An Introduction to Breast Cancer Translational Research

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What is translational medicine?

• The application of a discovery to the practice of medicine - from “bench” to “bedside”

Translational research cycle

1. Observation
2. Synthesis with Medical Knowledge
3. Hypothesis Testing
4. Discovery
5. Movement into Clinic
Timeline of translational oncology in the last 10 years

- **2004**: Oncotype Dx™
- **2006**: Glioblastoma multiforme genome completed
- **2008**: Intratumoral genomic heterogeneity
- **2010**: Large-scale screening for ‘actionable’ mutations in adult malignancies, and use of circulating tumour DNA to assess cancer burden and therapeutic efficacy
- **2012**: Immunotherapy: checkpoint inhibitors; genetically engineered T cells
- **2014**: Development of individually derived tumour models from circulating tumour cells for prospective drug testing
- **2016**: TCGA completed >10,000 tumours
Drugs developed on the basis of tumor biology

<table>
<thead>
<tr>
<th>Drug</th>
<th>Disease</th>
<th>Biology</th>
<th>Target</th>
<th>Mechanism of action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tamoxifen (Nolvadex)</td>
<td>Breast</td>
<td>Estrogen dependence</td>
<td>Estrogen receptor</td>
<td>Selective estrogen receptor modulator</td>
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<tr>
<td>Fulvestrant (Faslodex)</td>
<td>Breast</td>
<td>Estrogen dependence</td>
<td>Estrogen receptor</td>
<td>Degradation of estrogen receptor</td>
</tr>
<tr>
<td>Anastrazole (Arimidex),</td>
<td>Breast</td>
<td>Estrogen dependence</td>
<td>Aromatase</td>
<td>Aromatase inhibitor</td>
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<tr>
<td>letrozole (Femara),</td>
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<tr>
<td>exemestane (Aromasin)</td>
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<tr>
<td>Trastuzumab (Herceptin)</td>
<td>Breast</td>
<td>HER2/neu expression drives cell growth and</td>
<td>HER2/neu</td>
<td>Binds HER2/neu, inhibits signaling, induces antibody-dependent cellular cytotoxicity</td>
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<tr>
<td></td>
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<td>viability</td>
<td>extracellular</td>
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<td></td>
<td></td>
<td></td>
<td>domain</td>
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<td>Abiraterone acetate (Zytiga)</td>
<td>Prostate</td>
<td>Androgen dependence</td>
<td>CYP17A1</td>
<td>Inhibits activity of CYP17, decreasing androgens to subcastration concentrations</td>
</tr>
<tr>
<td>Bicalutamide (Casodex),</td>
<td>Prostate</td>
<td>Androgen dependence</td>
<td>Androgen receptor</td>
<td>Competitive inhibition of testosterone binding to androgen receptor</td>
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<td>flutamide (Eulexin),</td>
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<td>nilutamide (Nilandron)</td>
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<tr>
<td>Leuprolide (Lupron)</td>
<td>Prostate</td>
<td>Androgen dependence</td>
<td>Gonadotropin-</td>
<td>Decreases circulating androgens</td>
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<td>releasing hormone</td>
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<td>receptor agonist</td>
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<td>Imatinib (Gleevec)</td>
<td>Chronic myelogenous</td>
<td>Philadelphia chromosome</td>
<td>BCR-ABL tyrosine</td>
<td>TKI</td>
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<tr>
<td>Leukemia</td>
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<td></td>
<td>kinase abnormality</td>
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<td>produces oncogenic</td>
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<td>fusion protein</td>
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<td>BCR-ABL</td>
<td></td>
</tr>
<tr>
<td>Imatinib (Gleevec)</td>
<td>Gastrointestinal</td>
<td>cKIT drives proliferation and viability</td>
<td>cKIT tyrosine</td>
<td>TKI</td>
</tr>
<tr>
<td>Stromal Tumors</td>
<td></td>
<td></td>
<td>kinase</td>
<td></td>
</tr>
<tr>
<td>Dasatinib (Sprycel),</td>
<td>Chronic myelogenous</td>
<td>Mutations in BCR-ABL produce resistance to</td>
<td>BCR-ABL tyrosine</td>
<td>TKI</td>
</tr>
<tr>
<td>nilotinib (Tasigna)</td>
<td>Leukemia</td>
<td>imatinib</td>
<td>kinase</td>
<td></td>
</tr>
<tr>
<td>Rituximab (Rituxan)</td>
<td>Lymphoma</td>
<td>CD20 is a commonly expressed surface antigen</td>
<td>CD20</td>
<td>Binds CD20 and activates antibody-dependent cellular cytotoxicity</td>
</tr>
<tr>
<td>Ipilimumab (Yervoy)</td>
<td>Melanoma</td>
<td>Immunogenicity of melanoma</td>
<td>CTLA4</td>
<td>Binds CTLA4, releasing inhibitory checkpoint</td>
</tr>
</tbody>
</table>

Drugs whose activity was revealed by later understanding of tumor biology

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</thead>
<tbody>
<tr>
<td>Gefitinib (Iressa), erlotinib (Tarceva)</td>
<td>NSCLC</td>
<td>Activating mutations in EGFR increased dependence on target</td>
<td>EGFR</td>
<td>TKI</td>
</tr>
<tr>
<td>Bortezomib (Velcade)</td>
<td>Myeloma</td>
<td>Proteasomal inhibition in face of massive intracellular protein excess</td>
<td>Chymotrypsin-like β5 subunit of the catalytic chamber of the 20S proteasome</td>
<td>Proteasome inhibitor</td>
</tr>
</tbody>
</table>
Breast cancer translational medicine

• Multi-gene panels (e.g., OncoType Dx)
• CDK4/6 inhibitor – palbociclib
• Breast cancer immunotherapy
• Circulating tumor cells (CTCs) and circulating tumor DNA (ctDNA)
• Balancing translational research between treatment and prevention
Breast Cancer Intrinsic Subtypes

Microarray-Based Breast Cancer Subtype

- Luminal A: ER+ and/or PR+, HER2-
- Luminal B: ER+ and/or PR+, HER2+
- HER2+/ER-:
- Basal-like: ER-, PR-, HER2-, CK5/6+ and/or HER1+

Immunohistochemical Profile

Gene Expression (Fold Difference Relative to Median Level of Expression Across All Samples)

Lower | Median | Higher
---|---|---
5.6 | 4 | 2.8
2 | 1.4
1.4 | 1.4
2 | 2.8
4 | 5.6

JAMA. 2006;295:2492-2502
Intrinsic molecular subtypes

Carey 2006; JAMA 295:2492-2502
21-gene assay (Oncotype Dx)

Proliferation
- Ki67
- STK15
- Survivin
- CCNB1 (cyclin B1)
- MYBL2

HER2
- GRB7
- HER2

Estrogen
- ER
- PGR
- BCL2
- SCUBE2

GSTM1

Invasion
- MMP11 (stromolysin 3)
- CTSL2 (cathepsin L2)

CD68

BAG1

Reference
- ACTB (β-actin)
- GAPDH
- RPLPO
- GUS
- TFRC
21-gene assay (Oncotype Dx)

Ongoing trial - TAILORx

Schema: TAILORx

Node Negative, ER Positive Breast Cancer

Register Specimen Banking

- oncotype DX
  Breast Cancer Assay

RS ≤ 10
Hormone Therapy Registry

RS 11-25
Randomize Hormone Rx vs.
Chemotherapy + Hormone Rx

Primary study group

RS > 25
Chemotherapy + Hormone Rx

http://www.oncolink.org/types/article.cfm?id=9643
Kaplan–Meier Estimates in the Analyses of Invasive Disease–free Survival, Freedom from Recurrence of Breast Cancer at a Distant Site, Freedom from Recurrence at Any Site, and Overall Survival.
CDK4/6 inhibitor - palbociclib
CDK4/6 inhibitor - palbociclib

[Graphs showing progression-free survival and overall survival with palbociclib plus letrozole compared to letrozole.]

CDK4/6 inhibitor - palbociclib

Hazard ratio, 0.42 (95% CI, 0.32–0.56)
P<0.001

Palbociclib–fulvestrant (N=347)
Median progression-free survival, 9.2 mo (95% CI, 7.5–NE)

Placebo–fulvestrant (N=174)
Median progression-free survival, 3.8 mo (95% CI, 3.5–5.5)

<table>
<thead>
<tr>
<th>Month</th>
<th>0</th>
<th>2</th>
<th>4</th>
<th>6</th>
<th>8</th>
<th>10</th>
<th>12</th>
</tr>
</thead>
</table>

No. at Risk
Palbociclib–fulvestrant
347 279 132 59 16 6
Placebo–fulvestrant
174 109 42 16 6 1
Breast cancer immunotherapy

Breast cancer immunotherapy

  - overall response rate: 4.8% (8/168)
  - 5/8 had triple-negative cancer, and 4 had PD-L1 positive immune cells

  - ER-positive HER2 negative PD-L1 expressing
  - Of 25 evaluable patients, response rate 12%, plus 8% stable disease
SCALPEL-FREE BIOPSIES

Three different non-invasive techniques allow scientists to monitor tumours by performing ‘liquid biopsies’ on vials of blood.

Blood extracted from a person with cancer contains tumour information in the form of circulating tumour DNA and cells, and exosomes that are ejected by tumour cells.

Circulating tumour DNA

Blood sample is spun to separate plasma

Plasma

Blood

Filtration steps extract cell-free DNA from plasma

DNA fragments from malignant cells (red) are separated from normal DNA (blue) and analysed by next-generation sequencing or digital polymerization chain reaction (dPCR).

Circulating tumour cells

Circulating tumour cells are isolated from blood by cell-separation systems.

Blood sample

Red blood cell

Tumour cell

Tumour cells isolated

Cells are broken up to obtain tumour DNA that can be analysed by whole-genome sequencing.

Exosomes

Tumour exosomes are extracted from blood samples using different assays.

Chip sensor with antibodies

Antibody specific for exosome cell-surface protein

Tumour RNA

Tumour protein

The material inside the captured exosomes — RNA and/or proteins — is then analysed.
Clinical applications of CTC and ctDNA analyses in cancer care.
A pooled-analysis of CTC in breast cancer
Circulating Tumor Cells and Response to Chemotherapy in Metastatic Breast Cancer: SWOG S0500

Registered for screening (N = 624)

Excluded
- Initial CTC test not completed (n = 17)
- Ineligible after review (n = 12)

Initial CTC evaluation completed (n = 595)

Initial CTC < 5
Arm A (n = 276)
Low risk

Initial CTC ≥ 5
Arm B (n = 165)
Moderate risk

Excluded
- No day 21 CTC (n = 31)

Day 21 CTC evaluation completed (n = 288)

Day 21 CTC < 5
Arm C (n = 123)
High risk

Day 21 CTC ≥ 5
Arm D (n = 64)
Maintain therapy

Randomly assigned (n = 123)

Arm C1 (n = 64)
Maintain therapy

Arm C2 (n = 59)
Change therapy

Circulating Tumor Cells and Response to Chemotherapy in Metastatic Breast Cancer: SWOG S0500
Circulating Tumor Cells and Response to Chemotherapy in Metastatic Breast Cancer: SWOG S0500

A

Overall Survival (probability)

<table>
<thead>
<tr>
<th>Time Since Random Assignment (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
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- Arm C1: Maintain therapy 64 55 10.7
- Arm C2: Change therapy 59 52 12.5

Log-rank P = .98

B

Progression-Free Survival (probability)

<table>
<thead>
<tr>
<th>Time Since Random Assignment (months)</th>
</tr>
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<tbody>
<tr>
<td>0</td>
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</table>

- Arm C1: Maintain therapy 64 63 3.5
- Arm C2: Change therapy 56 56 4.6

Log-rank P = .64

Serial monitoring of circulating tumor DNA in patients with primary breast cancer for detection of occult metastatic disease
Mutation tracking in circulating tumor DNA predicts relapse in early breast cancer

- Core biopsy at diagnosis
- Neoadjuvant chemotherapy
- Blood sample at diagnosis
- Surgery
- Mutation confirmation (dPCR)
- Post-surgery Blood samples every 6 months
- Standard follow-up Blood samples
- Mutation tracking in post-surgery and follow-up blood samples
- Circulating tumor DNA detected
- MPS tumor sequencing to identify mutation
- High-throughput sequencing
- Mutant DNA
- Wild-type DNA
- Design-personalized mutation-specific dPCR assays
- MAF

Mutation tracking in circulating tumor DNA predicts relapse in early breast cancer.
Mutation tracking in circulating tumor DNA predicts relapse in early breast cancer

TP53 c.824G>T (C275F)
ANK3 c.3247G>C (E1083Q)
XIRP2 c.3473delA(Q1158fs)
Subclone + RB1 c.958C>T (R320*)
Two-way translational cancer research
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