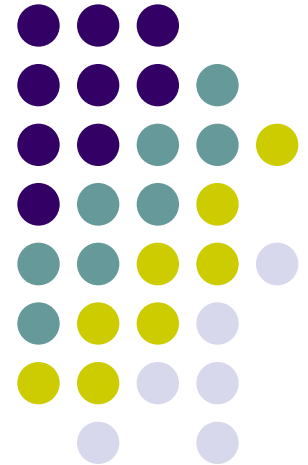# Translating Breast Cancer Genomics into Clinic

Prof. Miguel Martín
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Instituto de Investigación Sanitaria Gregorio Marañón

Disclosure

Dr. Martin has received speakers honoraria from Genomic Health, Nanostring, Agendia and Sividon and has participated in studies with Oncotype, Endopredict and Prosigna (PAM50). He is co-inventor in a PAM50-related patent.
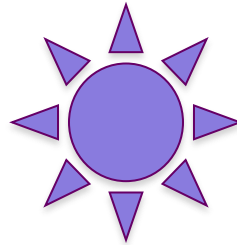
Genomic platforms: definition

- **Genomics** platforms are multigene profiles, based on DNA or RNA expression, aimed at prognosticating the outcome and/or predicting the response to systemic therapies

Genomic platforms: potential clinical applications in breast cancer
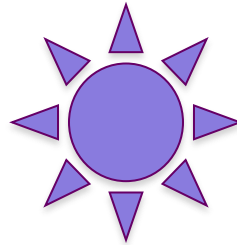
prognostication

Genomic platforms: potential clinical applications in breast cancer

prognostication
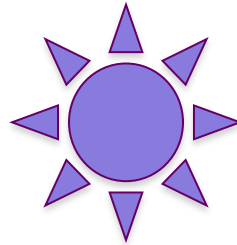
prediction of response to hormones

Genomic platforms: potential clinical applications in breast cancer

prognostication

prediction of response to hormones

prediction of response to chemotherapy

Genomic platforms: potential clinical applications in breast cancer

prognostication

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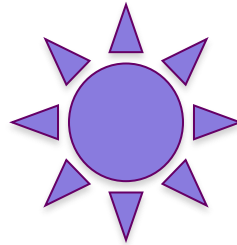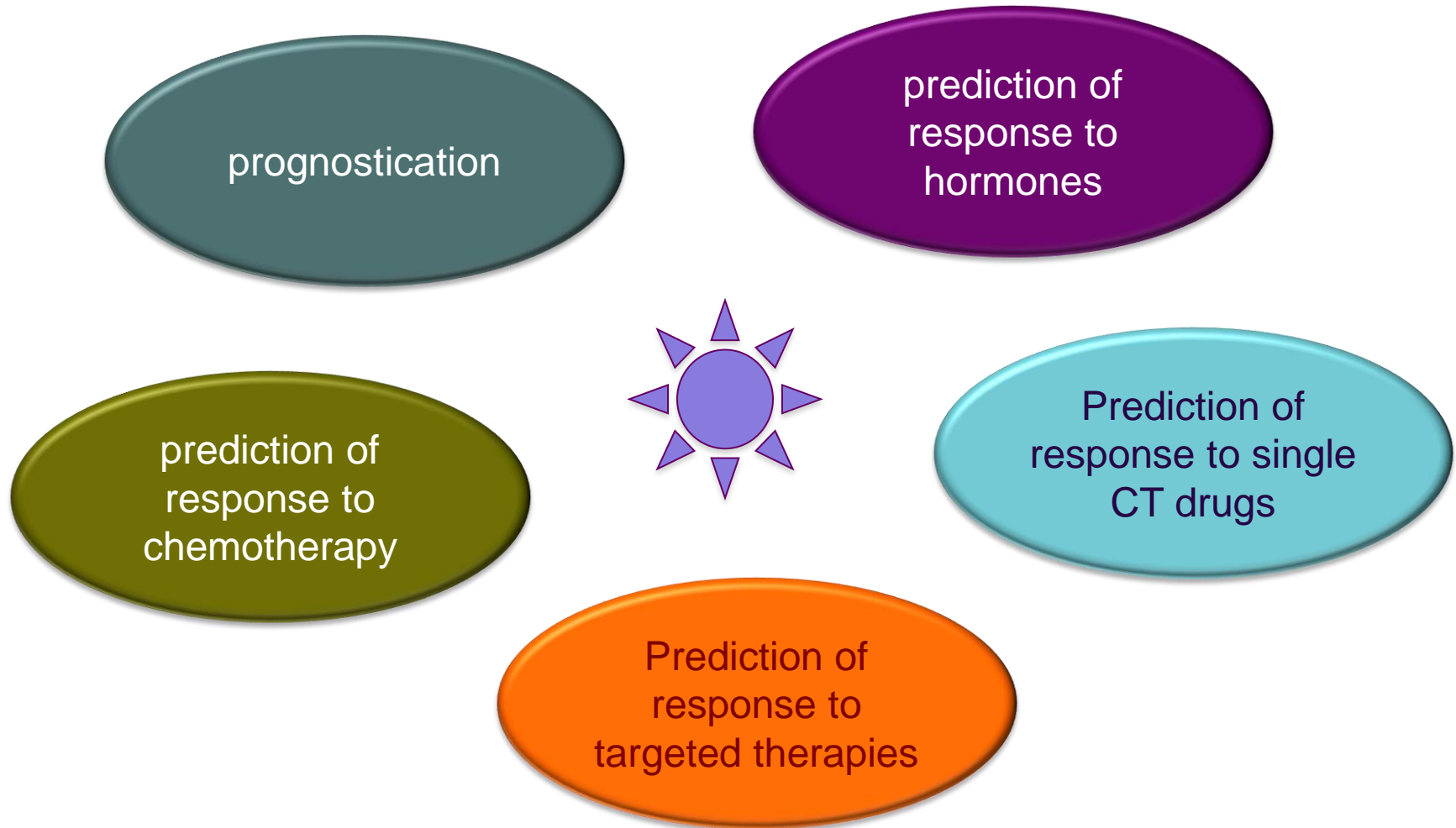Prediction of response to single CT drugs

Genomic platforms: potential clinical applications in breast cancer

prognostication

prediction of response to hormones

prediction of response to chemotherapy

Prediction of response to single CT drugs

Prediction of response to targeted therapies

Genomic platforms: potential clinical applications in breast cancer

prognostication

prediction of response to hormones

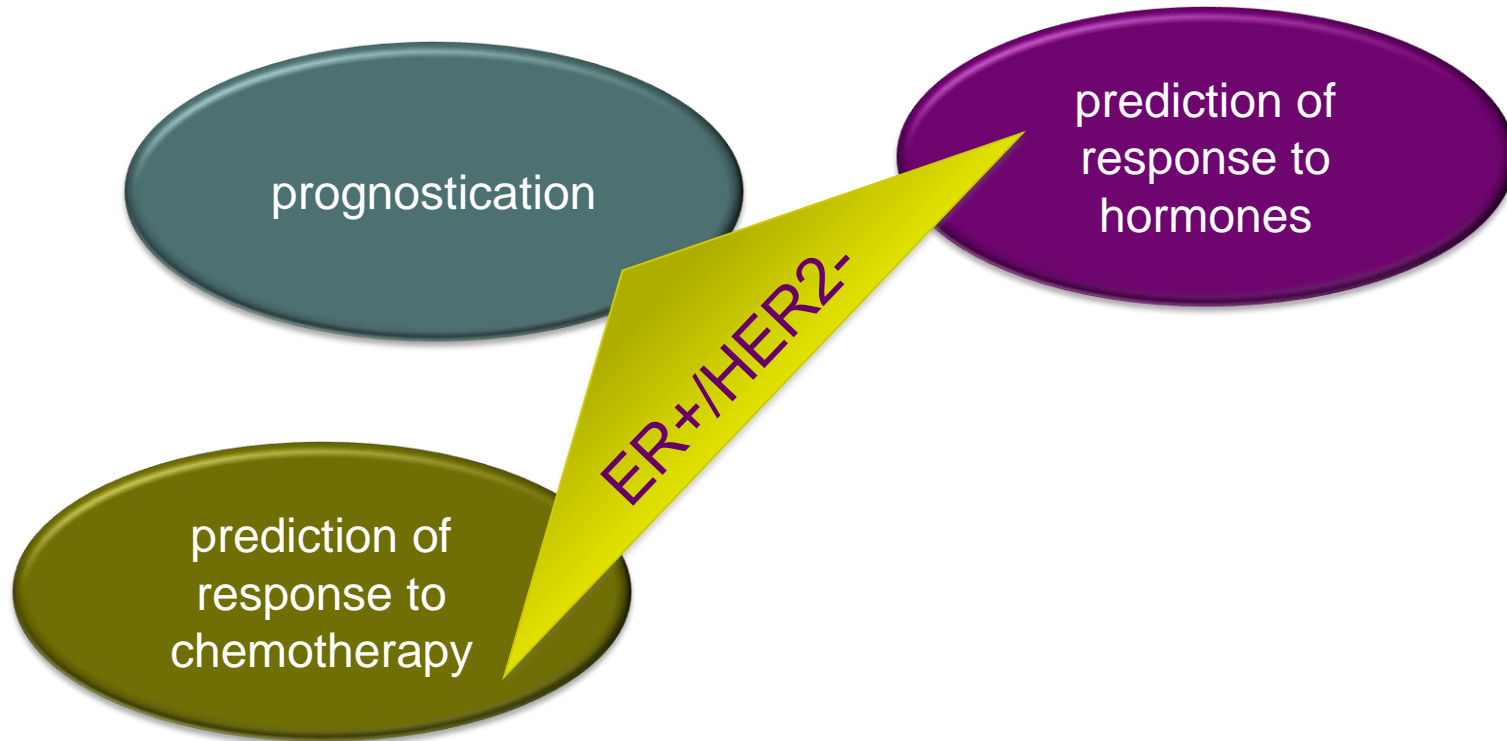prediction of response to chemotherapy

ER+/HER2-

Genomic platforms: potential clinical applications in breast cancer

prognostication

Making Predictions Less of an Art, More of a Science

- Analysts for … more than a dozen US government organizations depend on their ability to forecast national and global events to help ward off various threats to the country

- Old-style approaches can produce flawed results

- The effects (of deliberation) have led analysts to predict **events than didn´t occur, or miss events that did take place**

Pitfalls of old prognostication tools in breast cancer

- **Overtreatment** (low accuracy in identifying patients that actually do not benefit from chemotherapy)

- **Undertreatment** (insuficient accuracy in identifying patients with apparent low risk that actually benefit from chemotherapy)

Pitfalls of immunohystochemistry techniques

- Different antibodies
- Non-automaticized techniques
  - tissue sample fixation
  - deparaffinization
  - antigen retrieval
  - antibody staining
- Semiquantitative results
- Artificial cut-offs of positivity (i.e. ER, Ki67)
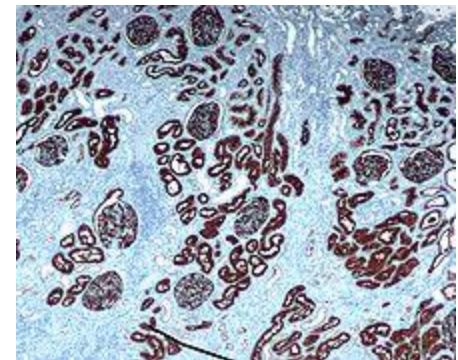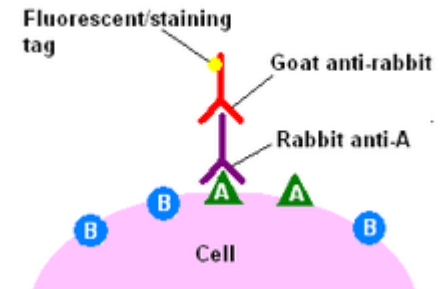
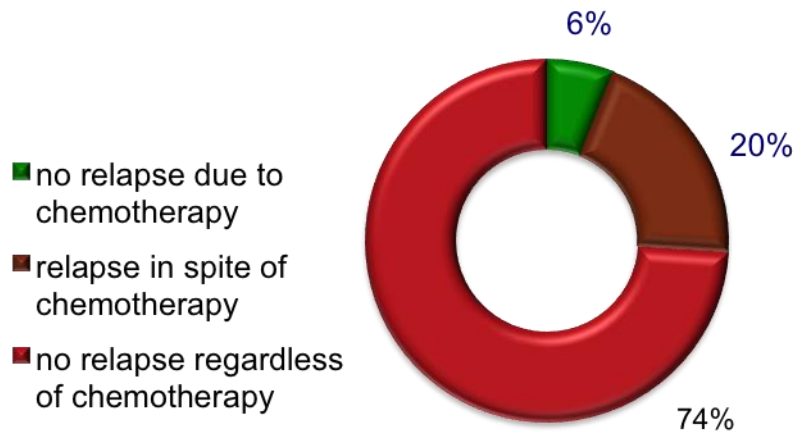# Absolute Benefit for Tamoxifen plus Chemotherapy vs Tamoxifen (5-year Recurrence Rate) in ER+ brast cancer*

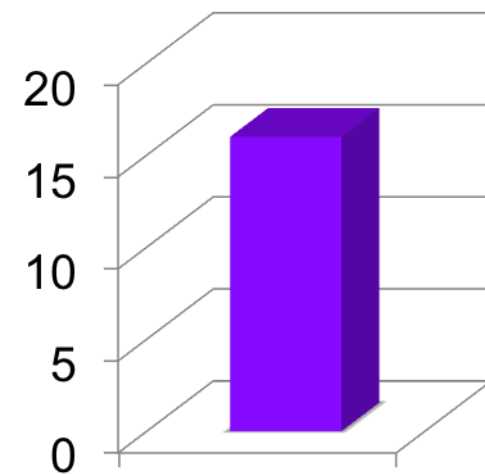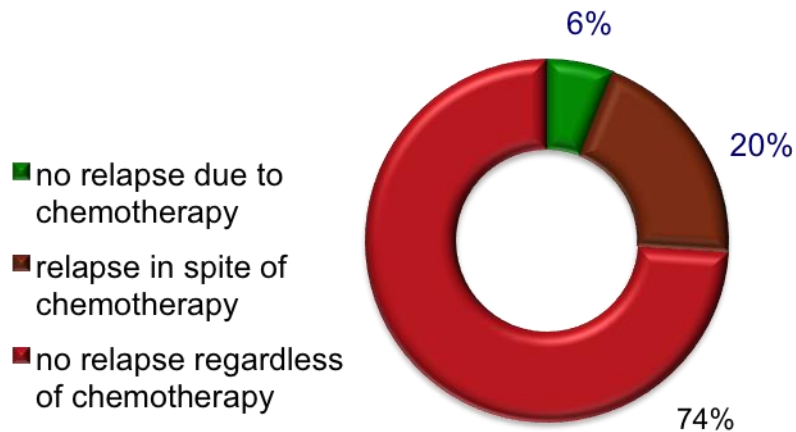| ER/N Status	Age	Comparison	Recurrence Endpoint	Absolute increase
ER+ (88%) or unknown N+ 73%	50-69	TAM alone vs TAM + CT	28.9% vs 24%	**4.9%**
ER+ (87%) or unknown N+ 34%	<50	TAM alone vs TAM + CT	21.6% vs 14%.	**7.6%**

*including HER2+ tumors

Absolute Benefit for Tamoxifen plus Chemotherapy vs Tamoxifen (5-year Recurrence Rate) in ER+ brast cancer*

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*including HER2+ tumors

- ■ no relapse due to chemotherapy
- ■ relapse in spite of chemotherapy
- ■ no relapse regardless of chemotherapy

6%

20%

74%

Absolute Benefit for Tamoxifen plus Chemotherapy vs Tamoxifen (5-year Recurrence Rate) in ER+ brast cancer*

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- ■ relapse in spite of chemotherapy
- ■ no relapse regardless of chemotherapy

6%
20%
74%

NNT to avoid a relapse by adding CT to TAM

Prognostication in early breast cancer

- We need better tools for prognostication of the risk of relapse

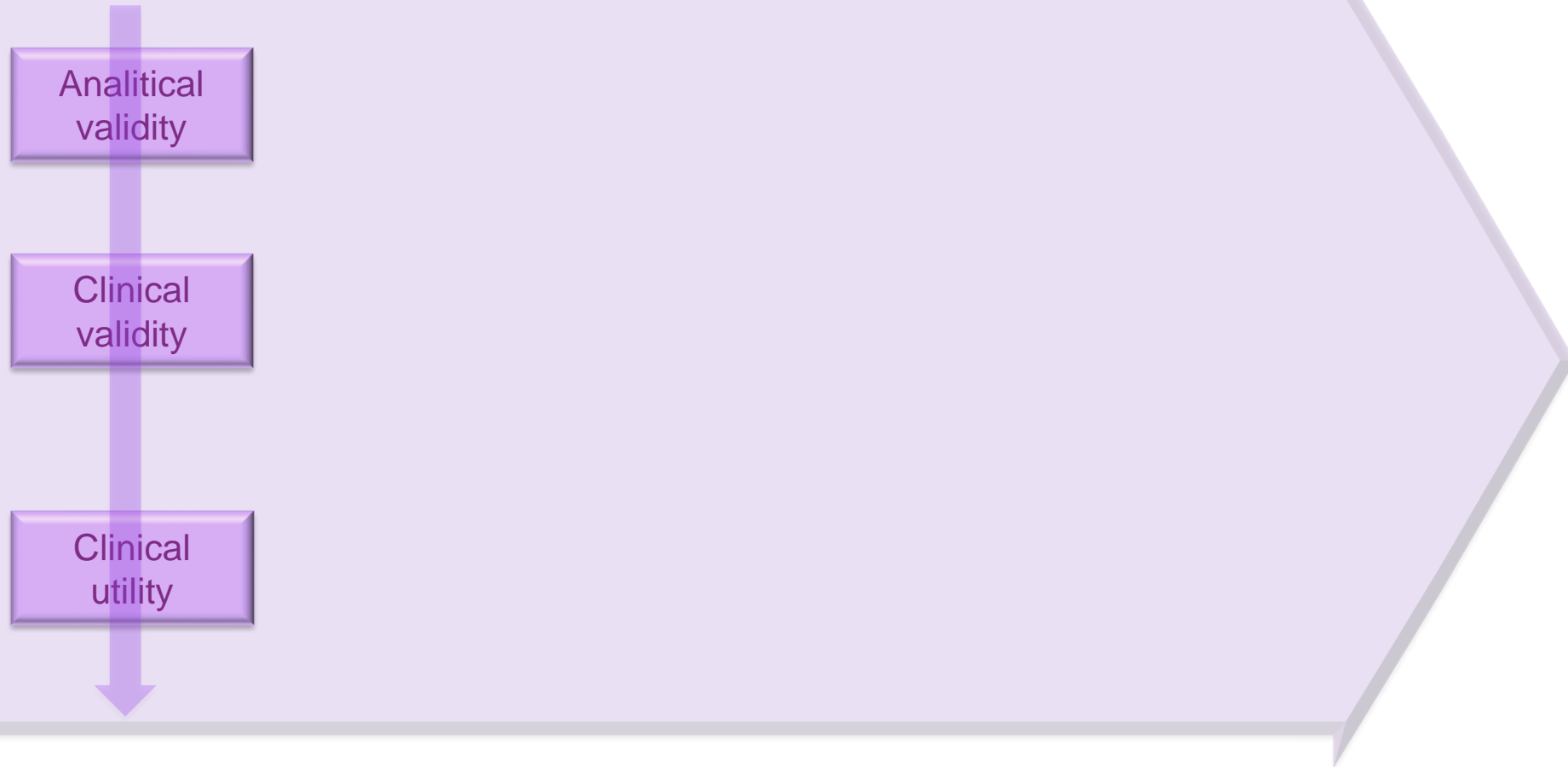- Can genomic mRNA-based test help stablishing a better therapeutic strategy in prognostication?

Fisrt/Second Generation Genomic Platforms

Evaluation and aims of genomic platforms

Analitical validity

Clinical validity

Clinical utility

Evaluation and aims of genomic platforms

Analitical validity

test's ability to accurately and reliably measure the genotype of interest

Clinical validity

test's ability to accurately and reliably predict a survival end point 5–10 years

Clinical utility

Improvement in measurable clinical outcomes and added value in clinical management and decision making compared with standard criteria

Evaluation and aims of genomic platforms

Analitical validity — test's ability to accurately and reliably measure the genotype of interest

Clinical validity — test's ability to accurately and reliably predict a survival end point at 5–10 years

Clinical utility — Improvement in measurable clinical outcomes and added value in clinical management and decision making compared with standard criteria

Evaluation and aims of genomic platforms

Analitical validity — test's ability to accurately and reliably measure the genotype of interest

Clinical validity — test's ability to accurately and reliably predict a survival end point at 5–10 years

Clinical utility — improvement in measurable clinical outcomes and added value in clinical management and decision making compared with standard criteria

Utility of prognostic genomic tests in breast cancer practice: The IMPAKT 2012 Working Group Consensus Statement[†]

- The IMPAKT 2012 Working Group proposed the following recommendations:

  - (i) a need to develop models that integrate clinicopathologic factors along with genomic tests

  - (ii) the creation of registries for patients who are subjected to genomic testing in the daily practice

  - **(iii) demonstration of clinical utility should be made in the context of a prospective randomized trial**

Use of Archived Specimens in Evaluation of Prognostic and Predictive Biomarkers

Richard M. Simon, Soonmyung Paik, Daniel F. Hayes

Table 1. Elements of tumor marker studies that constitute Levels of Evidence determination*

Category Element	A Prospective	B Prospective using archived samples	C Prospective/ observational	D Retrospective/ observational
Clinical trial	PCT designed to address tumor marker	Prospective trial not designed to address tumor marker, but design accommodates tumor marker utility	Prospective observational registry, treatment and follow-up not dictated	No prospective aspect to study
		Accommodation of predictive marker requires PRCT		
Patients and patient data	Prospectively enrolled, treated, and followed in PCT	Prospectively enrolled, treated, and followed in clinical trial and, especially if a predictive utility is considered, a PRCT addressing the treatment of interest	Prospectively enrolled in registry, but treatment and follow-up standard of care	No prospective stipulation of treatment or follow-up; patient data collected by retrospective chart review
Specimen collection, processing, and archival	Specimens collected, processed, and assayed for specific marker in real time	Specimens collected, processed, and archived prospectively using generic SOPs. Assayed after trial completion	Specimens collected, processed, and archived prospectively using generic SOPs. Assayed after trial completion	Specimens collected, processed and archived with no prospective SOPs
Statistical design and analysis	Study powered to address tumor marker question	Study powered to address therapeutic question and underpowered to address tumor marker question	Study not prospectively powered at all. Retrospective study design confounded by selection of specimens for study	Study not prospectively powered at all. Retrospective study design confounded by selection of specimens for study
		Focused analysis plan for marker question developed before doing assays	Focused analysis plan for marker question developed before doing assays	No focused analysis plan for marker question developed before doing assays
Validation	Result unlikely to be play of chance	Result more likely to be play of chance that A but less likely than C	Result very likely to be play of chance	Result very likely to be play of chance
	Although preferred, validation not required	Requires one or more validation studies	Requires subsequent validation studies	Requires subsequent validation

* PCT = prospective controlled trial; PRCT = prospective randomized controlled trial; SOPs = standard operating practices.

Use of Archived Specimens in Evaluation of Prognostic and Predictive Biomarkers

Richard M. Simon, Soonmyung Paik, Daniel F. Hayes

Table 2. Revised determination of Levels of Evidence using elements of tumor marker studies*

Level of evidence	Category from Table 1	Validation studies available
I	A	None required
I	B	One or more with consistent results
II	B	None or inconsistent results
II	C	2 or more with consistent results
III	C	None or 1 with consistent results or inconsistent results
IV–V	D	NA†

* Levels of Evidence (LOEs) revised from those originally proposed by Hayes et al. (3).

† NA = not applicable because LOE IV and V studies will never be satisfactory for determination of medical utility.

Onco*type* Dx: 21-gene recurrence score (ER+ tumors)

16 Cancer and 5 Reference Genes

PROLIFERATION
Ki-67
STK15
Survivin
Cyclin B1
MYBL2

ESTROGEN
ER
PR
Bcl2
SCUBE2

HER2
GRB7
HER2

REFERENCE
Beta-actin
GAPDH
RPLPO
GUS
TFRC

BAG1

GSTM1

INVASION
Stromolysin 3
Cathepsin L2

CD68

• Best RT-PCR performance and most robust predictions
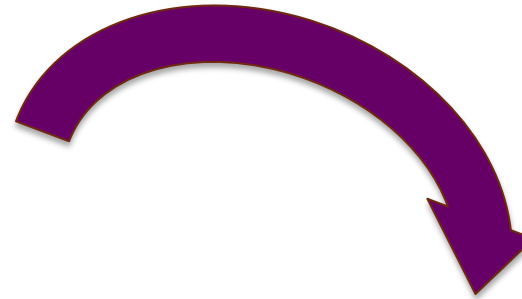
Recurrence Score =
+ 0.47 × HER2 Group Score
– 0.34 × Estrogen Group Score
+ 1.04 × Proliferation Group Score
+ 0.10 × Invasion Group Score
+ 0.05 × CD68
– 0.08 × GSTM1
– 0.07 × BAG1

The Onco*type* Dx® recurrence score is a continuous predictor of recurrence risk

Risk group	Recurrence Score
Low risk	< 18
Intermediate risk	18 - 30
High risk	≥ 31

Onco*type* DX® Clinical Validation:
B-14 Results – Distant Recurrence

Proportion without Distant Recurrence (y-axis)

Years (x-axis)

P <0.001

– 51% of population fell into the low-risk group
– 22% fell into the intermediate-risk group
– 27% fell into the high-risk group

RS <18 n = 338

RS 18-30 n = 149

RS ≥31 n = 181

Onco*type* DX® Clinical Validation: B-14 Results – Distant Recurrence

Chart: Proportion without Distant Recurrence (y-axis, 0%–100%) vs Years (x-axis, 0–16)

- RS <18 n = 338 (green)
- RS 18-30 n = 149 (orange)
- RS ≥31 n = 181 (pink)

P <0.001

Variable	Hazard Ratio	95% CI	*P* value
Age ≥50 years	0.71	(0.48, 1.05)	0.084
Size >2.0 cm	1.26	(0.86, 1.85)	0.231
Recurrence Score	3.21	(2.23, 4.61)	<0.001

Paik et al. N Engl J Med. 2004;351:2817-2826.

Mammaprint:
a 70-gene expression profile platfom

78 breast tumors
Age < 55 years, Tumor size < 5 cm
Lymph node negative & No adjuvant therapy

Distant metastases
within 5 years

No distant metastases
for at least 5 years

CLASSIFICATION THRESHOLD

**Low Risk
Signature**

**High Risk
Signature**

van 't Veer L et al., Nature, 2002; Van de Vijver M et al; N Engl J Med 347:1999,2002

Mammaprint:
a 70-gene expression profile platfom

78 breast tumors
Age < 55 years, Tumor size < 5 cm
Lymph node negative & No adjuvant therapy

Distant metastases within 5 years

No distant metastases for at least 5 years

Low Risk Signature

CLASSIFICATION THRESHOLD

High Risk Signature

A Gene-Expression Profiling

Good signature

Poor signature

P<0.001

Probability of Remaining Metastasis-free

Years

van 't Veer L et al., Nature, 2002; Van de Vijver M et al; N Engl J Med 347:1999,2002

Mammaprint: TRANSBIG Validation Results
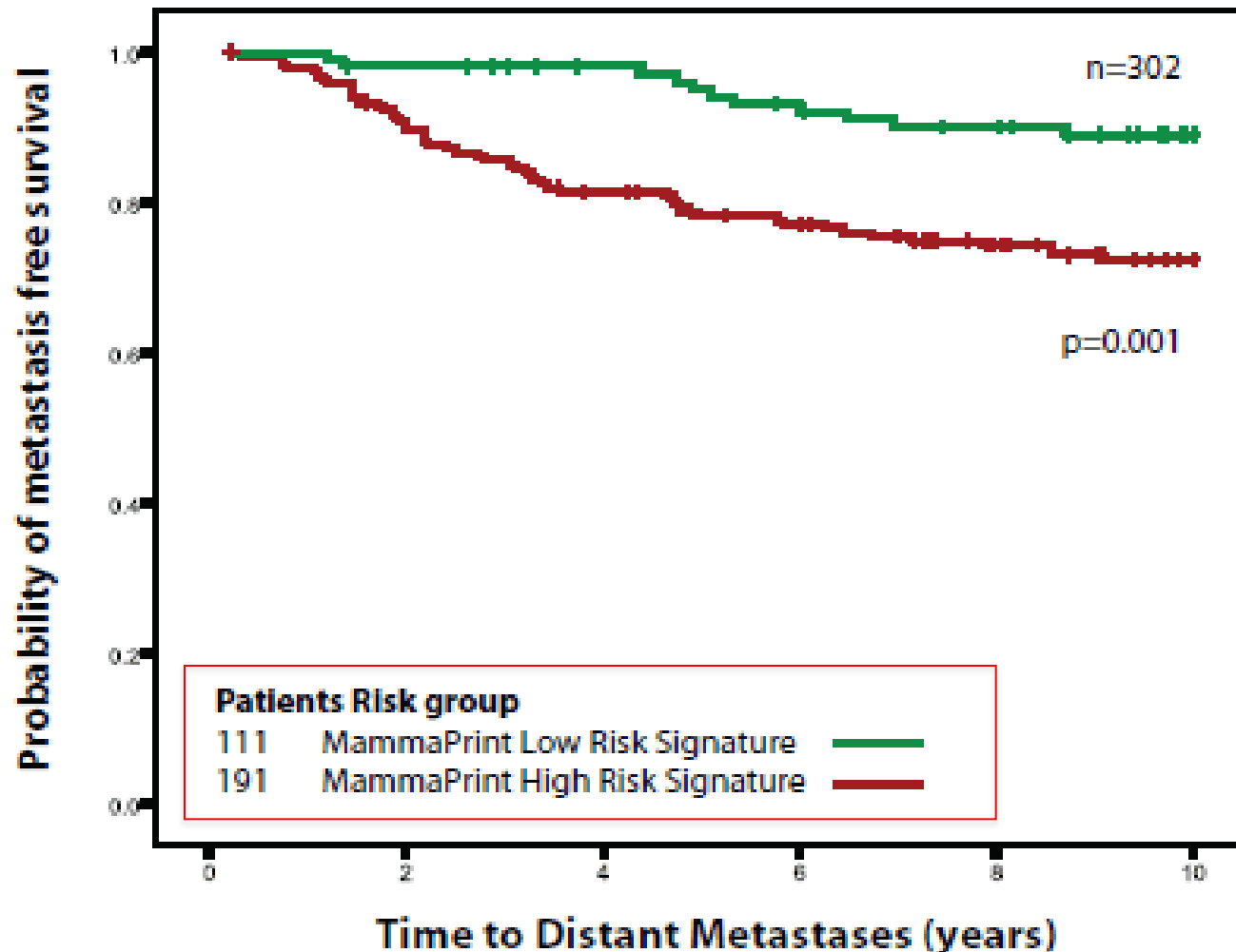
EndoPredict (Sividon Diagnostics)

- Decentralized test, currently, performed in 16 molecular labs in Germany, Switzerland and Austria

- 12 genes: 8 genes-of-interest, 3 normalization genes, 1 DNA control gene

- Two risk groups (low vs. high), no intermediate risk

- CE-IVD marks received as medical device
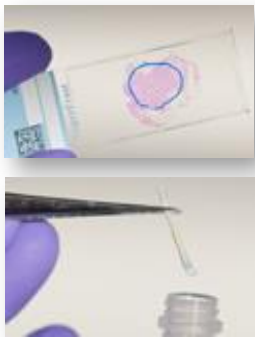
| Tumor sample | RNA isolation | EndoPredict-Test | Test result |
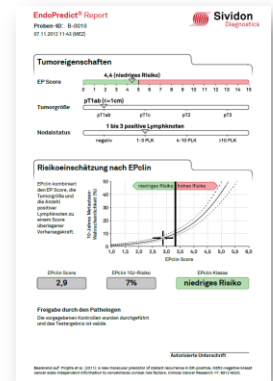
Turn-around-Time < 8 h

EndoPredict Report
Concise report showing relevant data

EP-Score
„molecular fingerprint"

+

Clinical-pathological parameters

tumor size + nodal status

=

EPclin-Score
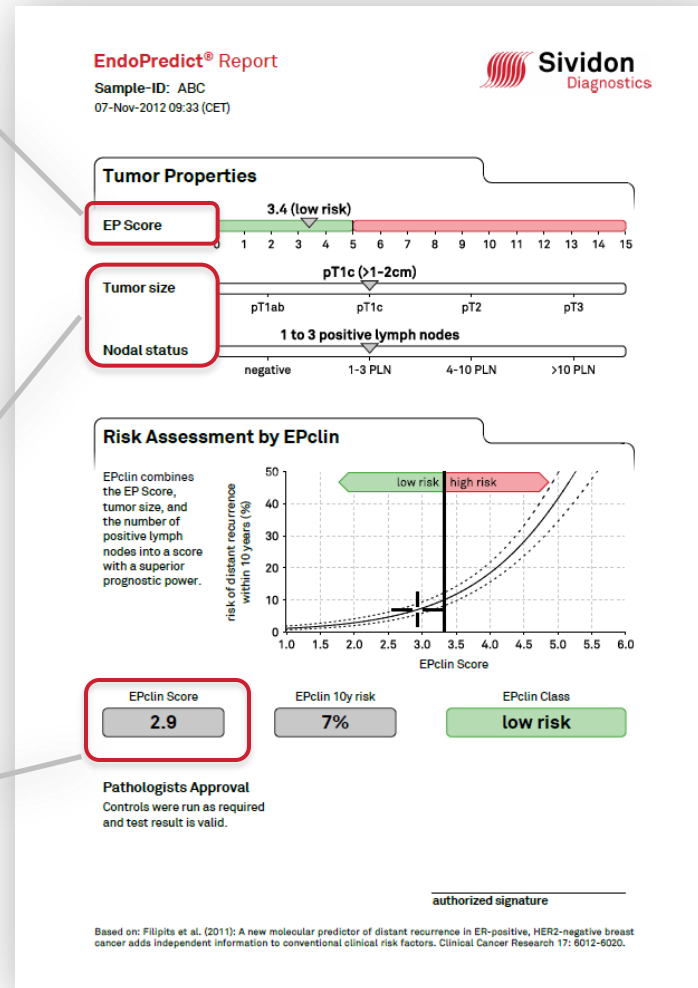
Scientific Validity of EndoPredict
Clinical validation trials

Endocrine therapy only   Adj. chemotherapy

Training

Multicenter
Tam Monotherapy
(n=964)

Validation I

ABCSG-6
TAM (n=378)

Validation II

ABCSG-8
TAM vs. TAM/Anast.
(n=1.324)

Validation III

GEICAM 9906
FEC vs. FEC-T
(n=555)
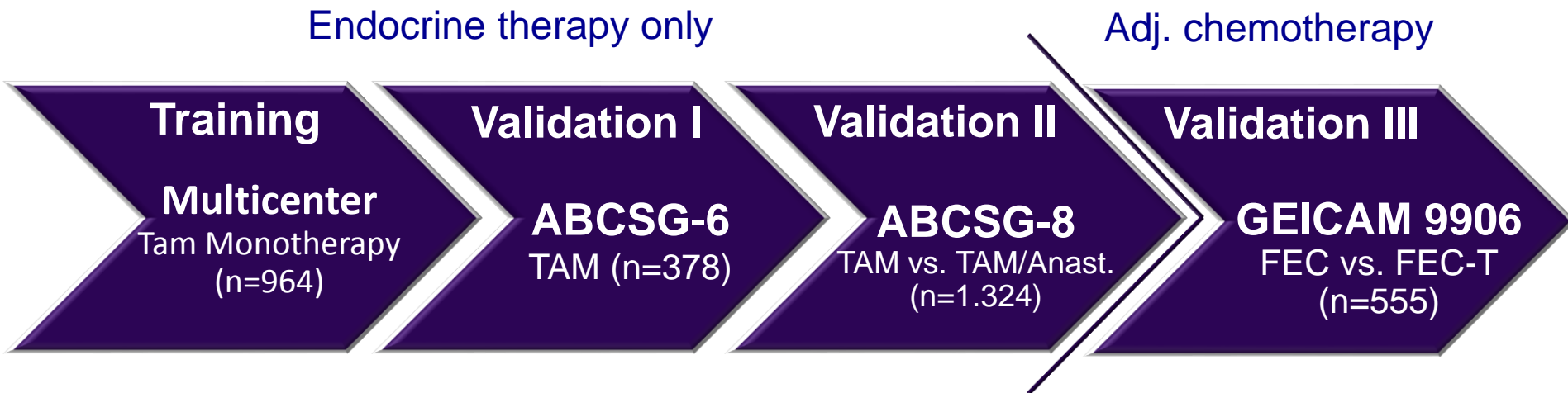
- **Clinically validated in two independent cohorts from two randomized clinical trials in 1.702 samples (ER+, HER2 neg.)**

- **Level of evidence of Ib according to Simon et al. (JNCI 2009)**

- **Successful validation in one further cohort from a randomized chemotherapy trial in 555 samples (ER+, HER2 neg.)**

Filipits et al. 2011; Dubsky et al. 2012; Martin et al. (SABCS 2012)

Clinical Validation
Validated for node positive and negative patients

Node negative — absence of distant recurrence vs months. P(LogRank)<0.001. EPclin low risk (green), EPclin high risk (red).
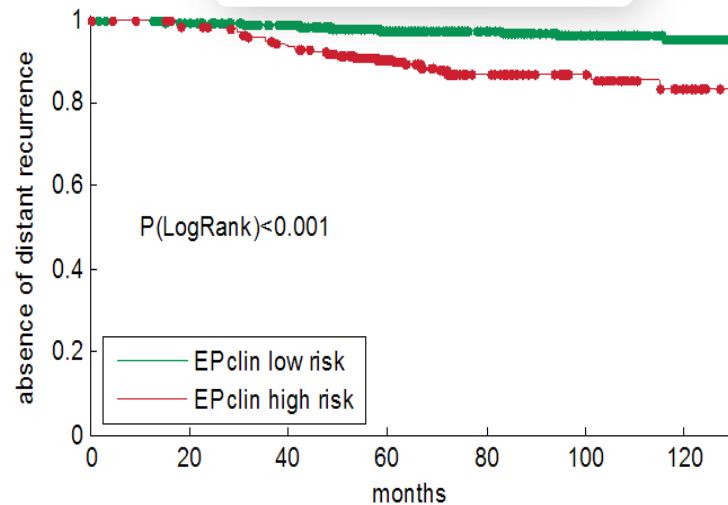
numbers at risk:
906	889	843	584	302	202	126
259	248	229	154	89	62	41

Node positive — absence of distant recurrence vs months. P(LogRank)<0.001. EPclin low risk (green), EPclin high risk (red).
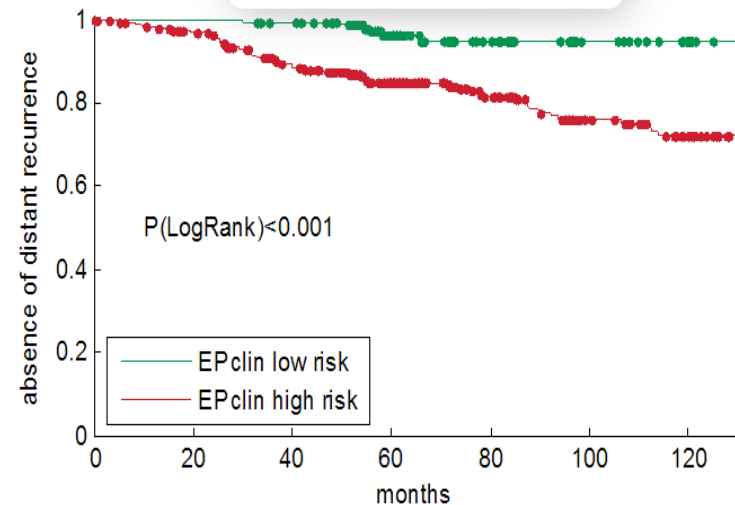
numbers at risk:
160	156	152	98	57	35	24
377	354	307	219	124	85	60

Filipits et al. Clin Cancer Res (2011)

Clinical validation of the EndoPredict test in node-positive, chemotherapy-treated ER+/HER2− breast cancer patients: results from the GEICAM 9906 trial
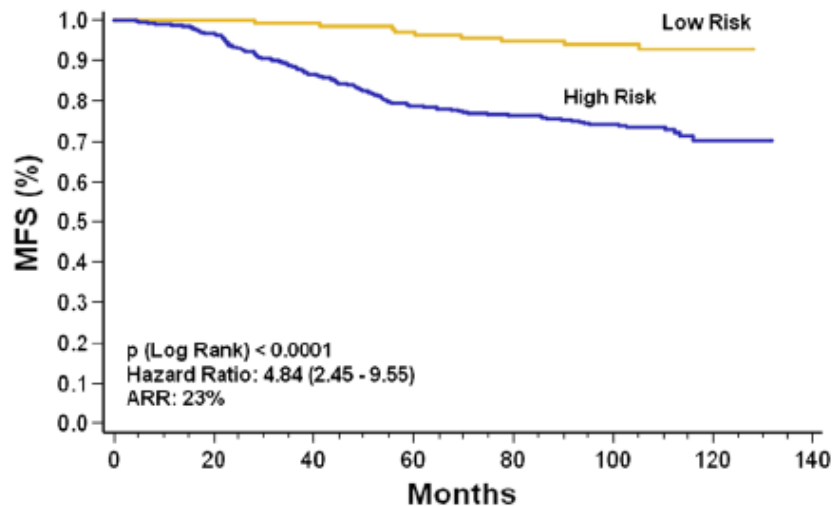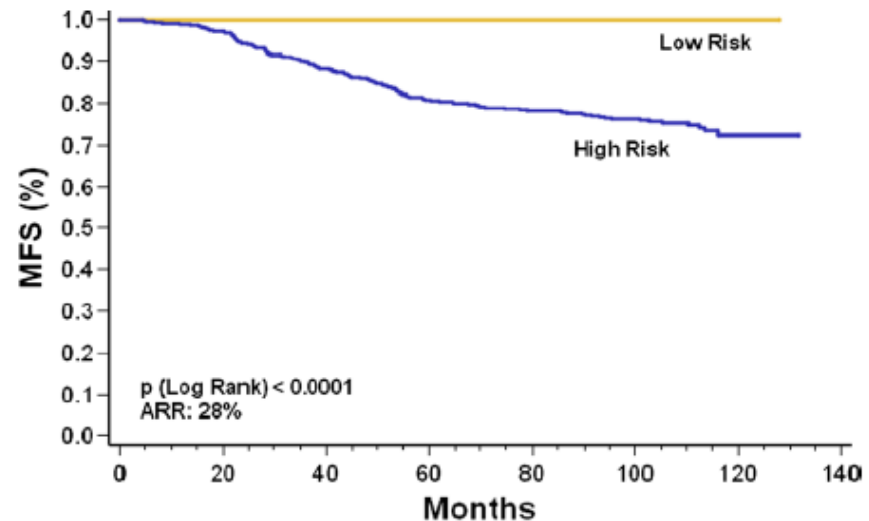
Figure 1 Kaplan-Meier metastasis-free survival curves for ER+/HER2− breast cancers. (A) Curves representing EndoPredict (EP) test results indicating estimated high and low risk of metastasis-free survival (MFS). The cutoff point was prespecified at 5. (B) Curves representing EPclin results indicating estimated high and low risk of MFS. The cutoff point was prespecified at 3.3. Numbers in parentheses indicate the 95% confidence intervals of the hazard ratios. ARR: Absolute risk reduction estimated at 10 years; ER+/HER2−: Estrogen receptor–positive/human epidermal growth factor receptor 2–negative. The MFS in the EP score–based low-risk category was 93% vs 70% in the EP score–based high-risk group. The MFS in the EPclin-based low-risk category was 100% vs 72% in the EPclin score–based high-risk group.

Prosigna (PAM50/nCOUNTER)

- 50-gene platform designed to identify breast cancer subtypes (LumA, LumB, Basal-like, HER2-E)
- Provides a ROR score (and ROR-C score) and 3 categories of risk
- Designed to be performed in local laboratories (nCOUNTER)

Extract RNA from FFPE tumor sample

Run RNA & PAM50 CodeSet on nCounter Analysis System

Capture patient expression profile
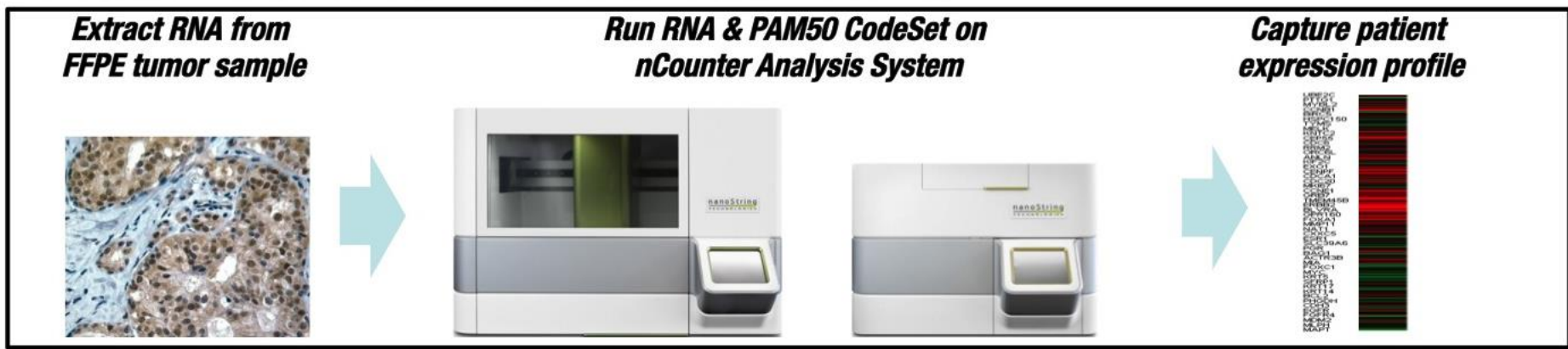
PAM50/nCOUNTER (Prosigna)

Analytical Validation

- **Reproducibility from tissue**
  - Multiple pathologists review tissue
  - Multiple techs processing tissue
  - Multiple lots of RNA extraction kits
- **Precision from RNA**
  - Multiple sites and operators
  - Multiple instruments
  - Multiple reagent lots

Analysis presented at USCAP March 2013

Clinical Validation

- **TransATAC**
  - 1007 patients from the ATAC trial
  - 10yr median follow up
  - Includes direct comparison to Oncotype Dx
- **ABCSG-8**
  - 1478 patients from study 8 re-consented
  - Tissue shipped to testing lab

- Shown to provide more prognostic information than RS and to categorize fewer patients as intermediate risk than RS in the transATAC population

- Validated as predicting prognosis more accurately than and beyond clinicopathological factors in ABCSG-8

- **Level of evidence of Ib according to Simon et al. (JNCI 2009)**

Parker et al, JCO 2009; Nielsen et al CCR 2010; Gnant et al, SABCS 2012; Cuzixk et al, ESMO 2012

Prosigna

Clinical Validation of the PAM50 Risk of Recurrence (ROR) score in ABCSG-8
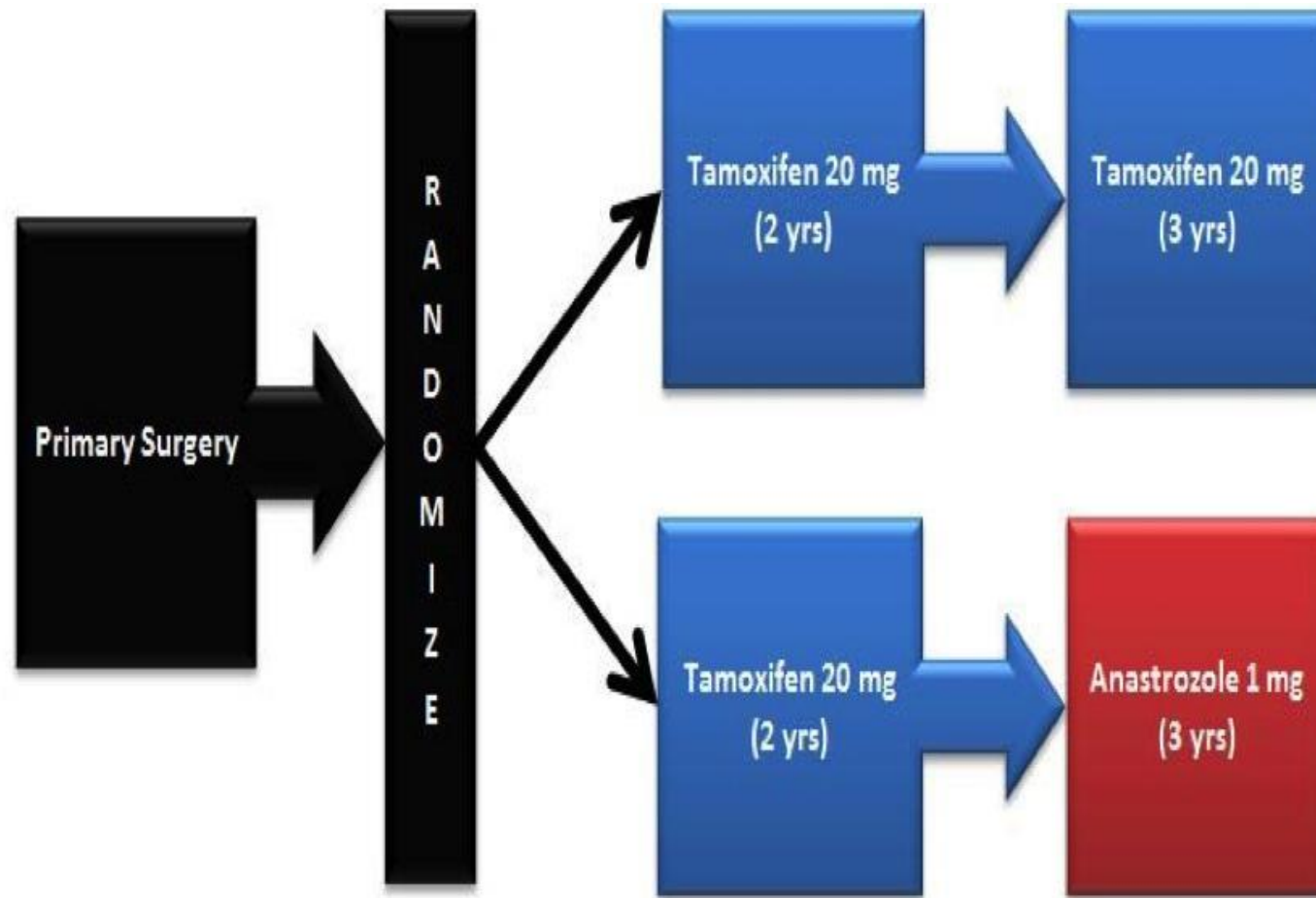
ROR Defined Risk Groups have statistically significant different outcomes at 10 years

ABCSG-8 trial

Low
Intermediate
High

96.7% (94.6-98.0)

91.3% (88.1-93.8)

79.9% (75.7-83.4)

Probability of distant recurrence-free survival (%)

Follow-up time (years)
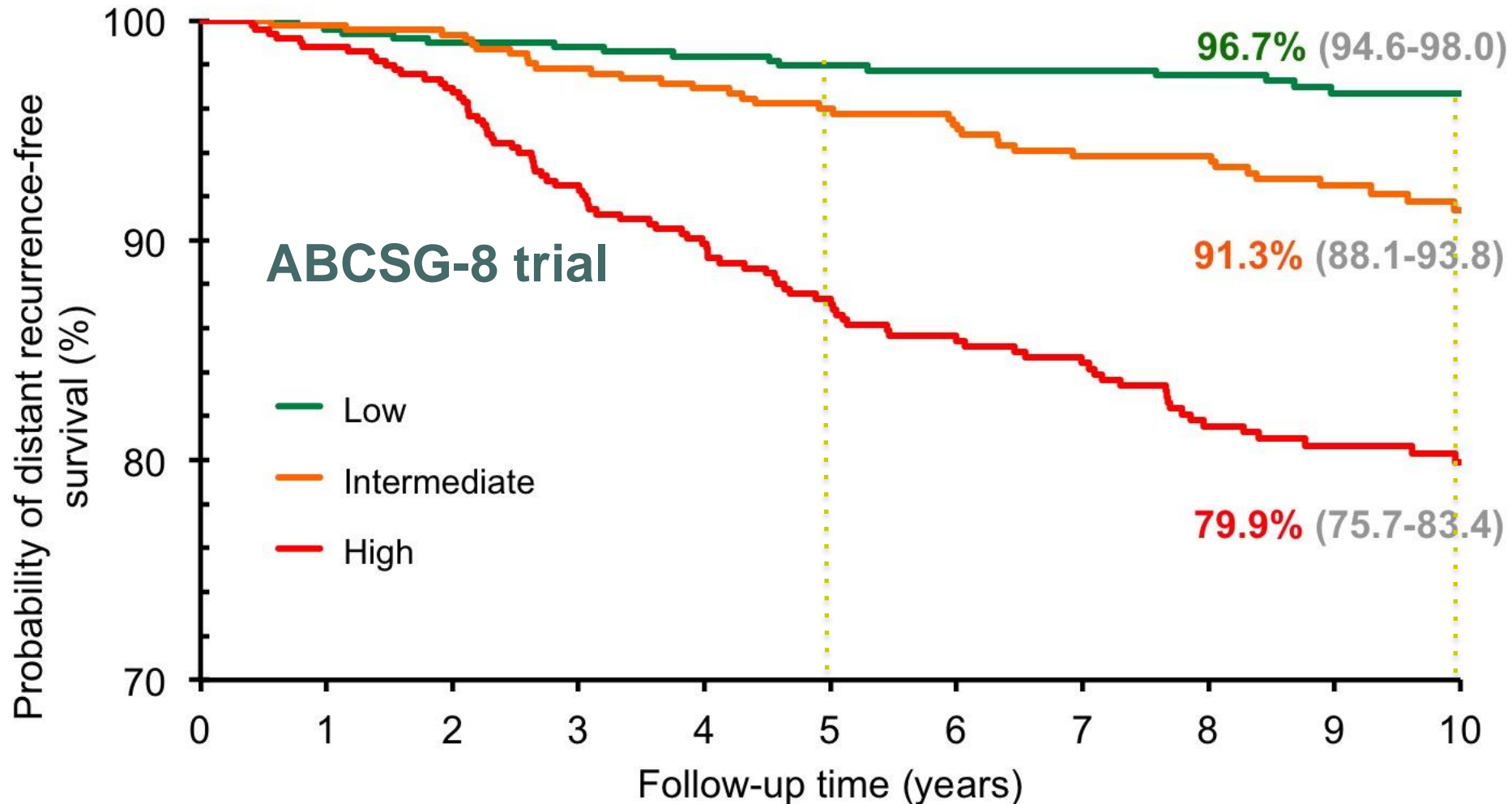
Late Relapse ROR Defined Risk Groups have significant different outcomes in the 2nd Quinquennium

ABCSG-8 trial

	10-yr DRFS (95% CI)	15-yr DRFS (95% CI)
low	98.7 (96.9 - 99.5)	97.6 (94.7 - 98.9)
intermediate	95.2 (92.3 - 97.0)	90.9 (85.9 - 94.2)
high	91.5 (87.8 - 94.1)	82.5 (74.8 - 88.1)

Distant recurence-free survival (%)
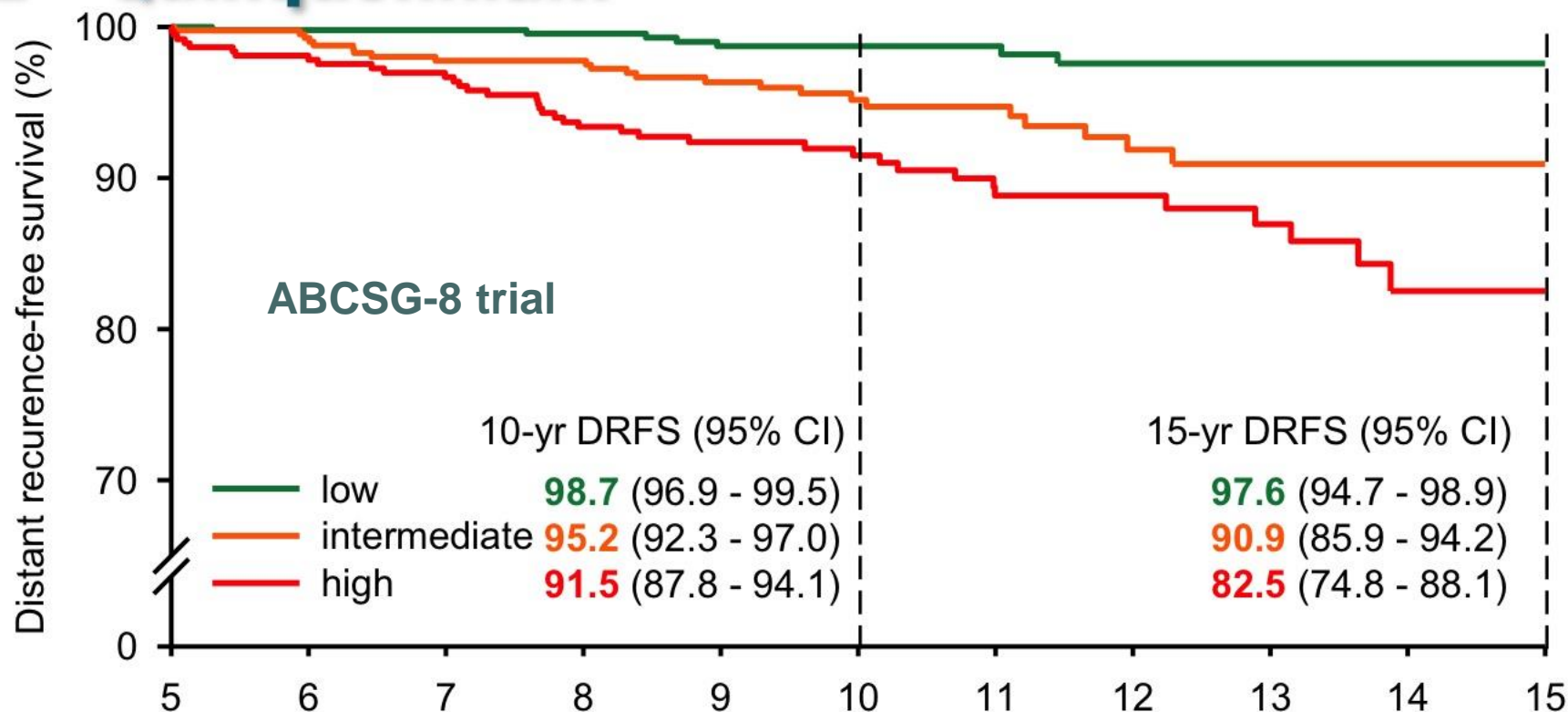
Follow-up time (years)

Patients at risk										
low	460	447	439	412	331	250	188	125	81	50	25
intermediate	416	400	387	370	289	220	161	109	71	41	14
high	370	347	330	301	238	198	153	119	82	43	24

Evaluation and aims of genomic platforms

Evaluation and aims of genomic platforms
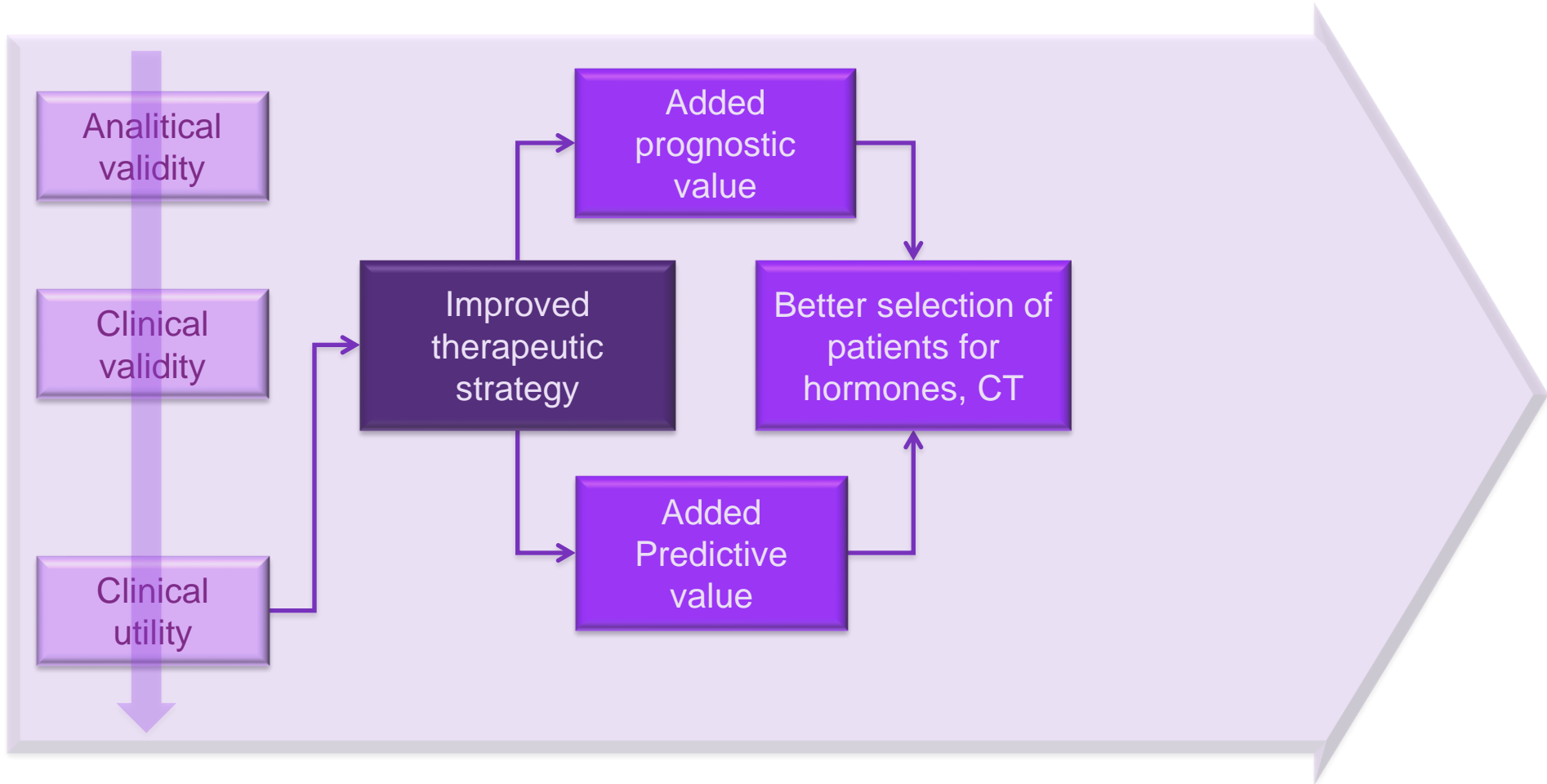
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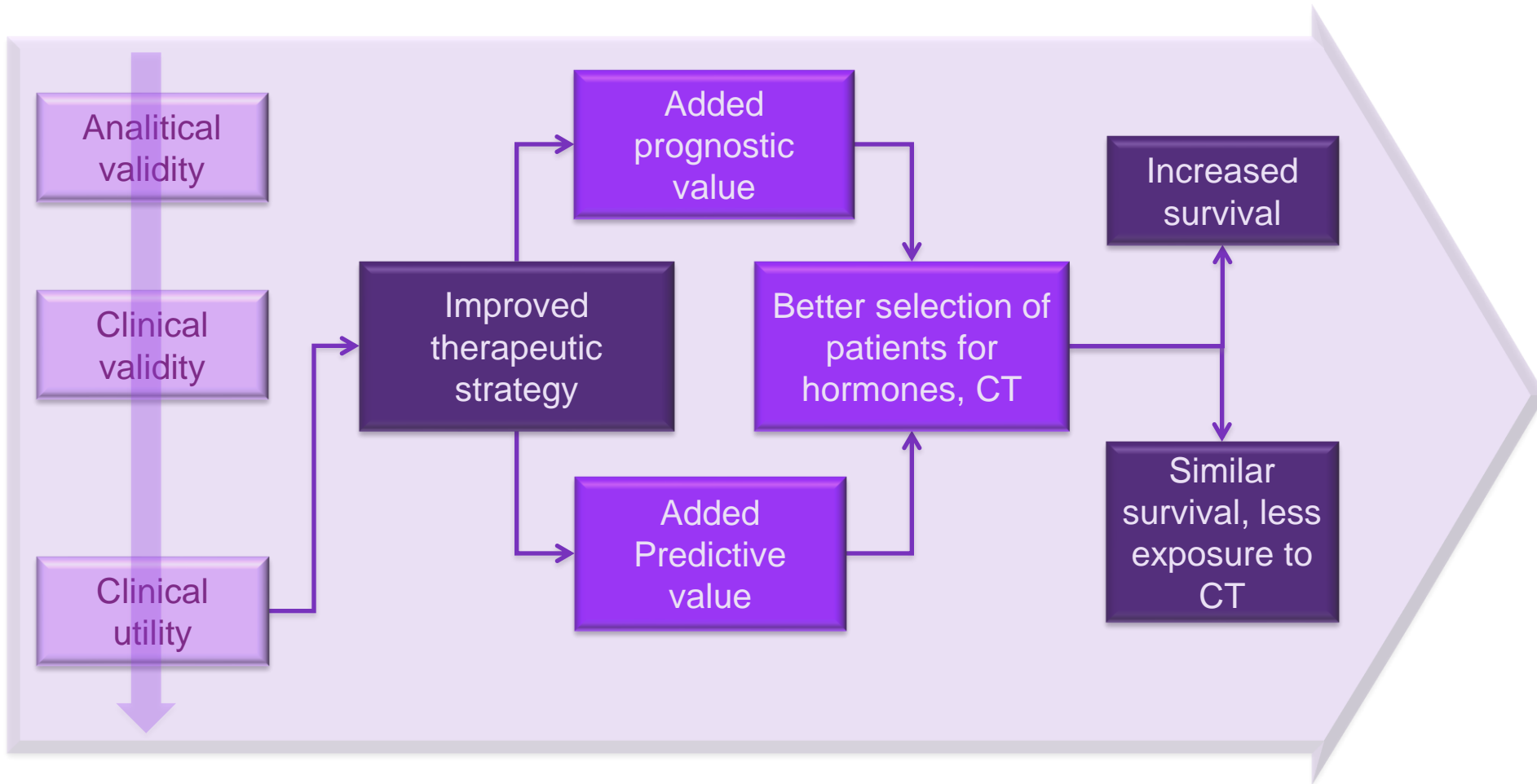
Evaluation and aims of genomic platforms
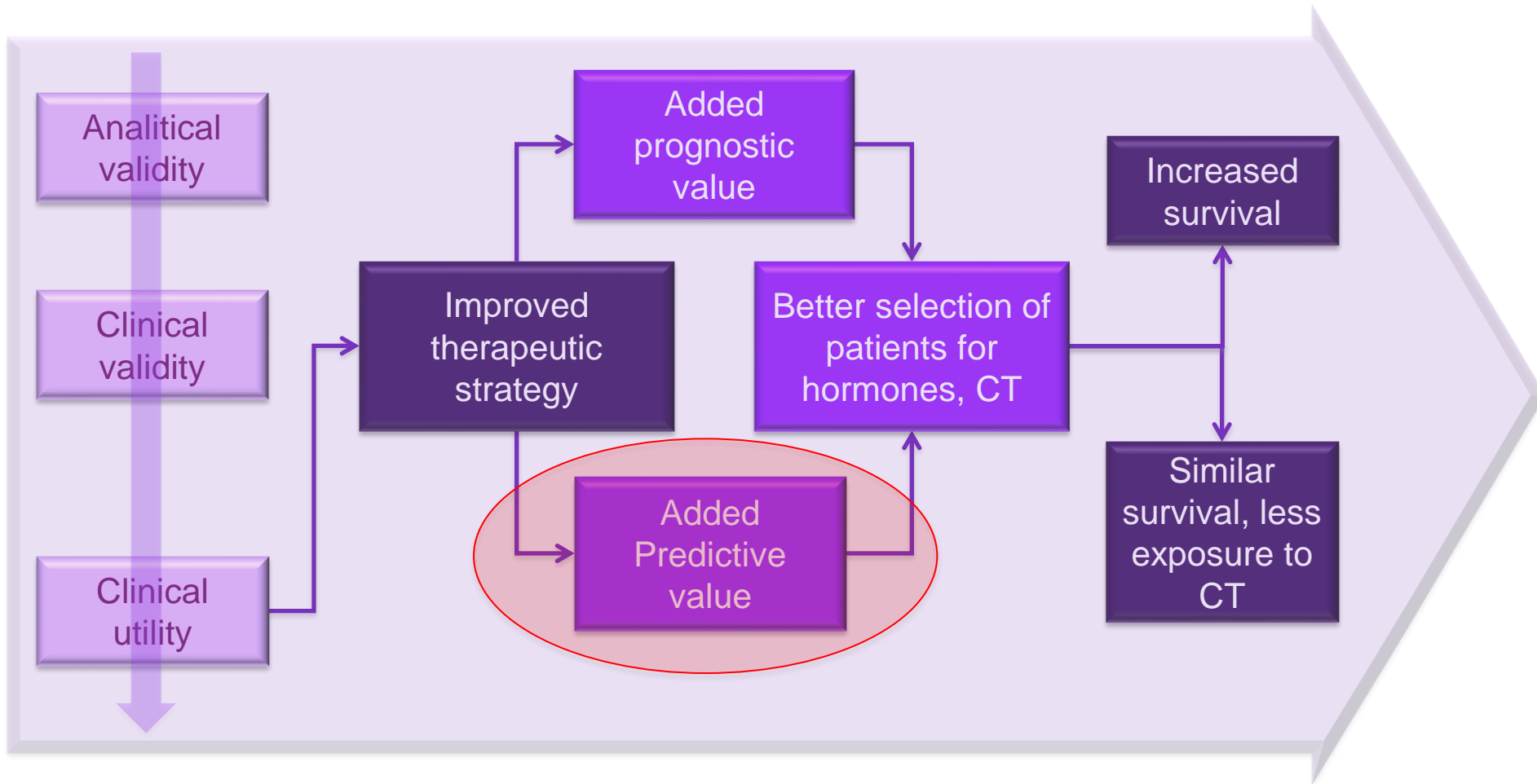
Genomic platforms: potential clinical applications in breast cancer

prognostication

prediction of response to hormones

prediction of response to chemotherapy
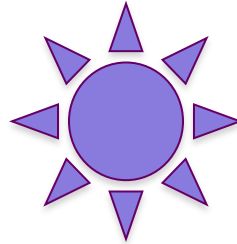
Are prognostication and prediction linked?

- Genomic platforms were designed for prognostication of risk of relapse

- Does risk of relapse according to genomic tests correlated with sensitivity to hormones, chemotherapy?

- Genomic tests are mainly based on ER-related and proliferation-related genes

Prognostication and prediction are linked in ER+/HER2- breast cancer
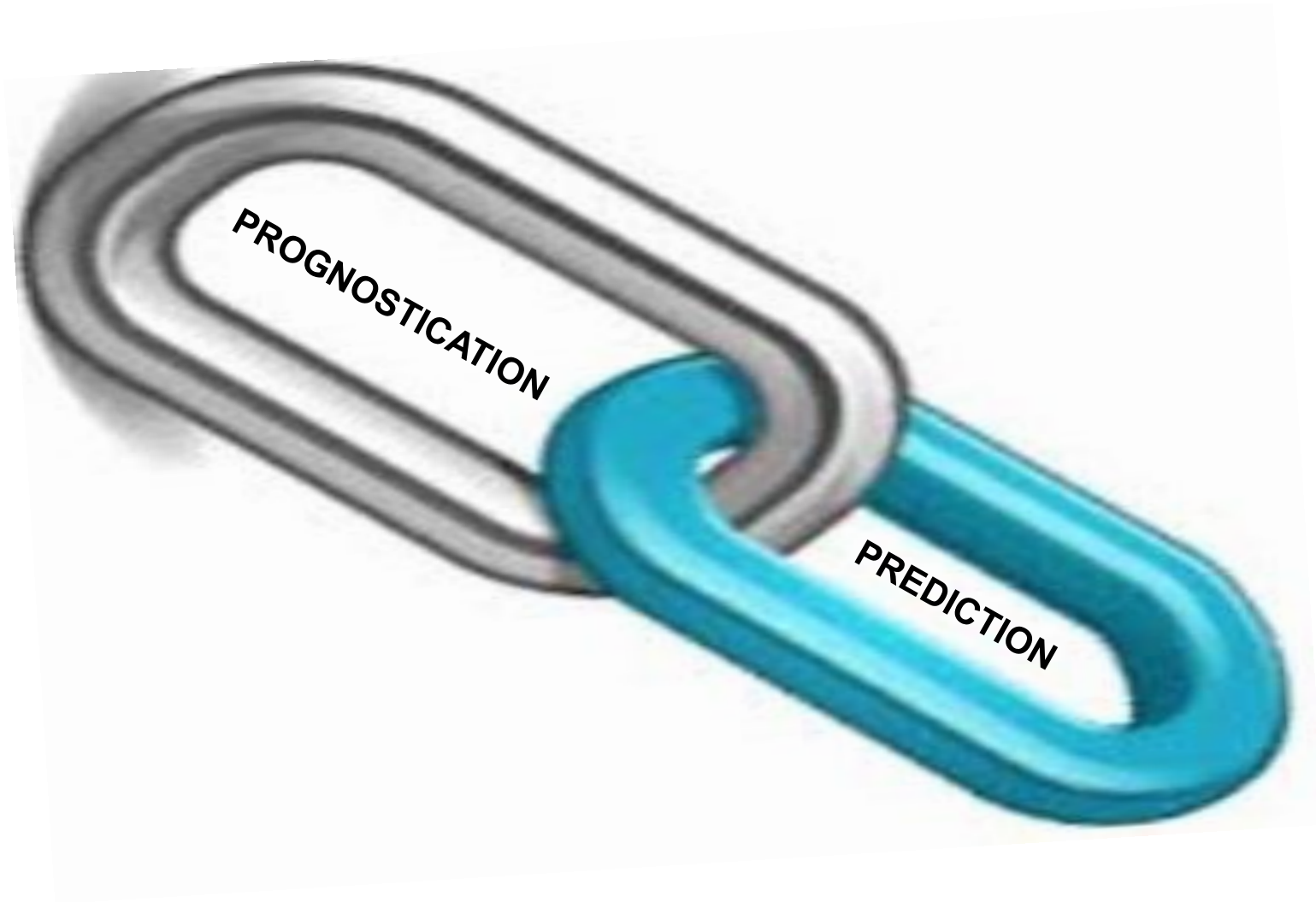
RS identifies patients in the B-14 study most likely to benefit from tamoxifen

DISTANT RECURRENCE FREE INTERVAL

Interaction *P* = 0.06
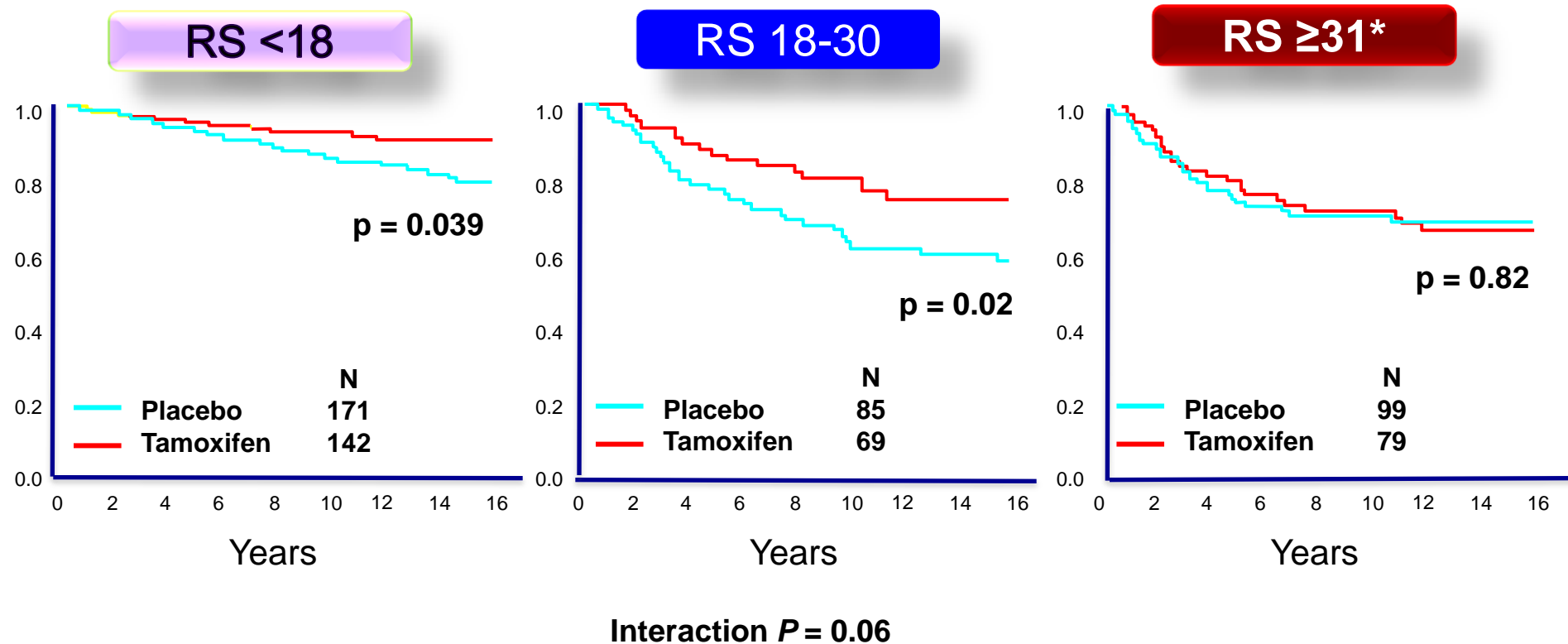
*Results should not be used to indicate that tamoxifen should not be given to the high-risk group

Paik S, et al. ASCO 2004; Abstract 510.

High Recurrence Score® result correlates with greater benefit from chemotherapy (NSABP B-20)

Overall, 4.4% absolute benefit from tamoxifen + chemotherapy

High Recurrence Score® result correlates with greater benefit from chemotherapy (NSABP B-20)

Overall, 4.4% absolute benefit from tamoxifen + chemotherapy

LOW RS GROUP
Recurrence Score <18

INTERMEDIATE RS GROUP
Recurrence Score 18-30

HIGH RS (>30)

28% absolute benefit

Paik S, et al. J Clin Oncol. 2006;24:3726-3734.
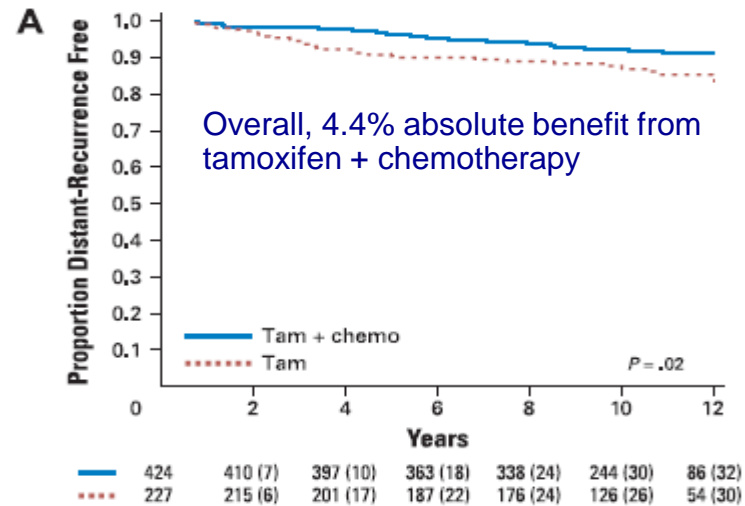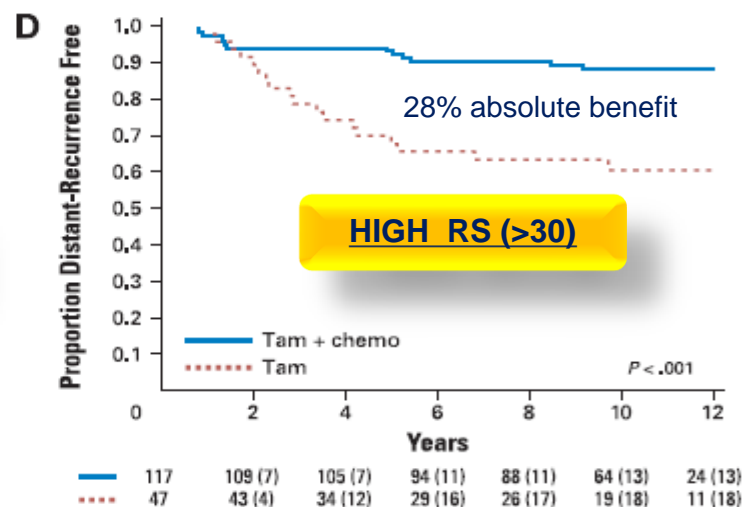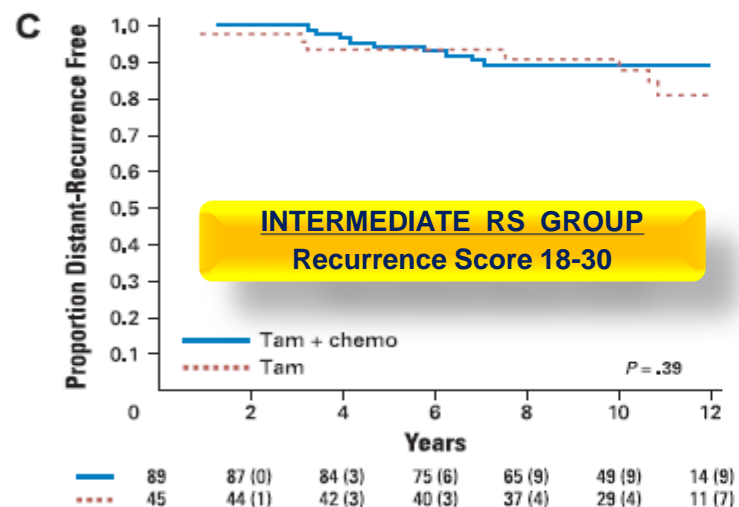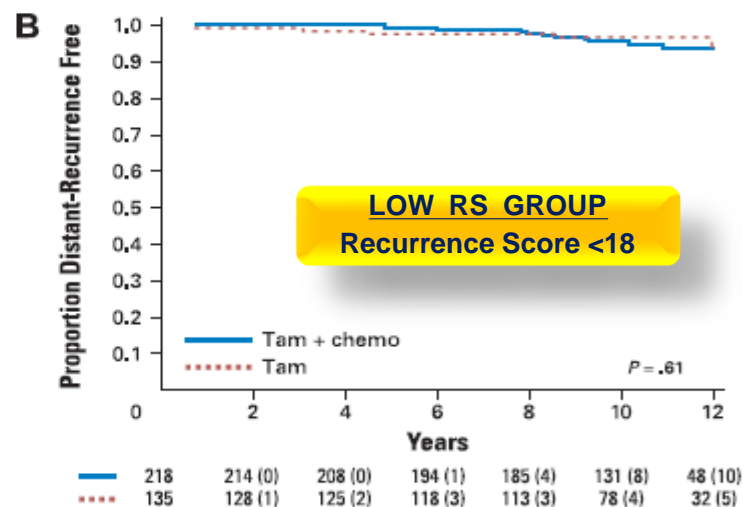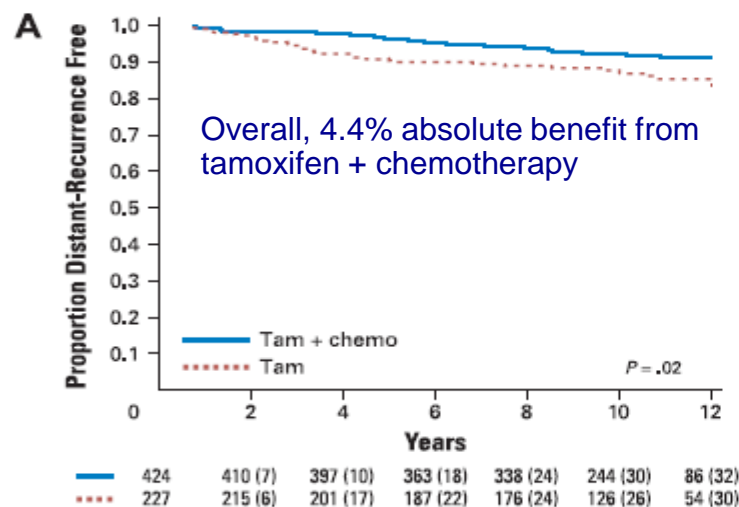
Number of Patients Needed to Treat (NNT) to Avoid a Distant Recurrence with tamoxifen + CT vs tamoxifen alone (NSABP B-20)
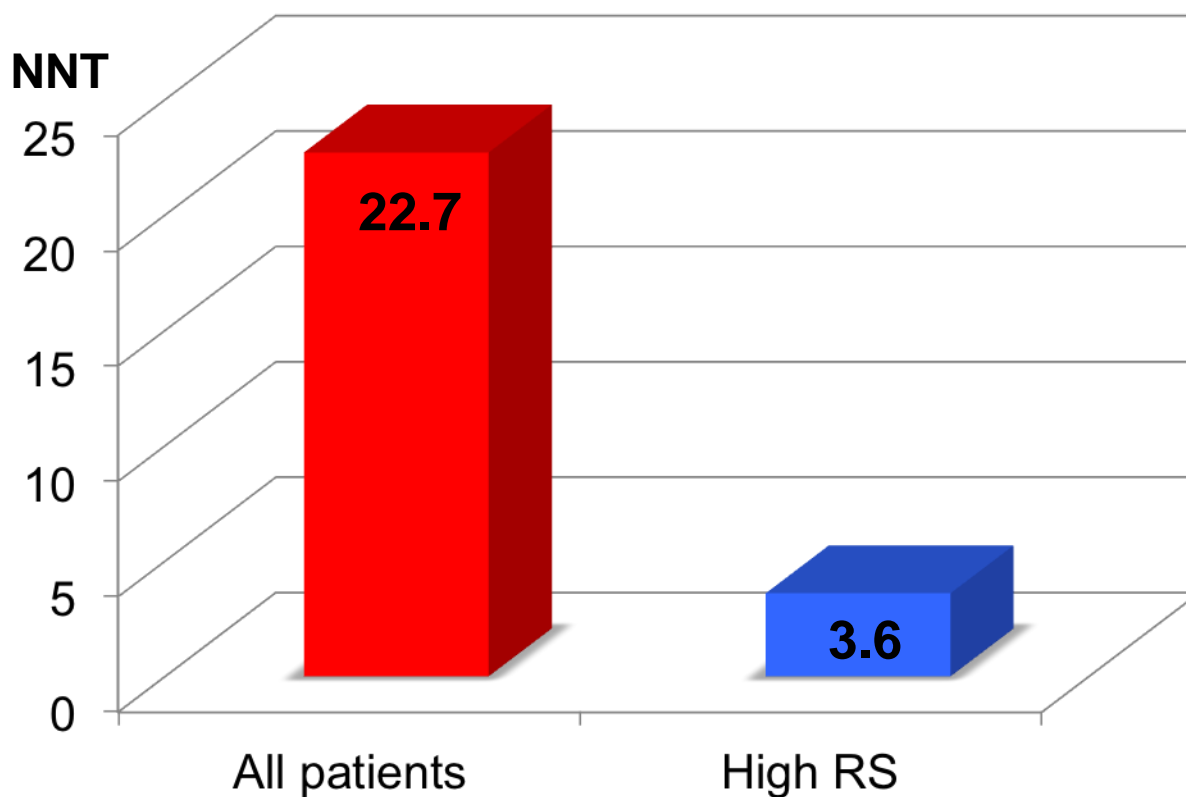
Population	Distant Recurrence Rate with tamoxifen	Distant Recurrence Rate with tamoxifen + chemotherapy
All patients	12%	8%
High RS	40%	12%

Ongoing Trials
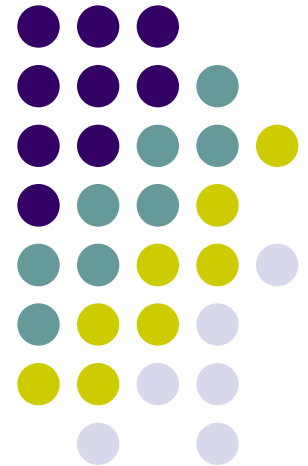
MINDACT: Optimizing decision-making for adjuvant chemotherapy

Assess clinical and genomic risk

Clinical and genomic BOTH HIGH RISK

DISCORDANT clinical and genomic risks

Clinical and genomic BOTH LOW RISK

RANDOMIZE decision-making

Use clinical risk

Use genomic risk

High risk

High risk

Low risk

Low risk

Chemotherapy

No chemotherapy
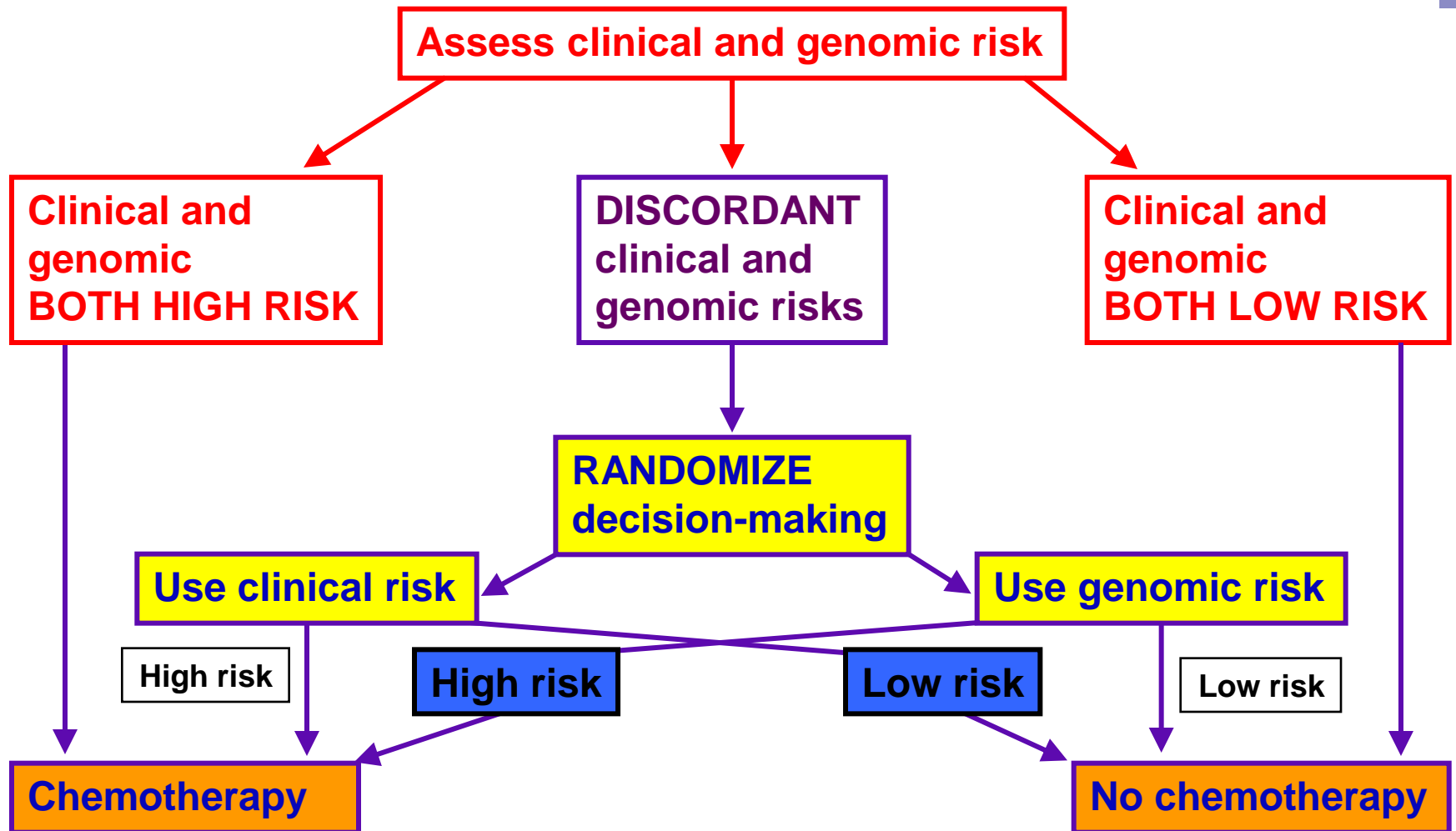
TAILORx Schema
Trial Assigning Individualized Options for Treatment

Patients with node-negative, hormone receptor-positive, HER2-negative breast cancer → **Onco*type* DX® Assay**

Recurrence Score <11
ARM A Hormone therapy registry

Recurrence Score 11-25
Randomize to:
- ARM B hormone therapy or
- ARM C chemo+hormone therapy

Recurrence Score >25
ARM D Chemotherapy + hormone therapy

Accrual goal n = 11,248
Initiated April 2006, recruitment completed October 2010

Primary endpoint: disease free survival

Sample size: n=4,390 for primary study group corresponding to a total accrual of n=11,248

Non-Inferiority design: decrease in 5-year DFS rate from 90% (with chemo) to 87% (without chemo) defined as unacceptable (one-sided type one error of 10% and 5% type II error)

PACCT-1 Intergroup Study: ECOG, SWOG, NCCTG, CALGB, NCIC, ACOSOG, and NSABP + study groups from Australia, Canada, Ireland, Peru ; Sponsor: NCI

Schema and Patient Flow

Node-positive (1-3 nodes) HR-positive and HER2-negative breast cancer

(N= 600)
RS already Available

(N= 8,800)
Patients consent to study-sponsored RS testing, discussion of potential trials, tumor tissue submission and linkage to cancer registry data

RS ≤ 25 ?

RS > 25

(N= 3,800)
Discuss alternative trials for high risk patients

RS ≤ 25

N= 5,600
Physician and patients discuss randomization knowing the RS

Accept

N= 4,000
Randomization stratified by
1. RS
   0-13 vs. 14-25
2. Menopausal status
3. Axillary node dissection vs. Sentinel node biopsy

N= 2,000
Chemotherapy; appropriate endocrine therapy

N= 2,000
No Chemotherapy; appropriate endocrine therapy

Refuse

N= 1,600
Record chosen therapy and followed for vital status through cancer registry

Southwest Oncology Group
A National Clinical Research Group

SWOG, and study group from Spain (GEICAM); Sponsor: NCI

Utility of prognostic genomic tests in breast cancer practice: The IMPAKT 2012 Working Group Consensus Statement[†]

- The IMPAKT 2012 Working Group proposed the following recommendations:

  - (i) a need to develop models that integrate clinicopathologic factors along with genomic tests

  - **(ii) the creation of registries for patients who are subjected to genomic testing in the daily practice**

  - (iii) demonstration of clinical utility should be made in the context of a prospective randomized trial

PREGECAM

(Programa de Predicción Genómica en Cáncer de Mama de la Comunidad de Madrid)

Population: 6,300.000 inh.
New breast cancers per year: 2.800

ER+/HER2-
N0 o Nmic, T > 1cm or
T<1 and G2-3 or KI67 >13%
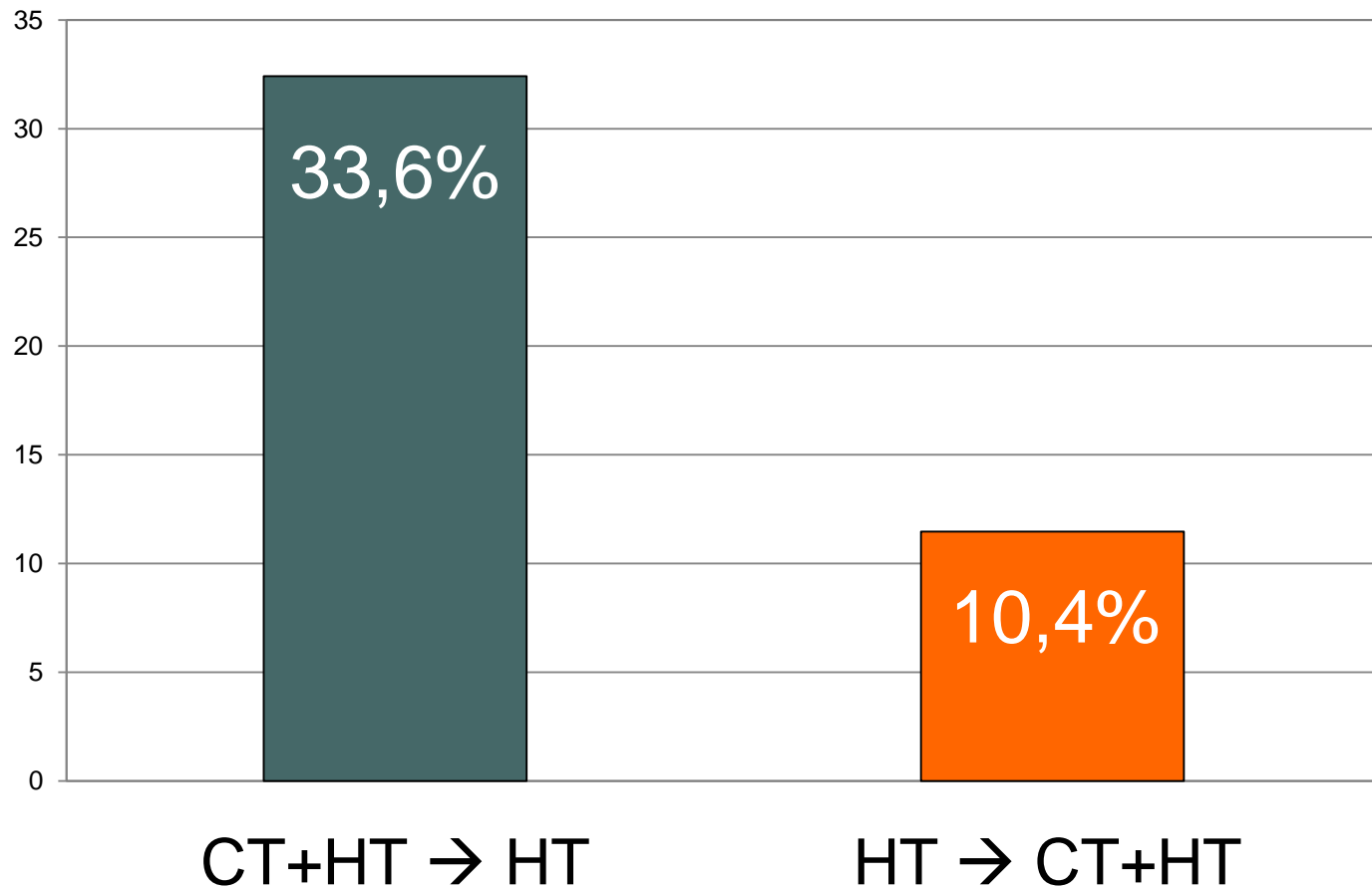**or lymphovascular invassion**

May 2012-April 2014

TEST	Nº. OF PATIENTS
Oncotype Dx®	255
Mammaprint	370
All	625

Prospective data collection including cost-efficacy analysis

PREGECAM (Madrid County, Spain)

switch in therapy (n=275)

PREGECAM (Madrid County, Spain)

switch in therapy (n=275)

44% change in treatment decissions, 22% reduction in the use of chemotherapy

CT+HT → HT: 33,6%

HT → CT+HT: 10,4%
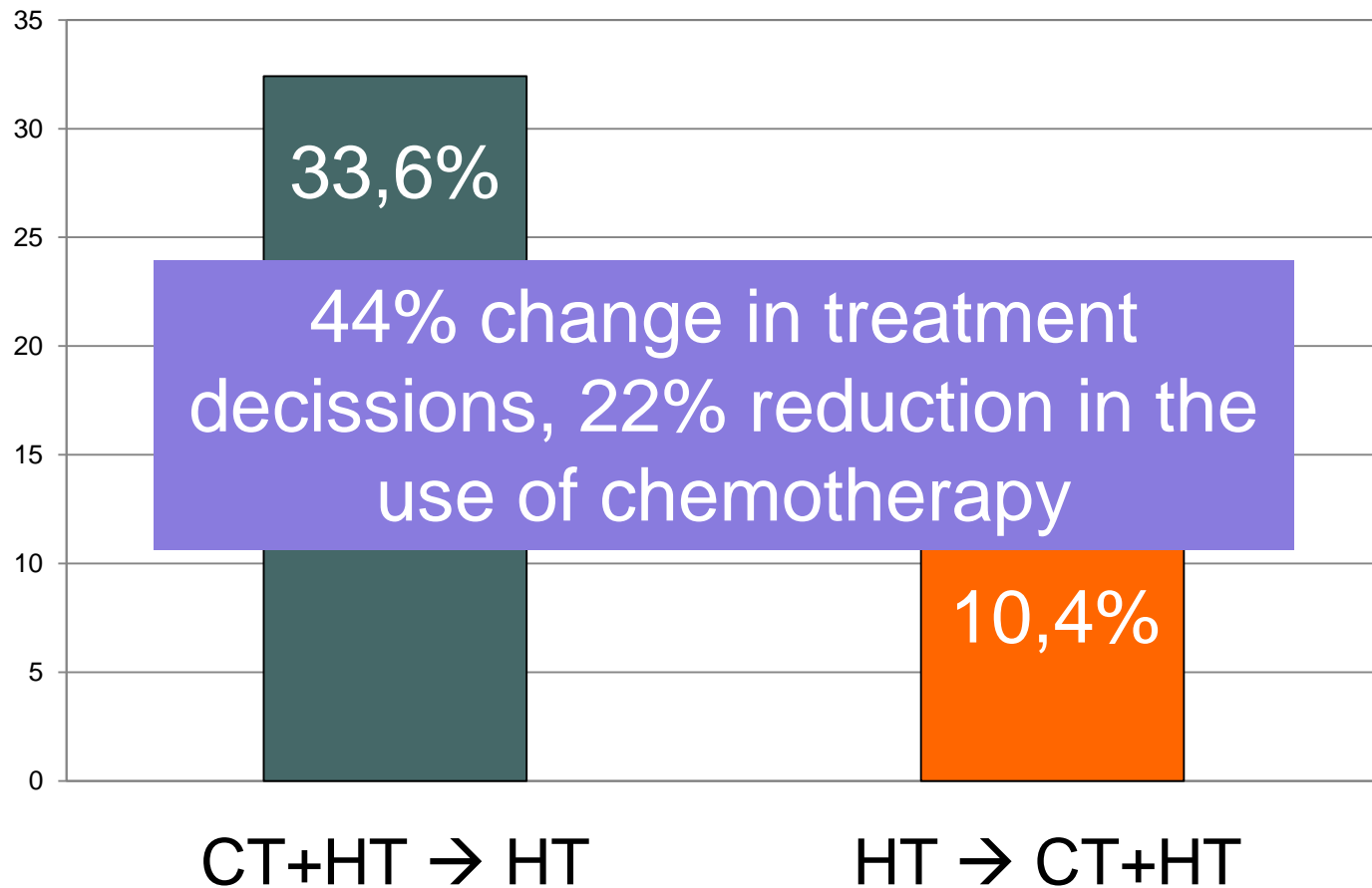
Conclusions

- Genomic platforms are contributing to an individualized therapeutic strategy in early breast cancer
- Genomic tests provide relevant prognostic information for ER+/HER2- early breast cancer patients
- Correlation between genomic prognostication and prediction of response to TAM/chemotherapy in ER+/HER2- tumors
- Debate about the need for prospective validation of clinical utility
- Registries necessary to evaluate the performance of the tests in the real life