Mechanisms of Resistance to Antineoplastic Agents

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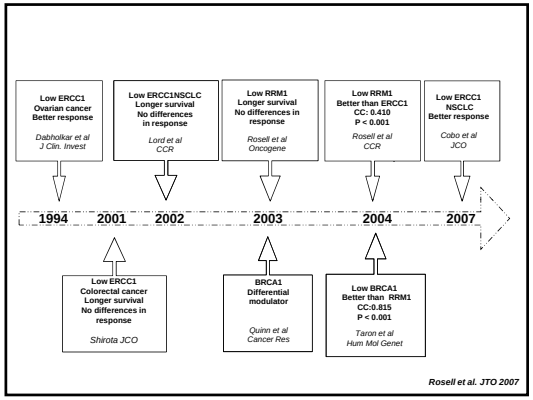
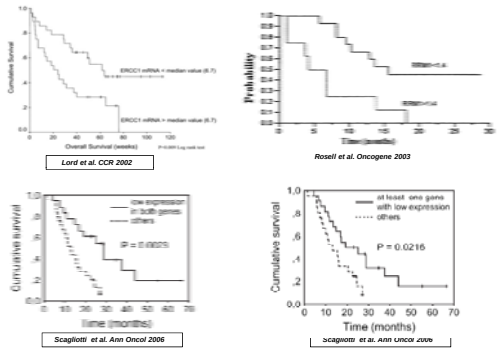


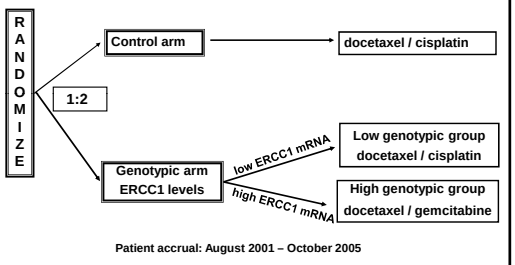
Rosell et al. JTO 2007

ERCC1 & RRM1 mRNA in gem/cis-treated NSCLC



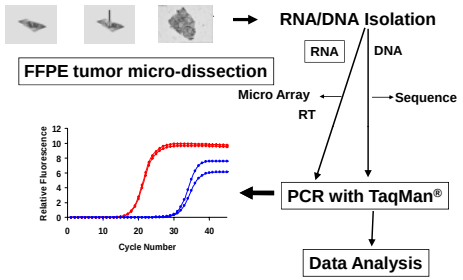
Lord et al. CCR 2002
Rosell et al. Oncogene 2003
Scagliotti et al. Ann Oncol 2006

ERCC1-based customized chemotherapy in NSCLC



Patient accrual: August 2001 – October 2005

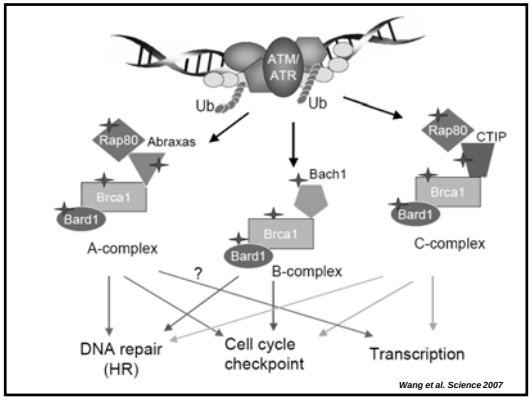
Cobo et al. JCO 2007

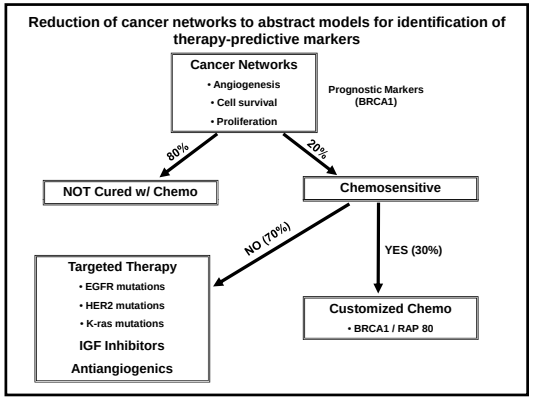


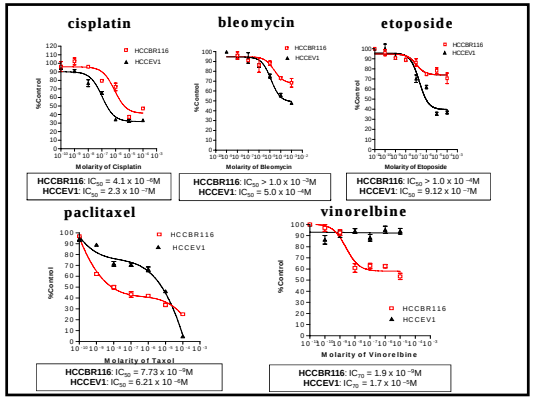
Treatment based on therapy-predictive markers

- standard or ERCC1-customized cisplatin/docetaxel *(Fossella et al. JCO 2003: Scagliotti et al. JCO 2008; Cobo et al. JCO 2007)*
  - MS = 10-11 mo
  - TTP = 5.1-6.7 mo
  - 2-y survival = 14-21%
- RAP 80 / BRCA1-customized cisplatin-based chemotherapy *(SLCG data; Rosell et al. ESMO 2008)*
  - MS = NR
  - TTP = 14 mo

  } in pts with low BRCA1 & RAP80 mRNA
- customized erlotinib in EGFR-mutant lung cancer in 217 pts
  - MS = 27 mo
  - TTP = 14 mo


ATM/
ATR
Ub
Ub
Rap80
Abraxas
Brca1
Bard1
A-complex
Bach1
Brca1
Bard1
B-complex
Rap80
CTIP
Brca1
Bard1
C-complex
?
DNA repair
(HR)
Cell cycle
checkpoint
Transcription
Wang et al. Science 2007

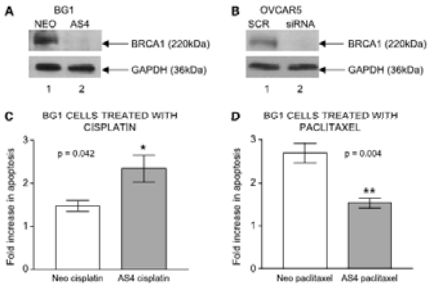

Reduction of cancer networks to abstract models for identification of therapy-predictive markers
Cancer Networks
• Angiogenesis
• Cell survival
• Proliferation
Prognostic Markers (BRCA1)
80%
20%
NOT Cured w/ Chemo
Chemosensitive
NO (70%)
YES (30%)
Targeted Therapy
• EGFR mutations
• HER2 mutations
• K-ras mutations
IGF Inhibitors
Antiangiogenics
Customized Chemo
• BRCA1 / RAP 80


cisplatin
bleomycin
etoposide
HCCBR116
HCCEV1
%Control
Molarity of Cisplatin
Molarity of Bleomycin
Molarity of Etoposide
HCCBR116: IC50 = 4.1 x 10 -4M
HCCEV1: IC50 = 2.3 x 10 -3M
HCCBR116: IC50 > 1.0 x 10 -2M
HCCEV1: IC50 = 5.0 x 10 -4M
HCCBR116: IC50 > 1.0 x 10 -4M
HCCEV1: IC50 = 9.12 x 10 -3M
paclitaxel
vinorelbine
Molarity of Taxol
Molarity of Vinorelbine
HCCBR116: IC50 = 7.73 x 10 -9M
HCCEV1: IC50 = 6.21 x 10 -4M
HCCBR116: IC70 = 1.9 x 10 -9M
HCCEV1: IC70 = 1.7 x 10 -9M

BRCA1 – differential modulator of chemosensitivity

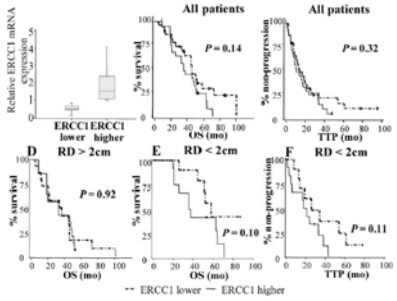
- **In breast and ovarian cancer cells, inducible expression of BRCA1 enhances paclitaxel sensitivity** *(Mullan et al. Oncogene 2001)*
- **siRNA of BRCA1 led to paclitaxel and docetaxel resistance, and reconstitution of BRCA1 enhanced sensitivity to paclitaxel and vinorelbine** *(Quinn et al. Cancer Res 2003; Chabalier et al. Cell Cycle 2006; Quinn et al. CCR 2007)*
- **3 retrospective studies in NSCLC and ovarian cancer** *(Taron et al. Hum Molec Genet 2004; Quinn et al. CCR 2007; Weberpals et al. IJC 2009)*
  - **low / intermediate BRCA1 → longer survival following platinum-based chemotherapy**
  - **high BRCA1 expression → survival increased following taxane-based therapy**

Abrogation of BRCA1 increases sensitivity to cisplatin and resistance to paclitaxel



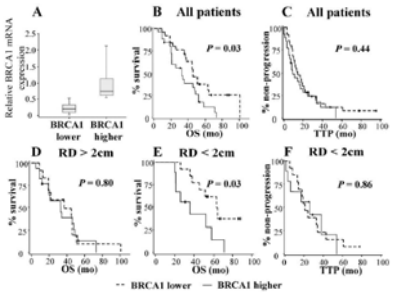
Quinn et al. CCR 2007

ERCC1 in sporadic ovarian cancer



Weberpals et al. Int J Cancer 2009

BRCA1 & ERCC1 in sporadic ovarian cancer



Weberpals et al. Int J Cancer 2009

Response in 96 gem/doc-treated stage IV NSCLC according to BRCA1 mRNA levels

	CR+PR	P	Univariate OR	p	Multivariate OR	p
BRCA1		0.002				
T1	8 (27.6)		0.31	0.03	0.54	0.40
T2	4 (13.8)		0.13	0.001	0.22	0.05
T3	17 (58.6)		1		1	
RRM1		0.56				
T1	12 (41.4)		1		1	
T2	9 (31)		0.65	0.43	1.43	0.62
T3	8 (27.6)		0.58	0.32	0.95	0.94
RRM2		<0.001				
T1	21 (72.4)		1		1	
T2	7 (24.1)		0.13	<0.001	0.20	0.02
T3	1 (3.4)		0.02	<0.001	0.02	<0.001
PS		0.02				
0	24 (82.8)		1		1	
1-2	5 (17.2)		0.28	0.02	0.44	0.23

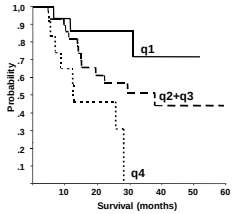
Boukavinas et al PLoS ONE 2008

PFS in 96 gem/doc-treated stage IV NSCLC stratified by RRM1

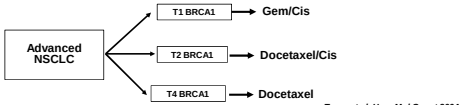
	RRM1 T1			RRM1 T2			RRM1 T3		
	N (%)	TTP mos	p	N (%)	TTP mos	p	N (%)	TTP mos	p
BRCA1			0.75			0.007			0.68
T1	16 (50)	4.17		10 (31.3)	2		6 (18.8)	2.40	
T2	6 (18.8)	8.50		14 (43.8)	2		12 (37.5)	3.60	
T3	10 (31.3)	9.87		8 (25)	12.80		14 (43.8)	3.57	
RRM2			0.49			0.06			0.76
T1	14 (43.8)	9.87		7 (21.9)	14.63		11 (34.4)	4.97	
T2	11 (34.4)	4.33		13 (40.6)	3.63		8 (25)	3	
T3	7 (21.9)	3.20		12 (37.5)	2.20		13 (40.6)	3	

Boukavinas et al PLoS ONE 2008

Survival to neoadjuvant gem/cis in NSCLC according to BRCA1 mRNA expression

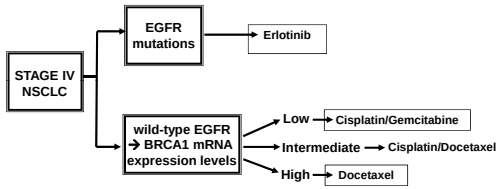


N	Median	P
q1=15	NR	0.014
q2+q3=28	37.80	
q4=12	12.70	

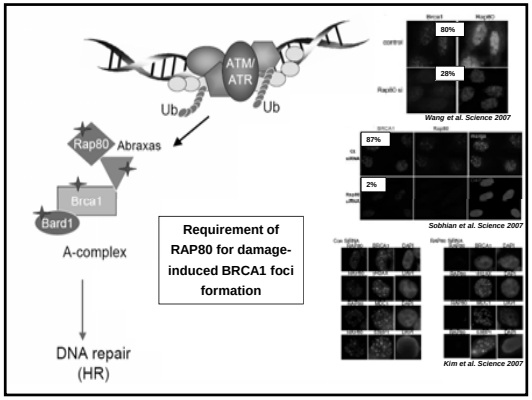


Taron et al. Hum Mol Genet 2004

BRCA1-customized phase II study in stage IV NSCLC (non-squamous)

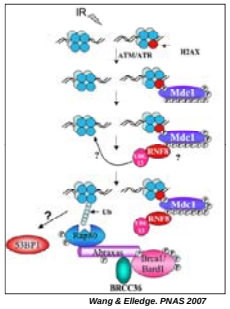


	All Patients (n=123)	EGFR Group (n=12)	Low BRCA1 Group (n=38)	Intermediate BRCA1 Group (n=40)	High BRCA1 Group (n=33)
Outcome	%	%	%	%	%
Complete response	3.3	16.7	0	2.5	3
Partial response	34.1	58.3	21.1	37.5	36.4
Stable disease	30.1	8.3	47.4	17.5	33.3
Progressive disease	20.3	0	15.8	30	21.2
Could not be determined	12.2	16.7	15.8	12.5	6.1
Overall response rate	43.6	90	25	45.7	41.9
Intent to treat	37.4	75	21.1	40	39.4
Survival					
Median, mo	12 mo	NR (>28 mo)	11 mo	9 mo	11 mo
1-year	49.2	91.7	47.8	41.1	42.4
2-year	31.5	73.3	41.2	15.6	0
28 months	24.5	73.3	35.3	0	0
Time to progression, mo	6 mo	13 mo	5 mo	5 mo	8 mo

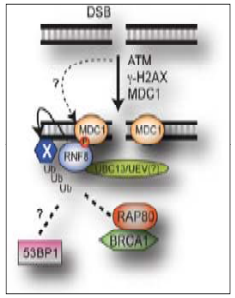


Requirement of RAP80 for damage-induced BRCA1 foci formation

BRCA1 assembly line of DNA damage response to DSBs



Wang & Elledge. PNAS 2007



Kolas et al. Science 2007

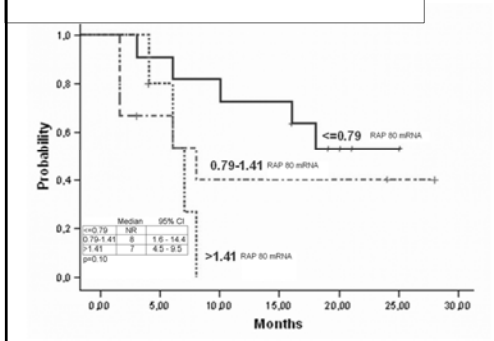
Median survival according to levels of BRCA1 & RAP80

	RAP80 LEVELS						
BRCA1 Levels	≤0.79		0.79-1.41		>1.41		
	N	months (95% CI)	N	months (95% CI)	N	months (95% CI)	P
Low	11	NR (-)	9	8 (1.6-14.4)	5	7 (4.5-9.5)	0.10
Intermediate	11	5 (3.4-6.6)	7	13 (10-15.9)	16	16 (5.5-26.5)	0.15
High	5	6 (1.8-10.1)	9	12 (9.3-14.6)	12	11 (8.2-13.8)	0.17

RAP80 by itself is able to translocate to IRIF in HCC1937 cells expressing truncated BRCA1 unable to migrate to IRIF

RAP80 alone could replace the BRCA1 function in cells lacking BRCA1
(Yan et al. Cancer Res 2007)

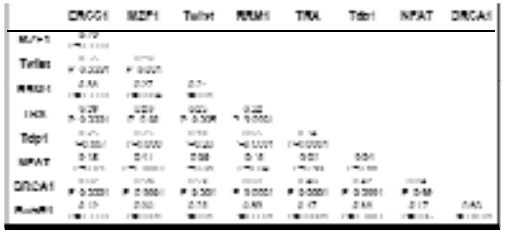
Survival in patients with low BRCA1 according to RAP80 levels



PFS according to RAP80 levels

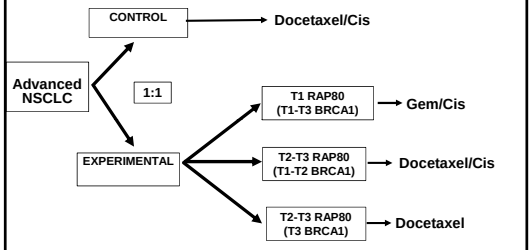
	RAP80 LEVELS						
	≤0.79		0.79-1.41		>1.41		
BRCA1	N	mo (95% CI)	N	mo (95% CI)	N	mo (95% CI)	P
Low	11	14 (5-22.9)	9	4 (2.8-5.1)	5	6 (-)	0.08
Intermediate	11	4 (3.1-4.9)	7	9 (2.5-15.5)	9	6 (3.1-8.9)	0.42
High	5	2 (0-4.1)	9	10 (7.3-12.6)	12	4 (1.7-6.3)	0.006

Correlation between expression levels of nine genes



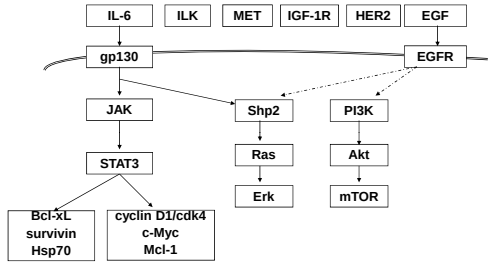
Rosell et al. PLoS ONE 2007

New international BREC (BRCA1 Expression Customization)



216 patients per arm – total: 432

Potential mechanisms of resistance in the BREC trial

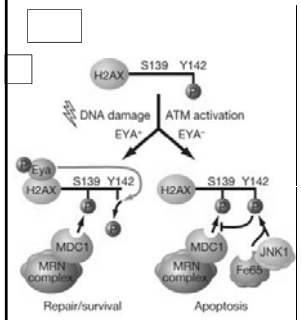


Modified from Rosell et al. Future Oncol 2009

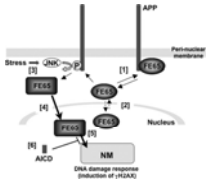
The hallmarks of cancer – the BREC trial

Survival	Longer	Shorter	Shortest
BREC customization	Low RAP80 & any BRCA1	Intermediate RAP80 & low/intermediate BRCA1	High RAP80 & BRCA1
Drug response	cisplatin +++ antimicrotubules – gem/cis	cisplatin + antimicrotubules ++ doc/cis	cisplatin - antimicrotubules +++ docetaxel
Drug resistance IL6 EGFR IGF-1R MACC/MET HER2	↑survivin ↑STAT3	↑survivin ↑STAT3	↑survivin ↑STAT3
Prognosis	cyclin D1/CDK4 CRKL	↑cyclin D1/CDK4 ↑CRKL	↑cyclin D1/CDK4 ↑CRKL

R Rosell

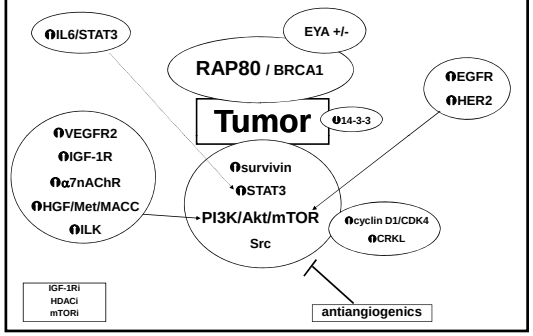


Cook et al. Nature 2009



Nakaya et al. J Biol Chem 2008

Reduction of cancer networks to abstract models for identification of antiangiogenic predictive markers



Conclusions

- **Customized chemotherapy based on RAP 80 / BRCA1 can improve PFS and overall survival**
- **Histology by itself is not predictive of pemetrexed efficacy in NSCLC. The levels of BRCA1 and RAP80 can accurately select adenocarcinomas and squamous cell carcinomas that are more sensitive to pemetrexed-platinum or other platinum-based combinations.**
- **Customizing EGFR TKIs based on EGFR mutations is the paradigm of targeted therapy in NSCLC**