In Search of the Drivers

PARTHA P. MAJUMDER
STORY # 1
STORY # 2
Doctor: Well, the patient has NSCLC. I need to put him on chemotherapy.

Patient’s Relative: Can the chemotherapy be done at home?

Doctor: No (pause) ... wait a minute, let us check out his EGFR mutation status.

(pause) If it turns out that he has EGFR mutations, then you can take him home and administer chemotherapy orally.
Cancer is a Disease of the Genome

CANCER CELLS ARE VERY DISORGANIZED

The hallmark of cancer is unregulated cell growth.
A turning point in cancer research: sequencing the human genome

“If we wish to learn more about cancer, we must concentrate on the cellular genome. ... We have two options: either to try to discover the genes important in malignancy by a piecemeal approach, or to sequence the whole genome ... it will be far more useful to begin by sequencing the cellular genome.”
**Exome:** ~ 180,000 exons constituting about 1% of the total genome, or 3 million nucleotides of DNA. Mutations in the **exome** are thought to harbor 85% of mutations that have a large effect on disease.
Whole exome capture/Sequencing pipeline

Whole exome capture array

Computational pipeline

Cluster images

Basecalling

Read data

Human genome

Read mapping

Aligned reads

Read trimming

Exomic reads

Basic statistics

SNVs/Indel calling

SNVs/Indels

Filtering and Annotation

Disease associated novel variants

Next-generation sequencing
Evolution of the Cancer Genome

Cancer usually involves multiple mutations
Evolution of the Cancer Genome

Cancer usually involves multiple mutations
Evolution of the Cancer Genome
Cancer usually involves multiple mutations

WHAT ARE THE GENES AND GENOMIC ALTERATIONS THAT ‘DRIVE’?
… that is, provide the cells a growth advantage.
Driver
Squamous Cell Carcinoma of the Oral Cavity: Epidemiological Features

- 8th most common cancer
- ~260,000 new cases annually
- 2/3rd in developing countries
- 128,000 deaths annually
- Accounts for ~1/3rd of all tobacco-related cancers in India.
- Between 20% and 60% of cancer of the oro-pharynx is associated with HPV
AGE AND SEX MATCHED RELATIVE RISK = 12.5%
GHOSH (1996) *Eur J Surg Oncol*

Slide courtesy: Dr. Anil D’Cruz, Tata Memorial Hospital, Mumbai
Most (88%) were male.

Most (72%) were middle-aged (40-55 years).

Most (86%) presented very late (Stage IV).

Most (99%) were exposed to tobacco; 80% for >10 years.

About a quarter (26%) harbored HPV infection.
EXOME and WHOLE GENOME RESEQUENCING

\[ n \sim 300 \]

Paired Blood and Tumor DNA
The Number of Single Nucleotide Variants is Highly Variable Across Patients
Tumor-suppressor gene

Normal growth-inhibiting protein

Cell division under control
Tumor-suppressor gene

Normal growth-inhibiting protein

Cell division under control

Mutated tumor-suppressor gene

Defective, nonfunctioning protein

Cell division not under control
Normal cell

mutated/damaged oncogene

Cancer cell

Normal genes regulate cell growth

Oncogenes accelerate cell growth and division
In order for cancer to occur

Tumor Suppressor Genes (TSGs) are turned ‘OFF’

Oncogenes are turned ‘ON’

TSG GENES

DNA Polymerase

Oncogenes
% of patients with mutations in Significantly Mutated Genes

**TEN GENES DRIVE**

% patients with mutations in Significantly Mutated Genes

Significant by at least 2 of 3 algorithms: GenomeMuSiC, MutSig2CV, OncodriveFM

<table>
<thead>
<tr>
<th>Genes</th>
<th>Mutated in at least 10% of patients</th>
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<tbody>
<tr>
<td>TP53 (&gt;65%)</td>
<td></td>
</tr>
<tr>
<td>CASP8</td>
<td></td>
</tr>
<tr>
<td>FAT1</td>
<td></td>
</tr>
<tr>
<td>NOTCH1</td>
<td></td>
</tr>
<tr>
<td>PIK3CA</td>
<td></td>
</tr>
<tr>
<td>HRAS</td>
<td></td>
</tr>
<tr>
<td>KMT28</td>
<td></td>
</tr>
<tr>
<td>CDKN2A</td>
<td></td>
</tr>
<tr>
<td>ARID2</td>
<td></td>
</tr>
<tr>
<td>USP9X</td>
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**Tumour Suppressors Drive**

% of patients with at least one non-silent mutation
Post-Treatment Disease-Free Survival Periods in Molecular Sub-Clusters of Patients
Patients with Mutations in *MLL4* Have Longer Disease Free Survival

With mutation (GREEN line): 20.4±3.1 months
Without mutation (BLUE line): 13.5±0.9 months

\( p = 0.047 \)
Mutational landscape of gingivo-buccal oral squamous cell carcinoma reveals new recurrently-mutated genes and molecular subgroups

India Project Team of the International Cancer Genome Consortium
Mutations of **BRAF** found in malignant melanoma and other cancers

Driver mutations of **BRAF** found in
- 70% malignant melanoma
- 30% thyroid cancer
- 10% colorectal cancer

- The B-Raf protein is involved in sending signals inside cells, which are involved in directing cell growth.

**Common mutation:** V600E

- **Oncogene** Vemurafenib – Inhibitor, if V600E is present
Personalised treatment with Vemurafenib selective inhibitor of mutated \textit{BRAF}.

\textbf{V600E} (Valine to Glutamic Acid) \textit{mutation} in \textit{BRAF} – gene inhibitor
Recurrence Frequency Distribution of Driver Genes Has a L-O-N-G Tail

Why bother about the long tail?
Free AA ... metabolized by three key enzymes, COX, LOX, or cytochrome P450 (CYP450) to generate lipid mediators, eicosanoids, which are involved in

- inflammation regulation,
- tumor progression
COX and LOX in Tumour

Cyclooxygenases & Lipoxygenases

Proliferation
- MAPKs
- PKC
- AKT

Anti-apoptosis
- p53
- Bcr-2
- PPARs

Angiogenesis
- VEGF
- MMPs

Invasion/Metastasis
- E-cadherin
- CD44
- MMP-2
- MMP-4

MAPKs: mitogen activated protein kinases; PKC: protein kinase C; Bcl-2: Bcell lymphoma2; PPARs: peroxisome proliferator-activated receptor; VEGF: vascular endothelial growth factor; MMP: matrix metalloproteinase.
CONCLUSION: Mutations in AAM pathway genes enhances DFS by 17 months, without any confounders.

Mutations in AAM pathway genes enhance DFS by 17 months, without any confounders.
Somatic mutations in arachidonic acid metabolism pathway genes enhance oral cancer post-treatment disease-free survival

Nidhan K. Biswas¹, Subrata Das¹, Arindam Maitra¹, Rajiv Sarin² & Partha P. Majumder¹
Conversation Overheard ....

Doctor: Well, the patient has NSCLC. I need to put him on chemotherapy.

Patient’s Relative: Can the chemotherapy be done at home?

Doctor: No (pause) … wait a minute, let us check out his EGFR mutation status.

(pause) If it turns out that he has EGFR mutations, then you can take him home and administer chemotherapy orally.
EGFR: a family of receptors which includes Her1 (erb-B1), Her2 (erb-B2), and Her 3 (erb-B3). EGFR is overexpressed in the cells of lung and breast cancers. This leads to inappropriate activation of the anti-apoptotic Ras signalling cascade, eventually leading to uncontrolled cell proliferation.

Mutations in the EGFR tyrosine kinase domain is responsible for activating anti-apoptotic pathways.
The LUX-Lung 3 study*

Afatinib a selective, orally bioavailable ErbB family blocker.

• **Response Rate:**
  - Oral (Afatinib) - 56%
  - Injectable (Cisplatin) - 23%

• **Progression free survival (months) among patients with EGFR mutation:**
  - Oral (Afatinib) - 11.1
  - Injectable (Cisplatin) - 6.9

Unleash the Power of Genomics to Effect Precision Medicine
And Turn Cancer into a Chronic Disease
In Search of Drivers of Lymph Node Metastasis in Oral Squamous Cell Carcinoma
• Metastasis is responsible for ~90% of all cancer-related deaths.

• **Oral cancer,**
  • late presentation
  • propensity for lymph node metastasis (LN+)
  • results in poor disease free and also overall survival (~50% reduction in survival).

• One of the primary routes for tumor cell dissemination from primary tumor is through local lymph nodes.

• **Lymph-node metastasis is an important adverse prognostic factor.**

• **Not all oral cancer patients exhibit lymph node metastasis.**
Disease free survival period is short for patients with lymph node metastasis.

N=137, each T4 stage
Study design

LN Negative (-) Group

- Tumor Stage → T4 only
- No Lymph Node Metastasis
  - n=35

LN positive (+) Group

- Tumor Stage → T1-T4
- Lymph Node Metastasis (N2b,c and N3)
  - n=37

High grade tumour
No lymph node met

Low to high grade tumour
With lymph node met
LN- vs. LN+: Profiles and numbers of somatic mutations similar

- Profiles and number of rare germline mutations also similar.

**Rare germline mutations:**

- Not present in dbSNP and EXAC cohorts
- Not present in a panel of 70 healthy Indians
Mutational Landscape of (LN+/−) Oral Tumours

Driver Genes for Oral Cancer

<table>
<thead>
<tr>
<th>TP53</th>
<th>NOTCH1</th>
<th>USP9X</th>
</tr>
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<tbody>
<tr>
<td>FAT1</td>
<td>MLL4</td>
<td>ARID2</td>
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<tr>
<td>CASP8</td>
<td>HRAS</td>
<td>PIK3CA</td>
</tr>
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</table>
Mutational Landscape of (LN+/-) Oral Tumours

Mutation frequencies of known oral cancer driver genes are similar.
### Hotspot somatic mutations in oral cancer driver genes?

- **TP53, CASP8, HRAS, PIK3CA**

<table>
<thead>
<tr>
<th>Hotspot mutations</th>
<th>LN (-)</th>
<th>LN (+)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>TP53</strong></td>
<td>14%</td>
<td>40%</td>
</tr>
<tr>
<td><strong>CASP8</strong></td>
<td>0%</td>
<td>10%</td>
</tr>
</tbody>
</table>
## Mutational Landscape of (LN+/-) Oral Tumours

**Rare germline mutations?**

<table>
<thead>
<tr>
<th>Rare Germline Mutations in Gene</th>
<th>LN (-)</th>
<th>LN (+)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BRCA2</td>
<td>2%</td>
<td>21%</td>
</tr>
<tr>
<td>FAT1</td>
<td>2%</td>
<td>21%</td>
</tr>
</tbody>
</table>
Mutation profiles – somatic and rare germline – of DNA repair genes between LN+ and LN- subgroups were compared.

<table>
<thead>
<tr>
<th>Pathway</th>
<th>LN Negative</th>
<th>LN Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>NHEJ pathway</td>
<td>8%</td>
<td>37%</td>
</tr>
</tbody>
</table>

\( p = 0.003 \)
BRCA2 (member of HR pathway) and genes of NHEJ pathway were significantly mutated in LN+ oral cancers.

What about **Copy number alterations** in the genes of HR and NHEJ pathways and their interacting genes?
Genes of homologous recombination pathway and its interacting partners were deleted in lymph node positive tumours in a significantly higher proportion of patients.

Mutational Landscape of (LN+/-) Oral Tumours

Copy Number Analysis

LN Negative (-) Group

LN Positive (+) Group

CNA DELETION

Copy number analysis
Multiple (>5) arm-level chromosomal deletions observed significantly more in lymph node positive oral tumours.

<table>
<thead>
<tr>
<th>Event</th>
<th>LN Negative</th>
<th>LN Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chromosomal Instability (CI)</td>
<td>20%</td>
<td>56.7%</td>
</tr>
</tbody>
</table>
Multiple (>5) arm-level chromosomal deletions observed significantly more in lymph node positive oral tumours.

<table>
<thead>
<tr>
<th>LN Negative (-) Group</th>
<th>LN Positive (+) Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>- Chromosomal instability</td>
<td></td>
</tr>
<tr>
<td>- Unstable micronuclei</td>
<td></td>
</tr>
<tr>
<td>- DNA into the cytosol.</td>
<td></td>
</tr>
<tr>
<td>- Cytosolic DNA-sensing pathway</td>
<td></td>
</tr>
<tr>
<td>- Inflammatory response via NF-κB pathway to promote metastasis.</td>
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</tbody>
</table>
Pathway Analysis of LN+ specific altered genes

Functional protein-protein interaction based pathway analysis of the LN+ specific altered genes

<table>
<thead>
<tr>
<th>GeneSet</th>
<th>FDR</th>
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</thead>
<tbody>
<tr>
<td>Mitotic G2-G2/M phases(R)</td>
<td>2.78E-15</td>
</tr>
<tr>
<td>Cilium Assembly (R)</td>
<td>2.78E-15</td>
</tr>
<tr>
<td>Tight junction (K)</td>
<td>1.07E-14</td>
</tr>
<tr>
<td>Nonhomologous End-Joining (NHEJ) (R)</td>
<td>5.45E-12</td>
</tr>
<tr>
<td>MHC class II antigen presentation (R)</td>
<td>1.47E-07</td>
</tr>
<tr>
<td>NCAM signaling for neurite out-growth (R)</td>
<td>0.002359</td>
</tr>
<tr>
<td>Fatty acid, triacylglycerol, and ketone body metabolism (R)</td>
<td>0.00237</td>
</tr>
</tbody>
</table>

**Significant enrichment of somatic and rare germline mutations in genes which promote genome instability**

**Mitotic G2-G2/M phase**
(At G2/M transition, duplicated centrosomes mature and separate and CDK1: cyclin B complexes become active, setting the stage for spindle assembly and chromosome condensation that occur in the prophase of mitosis).
Alterations in genes like (BRCA2, ATM, etc.) and pathways (NHEJ) are already proven to be attractive novel targets for treatment with DNA repair therapeutics like PARP-inhibitors (olaparib), platinum etc.
Our Findings Open Up Therapeutic Options

PARP-inhibitors (olaparib), platinum, etc.: Agents that impact on DNA repair pathways

PARP Inhibitors: The Cornerstone of DNA Repair—Targeted Therapies

By Jaydira del Rivero, MD and Elise C. Kohn, MD

Apr 15, 2017
Lymph node metastasis in oral cancer is strongly associated with chromosomal instability and DNA repair defects

Nidhan K. Biswas¹, Chitrarpita Das¹, Subrata Das¹, Arindam Maitra¹, Sudhir Nair², Tejpal Gupta², Anil K. D'Cruz², Rajiv Sarin² and Partha P. Majumder ³¹

¹National Institute of Biomedical Genomics, Netaji Subhas Sanatorium, Kalyani, West Bengal, India
²Tata Memorial Center, Advanced Centre for Treatment, Research and Education in Cancer, Navi Mumbai, Maharashtra, India
Comparing Drivers of Multiple Cancers

An Unfolding Story
• Cancer genomes evolve.

• By analyzing whole genomes of multiple cancer types, is it possible to identify commonalities of
  
  o evolutionary processes
  
  o evolutionary characteristics.
Landscape of Non-coding Mutations that Drive Cancer

Considerable heterogeneity in the burden of somatic mutations across patients and tumour types.
Processes leading to catastrophic events:
(i) **chromothripsis**, in which tens to hundreds of DNA breakages occur simultaneously, clustered on one or a few chromosomes;
(ii) **chromoplexy**, in which repair of co-occurring dsDNA breaks, typically on different chromosomes, results in shuffled chains of rearrangements;
(iii) **kataegis**, a focal hypermutation process leading to locally clustered nucleotide substitutions, biased towards a single DNA strand.
Kataegis events are non-clonal in most tumour types, implying late in mutational process.

Chromothripsis tended to be more clonal than subclonal. Implying early event.

Chromothripsis hits critical cancer genes in at least 30% of melanomas.
Tumour samples formed four distinct clusters

Cluster 1
(47 tumours)

Cluster 2
(42)

Cluster 3
(33)

Cluster 4
(2396)
Uneven Distribution of Somatic Driver Mutations Across the Four Clusters

C1: enriched for RB1 mutations or SVs

C3 and C4 tumours had relatively short telomere lengths

C3: enriched for TERT promoter mutations
Identifying drivers is providing

• insights on tumour evolution
• nature of alterations in genes and pathways in cancers
• complex alterations that result in spread
• handles on prediction, prevention and treatment
• learning new processes in biological evolution