![MINDACT logo] Microarray In Node-negative and 1 to 3 positive lymph node Disease may Avoid ChemoTherapy EORTC 10041 / BIG 3-04 TRANSBIG

# Can Surrogate Pathological Subtyping Replace Molecular Subtyping?
# Outcome Results from the MINDACT Trial

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## Background

Molecular subgroups within early breast cancer (EBC), such as Luminal A, Luminal B, HER2+, Basal-like may help to best to identify patients for specific treatment regimens. Controversy exists as to which methodology is best at identifying these molecular subgroups. Immunohistochemistry (IHC) may be used as a surrogate method to stratify patients. Molecular subtyping gene expression based tests, such as BluePrint, measure a greater number of genes than pathological criteria. ER, PgR, HER2 and Ki67 are measured individually at the protein level, while BluePrint is designed to capture the functional underlying biologic pathway regulated by these receptors.



Figure explaining different levels of Luminal-type classification: single gene mRNA is near 100% concordant with IHC ER assessment. While ER may be present, down-stream genes may be turned off, causing the tumor to be Basal-type, whereas IHC assessment had indicated the tumor to be hormone receptor positive.

## Methods

The MINDACT trial is an international, prospective, randomized, phase III trial which has proven the clinical utility of MammaPrint in selecting EBC patients who can safely avoid chemotherapy. Here we present the results of a preplanned MINDACT sub-study to compare outcome based on molecular subtyping to surrogate pathological subtyping as endorsed by 2013 St. Gallen Consensus. Molecular Subtyping (MS) data were obtained by MammaPrint (MP) and BluePrint classifying patients in the following subtypes: Luminal A (MP Low Risk); Luminal B (MP High Risk); HER2-type; and Basal-type. ER, PgR, HER2 and Ki67 protein status were centrally assessed by IHC/FISH. The primary hypothesis was that among Pathological Subtyping (PS) Luminal patients, patients with HER2 or Basal-type tumors by MS would have a decreased DMFS compared to MS Luminal patients. At α=5% with 220 events, the study has 80% power to demonstrate this for HR=2.44. Reported hazard ratios were adjusted for chemotherapy and endocrine therapy administration.

### Molecular Subtyping



### Surrogate definitions of subtypes

**Luminal A** — ER+ and PgR ≥20% and HER2- and Ki67 <20%

**Luminal B** — ER+ and PgR <20% and/or Ki67 ≥20% and HER2-

**HER2 enriched** — HER2+

**Triple Negative** — PgR- and ER- and HER2-

Schematic representation of Molecular Subtyping MammaPrint and 80-gene molecular subtyping profile BluePrint.

Goldhirsch et al, Ann Oncol 2013

# RESULTS

## Reclassification with molecular subtyping

Pathological Luminal A n=2747

Pathological Luminal B n=1971

Pathological HER2-enriched n=557

Pathological Triple Negative n=531



- Luminal A-type
- Luminal B-type
- HER2-type
- Basal-type

Molecular Subtyping (MS) classified 54% as Luminal A among the Luminal B by Pathological Subtyping (PS). MS classified 38% as Luminal (A and B) and 5% as Basal-type among the HER2+ by PS. MS classified 5% as Luminal (A and B) among the TN cases by PS.

## Surrogate Luminal classification

Among the PS Luminal patients, the Ki67 cut-off at 20% identified 69% patients with ki67 low (compared to 41% using the 14% cut-off) who had 96.0% 5yr DMFS (comparable to 97.5% for the 14% cut-off).

Note that this preplanned analysis shows how "improved" clinical classification, using centrally assessed pathological markers including Ki67 still overestimates the number of patients assigned to adjuvant chemotherapy.



## DMFS by central pathological subtyping and molecular subtyping



| Pathological Subtyping | % DMFS (95% CI) | HR (95% CI) |
|---|---|---|
| Lum A (n=2747) | 97.2 (96.4, 97.8) | 1.00 |
| Lum B (n=1971) | 93.7 (92.4, 94.8) | 2.30 (1.73, 3.06) |
| HER2+ (n=557) | 94.1 (91.6, 95.8) | 1.87 (1.23, 2.86) |
| TN (n=531) | 90.3 (87.3, 92.6) | 2.03 (1.27, 3.25) |

| Molecular Subtyping | % DMFS (95% CI) | HR (95% CI) |
|---|---|---|
| Lum A (n=3657) | 96.7 (96.0, 97.3) | 1.00 |
| Lum B (n=1169) | 92.7 (90.9, 94.2) | 2.29 (1.65, 3.16) |
| HER-2 (n=355) | 94.3 (91.2, 96.3) | 1.49 (0.90, 2.47) |
| Basal (n=625) | 90.4 (87.7, 92.5) | 1.80 (1.14, 2.83) |

Molecular Subtyping identifies 63% of patients as Luminal A, while Pathological Subtyping identifies 47% (Δ16%); 5yr DMFS for both methods was ≥ 96.0%.

PS Luminal cancers that were classified as HER-2 or Basal-type by MS had a lower 5yr DMFS (88.0% for HER-2+ and 90.2% for Basal), albeit non-significant, than those who were also Luminal by MS (95.9%): HR= 1.40, 95% CI = 0.75-2.60.

## Triple Negative classified as Luminal-type, and vice versa

In Triple Negative (TN) cancers, Molecular Subtyping identified 24 out of 531 patients (5%) as Luminal-type with excellent prognosis (5yr DMFS of 100% versus 71.4% for MS HER2+ or 90.1% for MS Basal-type).

This study also confirms earlier reports that ~1 in 50 IHC ER+ EBC patients are classified as Basal-type by BluePrint. Potentially explained by a dominant negative ERα-splice variant ERD7 in these tumors. BluePrint appears to measure ER activity independent of the ERα mRNA expression level itself. These tumors may lack a functional response to estrogen and consequently may not respond to hormonal therapy.



| Molecular Subtyping | % DMFS (95% CI) | HR (95% CI)* |
|---|---|---|
| Luminal (n=24) | 100 (not defined) | 1.00 |
| HER2+ (n=7) | 71.4 (25.8, 92.0) | 16.7 (0.82, 340.6) |
| Basal (n=500) | 90.1 (87.0, 92.5) | 5.64 (0.35, 91.3) |

* Firth's method was used; since classical estimation failed due to 0 events in the Luminal group.

# CONCLUSIONS

1) Molecular Subtyping was able to re-stratify 16% of patients to a low risk Luminal A-type group with an excellent outcome.

2) Centrally assessed Ki67 labeling index of 20% may be better than 14% cut-off for surrogate differentiation between Luminal A and B.

3) Among Triple Negative early breast cancer patients, 5% were classified as Luminal by Molecular Subtyping and had an excellent outcome.

4) The observed subtype discrepancies may have an impact on treatment decision making.

5) Albeit limited by low numbers of patients in each subgroup, this study suggest that Molecular Subtyping is better correlated with outcome than pathological classification.