Proliferation and apoptosis as measures of response

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NCI
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Proliferation and apoptosis as measures of response

1. Chemotherapy
2. Endocrine therapy

ΔProliferation (Ki67)
ΔApoptosis
1,000 cells scored % positive

14 gauge core-cut 2cm

3,000 cells scored % positive

Ki67

Apoptosis - TUNEL
Proliferation
- s-phase
- mitotic index
- thymidylate synthase
- BrDU
(Urruticoechea et al JCO, 2005, 23, 7212)

Cell death
- morphology
- activated caspase 3
- ? M30 (keratin 8/18)
- serum assay
- (also autophagy and necrosis)

Or:

14 gauge core-cut
2cm

or
FNA

Apoptosis - TUNEL

Ki67
Precision of measurements of Ki67 and AI in pairs of 14g core-cuts

- **Ki67 (%) vs. Ki67 (%)**
  - Mean: X=33%
  - Standard Deviation: SD=16%

- **AI (%) vs. AI (%)**
  - Mean: X=38%
  - Standard Deviation: SD=22%
Relationship between Ki67 and apoptosis (IMPACT baseline)

- **Ki67 (%)**
- **Apoptosis (%)**

- **Correlation:**
  - $\rho = 0.517$
  - $p = <0.0001$

- **Sample Size:** $n = 220$
Chemotherapy
High proliferation pretreatment:

good response to chemotherapy
but
poor long term outcome

NB pCR
Effect of 24 hours chemotherapy on apoptotic index and Ki67

**Al (%)**

- Pre: 0
- 24h: 5
- P < 0.001

**Ki67 (%)**

- Pre: 0
- 24h: 100
- P = 0.009
Change in Ki67 with neoadjuvant chemotherapy
Relationship of clinical response with change in Ki67 or apoptosis

<table>
<thead>
<tr>
<th></th>
<th>Δpre/24hr</th>
<th>Δ pre/21d</th>
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</thead>
<tbody>
<tr>
<td>Ki67↓</td>
<td>Non-significant</td>
<td>Significant:</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Makris et al 1998</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Chang et al 1999</td>
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<tr>
<td></td>
<td></td>
<td>Assersohn 2003</td>
</tr>
<tr>
<td>apoptosis↑</td>
<td>Significant: Chang et al 1999</td>
<td>Non-significant: Parton et al 2001</td>
</tr>
</tbody>
</table>
Pretreatment and excision (matched) Ki67 and RFS with neoadjuvant chemotherapy
Endocrine therapy
IMPACT: Per Protocol Ki67 Analyses
Anastrozole vs tamoxifen vs combination in ER+ patients

Presentation

Surgery

NR or R

excision

biopsy

n=241

2w

n=159

10w

n=236
ATAC: Kaplan–Meier Curves of Disease-free Survival in Receptor-positive Population

<table>
<thead>
<tr>
<th></th>
<th>Anastrozole</th>
<th>Tamoxifen</th>
<th>Combination</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proportion event free (%)</td>
<td>80</td>
<td>85</td>
<td>90</td>
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</tbody>
</table>

Curves truncated at 42 months

A > T = C

<table>
<thead>
<tr>
<th></th>
<th>HR</th>
<th>95.2% CI</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>AN vs TAM</td>
<td>0.78</td>
<td>0.65–0.93</td>
<td>0.0054</td>
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<tr>
<td>Comb vs TAM</td>
<td>1.02</td>
<td>0.87–1.21</td>
<td>0.7786</td>
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</tbody>
</table>
Apoptosis

Decreased with all 3 arms at 2 weeks (15-25%) and at 12 weeks for anastrozole (21%)
Proliferation Ki67 (%): Individual Patient Plots —
Anastrozole (Per-Protocol Population)
Percentage Ki67 Change (95% CI) from Baseline* During Treatment

* Via transformation of geometric mean proportion of baseline
Relationship Between Ki67 Change and Objective Clinical Response

2 Weeks

12 Weeks

<table>
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<tr>
<th>Treatment</th>
<th>2 Weeks</th>
<th>12 Weeks</th>
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<tbody>
<tr>
<td>Anastrozole</td>
<td>p=0.655</td>
<td>p=0.771</td>
</tr>
<tr>
<td>Tamoxifen</td>
<td>p=0.013</td>
<td>p=0.194</td>
</tr>
<tr>
<td>Combination</td>
<td>p=0.735</td>
<td>p=0.157</td>
</tr>
<tr>
<td>All</td>
<td>p=0.188</td>
<td>p=0.106</td>
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p-values indicate the statistical significance of the differences between responders and non-responders for each treatment group.
Ki67↓ PD

Ki67↓ PR
Effect of anastrozole on Ki67 in individual patients
Effect of anastrozole on Ki67 in individual patients
Effect of anastrozole on Ki67 in individual patients
Ki67 (%)

Weeks

Baseline

Prognostic

Pre-2wks

Predictive

2wks

Integrates

prognostic and predictive

2wk Ki67 should relate to outcome on anastrozole more closely than baseline Ki67
Relapse Free Survival by baseline LnKi67

Years since randomisation

<=2.25: N = 51, O = 5, E = 8.4
2.25-2.99: N = 55, O = 8, E = 9.9
3+: N = 52, O = 13, E = 7.7

Chi-squared = 4.68, df = 1, p = 0.03
Relapse Free Survival by 2 week LnKi67

Years since Randomisation

- \( \leq 0.8 \), \( N = 45 \), \( O = 3 \), \( E = 7.9 \)
- 0.81-1.99, \( N = 60 \), \( O = 9 \), \( E = 10.6 \)
- 2+, \( N = 54 \), \( O = 14 \), \( E = 7.4 \)

\( \chi^2 = 8.65 \), \( df = 1 \), \( p = 0.003 \)
# Multivariate analysis: RFS

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<th>p-value</th>
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<td>Tumour size per cm</td>
<td>1.67 (1.35 - 2.06)</td>
<td>&lt;0.001</td>
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<tr>
<td>Ki67 (2 wks) 2.7x increase</td>
<td>2.01 (1.28 - 3.15)</td>
<td>0.002</td>
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<td>ER (2 wks) 2.7x increase</td>
<td>0.79 (0.62 - 0.99)</td>
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## Multivariate analysis: RFS

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→ POETIC
Short-term presurgical treatment:
Biological characteristics of AI response

Presentation

Surgery

- 35 ER+ patients
- anastrozole or letrozole
- RNA T7 amplified
- cDNA microarray (17,468 features)

Incidental medication

biopsy

excision

2w
Selected clusters from heirarchical clustering based on 2680 most variable genes

HER2 cluster
TFF1 cluster
ER cluster
Global index of dependence on estrogen (GIDE)

- Number of genes which change [up or down] beyond a given threshold (x2)
- How does this index vary according to:
  - [ER]
  - HER2
<table>
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<tr>
<th>Patient</th>
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</table>
Biological disaggregation

- Malignant feature
  - Proliferation
  - Apoptosis/autophagy
  - Dormancy
  - Angiogenesis
  - Invasion
  - Metastasis
  - Others

Feature-specific gene signatures

GIDE
Biological disaggregation

Feature-specific gene signatures

Malignant feature
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- Others

GIDE

- Oestrogen dependency of feature
- Importance in clinical benefit from E-deprivation
- Determinants of response of feature to E-deprivation
Biological disaggregation

Malignant feature
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Feature-specific gene signatures

GIDE

- Oestrogen dependency of feature
- Importance in clinical benefit from E-deprivation
- Determinants of response of feature to E-deprivation

POETIC
Summary

Chemotherapy

• decreased proliferation is associated with response to treatment

• high residual Ki67 is associated with poor RFS

• increased apoptosis occurs in response to therapy but no close relationship with response
Summary
Endocrine therapy

• decreased proliferation may predict for treatment benefit but no close relationship with clinical response

• high 2 week Ki67 is associated with poor RFS

• increased apoptosis does not occur at 2 weeks or 12 weeks with tamoxifen or AI
Novel agents

Open-minded about effects on apoptosis and proliferation during early studies
Pre-chemotherapy Ki67 and Relapse Free Survival (matched)

- <14.9: N = 34, O = 17, E = 20.6
- 14.9 - 32.49: N = 35, O = 11, E = 18.3
- >=32.5: N = 34, O = 21, E = 10.2

Chi-squared trend = 7.3, df = 1, p = 0.000
Post-chemotherapy Ki67 and Relapse Free Survival (matched)

Years since presentation

- <2.2: N = 32, O = 12, E = 19.5
- 2.2 - 8.19: N = 36, O = 11, E = 20.7
- >=8.2: N = 35, O = 26, E = 8.8

Chi-squared = 23.53, df = 1, p < 0.00001