Validation Project 2008-2010

TRANSBIG

Collaboration within NKI-IGR-IEO-CRH-Agendia

Part 1.
Independent Validation Protocol for late metastases prognostic signatures developed by the Institut Gustave Roussy (IGR)

Part 2.
Validation of the 70-gene profile as predictor of disease outcome in breast cancer patients with 4-9 positive lymph nodes.

Part 3.
Predictive value of 70 gene-signature in the adjuvant chemotherapy setting for patients with breast cancer

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on behalf on the TRANSBIG/MINDACT Biotechnology Committee

Interim Report

Part 1. Independent Validation Protocol for late metastases prognostic signatures developed by the Institut Gustave Roussy (IGR)

Collection of MATERIAL completed :
Existing series of the NKI already used in other studies and for which clinical and genomic data have been previously collected will be explored.
Series of interest include:

- 295 NKI original series
- "Over 55 years" series
- "LN +" series
- "Tamoxifen response"series (patients treated with tam. at 1st relapse)

These series include patients of all ages, LN- and LN+; adjuvantly treated and untreated, with a follow-up of more than 5 years.
Patients were selected based on the selection criteria outlined in the protocol..

Selection NKI	M1<5yrs	M2 5-10yrs	M3 >10yrs	Profile
295 NEJM series (44K)				
Total, excl LN+	*49 M1*	*16M2*	*3M3*	*44K & MP*
"Over 55 years" series				
Total	*21M1*	*10M2*	*5M3*	
>55yrs TAM series	5 M1	4 M2	2M3	44K
>55yrs only	**16 M1**	**6 M2**	**3M3**	
"LN1-3 +" series :				
Total	*44M1*	*12M2*	*3M3*	*MP*
LNposNEJM	17M1	4 M2	3M3	44K & MP
LNpos only	**27M1**	**8 M2**	**0 M3**	**MP**
"TAM"only series	*24M1*	*19M2*	*2M3*	*44K*
Extra TAM series 13				
ERASMUS series 40				

LN 4-9 pos to be added

Total all	*138M1*	*57M2*	*13M3*	
TOTAL 44K available	95M1	43M2	10M3	44K
Remaining to be profiled + Controls (NR) to be profiled	**(43M1)**	**14M2**	**3M3**	

Note: the NEJMMahasti file does not include the 1-3N+ which are in the LNpos file !
Calculations for the NEJMMahasti file have been made on 189 patients only. The other samples are in the LNposMahasti file containing 347 patients.

Bioinformatics

Useability of IGR profiles (only 2 and 4 probes missing on NKI 44K) was checked with Agendia (MS provided genes and algorithm developed by IGR)

1- Quality control of data : done
2- Update of clinical data by MS : done
3- IGR predictors fine-tuning"

Profiling

In summary we already found in NKI series: 138 M1, 57 M2, 13 M3
Among those we have:
-95 M1 samples profiled on 44k
-43 M2 samples profiled on 44k
-10 M3 samples profiled on 44k

We need to further profile on 44k
-14 M2 samples
-3 M3 samples

A list of samples to be profiled on 44k was provided to agendia
RNAs are already available at Agendia
Agendia will perform the 44k profiling of the samples during Summer 2009
Agendia will provide the existing profiles of the rest of the samples included in the study

Database and statistics : to be done during the prolongation period

There will be one endpoint of interest in the validation, and all analyses will be performed on this endpoint: time to distant metastases as first event (TDM), defined as time from surgery to distant metastases, with all other events (local relapse, contralateral tumour, second primary, death) being censored observations.
All patients will be classified among various subgroups:
-no relapse at 10 years
-early distant metastasis before 5 years
-late distant metastasis after 5 years
-very late distant metastasis after 10 years
Survival time to distant metastases will be estimated for the different subgroups

Part 2. Validation of the 70-gene profile as predictor of disease outcome in breast cancer patients with 4-9 positive lymph nodes.

Part 2 is fully completed

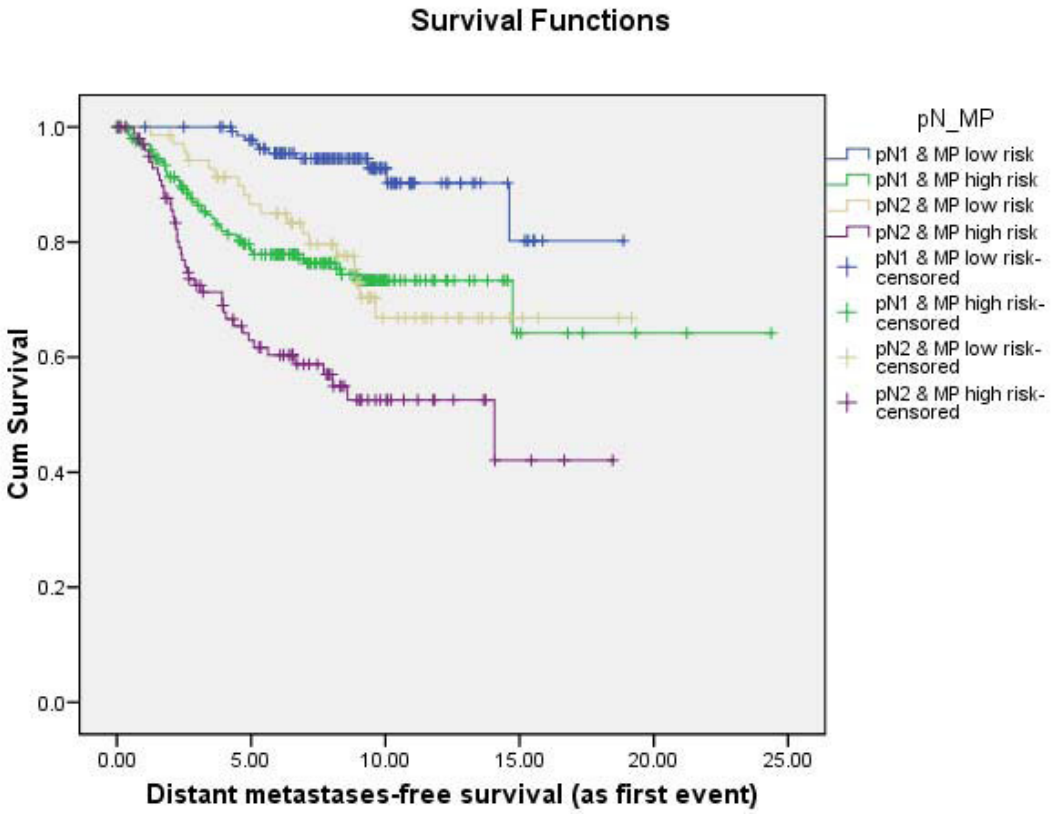
Poster presented at the St Gallen Breast Cancer Conference 2009-07-01

Abstract sent for the San Antonio Breast Cancer Conference (see below)

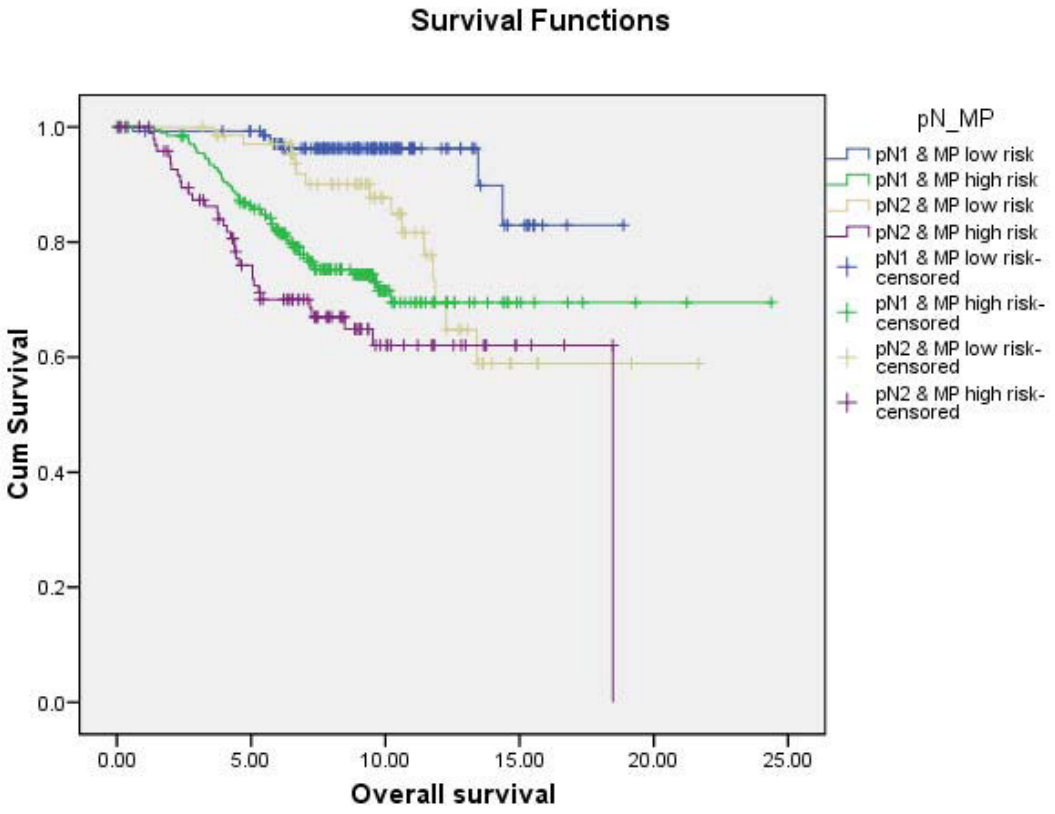
OBJECTIVES
The axillary lymph node (LN) status is considered to be one of the most important factors for chemotherapy decision-making of operable breast cancer patients (pts). It is commonly agreed that combination therapies with taxane-containing regimens should be recommended for these pts, whereas high-dose regimens have failed to provide further improvement for pts clinically considered at high-risk. It has previously been shown that the 70-gene profile (MammaPrint®TM), which was developed in node-negative patients is excellent in predicting disease outcome in pts with 1-3 positive nodes and similarly in pts with 4-9 positive-nodes. Further analysis based on adjuvant treatment received and pooled analysis of the 2 LN positive series was performed in order to assess the prognostic added-value of genomic profiling in LN positive pts.

METHODS Frozen tumor samples from breast cancer pts with positive LN coming from 2 hospitals were selected in consecutive series (1-3 LN, 4-9 LN; all female, diagnosed between 1984 and 1995, primary invasive breast carcinoma, unilateral T1, T2 or operable T3, mastectomy or breast-conserving therapy, no prior malignancies, fresh frozen tumor material available). Samples were evaluated by gene expression profiling for the 70-gene profile and were classified as genomic high risk (poor prognosis) or genomic low risk (good prognosis).

RESULTS A total of 519 pts have been analyzed: 346 with 1-3 positive LN (PN1) and 173 with 4-9 positive LN (PN2). Among them, 212 (41%) had the 70-gene good prognosis-profile and 307 (59%) had the 70-gene poor prognosis-profile (strictly equal proportions among the 2 LN groups). Median follow-up was 10.3 years: distant metastases occurred in 141 patients (116 as first event) and 103 (20 %) died of their disease. Distance metastases as first event and breast cancer specific survival according to LN group (PN) and genomic profile (MP) are shown in Figures 1 and 2.
[figure1]


Survival Functions
Cum Survival
1.0
0.8
0.6
0.4
0.2
0.0
0.00
5.00
10.00
15.00
20.00
25.00
Distant metastases-free survival (as first event)
pN_MP
pN1 & MP low risk
pN1 & MP high risk
pN2 & MP low risk
pN2 & MP high risk
pN1 & MP low risk-censored
pN1 & MP high risk-censored
pN2 & MP low risk-censored
pN2 & MP high risk-censored

[figure2]



CONCLUSION Our data show that the 70-gene profile is a strong prognostic marker of distant recurrence and breast specific death in breast cancer patients with positive LN. Combining nodal status (1-3 nodes vs. 4-9 nodes) and 70-gene profile (good vs. poor) allows stratifying patients among subgroups for whom tailored treatment strategies should be designed and assessed based on their very different outcome. Pts with elevated number of lymph nodes and high genomic risk have a very poor prognosis and might need to be considered for stronger treatment strategies.

Part 3 – Predictive value of 70 gene-signature in the adjuvant chemotherapy setting for patients with breast cancer

This part will be completed during the prolongation period.