National Cancer Institute

PREOPERATIVE THERAPY IN INVASIVE BREAST CANCER

Reviewing the State of the Science and Exploring New Research Directions

Importance of obtaining tissue for research – A case study in NSABP B-27

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National Institutes of Health

Current prognostic tools in adjuvant setting

- Can identify high risk patients
- High risk patients derive greater benefit from chemotherapy
- However, the tools are probabilistic
- The tools cannot tell who actually benefited from chemotherapy and who need more than chemotherapy after chemotherapy is administered

Oncotype DX 21 Gene Recurrence Score (RS) Assay

16 Cancer and 5 Reference Genes From 3 Studies

PROLIFERATION
Ki-67
STK15
Survivin
Cyclin B1
MYBL2

ESTROGEN
ER
PR
Bcl2
SCUBE2

GSTM1

BAG1

INVASION
Stromelysin 3
Cathepsin L2

CD68

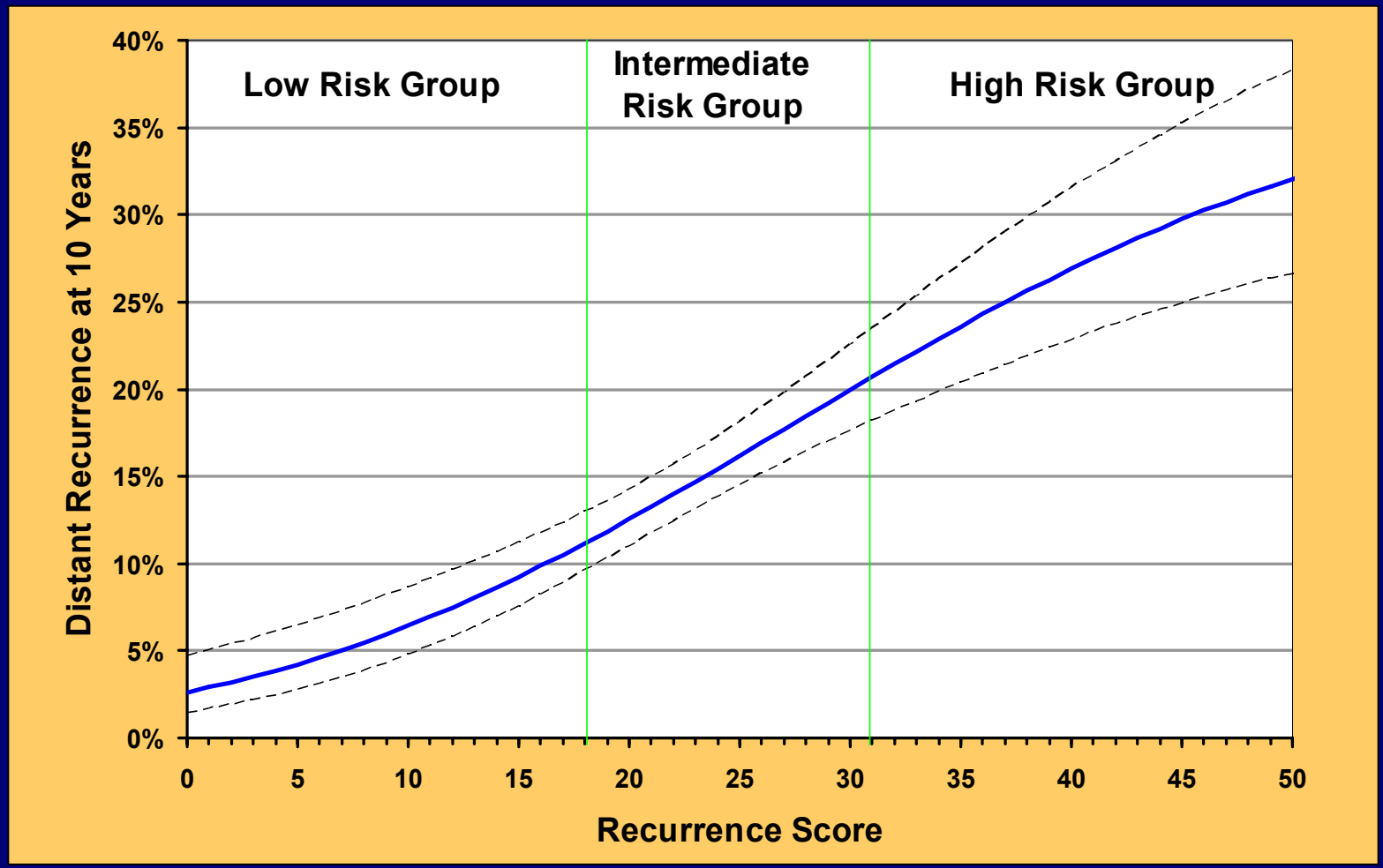
HER2
GRB7
HER2

REFERENCE
Beta-actin
GAPDH
RPLPO
GUS
TFRC

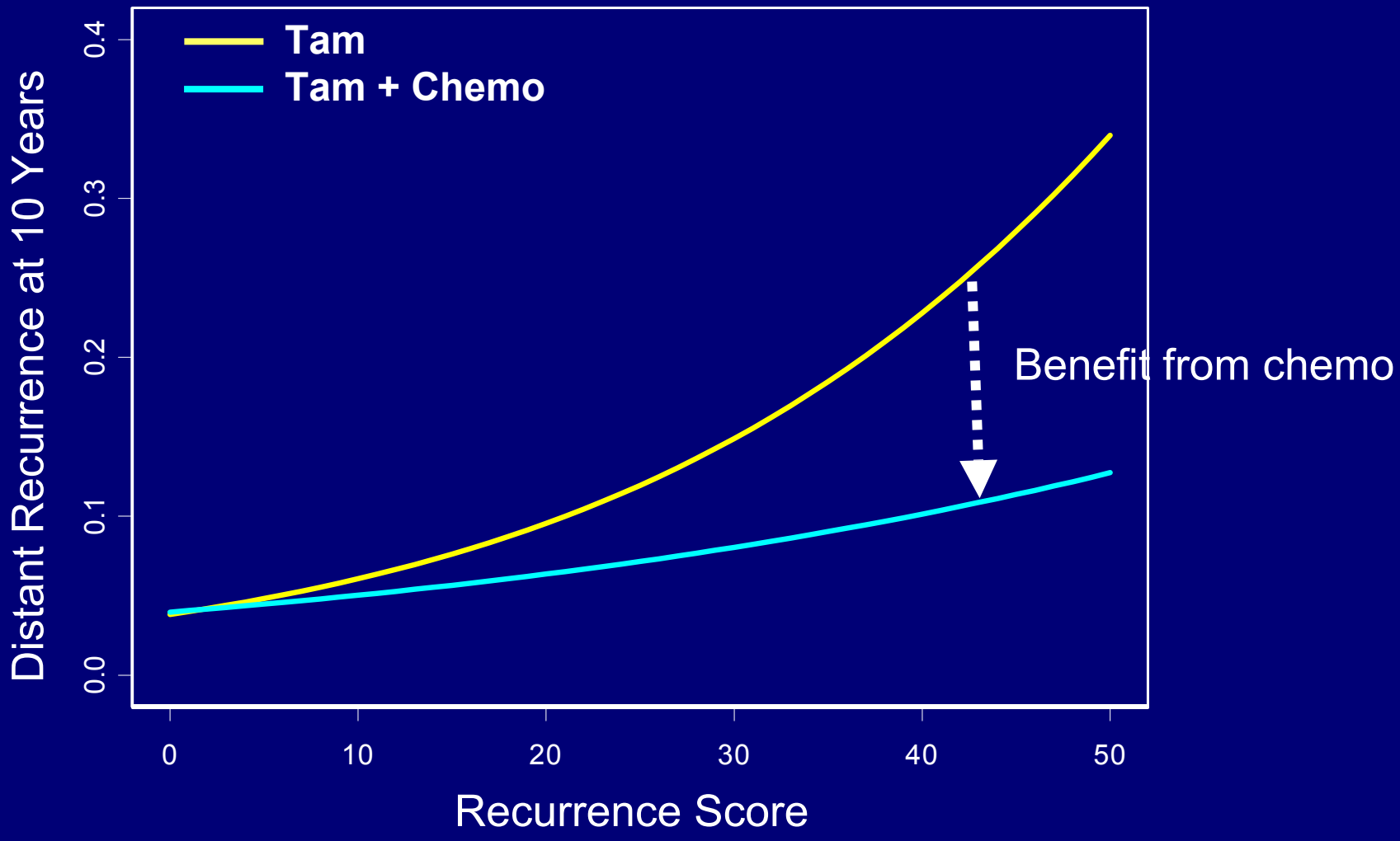
RS = + 0.47 x HER2 Group Score
- 0.34 x ER Group Score
+ 1.04 x Proliferation Group Score
+ 0.10 x Invasion Group Score
+ 0.05 x CD68
- 0.08 x GSTM1
- 0.07 x BAG1

Category	RS (0 – 100)
Low risk	RS < 18
Int risk	RS ≥ 18 and < 31
High risk	RS ≥ 31

Recurrence Score and prognosis (NSABP B-14 tamoxifen arm)

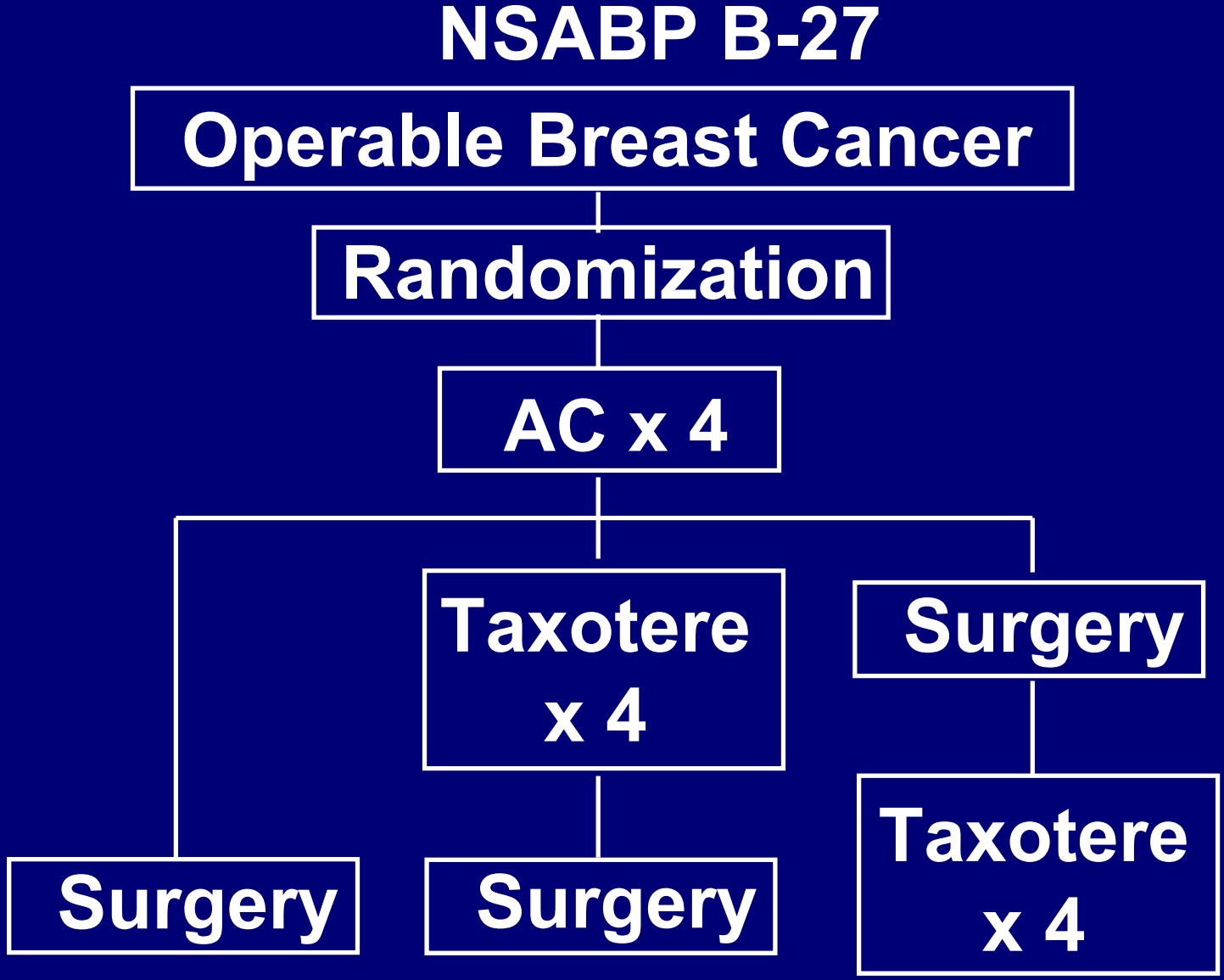


Higher risk = Greater benefit
(NSABP B-20)

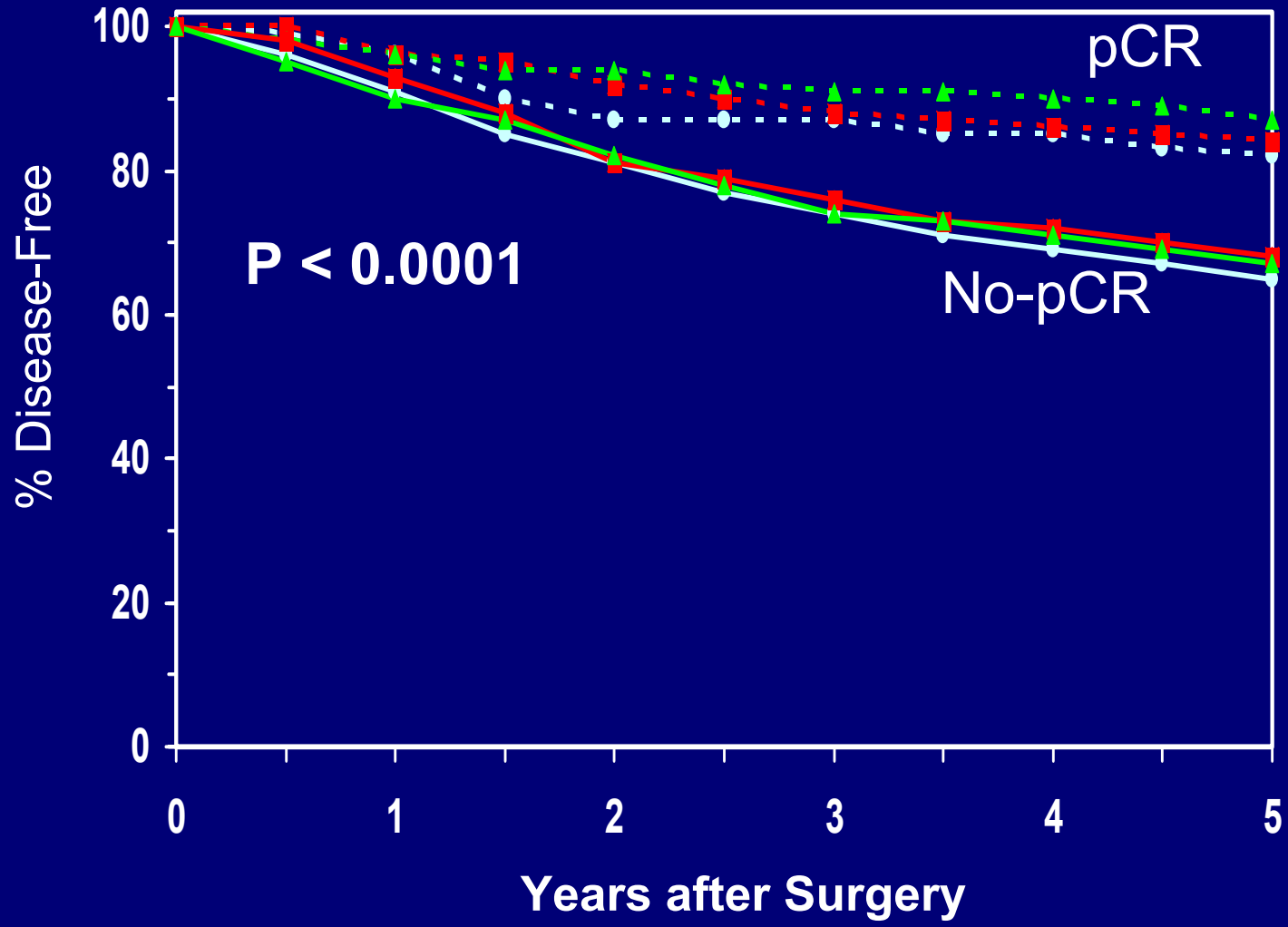


pCR provides patient specific in-vivo assessment of tumor response

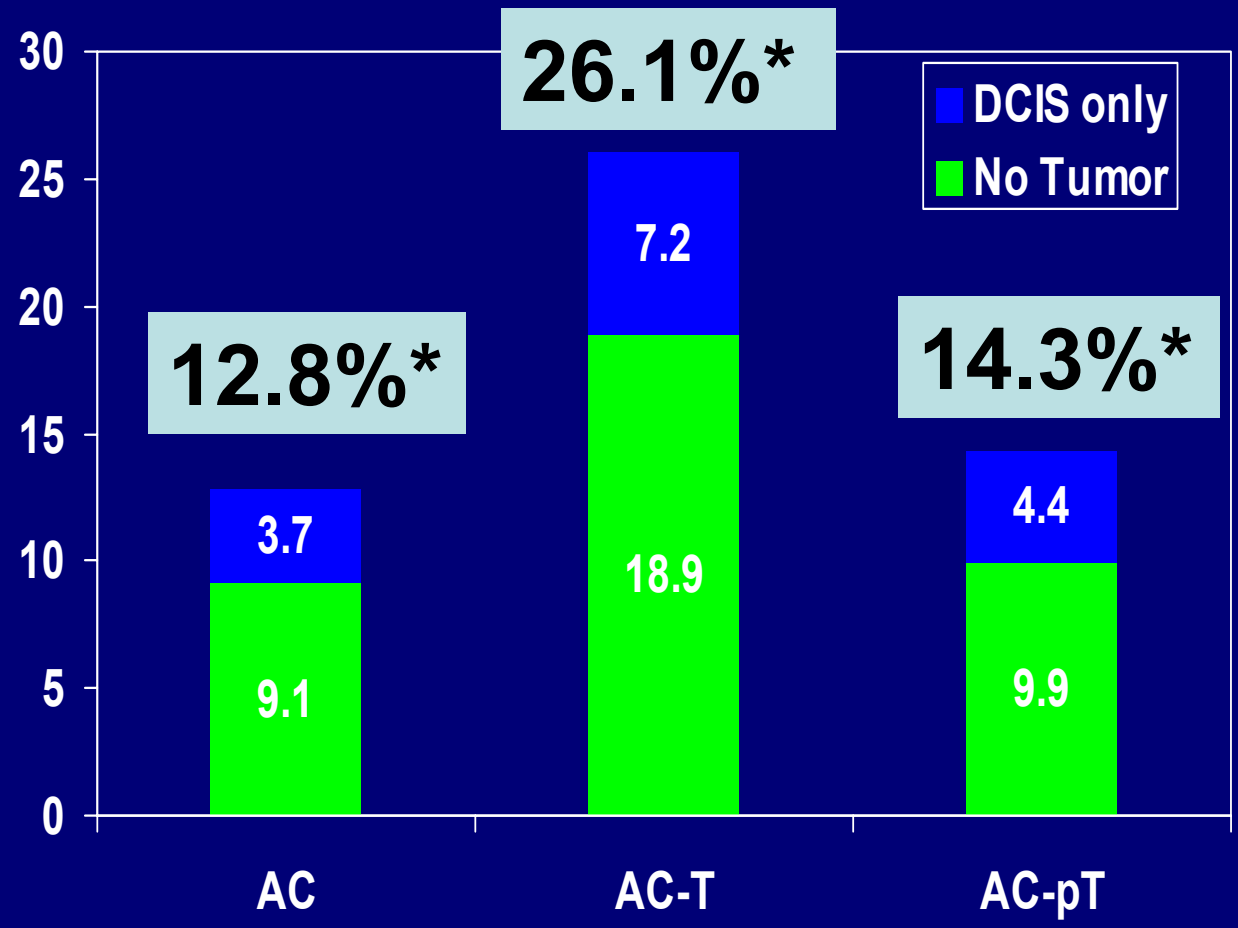
- However, not a perfect surrogate for survival endpoint
- Even doubling of pCR rate did not result in improvement in survival endpoint (NSABP B-27)
- Does not provide base-line risk assessment


NSABP B-27
Operable Breast Cancer
Randomization
AC x 4
Surgery
Taxotere x 4
Surgery
Surgery
Taxotere x 4

NSABP B-27: pCR as a surrogate for clinical end-points regardless of treatment



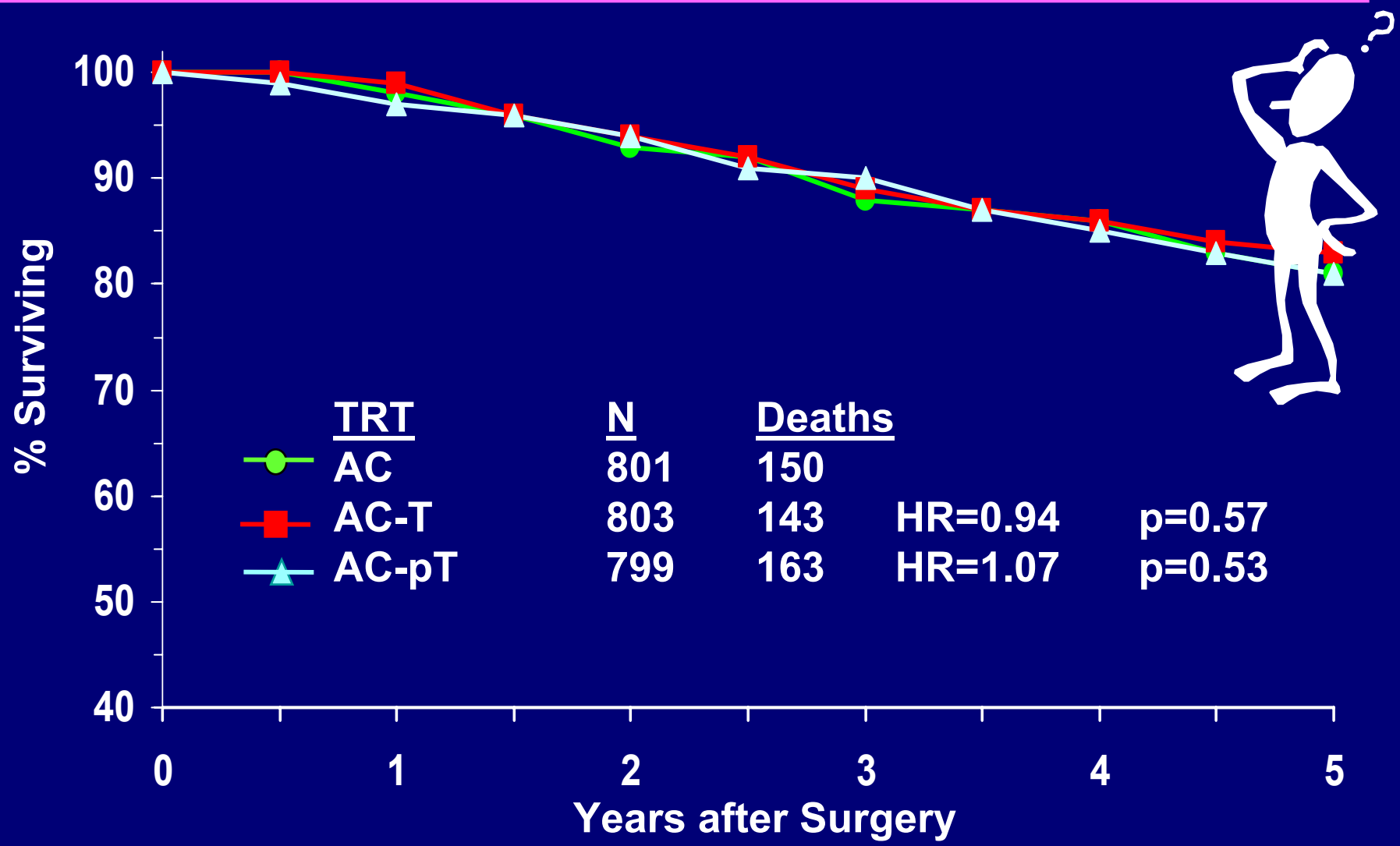
NSABP B-27
Doubling of pCR in AC-T vs AC



*p<0.001 for test of heterogeneity across groups

NSABP B-27

Doubling of pCR did not translate to clinical outcome differences

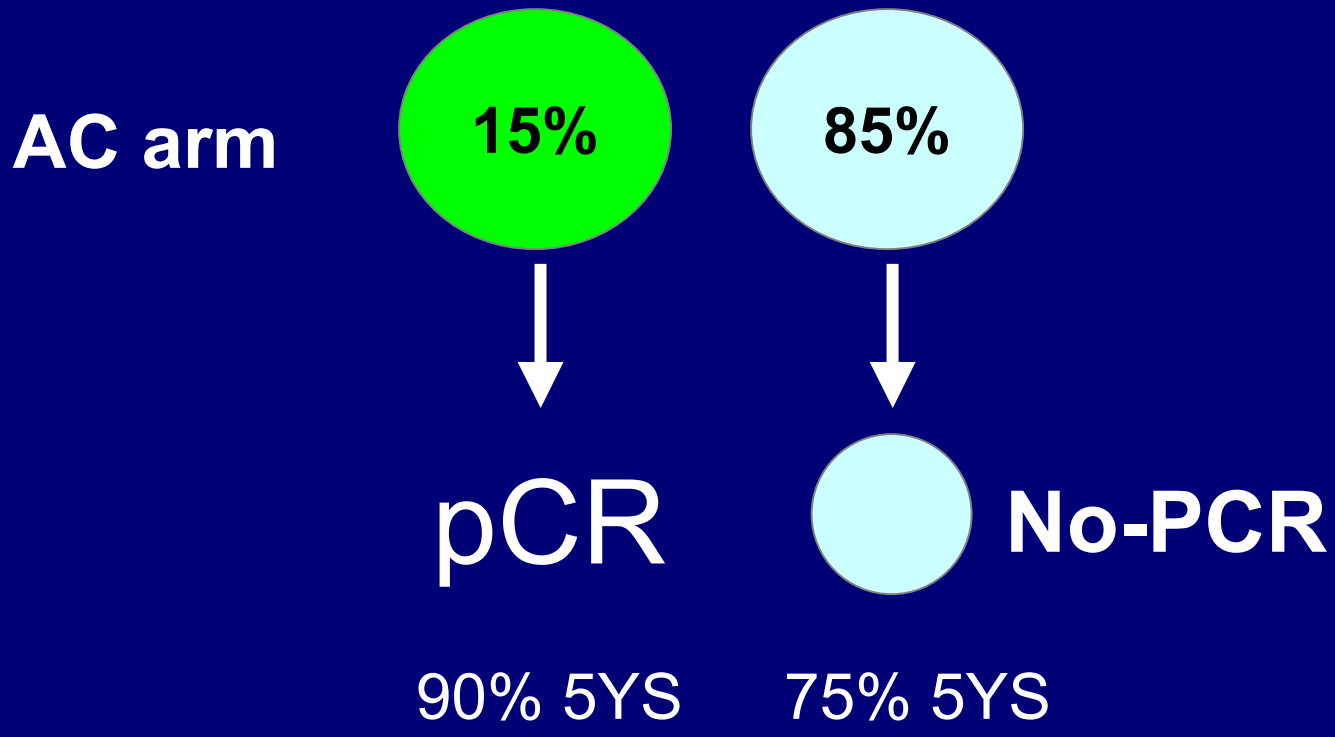


No perfect tools

- Current prognostic tools in adjuvant setting
  - Can identify high risk patients
  - High risk patients derive greater benefit from chemotherapy
  - However, the tools are probabilistic
  - The tools cannot tell who actually benefited from chemotherapy and who need more than chemotherapy
- pCR is a patient specific in-vivo assessment of tumor response
  - Not a perfect surrogate for survival endpoint
  - Even doubling of pCR rate did not result in improvement in survival endpoint (NSABP B-27)
  - Does not provide base-line risk assessment

Is pCR a valid surrogate endpoint?

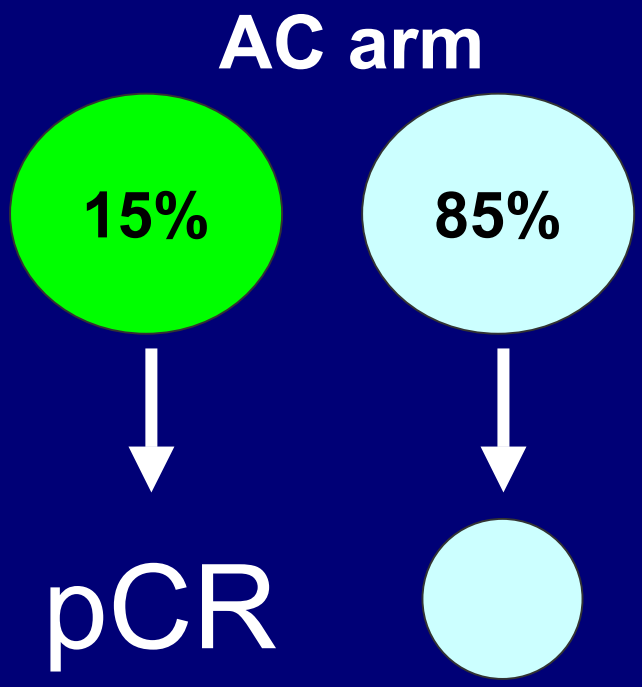
Extrapolation of B-18 data to B-27

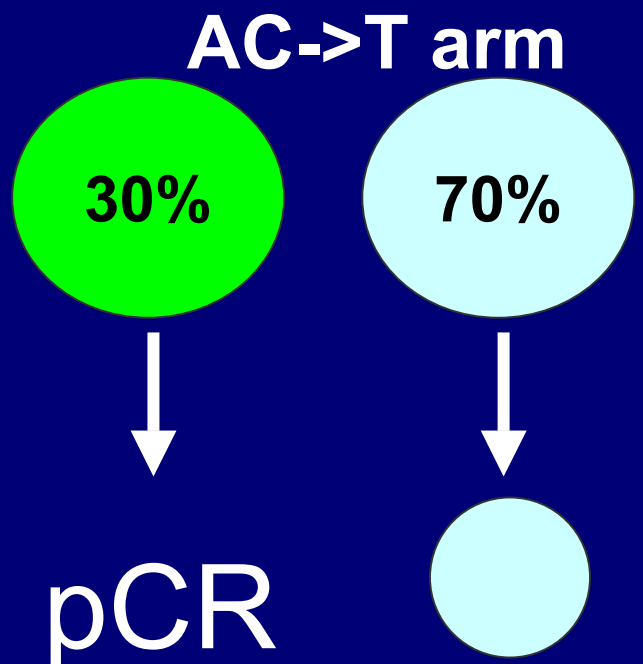


5YS of all patients in AC arm =

$$\frac{90\times15+75\times85}{100} = \mathbf{77.25\%}$$

Extrapolation of B-18 data predicted that B-27 clinical outcome data could not be robust





5YS of all patients in AC arm =

$$\frac{90\times15+75\times85}{100} = \textbf{77.25\%}$$

5YS of all patients in AT arm =

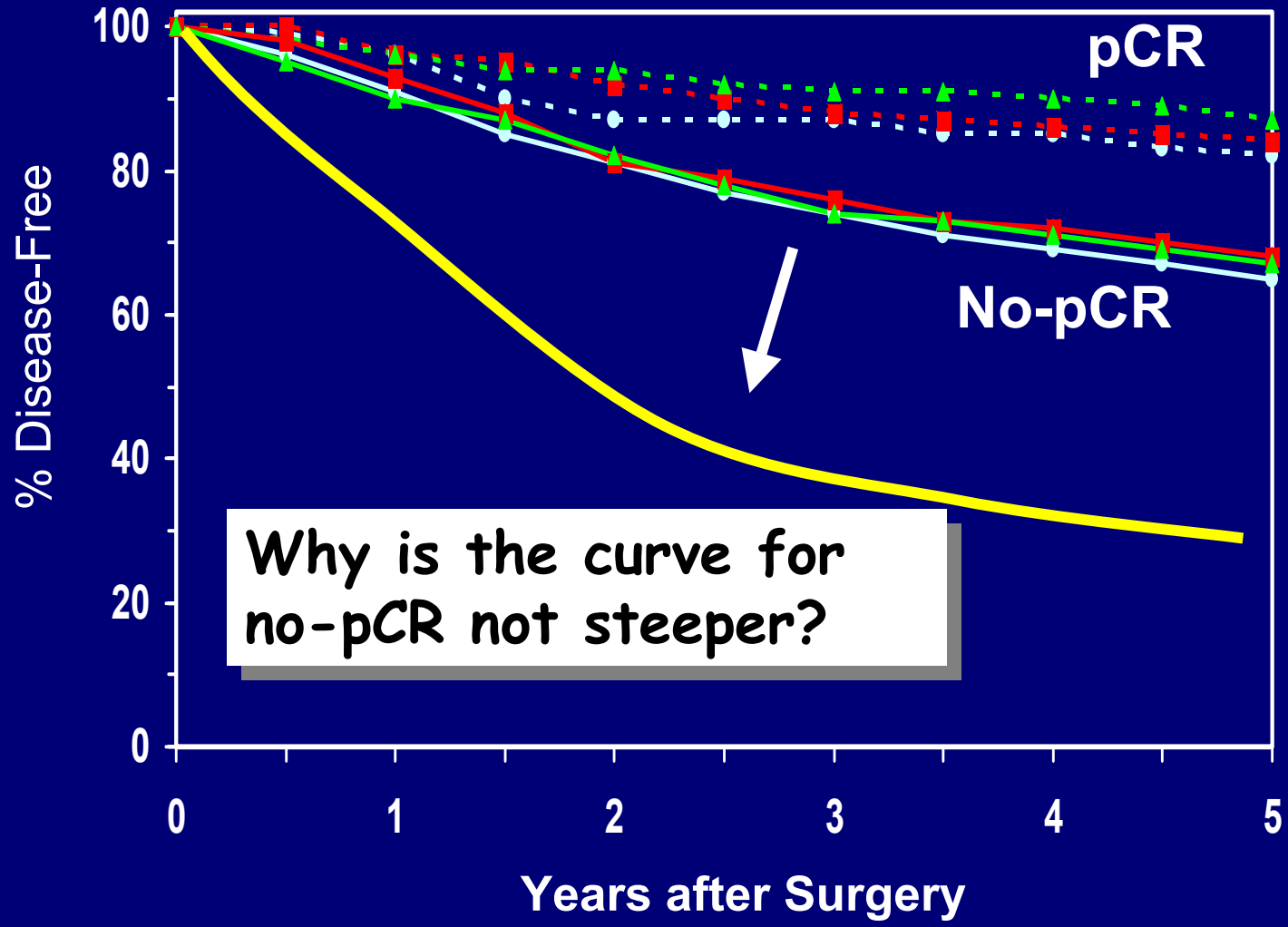
$$\frac{90\times30+75\times70}{100} = \textbf{79.50\%}$$

B-27 could not be robust for survival endpoint due to relatively good outcome of no-pCR patients

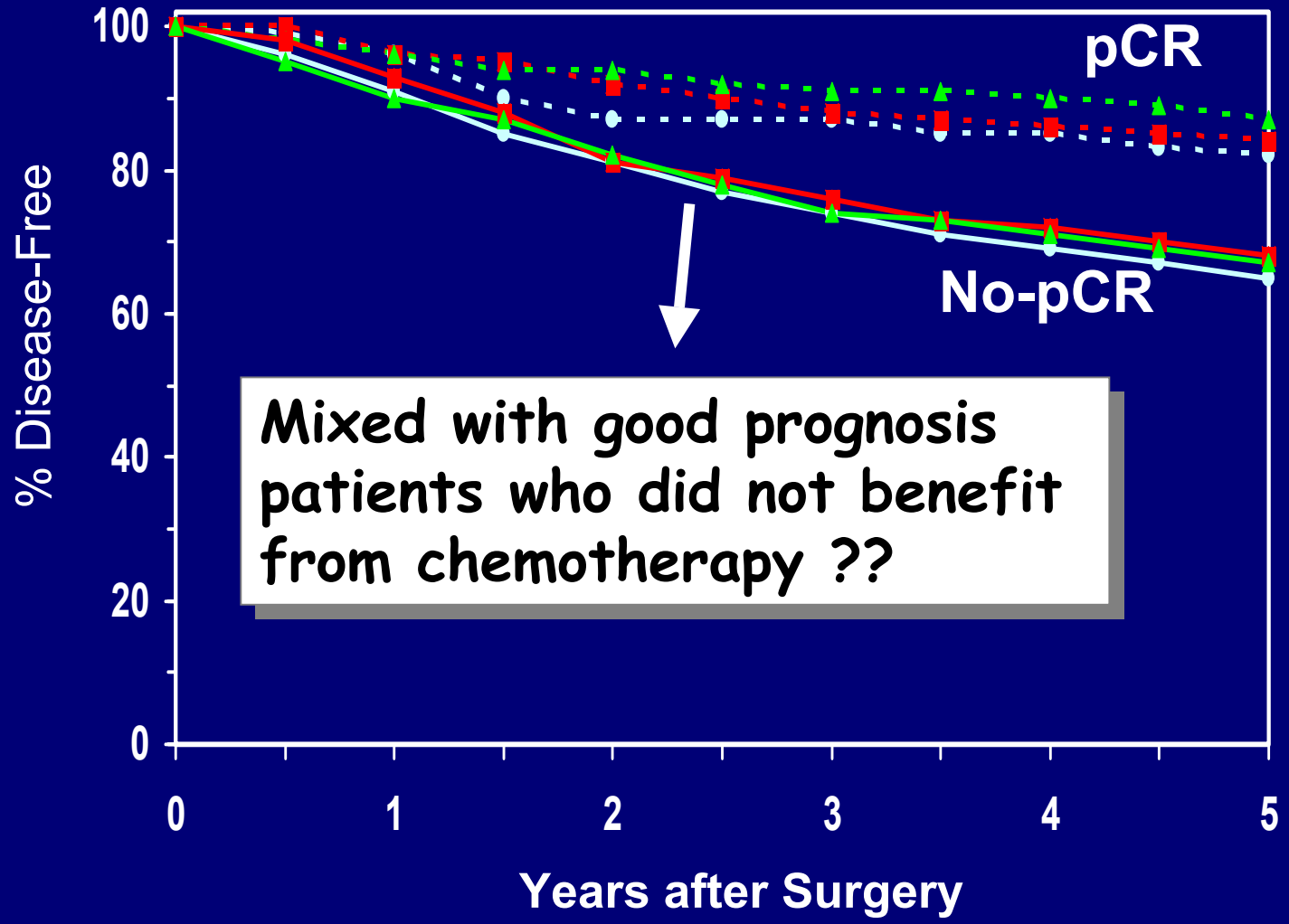
% pCR	expected 5YS
15	77.25%
30	79.5
50	82.5
60	84
80	87

NSABP B-27

pCR as a surrogate for clinical end-points



NSABP B-27
Problem of patient selection?



NSABP B-27 Pathology

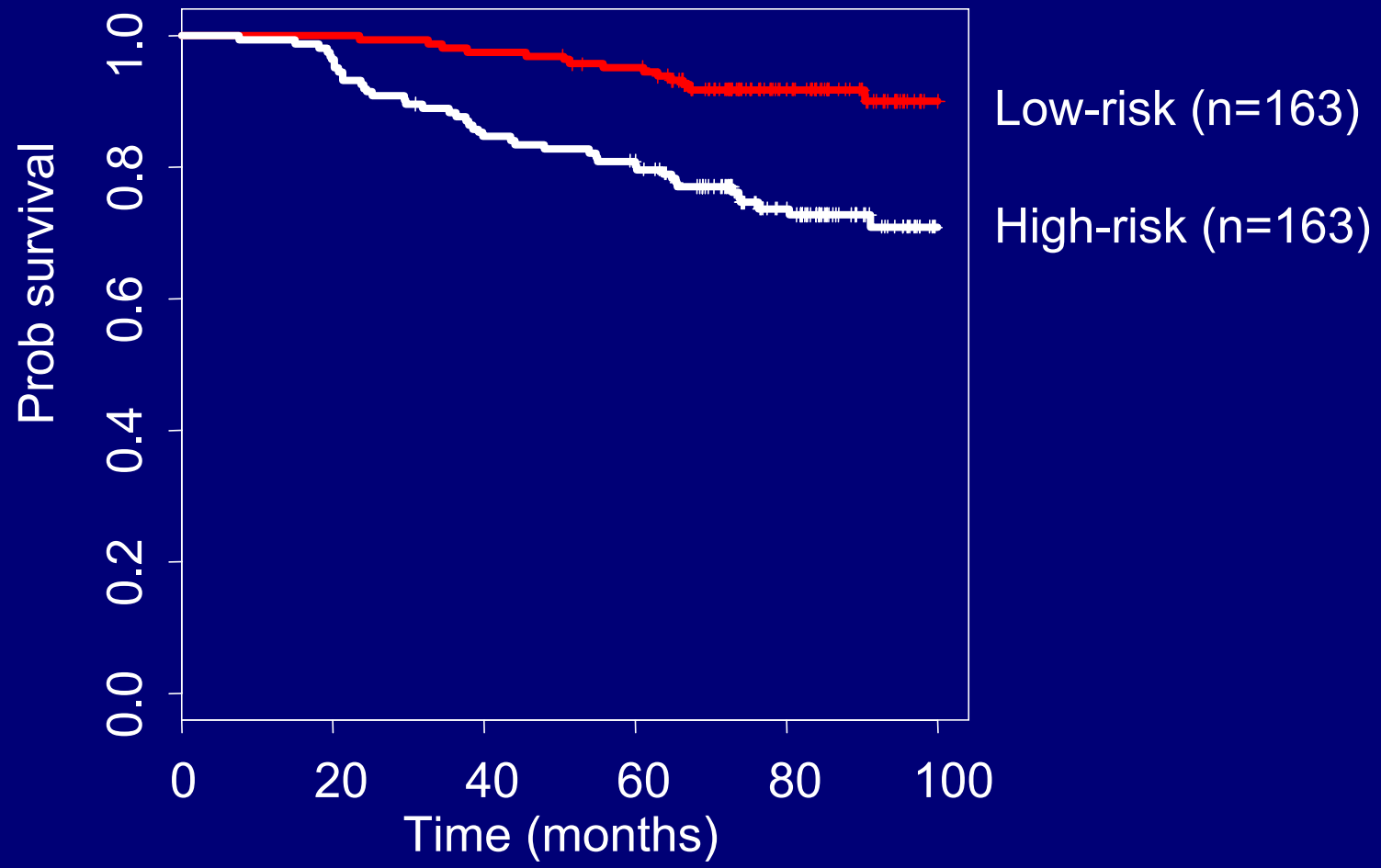
- Pretreatment core biopsy paraffin block procurement protocol (B-27.2) started one year after after initiation of the main trial (B-27)
- Initial planned markers – p53, Ki67, ER, PR, HER2 – but technology evolved
- Had to develop a new method for microarray gene expression profiling of paraffin embedded tumor tissue
- Affymetrix U133 2.0 plus GeneChip data available from 326 cases

Gene expression profiling of B-27 pre-treatment core biopsy specimens

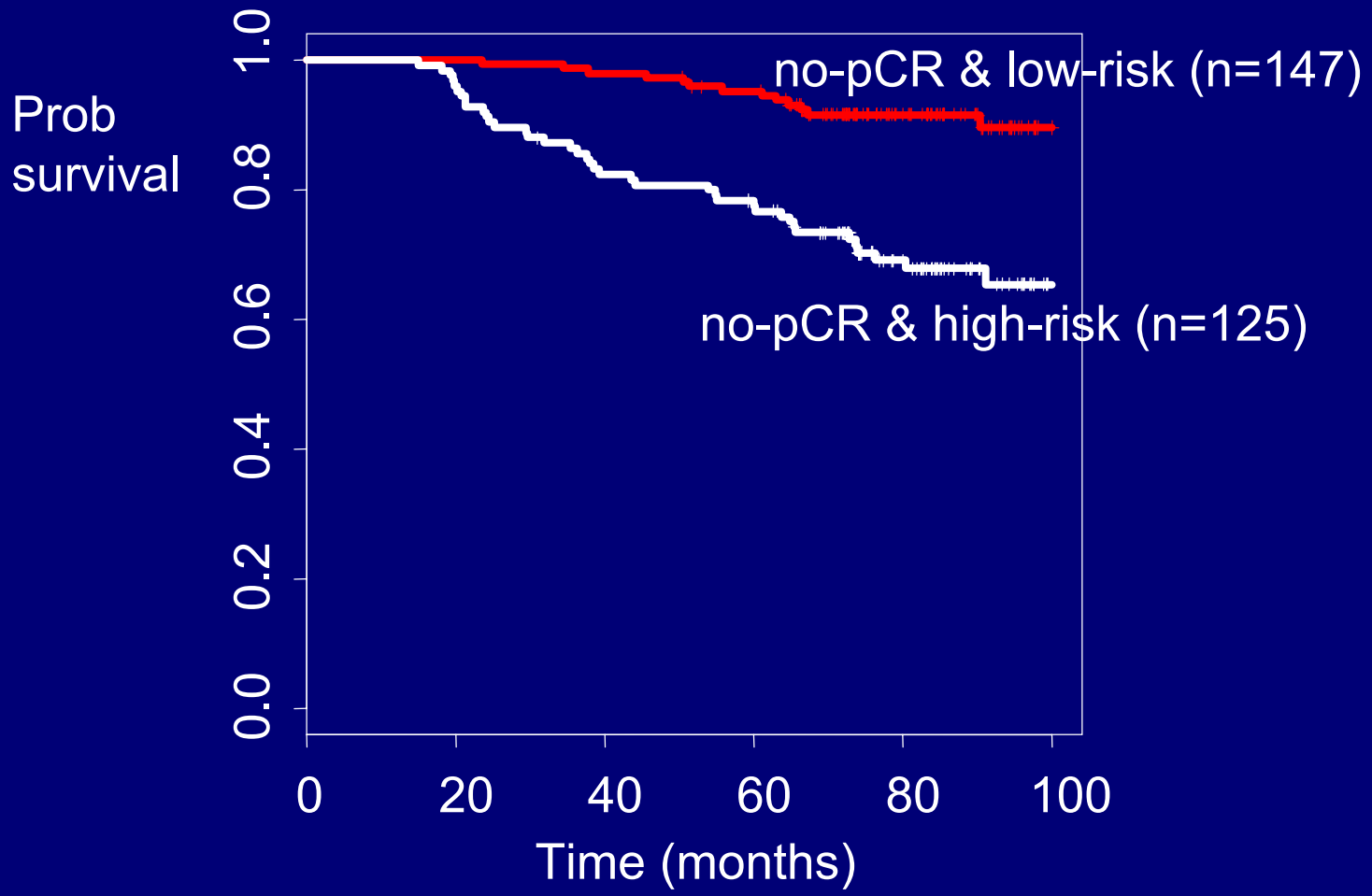
- RNA extraction using ROCHE kit
- 100 ng total RNA as starting material
- Hybridization to Affymetrix GeneChip U133 2.0 plus
- PAM and SUPERPC used for prediction of ER, pCR, and outcome

NSABP B-27

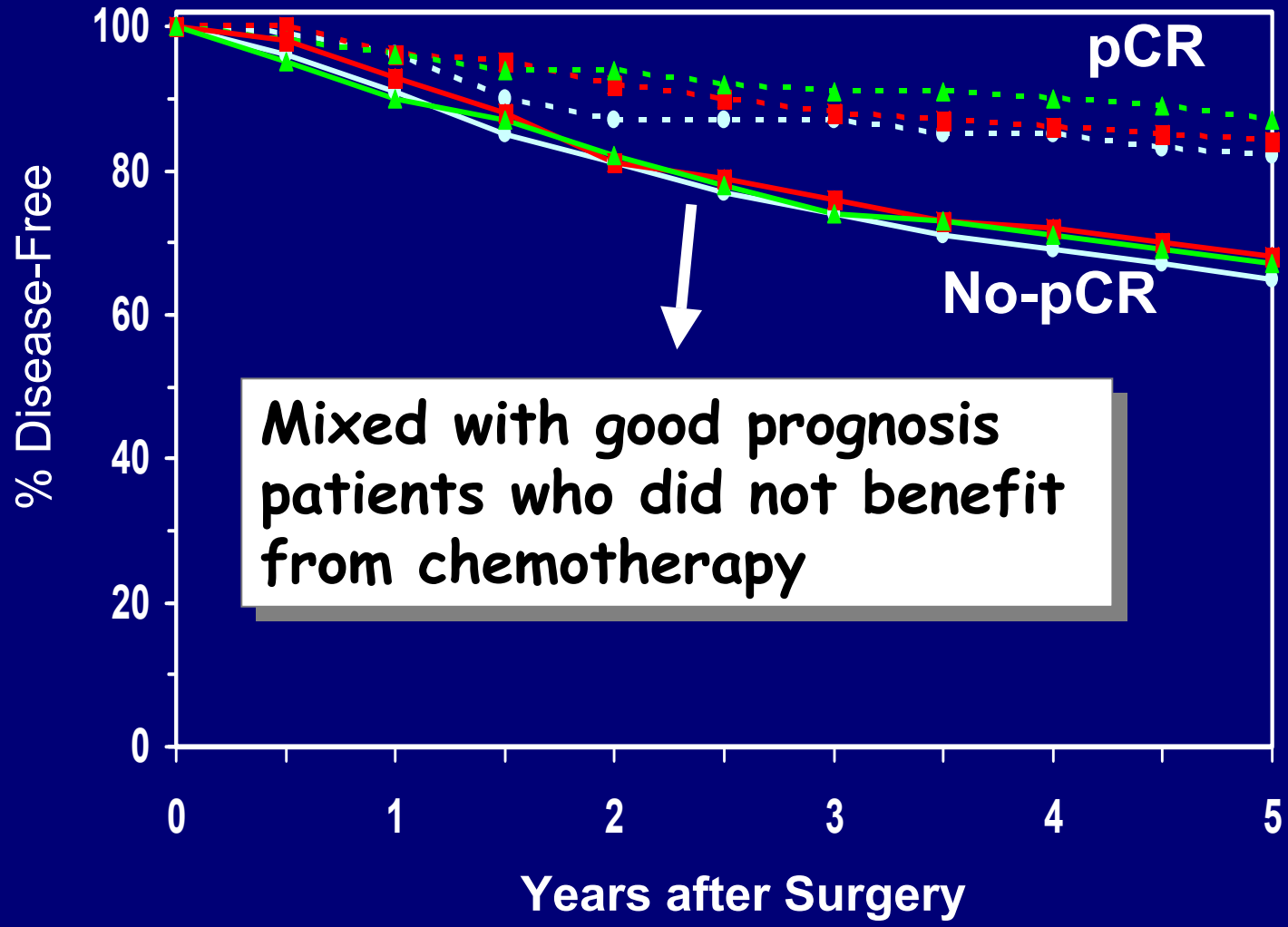
Gene expression and survival outcome



No-pCR group included both low and high risk patients



NSABP B-27
Problem of patient selection

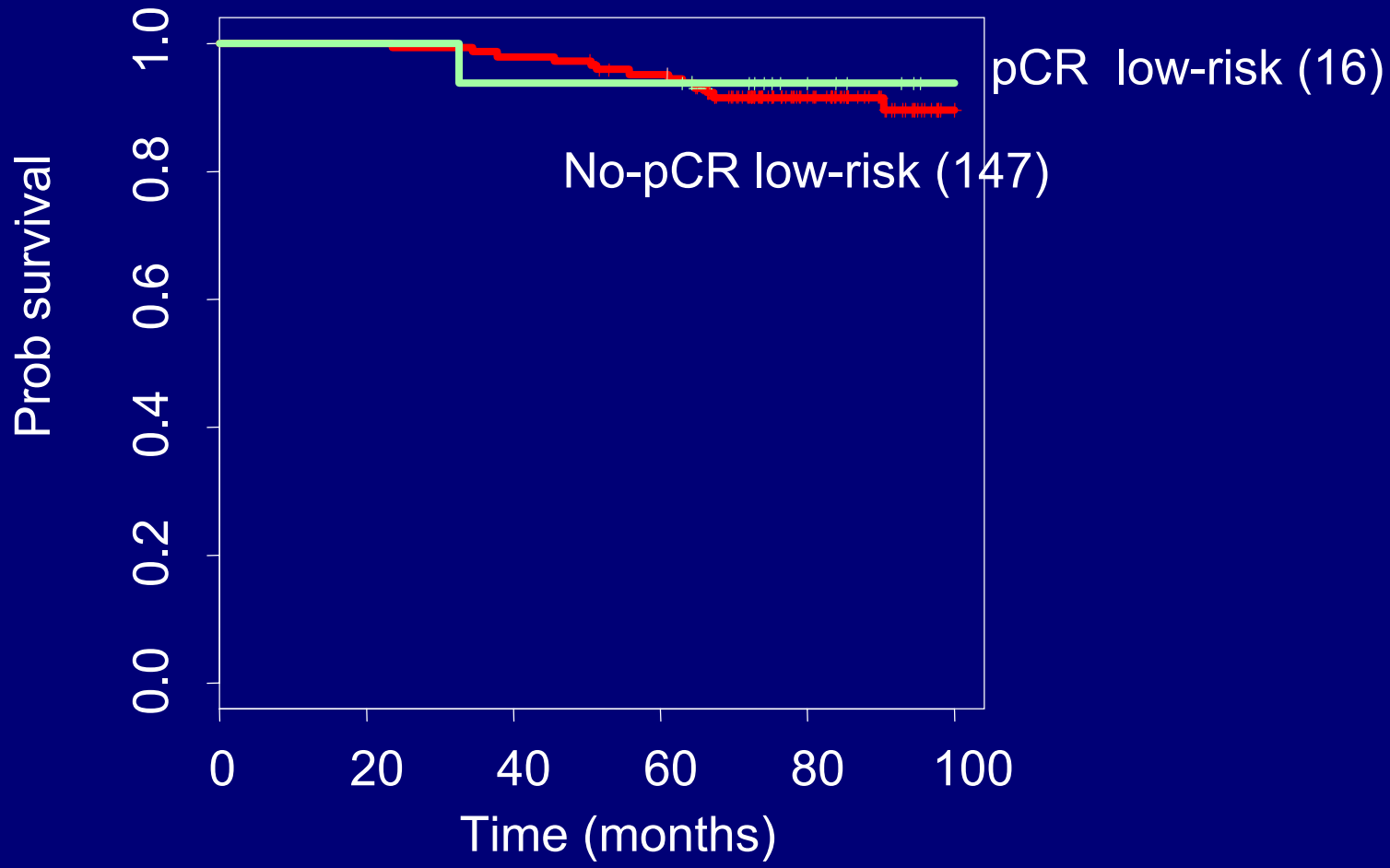


B-27 could have been more robust if only high-risk patients were enrolled

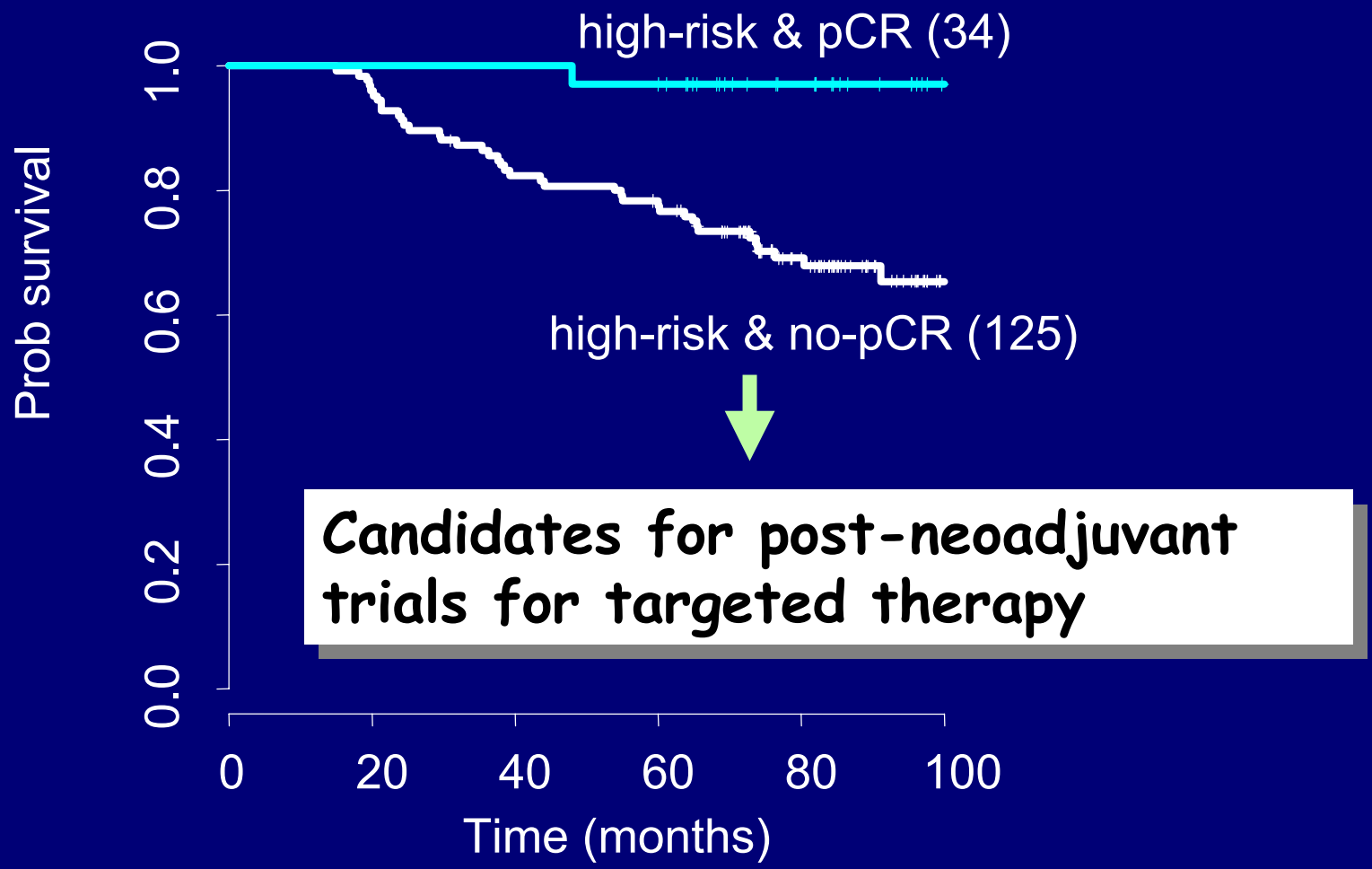
(no-pCR in high-risk has 65% rather than 75% 5YS)

% pCR	expected 5YS with no selection	expected 5YS with high-risk only
15	77.25%	68.8%
30	79.5	72.5
50	82.5	77.5

Low-risk patients had good outcome regardless of pCR



Combination of prognostic genes and pCR defines residual risk after chemotherapy



Can we predict pCR with gene expression profiling?

Prognostic Profile and pCR

	No-pCR	pCR
Low-risk	**147 (90%)**	**16 (10%)**
High-risk	**125 (79%)**	**34 (21%)**

The proportion of No-pCR in low-risk group is higher than expected (p-value=0.0067).

Microarray analysis of formalin fixed paraffin embedded B-27 core biopsy specimens

While prediction of ER status is very good……

		IHC (central lab)	
		ER-	ER+
Predicted by microarray	ER-	95	8
	ER+	4	206

Error rate = 3.8%

Microarray analysis of formalin fixed paraffin embedded B-27 core biopsy specimens

Prediction of pCR is poor

		Pathology	
		No pCR	**pCR**
Predicted by microarray	**No pCR**	**213**	**23**
	pCR	**59**	**27**

Error rate = 25.1%

Microarray analysis of formalin fixed paraffin embedded B-27 core biopsy specimens

Prediction of pCR in ER negative subset is better

		Pathology	
		No pCR	**pCR**
Predicted by microarray	**No pCR**	**67**	**9**
	pCR	**6**	**14**

Error rate = 15.6%

NSABP B-40

- Pre-treatment core biopsy mandatory
  - RNAlater for gene expression profiling
  - Formalin for validation and clinical adaptation of discovered expression profiles
  - Hank's buffer for In-vitro chemosensitivity assay

Conclusion

- Gene expression analysis of pre-treatment core biopsy provided biological explanation of NSABP B-27 data
- Combination of gene expression and pCR may identify patients who need more than chemotherapy
  - Validation study with ECTO and NSABP B-40