MEDICAL UNIVERSITY OF VIENNA

ABCSG
AUSTRIAN BREAST & COLORECTAL CANCER STUDY GROUP

Abstracts 120,130,200, 210

MicroRNA, DNA-Methylation, RNA-expression: "beating the old dog with a new stick?"

brief discussion

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COMPREHENSIVE CANCER CENTER VIENNA

Disclosures

- No relevant financial disclosures

- Collaborator and co-author of Ivana Sestak in related projects of the trans-ATAC/ABCSG group (Abstract 200).

Applying new tools to old questions

- What am I learning in terms of (breast) cancer biology? How is this relevant to clinically oriented research?

- What is the potential clinical relevance?
  - Analytic and clinical validity
  - Clinical utility
  - Is the information gained complementary ?

Molecular subtype of breast cancer metastases significantly influences patient post-relapse survival

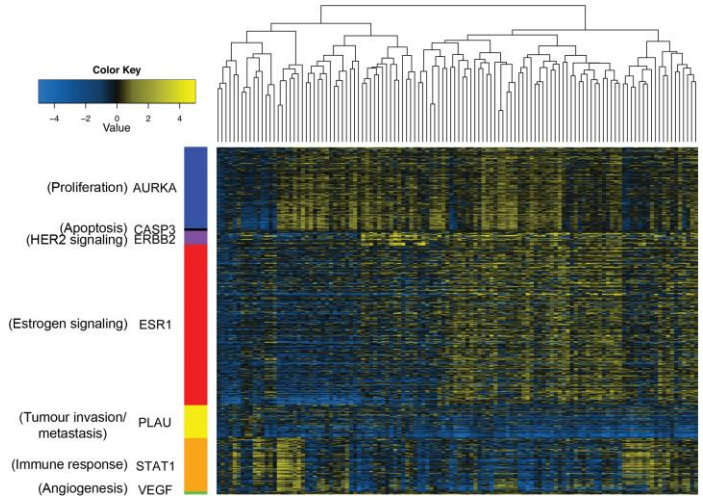
Nick Tobin, PhD

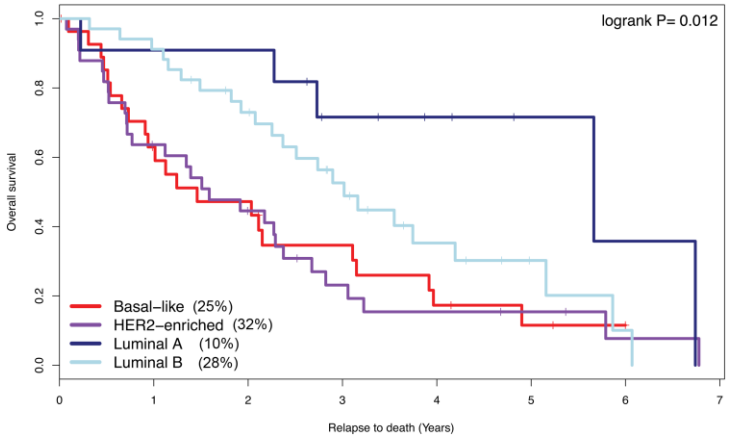
Nick Tobin, PhD
Karolinska Institutet

- Methods:
  - prospective collection of 120 FNA biopsies from metastatic sites
  - n=111; ET vs. TEX, phase III trial in recurrent BC
  - application of gene modules and PAM50 established in primary BC to metastatic samples
  - Descriptive analysis of relapses according to RNA expression patterns and survival analysis to detect prognostic relevance

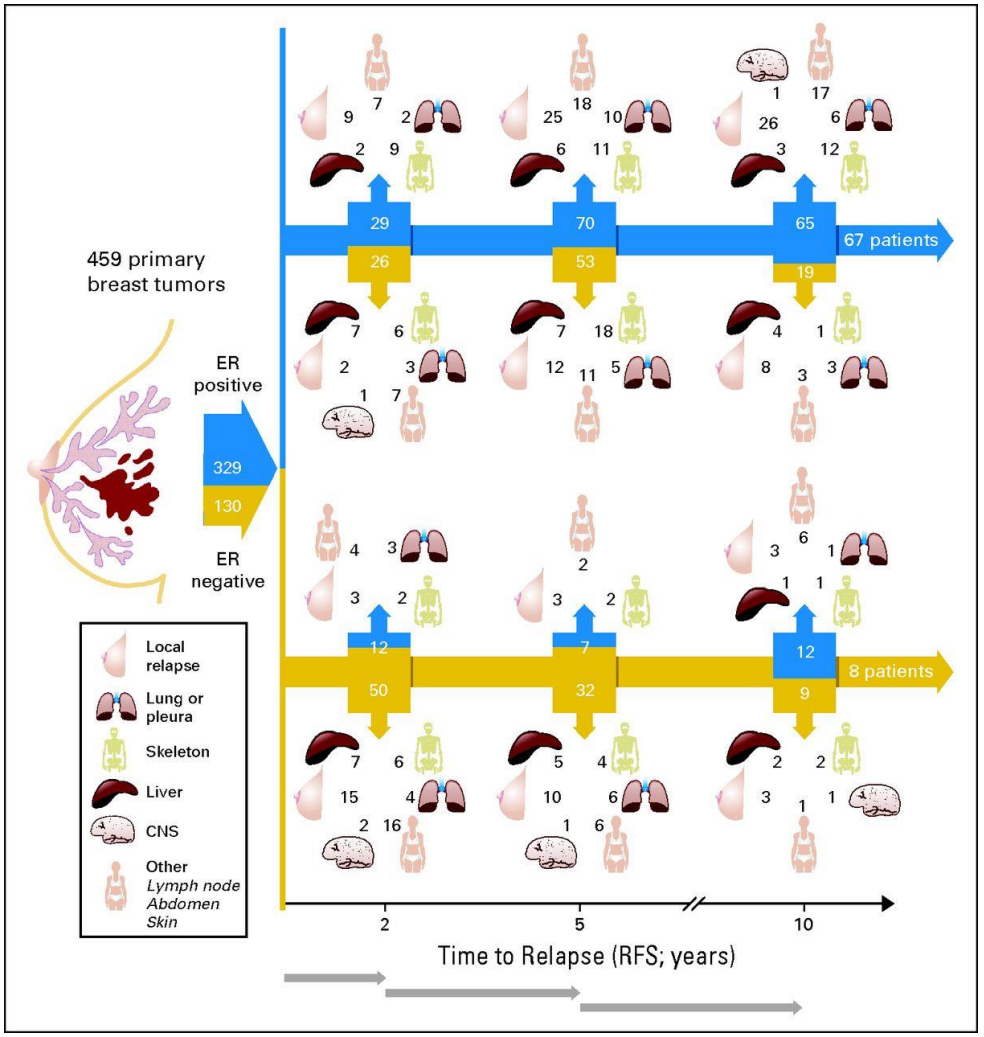
Main Findings

- Overrepresentation of:
  - low ER signaling, high proliferation, HER2 and angiogenic signaling
  - 25% basal, 32% HER2, 28% LUM B, 10% LUM A
- Both classifiers:
  - association with post-relapse overall survival



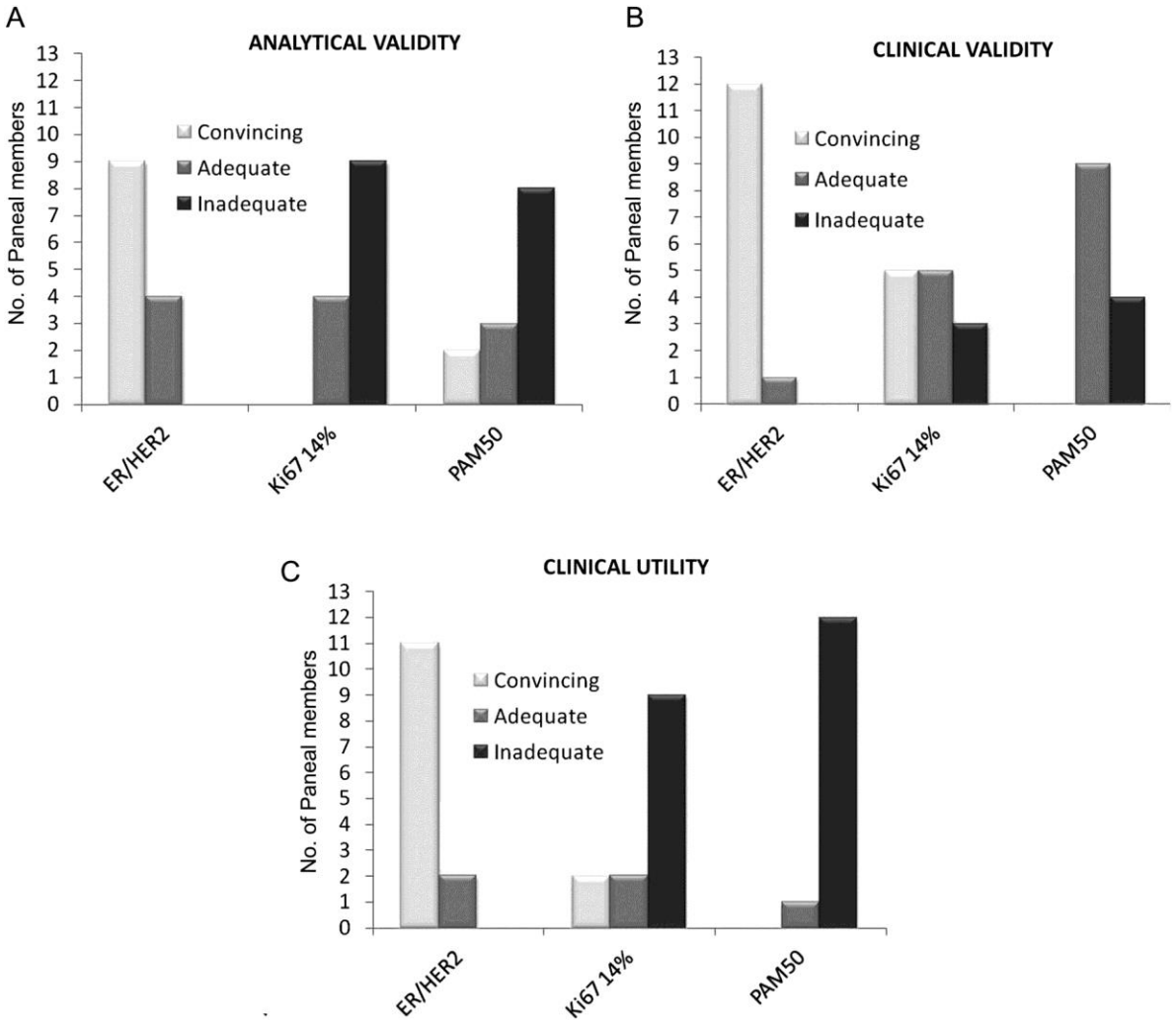


ER is unstable during disease progression….



Lindström L S et al. JCO 2012;30:2601-2608

Molecular subclasses of breast cancer: how do we define them? The IMPAKT 2012 Working Group Statement



Guiu S et al. Ann Oncol 2012;23:2997-3006

Annals of Oncology

Summary

- Elegant proof of principle: individual types of breast cancer change during disease progression. This data clearly adds detailed biology to what we know from IHC.

- Uncertain clinical validity:
  - Unlikely to detect more “actionable changes” than IHC
  - Little potential to find new actionable targets

IMPAKT BREAST CANCER CONFERENCE

IMProving cAre and Knowledge through Translational research

Evolutionary Patterns of microRNA expression through the Course of Disease and Treatment in Recurrent Breast Cancer

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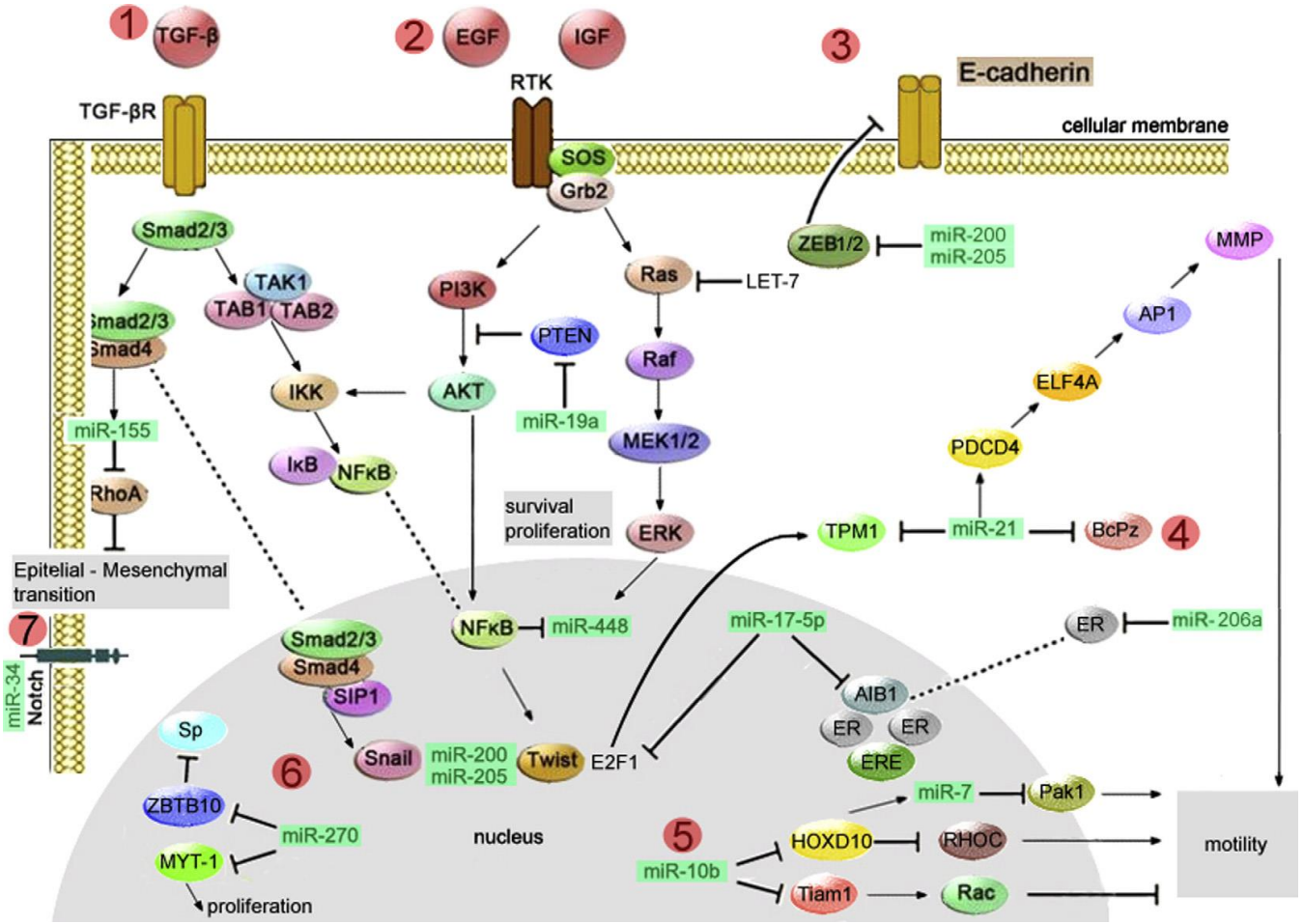
The Chaim Sheba Medical Center at Tel Hashomer · Est. 1948 · The Hospital of Israel

miRNAs interact with oncogenic pathways



Cancer Treatment Reviews, Serpicio et al. 2014

miRs are associated with subtype and therapy response

Table 3
miRNAs and breast cancer subtypes.

Signature*	Cancer subtype	Refs.
Up: miR-150, -142-3p, -142-5p, 148a, -106a/b, -18a, -93, -155, -25, -187, -135b	Basal-like	[58]
Up: miR-150, -142-3p, -142-5p, -148a, -106b, -93, -155, -25, -187, -375 Down: miR-125a and b	HER2 positive	[58,59,62]
Up: miR-191, -26, -126, -136, -100, -99a, -145, -146b, -10a, -199a/b, -130a, -30a-3p,-30a-5p, -224, -214, let-7a/b/c/f, -342 Down: miR-206, -15b, -107, -103	Luminal A	[7,58,59]
Up: miR-191, -26, -106a/b, -93, -25, -10a, -30a-3p, -30a-5p, -224, let-7b/c/f and -342, -15b, -107, -103 Down: miR-206, -100, -99a, -130, -126, -136, -146b	Luminal B	[7,58,59]
Up: miR-142-5p, -135b, -126, -136, -100, -99a, -145, -10a, -199a/b, -130a, -30a-3p, -214, -7a/c	Normal-like	[58]

* As compared with normal tissue or with parental cell lines in case of preclinical data.

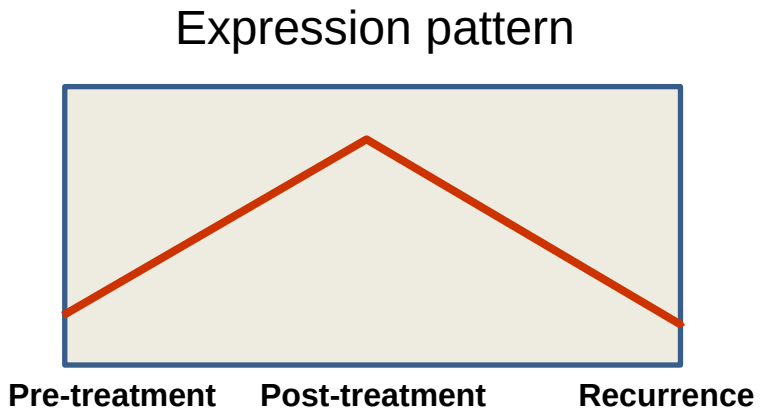
Table 4
miRNAs involved in resistance/sensitivity to current breast cancer therapeutic strategies.

miRNA	Drug		Anti-HER2	Mechanism	Type of evidence (Refs.)
miR-15a/16	Tamoxifen			Bcl2	Preclinical [72]
miR-21		Topotecan Paclitaxel	Trastuzumab		Preclinical Clinical [17,67,76]
miR-30c	Tamoxifen				Clinical [74]
miR-125b		Paclitaxel Anthracycline		Bak1	Preclinical Clinical [68,69]
miR-128	Letrozole			TGF-β	Preclinical [73]
miR-205			Trastuzumab Lapatinib	Akt	Preclinical [32]
miR-221/222	Tamoxifen Fulvestrant		Trastuzumab	p27Kip1 b-Catenin	Preclinical [70,71]
miR-301	Tamoxifen			Akt	Preclinical [63]
miR-326		Doxorubicin Etoposide		MRP1	Clinical [65]
miR-451		Doxorubicin			Preclinical [66]
miR-548d-3p/559			Trastuzumab	ERBB2	Preclinical [75]

Cancer Treatment Reviews, Serpicio et al. 2014

Excellent rationale: miRs to predict response and prognosis…

- Longitudinal observation to detect patterns
- of miR expression from FFPE matched samples
- on the nCounter miRNA expression assay
- n= 20 (10 with recurrence), pre/post NACT and metastasis.
- Example:

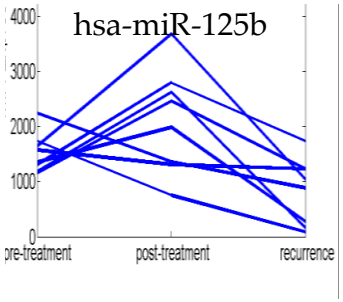


Main Findings I

- 21miRs shared down/up/down pattern
- 13/21 miRs differentially expressed in BC
- This set of miRs enriched in cell cycle pathways

Identify a significant set of miRNAs

13/21 significantly down-regulated in breast cancer	Significantly enriched for cell cycle pathway



Main Findings II

- In each patient the Λ pattern correlates with response to NACT, the absence of Λ to resistance
- Recurrence invariably has the lowest expression of the 13miR set in comparison to pre and post-NACT sample
- Finally: absolute expression of the 13miR set at diagnosis is a prognostic marker

Biology

- Pattern analysis (as opposed to absolute expression levels) reveals miRs that are clearly informative about
  - a) response and b) prognosis
- This data helps direct miRNA research toward breast cancer biology
- This data reveals how miRNA interacts with known biologic pathways

Clinical validity

- Can this data add information to other models/markers of
  - response and prognosis
  - such as ER, Grading, RNA/DNA based markers?
  - or algorithms thereof

- Would a longitudinal miR approach like this work with liquid biopsy- and thus add prognostic or predictive information for post-treatment survival?

Analysis of multigene scores for the prediction of distant recurrence according to non-clinical baseline factors

Ivana Sestak
Mitch Dowsett, Sean Ferree , J. Wayne Cowens,
Frederick L. Baehner, Jack Cuzick
on behalf of the ATAC/LATTE Trialists' Group

Centre for Cancer Prevention, Queen Mary University of London, London, UK
Royal Marsden Hospital, London, UK
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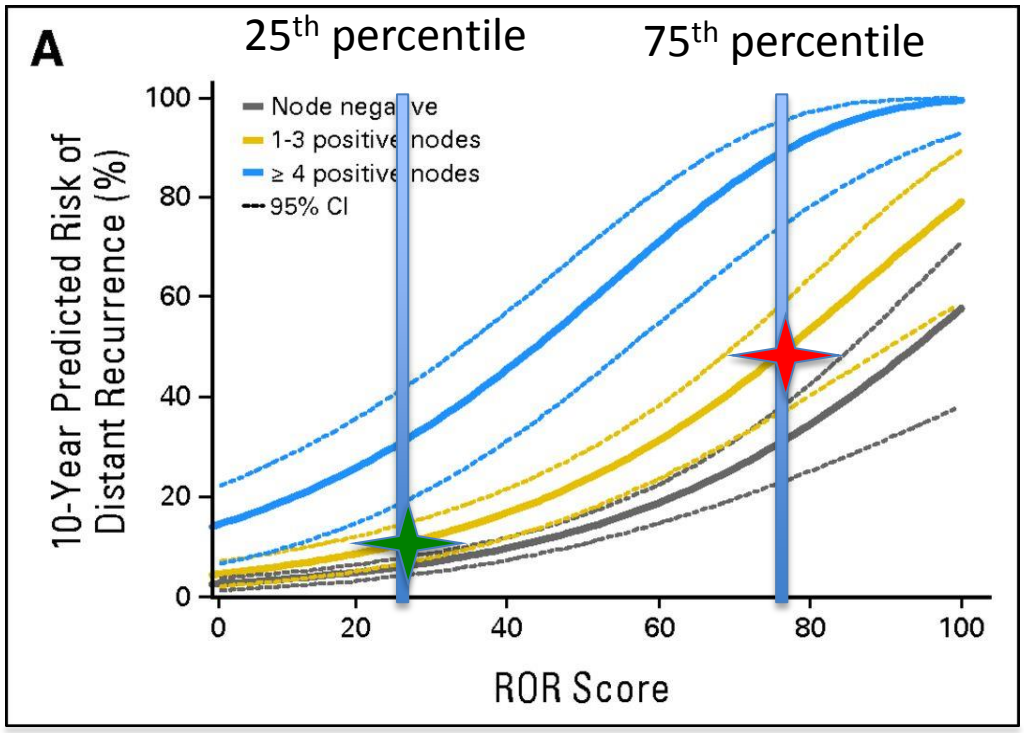
Genomic Health, Redwood City, CA, USA

Do non-tumour derived factors influence prognostic scores?

- Methods:
  - 940 ER+ postmenopausal women- from ATAC
  - AI or TAM-treated in the absence of chemotherapy
  - primary endpoint: distant recurrence
  - HRs from Cox models to describe impact of:
    - Age, BMI, prior HRT, Smoking, Hysterectomy, RT, Mastectomy
  - ROR, RS, IHC4 as continuous variables and all adjusted for CTS

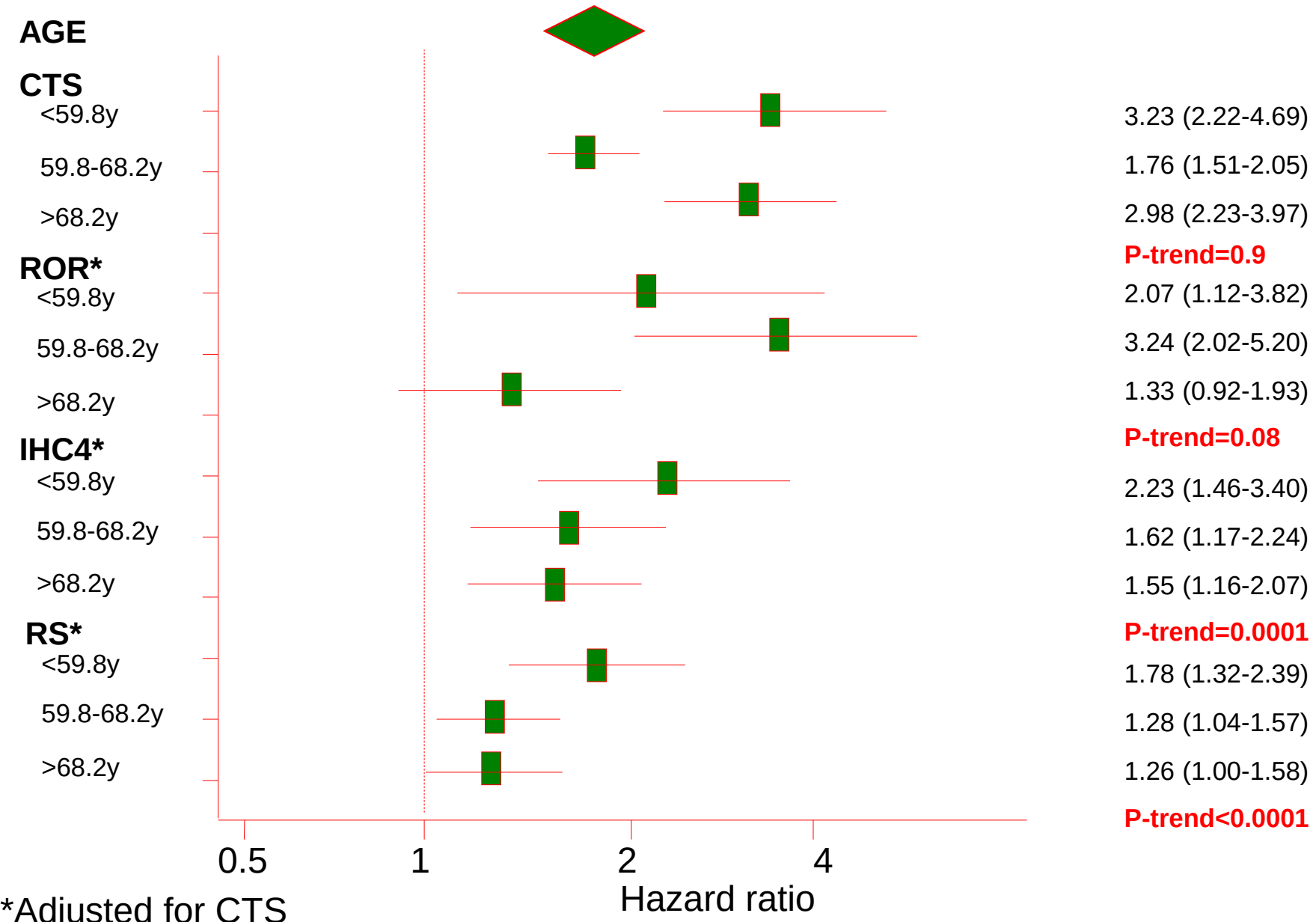
Dowsett M et al. JCO 2013;31:2783-2790



= 4x

Impact of age on scores for all patients

Hazard Ratios (95% CI)



*Adjusted for CTS

Main Findings

- No detectable influence of HRT, Smoking, Hysterectomy, Radiotherapy, Mastectomy
- CTS and molecular scores work less well in women >68 years.
- Most prognostic information is added in normal and overweight women, but less in obese women

Biology…What could be behind age?

- Features of "Immunosenescence"[1]
  - Impaired ability to respond to new antigens
  - Unsustained memory responses
  - Increasing incidence of immune disorder
  - Sustained, low-grade inflammation
- Angiogenesis/wound healing ?
- metabolic factors associated rather with age than BMI?

[1]Goronzy and Weyand, Nat. Immunol. 2013

Clinical validity

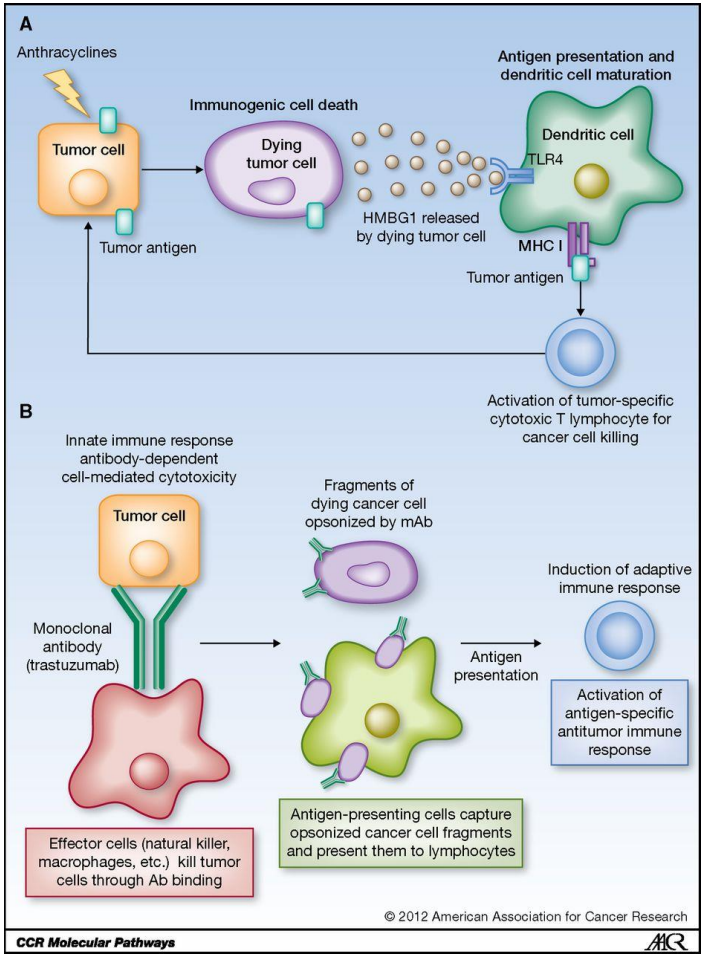
- Retrospective unplanned analyses
- Validation in similar populations needed
- “Host factors” are often disregarded in MDT meetings
- Both tumor derived and patient derived factors are likely to influence the decision to a) order a molecular score b) decision-making concerning adjuvant therapy.

IMPAKT Breast Cancer Conference

A novel methylation signature that reflects intratumoral lymphocyte infiltration in breast cancer and predicts for response to anthracycline treatment

J. Jeschke, M. Bizet, C. Desmedt, M. Defrance, S. Dedeurwaerder, E. Calonne, C. Sotiriou, F. Fuks

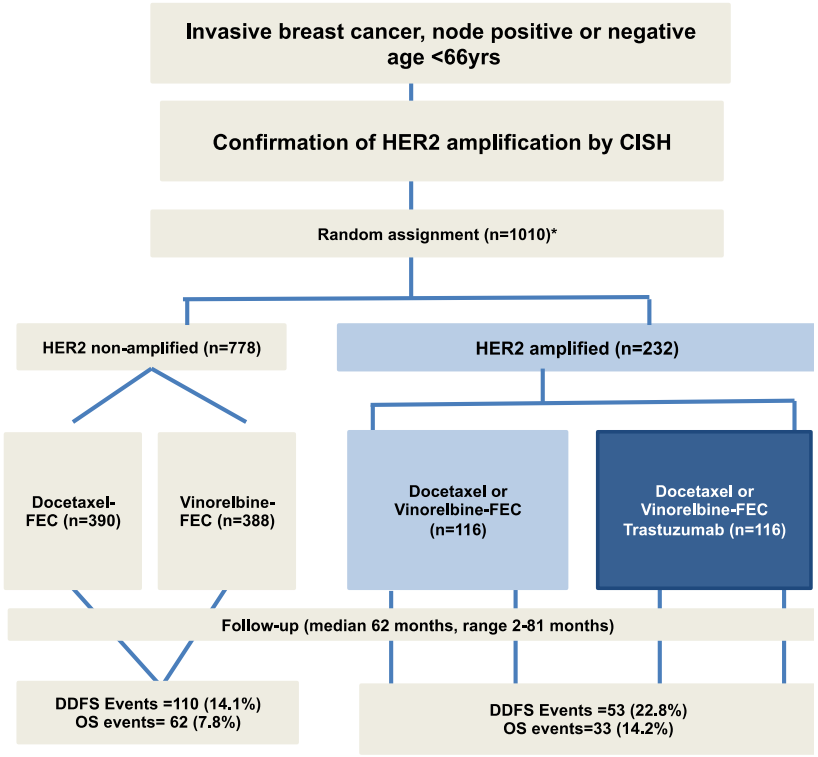
Immune infiltrates have IMPAKT



Fabrice Andre, Maria V. Dieci, Peter Dubsky, Christos Sotiriou, Giuseppe Curigliano, Carsten Denkert, and Sherene Loi.
Clin Cancer Res 2013;19:28-33

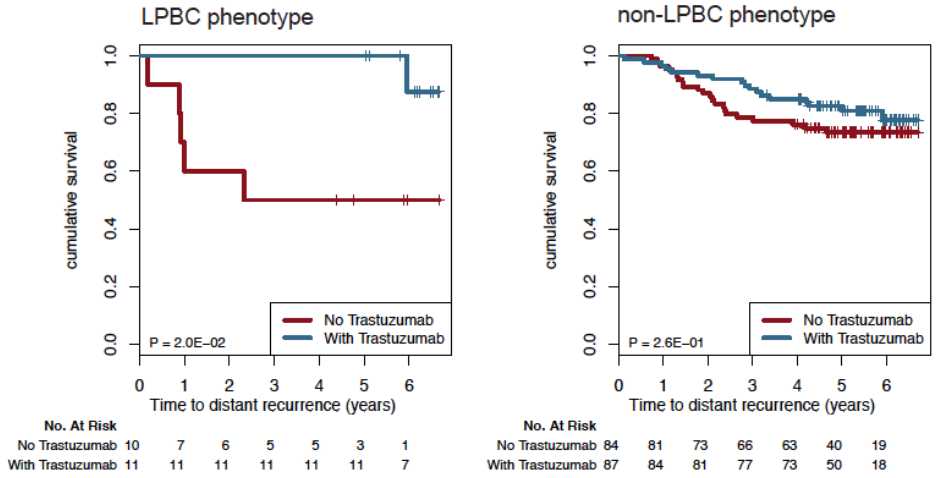
FinHER Study Schema

Joensuu et al, NEJM 2006



Note: all patients that were ER-positive received Tamoxifen/Aromatase inhibitor for 5yrs

Lymphocyte Predominant Breast Cancer Phenotype (LPBC)
>50% infiltration



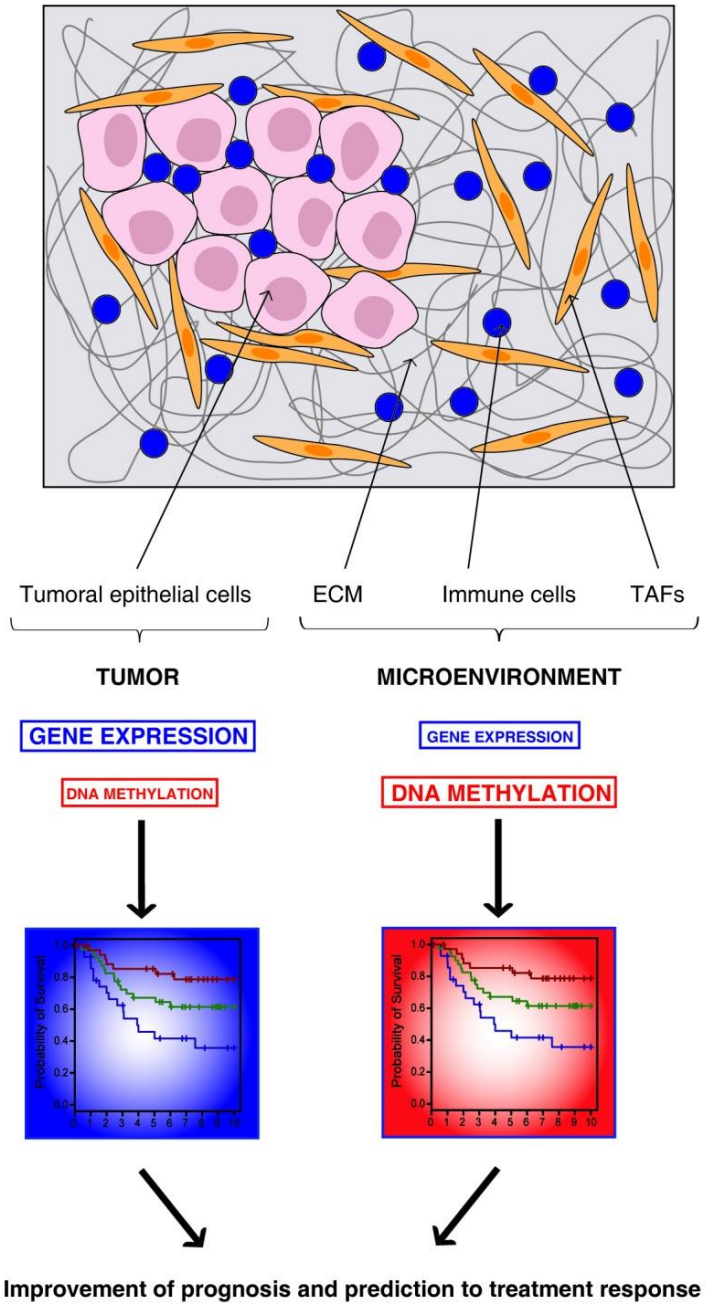
Loi et al. ASCO 2013

Interaction LPBC and Trastuzumab!!

Validation in GeparQuatro , SABCS 2013 !

SABCS 2013- Level I Evidence

	Loi et al	Adams et al
Randomized Ph III trial	BIG 02-98; 8-year f/u	E2197, E1199; 10.6- year f/u
	256 TNBC, all LN pos	481 TNBC, 59% LN pos
Methods	REMARK & pre-specified analysis	REMARK & pre-specified analysis
	H&E full section	H&E full section
	2 pathologists independently	2 pathologists jointly
	Analyzed in 10% increments + binary	Analyzed in 10% increments + binary
TIL%	Median: 20 sTIL, 5 iTIL	Median:10 sTIL, 0 iTIL
	LPBC: 10.6	LPBC: 4.4
LPBC vs <50% TIL	HR 0.31 (p=0.02, DFS)	HR 0.58 (p=0.18, DFS)
Intraepi TIL, 10% increase	HR 0.83 (p=0.1, DFS)	HR 0.72 (p=0.06)
	HR 0.73 (p=0.03, OS)	HR 0.64 (p=0.08)
Stromal TIL, 10% increase	HR 0.84 (p=0.02, DFS)	HR 0.86 (p=0.02, DFS)
	HR 0.82 (p=0.02, OS)	HR 0.81 (p=0.01, OS)
	HR 0.85 (p=0.02, DFS multivariate)	HR 0.84 (p=0.005, DFS multivariate)
	HR 0.83 (p=0.02, OS multivariate)	HR 0.79 (p=0.003, OS multivariate)

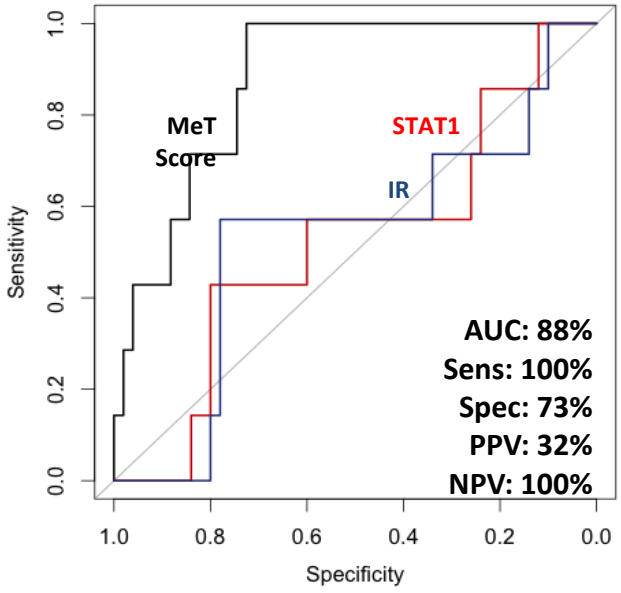


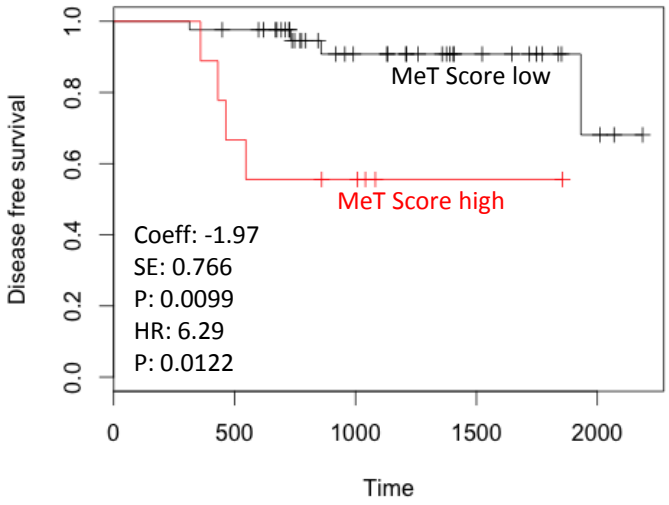
- DNA methylation profiling is a sensitive tool to capture BC infiltration of immune cells.
- Specifically T-cell marker genes correlate with clinical outcomes.

Dedeurwaerder and Fuks, Oncoimmunology 2013

Methods and Findings

- MeT signature derived from breast cancer cell lines versus T-cell lines
- Validated in the TOP cohort
- Optimized in the same cohort

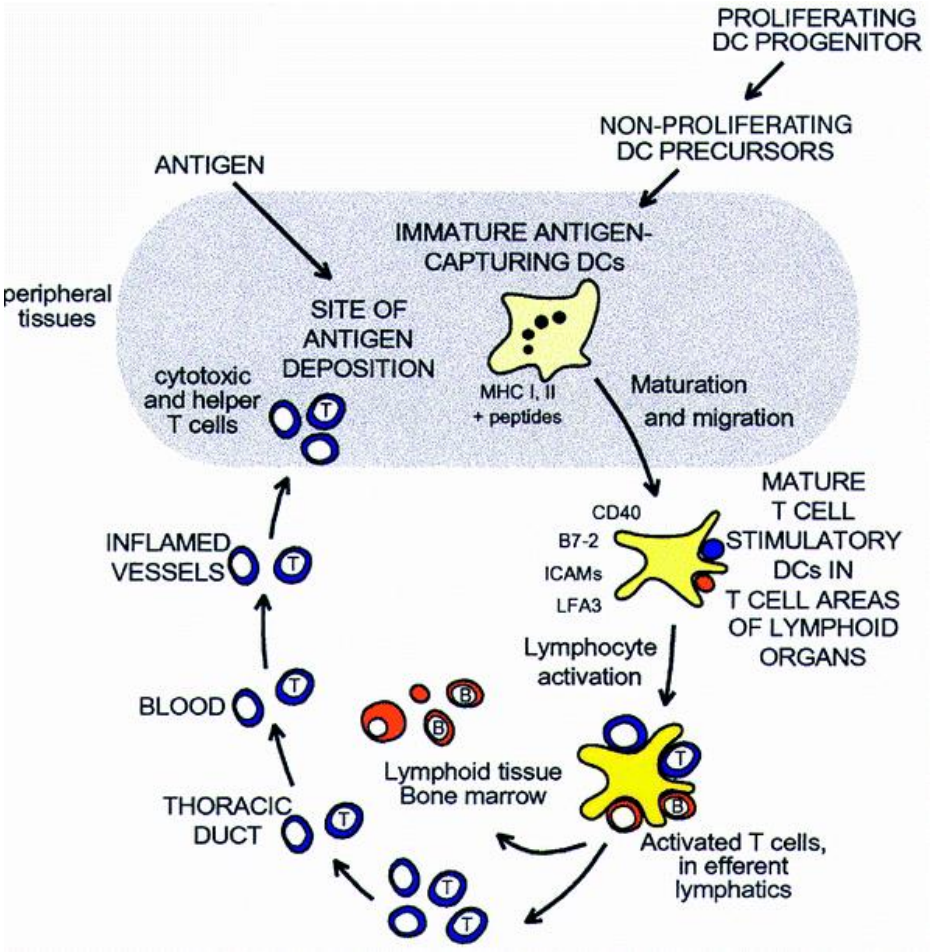




Clinical Impact: Anthracyline response

- Analytic validity:
  - H&E staining of TILs vs. Methylation arrays?
- Clinical validity:
  - H&E measure of sTILs is validated in TNBC.. How would a methylation array perform? Is the information complementary?
- Clinical utility:
  - Potentially this technology could be combined with IHC of CEP17 and TOP2A to predict benefit from Anthracylines…

Enriching classic Immunology using new biotech…



Banchereau and Steinman 1998

Biology

- Epigenetic activation and silencing is complementary to the current understanding of cancer immunology
- Epigenetics clearly ad a layer of complexity to the interaction of T-cell activation via APC
- What are the links between the biochemistry of methylation and the (co)-stimulatory activation via dendritic cells? This is relevant to therapies involving costimulatory blockade but also to mAb targeting HER2, EGFR etc.

Thank You