Biomarqueurs et analyses moléculaires en Oncologie

Paris Jeudi 14 et Vendredi 15 Juin 2018

Atelier 2 – Cancer du Sein

Quels profils moléculaires rechercher ?

Gérard MILANO
Oncopharmacologie – UCA EA 3836
Expert INCa, UNICANCER, Cancéropôles
Centre Antoine Lacassagne, Nice
gerard.milano@nice.unicancer.fr

Cancer du sein invasif.
Besoin d'un critère objectif et mesurable de discrimination

(Cardoso F. et al, Ann Oncol 2017)

- 90 % sont classés M0.

- 60 % sont classés N-.

- Parmi ces patientes M0 N-, seules 20 % récidiveront à 10 ans.

- Pourtant aujourd'hui 90 % reçoivent un traitement adjuvant.

Biomarqueurs :
Critères requis pour une utilité en pratique clinique

1) Validation Analytique

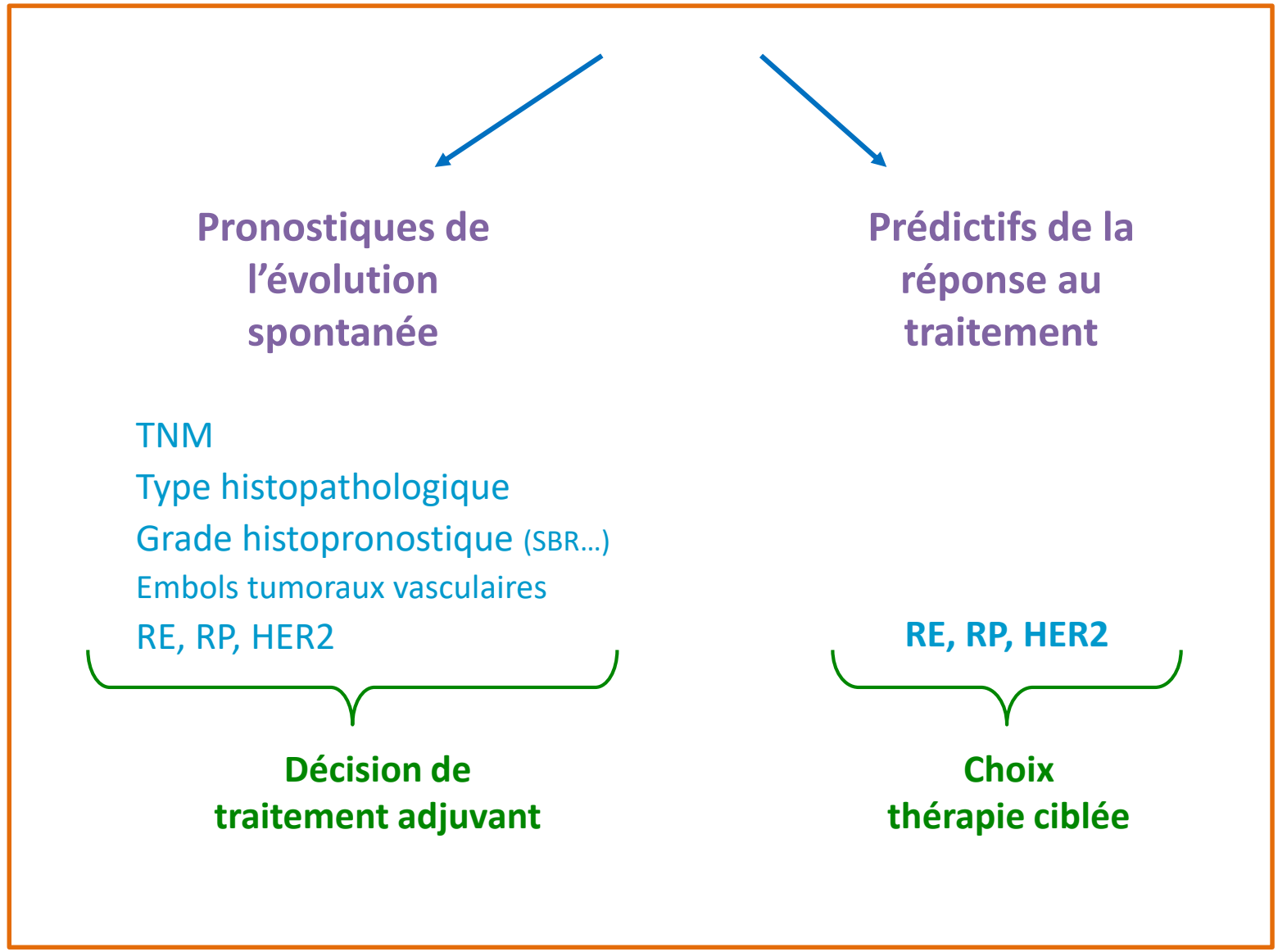
- Contrôle des procédures pré-analytiques
- Technique précise, sensible, spécifique, reproductible, robuste
- Standardisation des procédures analytiques et bioinformatiques
- Nécessité de Contrôles Qualités Externes

2) Validation Clinique ➔ Niveau de preuve I (Level Of Evidence, Grille de Simon)

LOE I	AB	Niv de preuve élevé (études prospectives ou prospective-rétrospectives)
LOE II	BC	Niv de preuve intermédiaire (études pros-rétro ou pros-observationnelles)
LOE III	C	Niv de preuve intermédiaire faible (études pros-observationnelles)
LOE IV-V	D	Niv de preuve bas (études rétrospectives observationnelles)

3) Aspects logistiques et coûts

BIOMARQUEURS validés de 1ère génération (LOE I)



BIOMARQUEURS de 2ème génération

		Pronostique	Prédictif efficacité	Prédictif toxicité
Tumoraux	Signatures moléculaires (profils d'expression):	?	?	
	- **Oncotype DX®**			
	- **MammaPrint®** …			
	Marqueurs d'invasion:			
	uPA/PAI1	validé	?	
	Marqueurs de prolifération:			
	Ki67	validé	?	
	Marqueurs moléculaires:			
	PIK3CA, PTEN	?	?	
	BRCA1/2	?	?	
	TOP2A	?	?	
Circulants	**Cellules tumorales circulantes**	?		
	Polymorphismes génétiques		?	?
Autres	**Ganglions sentinelles**	validé		
	Micrométastases médullaires	validé		

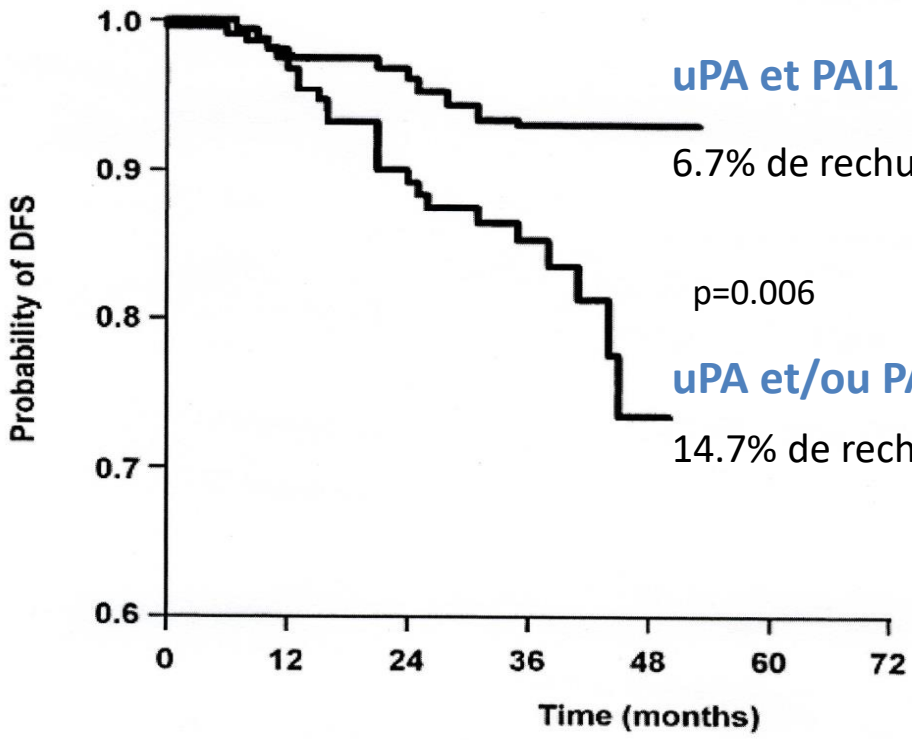
Guidelines on the use of biomarkers in patients with invasive breast cancer. EGTM recommendations. BCI, Breast Cancer Index; LOE, (level of evidence) based on Ref. [6] SOR (strength of recommendation) based on Ref. [7].

Biomarker	Recommendation	LOE	SOR
ER	For predicting the response to endocrine therapy in patients with early or advanced breast cancer. Mandatory in all patients.	IA	A
PR	In combination with ER for predicting response to endocrine therapy in patients with early or advanced breast cancer. Mandatory in all patients.	IB	A/B
HER2	For predicting response to anti-HER2 therapy in patients with early or advanced breast cancer. Mandatory in all patients.	IA	A
Ki67	In combination with established clinical and pathological factors for determining prognosis in patients with newly diagnosed invasive breast cancer, especially if values are low or high.	IB	A/B
uPA/PAI-1	For determining prognosis and aiding decision-making for the administration of adjuvant chemotherapy to patients with ER-positive, HER2-negative, lymph node−negative disease.	IA	A
Oncotype DX	For determining prognosis and aiding decision-making for the administration of adjuvant chemotherapy in patients with ER-positive HER2-negative lymph, node−negative and lymph node −positive (1−3 nodes) disease.	IB	A
MammaPrint	For determining prognosis and aiding decision-making for the administration of adjuvant chemotherapy to patients with ER-positive, HER2-negative, lymph node−negative and lymph node−positive (1−3 nodes) disease.	IA	A
Prosigna	For determining prognosis and aiding decision-making for the administration of adjuvant chemotherapy to patients with ER-positive HER2-negative, lymph node−negative and lymph node −positive (1−3 nodes) disease.	IB	A
EndoPredict	For determining prognosis and aiding decision-making for the administration of adjuvant chemotherapy to patients with ER-positive HER2-negative lymph node−negative and lymph node −positive (1−3 nodes) disease.	IB	A
BCI	For determining prognosis and aiding decision-making for the administration of adjuvant chemotherapy in patients with ER-positive, HER2-negative, lymph node−negative disease.	IB	A

(Duffy MJ et al., Eur J Cancer 2017)

Valeurs pronostique et prédictive de uPA et PAI1

Valeur pronostique de uPA/PAI1 chez les patientes non traitées



	Analyse intermédiaire	Analyse finale
uPA et PAI1 bas	6.7% de rechutes à 3 ans	**12.9% de rechutes à 10 ans**
	p=0.006	**p=0.011**
uPA et/ou PAI1 élevé	14.7% de rechutes à 3 ans	**23% de rechutes à 10 ans**

(Jänicke JNCI 93, 2001)

(Harbeck JCO 27, 2009)

Autres signatures multigéniques

TABLE 1. Most Common Commercially Available Prognostic Gene Signatures for Breast Cancer

	MammaPrint	Oncotype DX	Breast Cancer Index	Mapquant DX	PAM 50 ROR	EndoPredict
Provider	Agendia	Genomic Health	Biotheranostics	Ipsogen	NanoString	Sividon
Type of Assay	70-gene assay	21-gene recurrence score	2-gene ratio (H/I) and molecular grade index	Genomic grade	50-gene assay	12-gene assay
Type of Sample	Fresh or frozen or FFPE	FFPE	FFPE	Fresh or frozen or FFPE	FFPE	FFPE
Technique	DNA microarray or qRT-PCR	qRT-PCR	qRT-PCR	DNA microarray or qRT-PCR	qRT-PCR	qRT-PCR
Clinical Application	Prognosis of N0, < 5 cm, stage I/II, age < 61	Prediction of recurrence risk in ER+ and N0 treated with TAM	Prognostic in ER+, prediction of response to TAM	Molecular grading for ER+, histologic grade II disease	Originally for intrinsic subtyping, recurrence prediction	Recurrence prediction for ER+ HER2−
Results Presentation	Dichotomous, good or poor prognosis	Continuous variable	Continuous variable	Dichotomous, GGI I or GGI III	Continuous variable	Dichotomous, low or high risk

- Niveaux de preuve plus bas (II et plus)
- Pas d'études prospectives dédiées
- Evaluation ++++++
- Contexte du RIHN

Oncotype DX ®
Test du score de récidive (SR)

16 gènes candidats + 5 gènes de référence

PROLIFERATION
Ki-67
STK15
Survivine
Cycline B1
MYBL2

OESTROGENES
RE
RP
Bcl2
SCUBE2

GSTM1

BAG1

INVASION
Stromelysine 3
Cathepsine L2

CD68

HER2
GRB7
HER2

REFERENCE
Beta-actine
GAPDH
RPLPO
GUS
TFRC

SR = + 0,47 x score du groupe HER2
- 0,34 x score du groupe RE
+ 1,04 x score du groupe prolifération
+ 0,10 x score du groupe Invasion
+ 0,05 x CD68
- 0,08 x GSTM1
- 0,07 x BAG1

Catégorie	SR (0 -100 %)
Risque faible	SR <18
Risque intermédiaire	SR 18 - 30
Risque élevé	SR ≥ 31

(Paik, N Engl J Med 351, 2004)

Essais randomisés en cours
Utilisant les signatures OncotypeDX ou MammaPrint

	TAILORx	MINDACT
Coordination	ECOG	EORTC
Critères d'inclusion	**N- RH+** Stades I-II	**N- et N+**
Signature utilisée	**Oncotype Dx**	**MammaPrint**
Nb patiente screenées	10 500	6000
Critère de sélection pour la randomisation	Patientes avec risque intermédiaire (SR entre 11 et 25)	Patientes avec risque discordant entre MammaPrint et Adjuvant On Line
Nb patientes randomisées	4390	1920
Traitement par bras	Hormono *vs* Chimio+hormono	Recommandation MammaPrint *vs* Recommandation Adjuvant On Line
Critère de jugement	DFS	Survie sans métastase
Etat d'avancement	Inclusions terminés Résultats attendus 2015	Inclusions terminées Résultats attendus 2019

The NEW ENGLAND JOURNAL of MEDICINE

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Prospective Validation of a 21-Gene Expression Assay in Breast Cancer

J.A. Sparano, R.J. Gray, D.F. Makower, K.I. Pritchard, K.S. Albain, D.F. Hayes, C.E. Geyer, Jr., E.C. Dees, E.A. Perez, J.A. Olson, Jr., J.A. Zujewski, T. Lively, S.S. Badve, T.J. Saphner, L.I. Wagner, T.J. Whelan, M.J. Ellis, S. Paik, W.C. Wood, P. Ravdin, M.M. Keane, H.L. Gomez Moreno, P.S. Reddy, T.F. Goggins, I.A. Mayer, A.M. Brufsky, D.L. Toppmeyer, V.G. Kaklamani, J.N. Atkins, J.L. Berenberg, and G.W. Sledge

Event Rates at 5 Years, According to Histologic Grade.*

Tumor Grade	Invasive Disease–free Survival (95% CI)	Freedom from Distant Recurrence (95% CI)	Freedom from Any Recurrence (95% CI)	Overall Survival (95% CI)
All grades	93.8 (92.4–94.9)	99.3 (98.7–99.6)	98.7 (97.9–99.2)	98.0 (97.1–98.6)
Low grade	95.8 (93.5–97.3)	99.8 (98.3–100)	99.8 (98.3–100)	98.7 (97.0–99.4)
Intermediate grade	93.6 (91.7–95.1)	99.0 (98.0–99.5)	98.2 (97.0–99.0)	97.9 (96.8–98.7)
High grade	91.3 (83.9–95.4)	100 (NC–NC)	98.7 (91.1–99.8)	97.3 (91.9–99.1)

ONCOTYPE-DX

- **Bonne indication de marqueur pronostique**
- **Niveau de preuve insuffisant**
- **Existence d'une zone « grise ».**

(Sparano JA et al, NEJM 2015)

PFS RS intermédiaire (11 à 25)

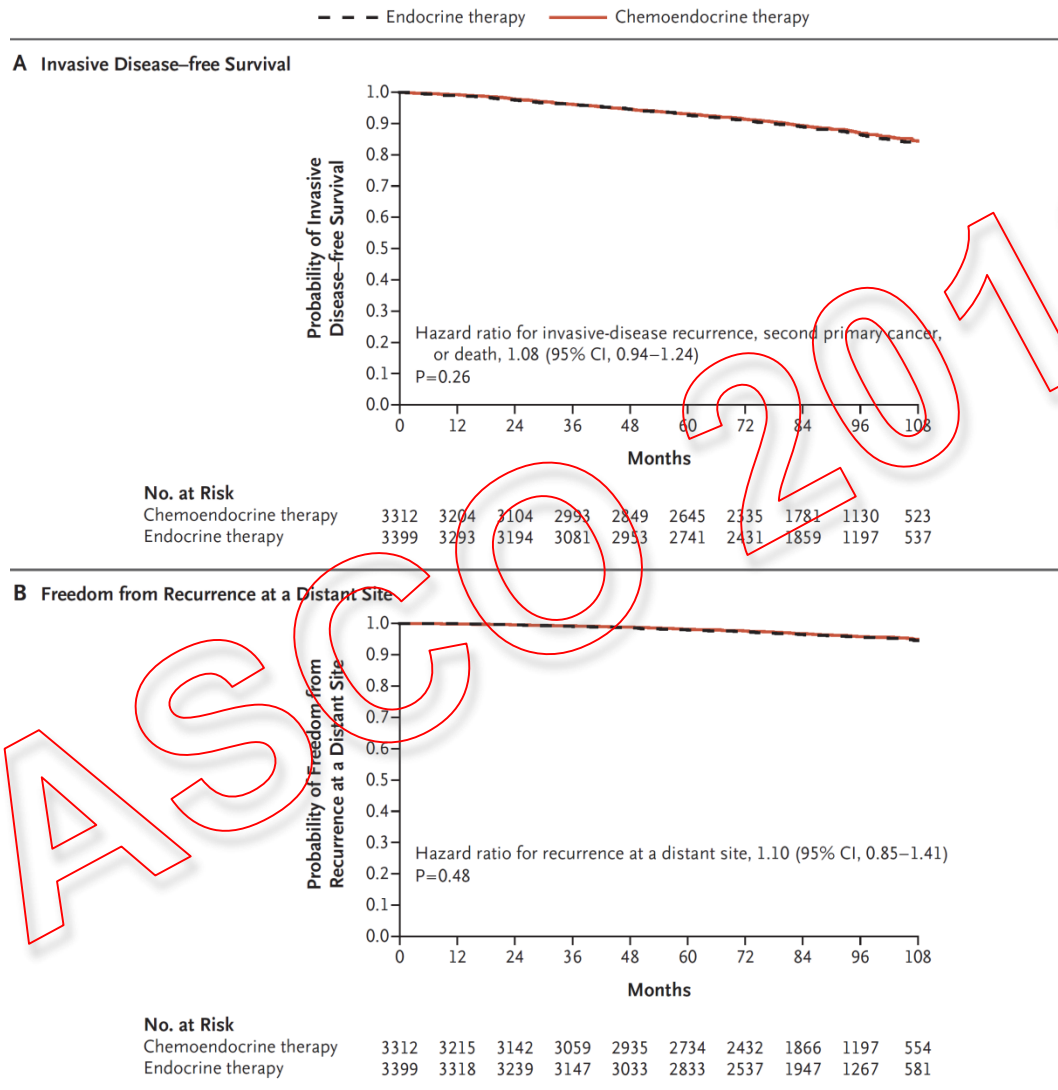
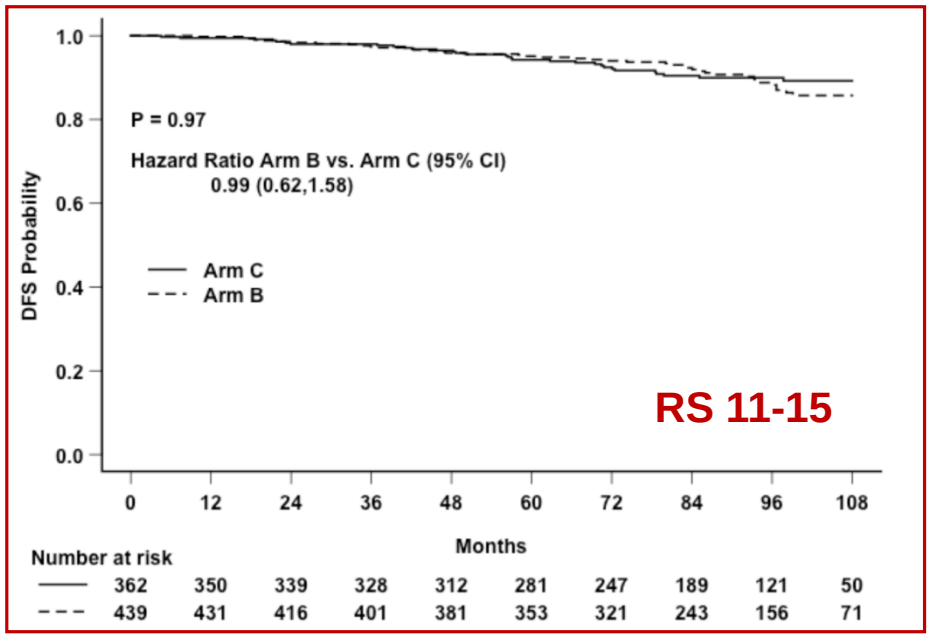
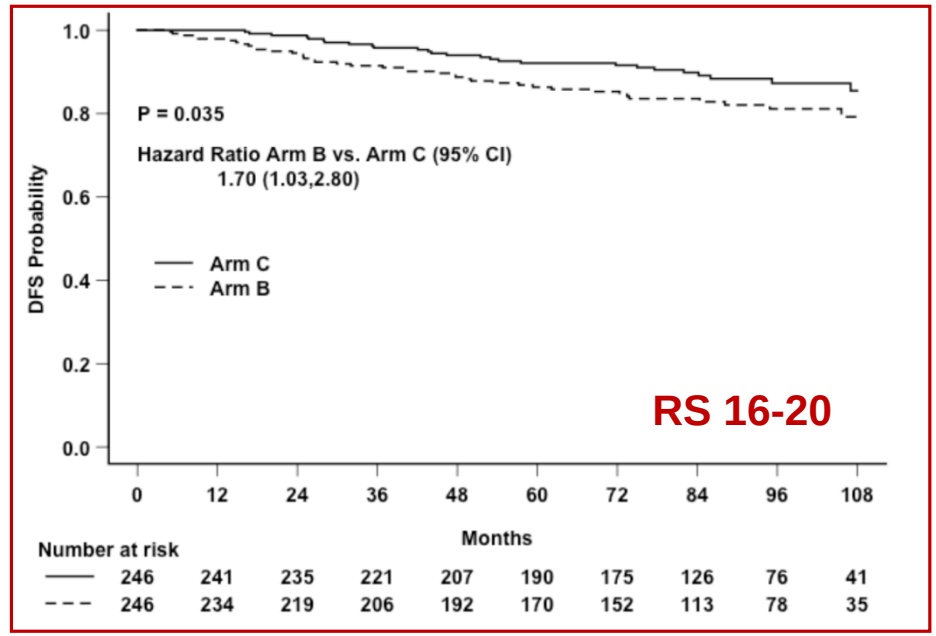


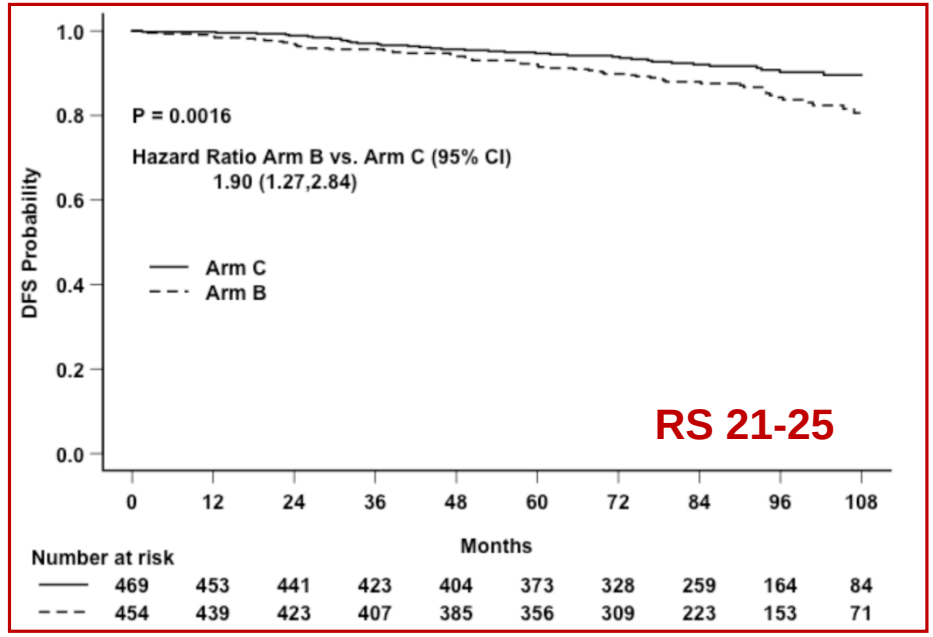
Figure 2. Clinical Outcomes among Patients with a Recurrence Score of 11 to 25.

Kaplan–Meier estimates of survival rates in the analysis according to the assigned treatment group are shown for the group that received endocrine therapy alone and the group that received chemoendocrine therapy in the intention-to-treat analysis of invasive disease–free survival (defined as freedom from invasive disease recurrence, second primary cancer, or death) and freedom from recurrence of breast cancer at a distant site. The hazard ratios are for the endocrine-therapy group versus the chemoendocrine-therapy group.

Patientes </= 50 ans





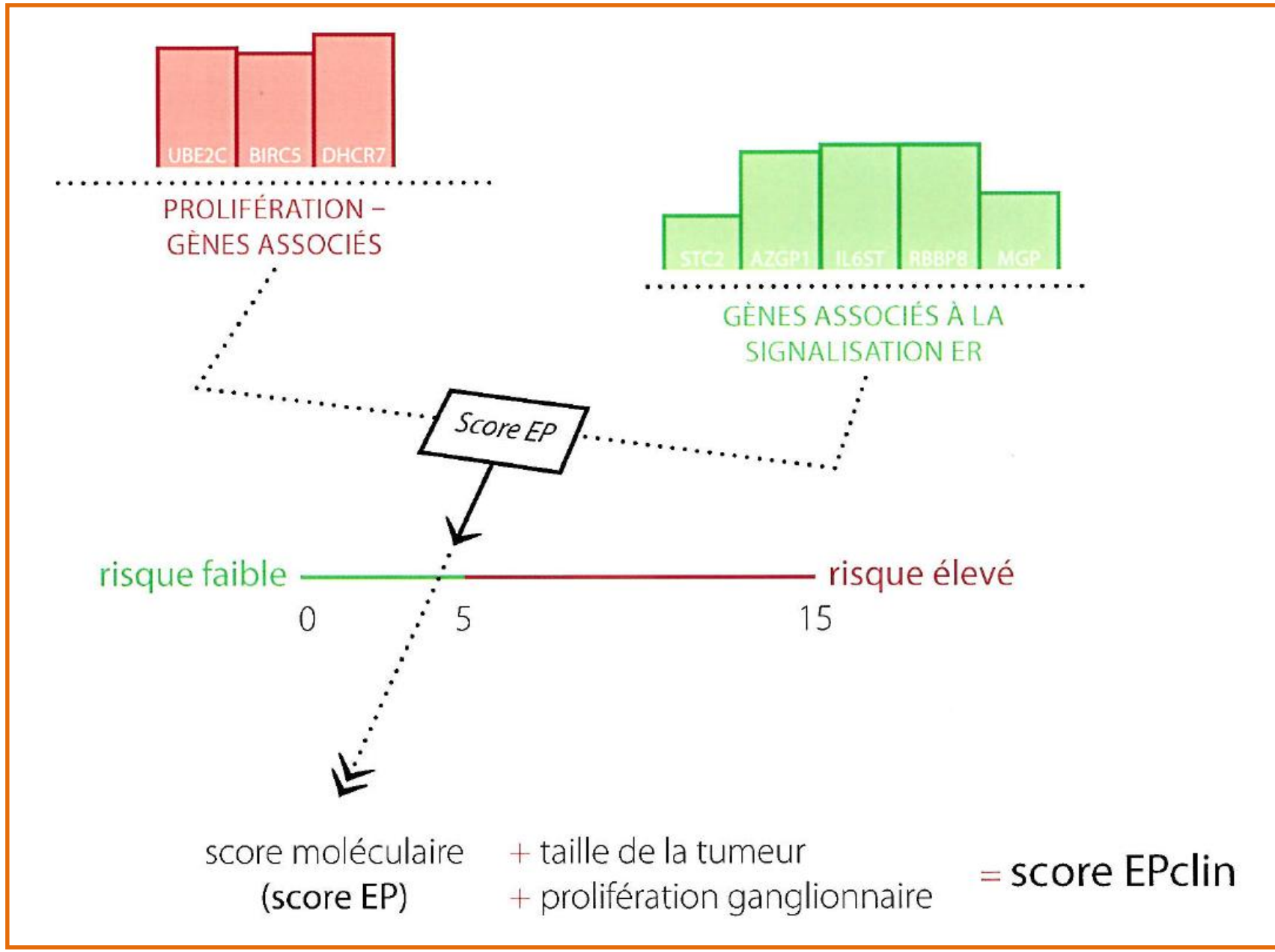


(Sparano JA et al, N Engl J Med 2018)

ENDOPREDICT : 8 GÈNES INFORMATIFS

Gène	Processus biologiques affectés
UBE2C	Dégradation des protéines, division cellulaire
BIRC5	Anti-apoptose, division cellulaire, cytokinèse, localisation chromosomique
DHCR7	Biosynthèse du cholestérol
STC2	Communication intercellulaire
AZGP1	Adhésion cellulaire
IL6ST	Diverses voies de transduction des signaux, prolifération cellulaire, prolifération des cellules T
RBBP8	Réparation de l'ADN
MGP	Régulation de la transcription

EndoPredict : le principe



Inclusion dans le score Epclin de critères clinico-pathologiques

Biologie de la tumeur
(Agressivité)

- **Test d'expression génomique (signature moléculaire)**

Masse tumorale
(Taille et dissémination)

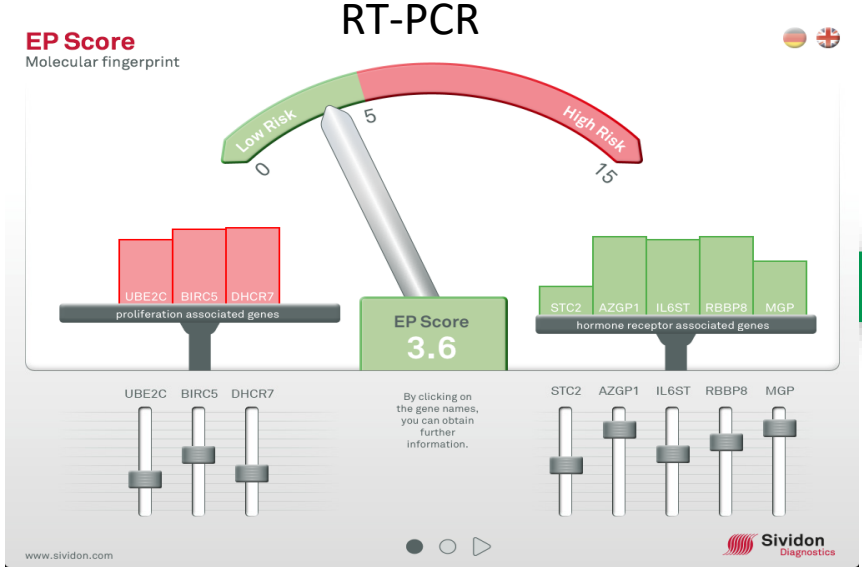
- **Taille de la tumeur (pT)**
- **Nombre de ganglions envahis (pN)**

Score Epclin
EndoPredict

Design d'EndoPredict
Epclin score : score moléculaire-clinico-pathologique

EP Score
(score moléculaire – 12 gènes)

RT-PCR

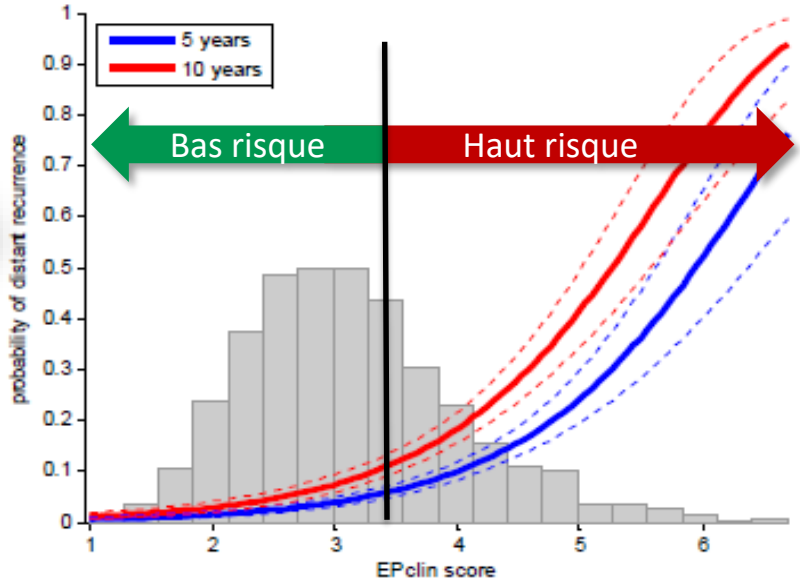


algorithme

(pN) + (pT)

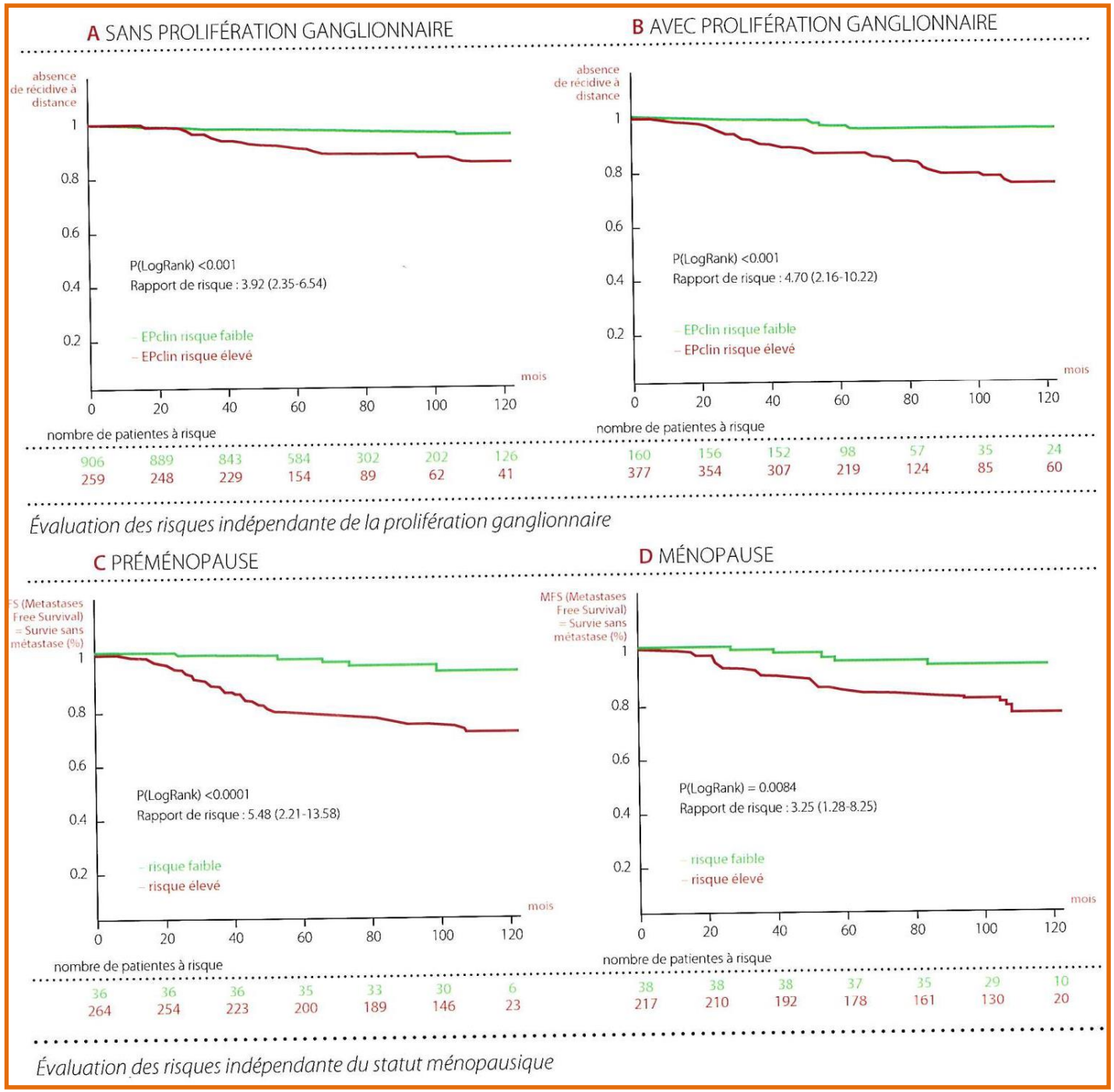
EPclin Score

Valeur du cut off
10% récidive à distance à 10 ans



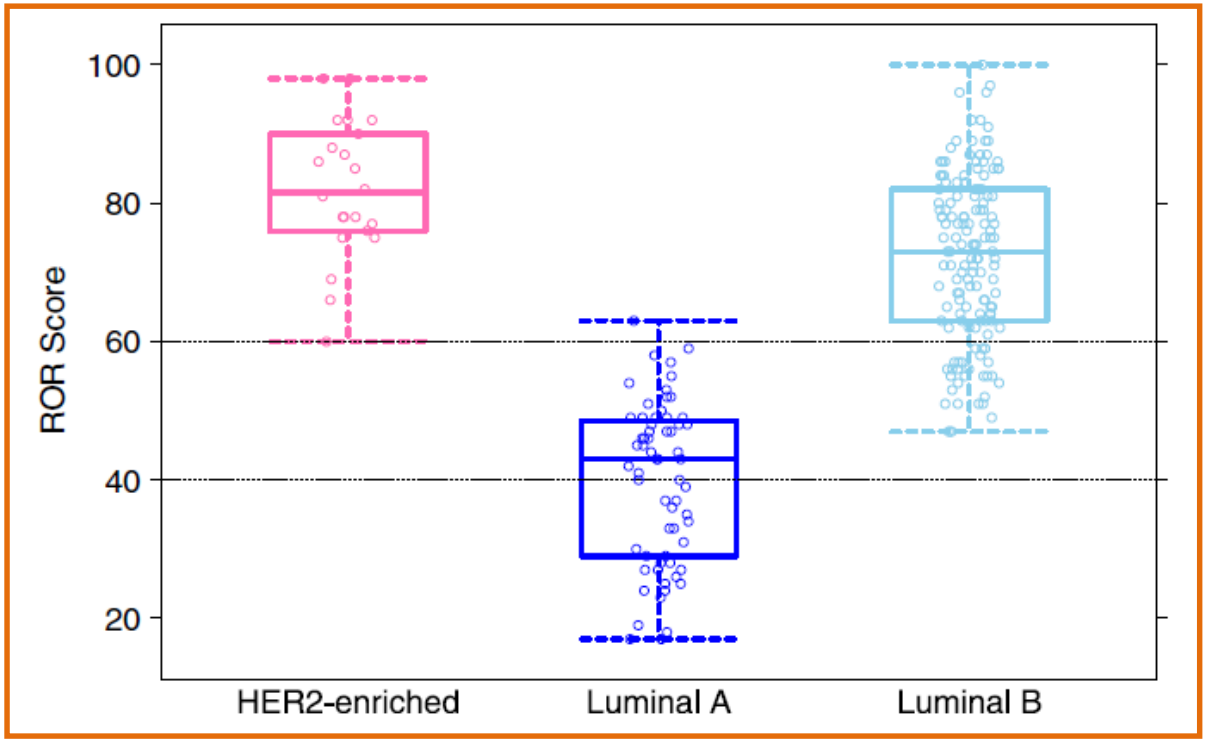
➔ 2 catégories de risque
➔ Valeur pronostique à 10 ans

EndoPredict : les performances pronostiques



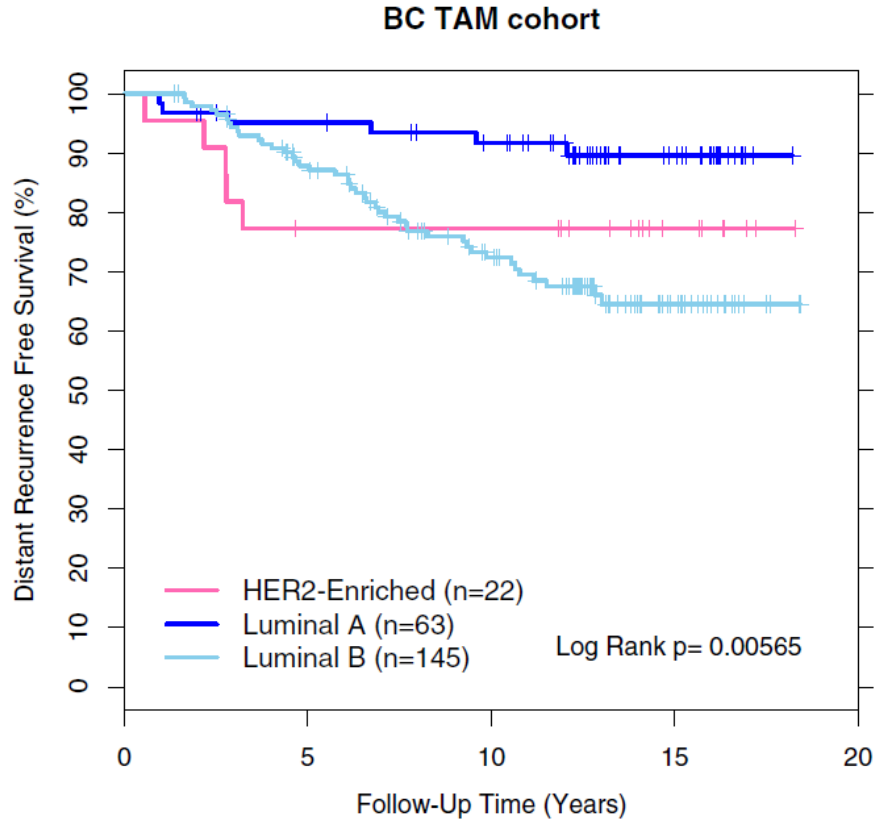
Valeur pronostique du PAM50 – Plateforme Nano String

Distribution du score selon les classes



Applicabilité : problème posé par une zone « grise », résultat sans décision en regard.

Confirmation valeur pronostique



(Wallden B et al, BMC Med Genomics 2015)

JAMA Oncology | Original Investigation

Comparison of the Performance of 6 Prognostic Signatures for Estrogen Receptor–Positive Breast Cancer
A Secondary Analysis of a Randomized Clinical Trial

Ivana Sestak, PhD; Richard Buus, PhD; Jack Cuzick, PhD; Peter Dubsky, MD; Ralf Kronenwett, MD; Carsten Denkert, MD; Sean Ferree, PhD; Dennis Sgroi, MD; Catherine Schnabel, PhD; Frederick L. Baehner, MD; Elizabeth Mallon, PhD; Mitch Dowsett, PhD

IMPORTANCE Multiple molecular signatures are available for managing estrogen receptor (ER)–positive breast cancer but with little direct comparative information to guide the patient's choice.

OBJECTIVE To conduct a within-patient comparison of the prognostic value of 6 multigene signatures in women with early ER-positive breast cancer who received endocrine therapy for 5 years.

DESIGN, SETTING, AND PARTICIPANTS This retrospective biomarker analysis included 774 postmenopausal women with ER-positive *ERBB2* (formerly *HER2*)–negative breast cancer. This analysis was performed as a preplanned secondary study of data from the Anastrozole or Tamoxifen Alone or Combined randomized clinical trial comparing 5-year treatment with anastrozole vs tamoxifen with 10-year follow-up data. The signatures included the Oncotype Dx recurrence score, PAM50-based Prosigna risk of recurrence (ROR), Breast Cancer Index (BCI), EndoPredict (EPclin), Clinical Treatment Score, and 4-marker immunohistochemical score. Data were collected from January 2009, through April 2015.

MAIN OUTCOMES AND MEASURES The primary objective was to compare the prognostic value of these signatures in addition to the Clinical Treatment Score (nodal status, tumor size, grade, age, and endocrine treatment) for distant recurrence for 0 to 10 years and 5 to 10 years after diagnosis. Likelihood ratio (LR) statistics were used with the χ^2 test and C indexes to assess the prognostic value of each signature.

RESULTS In this study of 774 postmenopausal women with ER-positive, *ERBB2*-negative disease (mean [SD] age, 64.1 [8.1] years), 591 (mean [SD] age, 63.4 [7.9] years) had node-negative disease. The signatures providing the most prognostic information were the ROR (hazard ratio [HR], 2.56; 95% CI, 1.96-3.35), followed by the BCI (HR, 2.46; 95% CI, 1.88-3.23) and EPclin (HR, 2.14; 95% CI, 1.71-2.68). Each provided significantly more information than the Clinical Treatment Score (HR, 1.99; 95% CI, 1.58-2.50), the recurrence score (HR, 1.69; 95% CI, 1.40-2.03), and the 4-marker immunohistochemical score (HR, 1.95; 95% CI, 1.55-2.45). Substantially less information was provided by all 6 molecular tests for the 183 patients with 1 to 3 positive nodes, but the BCI (ΔLR χ^2 = 9.2) and EPclin (ΔLR χ^2 = 7.4) provided more additional prognostic information than the other signatures.

CONCLUSIONS AND RELEVANCE For women with node-negative disease, the ROR, BCI, and EPclin were significantly more prognostic for overall and late distant recurrence. For women with 1 to 3 positive nodes, limited independent information was available from any test. These data might help oncologists and patients to choose the most appropriate test when considering chemotherapy use and/or extended endocrine therapy.

TRIAL REGISTRATION isrctn.com Identifier: ISRCTN18233230

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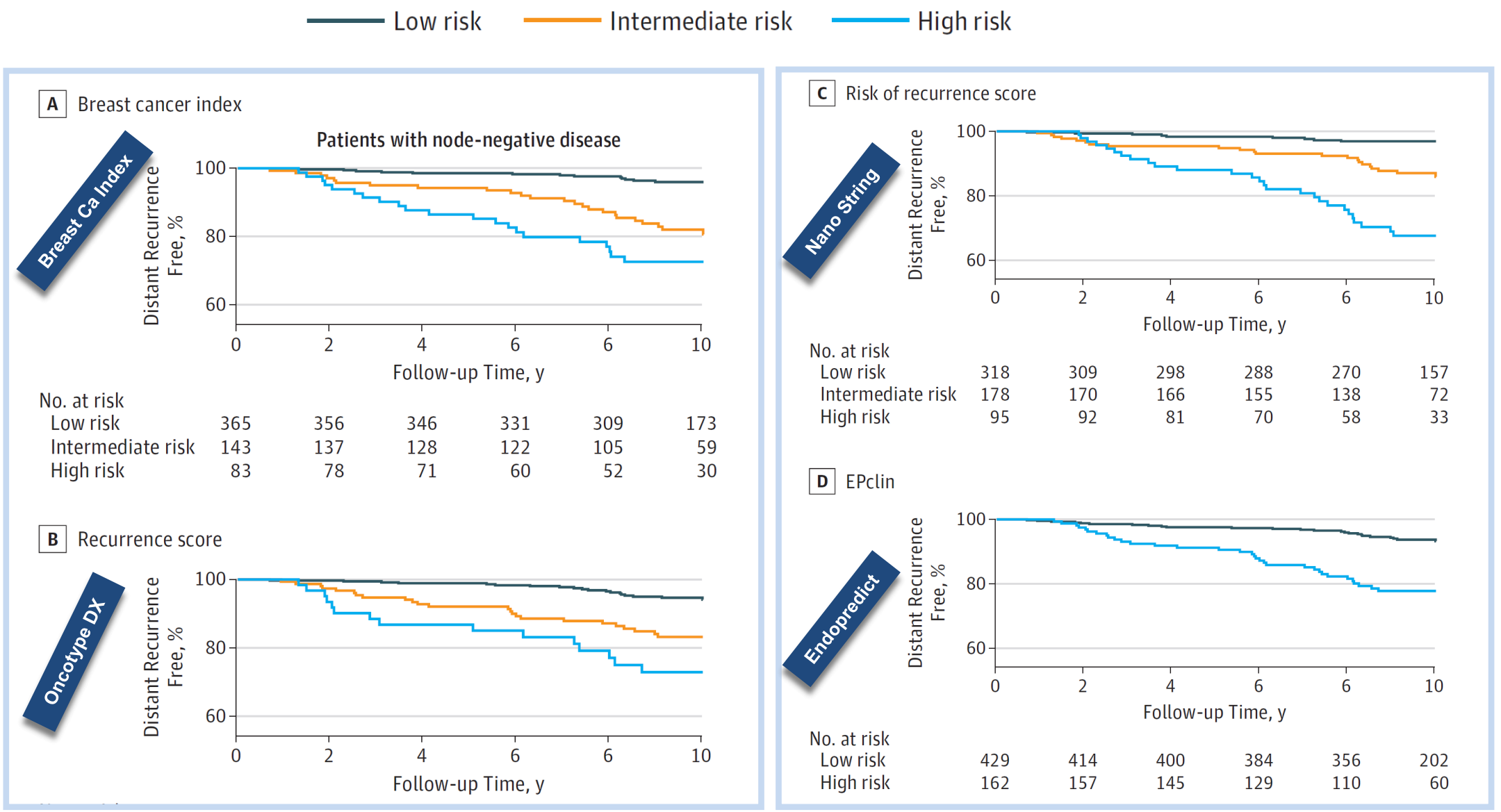
Supplemental content

Author Affiliations: Author affiliations are listed at the end of this article.

Corresponding Author: Ivana Sestak, PhD, Centre for Cancer Prevention, Wolfson Institute of Preventive Medicine, Queen Mary University London, Charterhouse Square, London, EC1M 6BQ, England (i.sestak@qmul.ac.uk).

Kaplan-Meier Curves for Recurrence During Years O to 10



(Sestak I, JAMA Oncol 2018)

Quel test choisir ?

- 4 tests ont une valeur pronostique élevée.
- Utilisation avant tout dans les N-.
- Les tests restent imparfaits, les catégories intermédiaires sont difficiles à utiliser.

- Tests décentralisés : maîtrise du pr+ocessus.
- Contrôle de qualité.
- Décision clinique avec l’ensemble des résultats.