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MD Anderson Cancer Center
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Influence of the gut microbiome upon anti-PD-1 and immuno-oncology responses

Jennifer A. Wargo MD MMSc
Associate Professor,
Departments of Genomic Medicine & Surgical Oncology
UT, MD Anderson Cancer Center

Society for the Immunotherapy of Cancer

Workshop on Single Cell Techniques in Immunology and Cancer Immunotherapy

Session III: Predictors of Response and Liquid Biopsy

November 9, 2017

Disclosure information
SITC Workshop on Single Cell Techniques in Immunology and Cancer Immunotherapy
Session III: Predictors of Response and Liquid Biopsy
November 8, 2017

Influence of gut microbiome upon anti-PD-1 immuno-oncology responses

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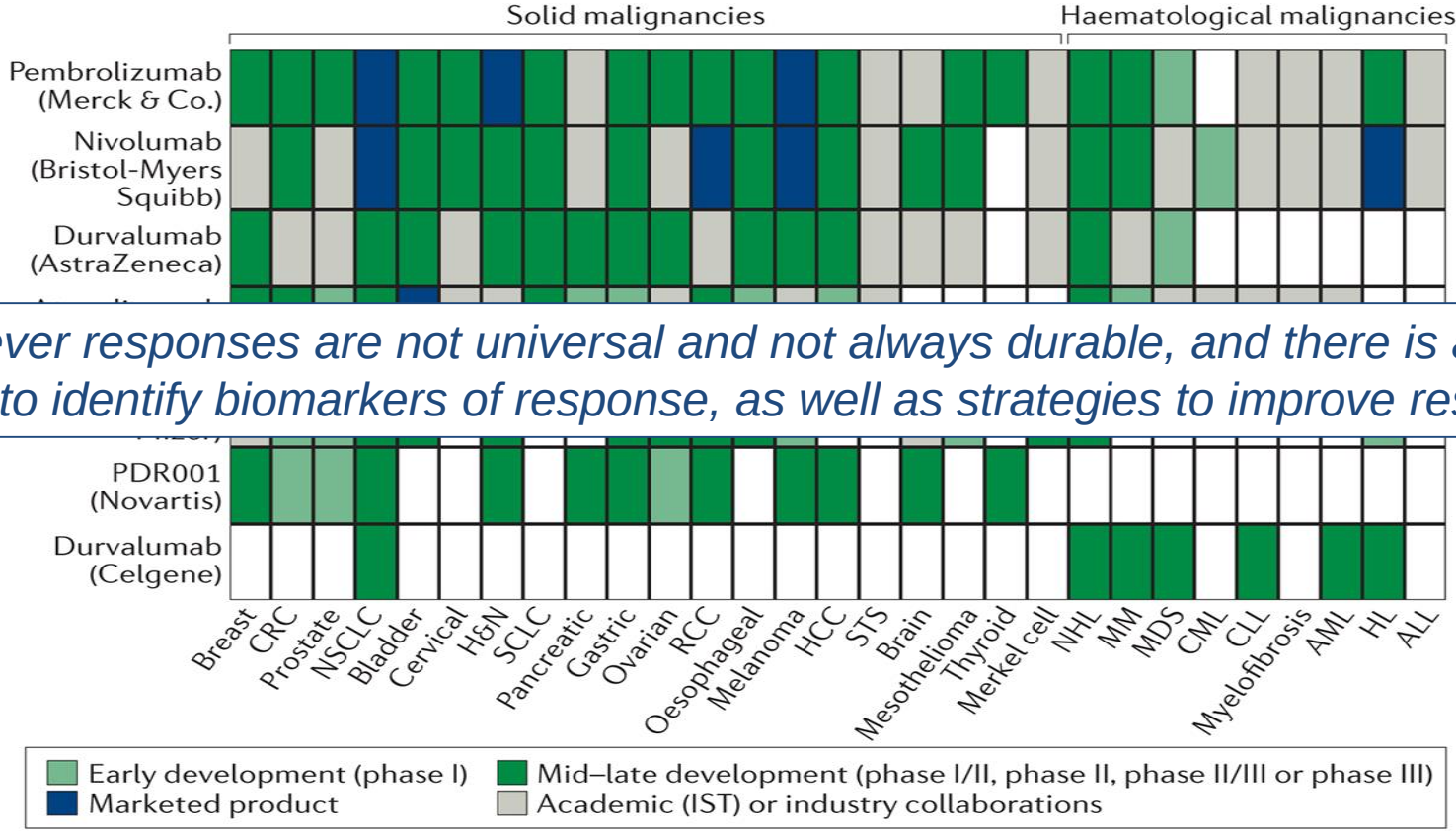
- I have the following financial relationships to disclose:

- 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 an 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)*

We have made major advances in the treatment of cancer through the use of immunotherapy (immune checkpoint blockade)



However responses are not universal and not always durable, and there is a critical need to identify biomarkers of response, as well as strategies to improve responses

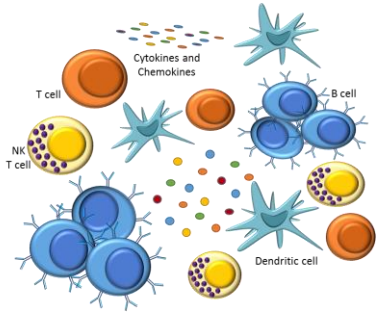
Cavnar et al, Nature Reviews of Drug Discovery, 2017

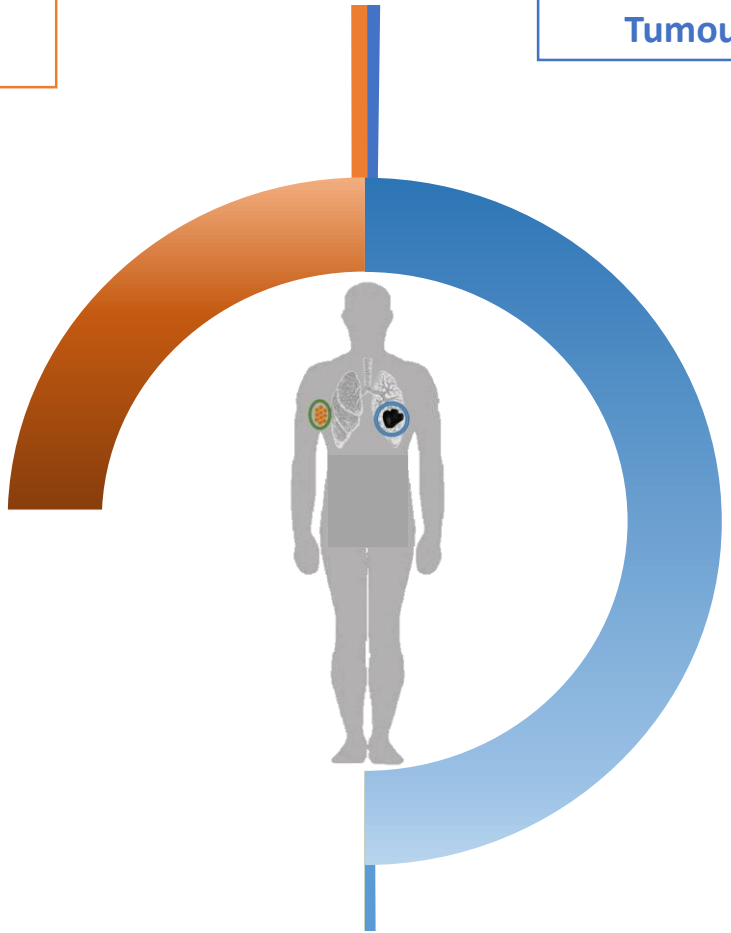
Nature Reviews | Drug Discovery

How can we better understand responses to therapy and optimize treatment regimens?

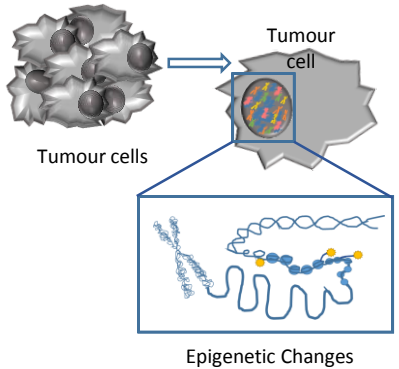
Responses are dependent on factors shaping tumor growth and immunity

Systemic Immunity
Innate and Adaptive

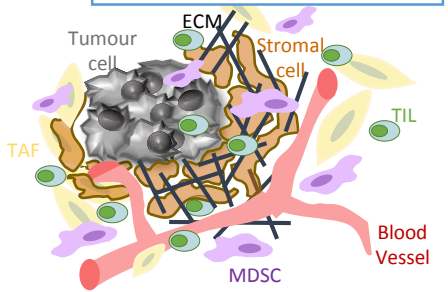




Tumour Genome and Epigenome

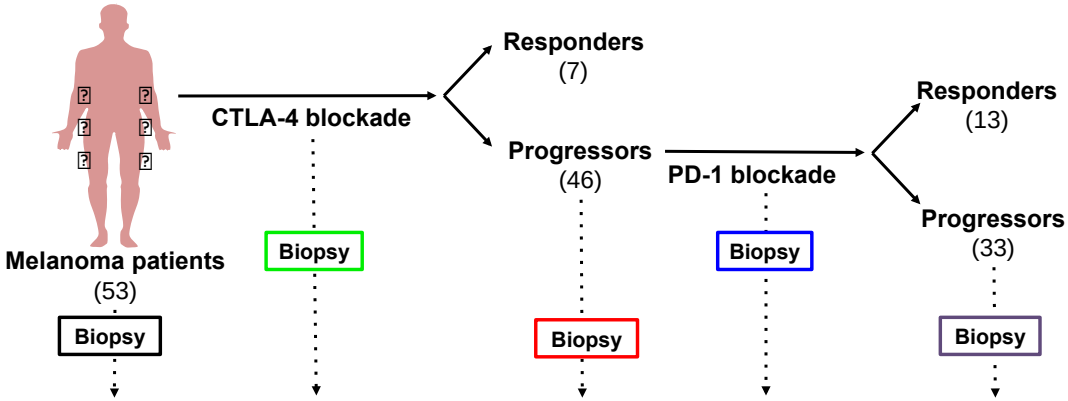


Tumour Microenvironment



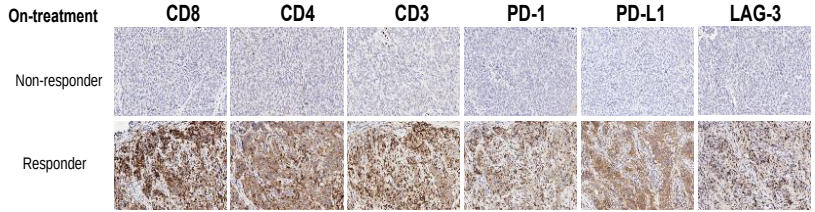
Cogdill, Andrews, Wargo - *British Journal of Cancer May 2017*

Translational research in tumors of patients on checkpoint blockade reveals molecular and immune mechanisms of response & resistance

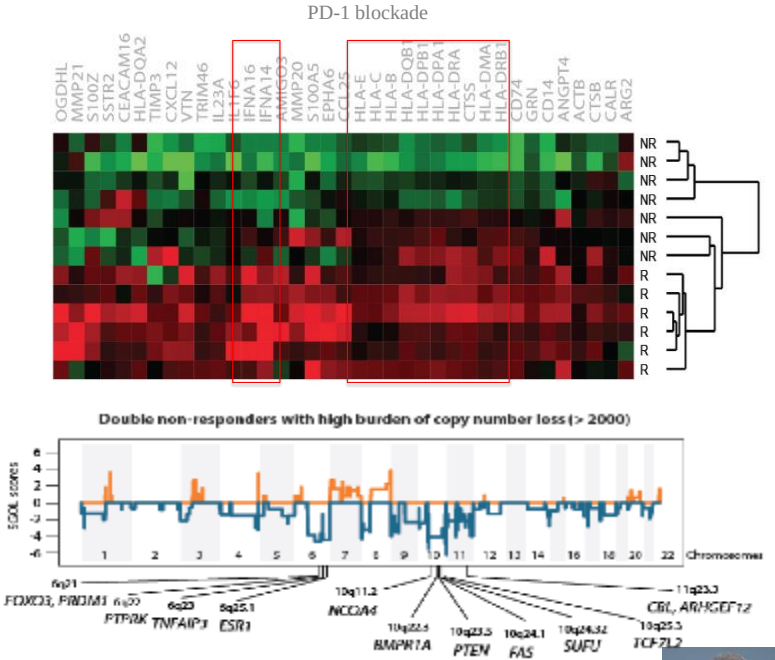


Molecular and immune profiling performed in longitudinal tumor samples during therapy

These studies also revealed that adaptive immune signatures in early on-treatment biopsies are highly predictive of response



Mechanisms of resistance were identified, with defects in IFN signaling & antigen processing / presentation, As well as a high burden of copy number loss



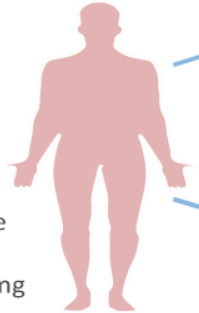
Chen et al, Cancer Discovery 2016
Roh et al, Science Translational Medicine 2017

With Jim Allison, Pam Sharma, Andy Futreal, Lynda Chin, Arlene Sharpe, and others

Translational research in novel clinical trials is also providing insights into mechanisms of response and resistance to checkpoint blockade

Inclusion Criteria
- Resectable stage IIIB/IIIC, oligometastatic stage IV melanoma
- >1 site amenable to biopsy

Exclusion Criteria
- Brain, bone mets, LMD
- Active, known autoimmune disease
- Systemic steroid use (>10 mg prednisone daily)
- Prior anti-PD1/PD-L1, CTLA-4 treatment



Stratify by Stage and PDL1 Status

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)

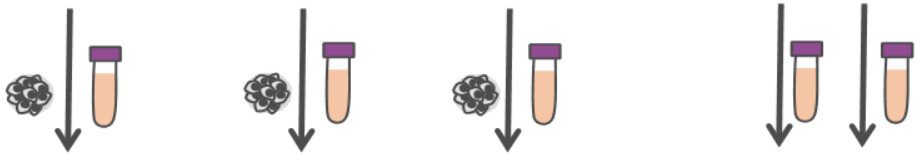
Surgery

Primary Endpoint: Path CR rate at surgical resection

Secondary Endpoints:
-RECIST 1.1 measurement
-Toxicity

Nivo 3 mg/kg q 2 wks x 6 months

Secondary Endpoints:
-RFS
-DMFS
-OS
-Toxicity
-Immune Monitoring



Molecular & immune profiling in longitudinal tissue and blood samples

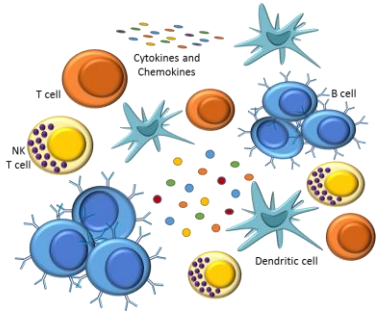
PIs: Rodabe Amaria & Jennifer Wargo

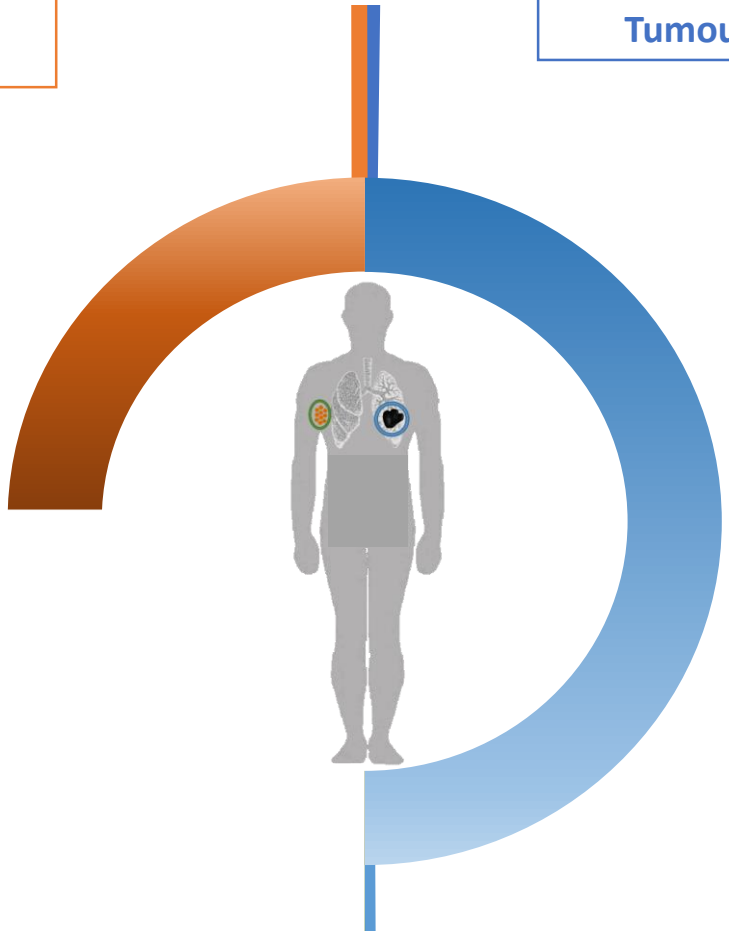
NCT02519322

To be presented by Dr. Sangeetha M. Reddy on Saturday November 11 at The Presidential Session (102), 2:20 – 2:35 pm Abstract # 015
Presidential award nominee

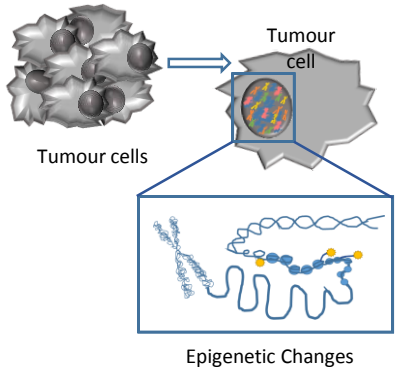
Environmental factors (such as the **microbiome**) may also impact responses

Systemic Immunity
Innate and Adaptive

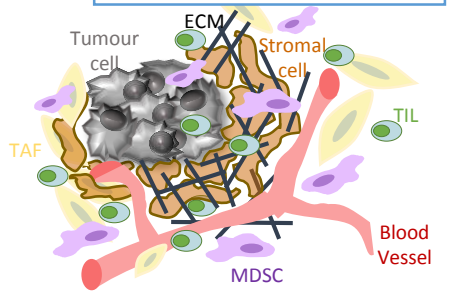




Tumour Genome and Epigenome



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

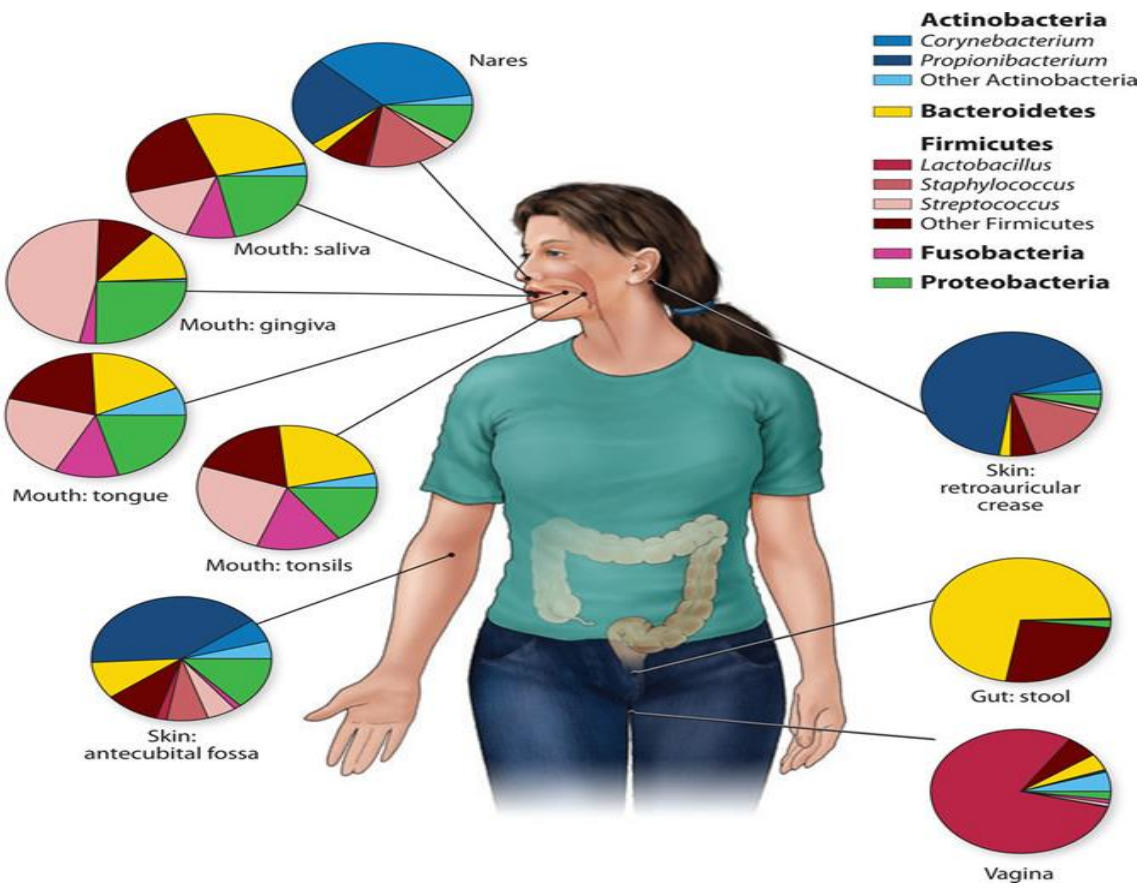
10-100X microbial : human genes

largest # microbes – GI tract

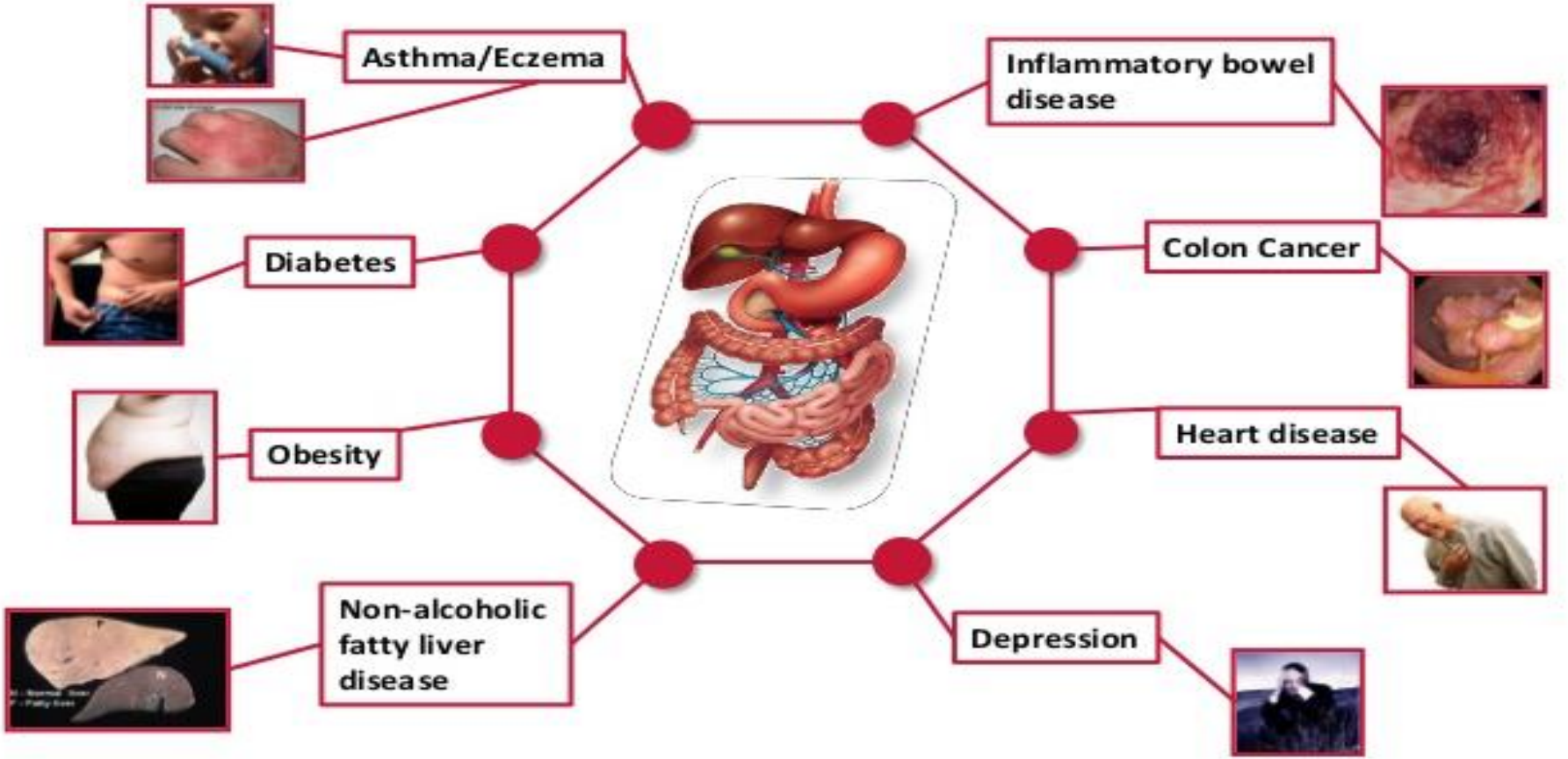
Slide credit: Ami Bhatt and Robert Jenq

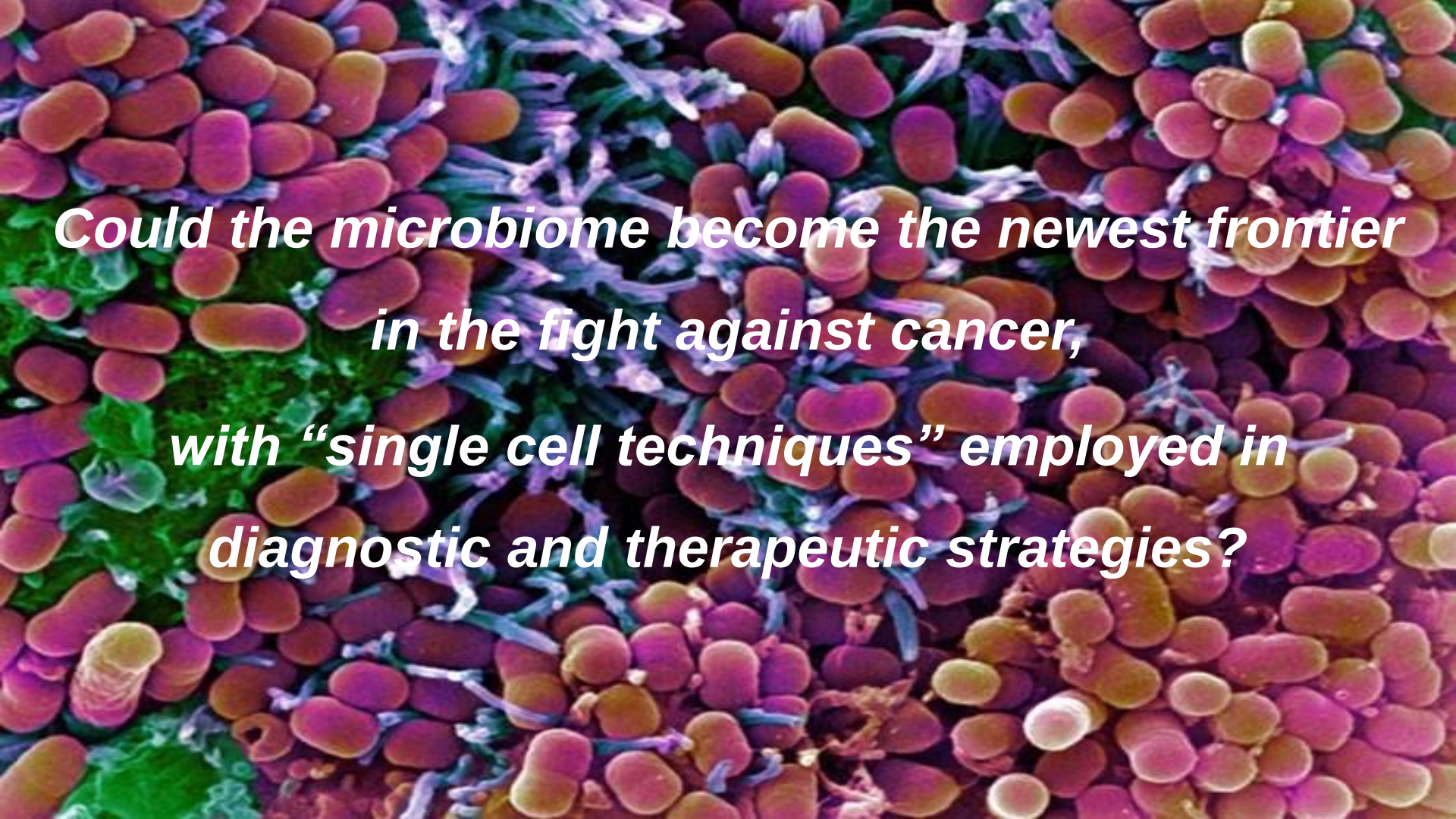
There Human Microbiome Project (HMP) helped to define the composition of the microbiome in healthy individuals

NIH HUMAN MICROBIOME PROJECT



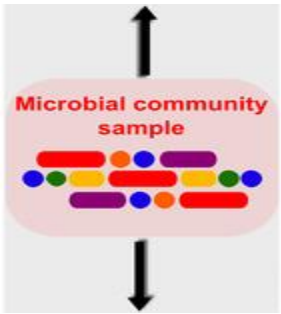
Disturbances of the gut microbiome (dysbiosis) are implicated in a large number of diseases




Could the microbiome become the newest frontier
in the fight against cancer,
with “single cell techniques” employed in
diagnostic and therapeutic strategies?

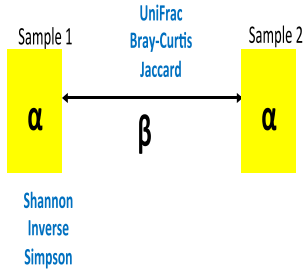
How can we characterize the microbiome?

16s and whole genome shotgun sequencing (WGS) are useful tools in characterizing the microbiome

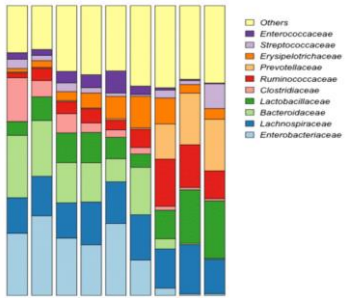


Metrics:

- **Diversity:** elucidates distribution and assembly patterns of microbial communities
- **Types of diversity**
  - **α** - intra-sample
  - **β** - inter-sample



- **Relative abundance:** bacterial communities analyzed as discrete OTUs, and frequency of an OTU relative to all others is quantified



Morgan XC, doi:10.1371/journal.pcbi.1002808

What is the role of the microbiome in cancer?

There is a growing appreciation of the role of the microbiome in cancer

DOI: 10.1093/jnci/djs003
Advance Access publication on January 23, 2012.

Published by Oxford University Press 2012.

BRIEF COMMUNICATION

Fifteen-Year Effects of *Helicobacter pylori*, Garlic, and Vitamin Treatments on Gastric Cancer Incidence and Mortality

Jun-Ling Ma, Lian Zhang, Linda M. Brown, Ji-You Li, Wei-Dong Liu, Yuanreng Hu, David Pee, William

New written informed consents were obtained for the extended follow-up phase from May 2, 2003, to August 1, 2010. Data from 3365 eligible

Report

LETTER

Proinflammatory CD4^{+}CD45RB^{hi} Lymphocytes Promo and Intestinal Carcinogenesis in *Apc*$^{Min/+}$ Mice

Varada P
Bruce H

Zhongming Ge,[1] Prashant R. Namb

Adenoma-linked barrier defects and microbial products drive IL-23/IL-17-mediated tumour growth

doi:10.1038/nature11465

Sergei I. Grivennikov[1]*, Kepeng Wang[1,3]*, Daniel Mucida[2,4], C. Andrew Stewart[5], Bernd Schnabl[6], Dominik Jauch[1], Koji Taniguchi[1,7], Guann-Yi Yu[1], Christoph H. Österreicher[8,9], Kenneth E. Hung[9], Christian Datz[10], Ying Feng[11], Eric R. Fearon[11], Mohamed Oukka[12], Lino Tessarollo[13], Vincenzo Coppola[14], Felix Yarovinsky[15], Hilde Cheroutre[2], Lars Eckmann[6], Giorgio Trinchieri[5] & Michael Karin[1]

Rese

MyD88 inhibition amplifies de cell capacity to promote pancreatic carcinogenesis via Th2 cells

Cel PRESS

The In
Inflamn

Atsuo Ochi,[1] Andrew H. Nguyen,[2] Andrea S. Bedrosian,[1] Harry M. Mushlin,[2] Saman Zarbakhsh,[1] Rocky Barilla,[1] Constantinos P. Zambirinis,[1] Nina C. Fallon,[1] Adeel Rehman,[1] Yuliya Pylayeva-Gupta,[3] Sana Badar,[1] Cristina H. Hajdu,[4] Alan B. Frey,[2] Dafna Bar-Sagi,[3] and George Miller[1,2]

Grace Y. Chen,[1,3] Michael H. Shaw,[2,3] Gloria Redondo,[2,3] and Gabriel

Division of Hematology and Oncology, Department
Cancer Center, University

Intestinal Neoplasia in the *Apc*^{Min} Mouse: Independence from the Microbial and Natural Killer (*beige* Locus) Status[1]

William F. Dove,[2] Linda Clipson, Karen A. Gould,[3] Cindy Luongo,[4] David J. Marshall, Amy R. Moser,[5] Michael A. Newton, and Russell F. Jacoby

McArdle Laboratory for Cancer Research [L. C., A. R. M.] and Laboratory of Genetics [W. F. D., K. A. G., C. L.], University of Wisconsin, Madison, Wisconsin 53706; Department of Medicine, Division of Gastroenterology [D. J. M., R. F. J.] and Department of Biostatistics, Comprehensive Cancer Center [M. A. N.], University of Wisconsin, Madison, Wisconsin 53792

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Immunoproliferative Small Intestinal Disease Associated with *Campylobacter jejuni*

Martin, M.D.,

Promotion of Hepatocellular Carcinoma by the Intestinal Microbiota and TLR4

Dianne H. Dapito,[1,2,10] Ali Mencin,[3,10] Geum-Youn Gwak,[1,7,10] Jean-Philippe Pradere,[1,10] Myoung-Kuk Jang,[1] Ingrid Hossein Khabanian,[4,5] Adebowale Adeyemi,[3] Ramon Bataller,[6] Jay Raul Rabadan,[4,5]

Chronic Active Hepatitis and Associated Liver Tumors in Mice Caused by a Persistent Bacterial Infection With a Novel *Helicobacter* Species

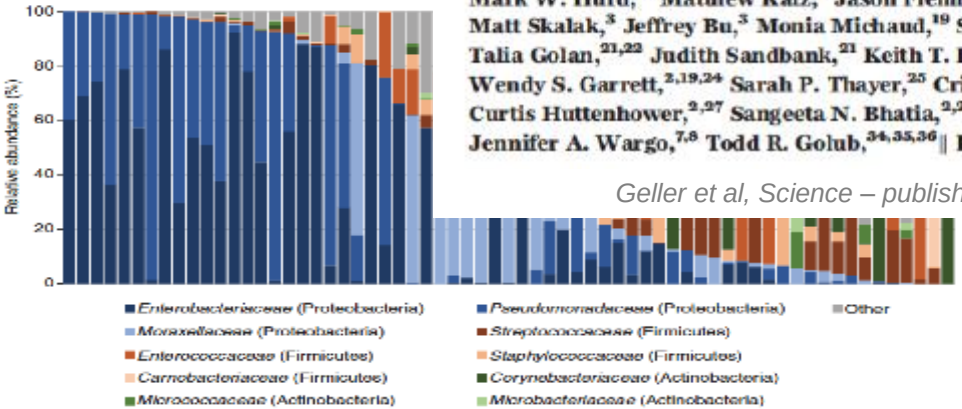
*Jerrold M. Ward, James G. Fox, Miriam R. Anver, Diana C. Haines, Cathi V. George, Michael J. Collins, Jr., Peter L. Gorelick, Kunio Nagashima, Matthew A. Gonda, Raymond V. Gilden, Joseph G. Tully, Robert J. Russell, Raoul E. Benveniste, Bruce J. Paster, Floyd E. Dewhirst, John C. Donovan, Lucy M. Anderson, Jerry M. Rice**

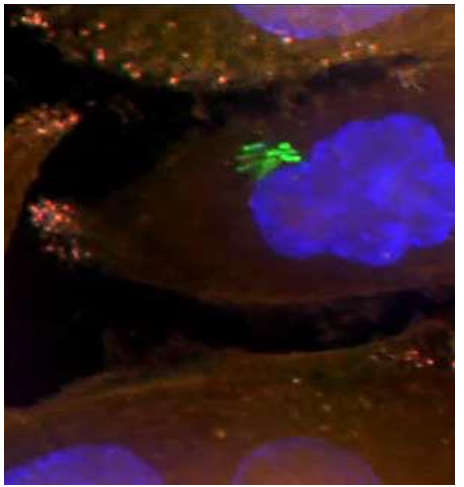
Bacteria in tumors of cancer patients may mediate resistance to therapy

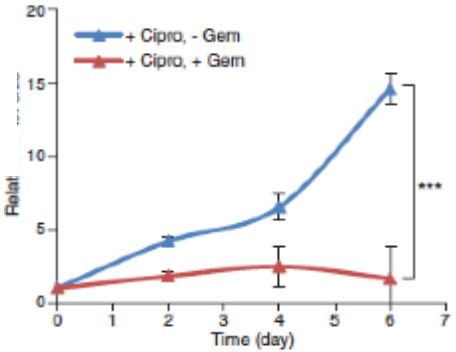
CANCER

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

Leore T. Geller,[1†] Michal Barzily-Rokni,[2*] Tal Danino,[3†] Oliver H. Jonas,[4,5] Noam Shental,[6] Deborah Nejman,[1] Nancy Gavert,[1] Yaara Zwang,[1] Zachary A. Cooper,[7,8‡] Kevin Shee,[2] Christoph A. Thaiss,[9] Alexandre Reuben,[8] Jonathan Livny,[2] Roi Avraham,[10] Dennie T. Frederick,[11] Matteo Ligorio,[12] Kelly Chatman,[13] Stephen E. Johnston,[2] Carrie M. Mosher,[2] Alexander Brandis,[14] Garold Fuks,[15] Candice Gurbatri,[16] Vancheswaran Gopalakrishnan,[8] Michael Kim,[8] Mark W. Hurd,[17] Matthew Katz,[8] Jason Fleming,[8] Anirban Maitra,[18] David A. Smith,[2] Matt Skalak,[3] Jeffrey Bu,[3] Monia Michaud,[19] Sunia A. Trauger,[13] Iris Barshack,[20,21] Talia Golan,[21,22] Judith Sandbank,[21] Keith T. Flaherty,[12] Anna Mandinova,[2,23] Wendy S. Garrett,[2,19,24] Sarah P. Thayer,[25] Cristina R. Ferrone,[26] Curtis Huttenhower,[2,27] Sangeeta N. Bhatia,[2,28,29,30,31,32,33] Dirk Gevers,[2]§ Jennifer A. Wargo,[7,8] Todd R. Golub,[34,35,36]‖ Ravid Straussman[1]‖¶







Geller et al, Science – published September 15, 2017

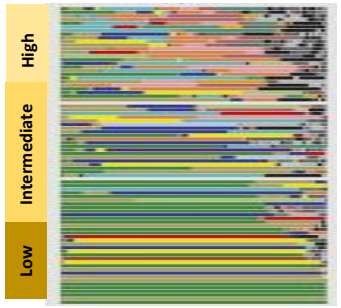
With Ravid Straussman Todd Golub, Keith Flaherty, Curtis Huttenhower et al

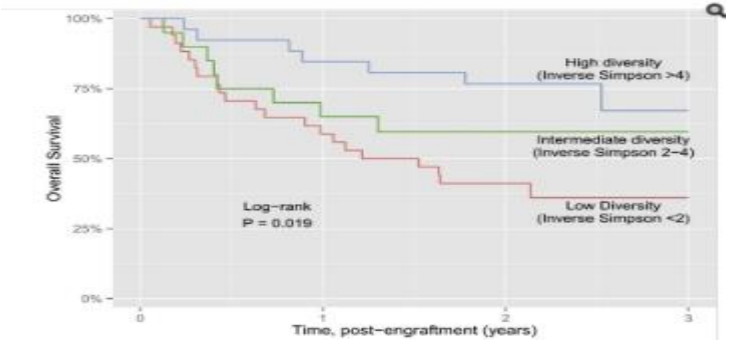
There is also strong evidence that bacteria in the gut may influence responses to cancer therapy

(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



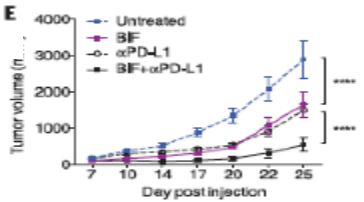


Taur…Pamer Blood 2014

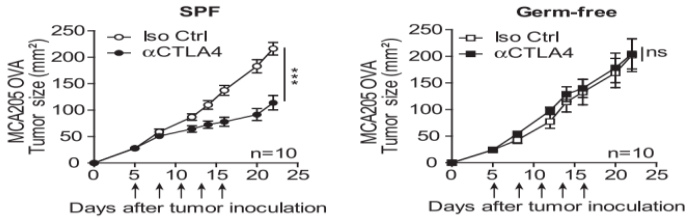
Composition of the gut microbiome is associated with differential responses to checkpoint blockade in murine models

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

Ayclet Sivan,[1*] Leticia Corrales,[1*] Nathaniel Hubert,[3] Jason B. Williams,[1] Keston Aquino-Michaels,[2] Zachary M. Earley,[2] Franco W. Benyamin,[1] Yuk Man Lei,[2] Rana Jabri,[3] Maria-Luisa Alegre,[3] Eugene B. Chang,[3] Thomas F. Gajewski[1,2,3]†



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



Sivan…Gajewski Science 2015, Vetizou…Zitvogel Science 2015

Based on this evidence, we wanted to better understand the role of the gut microbiome in response to checkpoint blockade in patients with melanoma

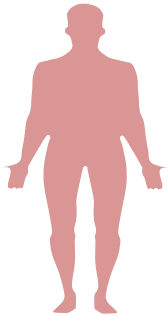
Hypothesis

- Differential bacterial “signatures” exist in responders versus non-responders to immune checkpoint blockade

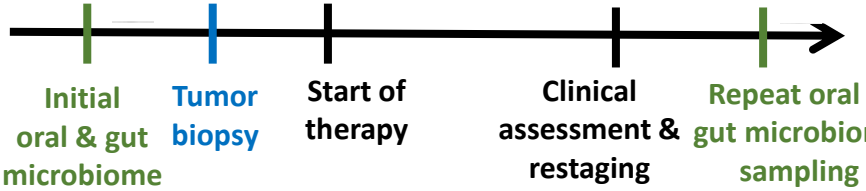
- Favorable signatures will be associated with an enhanced anti-tumor immune response (with increased CD8+ T cells, as well as evidence of an enhanced innate immune response)

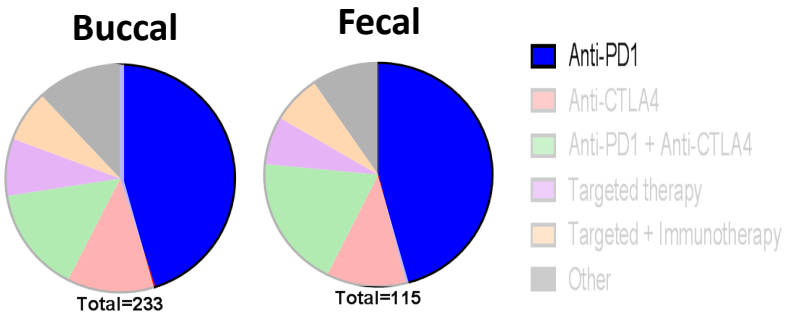
- Insights gained could lead to strategies to enhance responses to therapy (through modulation of the microbiome)

We studied oral and gut (fecal) microbiome in a large cohort of patients with metastatic melanoma going onto systemic therapy



n = 233 patients





Microbiome sequencing

16S sequencing
Whole genome sequencing

Molecular Profiling

Whole Exome Sequencing

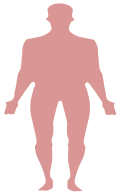
Immune Profiling

Immunohistochemistry
Flow cytometry
T cell Receptor Sequencing
Cytokine Analysis

Gopalakrishnan et al, Science (in press) (First Release November 2 2017)

Is there an association between the *diversity* of the microbiome and response to anti-PD-1 in patients with metastatic melanoma?

The *diversity* of the oral and gut microbiome were analyzed in responders versus non-responders to PD-1 blockade



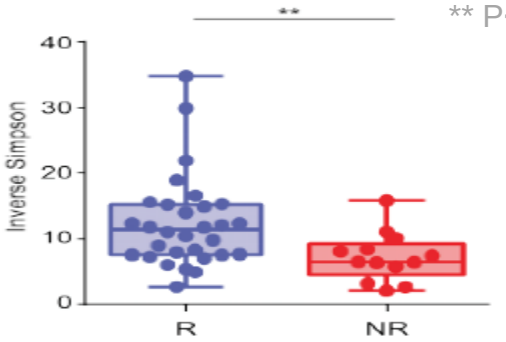
Patients with metastatic melanoma going onto aPD-1 were enrolled onto p
mic
pr

Restatging scans performed at week 12 & RECIST responses were measured

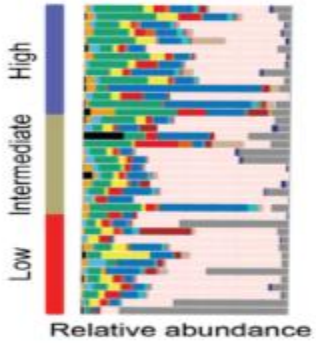
"Responders" CR, PR, SD for at least 6 months

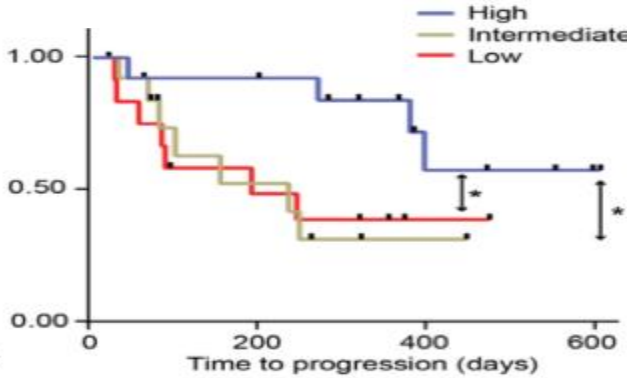
"Non-responders" PD

PFS also assessed



Responders to anti-PD-1 therapy had a higher diversity in their gut microbiome than non-responders
(with no differences noted in the the oral microbiome)





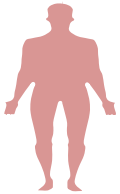
* p<0.05

Higher diversity of the gut microbiome is associated with improved PFS on melanoma patients on anti-PD-1 therapy
(no difference in oral microbiome)

Gopalakrishnan et al, Science (in press) (First Release November 2 2017)

Is there an association between the *composition* of the microbiome and response to anti-PD-1 in patients with metastatic melanoma?

The *composition* of the oral and gut (fecal) microbiome were analyzed in responders versus non-responders to PD-1 blockade

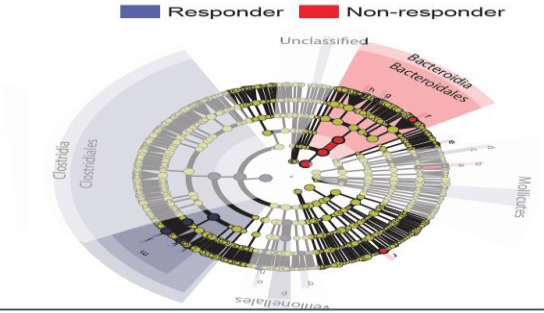


Patients with metastatic melanoma going on to receive PD-1 blockade

Restatging scans performed at week 12 & RECIST responses were measured

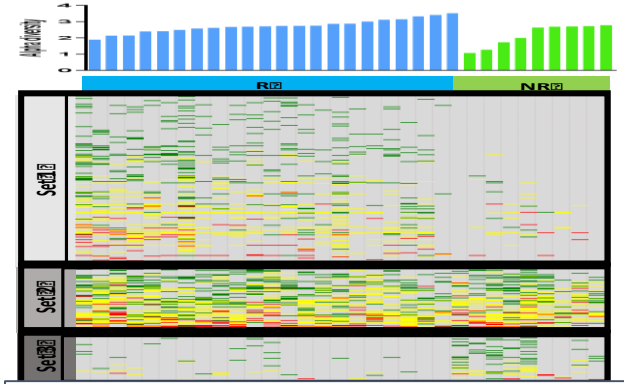
"Responders" CR, PR, SD for at least 6 months

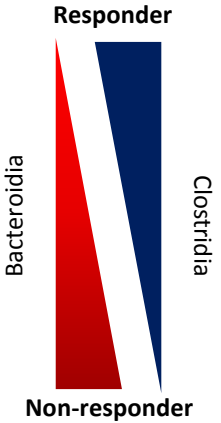
"Non-responders" PD

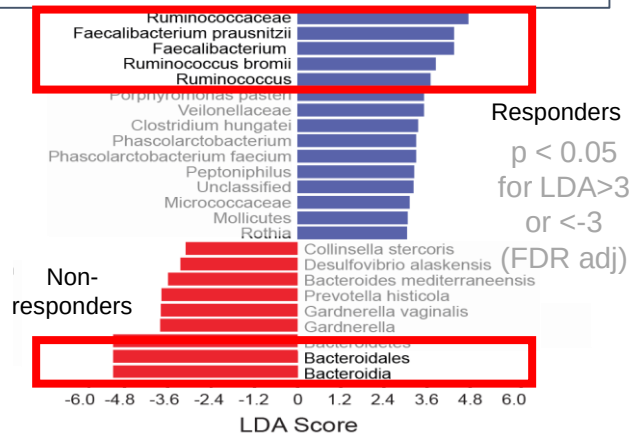


Significant differences were noted in composition in the gut microbiome of responders vs non-responders

microbiome protocol





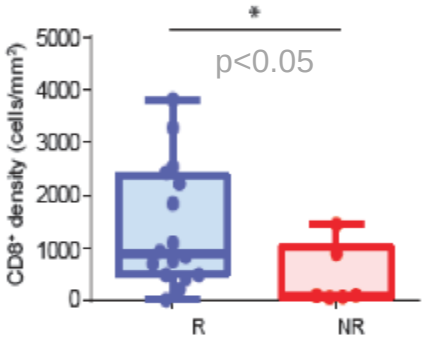


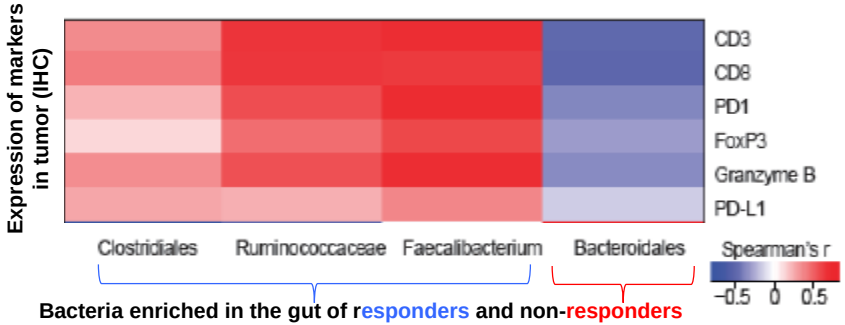
No substantial differences were noted in composition in the oral microbiome of responders vs non-responders

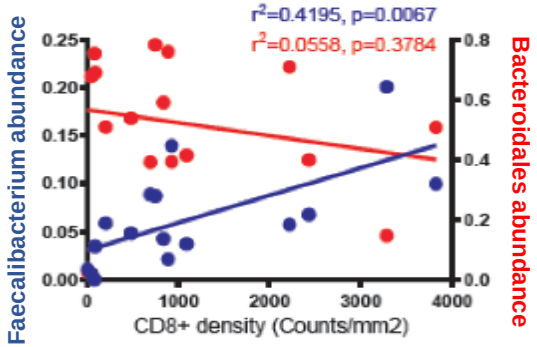
Gopalakrishnan et al, Science (in press) (First Release November 2 2017)

What is the relationship between the gut microbiome and anti-tumor immunity in this cohort?

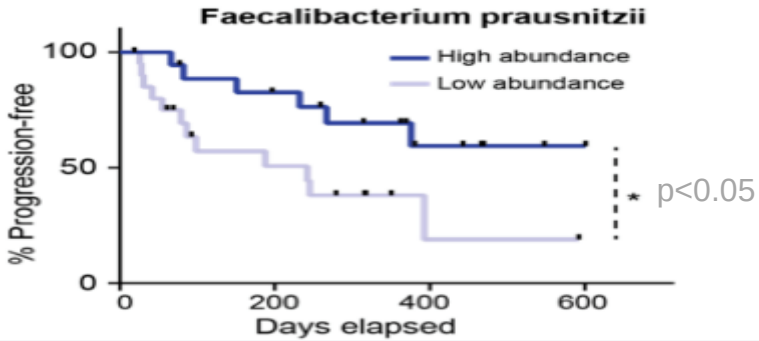
Anti-tumor immune responses were assessed and were compared to the composition of the gut (fecal) microbiome in patients on anti-PD-1

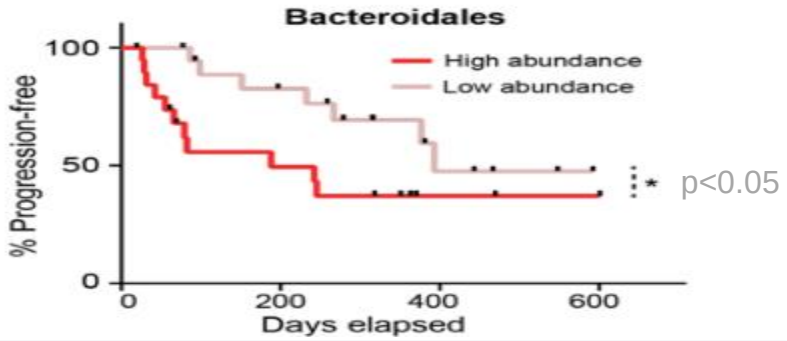






High abundance of Ruminococcus & Faecalibacteria in the gut was associated with cytotoxic T cells in TME





Differences in composition of the gut microbiome were associated with differences in PFS on aPD-1

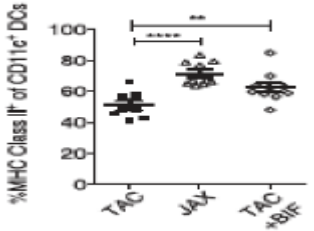
Gopalakrishnan et al, Science (in press) (First Release November 2 2017)

What is the mechanism through which a “favorable” gut microbiome may enhance responses to checkpoint blockade?

We are gaining insight into how the gut microbiome influences responses

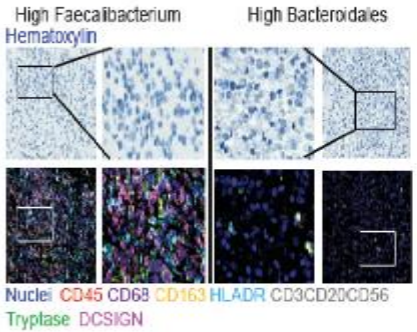
Commensal *Bifidobacterium* promotes antitumor immunity and facilitates anti–PD-L1 efficacy

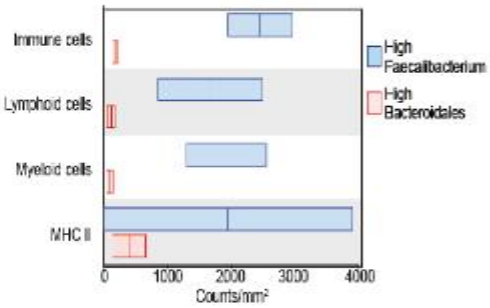
Ayelet Sivan,[1*] Leticia Corrales,[1*] Nathaniel Hubert,[1] Jason B. Williams,[1] Keston Aquino-Michaels,[1] Zachary M. Earley,[1] Franco W. Benyamin,[1] Yuk Man Lei,[1] Rana Jabri,[1] Maria-Luisa Alegre,[1] Eugene B. Chang,[1] Thomas F. Gajewski[1-3†]



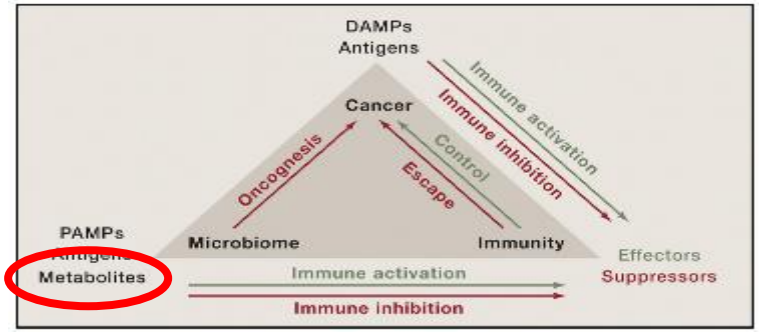
Sivan…Gajewski Science 2015

Mice with a "favorable" gut microbiome and enhanced response to checkpoint blockade had more functional antigen-presenting cells (DCs) capable of priming an antigen-specific T cell response

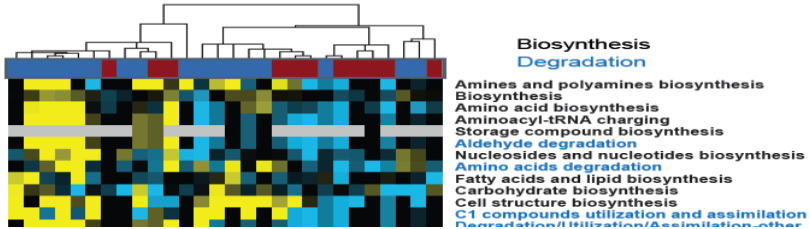




Similar findings in our patients treated with anti-PD-1
(multiplex IHC panel developed by Lisa Coussens PhD, Staining performed by Alex Reuben PhD)



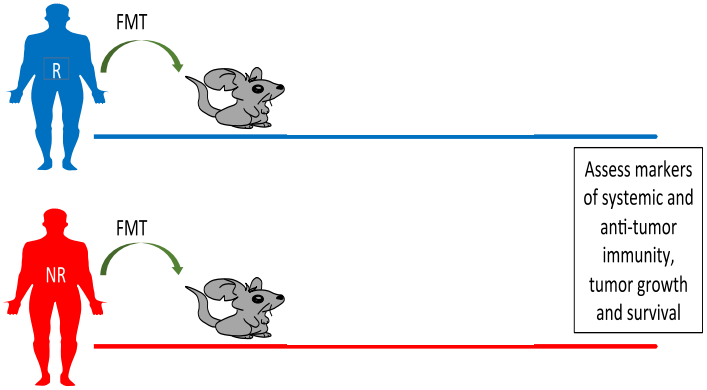
Zitvogel et al, Cell 2016

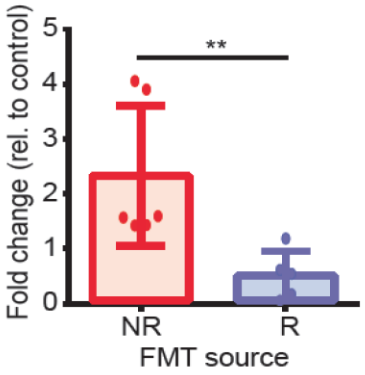


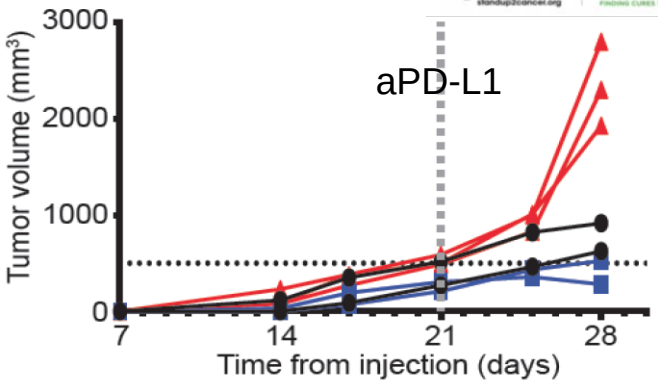
Differences in the metabolic profiles of gut bacteria in responders versus non-responders to anti-PD-1 were noted in our cohort

Gopalakrishnan et al, Science (in press) (First Release November 2 2017)

We are now building on these studies to further investigate the mechanism and to test strategies to enhance therapeutic responses



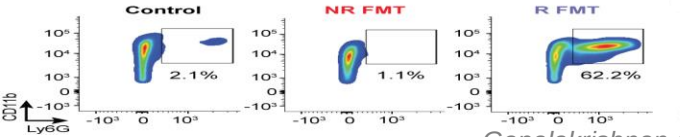




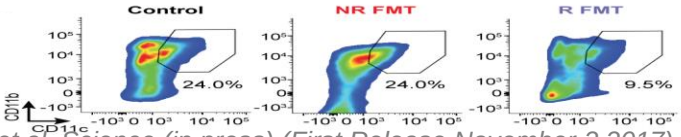
Germ-free mice receiving FMT from responders have delayed tumor growth and enhanced response to aPD-L1

To be presented by Dr. Vancheswaran (Deepak) Gopalakrishnan on Friday November 10 at the Science Behind the Therapy Session (102) 11:45 am – 12:00 pm, Abstract # 030

Tumors in mice receiving R FMT are more infiltrated



The gut of mice receiving R FMT is also more infiltrated



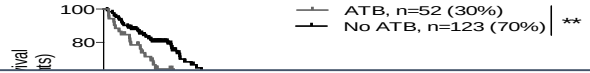
Other important differences in markers of systemic and anti-tumor immunity were noted (AP Cogdill J Allison et al)

Gopalakrishnan et al, Science (in press) (First Release November 2 2017)

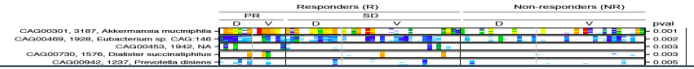
Importantly, other groups have made similar observations in other cancer types

Routy B.,.. L. Zitvogel. **Gut microbiota determines efficacy of PD-1 blockade against lung and renal carcinoma.**

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



Ruminococcaceae and Akkermansia muciniphila contrasted responders from NR (shotgun MG in 107 pts)

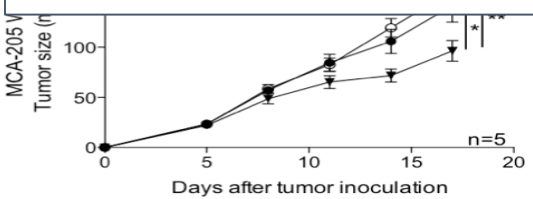


Other studies are emerging on gut microbiome and response to checkpoint blockade in patients…

Combination immunotherapy approaches through understanding the tumor, host, and the microbiota (Gajewski – AACR Annual Meeting 2017)

Metagenomic shotgun sequencing to identify specific human gut microbes associated with immune checkpoint therapy efficacy in melanoma patients (Koh Neoplasia 2017)

Baseline gut microbiota predicts clinical response and colitis in metastatic melanoma patients treated with ipilimumab – Chaput et al, Annals of Oncology 2017



FMT from NSCLC pts in GF mice predicted response to αPD-1, and oral gavage of specific bacteria (A. muciniphilia) restored sensitiviy to aPD-1

Routy et al, Science (in press) (First Release November 2 2017)

University of Colorado Cancer Center

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Cancer and the Microbiome

Does your gut hold the key to your cancer?

Can we modulate the gut microbiome to enhance responses to immunotherapy?

YES!

We are working with the Parker Institute for Cancer Immunotherapy and others to implement a clinical trial to test the hypothesis that modulation of the gut microbiome will enhance responses

(using FMT and other strategies)

IT'LL TAKE AN ARMY TO KILL THE EMPEROR

Detweiler J Popular Mechanics June 2017

Conclusions and potential implications of these findings:

- There is increasing evidence for the role of the microbiome in health and disease, and evidence that the gut microbiome may influence responses to cancer therapy
- This raises important questions in the context of immunotherapy
  - should we be profiling the microbiome of patients going onto therapy?
  - should we also be limiting (or closely monitoring) antibiotic use in these patients?
  - do we need to consider diet / pro-biotic intake in these patients?
  - do we also need to consider the role of the microbiome in pre-clinical models?
- There is also now strong evidence to suggest that modulation of the microbiome can enhance responses to immune checkpoint blockade and other forms of immunotherapy, though this needs to be tested carefully in the context of clinical trials
- There is still a great deal to learn, and the strongest gains are made through collaboration (*and we owe this to our patients*)

Acknowledgements

Patients and their families

SITC / Workshop Organizers, Speakers, Attendees

Laboratory Investigation (Wargo lab members)

- **Vancheswaran Gopalakrishnan MS (PhD candidate)**
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- **Miles Cameron Andrews MD PhD**
- **Luigi Nezi PhD**
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- Robert Szczepaniak-Sloane BS (PhD candidate)
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- Sangeetha Reddy MD PhD
- **Liz Burton MBA**

Other key collaborators

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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**
- Jeff Lee MD, Merrick Ross MD, other Surg Onc Faculty / Staff
- Andrew Futreal PhD, Giulio Draetta MD PhD
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Parker Institue for Cancer Immunotherapy