Understanding responses to cancer immunotherapy: the tissue is the issue, but the scoop is in the poop

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Understanding responses to cancer immunotherapy: the tissue is the issue, but the scoop is in the poop

Jennifer A. Wargo MD MMSc

- I have the following financial relationships to disclose:
  - Speaker's bureau: Imedex, Dava, Omniprex, Illumina, BMS
  - Advisory board member: Roche - Genentech, GSK, Novartis, Astra-Zeneca
  - Clinical trial support: Roche - Genentech, GSK, BMS, Novartis
- I am a scientific advisor to Microbiome DX
- I am co-Inventor on patent submitted by The University of Texas MD Anderson Cancer Center to the US Patent and Trademark Office based on this work (Patent # PCT/US1/53717)
It is great to be at the Yale Center for Immuno-Oncology!

MGH Surgery residency
1998 - 2000

MGH Faculty
2008 - 2013

Research Fellow 2001-2003
With Toni Ribas (UCLA)

NIH Surgery Branch Fellow 2006 - 2008 (with Steve Rosenberg)

MD Anderson Faculty
2013-present
(with Patrick Hwu, Jim Allison, and others)

It is an honor to speak here today amidst the leaders in Immuno-Oncology at Yale
We have made major advances in the treatment of melanoma and other cancers with targeted therapy and immunotherapy.

**FDA-approved agents for stage IV melanoma**

- Dacarbazine (1976)
- High-dose IL-2 (1998)
- Ipilimumab (2011)
- Vemurafenib (2011)
- Dabrafenib (2013)
- Trametinib (2013)
- Dabrafenib + Trametinib (2014)
- Pembrolizumab (2014)
- Nivolumab (2014)
- Nivolumab + Ipilimumab (2015)
- Vem + Cobi (2015)
- TVEC (2015)

**1-year OS**
- 25–35%

**2-year OS**
- 64% (Ipi Nivo ph II)

Dab, dabrafenib; FDA, Food and Drug Administration; IL-2, interleukin 2; Tram, trametinib – www.FDA.gov
Despite these advances responses are heterogeneous and are not always durable, and toxicity can be an issue...

There is a critical need to better understand who will benefit from therapy, as well as proper timing, sequence and combination of different therapeutic agents.

Menzies Cancer 2015, Larkin NEJM 2015
How can we better understand responses to therapy and optimize treatment regimens?
A powerful way to better understand responses is via “reverse translation” – where findings go from bedside to bench, and back again.

Translational research in patients with analysis of longitudinal tissue and blood samples.

- Control
- Treatment 1
- Treatment 2
- Treatment 1 + treatment 2

Mechanistic studies and therapeutic optimization in murine models.

Murine models (GEMM, PDX, etc)
Responses are dependent on factors shaping tumor growth and immunity.

Cogdill, Andrews, Wargo - *British Journal of Cancer* 2017
Lesson # 3:

be open to new opportunities
and to opportunities for your family

Part I:

“The tissue is the issue”
The impact of oncogenic mutations on anti-tumor immunity
Oncogenic BRAF mutations are found in over half of patients with melanoma, and this mutation can be targeted therapeutically (BRAF/MEK inhibitors).
We wanted to better understand mechanisms of response and resistance to targeted therapy, and studied this in patients during treatment. Several molecular targets were identified. We also know that oncogenic mutations may lead to immune evasion and blocking them can make tumors more immunogenic.
Addition of immune checkpoint blockade to targeted therapy enhanced immune infiltrate in a murine model

Multiple trials combining targeted therapy and immune checkpoint blockade are currently underway (for melanoma and other cancers) (also targeting other oncogenic mutations)

Cooper et al. *Cancer Immunol Res.* 2014 (with Arlene Sharpe et al)
The role of tumor stroma in mediating therapeutic resistance

(and the role of the tumor microbiome)
We used a model to study stromal-mediated resistance in melanoma

Certain stromal cells were capable of mediating resistance to targeted therapy

Tumor microenvironment induces innate RAF-inhibitor resistance through HGF secretion

Straussman et al, Nature 2012
We used the model to study stromal resistance in other cancers \((\text{with a twist})\)

The same strategy was employed to study resistance to chemotherapy in colorectal cancer and pancreatic cancer.

CRC+ Pancreatic Cell lines

<table>
<thead>
<tr>
<th>Cell line</th>
<th>CRC+</th>
</tr>
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<tbody>
<tr>
<td>Colo-205</td>
<td>1</td>
</tr>
<tr>
<td>DLD-1</td>
<td>1</td>
</tr>
<tr>
<td>HCT-116</td>
<td>1</td>
</tr>
<tr>
<td>HT-29</td>
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</tr>
<tr>
<td>LS411N</td>
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<tr>
<td>BxPC-3</td>
<td>1</td>
</tr>
<tr>
<td>CFPAC-1</td>
<td>1</td>
</tr>
</tbody>
</table>

In these studies, one cell line rescued cancer cells from gemictabine.

Mycoplasma is responsible for rescue from Gemcitabine:

- Eradication of mycoplasma → no rescue
- Infection of another cell line → rescue
- WGS of HDF-pre-conditioned media → mycoplasma
- Bacteria were breaking down gemcitabine into inactive form
We validated findings in human samples and mouse models, suggesting that intra-tumoral bacteria may mediate resistance to chemotherapy.

Geller et al, Science – published September 15, 2017

Potential role of intratumor bacteria in mediating tumor resistance to the chemotherapeutic drug gemcitabine

With Ravid Straussman Todd Golub, Keith Flaherty, Dirk Gevers, Curtis Huttenhower et al
Biomarkers of response to immunotherapy in melanoma
Through the outstanding work of others, biomarkers of response to immune checkpoint have been identified, but are not perfectly predictive.

- **Mutational load and neoantigens may help explain varied response to therapy**
- **The density and distribution of CD8+ T cells at baseline can help predict response (baseline)**
- **PD-L1 tumors are more likely to respond to checkpoint blockade**
- **T cell “inflamed” tumors are more likely to respond to immune checkpoint blockade**


Taube et al. 2014.
We studied a cohort of patients treated with sequential immune checkpoint blockade (first with CTLA-4 blockade followed by anti-PD-1 blockade)

Molecular profiling (whole exome sequencing, nanostring, RPPA)
Immune profiling (IHC, flow cytometry, TCR sequencing) at each time point

With Jim Allison, Pam Sharma, Mike Davies, MDACC Melanoma Medical Oncology, and Moon Shot team
Immune signatures in pre-treatment biopsies largely failed to predict therapeutic response  
**but signatures in on-treatment biopsies were highly predictive**

![Image of immune signatures in pre- and on-treatment biopsies.](image)

Pei-Ling Chen MD PhD

Pei-Ling Chen MD PhD, *Cancer Discovery 2016*

Alex Reuben PhD
We may have acceptable predictive biomarkers at present but may simply be looking at the wrong time point. Perhaps rather than putting an emphasis on pre-treatment markers for checkpoint therapy, we should be looking at adaptive immune responses in early on-treatment samples (at least until we identify better pre-treatment biomarkers).
Studying responses to cancer therapy in neoadjuvant “window” trials
Targeted therapy and immunotherapy are being used in the adjuvant setting, and there is a strong rationale to use this in the neoadjuvant setting.

Upfront surgery is currently the standard of care for these patients, but up to 70% of patients treated in this manner will relapse and die of disease.

Pre-clinical models suggest improved outcomes in neoadjuvant vs. adjuvant treatment.
Phase II trial to test the hypothesis that treatment with neoadjuvant (+ adjuvant) BRAF/MEK inhibitors would improve RFS over SOC upfront surgery

**Arm A**
SOC - Upfront surgery

**Arm B**
Neoadjuvant BRAFi/MEKi x 8 weeks

**Surgical resection**

**Pathologic assessment (pCR rate)**

**CT scans with RECIST, Surgical resection**

**Adjuvant BRAF/MEK x 44 weeks**

**Clinical and radiographic follow up**

Assess relapse-free survival, overall survival, toxicity

**Biopsy & blood draw**

**Biopsy & blood draw on-treatment (wk 3, 5)**

**Biopsy & blood draw at surgery**

**Blood draws q 3 months with restaging**

**Biopsy & blood draw on relapse**

Molecular & immune profiling in longitudinal tissue and blood samples

PIs: Amaria & Wargo

Amaria et al, Lancet Oncology 2018

NCT02231775
Treatment with neoadjuvant BRAF/MEKi was associated with a high RECIST and path CR rate, and improved RFS over SOC surgery.

RECIST response rate 85%

No correlation between RECIST and path responses

Path CR rate 58%

Amaria et al, Lancet Oncology 2018
Correlative studies on longitudinal tumor samples revealed potential predictors / targets of therapeutic resistance

Patients with < pCR had a higher frequency of known resistance conferring mutations (activating MAPK)

Immune mechanisms of therapeutic resistance were also identified, with high expression of PD-1, Tim-3, Lag-3 in TILs of pts with < pCR and failure to induce an immune infiltrate in early on-treatment biopsies
Correlative studies also highlighted provocative findings that were validated in additional cohorts and in murine models.

<table>
<thead>
<tr>
<th>Gender</th>
<th>RECIST</th>
<th>pCR vs &lt;pCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>M</td>
<td>PR</td>
<td>NR</td>
</tr>
<tr>
<td>M</td>
<td>SD</td>
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<td>F</td>
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<td>pCR</td>
</tr>
<tr>
<td>F</td>
<td>PR</td>
<td>pCR</td>
</tr>
</tbody>
</table>

We now have insight into the mechanism behind this and potential targets.
We also ran a phase II trial using neoadjuvant (+ adjuvant) checkpoint blockade in patients with high risk resectable metastatic melanoma.

Ipi 3mg/kg + Nivo 1 mg/kg q 3 wks x 3 doses (n=20)

Nivo 3mg/kg q 2 wks x 4 doses (n=20)

Patients with resectable stage IIIB/IIIC melanoma, no brain mets or prior ICB

Primary Endpoint:
Path response

Secondary endpoints:
- RECIST
- RFS
- DMFS
- OS
- Toxicity
- Correlatives

Molecular & immune profiling in longitudinal tissue and blood samples

Trial was stopped early due to concerns re: progression / toxicity concerns

NCT02519322

PIs: Amaria & Wargo
Treatment with neoadjuvant Ipi Nivo was associated with a higher RECIST / pCR rate, and improved RFS over Nivo monotherapy.

Roda Amaria MD

Amaria, Reddy et al, confidential unpublished data, submitted * DO NOT POST *

Sangeetha Reddy MD MS
However treatment with combined therapy was associated with a high rate of adverse events

<table>
<thead>
<tr>
<th>Select Treatment Related Adverse Events During Neoadjuvant Treatment</th>
<th>Nivolumab (n=12)</th>
<th>Ipilimumab + Nivolumab (n=11)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Any Grade, %</strong></td>
<td><strong>Grade 3-4, %</strong></td>
<td><strong>Any Grade, %</strong></td>
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<tr>
<td>Any Treatment Related Adverse Events</td>
<td>92</td>
<td>8</td>
</tr>
<tr>
<td>Fatigue</td>
<td>67</td>
<td>0</td>
</tr>
<tr>
<td>Rash</td>
<td>17</td>
<td>0</td>
</tr>
<tr>
<td>Fevers/chills/flu like</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>Weight loss/anorexia</td>
<td>17</td>
<td>0</td>
</tr>
<tr>
<td>Transaminitis</td>
<td>17</td>
<td>0</td>
</tr>
<tr>
<td>Colitis/diarrhea</td>
<td>17</td>
<td>0</td>
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<tr>
<td>Hyperthyroidism</td>
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<td>0</td>
</tr>
<tr>
<td>Hypothyroidism</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Myositis/myalgias</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>Pain</td>
<td>25</td>
<td>8</td>
</tr>
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</table>
Correlative analyses on samples from the neoadjuvant checkpoint blockade trial reveal known and novel biomarkers/targets for therapeutic resistance. Similar biomarkers of response (inflamed tumors) are enriched in responders to checkpoint blockade. Tertiary lymphoid structures are enriched in responders to checkpoint blockade.

Sangeetha Reddy MD MS  Amaria, Reddy et al, confidential unpublished data, submitted * DO NOT POST * Michael Tetzlaff MD PhD
Importantly, we are working with others to better understand the implications of these findings and inform next steps.

We are working with others globally to form an International Neoadjuvant Melanoma Consortium.
Part 2:

“The scoop is in the poop”
The role of the gut microbiome in response to immune checkpoint blockade
Responses are dependent on factors shaping tumor growth and immunity.

Systemic Immunity
Innate and Adaptive

Tumour Genome and Epigenome

Tumour Microenvironment

Cogdill, Andrews, Wargo - British Journal of Cancer May 2017
THE HUMAN MICROBIOME

- 100 trillion microbes
- 3% human body mass
- 1-10X microbes : human cells
- 10-100X microbial : human genes
- largest # microbes – GI tract

Slide credit: Ami Bhatt and Robert Jenq
There is strong evidence that bacteria in the gut may influence responses to cancer therapy (particularly immunotherapy).
The gut microbiome may influence responses to SCT and checkpoint blockade

Diversity of the gut microbiome is associated with differential outcomes in the setting of stem cell transplant in patients with AML

Composition of the gut microbiome is associated with differential responses to checkpoint blockade in murine models

Commensal *Bifidobacterium* promotes antitumor immunity and facilitates anti-PD-L1 efficacy

Anticancer immunotherapy by CTLA-4 blockade relies on the gut microbiota

Taur...Pamer Blood 2014

Sivan...Gajewski Science 2015, Vetizou...Zitvogel Science 2015
We studied oral and gut (fecal) microbiome in a large cohort of patients with metastatic melanoma going onto systemic therapy. Microbiome sequencing & immune profiling was performed.

Responders to anti-PD-1 had a higher diversity of gut bacteria associated with prolonged PFS (along with additional compositional differences).

Deeapk Gopalakrishnan PhD
Gopalakrishnan et al, Science 2018
In our cohort, we identified a gut microbiome “signature” with a high likelihood of response to anti-PD-1 (with subsequent validation in a larger cohort) suggesting that the gut microbiome could be used as a biomarker of response to immune checkpoint blockade, with patients with a “type I” signature more likely to respond.

Gopalakrishnan et al, Science 2018
Importantly, “favorable” signatures in the gut microbiome were associated with enhanced immune responses in the tumor microenvironment.

And mechanistic studies in germ free mice showed that fecal transplant could recapitulate the phenotype.

Mechanistic insights suggest that this is mediated both at the level of the gut and mesenteric lymph node, and also via metabolites produced by gut microbes potentially mediating distant effects (needs validation).

Gopalakrishnan et al, Science 2018
Numerous studies in human cohorts now support a link between the gut microbiome and response and toxicity to immune checkpoint blockade.

However bacterial taxa associated with response differ across the cohorts, which is in part related to different approaches for profiling – highlighting the need to standardize approaches.

Setting standards for reproducibility in gut microbiome research nature.com September 2018
Matson et al, Routy et al, Science 2018
Gopalakrishnan, Helmink et al, Cancer Cell 2018
Can we modulate the gut microbiome to enhance responses to immunotherapy? (and/or to abrogate toxicity)

YES!
We are working with the Parker Institute for Cancer Immunotherapy and Seres Therapeutics to run a clinical trial to test the hypothesis that modulation of the gut microbiome will enhance immunotherapy responses (using FMT and other strategies).
PICI-0014: A randomized trial to evaluate the impact of gut microbiome modulation in patients going on to treatment with immune checkpoint blockade

**Patients with metastatic cancer (melanoma) going onto immune checkpoint blockade (anti-PD-1)**

- **Arm A**
  - FMT from complete responders (CRs) to aPD-1 (n=20)
- **Arm B**
  - Ser-401 (Live bacterial product – spore prep with Type 1 signature) (n=20)
- **Arm C**
  - Placebo (n=20)

**All patients = CT scans with RECIST week 12**

**Primary endpoint = safety and tolerability**

**Secondary endpoints: engraftment, response and correlative studies (immune correlates in blood and tumor, metabolites)**

**Key drivers: Burton & LaValle**
These approaches may also be helpful in treating immunotherapy toxicity.

50 yo female with metastatic urothelial cancer was treated with aCTLA-4 + a PD-1 and developed colitis refractory to steroids and aTNF. She was treated with FMT from a healthy donor and had complete resolution of all symptoms.
What other factors influence the microbiome that we should monitor (and potentially modulate)?
In Zitvogel cohort, use of antibiotics in the setting of treatment with checkpoint blockade was associated with impaired survival

**Antibiotics (ATB) taken 2 months before and/or 1 month after the 1st administration of aPD1 Ab or aPD-L1 Ab.**

- ATB, n=52 (30%)
- No ATB, n=123 (70%)

**Antibiotic use was associated with lower diversity of the gut microbiome in a cohort of melanoma patients (MDACC)**

- Median OS ATB: 12.1 months
- Median OS No ATB: 20.8 months

**We know from published literature that other medications can also impact the microbiome (Maier et al Nature March 2018 among other papers)**

Routy et al Science 2018; Spencer et al, confidential unpublished data *PLEASE DO NOT POST* Christine Spencer PhD
In our cohort, we also studied the influence of diet and lifestyle factors (such as stress and depression) on the microbiome and response.

Patients with a high fiber diet had higher diversity in the gut microbiome (with higher abundance of “favorable” gut bacteria).

42% of our patients reported taking probiotics, and this was associated with a LOWER diversity in the gut microbiome.

Spencer et al, confidential unpublished data * PLEASE DO NOT POST*
The Melanoma Moon Shot Program

These efforts are going on worldwide through outstanding research studies and the strongest gains are made through collaboration.
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Patients and their families
Yale Center for Immuno-Oncology / conference organizers and participants

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• Beth Helmink MD PhD
• Alexandria P. Cogdill MS (PhD candidate)
• Robert Szczepaniak-Sloane BS (PhD candidate)
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• Luigi Nezi PhD, Wei Shen Chen (alumni)

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• Ravid Straussman MD PhD

MDACC Collaborators
• Jim Allison PhD, Pam Sharma MD PhD
• Michael Davies MD PhD, Jeff Gershenwald MD
• Patrick Hwu MD, other Melanoma Med Onc Facutly / Staff
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