Overview

• Background

• 3 studies using MA27 trial
  - Musculo-Skeletal Adverse Events
    • Study Design and Statistical Analysis
    • Pharmacogenomics Functional Studies
  - Bone Fracture : Osteoporosis
    • Study Design and Statistical Analysis
    • Pharmacogenomics Functional Studies
  - Breast Cancer Recurrence
    • Study Design and Statistical Analysis
    • Pharmacogenomics Functional Studies
  - Conclusions and future work
Exemestane Versus Anastrozole in Postmenopausal Women With Early Breast Cancer: NCIC CTG MA.27—A Randomized Controlled Phase III Trial


- Largest trial examining aromatase inhibitors as adjuvant therapy for early stage hormone receptor positive breast cancer (n=7,576 patients)
- No difference between exemestane and anastrozole
- Majority (79.5%, 5,427 of 6827 North American patients) of patients consented to collection and use of DNA for genetic studies
Activated: May 26, 2003  
Accrual completed: July 31, 2008

December 21, 2004: closure of celecoxib:placebo randomization after entry of 1622 patients

*The Breast Cancer Intergroup of North America: NCIC CTG, CALGB, ECOG, NCCTG, SWOG
Introduction

• Aromatase inhibitors (AI)
  • Postmenopausal patients with ER+ breast cancer are treated with AI drugs

• Side effects
  • About one-half of patients have joint-related complaints with AI therapy (Crew, JCO, 2007; 25:3877)
  • Bone Fractures
Aromatase Inhibitors are important in the management of postmenopausal women with early stage breast cancer

American Society of Clinical Oncology
Clinical Practice Guideline, 2010

“consider incorporating aromatase inhibitor therapy at some point during adjuvant treatment, either as up-front therapy or as sequential treatment after tamoxifen.”

AI therapy advantages

- AIs are even more effective than Tam monotherapy in preventing recurrence and breast cancer death.
GWAS and Functional Follow-up of Muscular Skeletal Events

Hypothesis  PGRN-RIKEN-MA.27 Study

A genome-wide association case control study will identify single nucleotide polymorphisms associated with musculoskeletal adverse events (MS-AEs) in women receiving aromatase inhibitor adjuvant therapy for early breast cancer
Design

• This study was blinded for Treatment arm and Celecoxib allocation

• A nested matched case-control study with two controls for each case. Matching on the following factors:
  • Treatment arm (exemestane vs. anastrozole)
  • Prior chemotherapy (yes/no)
  • Age at treatment (+/- 5 years)
  • Celecoxib allocation (yes/no)

• Restricted to self-identified Caucasians (94% of accrued patients)
NCI Common Terminology Criteria for Adverse Events (Version 3.0)
Arthralgia

- Grade 1: Mild pain not interfering with function
- Grade 2: Moderate pain; pain or analgesics interfering with function, but not interfering with activities of daily living (ADL)
- Grade 3: Severe pain; pain or analgesics severely interfering with ADL
- Grade 4: Disabling
Case Selection

- Case definition: grade 3-4 MS-AE or off-treatment for any grade of MS-AE
- MS-AE must occur within the first two years
- Exclude from the case group subjects who met the case definition while on celecoxib or in the three months after stopping celecoxib
- Available DNA and consent
Control Selection

- No report of any grade MS-AE
- Followed six months longer than the matched case
- Off celecoxib for at least six months
# Patient Characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Cases (n=293)</th>
<th>Controls (n=585)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>63.3</td>
<td>64.1</td>
</tr>
<tr>
<td>Range</td>
<td>46.1-86.9</td>
<td>45.1-84.4</td>
</tr>
<tr>
<td><strong>Treatment, %</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>56</td>
<td>56</td>
</tr>
<tr>
<td>B</td>
<td>44</td>
<td>44</td>
</tr>
<tr>
<td><strong>Prior chemo, %</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>68</td>
<td>69</td>
</tr>
<tr>
<td>Yes</td>
<td>32</td>
<td>31</td>
</tr>
<tr>
<td><strong>Celecoxib, %</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>75</td>
<td>73</td>
</tr>
<tr>
<td>D</td>
<td>25</td>
<td>28</td>
</tr>
<tr>
<td><strong>Prior HRT, %</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unknown</td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td>No</td>
<td>35</td>
<td>53</td>
</tr>
<tr>
<td>Yes*</td>
<td>65</td>
<td>47</td>
</tr>
<tr>
<td><strong>BMI at baseline</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>28.2</td>
<td>27.9</td>
</tr>
<tr>
<td>Range</td>
<td>17.7-56.8</td>
<td>16.9-50.8</td>
</tr>
</tbody>
</table>

*extended Fisher’s exact test, p<0.001 **291 cases, 577 controls
Genotype Quality Control and SNPs for Analyses

• Call Rates: 906 of 912 (99.3%) samples (cases, controls, duplicates, CEPH trios) with call rate >0.98
• Received genotyping data on 580,955 SNPs
• In pool of cases and controls, MAF < 1% in 29,478 SNPs (removed from analysis)
• Hardy-Weinberg in controls, P < 10^-6
  82 SNPs (removed from analysis)
• Number of SNPs in analyses: 580,955 – 29,478 – 82 = 551,395
Conditional Logistic regression adjusted for 8 Eigenvectors

2 SNPs in high linkage disequilibrium

adjusted for 8 eigenvectors
Fine mapping of +/- 200 kb region

-\log_{10}(p-value)

-\log_{10}(p-value)

<table>
<thead>
<tr>
<th>Fine-Map, N = 29</th>
</tr>
</thead>
<tbody>
<tr>
<td>Imputed, N = 530</td>
</tr>
<tr>
<td>Observed, N = 133</td>
</tr>
</tbody>
</table>
Imputation & Fine mapping

- SNPs were imputed within 300 kb of the smallest P value on Chr 14 showed an additional SNP
  - MACH 1.0 with white CEPH European Ref panel

- Fine mapping within 200kb region of the imputed data was done on 29 SNPs

- Based on LD we picked 20kb region including the 4 SNPs of interest.

- Re-sequencing did not find SNPs with stronger association than rs11849538 (70 dbSNPs & 40 novel).
## SNPs with Lowest P values

<table>
<thead>
<tr>
<th>SNP</th>
<th>MAF</th>
<th>OR</th>
<th>P-Value</th>
<th>Type</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cases</td>
<td>Controls</td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs11849538*</td>
<td>0.172</td>
<td>0.091</td>
<td>2.21</td>
<td>6.67E-07 Imputed &amp; Finemapped</td>
</tr>
<tr>
<td>rs7158782</td>
<td>0.190</td>
<td>0.110</td>
<td>2.16</td>
<td>7.74E-07 Genotyped</td>
</tr>
<tr>
<td>rs7159713</td>
<td>0.190</td>
<td>0.110</td>
<td>2.16</td>
<td>7.74E-07 Genotyped</td>
</tr>
<tr>
<td>rs2369049</td>
<td>0.180</td>
<td>0.100</td>
<td>2.08</td>
<td>2.23E-06 Genotyped</td>
</tr>
</tbody>
</table>
Chromosome 14, MA.27 GWAS signal
Challenges

- SNP function
- Relating SNPs to genes
- Relating genes to drug effect
Estrogen induced TCL1A expression variation

- E2 induces *TCL1A* expression in U2OS cells transfected with ERα or ERβ
- Most significant SNP (rs11849538) creates an estrogen response element
- Lymphoblastoid cells transfected with ERα: *TCL1A* expression greater for variant than wild type
Pharmacogenomics Model System

“Human Variation Panel”
300 LCL Cell Lines

• 100 EA, 100 AA, 100 HCA
• 1.3 million SNPs/cell line (~7 million after imputation)
• 54,000 expression array probes/cell line
• Genome-wide CpG methylation

Liewei Wang, M.D., Ph.D.
SNP-related Differences in TCL1A Expression to Estrogen Response in Three Ethnic Groups in ERα-Transfected “Human Variation Panel” Cells
Conclusions

• This GWAS identified 4 SNPs in linkage disequilibrium on Chr14 associated with musculoskeletal adverse events in women receiving aromatase inhibitors.

• These SNPs appear to be functionally significant based on EMSA, ChIP assays and their association with TCL1A expression.

• Women with a musculoskeletal adverse event after AI therapy are more likely to have a variant on Ch14 that creates an ERE for ERα.

• WT and variant SNP sequences had differing effects on the estrogen-dependent expression of TCL1A.
GWAS and Functional Follow-up of Fragility Fractures

Estrogen Levels in Women and Men

Bioavailable E2, pmol/L

- Premenopausal women
- Postmenopausal women
- Aromatase Inhibitor Therapy
- Stop AI
- Normal men

Adapted from: Khosla et al. J Clin Endocrinol Metab 2001;86:3555-61
Primary objective of GWAS

• To identify genetic variation as measured by SNPS associated with fragility fractures in women treated with aromatase inhibitors as adjuvant therapy for early stage breast cancer
• Note: this is not an osteoporosis study
Definition of Fragility Fracture

Sites of fractures that would be expected to be related to AI-associated bone loss, specifically those in the
• spine
• forearm
• humerus
• proximal femur/hip
Rationale for Pharmacogenomic study of Bone Fractures in MA.27

- There is a direct relationship between serum estrogen concentrations and osteoporosis risk

- AIs greatly decrease serum estrogen levels in post menopausal women

- Bone loss with clinical fracture is a potentially life-threatening adverse event of AI therapy

- Identifying those at risk for clinical fractures would improve the therapeutic index of AIs
Example Case-Cohort Sampling

genotyping of:
(1) a random subcohort selected independent of definition of cases
(2) all cases outside the subcohort,

union of (1) and (2) = case-cohort
Selection of Subjects for Analyses

Planned
Random Sub-Cohort (n=900)
Additional Fracture Cases (n=231)
Total (n=1131)

Received (n=1115)

Removed: Failed genotyping (n=5)
Removed: Sample mix-up (n=2)
Removed: Cancer recurrence prior to fracture (n=11)
Removed: Cases missing time of fracture (n=25)
Removed: Control with no follow-up (n=1)

Analysis (n=1071)
<table>
<thead>
<tr>
<th>Patient Characteristics</th>
<th>Cases (N=231)</th>
<th>Controls (N=840)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age: Med (Range)</td>
<td>68.7 (46.1 – 89.8)</td>
<td>64.2 (35.9 – 88.9)</td>
</tr>
<tr>
<td>Prior Fracture (10 yrs)</td>
<td>45 (19.5%)</td>
<td>82 (9.8%)</td>
</tr>
<tr>
<td>Prior Chemotherapy</td>
<td>57 (24.7%)</td>
<td>255 (30.4%)</td>
</tr>
<tr>
<td>BMI</td>
<td>N=227</td>
<td>N=836</td>
</tr>
<tr>
<td>Median (Range)</td>
<td>28.6 (17.4 – 66.8)</td>
<td>28.4 (16.5 – 61.3)</td>
</tr>
<tr>
<td>RACE</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asian</td>
<td>2 (0.9%)</td>
<td>12 (1.4%)</td>
</tr>
<tr>
<td>Black</td>
<td>5 (2.2%)</td>
<td>22 (2.6%)</td>
</tr>
<tr>
<td>Hawaiian or Pacific Islander</td>
<td>0 (0.0%)</td>
<td>1 (0.1%)</td>
</tr>
<tr>
<td>Unknown</td>
<td>0 (0.0%)</td>
<td>3 (0.4%)</td>
</tr>
<tr>
<td>White</td>
<td>224 (97.0%)</td>
<td>802 (95.5%)</td>
</tr>
</tbody>
</table>
Methods: Genotyping

- 887 (83%) on Omni chip
- 184 (17%) on Human610 Quad Beadchip (previously genotyped in AI MS-AE GWAS)
Screening covariates one at a time

<table>
<thead>
<tr>
<th>Covariate</th>
<th>RelRisk</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment Exemestane</td>
<td>0.994</td>
<td>0.970</td>
</tr>
<tr>
<td>chemoYes</td>
<td>0.755</td>
<td>0.094</td>
</tr>
<tr>
<td>age.65 (65, 89.8]</td>
<td>2.273</td>
<td>0.000</td>
</tr>
<tr>
<td>ECOG</td>
<td>1.386</td>
<td>0.038</td>
</tr>
<tr>
<td>surgeryPartial Mastectomy</td>
<td>1.006</td>
<td>0.967</td>
</tr>
<tr>
<td>riken1Yes</td>
<td>0.774</td>
<td>0.206</td>
</tr>
<tr>
<td>FracPriorYes</td>
<td>2.351</td>
<td>0.000</td>
</tr>
<tr>
<td>RaloxUseYes</td>
<td>1.016</td>
<td>0.977</td>
</tr>
<tr>
<td>BisphosUseYes</td>
<td>2.400</td>
<td>0.000</td>
</tr>
<tr>
<td>bmi</td>
<td>1.006</td>
<td>0.657</td>
</tr>
<tr>
<td>stageTNMII</td>
<td>1.197</td>
<td>0.259</td>
</tr>
<tr>
<td>stageTNMIII</td>
<td>1.558</td>
<td>0.102</td>
</tr>
<tr>
<td>EVEC.1</td>
<td>0.098</td>
<td>0.376</td>
</tr>
</tbody>
</table>
Statistical Analysis

• Primary covariates:
  • age
  • Baseline BMI
  • Bisphosphonate use
  • First 3 eigenvectors
• Primary analysis based on a weighted Cox proportional hazard model to account for the case-cohort design
• SNP genotypes analyzed as log-additive effects on risk of an event
Adj for Clinical & Eigenvect, MAF > .01
(Observed + Imputed: N=7,560,631)
Validation Decision Cascade

20 SNP Signals and 22 Genes (combined from all GWAS data)

- Removed 6 signals and 1 gene, all SNPs were imputed or mapped to a gene desert

14 SNP Signals, 21 Genes

- Removed 11 SNP signals and 14 genes, not expressed in LCLs

3 SNP Signals, 7 Genes

- Removed 1 gene during functional validation
  THIL, no SNP-dependent effect

MAP4K4
TRAM2-TMEM14A
CTSZ-SLMO2-ATP5E

Functionally validated 3 SNP signals and 6 genes

Figure 2
Challenges

- SNPs
- Function
- Genes
- Drug Effect
- Clinical Phenotype

Genome-wide meta-analysis identifies 56 bone mineral density loci and reveals 14 loci associated with risk of fracture

- Meta-analysis on lumbar spine and femoral neck BMD
- 17 GWAS involving 32,961 individuals of European and east Asian ancestry
- Top BMD-associated markers tested in 50,933 independent subjects, and
- For association with risk of low-trauma fracture in 31,016 cases (with fracture) and 102,444 controls
MA.27 GWAS Gene Expression Correlated with Expression in LCLs of Published Osteoporosis GWAS Genes

<table>
<thead>
<tr>
<th>Genes MA.27 GWAS</th>
<th>Genes Osteoporosis GWAS</th>
<th>r</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>LMNA</td>
<td>CRTAP</td>
<td>0.358</td>
<td>6.77E-10</td>
</tr>
<tr>
<td>LMNA</td>
<td>SLC25A13</td>
<td>-0.26</td>
<td>1.02E-05</td>
</tr>
<tr>
<td>LMNA</td>
<td>SPTBN1</td>
<td>-0.337</td>
<td>6.63E-09</td>
</tr>
<tr>
<td>LMNA</td>
<td>MARK3</td>
<td>-0.274</td>
<td>3.23E-06</td>
</tr>
<tr>
<td>MANEA</td>
<td>SPTBN1</td>
<td>0.302</td>
<td>2.43E-07</td>
</tr>
<tr>
<td>MANEA</td>
<td>SLC25A13</td>
<td>0.333</td>
<td>1.10E-08</td>
</tr>
<tr>
<td>MANEA</td>
<td>CRTAP</td>
<td>0.375</td>
<td>8.20E-11</td>
</tr>
<tr>
<td>FXC1</td>
<td>SPTBN1</td>
<td>-0.265</td>
<td>6.88E-06</td>
</tr>
<tr>
<td>FXC1</td>
<td>MARK3</td>
<td>0.297</td>
<td>3.94E-07</td>
</tr>
<tr>
<td>ARFIP2</td>
<td>TNFRSF11A</td>
<td>-0.38</td>
<td>4.46E-11</td>
</tr>
<tr>
<td>ARFIP2</td>
<td>SLC25A13</td>
<td>-0.427</td>
<td>6.72E-14</td>
</tr>
<tr>
<td>ARFIP2</td>
<td>SPTBN1</td>
<td>-0.459</td>
<td>4.59E-16</td>
</tr>
<tr>
<td>ARFIP2</td>
<td>CRTAP</td>
<td>-0.314</td>
<td>7.55E-08</td>
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<tr>
<td>ARFIP2</td>
<td>PPIB</td>
<td>0.409</td>
<td>9.44E-13</td>
</tr>
<tr>
<td>SLC36A4</td>
<td>SPTBN1</td>
<td>-0.296</td>
<td>4.27E-07</td>
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<tr>
<td>SLC36A4</td>
<td>CRTAP</td>
<td>0.277</td>
<td>2.36E-06</td>
</tr>
</tbody>
</table>
Figure 5

(A) Relative mRNA to ERα

- CTSZ
- SLOM2
- ATP5E

(B) Relative mRNA to ERα

- OPG
- THIL
- TMEM14A
- TRAM2

(C) Relative mRNA to ERα

- MAP4K4
- OPG

Graphs illustrate the changes in relative mRNA levels of various genes (CTSZ, SLOM2, ATP5E, OPG, THIL, TMEM14A, TRAM2, MAP4K4, OPG) in response to estradiol (nM) exposure, comparing VV and WW conditions. Statistical significance is indicated by symbols: * P < 0.05, ** P < 0.01, *** P < 0.001 compared to 0 nM E2.
## MAF Values of SNPs in Candidate Genes

<table>
<thead>
<tr>
<th>Human Variation Panel MAF</th>
<th>1000 Genomes Data MAF</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA</td>
<td>CA</td>
</tr>
<tr>
<td>MANEA</td>
<td>22%</td>
</tr>
<tr>
<td>LMNA</td>
<td>49%</td>
</tr>
<tr>
<td>FXC1/AR</td>
<td></td>
</tr>
<tr>
<td>FIP2</td>
<td>20%</td>
</tr>
<tr>
<td>SLC36A4</td>
<td>26%</td>
</tr>
</tbody>
</table>
Conclusions

• The four genes observed during our fracture GWAS were related to osteoporosis gene expression after estrogen exposure in a SNP-dependent fashion.

• The SNPs identified have very small MAFs in Whites (the focus of our GWAS) but were common variants in African Americans and Han Chinese.

• Further study of our “Fracture SNPs and genes” in Blacks and Asians is indicated.

• These findings may provide novel insights into the biology of osteoporosis.
GWAS and functional follow-up of Breast Events in MA27 study
Breast Events GWAS

• **Primary objective:** To identify SNPs related to time to a breast event (BCFI) in women receiving aromatase inhibitors on MA.27
Breast Events GWAS

Patients in GWAS from 3 cohorts of patients entered on MA.27
1. MS-AE GWAS: 843 pts genotyped with Human610 Quad BeadChip
2. Fractures GWAS: 887 pts genotyped on Omni in 2012
3. Breast Events GWAS: 2,927 pts genotyped on OmniExpress in 2013
Final Race Classification, n=4657

Caucasian = 4449
Africans = 152
Han Chinese = 56
Genotyped SNPs in MA27 Studies
n=899,848

MSKE

227111

11771

Fractures

16202

301,083

Breast Events

11366

299,819

32496

n=899,848
Imputed SNPs in MA27 Studies
MAF>0.01, R2>0.8

- MSKE
  - 84,701
- Fractures
  - 44,659
- Breast Events
  - 7,430,235
  - 54,252
  - 27,404
  - 50,119
  - 235,759
### Genotype QC Summary

#### Genotype QC on observed SNPs

<table>
<thead>
<tr>
<th># SNPs</th>
<th>excluded</th>
<th>Remaining</th>
<th>Reason</th>
</tr>
</thead>
<tbody>
<tr>
<td>964193</td>
<td>1506+218+1199</td>
<td>961270</td>
<td>Chr Y, MT, and unplaced</td>
</tr>
<tr>
<td>961270</td>
<td>40631</td>
<td>920639</td>
<td>Failed SNPs</td>
</tr>
<tr>
<td>920639</td>
<td>250843</td>
<td>669796</td>
<td>MAF ≤ 0.01</td>
</tr>
<tr>
<td>669796</td>
<td>0</td>
<td>669796</td>
<td>call rate ≤ 95%</td>
</tr>
<tr>
<td>669796</td>
<td>460</td>
<td>669336</td>
<td>HWE</td>
</tr>
</tbody>
</table>

### Genotype QC after imputation

- After imputation, removed SNPs with MAF < 0.01 and $R^2 < 0.8$ in all 3 cohorts
- Final number of SNPs for analysis from imputation: **7,430,235**

**Final number SNPs in analysis:** **8,099,571**
Analysis

- 254 Events (breast recurrence)
- 4403 No Event
- Cox Proportional Hazard regression, adjusted for significant covariates.
Future Directions

- GWAS complete
- Functional follow-up of Candidates
- The Cancer Genome Atlas data
- Breast Cancer Genome-Guided Therapy study (BEAUTY)
Acknowledgements

• MA27 clinical trial group
• Drs. Ingle and Goetz
• Drs. Weinshilboum and Wang
• Dr. Schaid
• Poulami Barman and Erin Carlson
• Greg Jenkins and Tony Batzler
Thank you

Questions?