Gene expression based novel prognostic factors in breast cancer

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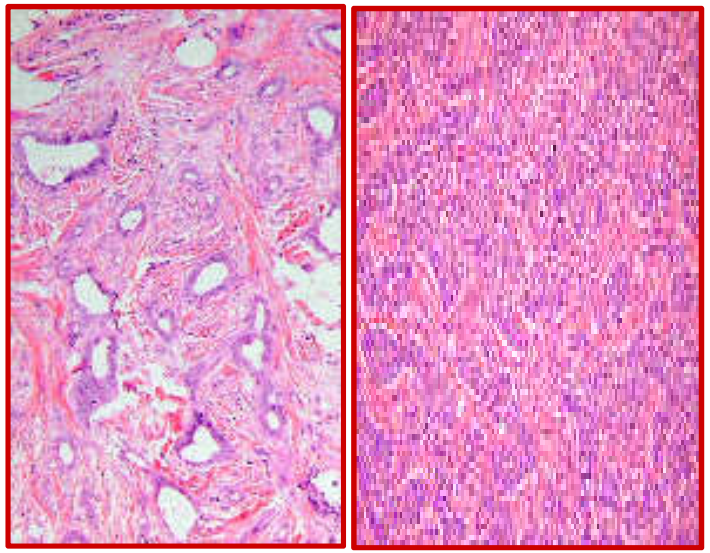
Prognosis = prediction of risk of recurrence

- Historically, prognosis was primarily determined by stage at diagnosis
  - (TNM = tumor size, lymph node spread, metastatic disease)
- < 6% of patients have distant metastasis at time of initial diagnosis.
- Death rate by stage if treated with surgery alone
  - DCIS 1-2%
  - Invasive / lymph node neg 30%
  - Invasive / lymph node pos 75%

Additional prognostic factors

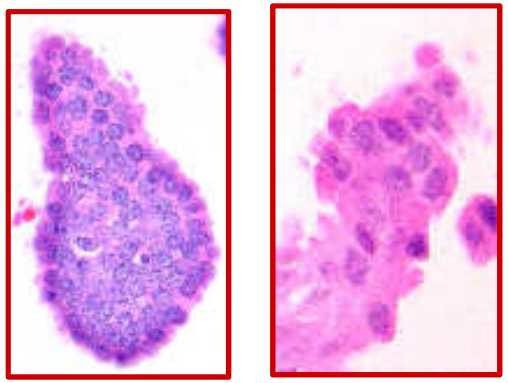
- p53 mutation
- S-phase fraction, Ki-67 index, mitosis
- histologic grade:

Tubule formation + Nuclear Grade + Mitotic index



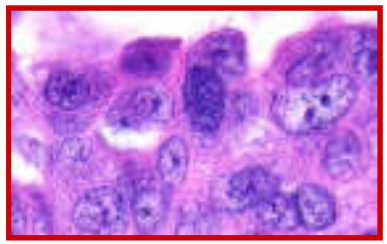
Lots of tubules

No tubules

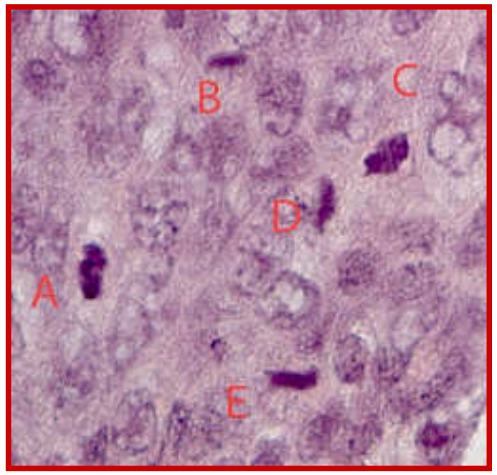


Low

Intermediate



High



Number of mitosis per 10 high power fields

Important points to keep in mind

- Stage at diagnosis and tumor grade are largely independent (i.e. a high grade tumor can present at low stage, a low grade tumor can present at high stage)

- Tumor grade likely stays consistent during the life of the tumor (i.e. a low grade tumor stays low grade even when it metastasizes). Is that true?

- High tumor grade, p53 mutation, high S-phase fraction, mitotic rate are poor prognostic factors that are all highly correlated with each other.

Current prognostic markers in the clinic

- Patient age
- Tumor size
- Histological type
- Grade (tubule formation, nuclear grade, mitotic index)
- Nodal status
- Stage
- Hormone receptor status
- Her2 status
- Proliferation markers (Ki-67, others)

- Nottingham Prognostic Index (NPI)
- Subtype (derived from ER, PgR, Her2, Ki67)
- !AdjuvantOnline
- Response to Chemotherapy in NEOADJUVANT setting

Prognostic factors (cont.)

- Myriad of biomarkers (with no / questionable validation)
- BRCA
- NPI+
- ERPI
- Multigene tests
  - Ancillary first-generation tests (Mammaprint, Oncotype)
  - Second-generation tests (PAM50)

Multigene tests

Tab. 1: The available assays for prediction of prognosis in breast cancer

Method/level	Test	Material used	Reference
Sequencing/DNA	BRCA1/2	Blood	[24]
FISH	HER2	FFPE	[6]
	3-gene (CYP24, PDCD6IP, and BIRC5)	FFPE	[25]
Expression array/RNA	70-gene Amsterdam	Frozen	[26]
	76-gene Rotterdam	Frozen	[15]
	97-gene Genomic Grade Index	Frozen	[13]
	163-gene Stroma-derived Prognostic Predictor	Frozen	[21]
	459-gene Wound-response signature	Frozen	[19]
qPCR/RNA	21-gene (Recurrence Score)	FFPE	[11]
	4-gene (MYBL2, KPNA2, CDC2, CDC20)	FFPE	[27]
	5-gene (HOXB13, IL17BR, CHDH, MIB1, MKI67)	FFPE	[28]
	8-gene (TOPFOX)	FFPE	[22]
	14-gene (Metastasis Test)	FFPE	[16]
	50-gene (PAM50)	FFPE	[9]
IHC/protein	ER	FFPE	
	PgR	FFPE	
	HER2	FFPE	[7]
	Ki67	FFPE	[29]
	IHC4 (ER, PgR, HER2, Ki67)	FFPE	
	5-marker (p53, NDRG1, CEACAM5, SLC7A5, and HTF9C)	FFPE	[30]

F. R. Stoddard, A. M. Szasz, B. Szekely, A.-M. Tokes, J. Kulka. **memo** (2011) Vol. 4: 1–5

Conclusions about supplementary prognostic tests

- The genes in molecular prognostic signatures are highly **correlated with proliferation** and work by identifying the highly proliferative subtypes of tumors.
- **ER- (and HER2+) tumors** are almost all high grade, high S-phase, high mitotic rate, frequent p53 mutation.
- However, **ER+/HER2- tumors** can be low or high grade and have a broad range of S-phase fraction and mitotic rate.
- **ER alone** is not a reliably strong prognostic marker
  - Although ER- tumors are high proliferation / ”poor” prognosis, the converse is not true. About **third of ER+ tumors** are equally high grade, high proliferation, and “poor” prognosis.
- **HER2 alone** is not a reliably strong prognostic marker
  - Similar issue: Although, HER2+ tumors are high proliferation / “poor” prognosis, the comparison **HER2- group** is not uniformly good prognosis. HER2 “negative” includes high grade triple negative tumors, and high grade ER+ tumors as well.

frontiers in GENETICS

ORIGINAL RESEARCH ARTICLE
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Current composite-feature classification methods do not outperform simple single-genes classifiers in breast cancer prognosis

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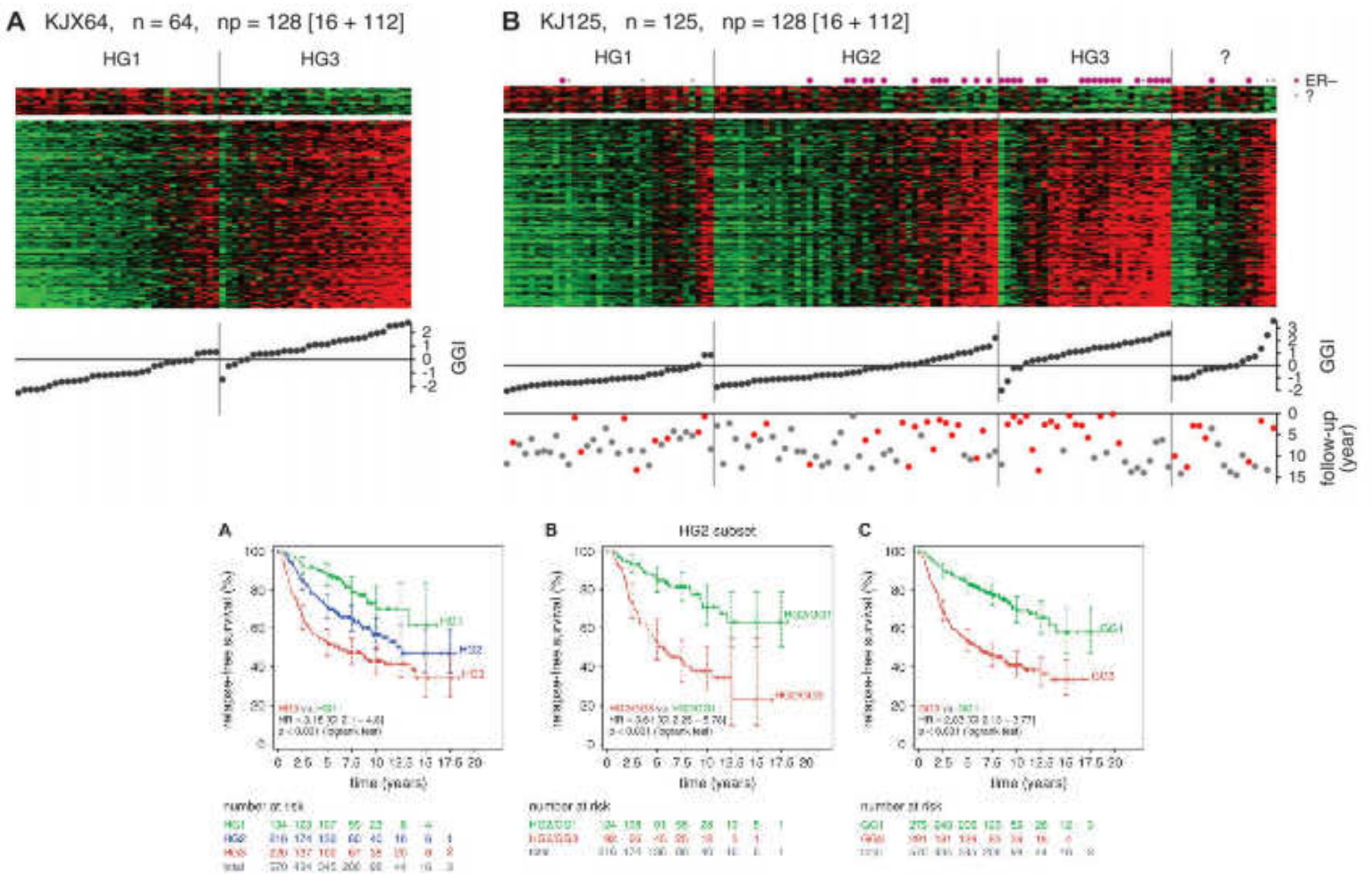
„ANY of the many thousands of genes that correlate with tumor proliferation and subtype will be equally good prognostic markers.”

Genomic Grade Index



Sotiriou et al. J Natl Cancer Inst 2006

CIN4 signature
(= TOP2A-FOXM1-TPX2-AURKA)

OPEN ACCESS Freely available online

PLOS | ONE

The CIN4 Chromosomal Instability qPCR Classifier Defines Tumor Aneuploidy and Stratifies Outcome in Grade 2 Breast Cancer

Attila Marcell Szász[1], Qiyuan Li[2], Aron C. Eklund[2], Zsófia Sztupinszki[3], Andrew Rowan[4], Anna-Mária Tőkés[1,5], Borbála Székely[1], András Kiss[1], Miklós Szendrői[6], Balázs Győrffy[3], Zoltán Szállási[2,7]*, Charles Swanton[4,8]*, Janina Kulka[1,9]*

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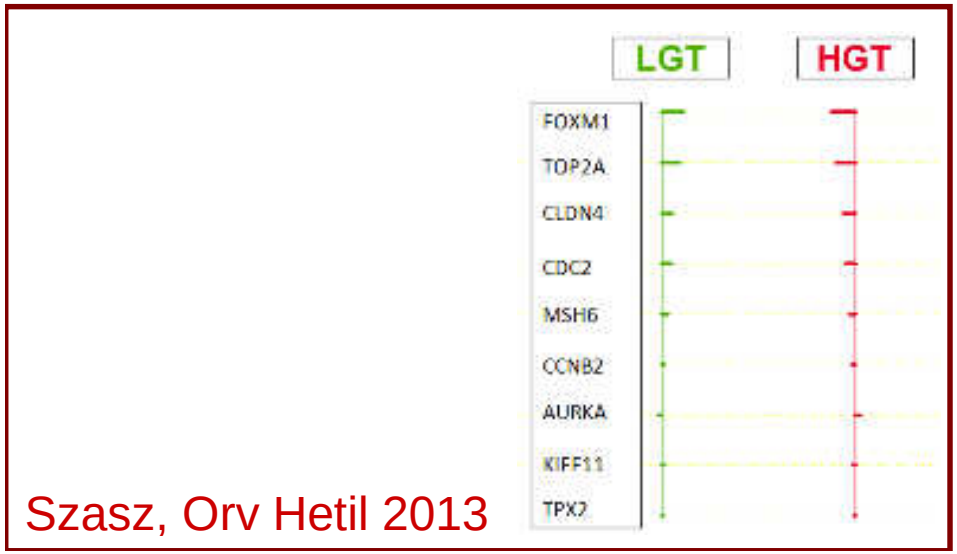
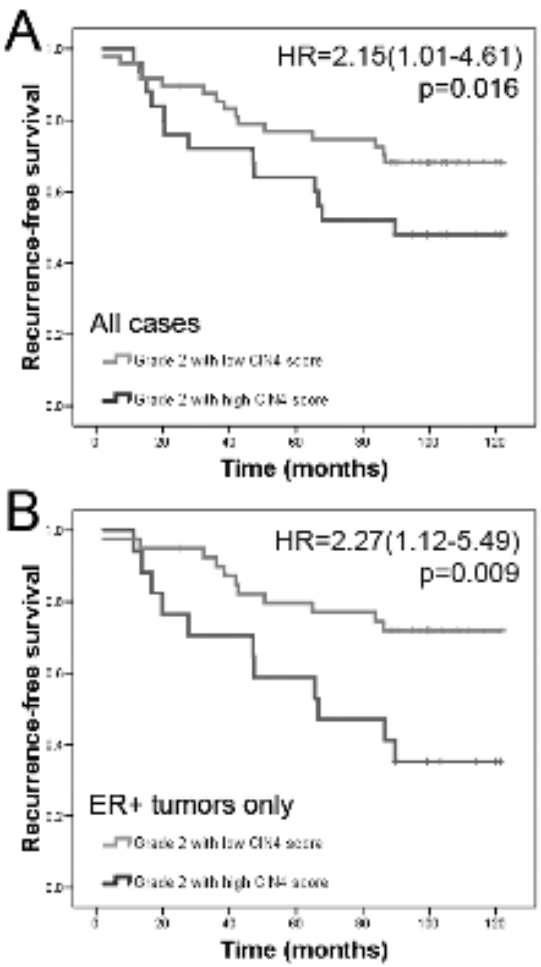


Szasz, Orv Hetil 2013



Szasz, PLoS1 2013

TOP2A-FOXM1-MKI67 signature

Breast Cancer Res Treat
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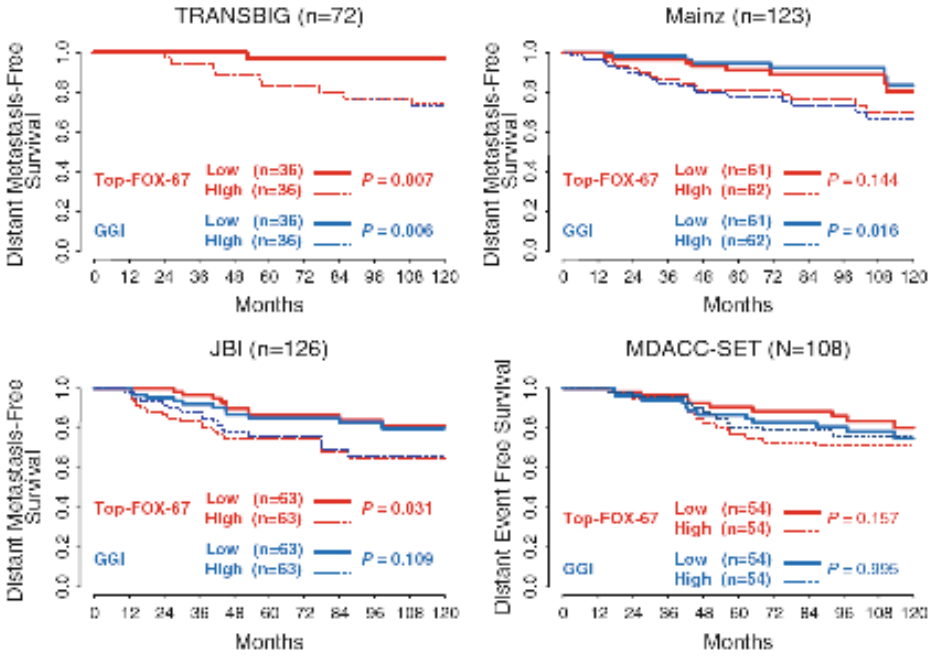
PRECLINICAL STUDY

A 3-gene proliferation score (TOP-FOX-67) can re-classify histological grade-2, ER-positive breast cancers into low- and high-risk prognostic categories

Borbala Szekely · Takayuki Iwamoto · A. Marcell Szasz · Yuan Qi · Junji Matsuoka · W. Fraser Symmans · Anna-Maria Tokes · Janina Kulka · Charles Swanton · Lajos Pusztai

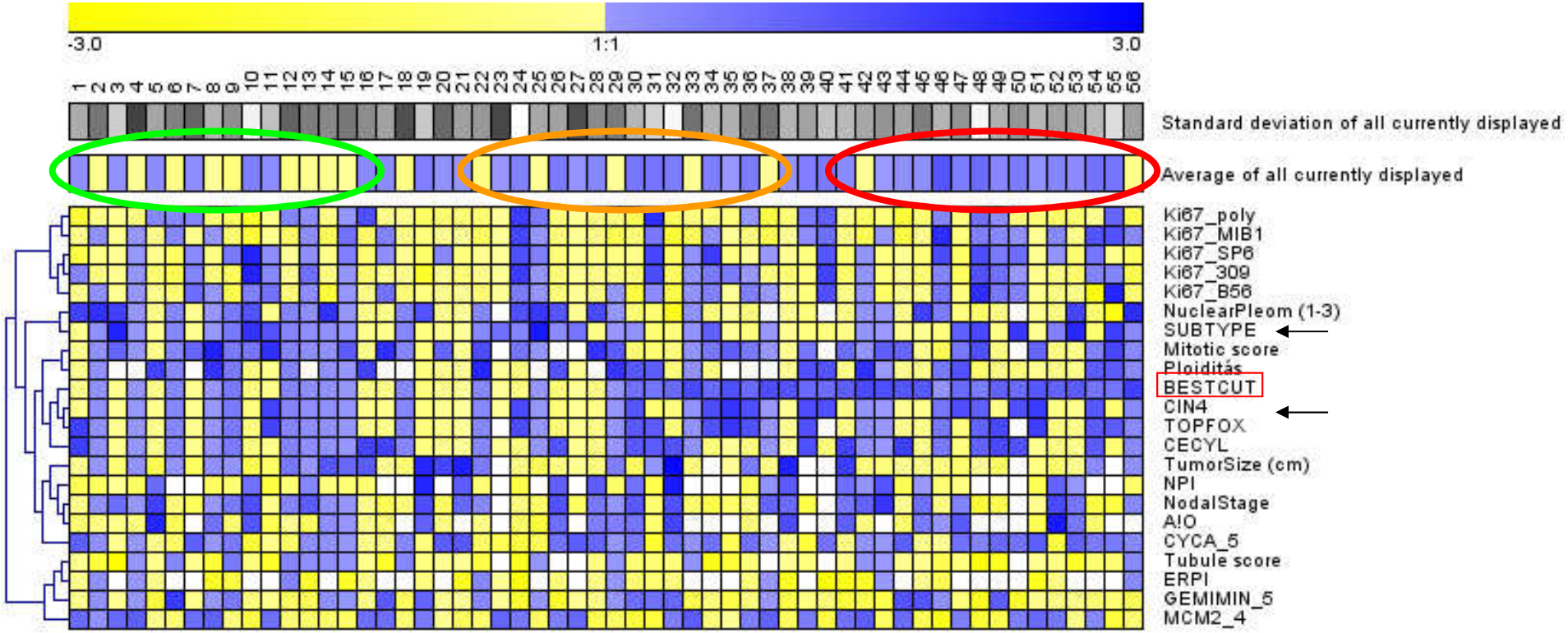
TOP-FOX-67 outperforms GGI in multivariate analysis.



Szekely, Breast Cancer Res Treat 2013

Previous studies (2007-2014) compared (GRADE 2)

← GOOD **prognózis** POOR→



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Charles Swanton (UK)

Zoltán Szállási
(DK/USA)

Janina Kulka

Balázs Győrffy

Anna-Mária Tőkés

Thank you

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