A211102

Testing for Atypia in Random Periareolar Fine Needle Aspiration (RPFNA) Cytology after 12 months Metformin Chemoprevention versus Placebo Control

Victoria Seewaldt, M.D.
Duke University
Pre-registration
- BMI > 25
- Premenopausal
- High-risk
  > 20% Gail, biopsy atypia, DCIS, LCIS
  BRCA mutation

\[ \text{RPFNA } t=0 \]
Atypia, Masood Cytology \( \geq 14 \)

\[ \text{Metformin} \]
850 po bid
(ramp up)
Can back down to 850 qd

\[ \text{Placebo} \]
850 po bid
Unblinded at 12 mos
Can opt for drug

\[ \text{RPFNA } t=12 \text{ months} \]

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Readout
- pAMPK
- pAkt/mTor
- pS6K
**Inclusion:**
Increased risk for breast cancer
  - Gail > 1.6 five year
  - Biopsy atypia, DCIS/LCIS
  - Family history breast and/or ovarian cancer
Atypia on RPFNA in non-excised breast tissue (Masood 14-17).
Subjects may have a *new* diagnosis of DCIS or LCIS, however, in subjects with DCIS only the nonradiated breast can be aspirated.

Premenopausal
Age 25 to 55.
BMI > 25
Mammogram within the last 3 months.
At least one year from pregnancy, lactation or chemotherapy.

**Exclusions:**
Metastatic malignancy of any kind.
Subjects on warfarin or other anticoagulants.
Bilateral breast implants or tram flap reconstruction.
Radiation to both breasts.
Elevated Cr.
Primary Outcomes

Test for the presence or absence of atypia in RPFNA after 12, and 24 months Metformin versus placebo control.
Secondary Outcomes

Test the reproducibility of RPPM in duplicate RPPM determination from single RPFNA specimen.

RPPM analysis of the initial RPFNA cytology in women with disappearance of atypia versus persistent atypia after 12, and 24 mos Metformin.

Determine the change in percent breast density from prior to the initiation of metformin or placebo treatment through one year of therapy in subjects.
Correlative Research

Surrogate biomarkers of breast cancer risk:
- Atypia - surrogate endpoint biomarker/risk biomarker
- Mammographic density – surrogate/risk biomarker
- Phosphoprotein expression – exploratory biomarker
- Circulating insulin growth factor and other cytokines – surrogate biomarker and risk biomarker

Markers of obesity and insulin resistance:
- Waist to hip ratio
- Fasting serum insulin, fasting serum glucose
- Circulating Homeostatic Model Assessment (HOMA)

Other Measures:
- Tissue/serum Metformin levels – exploratory
- Estrogen/Progesterone – exploratory biomarker.
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Atypia, Masood Cytology ≥14

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Contact Victoria Seewaldt, M.D. for RPFNA training
seewa001@mc.duke.edu
subject line “RPFNA TRAINING”

Training options
- Skype
- Visit Duke – we can make this easy

Best patient – aspirate in OR during DCIS surgery:
35-45 years old, pre-menopausal, BMI 25-30
Contralateral breast – biopsy proven DCIS
and low suspicion for invasive cancer
Aspirate contralateral breast - >90% chance atypia
Has to refuse tamoxifen or not be eligible
Can be BRCA mutation carrier
Phosphoprotein analysis of RPFNA cytology

50 proteins in triplicate
20,000 epithelial cells

Cytospin.
Microdissection of epithelium and stroma

Cytological Sampling of High-Risk women using Random Periarolar FNA

5 institutions, n=78; Not affected by: Age p=0.249, BMI p=0.115, Gail p=0.938, BRCA p=0.2151, 1° family p=0.4022.

Atypia in RPFNA predicts risk for progression to cancer in women from non-BRCA high-risk families

Atypia n= 68
No Atypia n= 186
Total n= 254 15% African American (34% AA entire cohort)

Hazard Ratio = 6.3  p=0.007

Peterson et al. in revision JNCI, 2013
Atypia Cluster 1 – 31 high risk AA women
Akt/mTor, p53/stress, Wnt/AMPK

CLUSTER 1
ErbB3 Y1289
PKC
Paxillin-Y118
Vimentin
IGF1R-1131
Wnt10B
p70S6K-T389
pChk1-S346
p90RSK-S380
p53
mTOR-Ser2448
pS6K-Thr389
pATPCL-Ser454
pACC-Ser79
p38MAPK-Thr180/Tyr182
14-3-3-zeta

Lopez-Contreras… Fernandez-Capetillo
An allele Chk1 limits oncogene replicative stress and promotes transformation  JEM, 2012
Low Masood

High Masood

BMI < 30

BMI > 30

vimentin negative

vimentin positive

CLUSTER 1

ErbB3 Y1289

PKC

Paxillin-Y118

Vimentin

IGF1R-1131

pStat3

p70S6K-T389

pChk1-S346

p90RSK-S380

Snail1

mTOR-Ser2448

pS6K-Thr389

pATPCL-Ser454

pACC-Ser79

p38MAPK-

Thr180/Tyr182

14-3-3-zeta
DU-368 – Markers of EMT

vimentin(+)            snail1(+)            pericentrin

[Image of tissue sections with vimentin, snail1, and pericentrin markers highlighted]
Non-BRCA1 mutation carrier
40 yr old woman. First pregnancy age 37, breast cancer R found after weaning.

Radiated breast

Atypia/one foci DCIS

Arimidex

Masood=11
Masood=12
Masood=10
Masood=10
Masood=16
Masood=17
Masood=10
Breast MRI in High-Risk Women

42 yr old woman. Sister died metastatic TNBC breast cancer (normal mammogram). No BRCA mutation.

R Masood=14
L Masood=12

R Masood=16
L Masood=17