Annotating MammaPrint and BluePrint gene profile to the Hallmarks of cancer and understanding the biology of MammaPrint extreme risk groups

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Background

MammaPrint® (MP) is a 70-gene based assay that stratifies early-stage breast cancer (EBC) patients into low and high-risk of relapse. BluePrint® (BP) is an 80-gene based assay that stratifies EBC patients into 3 molecular subtypes (Basal, Luminal and HER2). Previously, we showed that the MP genes reflect the six hallmarks of cancer (HoC) as defined by Hanahan and Weinberg[1]. Later, these HoC were extended to ten[2]. In this study we annotated the MP 70- and BP 80-genes with respect to the ten HoC.

In addition, further stratification of the MP risk results identified ultra low- and high-risk subgroups with specific prognostic[3,4] and predictive outcomes[5]. To gain more insight into their biological significance we related gene expression profiles of the ultra low/high MP subgroups to the ten HoC per BP subtype.

Methods

To associate the MP and BP genes to the HoC we used the Cancer Hallmarks Analytics Tool (CHAT)[6]. For expression analysis, we selected full-transcriptome microarray data from 600 FFPE samples that were archived at Agendia. MP subgroups (Ultra high (UH) vs High risk (HR) and Ultra Low (UL) vs Low risk (LR)) from each BP subtype where applicable were compared to further understand their biological characteristics by use of Limma and subsequent pathway analysis with GSEA 3.0. Gene sets were filtered based on an FDR q-value < 0.05 and associated with HoC as described by Dhawan et al.[7]

Results

MP and BP gene functions reflected all ten HoC. A large number of MP and BP genes were associated to “sustaining proliferative signaling”, followed by “genome instability and mutation”, “evading growth suppressors”, “invasion and metastasis”, “resisting cell death” and “inducing angiogenesis”. **Figure 1** shows that the 70 MP genes are implicated in multiple functions, from uncontrolled proliferation to evasion of anti-tumor immunity.

Gene expression comparison of LR *versus* UL identified 48 genes (mapping to 63 microarray probes) that were differentially expressed with q value < 0.01 and a fold change higher than 2. For the UH versus HR comparison 73 genes (mapping to 88 microarray probes) were identified as differentially expressed in all BP subtypes. Supervised hierarchical clustering of these 88 probes revealed 4 main clusters of which one contains the majority of UH samples and one cluster contains most of the UL samples (**Figure 2**). The 2 remaining clusters contain LR and HR samples.

Based on GSEA, UL and UH subgroups were enriched, with opposite normalized enrichment scores, (meaning downregulated in the UL group and upregulated in the UH group), in pathways reflecting proliferative and metastatic features. Additionally, the UH subgroup was enriched in “evading growth suppressors”, “genome instability and mutation” and “enabling replicative immortality pathways”, highlighting genetic diversity of the UH compared to other groups. Notably, the UH HER2 subgroup was enriched in several immune signaling pathways (**Figure 3**).

Figure 1

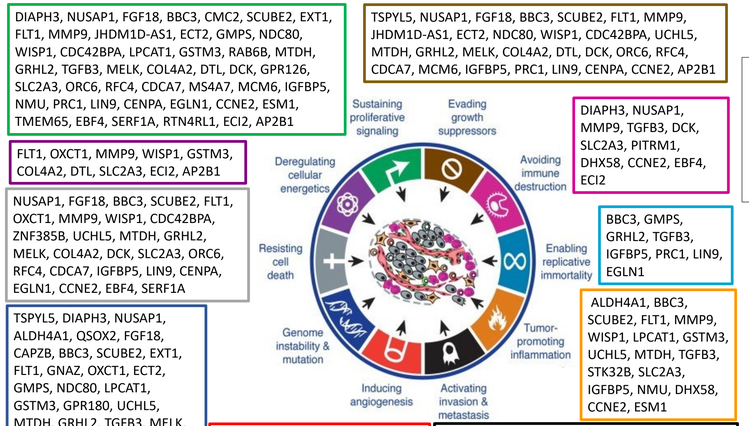


Figure 2

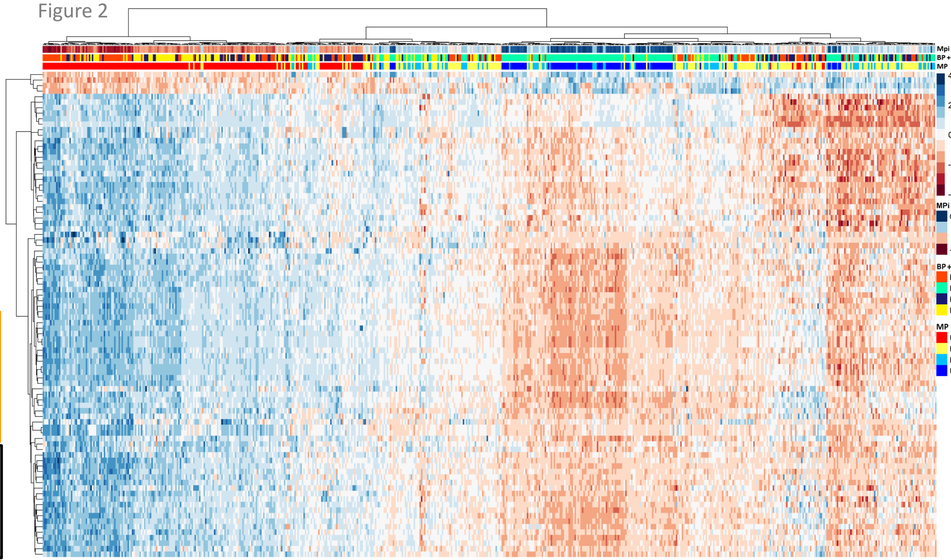
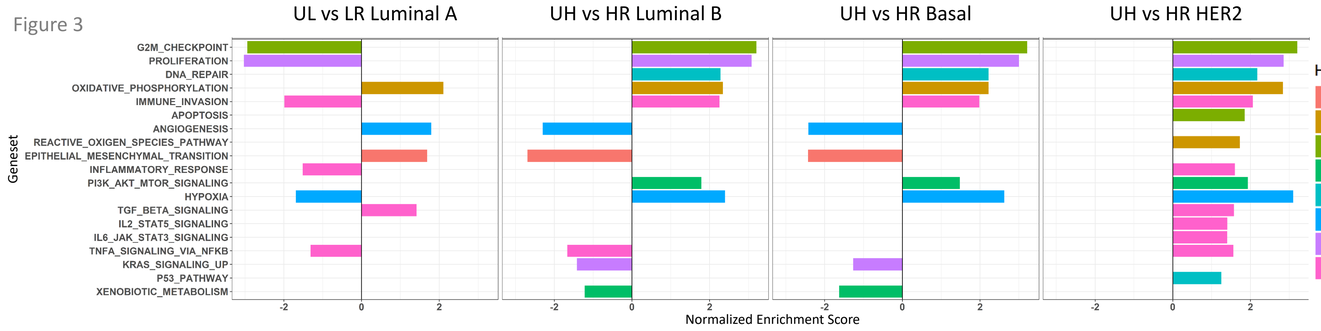


Figure 3



Conclusion

In this study we updated the 70 MP and 80 BP gene annotation and mapped them to the latest 10 Hallmarks of Cancer. The MP and BP genes reflect all 10 HoC highlighting that these signatures capture all steps of cancer progression that drive normal cells into malignant cells that survive, proliferate and spread. Dissecting and understanding the biological processes of early breast cancer at extreme high risk of relapse, might guide relevant treatment decisions and therefore improve patient care.

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ESMO 2020, abstract number 2294; Conflict of interest: JC Haan, R Bhaskaran, L Mittempergher, E Lujinovic, W Audeh and AM Glas are employed at Agendia

VIRTUAL 2020 ESMO congress

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Avoid systemic overtreatment of postmenopausal breast cancer patients with ultralow MammaPrint result

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Background

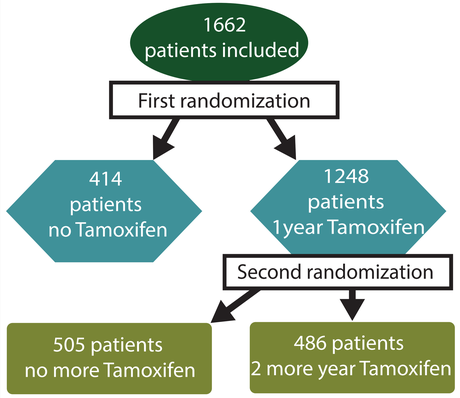
Adjuvant tamoxifen is widely used as endocrine treatment for oestrogen receptor positive (ER+) breast cancers (BC).
Guidelines recommend the use of tamoxifen up to 10 years.
Tamoxifen can cause serious side effects and not all patients need adjuvant tamoxifen to have an excellent prognosis.
To avoid overtreatment, a test that identifies these patients is necessary.

The 70-gene FDA-approved MammaPrint has potential to select patients that have an excellent survival without chemotherapy and only limited or no tamoxifen treatment.
Three thresholds are predefined and indicate the expected benefit of tamoxifen and chemotherapy.[1,2]

	Ultralow risk	Low risk	High risk
Endocrine	limited	Yes	Yes
Chemotherapy	NO	NO	Yes

Randomized controlled trial

Between 1982 and 1994, a total of 1662 postmenopausal patients with stage I to III BC were randomized for no, 1 or 3 years adjuvant tamoxifen treatment. [3]
After 1989 lymph node-positive patients always received at least 1 year of tamoxifen (30 mg/day).
All received surgery but no chemotherapy.



AIM: validate whether the MammaPrint ultralow threshold can select postmenopausal BC patients with an excellent prognosis after only limited or no tamoxifen treatment.

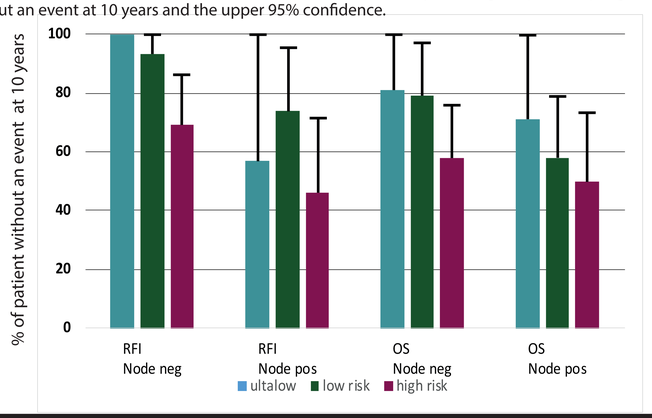
Patients

For 736 out of the 1662 patients we collected FFPE tumor material [4]. From the stored FFPE blocks, 482 were from ER+ HER2- stage I-III patients and of these 346 had sufficient material left for RNA isolation and MammaPrint test. This resulted in reliable scores for 135 patients. In the table below also the 347 ER+ HER2- stage I-III without a MammaPrint score are shown.

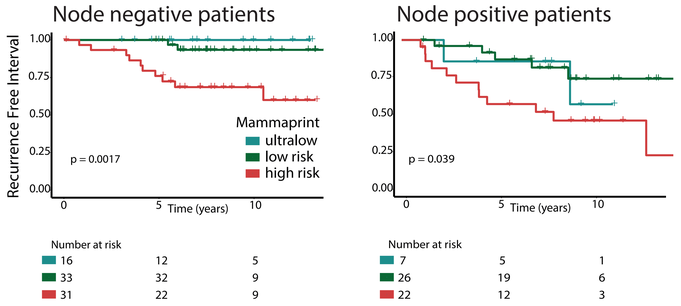
	Ultralow	Low risk	High risk	Not tested ER+HER2-
Number of patients	23	59	53	347
Age [<65 or >65 years]	52% 48%	47% 53%	55% 45%	47% 53%
Nodal status [neg or pos]	70% 30%	56% 44%	58% 42%	56% 44%
T-size [T1, T2 or T3]	17% 74% 9%	35% 58% 7%	22% 74% 4%	33% 59% 8%
Grade [I, II or III]	65% 35% 0	39% 37% 24%	13% 36% 51%	30% 40% 30%
Tamoxifen [0,1 or 3 years]	22% 43% 35%	22% 36% 42%	30% 42% 28%	24% 50% 26%

Survival analysis

Recurrence Free Interval (RFI) was defined as time from the first randomization to the occurrence of a local, regional or distant recurrence or breast cancer-specific death. Patients with a secondary contralateral breast tumor were censored at the time of the contralateral diagnosis. Median follow-up was 8 years for RFI and 13 year for overall survival (OS). Shown are the percentage of patients per group without an event at 10 years and the upper 95% confidence.

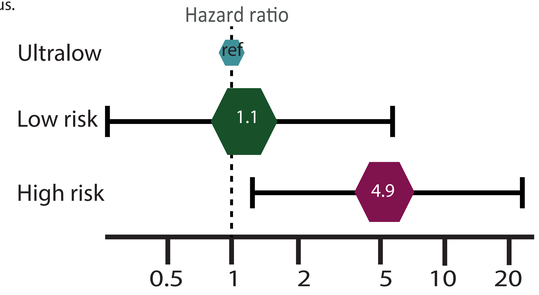


Kaplan Meier plots for Recurrence free interval



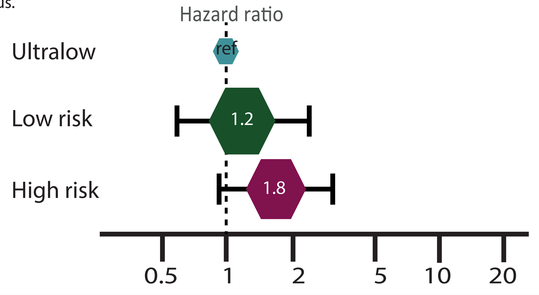
Cox proportional hazard model of Recurrence Free interval

stratfied for nodal status.



Cox proportional hazard model of Overall Survival

stratfied for nodal status.



CONCLUSION: Postmenopausal node negative patients with an Ultralow MammaPrint score have an excellent RFI with ≤3 years of endocrine treatment.

Discussion: Although the number of patients is small, this result is supported by the results of the STO-3 randomized clinical trial.[5] Clinicians should consider limiting endocrine treatment duration for this specific group of patients.

Future plan: Increase follow-up to 20 years to gain more evidence that patients with Ultralow MammaPrint results do not need long term endocrine treatment.

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Acknowledgments
All patients and their families for participating in the study, all participating hospitals and the Core Facility Molecular Pathology & Biobanking (CFMPB) of the Netherlands Cancer Institute.

Funding sources
A Sister's Hope and Agendia

Declaration of interest
Miranda Kleijn and Anuska Glas employed full-time by Agendia. All other authors declare no conflict of interest regarding this project.

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BluePrint Molecular Subtyping Recognizes Single and Dual Subtype Tumors with Implications for Therapeutic Guidance

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INTRODUCTION

BluePrint is an 80-gene molecular subtyping test that classifies early-stage breast cancer (EBC) patients into Basal, Luminal, and HER2 subtypes [1]. Luminal can be further stratified into Luminal A and Luminal B, based on the MammaPrint risk outcome, a 70-gene test for either Low- or High-risk of distant recurrence [2]. BluePrint calculates scores for each of the three subtypes based on an 80-gene signature to determine the subtype with the highest score. A small proportion of tumors exhibit a secondary subtype with a relatively high BluePrint score, i.e., a dual subtype. This study identifies and examines samples with features of dual subtypes using BluePrint scores to understand tumor biology and possible implications on treatment recommendations.

METHODS

We collected a dataset of 9573 EBC samples with pathological receptor status for ER, PR, and HER2 and a dataset of 7985 EBC samples with full-genome microarray expression data. To identify the samples which exhibit more than one activated subtype, a Maximum Allowable Difference (MAD) value was determined using repeated control sample measurements. The MAD value was applied within an area of interest (Figure 1). For a sample to be classified as a dual subtype, two of the BluePrint scores should fall within the determined MAD range. Differential expression analysis (DEA) was performed to determine biological differences between dual subtypes and their respective single subtypes. Additionally, the BluePrint single and dual Basal samples were classified based on Burstein's algorithm into four subtypes: the Basal-Like Immune Activated (BLIA), the Basal-like Immune Suppressed (BLIS), the Luminal/Androgen Receptor (LAR), and the Mesenchymal (MES) [3]. Analyses were performed in R using limma and GSEA packages.

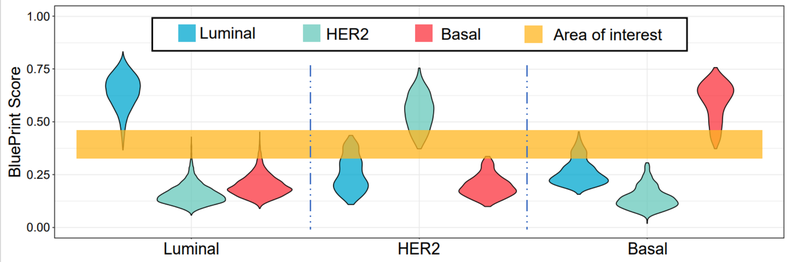

Figure 1: BluePrint scores expressed using MAD. Area of interest indicates where the score of the highest scoring subtype is overlapping with the scores of the lower scoring subtypes

RESULTS

Figure 2: Sankey plot indicating the distribution of single and dual subtypes. Samples with BluePrint outcome were further classified into single (Luminal A, Luminal B, HER2, or Basal) or dual (Luminal B/ HER2, Luminal B/ Basal) subtype. The size of the boxes and colored bars are not representative of sample size.

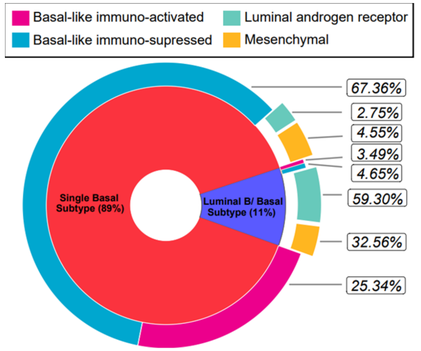

Figure 3: Burstein's classification of the BluePrint Basal samples. This chart shows that most single basal samples correspond with the BLIA or BLIS classification and the Luminal B/ Basal dual subtype with the LAR and MES classification. LAR and MES are highlighted to illustrate the difference in proportions of the single and dual classification.

Out of our 9573 EBC samples, about 98% were classified as a single subtype. The remaining 2% were either dual or triple subtypes (samples with similar scores for all three BluePrint subtypes). The two most frequently encountered dual subtypes were Luminal B/Basal (N = 96) and Luminal B/ HER2 (N = 87) (Figure 2), and they were further characterized using global gene expression analyses. The HER2/ Basal (N = 24) and the triple (N = 17) subtypes were not analyzed further owing to the limited sample size.

Luminal B/ Basal Subtype

The majority (71%) of Luminal B/ Basal dual samples had positive ER status and no amplification of HER2, as measured by IHC/FISH. Interestingly, when using the Burstein classification, the Luminal B/ Basal dual samples most often classified as LAR or MES (Figure 3) while the single Basal samples classified almost always as either BLIA or BLIS. As the BLIA and BLIS types relate to more proliferative tumors, we hypothesize that the single BluePrint Basal subtype will benefit more from chemotherapy, whereas the dual Luminal B/ Basal might have a greater benefit from both chemo- and hormonal-therapy.

Luminal B/ HER2 Subtype

Luminal B/ HER2 dual subtype samples had features of both Luminal B and HER2, with 97% being ER or PR positive and 48% having a HER2 positive status. DEA comparing the dual Luminal B/HER2 with the single HER2 subtype samples showed downregulation of MAPK/ Akt pathways in the dual subtype (Table 1). As MAPK and Akt inhibit ER signaling, their down-regulation allows for co-expression of ER and HER2, which might result in increased resistance to targeted therapies [4].

Table 1: Akt and MTOR, and MAPK related (MEK and RAF) gene sets. Gene sets from the molecular signature database containing genes up- and down-regulated, specific for oncogenic pathway functioning in MCF-7 cells (breast cancer) cell lines. Enrichment score (ES), P-value, and FDR adjusted P-value are given for each gene set.

Gene set	ES	P-value	P-adjust
Up-regulation			
Akt & MTOR	-0.3559	0.0237	0.1700
MEK	-0.3798	0.0064	0.0671
RAF	-0.4285	0.0004	0.0156
Down-regulation			
Akt & MTOR	0.3315	0.0415	0.2450
MEK	0.3593	0.0140	0.1104
RAF	0.4991	<0.0001	<0.0001

CONCLUSION

In BluePrint diagnostic testing, most samples analyzed show a single functional subtype; however, a small proportion of samples display a dual BluePrint subtype. DEA shows that these dual subtypes have distinct genomic characteristics that might help to elucidate the biology of these tumors and further improve their treatment recommendations.

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EBCC conference, 2nd & 3rd October