Conflict of interest: None

ER+/HER2+ and ER-/HER2+ breast cancers are molecularly distinct but immune gene signatures are prognostic and predictive in both groups

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Background

- In HER2-positive breast cancer, estrogen-receptor (ER) positive and negative tumors represent molecularly distinct entities (Loi S et al *IMPAKT* 2010; Bianchini G et al. *ASCO* 2011)

- ER+/HER2+ and ER-/HER2+ tumors showed a different clinical behavior
  - The pCR rate after neoadjuvant chemotherapy alone or in combination with targeted agents was significantly lower in ER+ tumors (Gianni L Lancet 2010; von Minckwitz G JCO 2012, Untch M Lancet Oncol 2012, Gianni L Lancet Oncol 2012, *Baselga J Lancet 2012)*
  - pCR was associated with improved DFS in ER-/HER2+ but not ER+/HER2+ tumors (von Minckwitz G JCO 2012)

- In HER2-negative tumors, different biological processes are associated with prognosis and chemotherapy response in ER+ and ER- (Iwamoto T. JNCI 2011). This aspect has not been addressed in HER2-positive tumors

Aims

- We examined gene expression differences among HER2 positive breast cancers by ER status

- We also assessed the prognostic and chemotherapy response predictive values of over 3000 *a priori* defined gene sets separately in HER2+/ER positive and HER2+/ER negative tumors

Publically available, clinically annotated Affymetrix gene expression data sets used in this analysis

	Mixed Therapies†		Pure Prognostic‡	Predictive (Neoadjuvant chemo) §	
	Cohort 1 (U133a)	Cohort 2 (Plus2)	(Node negative, untreated)	Taxane	Anthracycline
Overall	594	1291	781	613	114
HER2-neg					
ER-pos	381	739	495	228	1
ER-neg	169	425	168	324	86
HER2-pos					
ER-pos	44	127	58	27	2
ER-neg	31	131	60	34	25

† GEO: GSE1456, GSE1561, GSE2603, GSE31519, GSE3494, GSE10780, GSE12276, GSE12763, GSE13787, GSE16391, GSE19615, GSE20711, GSE2109, GSE21653, GSE3744, GSE5460

‡ GEO: GSE11121, GSE2034, GSE2990, GSE5327, GSE7390

§ GEO: GSE20194, GSE20271, GSE23988, GSE25066, GSE16464

Methods

- MAS5 normalization was performed to a median target array intensity of 600 and data was transformed to log2 values

- ER and HER2 status were defined by gene expression data as previously reported (Gong Lancet Oncol 2007, Bianchini JCO 2010, Iwamoto JNCI 2011)

- We assessed prognostic and predictive values of ~3000 gene pathways from Gene Ontology, KEGG, BioCarta, and Lymphoid signatures using the Efron's Tibshirani method implemented in BRB-ArrayTools (developed by Richard Simon and BRB-ArrayTools Development Team)

- Distant Metastasis Free Survival was the endpoint used to assess gene sets associated with prognosis

- Pathological complete response in breast and axilla was the endpoint used to assess the predictive value of gene sets

Gene expression differences between ER+/HER2+ and ER-/HER2+ breast cancers

- Gene expression differences between ER+ and ER- cancers were defined in two independent discovery datasets for which outcome annotation was not available (put here the GEO accession numbers)

- Probe sets were defined as differentially expressed if $p<0.001$ in both discovery datasets (combined p value $\leq$ 1.00E-05 by Fisher's Chi-squared combined probability test)
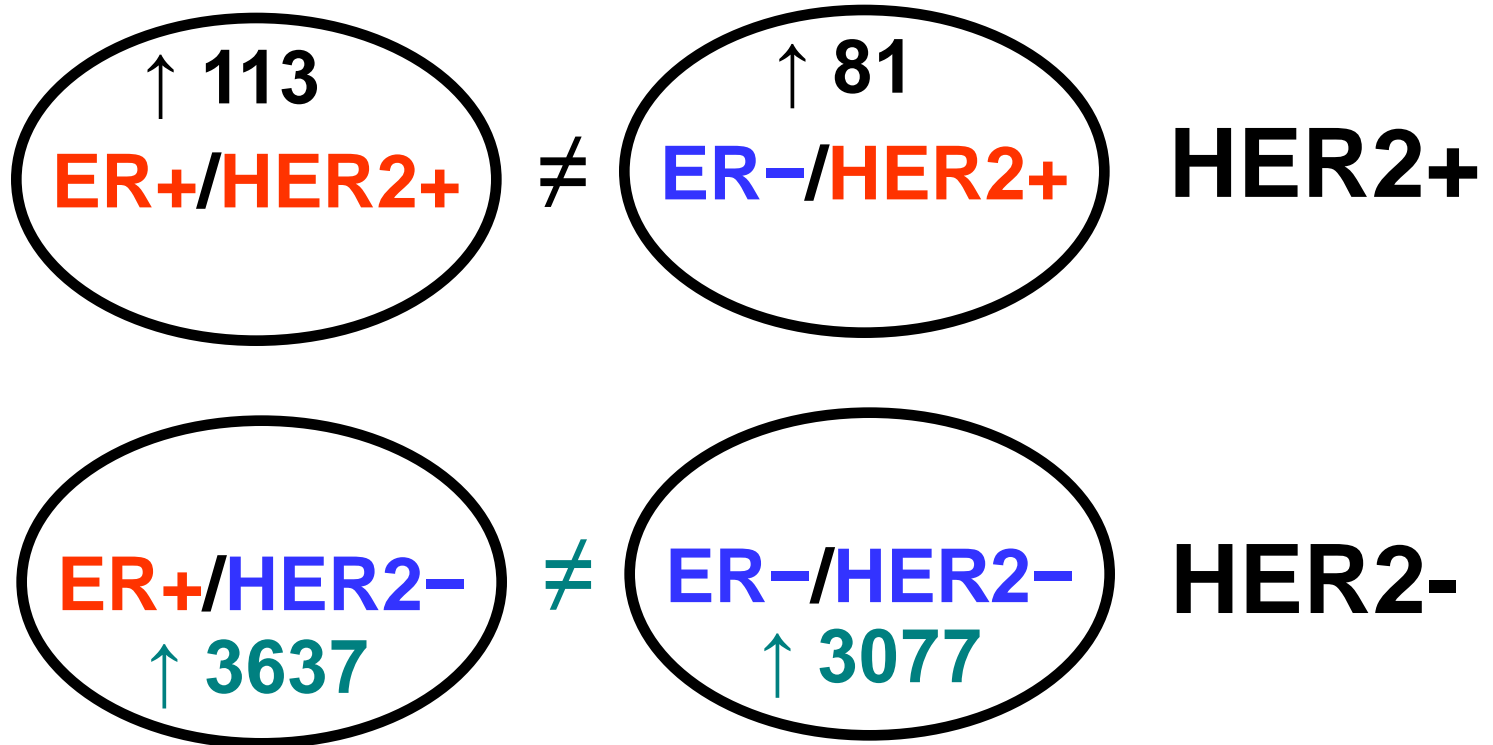
Gene expression differences between ER+/HER2+ and ER-/HER2+ breast cancers
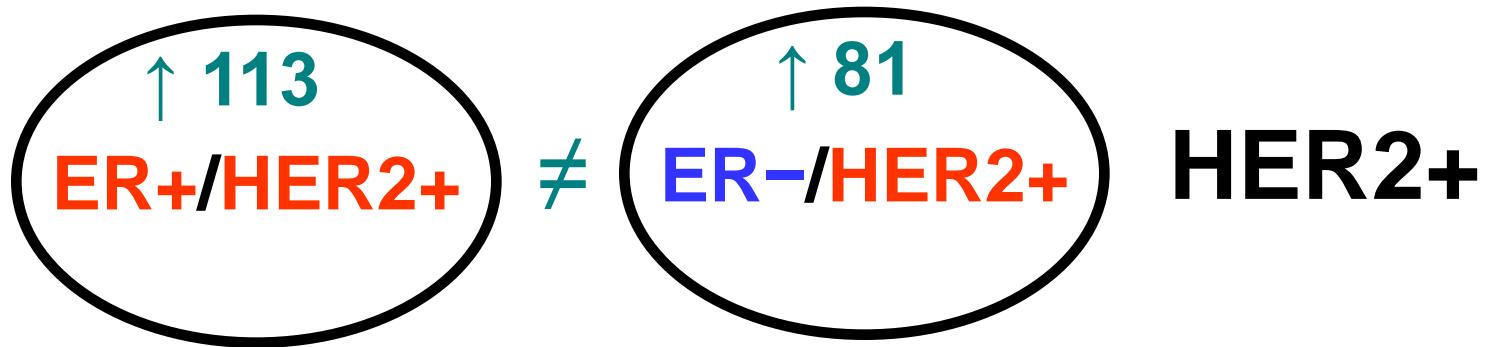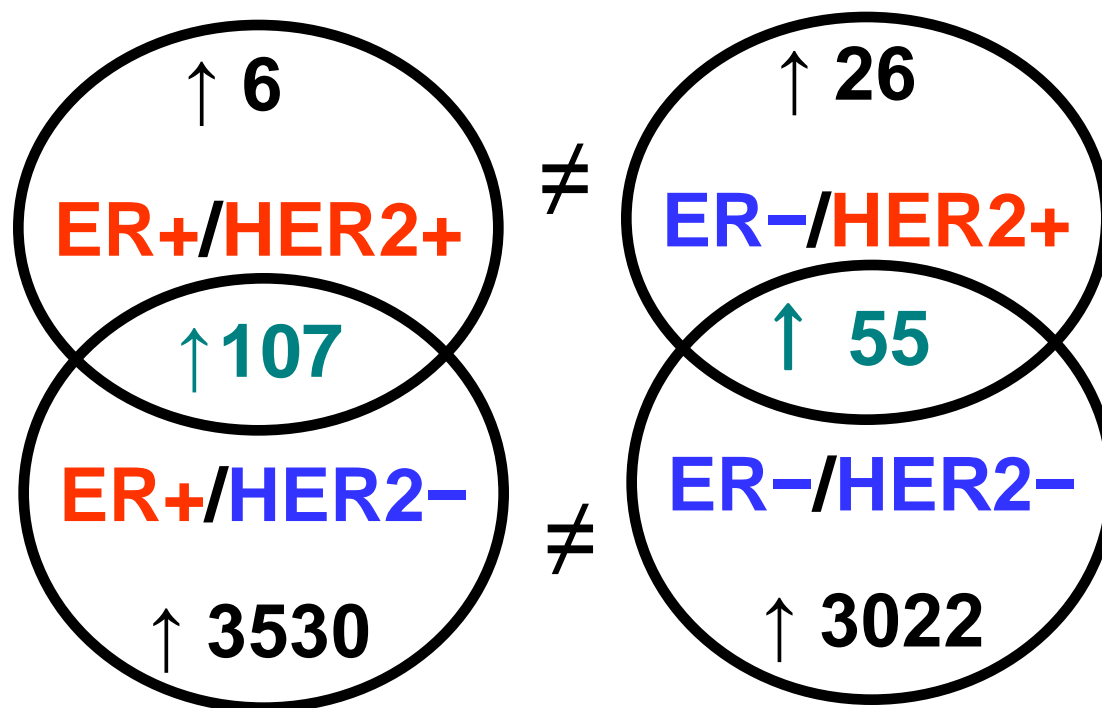
194 probes

↑ 113

ER+/HER2+ ≠ **ER−/HER2+** **HER2+**

↑ 81

Gene expression differences between ER+/HER2- and ER-/HER2- breast cancers

194 probes

↑ 113
ER+/HER2+ ≠ **ER−/HER2+**   **HER2+**
↑ 81

ER+/HER2− ≠ **ER−/HER2−**   **HER2-**
↑ 3637   ↑ 3077

6714 probes

Genes that distinguished ER+ from ER- cases significantly overlapped for HER2+ and HER2- cancers

194 probes

↑ 6

ER+/HER2+   ≠   **ER−/HER2+**

↑107   ↑ 55

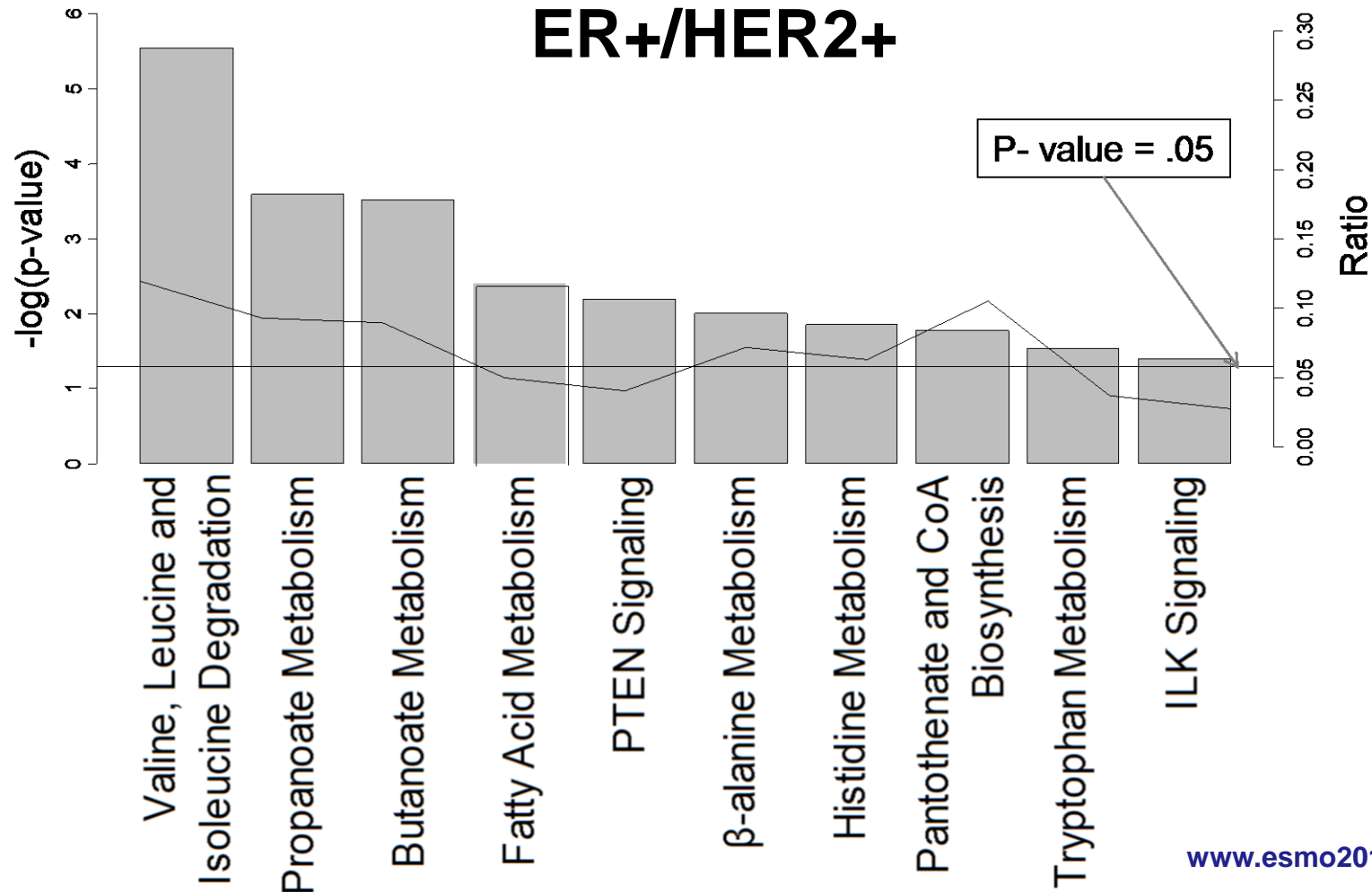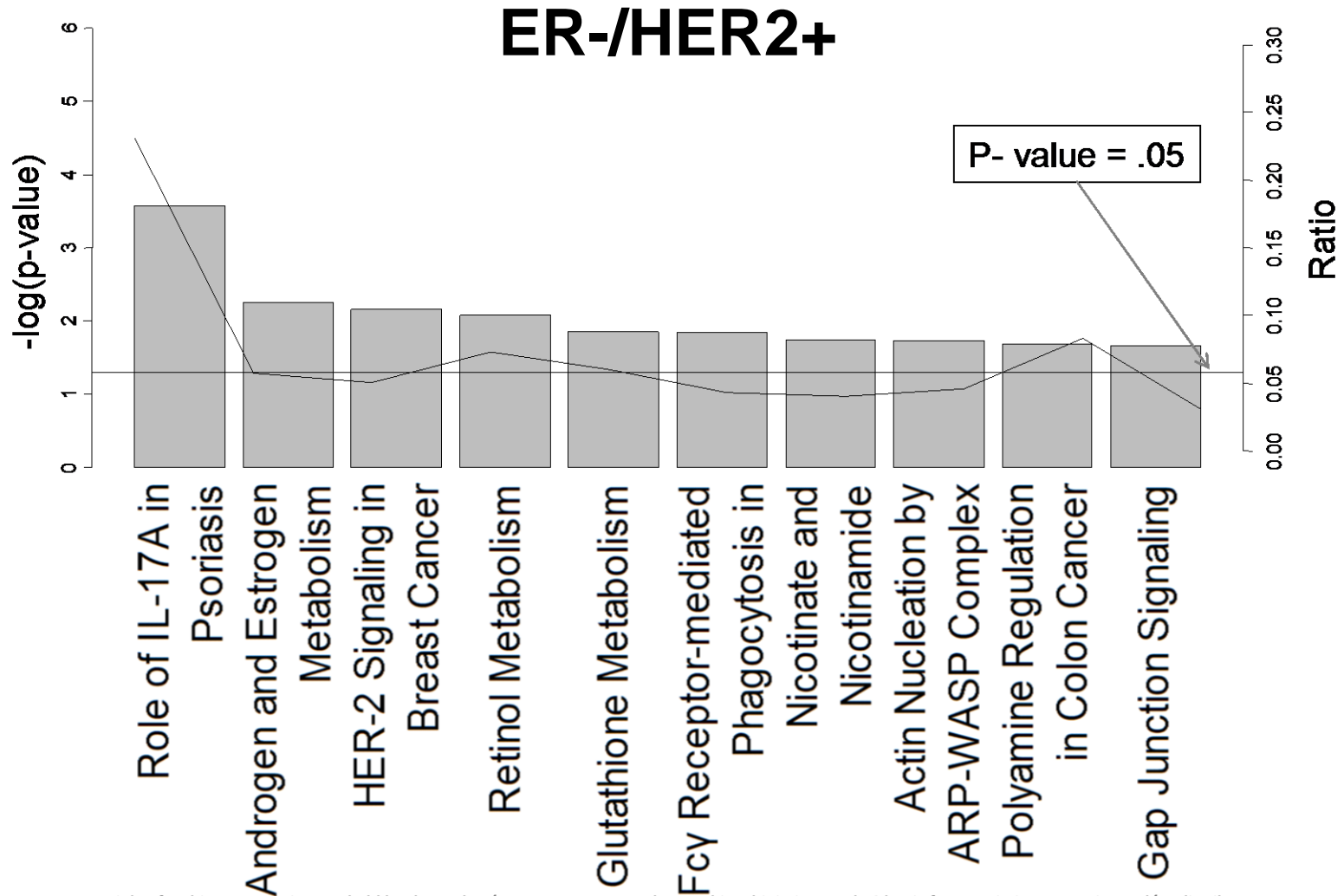**ER+/HER2−**   ≠   **ER−/HER2−**

↑ 3530   ↑ 3022

↑ 26

162/194 (84%) of the probes are in common

6714 probes

Canonical pathways (using IPA® software) for genes overexpressed in ER+/HER2+

ER+/HER2+

P- value = .05

-log(p-value)

Ratio

Valine, Leucine and Isoleucine Degradation
Propanoate Metabolism
Butanoate Metabolism
Fatty Acid Metabolism
PTEN Signaling
β-alanine Metabolism
Histidine Metabolism
Pantothenate and CoA Biosynthesis
Tryptophan Metabolism
ILK Signaling

Canonical pathways (using IPA® software) for genes overexpressed in ER-/HER2+

ER-/HER2+

P- value = .05

Gene expression differences
between HER2+ and HER2- by ER status

ER+

ER-

↑ **141**

ER+/HER2+

↑ **769**

ER−/HER2+

242 probes

≠

1200 probes

≠

ER+/HER2−

↑ **101**

ER−/HER2−

↑ **431**

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Gene expression differences between HER2+ and HER2- by ER status

242 probes

↑ **94**

ER+/HER2+

↑**47**

27 probes from chromosome 17 (co-amplified with HER2)

↑ **722**

ER−/HER2+

FGFR4, PPP1R10, G6PD, SOX11, KMO, HOXC11, ATP2A2, GGT1, C14orf147, PRODH, TNEM132A, ESRP2, FA2H, C2orf54, CATSPERB

≠

ER+/HER2−

↑**3**

ER−/HER2−

↑ **98**

↑ **428**

FGFR2, RBBP8, CAND2
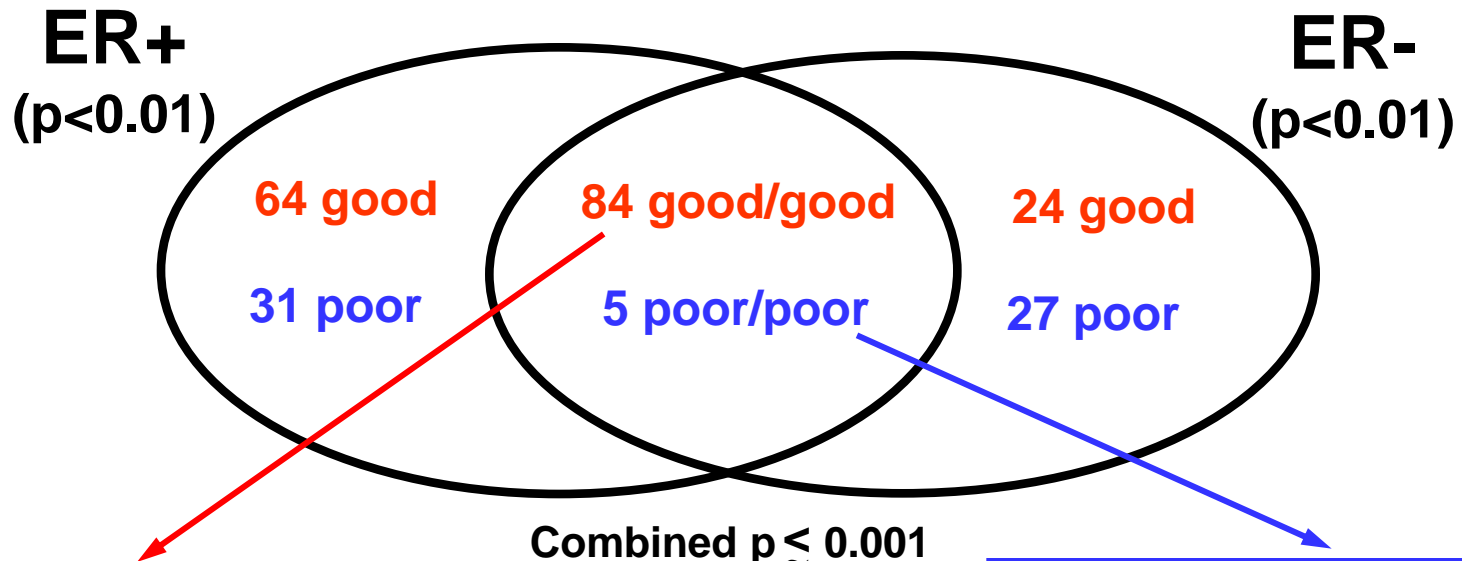
Only 50 probes are in common

Consistency of differentially expressed genes in Independent validation cohort

- Reproducibility of differentially expressed genes by ER and HER2 status was tested in independent validation cohorts, the prognostic data sets

- 90 to 100% of probes identified as differentially expressed in our discovery cohorts were also significantly differentially expressed in the validation sets

Class comparison	Discovery No. of probes	Validation cohort Probes with p<0.05	%
ER+ vs ER- (HER2+)	194	184	94.8
ER+ vs ER- (HER2-)	6750	6169	91.3
ER+ vs ER- (HER2+ and HER2-)	162	156	96.3
HER2+ vs HER2- (ER+)	242	216	89.2
HER2+ vs HER2- (ER-)	1200	1149	95.7
HER2+ vs HER2- (ER+ and ER-)	50	50	100

Gene Set analysis to define prognostic biological processes in ER+ and ER-, HER2+ cancers

ER+
(p<0.01)

ER-
(p<0.01)

64 good

31 poor

84 good/good

5 poor/poor

24 good

27 poor

Combined p ≤ 0.001
(Expected by chance ~3)

Immune related functions
(i.e. positive regulation of T cell, NK and B cell; differentiation and activation of T cell, NK; Plasma cell production; Adhesion and diapedesis of lymphocytes; JAK-STAT cascade; Interferon-gamma production)

• PTEN dependent cell cycle arrest and apoptosis
• Cytokinesis and cell division site part
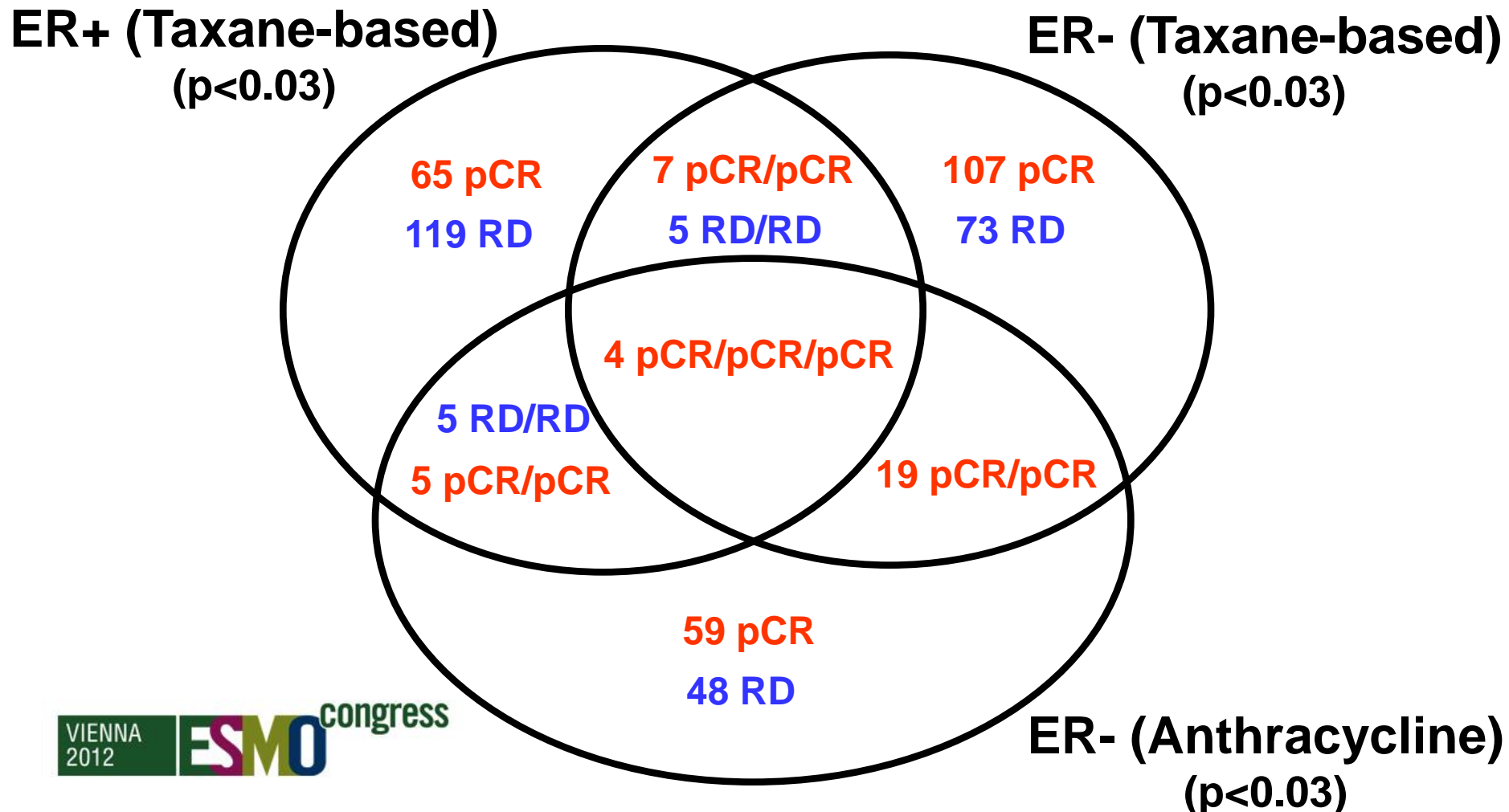• Carbohydrate transport

Cox univariate analysis for association with prognosis of previously published immune metagenes

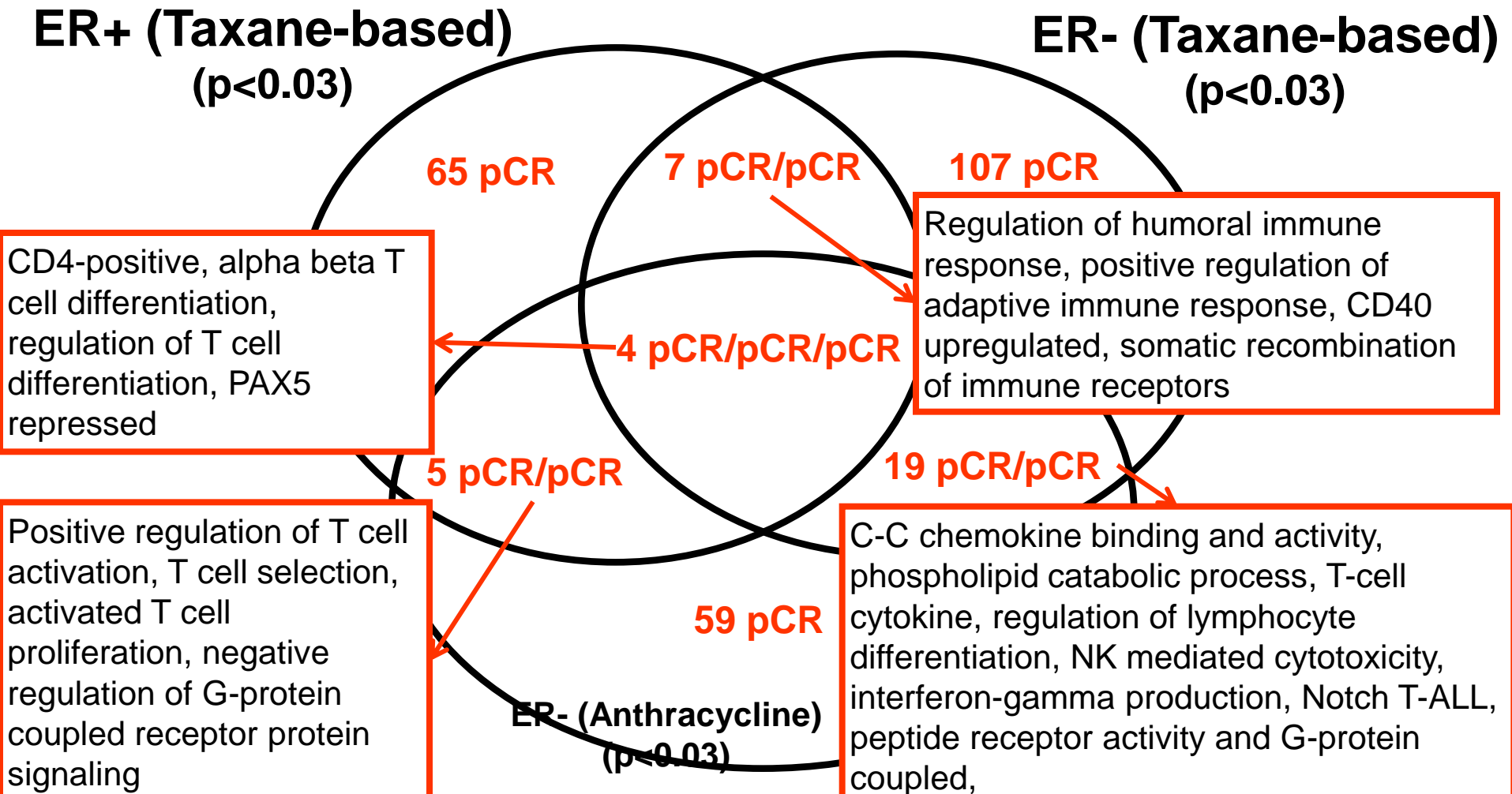| Metagene	HER2+ (N=118)			ER+ / HER2+ (N=58)			ER- / HER2+ (N=60)		
	HR	95%CI	p*	HR	95%CI	p*	HR	95%CI	p
Dendritic†	0.58	0.41 - 0.83	**0.003**	0.62	0.40 - 0.96	**0.033**	0.52	0.28 - 0.99	**0.047**
B-cell/Plasma cell†	0.64	0.50 - 0.80	**0.0001**	0.67	0.47 - 0.94	**0.022**	0.60	0.44 - 0.84	**0.003**
CD8A (~NK/CD8)	0.54	0.38 - 0.76	**0.0004**	0.61	0.40 - 0.93	**0.022**	0.45	0.25 - 0.81	**0.008**
GZMK (~NK/CD8)	0.58	0.41 - 0.82	**0.002**	0.60	0.40 - 0.92	**0.018**	0.55	0.30 - 1.01	*0.053*
Interferon inducible	0.50	0.34 - 0.74	**0.001**	0.41	0.22 - 0.76	**0.005**	0.51	0.28 - 0.91	**0.024**
MHC1	0.42	0.26 - 0.69	**0.001**	0.38	0.19 - 0.74	**0.004**	0.48	0.23 - 1.04	*0.062*
MHC2	0.62	0.44 - 0.88	**0.008**	0.64	0.42 - 0.98	**0.042**	0.62	0.33 - 1.14	0.122
STAT1	0.61	0.43 - 0.85	**0.003**	0.58	0.38 - 0.90	**0.015**	0.64	0.38 - 1.08	0.097

† Metagenes adopted from Bianchini G et al. JCO 2010

* Cox univariate analysis. Metagenes were considered as continuous variables

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Gene sets associated with response to neoadjuvant chemotherapy in ER+ and ER-, HER2+ breast cancer

ER+ (Taxane-based)
(p<0.03)

ER- (Taxane-based)
(p<0.03)

ER- (Anthracycline)
(p<0.03)

65 pCR
119 RD

7 pCR/pCR
5 RD/RD

107 pCR
73 RD

4 pCR/pCR/pCR

5 RD/RD
5 pCR/pCR

19 pCR/pCR

59 pCR
48 RD

In HER2-positive cancers immunological functions are consistently associated with pCR regardless of treatment regimen and ER status

ER+ (Taxane-based)
(p<0.03)

ER- (Taxane-based)
(p<0.03)

65 pCR

7 pCR/pCR

107 pCR

CD4-positive, alpha beta T cell differentiation, regulation of T cell differentiation, PAX5 repressed

4 pCR/pCR/pCR

Regulation of humoral immune response, positive regulation of adaptive immune response, CD40 upregulated, somatic recombination of immune receptors

5 pCR/pCR

19 pCR/pCR

Positive regulation of T cell activation, T cell selection, activated T cell proliferation, negative regulation of G-protein coupled receptor protein signaling

59 pCR

ER- (Anthracycline)
(p<0.03)

C-C chemokine binding and activity, phospholipid catabolic process, T-cell cytokine, regulation of lymphocyte differentiation, NK mediated cytotoxicity, interferon-gamma production, Notch T-ALL, peptide receptor activity and G-protein coupled,

Diverse metabolic functions associated with residual disease (lesser chemotherapy sensitivity)

ER+ (Taxane-based)
(p<0.03)

ER- (Taxane-based)
(p<0.03)

119 RD

5 RD/RD

73 RD

5 RD/RD

Positive regulation of carbohydrate biosynthetic process, regulation of carbohydrate biosynthetic process, cellular response to oxidative stress, mitochondrial membrane organization, neuron death

Cell-cell junction organization, Myofibril and contractile fiber, neuron projection terminus, axon part

48 RD

ER- (Anthracycline)
(p<0.03)

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Conclusions

- Among HER2+ tumors, ER- and ER+ cancers represent distinct molecular subtypes.

- Immune signatures strongly and consistently predict for good prognosis and to a lesser extent for higher chemotherapy sensitivity in HER2+ cancers regardless of ER status.

- Tumor immune infiltration was associated with pCR as well as good prognosis.

- Biosynthetic and metabolic processes are associated with poor prognosis and lesser response to chemotherapy that warrant further study in the laboratory.

Acknowledgments