Molecular correlates of response to immune checkpoint inhibitors

David Coffey | Hootie Warren

January 9, 2015
Selected publications

Nature 2014; 515: 568–571

NEJM 2014; 371: 2189-2199

Science Translational Medicine 2014; 6: 1-9

The New England Journal of Medicine
Immune checkpoint inhibitors

**Diagram:**
- **Tumour-specific T cell**
- **PD-1**
- **PD-L1**
- **CTLA-4**
- **CD28**
- **MHC**
- **Antigen**
- **Tumour cell or antigen-presenting cell**

**References:**
Strategies for detecting an immune response to checkpoint inhibitors

- Measurements of the T cell receptor repertoire
- Immunophenotyping of the tumor and T cell
- Quantifying tumor mutational load
- Discovering neoantigen signatures
- Measuring absolute lymphocyte count
- Monitoring markers of T cell activation
- Detecting changes within the tumor microenvironment
- Gene expression profiling
RESEARCH ARTICLE

CANCER

Improved Survival with T Cell Clonotype Stability After Anti–CTLA-4 Treatment in Cancer Patients

Edward Cha,¹ Mark Klinger,² Yafei Hou,¹ Craig Cummings,² Antoni Ribas,³ Malek Faham,² Lawrence Fong¹*

Science Translational Medicine 2014; 6: 1-9
Study design

**Hypothesis:** anti-CTLA4 therapy may expand the range of T cells bearing low-affinity T cell receptors (TCRs) or reduce diversity by narrowing the scope of reactive clones to those bearing high-affinity TCRs.

1. Deep sequencing of the TCR was performed on 25 patients with metastatic prostate cancer receiving ipilimumab, 21 patients receiving tremelimumumab, and 9 untreated healthy controls.

2. TCR repertoire was analyzed for changes in clonality and diversity following treatment.
T cell receptor (TCR)

α chain

β chain

Peptide

Complimentary determining regions

MHC class I
Next generation sequencing of the TCR

V genes
- TCRα: 70
- TCRβ: 52

D genes
- TCRβ: 2

J genes
- TCRα: 61
- TCRβ: 13

Constant genes

βCDR3 amino acid sequence | Count | Frequency |
--- | --- | --- |
CASSMDRADTQYF | 3428 | 3% |
CASRGGSYEQYF | 3378 | 2.5% |
CASTPRGAPRFF | 3129 | 2.1% |
Patient who received anti-CTLA4 treatment developed increases and decreased in absolute clonotype counts.
Repeated dosing of anti-CTLA-4 leads to continuous remodeling of the TCR repertoire over time
TCR repertoire becomes more diverse following anti-CTLA4 therapy
Repertoire diversification is driven by the overall contribution of clonotype gain rather than loss.
Improved survival is associated with maintenance of high-frequency clones.
Expanded T cell clones did not exhibit a naive phenotype
Anti-tumor T cell responses are private

- Tumor-specific T cells could not be isolated by tetramer staining for MART1, tyrosinase, gp100, PSA, and PAP

- Patients may develop “private” immune response to their cancer that may include tumor-specific antigens rather than developing responses to shared, common antigens
Conclusions

1. The TCR repertoire becomes more diverse with treatment
2. Repertoire diversification is driven by the overall contribution of clonotype gain rather than loss
3. Improved survival is associated with maintenance of high-frequency clones
LETTER

PD–1 blockade induces responses by inhibiting adaptive immune resistance

Paul C. Tumeh1,2, Christina L. Harview1, Jennifer H. Yearley3, I. Peter Shintaku1, Emma J. M. Taylor1, Lidia Robert1, Bartosz Chmielowski1,2, Marko Spasic1, Gina Henry1, Voicu Ciobanu1, Alisha N. West1, Manuel Carmona1, Christine Kivork1, Elizabeth Seja1, Grace Cherry1, Antonio J. Gutierrez1, Tristan R. Grogan1, Christine Mateus4, Gorana Tomasic4, John A. Glaspy1,2, Ryan O. Emerson5, Harlan Robins5,6, Robert H. Pierce3, David A. Elashoff1,2, Caroline Robert4 & Antoni Ribas1,2

Nature 2014; 515: 568–571
Study design

Hypothesis: pre-existing cytotoxic T cells at the tumor margin and bearing expression of PD-1 are necessary for response to anti-PD-1 therapy

1. Immunohistochemistry for PD-1, PDL-1 and T cell markers and deep sequencing of tumor infiltrating T cells was performed on 46 patients with metastatic melanoma before and during treatment with pembrolizumab
Higher CD8+ cell densities were present at the tumor margin before and after anti-PD-1 treatment in patient who responded.
A significant correlation was observed between the proximity of PD-1 and PD-L1 and response to therapy.
TCR sequencing of tumor-infiltrating lymphocytes reveal a less diverse repertoire which is more clonal in nature.

**Figure:**

- **Left panel:**
  - Graph showing clonality with different symbols and error bars.
  - Two groups labeled as 'Response' and 'Progression'.

- **Right panel:**
  - Bar graph showing the number of expanded T cell clones.
  - Two bars labeled 'Progression' and 'Response'.
  - Statistical significance indicated with 'p = 0.006'.
Conclusions

• Pre-existing CD8+ T cells distinctly located at the invasive tumor margin are associated with the expression of PD-1/PDL-1 immune inhibitory axis and may predict response to therapy

• Tumor-infiltrating lymphocytes reveal a less diverse repertoire which is more clonal in nature
Genetic Basis for Clinical Response to CTLA-4 Blockade in Melanoma

Alexandra Snyder, M.D., Vladimir Makarov, M.D., Taha Merghoub, Ph.D., Jianda Yuan, M.D., Ph.D., Jesse M. Zaretsky, B.S., Alexis Desrichard, Ph.D., Logan A. Walsh, Ph.D., Michael A. Postow, M.D., Phillip Wong, Ph.D., Teresa S. Ho, B.S., Travis J. Hollmann, M.D., Ph.D., Cameron Bruggeman, M.A., Kasthuri Kannan, Ph.D., Yanyun Li, M.D., Ph.D., Ceyhan Elipenahli, B.S., Cailian Liu, M.D., Christopher T. Harbison, Ph.D., Lisu Wang, M.D., Antoni Ribas, M.D., Ph.D., Jedd D. Wolchok, M.D., Ph.D., and Timothy A. Chan, M.D., Ph.D.

NEJM 2014; 371: 2189-2199
Study design

**Hypothesis:** a neoepitope signature derived from tumor somatic mutations may predict response to anti-CTLA4 therapy

1. Tumor tissue was obtained from 64 patients receiving anti-CTLA4 blockade with either ipilimumab or tremelimumab
2. Whole-exome sequencing was performed on tumors and matched blood samples
3. Somatic mutations and candidate neoepitopes were identified using a bioinformatics approach
Neoepitope
1,353,506 point mutations and indels in 25 tumor-normal tissue pairs

Exome analysis pipeline

BWA
GATK
Somatic Sniper

1,353,506 point mutations and indels in 25 tumor-normal tissue pairs

1000 Genomes

Exclude 493,525

Exclude 2,073

Exclude 70,731

ESPS400

dBSNP 132

ANNOVAR

Intronic/Intergenic
Noncoding
Synonymous Coding

Exclude 293,442
Exclude 25,786
Exclude 5,029

Minimum 11x depth
Manual IGV Curation

9291 non-synonymous, coding point mutations and indels
371 per tumor-normal tissue pair

Exclude 4801
Neoantigen analysis pipeline

Exome pipeline \(\rightarrow\) Somatic mutations

NASeek for 17mers\(^*\): iterative scanning analysis

Wild type \(\rightarrow\) Mutant \(\rightarrow\) Tetrapeptide
Neoantigen analysis pipeline

Exome pipeline → Somatic mutations

NASeek for 17mers^: iterative scanning analysis

MHC Class I binding prediction for all patient-specific HLA

NASeek for shared amino acid stretches

IEDB for substrings of or homology to known epitopes from pathogens/antigens

Peptides predicted to:
- bind MHC Class I
- Homology to known epitope

T cell interaction prediction**

Peptides predicted to:
- bind MHC Class I
- interact with T cell

Candidate Neoantigens
The mutant peptide is more likely to bind MHC Class I than the corresponding wild type peptide.
High mutational load was significantly correlated with improved overall survival.
A neoepitope signature was identified in patients with a long-term benefit but absent in patients with a minimal or no benefit.
Neoepitope signature was associated with improved overall survival in the discovery and validation sets.
*In vitro* T cells responses were detected to mutant but not wild-type peptide
Conclusions

1. High mutational load was significantly correlated with improved overall survival
2. The presence of a neoepitiope signature was associated with improved overall survival
3. Neoepitopes common to patients with long term benefit were homologous to viral and bacterial antigens
Summary

• Checkpoint inhibitor therapy leads expansion of the T cell repertoire, which is more diverse in the peripheral compartment and clonal in the tumor microenvironment.

• Responders to checkpoint inhibitors are more likely to have pre-primed T cells located at the tumor interface.

• A neoepitope signature derived from tumor mutations can be identified in responders to checkpoint inhibitors.