Monitoring the response to immunotherapy through the use of liquid biopsy

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## Checkpoint Blockade Antibodies

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<th>Therapy</th>
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<td>Pembrolizumab</td>
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Approved by the FDA for treatment of patients with:
- metastatic melanoma
- non-small cell lung cancer
- renal carcinoma,
- bladder cancer,
- head and neck cancer,
- gastric cancer
- Relapsed/refractory Hodgkin’s lymphoma
- tumors displaying microsatellite instability (MSI)-high and mismatch repair deficiency (MRD)
- Liver cancer

- How many autologous T-cell clones are needed for this response?
- Which tumor antigen(s) are being recognized by these T-cell clones?
- Which tumor characteristics are driving (or limiting) this response?
Chimeric Antigen Receptor (CAR) T-cells induce clinically effective antitumor responses

August 30, 2017: FDA Approves tisagenlecleucel (formerly CTL019) for the treatment of children and young adults (up to 25 years of age) with B-cell precursor acute lymphoblastic leukemia (ALL) that is refractory or in second or later relapse.

October 19, 2017: FDA Approves axicabtagene ciloleucel (formerly KTE-C19) for the treatment of adult patients with certain types of large B-cell lymphoma who have not responded to or who have relapsed after at least two other kinds of treatment.

May 1st, 2018: FDA Approves tisagenlecleucel for the treatment of adult patients with relapsed or refractory large B-cell lymphoma after two lines of systemic therapy (DLBCL, high grade B-cell lymphoma and DLBCL arising from FL)

How many genetically modified T-cells clones are needed for an effective antitumor response?
Despite growing success, many patients do not respond to Immunotherapy

- Response rates for the 6 currently approved checkpoint inhibitors typically range from ~10-30% for solid tumors (Brahmer et al., 2010; Brahmer et al., 2012; Hodi et al., 2010; Le et al., 2015; Topalian et al., 2015)

- Complete and durable responses to CAR T-cells (three different platforms) are seen in approximately 30-40% patients (ASH 2018)

• Moving forward…
  • Identification of biomarkers of response (…or resistance)
    • Tumor cells and their microenvironment
    • Lymphoid organs
    • Bone marrow
    • Peripheral blood

“Classic biopsies”

“Liquid biopsy”
Biomarkers of response in tumor biopsies

- PD1/PD-L1 expression
- Tumor infiltrating lymphocytes (TIL)
- Tumor Mutation Burden (TMB)

Given the complexity of the immune response and tumor biology, it is unlikely that a single biomarker will be sufficient to predict clinical outcomes in response to immunotherapy.

Given the challenges in obtaining serial tumor biopsies, it is unlikely that a single biopsy will “tell us the whole story” of what it is a very dynamic interaction between immune cells and tumors.
Liquid Biopsy

Serial liquid biopsies might allow us to better “visualize” the dynamics of antitumor immune responses before (pre-existing), during, and after treatment with checkpoint blockade or CAR T-cells.

….Are circulating immune cells (or their products, ie, cytokines) in peripheral blood a good reflection of the immune reaction occurring in the tumor microenvironment?
Emerging Biomarkers of Response to Immunotherapy in Liquid Biopsies

- Tumor mutation burden (TMB)

- Pre-existent tumor-antigen specific T-cell clones
Tumor Mutation Burden in Liquid Biopsies

- 69 patients with various malignancies who received checkpoint inhibitor immunotherapy

- Simple blood test to detect circulating tumor DNA alterations by NGS

- Patients (29%) with more than 3 variants of unknown significance (VUS) had a response rate of 45%, compared to a 15% response rate in those patients with three or less VUS (71% patients)

- PFS: 23 months versus 2.3 months

- Patients with a higher VUS alterations status had a significant increased median OS rates (not reached versus 11 months)

Emerging Biomarkers of Response to Immunotherapy in Liquid Biopsies

- Tumor mutation burden (TMB)

- Pre-existent tumor-antigen specific T-cell clones
Emerging evidence suggest that only few T-cell clones seems to be needed for a clinically effective antitumor effect in response to immunotherapy.

“Holy Grail”: Identification of those antigen-specific T-cells
- **Goal**: Expansion and/or genetic manipulation of these clones (more selective and powerful “army” of T-cells)

However, their frequency is low……
- **Novel immune technologies and sophisticated bio-informatics and computational biology tools** are needed for identification of those T-cells (in tumor biopsies and/or liquid biopsies)
Next generation sequencing (NGS) of patient’s tumor is allowing the Identification of somatic mutations (encoding tumor antigens) that can be recognized by T-cells.
Identification of tumor neo-antigens and T-cells that can recognize them

- Neoantigen load/signature related to clinical response (Brown Gen Res 2014; Champiat Oncoimmunology 2014; Snyder NEJM 2014)


- Neoantigen-specific T cells kill tumors in vivo
Identification and tracking of mutation-associated neo-antigen specific T-cells in patients treated with anti-PD1

Neoadjuvant PD-1 Blockade in Resectable Lung Cancer


- Patients with untreated, surgically resectable stage I, II, or IIIA NSCLC.

- Two preoperative doses of Nivolumab (at a dose of 3 mg/ kg, IV every 2 weeks). Surgery planned approximately 4 weeks after the first dose.

- **Primary end point:** Safety and feasibility.

- **Secondary endpoints:** Tumor pathological response, expression of PD-L1, mutational burden, and mutation-associated, neoantigen-specific T-cell responses.
Neoadjuvant PD-1 blockade in resectable Lung cancer
MANAFEST: Mutation Associated Neo-Antigens + Functional Expansion of Specific T-cells

- Whole Exome Sequencing (WES) in pre-treatment tumors and matched normal tissue: tumor-specific alterations are analyzed using a neo-antigen prediction pipeline to identify candidate MANAs specific to the patient's HLA haplotype (Bioinformatics and computational science)

- T cells are isolated from patients 4 weeks after initiation of anti-PD1 and cultured for 10 days with putative MANAs

Resulting expanded CD8β T cells are isolated for TCR Vβ CDR3 sequencing and MANAFEST analysis
MANAFEST: Tracking of mutation-associated, neo-antigen specific T-cells in patients treated with checkpoint blockade
“Immune” Liquid Biopsy

Are circulating immune cells (or their products, ie, cytokines) in peripheral blood a good reflection of the immune reaction occurring in the tumor microenvironment?

....The answer seems to be....YES
Identification and quantitation of antigen specific T-cells (clonotypes)

- Next generation sequencing (NGS) of T cell receptor (TCR) b chain enables identification and quantitation of all rearranged T cell antigen receptors, or clonotypes within a sample

Clonal expansion of CD8 T cells in the systemic circulation precedes development of ipilimumab-induced toxicities

Sumit K. Subudhi, Ana Aparicio, Jianjun Gao, Amado J. Zurita, John C. Araujo, Christopher J. Logothetis, Salahaldin A. Tahir, Brinda R. Korivi, Rebecca S. Slack, Luis Vence, Ryan O. Emerson, Erik Yusko, Marissa Vignali, Harlan S. Robins, Jingjing Sun, James P. Allison, and Padmanee Sharma
Clonal expansion of CD8+ T-cells and development of Ipilimumab toxicities

- 27 patients with metastatic prostate cancer treated with androgen deprivation therapy plus ipilimumab

- Grade 3 toxicities in >40% of treated patients which led to early closure of the study.

- Sequencing of the T-cell receptor β-chains in purified T cells from peripheral blood revealed clonal expansion of CD8 T cells before the onset of grade 2–3 irAEs.
  - Expansion of ≥55 CD8 T-cell clones preceded the development of severe irAEs.

- CD8 T-cell clonal expansion may be a biomarker to enable close monitoring and early intervention for patients receiving ipilimumab.
Moving forward….Immunosequencing in combination with bio-informatics/computational biology tools to identify disease-associated T-cell clones

Immunosequencing identifies signatures of cytomegalovirus exposure history and HLA-mediated effects on the T cell repertoire

Ryan O Emerson¹,⁴, William S DeWitt¹,²,⁴, Marissa Vignali¹, Jenna Gravley³, Joyce K Hu¹, Edward J Osborne¹, Cindy Desmarais¹, Mark Klinger¹, Christopher S Carlson³, John A Hansen³, Mark Rieder¹,⁵ & Harlan S Robins¹,²,⁵

An individual's T cell repertoire dynamically encodes their pathogen exposure history. To determine whether pathogen exposure signatures can be identified by documenting public T cell receptors (TCRs), we profiled the T cell repertoire of 666 subjects with known cytomegalovirus (CMV) serostatus by immunosequencing. We developed a statistical classification framework that could diagnose CMV status from the resulting catalog of TCRβ sequences with high specificity and sensitivity in both the original cohort and a validation cohort of 120 different subjects. We also confirmed that three of the identified CMV-associated TCRβ molecules bind CMV in vitro, and, moreover, we used this approach to accurately predict the HLA-A and HLA-B alleles of most subjects in the first cohort. As all memory T cell responses are encoded in the common format of somatic TCR recombination, our approach could potentially be generalized to a wide variety of disease states, as well as other immunological phenotypes, as a highly parallelizable diagnostic strategy.
Identification of disease-associated antigen-specific T-cell clones

Implications for Cancer Immunotherapy

• High-throughput sequencing of TCRs captures all T cell responses equally (…including to tumor antigens)

• …and host’s store immunological memory to antigens

• “Reading T-cell memory” ….in liquid biopsies and with a simple unified assay will be a valuable strategy to identify baseline frequency of antigen specific T-cells and their dynamic response to immunotherapies…..

“Holy Grail”: Expansion and/or genetic manipulation of these clones (more selective and powerful “army” of T-cells)
Conclusions

• There is so much that we can learn about tumor-specific immune responses in peripheral blood....

• Blood is like an “history book” of past encounters of immune cells with a variety of antigens (microbial, tumor....)

• We are “just learning” to identify and track in liquid biopsies mutation-associated, neoantigen-specific T-cells and its association with response and/or side effects to checkpoint blockade

• The future is promising, but significant challenges ahead, in particular dealing with big data sets....
Immunotherapy Fueling Transformational Advances in Cancer

Former President Jimmy Carter Says He Is Free of Cancer

A New Cancer Immunotherapy Leads to Remissions

The New York Times

Harnessing the Immune System to Fight Cancer