Genomics and Personalized Cancer Treatment

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Basic Research and Health Care Needs

• There is still a great need for basic or fundamental research: in basic or fundamental research the specific applicability is unknown and thus researchers should not be obliged to declare a translational pathway.

• There are many flavors of translational research: in all the health care needs must be defined and a translational pathway predetermined.

• Our health care ‘system’ is built for the 1950’s and not well equipped for translation and transfer of knowledge.
The British Columbia Personalized Medicine Initiative

From individualized health solutions to better health for all

Operations Group: Pieter Cullis, David Huntsman, Michael Hayden, Bruce McManus, Michael Burgess, Jim Russell

Operations Officer: Rob Fraser (robertfr@mail.ubc.ca)
Cancer as a genetic disease

Stratton Nature, 2009
Cancer: A tale of two Genomes

Germline SNP’s and mutations

Somatic mutations

Mutation in egg or sperm

Parent

Child

Somatic mutations
• For biomarker research a refined understanding of the clinical question and a defined translational pathway should be in place prior to commencement.
The first disruptive technology in diagnostic pathology since the 1850’s
Sanger sequencing

Massively parallel sequencing

OVCARe
BC's Ovarian Cancer Research Team
Paired-End Sequencing

Primary RNA or DNA

Fragment at random and sequence both ends of each DNA fragment

Align fragments to reference genome sequence

**ATGCCGCG**
**ATGTTCGCG**
single nucleotide variants (SNVs)

**ATCGG . .CGGATG**
**ATCGG . .TATTCA**
chr20 Translocations, inversions

**ATCGGGCGGATG**
**ATCGG--GATG**
indels

SNV’s called with SNV mix Goya et al. Bioinf, 2010
Computational interpretation of cancer genomes

- Tumour RNA-seq
  - Gene expression
  - Gene fusions
    - defuse*
    - RPKM
    - DriverNet*
  - Mono-allelic expression
  - Driver mutations

- Tumour genome
  - Copy number profile
    - HMMCopy
    - Apollo*
  - LOH profile

- Normal genome
  - JointSNVMix*
  - mutationSeq*
  - Ultradep amplicon sequencing
  - Validated somatic mutations
  - Mutational landscape
  - Clonal population structure
  - Mutational processes

- Genomic instability

OVCARE
BC's Ovarian Cancer Research Team
What to sequence?

WHAT DO YOU WANT TO LEARN?

Note
Powerful technology can not overcome poor experimental design
Three flavors of cancer: an approach to cancer genomics

- High grade cancers
- High grade serous cancer
- Pathognomonic mutations unlikely

- Moderate grade cancers
- Clear cell cancer
- Mutations in specific pathways that will be important in other cancers

- Unusual tumours with pathognomonic features
- Granulosa cell tumor of the ovary
- Pathognomonic mutations
Type 3 Cancers; Example  Granulosa cell tumours
FOXL2 mutation in all 4 granulosa cell tumors of the ovary

FOXL2 IHC and mutational analysis as a standard diagnostic (Kommoss et al Mod Path in press)

Confirmation of FOXL2 aGCT specific c.402C>G mutation by Sanger Sequencing

TaqMan based digital mutation assay for FOXL2 aGCT specific c.402C>G mutation
Subtype driven approach to ovarian cancer research: Clear cell carcinoma of the ovary

- 2\textsuperscript{nd} most common ovarian carcinoma subtype in NA (12%) and more frequent in Asia
- Do not respond to standard ovarian chemotherapy
- No other treatments available
- Molecular basis little understood
- Weird cousins of renal CCC
- Relatively genominally stable (hence small study should yield)
• Clear cell carcinomas are strongly associated with endometriosis
• ARID1A mutations predate the transformation of endometriosis into cancer
Question 1: Beyond PIK3CA, ARID1a and MET, what events cause endometriosis to turn bad?
Question 2: prognostic markers for stage 1 OCCC

P Hoskins et al JCO 2012
Question 3: markers for radiation response IC and II

Fig 4. Impact of irradiation. (A) Stage I/II and IC defined by rupture alone (gold, with irradiation, n = 57; blue, no irradiation, n = 63). (B) All other stage IC and stage II (gold, with irradiation, n = 59; blue, no irradiation, n = 62). RR, relative risk.

P Hoskins et al JCO 2012
Ovarian CCC outcomes

A. (OS) Stage I/II

B. (PFS) Stage I/II

C. (OS) Stage III/IV

D. (PFS) Stage III/IV
Question 4: how to treat late stage OCCC

- New therapeutic targets needed
- Targets need evaluation in CCC context (CCC model systems. Mike Anglesio)
- Could ARID1A loss uncover an effective target (Kim Wiegand)
## Subtype specific cell line models: essential for translation of subtype focused research

<table>
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<tr>
<th>Cell Line</th>
<th>Reported Histotype in Literature</th>
<th>COSP Markers</th>
<th>COSP Prediction (Clinical)</th>
<th>Mutational Profile</th>
<th>Validated Histotype based on immuno- and mutational profiles</th>
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<td>ENOCa**</td>
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<td>LGSC*</td>
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Changing Paradigms for Cancer Treatment

- **Generic Cancer Treatment** (1990)
- **Stratified Cancer Treatment** (2010)
- **Individualized Cancer Treatment** (2020?)

Breast cancer
Lymphoma
Uterine cancer
Ovarian cancer
Pancreatic cancer
Generic Site Based Cancer Treatment

- **Disease Type A**
  - Site Specific Rx

- **Disease Type B**
  - Site Specific Rx

- **Disease Type C**
  - Site Specific Rx
Stratified Cancer Treatment: 2000-20?? AD

Many successes but too crude
Gastric Cancer: 200 cases analyzed for 700 common mutations: Kennecke, Lim, Yip, and Huntsman (funded by BCCF)

Ion AmpliSeq™ Cancer Panel: Content
46 genes, 739 mutations

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<tr>
<th>KRAS</th>
<th>BRAF</th>
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<th>TP53</th>
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</table>

For Research Use Only. Not intended for any animal or human therapeutic or diagnostic use.
Breakdown of mutations in FFPE gastric carcinoma specimens

- **TP53**
- **PIK3CA**
- **MET**
- **CTNNB1**
- **KRAS**
- **HNF1A**
- **SMAD4**
- **APC**
- **STK11**
- **JAK3**
- **MLH1**
- **CDH1**
- **CDKN2A**
- **GNAS**
- **RB1**
- **IDH1**
- **STK11**
- **JAK3**
- **MLH1**
- **CDKN2A**
- **APC**
- **STK11**
- **JAK3**
- **MLH1**
- **RB1**
- **IDH1**

2 cases – R132C & R132H

% mutations
Application of full scale genomics in the clinic

Evolution of an adenocarcinoma in response to selection by targeted kinase inhibitors

Stratified Cancer Treatment 2012 and the promise of genomics—finer stratification

Stratification defined by molecular features
Problems: How many subtypes are there and how to test treatment options?
Mucinous Carcinomas of the Ovary

How to treat relapsed mucinous carcinomas (do not respond to current therapy?)
HER-2 amplification seen in 22% of Mucinous ovarian carcinomas

Case 1

Case 2

Case 3

McAlpine BMC Cancer, 2010 and Anglesio J Path 2012
Example: HER-2 is amplified in 20% of mucinous carcinomas, anecdotal evidence that these cancers respond to Trastuzumab.

Problem: How do you mount a trial for 20% of 4% of ovarian cancers?

McAlpine BMC Cancer, 2010
Stratified Cancer Treatment 2012 and the promise of genomics – finer stratification

Fortunately targetable molecular features used to stratify are often shared between different cancers types.
Is a mutation based taxonomy for cancers possible or desirable?

Advantage: tractable sized patient populations to test treatments
Mutation-Based Treatment Stratification

Disease Type A

Somatic Mutation Identified

Treatment Y of Proven Benefit

Favourable Response

Disease Type B

Somatic Mutation Identified

Treatment Y of Unknown Benefit

Favourable or Poor Response
Challenges (beyond finding the right drug to match a mutation/feature) of using heterogeneous molecular features to drive treatment

1. Intratumoral heterogeneity - is the mutation clonally dominant in the cancer today?
2. Is the mutation active in the cancer?
3. Is the mutation targetable in the context of the cellular origins of the cancer?

Just when we thought it was safe to go back to the water
Cancer: A tale of many Genomes

Germline SNPs and mutations  Somatic mutations

Germline Genome  Cancer Genomes
The clonal evolution of tumor cell populations: Peter Nowell—Science 1976

Intra-tumoral heterogeneity is better than a new idea it is a road tested idea that has found its time.
Intratumoral heterogeneity has implications for how we sample and how we treat cancers.
Mutational profiling of multi-region anatomic sites

DNA extraction

- Affymetrix SNP 6.0
  - Copy number genome architecture
- Whole exome sequencing
  - Somatic point mutations
- Deep targeted resequencing
  - Clonal population structure

Project lead and Informatics (Dr. S. Shah)
Multiregion sampling (Dr. J. McAlpine)
Histopathology (Dr. C.B. Gilks)
Molecular pathology (Dr. D. Huntsman)
Regional diversity of mutational profiles

- 6 cases complete
- 52% +/- 31% of mutations present in all samples
- 91% in primary-recurrence comparison
- 10% in most diverse case
- TP53 always in all samples
- Driver mutations PIK3CA, CTNNB1, NF1, PDGFRB not present in all samples
Sadly even low grade cancers are heterogeneous

**LGSC-10P**  
*(Oct27-05, 57Y)*  

- 31% (1456 variant/4723 total)

**LGSC-10R**  
*(July13-09)*

- *KRAS*
  - chr12: 25,398,284 C>A; p.G12V (COSM520)

N/A

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A Tone unpublished
Is the mutation actively driving the cancer or is it a deadbeat?

• Mutations may critical in the early development of a cancer may no longer be active
• Mutations that are invariably active in one cancer type may no longer be active in another
• Fortunately tools exist to determine the activation status of mutations in cancer

To stop the vehicle – shoot the driver
The functional impact of mutations can be detected in expression profiles
Mutational landscapes can be defined by the most active mutations

Sohrab Shah, Ali Bashashati, Jiarui Ding and team
Improved Survival with Vemurafenib in Melanoma with BRAF V600E Mutation

Same mutation, same drug so why no response in colon cancer?

- In CRC but not melanoma BRAF inhibition leads to a surge in EGFR expression and pAKT
- Dual treatment could work
- Prahallad et al. Nature 2012
Contextual Genomics-Based Treatment Stratification

Disease Type A
Somatic Mutation Identified
Favourable Response

Disease Type B
Somatic Mutation Identified
Driver? Clonal Dominance?
YES
NO

Context Specific \textit{in vitro} validation
YES

Treatment Y
Favourable Response

NO

Spared effects of ineffective treatment
What about relapsed disease?

• Considering the context of mutations at time zero is not enough – how can we adapt our decisions over time to manage the emergence of resistant disease?

• Considering that single biopsies may not provide information relevant for the whole tumor - is there a better way of sampling cancers for relevant mutations?
1950’s Diagnostics

• Kindly physician compiles and interprets data for admiring patient
Today’s Diagnostics

• There is a little too much data for effective on the fly integration
Tomorrow’s Diagnostics

• As genomics and bioinformatics become commonplace as decision support tools integration of all diagnostic data will be impossible in the clinic
A Priori integration of diagnostics may optimize utility of genomics in the clinic.
Ready to hit the runway - definition #1
Today’s fashion - by nature transient and disposable
Ready to hit the Runway Definition #2
Ready to take off- a meaningful departure from the status quo
But if we are rash in our approach to personalizing cancer control

The 200-ton Spruce Goose flies just above the water off Long Beach on Nov. 2, 1947. The flight, the plane's only one, lasted about a minute.