SCAN-B: Sweden Cancerome Analysis Network – Breast

NCI/NIH Informatics Technology for Cancer Research WG

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http://www.med.lu.se/saalgoup
Lund University, Sweden

Lund University Cancer Center
at Medicon Village
~30 groups, ~200 cancer researchers
Dept of Oncology & Pathology
Dept of Immunotechnology
Dept of Translational Cancer Research

Translational Oncogenomics Unit
http://www.med.lu.se/saalgroup

- Breast cancer, gene expression, & mutation profiling
- Circulating tumor DNA
- PTEN/PI3K pathway biology, function, therapy

Investigation → Translation → Clinical practice
Breast cancer diagnosed in 1 of 9 women

BREAST CANCER 5-year survival (%)

OECD Health Data 2011
Room for Improvement

**BREAST CANCER 5-year survival (%)**

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<thead>
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<td>United States</td>
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<tr>
<td>Slovenia</td>
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</tbody>
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**OECD Health Data 2011**

- **Uterine**
  - Uterine cancer “Cured” at 5-years

- **Breast**
  - Breast Cancer
  - 25% have late mortality after 5-years

Brenner and Hakulinen, JCO 2002
Room for Improvement

Selecting the appropriate treatment

![Survival Free of Recurrence (% of patients)](image)

- Treatment B
  - Hazard ratio, 0.58 (95% CI, 0.40–0.85)
  - P=0.005

- Treatment A
Selecting the appropriate treatment

Identify patients who would benefit from alternative treatment

Hazard ratio, 0.58 (95% CI, 0.40–0.85)
P=0.005
Room for Improvement

Selecting the appropriate treatment

Identify patients resistant to treatment

Hazard ratio, 0.58 (95% CI, 0.40–0.85)
P=0.005
Room for Improvement

Selecting the appropriate treatment

Identify patients who are potentially overtreated
Primary “scan” – patient, general practitioner, ob/gyn, radiologist, surgeon, pathologist, oncologist, clinical geneticist

**Diagnosis**

- Self-exam, clinical exam, imaging
- Surgery, node dissection
- Pathological evaluation
  - Histopathology (histotype, grade, nodes, invasion/infiltration, etc…)
- TNM staging
- Conventional biomarkers: ER, PgR, HER2, Ki67
- Genetic evaluation
  - BRCA screening
- Molecular profiling
  - none (OncotypeDX, MammaPrint)

**Prognosis and Treatment**

[Image: Gene Profile Result - Low Risk]

[Image: Oncotype DX Clinical Validation: Recurrence Score as Continuous Predictor]
Gene expression and genomic tumor profiling in the clinical routine for breast cancer for improved tumor classification, diagnosis, prognostication and prediction of treatment effects, and better understanding of tumor biology aiming at health care implementation, clinical trials, cooperation with drug and biotech industry and accelerated pipeline towards precision medicine.
SCAN-B (Sweden Cancerome Analysis Network – Breast)

Network among surgeons, pathologists, oncologists, radiologists, nurses, biologists

Network Sites: South Sweden Breast Cancer Group (SSBCG)
Lund
Malmö
Halmstad
Helsingborg
Karlskrona
Kristianstad
Växjö
Uppsala
Jönköping

Inclusion: Dx primary breast cancer
(2016: + metastatic breast cancer)

Catchment: ~1500 patients/year
(20-30 per week)

Analysis: RNA-seq on Illumina
(other genomic modalities)

Sample collection and processing

**Surgery:** Informed consent; surgery by normal procedures

**Chemistry:** Pre-operative blood & plasma sample
   Post-op blood & plasma sampling at regular intervals

**Pathology:** 1st routine evaluation; any remainder specimens saved
   Shipped in RNAlater preservative to our lab
   Pre-operative core biopsies

**Lund lab:** BASE LIMS + data from INCA national clinical registry
   (Saal et al, Genome Biology 2002; Vallon-Christersson et al, BMC Bioinformatics 2009)

**AllPrep:** isolation of total RNA, DNA, small RNA, and protein

**Quality assurance:**
   % cancer cells
   tissue macroarrays

**Nucleic acid integrity**

Surg-to-RNAlater: 46 min
Specimen size: 63 mg

TMA

30 mg 10 mg
Reserve

10 mg

Enrolled patients: 9,379 patients consented (~85% of eligible) population-based

99% with pre-operative blood sample

6,593 (70%) with surgical tumor specimen [very small tumors under-sampled]

~5900 tumors in tissue macro-array

~500 pre-op biopsies (70% with op specimen)
Enrolled patients: 9,379 patients consented (~85% of eligible) population-based

99% with pre-operative blood sample

6,593 (70%) with surgical tumor specimen

[very small tumors under-sampled]

~5900 tumors in tissue macro-array

~500 pre-op biopsies (70% with op specimen)

SCAN-B Laboratory: All tumors processed

7,463 libraries RNA-sequenced (~800 replicates)

“Real-time” analysis as of September 2015

(1 week turn-around time, surgery → RNA-seq)
SCAN-B (ClinicalTrials.gov ID: NCT02306096)

Enrollment
Patients are enrolled during pre-surgery visit at surgery/oncology clinic

Surgery
Surgery follows clinical routine; time of ischemia for surgical specimen is noted

Pathology
Tumor samples taken after routine assessment and placed in pre-aliquoted RNA later

Blood
Study blood samples taken in conjunction with routine pre-operative blood sampling

SCAN-B laboratory
Samples and referral forms registered together with accrued accessory information

Partitioning
Biopsies sectioned in preparation for extraction and TMA

Extraction
Tissue lysis and purification of nucleic acids (AllPrep)

Sequencing
Preparation of sequencing libraries and sequencing

Sequencing: NextSeq 500
Library prep: moving to NeoPrep

RNA-sequencing
- mRNA purification
- Fragmentation
- cDNA synthesis
- dUTP tagged ds-cDNA
- End repair
- 3’A-tailing
- TruSeq adaptor ligation
- Adapter ligated ds-cDNA
- Size selection
- 2nd strand digestion
- Amplification
- Library clean-up
- HiSeq sequencing

Raw data processing
Demultiplexing, filter against ribosomal sequences, RepeatMasker track

Alignment
TopHat2, GRCh37/hg19 + decoy reference genome, UCSC knownGenes

Expression counts
CuffLinks 2: fragments per kilobase of exon per million mapped reads (FPKM)

Evaluating: Kallisto, HISAT
RNA-seq gene expression profiling (>6500 tumors to date)
Implementing SCAN-B into clinical practice

Prospective collection

Biomarker validation

Biomarker Development

Individualized tumor scores in “real-time” (1 week)

>6500 cases with clinical FU

Biomarker signatures for:
- Good vs poor prognosis
- Response to therapy
- Resistance to therapy
- Mutation status
- Novel biology
Implementing SCAN-B into clinical practice

new tools for clinical decision-making
(diagnosis, prognosis, therapy selection, predicting effects, clinical trials, ...)

Biomarker validation

Biomarker Development

Individualized tumor scores
in "real-time" (1 week)
Scan-B Projects

Strengths of Scan-B:

- Large, population-based, contemporary & relevant Tx RNA-seq data on all available tumors
- Tissue macro-array of adjacent piece
- Linked to clinicopathological information
- Available tumor RNA, DNA, and AllPrep lysates
- Available tumor tissue (RNAlater, frozen, FFPE)
- ~4000 cohort with up to date Overall Survival
- Invasive disease-free survival being updated
- Subgroups with exome data, SNP data

37 approved projects to date

We are open minded towards collaborations

Project proposals must be approved by the Steering Committee

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