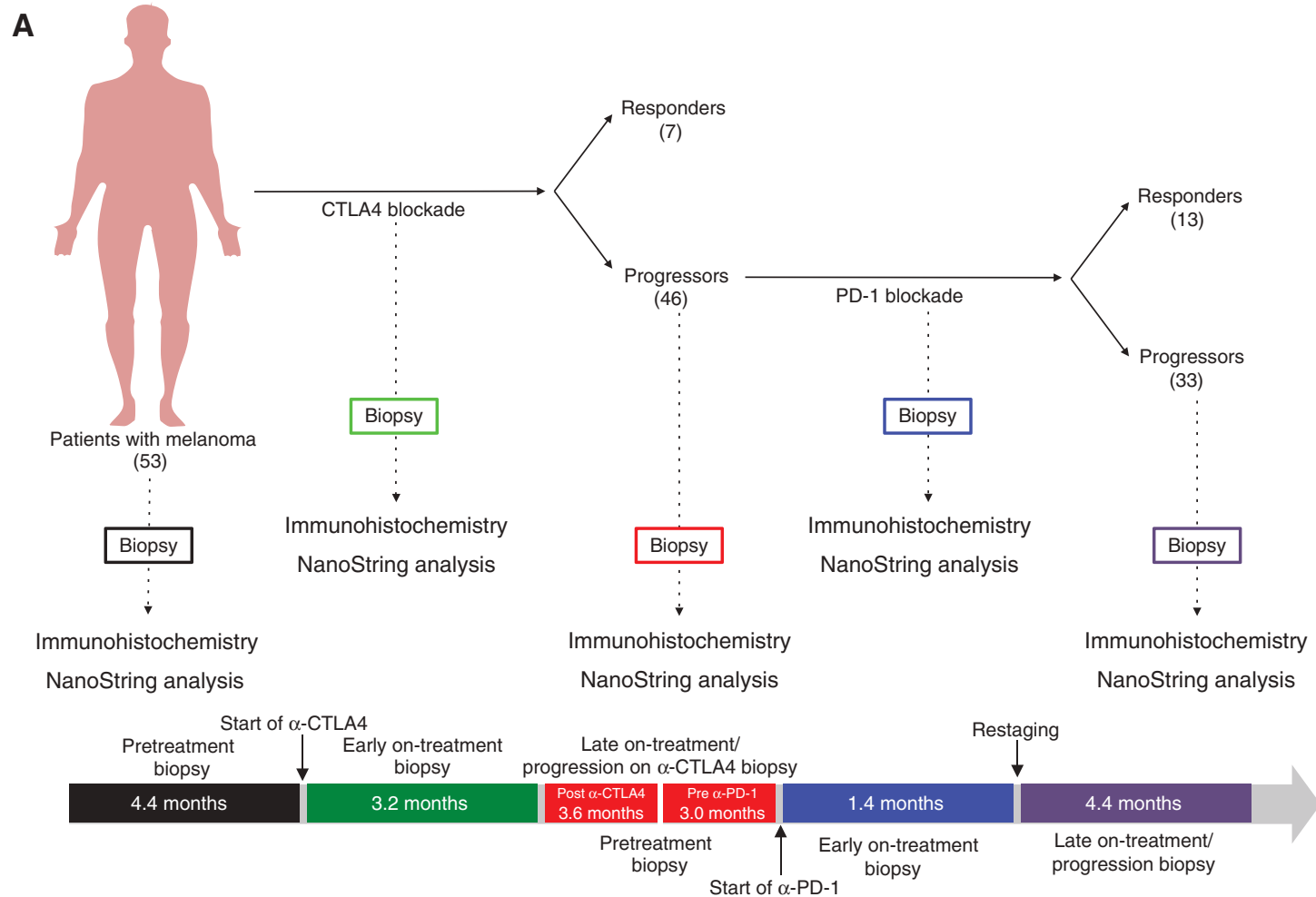
⁹Taube et al., *Clin Cancer Res* 2014

¹⁰Van Allen et al., *Science* 2015

¹¹Snyder et al., *N Engl J Med* 2014

¹²Gubin et al., *Nature* 2014

Patients and methods



Patients and methods

- **Biopsies:**
- Of the most safely accessible sites at different time points

- **IHC: (88 FFPE)**
- CD3, CD4, CD8, FOXP3, Granzyme B, CD57, CD20, CD45RO, LAG3, PD-1, PD-L1, CD14, CD33, CD68, CD163, CD206
- → all were calculated by positive cells/mm²
- → PD-1: H score (0-300) = +cells with membrane staining (%) / intensity of staining

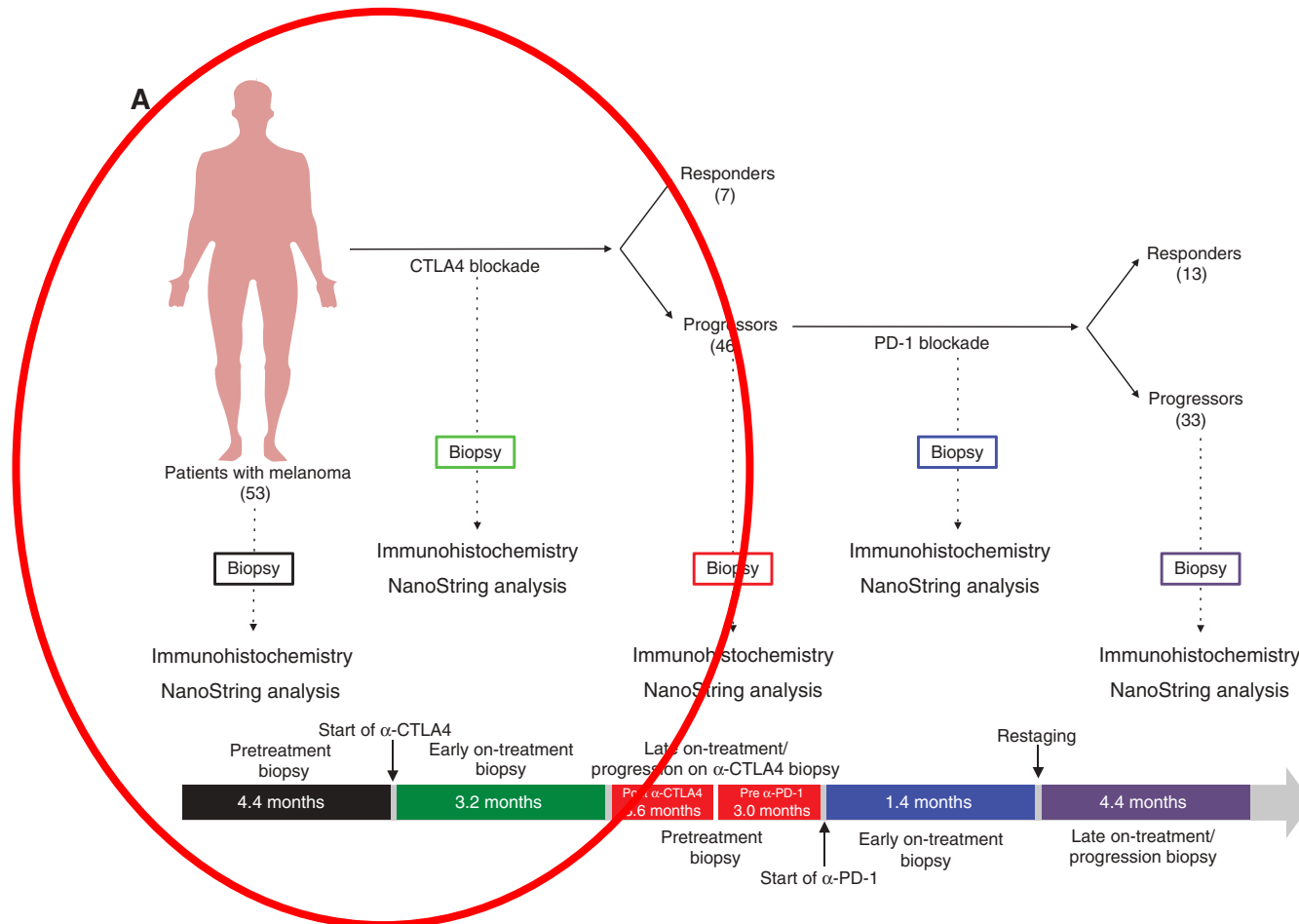
- **Immunofluorescence: (19 samples)**
- DAPI, CD8, CD68 (potential myeloid-T cell interaction?)

Patients and Methods

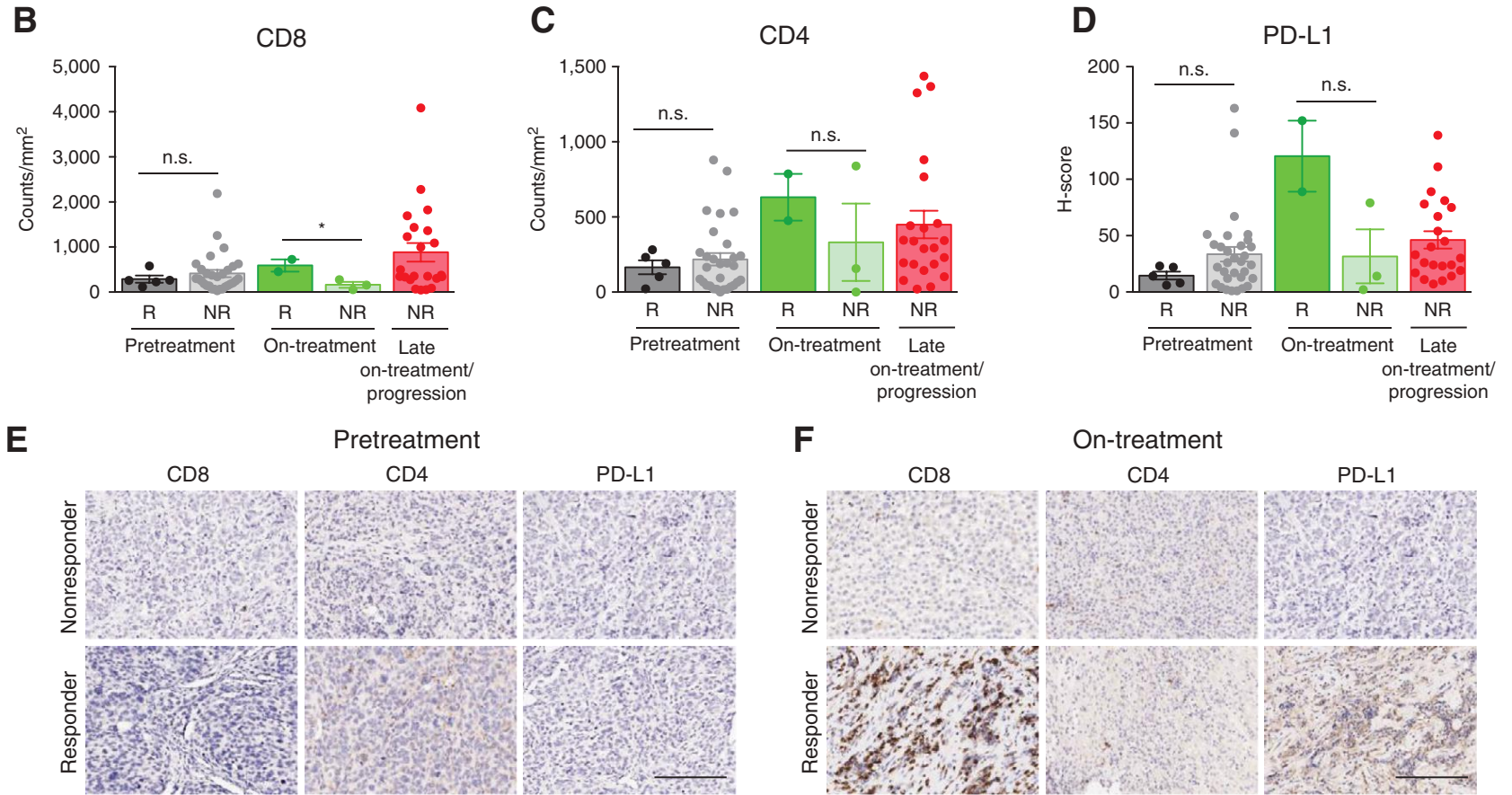
- **NanoString analyses: (54 samples following immune profiling)**
 1. FFPE → RNA extraction (1 μg tissue RNA / string assay)
 2. Mixed with specific NanoString code set mix → hybridization overnight
 3. Loading on nCounter Prep Station (binding + washing)
 4. Scanning / analyzing / data collection
- Gene expression profiling (GEP) analysis: 795-probe codeset
- Immune related genes; common cancer signaling related genes

Results

Immune profiling in early on-treatment biopsies is predictive of response to CTLA-4 blockade

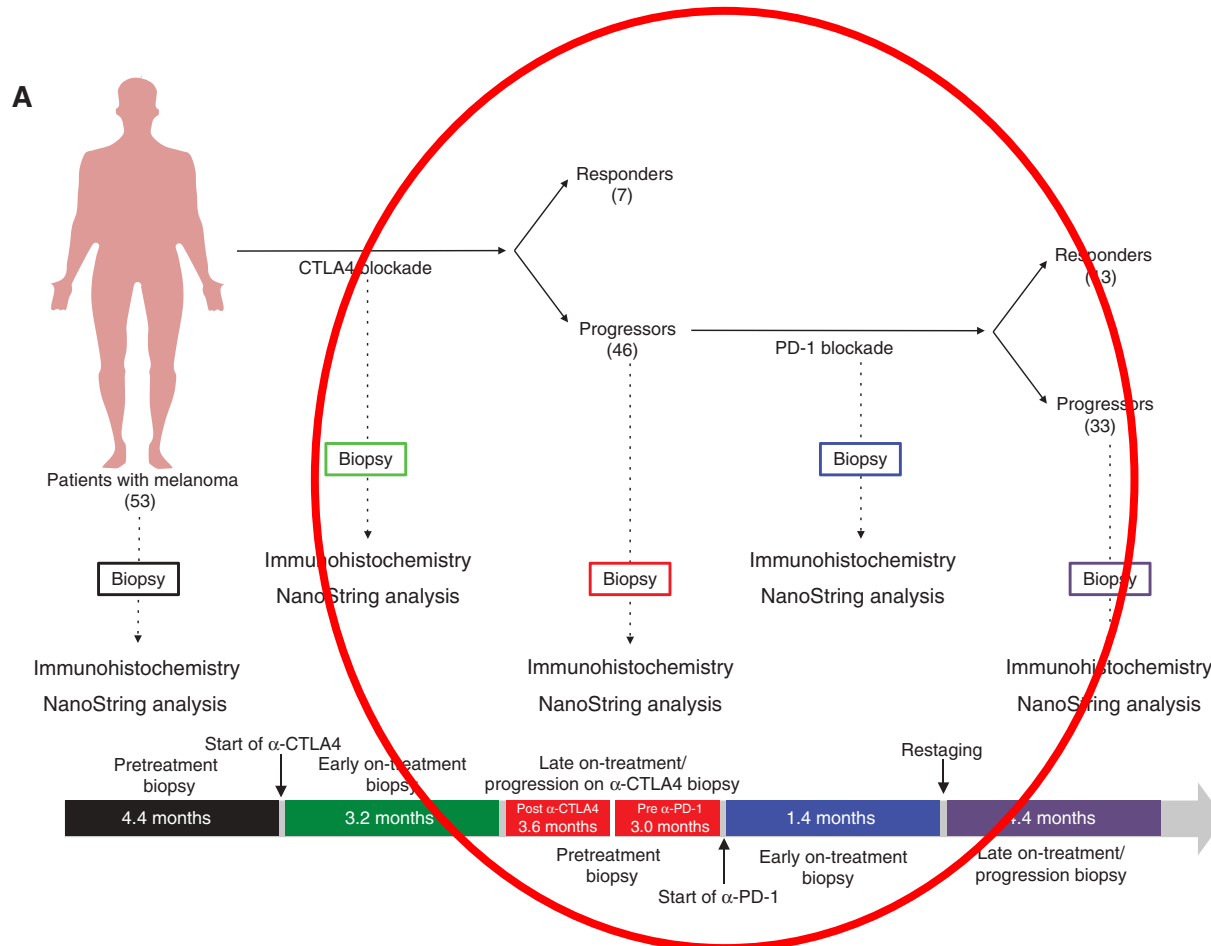


Results

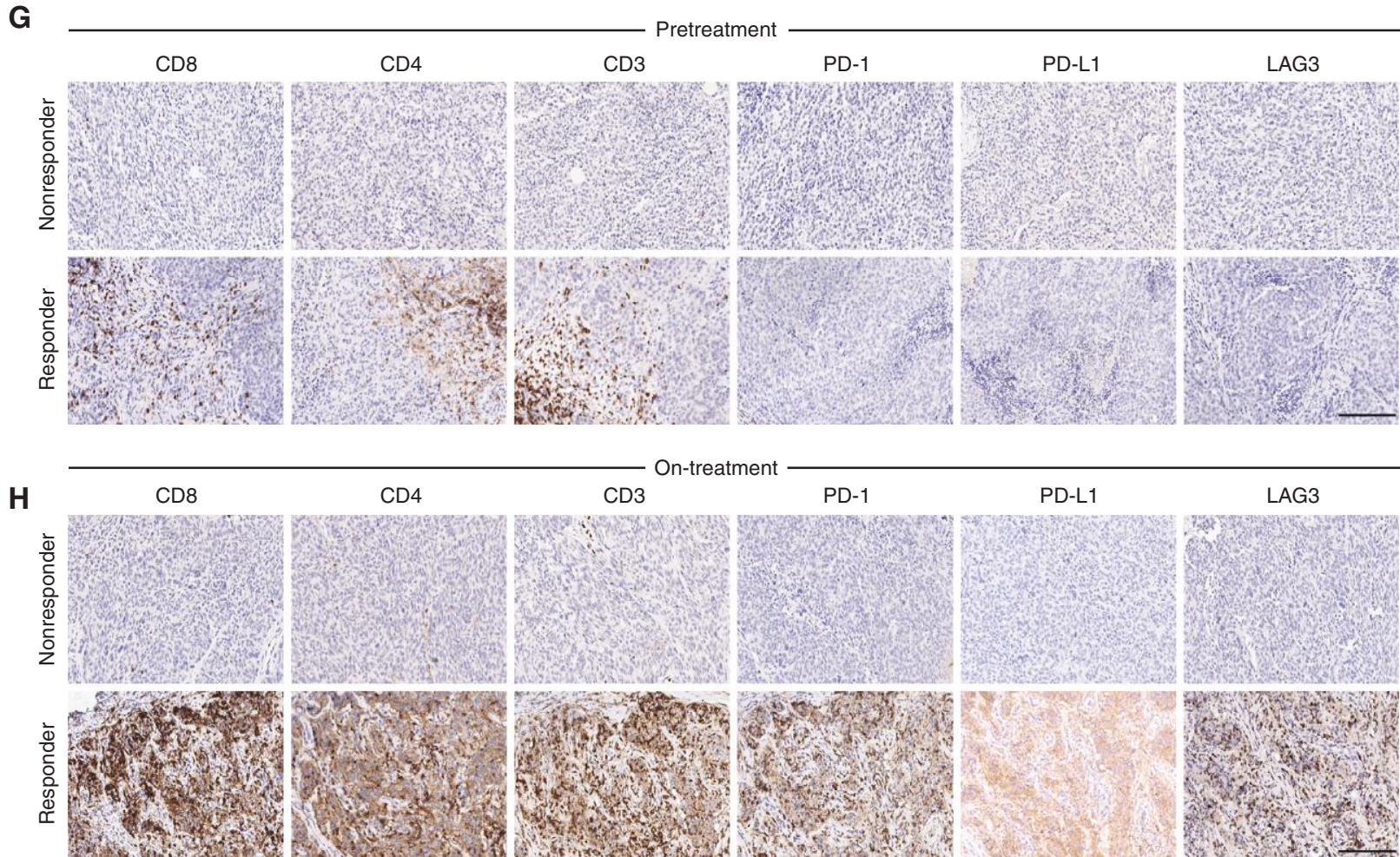


Results

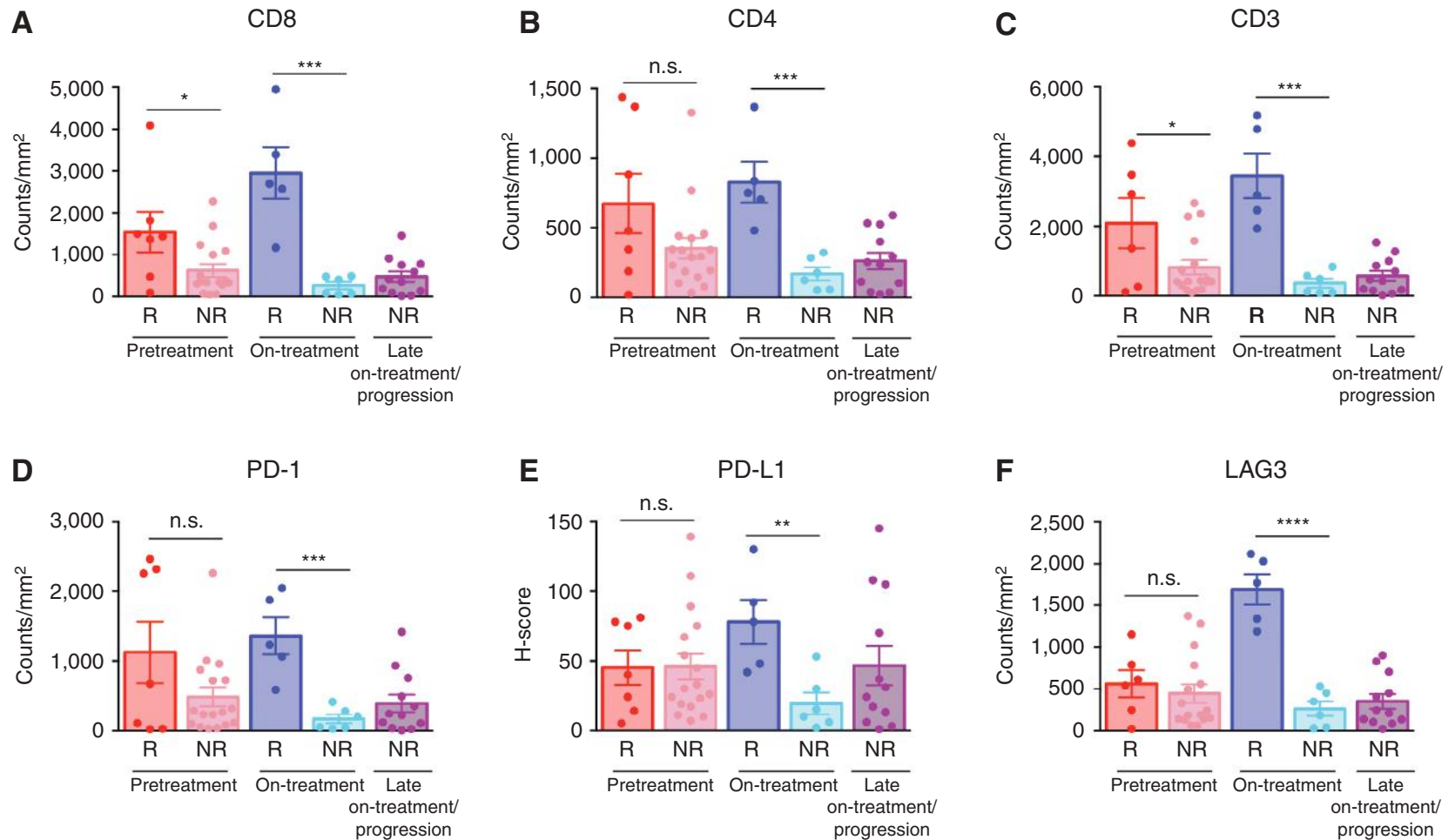
Immune profiling in early on-treatment biopsies is highly predictive of response to PD-1 blockade



Results

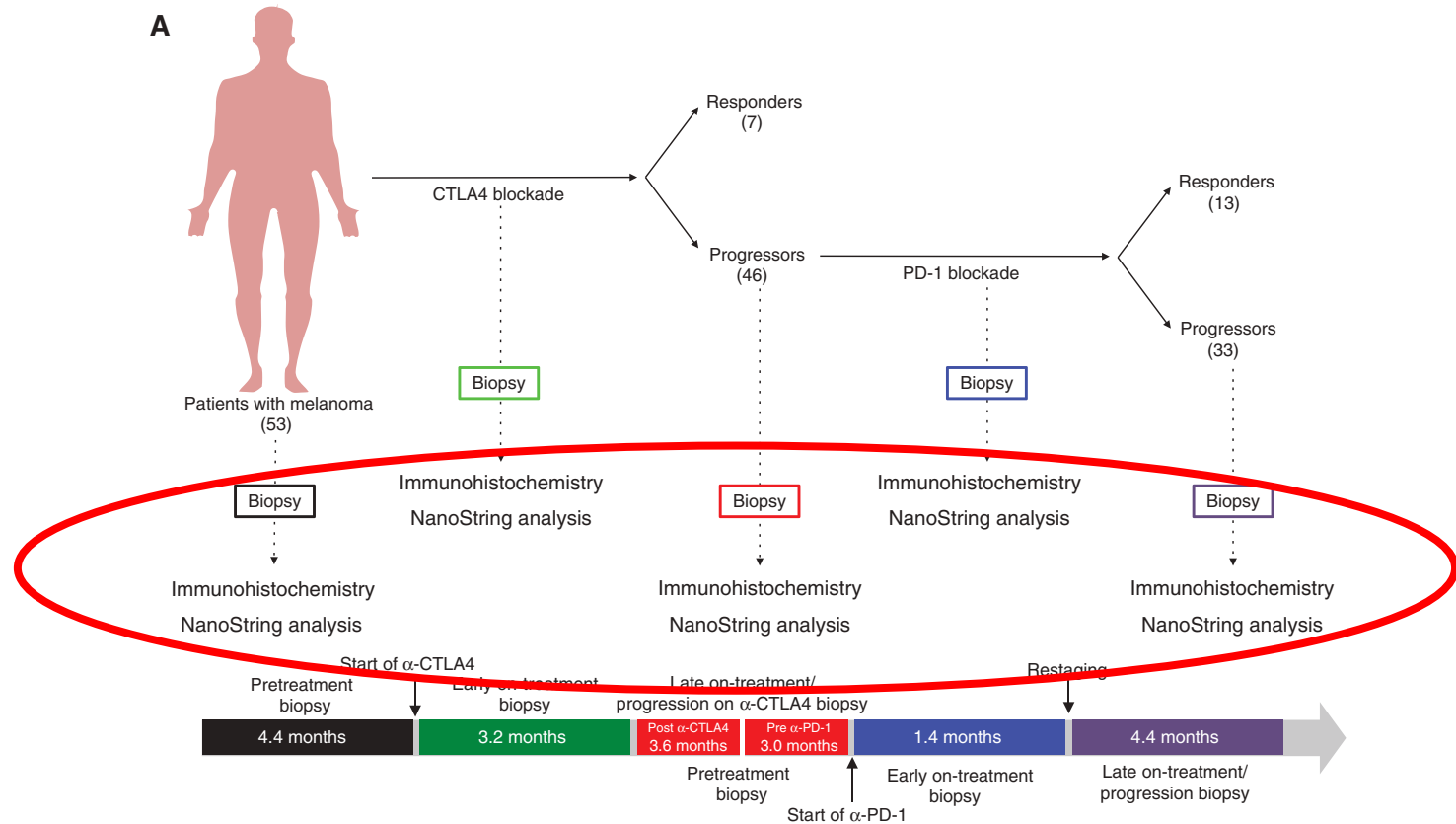


Results

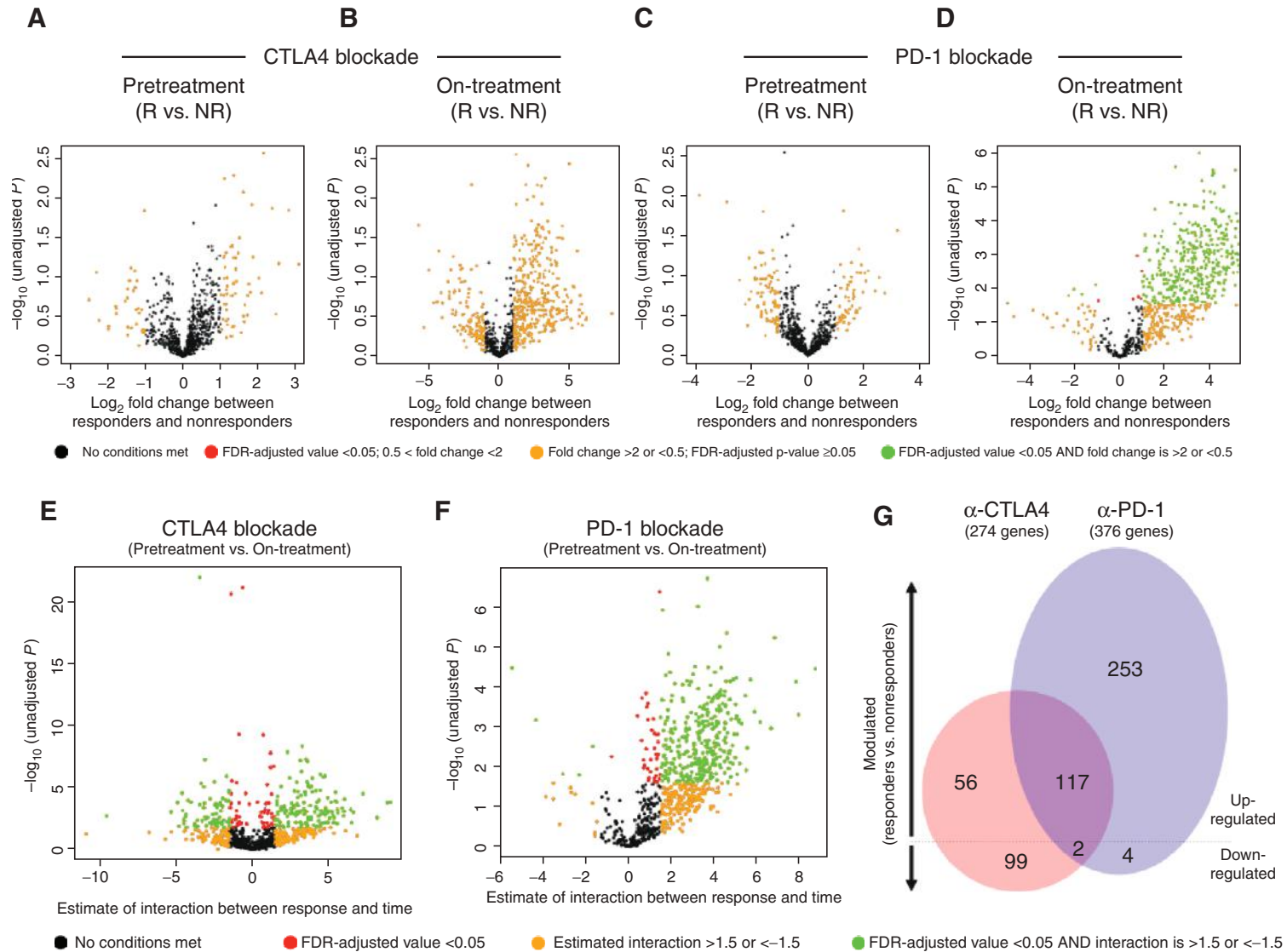


Results

Gene expression profiling in longitudinal tumor biopsies is predictive of response

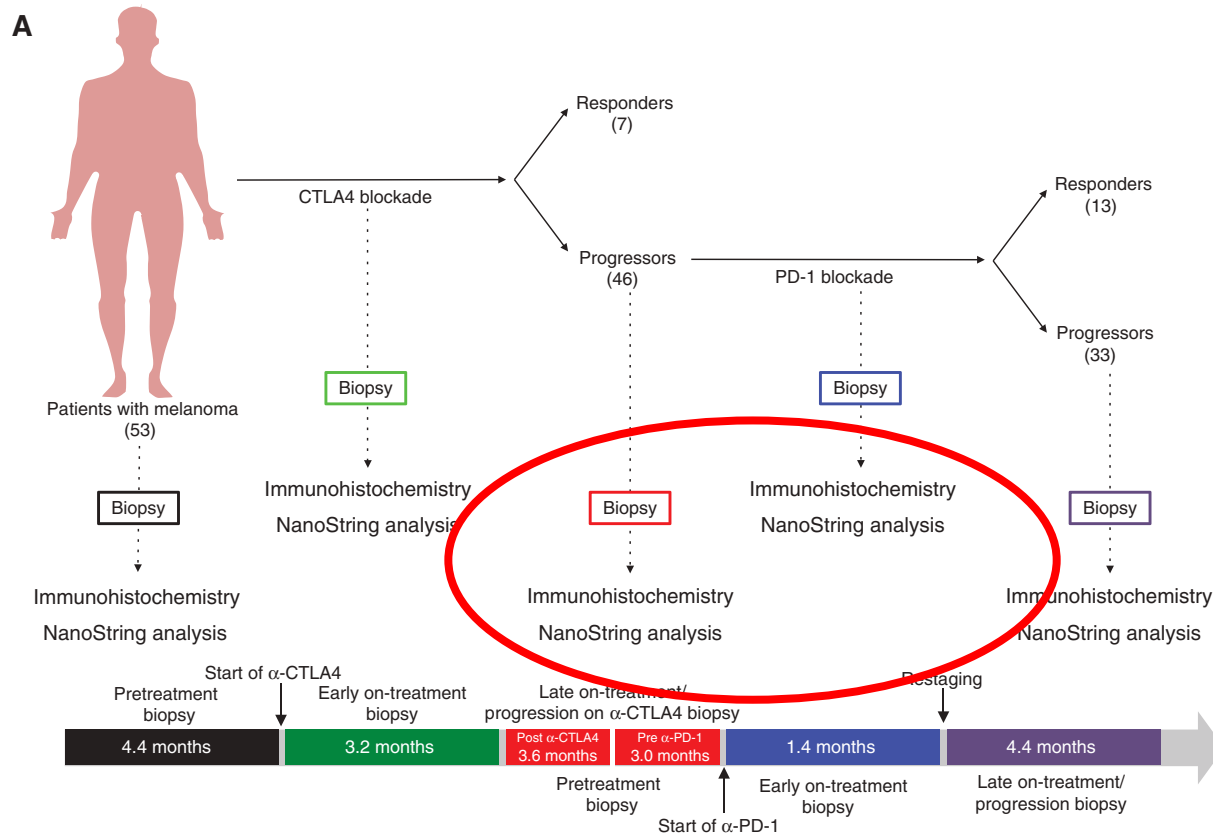


Results

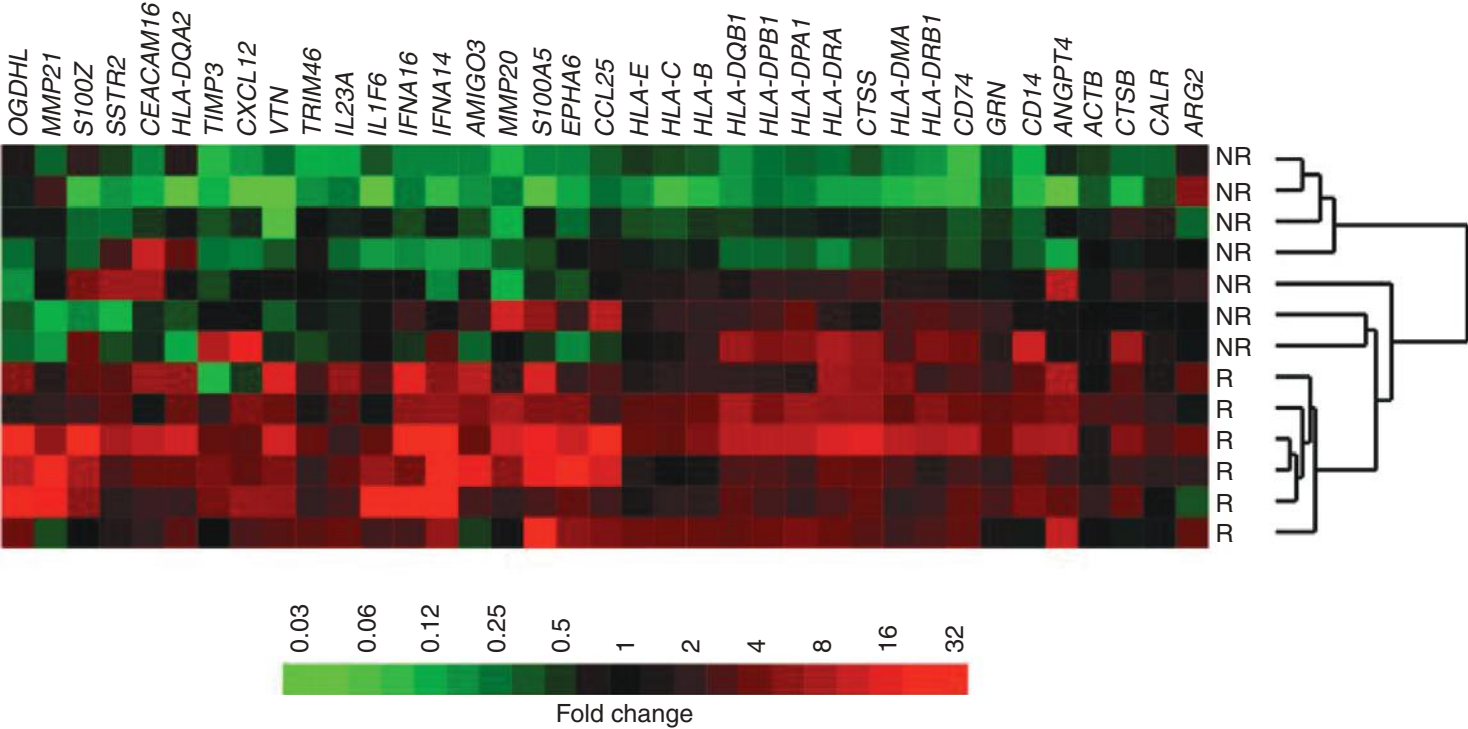


Results

NanoString paired analysis



Results



Results - Summary

- Early-on CTLA-4 blockade: CD8 (R vs NR)
- Early-on PD-1 blockade: CD8, CD4, CD3, PD-1, PD-L1, LAG3 (R vs NR)
- **GEP:**
- Pre-CTLA-4; Early-on CTLA-4; Pre-PD-1: no differences in R vs NR
- Early-On PD-1: >400 up-expressed genes in R vs NR
- Pre-PD-1 versus Early-on PD-1: 370 dynamic changes in gene expression
- **Paired biopsies:** (Pre-PD-1 versus Early-On PD-1)
- Stratification of patients in NR and R based on the the GEP-changes

**IMMUNE SIGNATURES IN TUMOR BIOPSIES EARLY-ON TREATMENT ARE
HIGHLY PREDICTIVE OF RESPONSE TO CIS**

Discussion

- Only a fraction of patients benefits from immunotherapy
- Current approaches focus on assessing immune markers in pretreatment tissue
- Immune signatures in biopsies should be evaluated early after treatment initiation rather than in pre-treatment tissue
- Immune signatures in early on treatment rather a consequence of immune response to checkpoint-inhibitor than of therapeutic response?

Personal opinion

- Fluently written, easy to understand
- Clinical relevance? (Early on treatment?)
- Early-on biopsies 1.4 months too early to assess response?
- GEP (37 genes) in each patient during treatment?
- Dynamic GEP only in 13 patients?
- In MM easy to perform re-biopsy, but for NSCLC? And other tumors?
- Liquid biopsy?