Advancing Research in Tumor Cell Heterogeneity

NCI-Frederick Advisory Committee (NFAC)

February 4, 2014
Tumor Cell Heterogeneity Think Tank
December 2-3, 2013

- Steven Altschuler
- Mike Barrett
- Joan Brugge
- Jeff Engelman
- Sui Huang
- Joe Gray
- Garry Nolan

- William Pao
- Jennifer Pietenpol
- Sylvia Plevritis
- Kornelia Polyak
- Vito Quaranta
- Charles Sawyers
- Lani Wu

- Dinah Singer, Dan Gallahan, Suresh Mohla
Data and concepts brought to you by:

Integrative Cancer Biology Program:
Centers for Cancer Systems Biology

Vanderbilt-Ingram Cancer Center

Current Topics in Cancer Systems Biology:
Tumor Cell Heterogeneity Workshop
December 2-3, 2013
Summary Observations From the Tumor Cell Heterogeneity Think Tank

• Heterogeneity likely arises from epigenomic and genomic events intrinsic to tumors and regulatory signals from diverse micro-environments.

• Tumor heterogeneity is a fundamental driver of therapeutic resistance in most human cancers. Understanding this is an urgent and unmet need in cancer treatment.

• Recent advances in measurement technology, data analytics and biological models enable new approaches to studies of tumor heterogeneity.
Summary Observations From the Tumor Cell Heterogeneity Think Tank

- Heterogeneity likely arises from epigenomic and genomic events intrinsic to tumors and regulatory signals from diverse micro-environments.

- Tumor heterogeneity is a fundamental driver of therapeutic resistance in most human cancers. Understanding this is an urgent and unmet need in cancer treatment.

- Recent advances in measurement technology, data analytics and biological models enable new approaches to studies of tumor heterogeneity.
Illustrative example
Heterogeneity in TNBC

Joe Gray
Genomic aberrations are well established as mechanisms of therapeutic resistance (NSCLC). Gefitinib/Erlotinib (+ Afatinib) as an example

- Activation of other receptor tyrosine kinases? (e.g. ERBB2 amplification)
- FAS/NFκB activation?
- Epithelial-mesenchymal transition? (AXL, Slug activation?)
- Loss or spliced variant of BIM?
- Other? (e.g. CRKL amplification)
  - ~1% BRAF mutations
  - ~5% SCLC transformation
  - ~5% PIK3CA mutations
  - 5-10% MET amplification

- +/- Pharmacokinetic failure
- +/- Exogenous factors e.g. HGF, IL-6

~60% Second-site EGFR mutations (mostly T790M)

Ohashi et al ‘13

William Pao
Jeff Engelman Persister cells evolve through drug treatments; can generate a pie chart for each patient
Both the breast cancer and lymph node have Her2 staining.
Note, abrupt boundaries of expression.
Where the core biopsy is taken makes a difference – tumor sampling bias.
The degree of heterogeneity can vary substantially between TNBC tumors; what are the drivers

The degree of heterogeneity has therapeutic implications

Understanding the biology and therapeutic responses of patients with TNBC will require the determination of individual tumor clonal genotypes

Jennifer Pietenpol
Summary Observations From the Tumor Cell Heterogeneity Think Tank

- Heterogeneity likely arises from epigenomic and genomic events intrinsic to tumors and regulatory signals from diverse micro-environments.

- Tumor heterogeneity is a fundamental driver of therapeutic resistance in most human cancers. Understanding this is an urgent and unmet need in cancer treatment.

- Recent advances in measurement technology, data analytics and biological models enable new approaches to studies of tumor heterogeneity.
Model systems display intrinsic heterogeneity and can be used to study multi-drug steering strategies and mechanisms.

HCC1143 (not actual starting density)

CK14, Vimentin, CK19

Selection versus steering to a more homogenous state?

Sequential single agents vs combinations

Genotype or state of differentiation?

Rosalie Sears, Joe Gray
Both genomic and epigenomic mechanisms influence therapeutic response evolution clinically.

Changing Landscape of Castration Resistant Prostate Cancer (CRPC)

- Rising PSA (M0)
- Metastasis (M1)
- Chemo-refractory CRPC

Primary CRPC

AR pathway

PTEN/PI3K pathway

Transdifferentiation/Lineage switching

Next generation CRPC

Charles Sawyers
Extrinsic signals from the microenvironment also drive heterogeneity

Hanahan & Coussens, Cancer Cell (2012)
Biological systems can be engineered to study the impact of specific extrinsic signals

- Growth on thousands of combinations of ECM and signaling proteins - cancer cell lines are adhered to array spots
- Heterogeneous “printed” 3D tissue structures and PDX models derived from tumor biopsies from patients on clinical trials
Selected microenvironment proteins influence therapeutic response

EdU incorporation 48 h after lapatinib in ERBB2\textsuperscript{AMP} cells

Resistant environments

<table>
<thead>
<tr>
<th>CEACAM6 / Trance</th>
</tr>
</thead>
<tbody>
<tr>
<td>CEACAM6 / SCF</td>
</tr>
<tr>
<td>HMW HyA / Leptin</td>
</tr>
</tbody>
</table>

Responsive environments

<table>
<thead>
<tr>
<th>ICAM 1 / Trance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Osteoadherin / Osteoactivin</td>
</tr>
<tr>
<td>Osteoadherin / SDF1A</td>
</tr>
</tbody>
</table>

EdU incorporation vs. DAPI

*Watson, Korkola, Gray*
Competition within a tumor environment - Barcode system to quantitatively analyze interactions between clones

Ovarian Ascites

Equal ratio mix
Injection to mice

Collect tumors

Genomic DNA

PCR- to amplify the Barcode region

High throughput sequencing (Ion torrent)

- Provides evidence that crosstalk between clonal populations can promote cancer progression and aggressiveness.

Joan Brugge
Summary Observations From the Tumor Cell Heterogeneity Think Tank

- Heterogeneity likely arises from epigenomic and genomic events intrinsic to tumors and regulatory signals from diverse micro-environments.
- Tumor heterogeneity is a fundamental driver of therapeutic resistance in most human cancers. Understanding this is an urgent and unmet need in cancer treatment.
- Recent advances in measurement technology, data analytics and biological models enable new approaches to studies of tumor heterogeneity.
New experimental tools to facilitate study of heterogeneity

- Vital imaging to study dynamic changes in population composition
- Mass cytometry for high dimensional assessment of heterogeneity (CyTOF)
- Multi-color super resolution fluorescence microscopy
- Nanometer resolution 3D electron microscopy
- Relatively low cost, single cell sequencing
- Computational-based tools
Overall conclusion - heterogeneity influences most aspects of tumor biological and clinical behavior

- Tumors are heterogeneous in every way possible
- Drivers of heterogeneity not understood
- Heterogeneity affects response to Rx
- Need to control heterogeneity for durable response to therapy
- Tools and models available to study; need to leverage resources to accelerate discoveries
Questions from research community

• What are the intrinsic and extrinsic mechanisms that drive heterogeneity?

• What are the dominant heterogeneity drivers? Genome instability? Epigenomic instability? Extrinsic influences?

• How do mechanisms that influence heterogeneity interact/synergize?

• Does treatment cause state change via selection or epigenomic state change or both?

• What are the best strategies to counter heterogeneity?

• What are the resistance states and how do we target them?
PQB – 4: What methods can be devised to characterize the functional state of individual cells within a solid tumor?

PQC – 4: What in vivo imaging methods can be developed to portray the "cytotype" of a tumor — defined as the identity, quantity, and location of each of the different cell types that make up a tumor and its microenvironment?

PQD – 4: What are the mechanistic bases for differences in cancer drug metabolism and toxicity at various stages of life?

PQE – 4: What are the best methods to identify and stratify subgroups of patients with particular co-morbidities who will benefit from defined cancer therapies?
FNLCR as an integrator and enabler of efforts to understand and manage tumor heterogeneity

- Develop and offer tools to study heterogeneity (imaging, biological models, experimental & computational methods, antibodies, reagents)

- Coordinate clinical trials to enable analysis of mechanisms that influence heterogeneity-mediated resistance (e.g. NCI-MATCH)

- Establish a national “clearing house” to collect, organize, and disseminate clinical and basic science data applicable to the study of tumor heterogeneity (e.g. in depth analysis of cell lines, PDXs, GEMMs and tumors from pre-clinical and clinical studies)

- Facilitate collaborative, pre-clinical and clinical studies across the national cancer program, aimed at deciphering and targeting heterogeneity-based resistance
Current Topics in Cancer Systems Biology:
Tumor Cell Heterogeneity Workshop
December 2-3, 2013

Data and concepts brought to you by:

Integrative Cancer Biology Program:
Centers for Cancer Systems Biology

Vanderbilt-Ingram Cancer Center