National University Cancer Institute, Singapore

NUH National University Hospital
A member of the NUHS

Molecular Profiling of Ovarian Cancers to Optimize Therapy

Dr David SP Tan

MBBS(Hon), BSc(Hon), PhD, MRCP(Med Onc), FRCP(Edin)

National University Cancer Institute, Singapore
Yong Loo Lin School of Medicine, NUS

Presenter Disclosures

Research Support	Karyopharm Therapeutics
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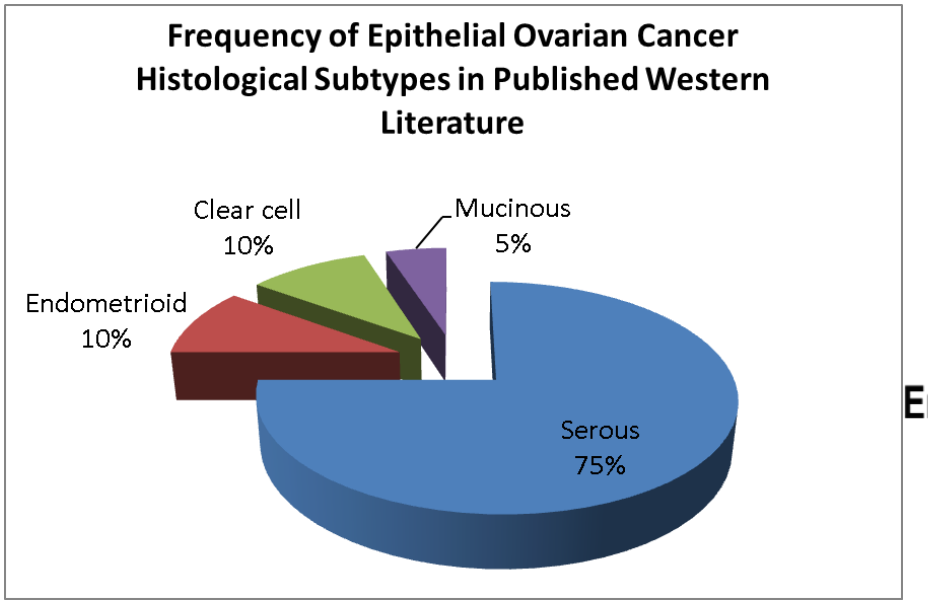
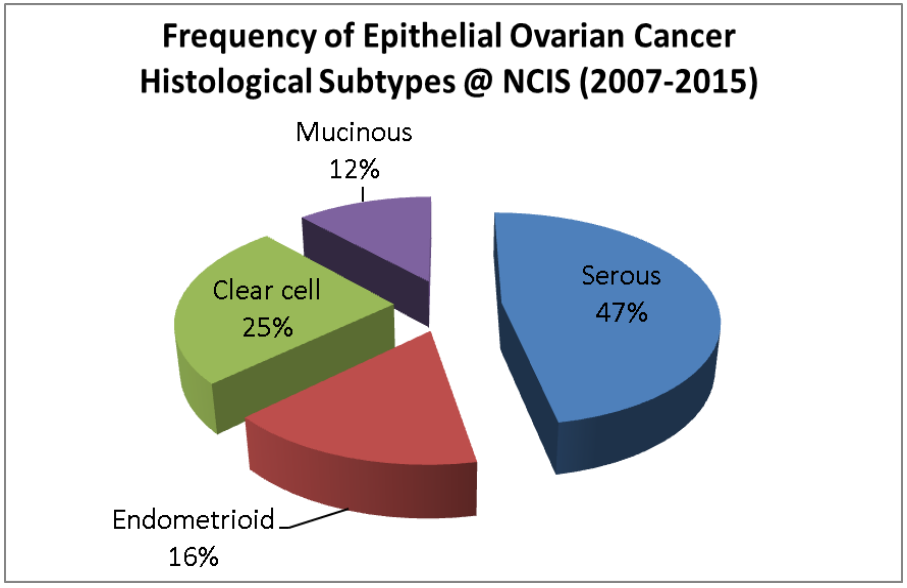
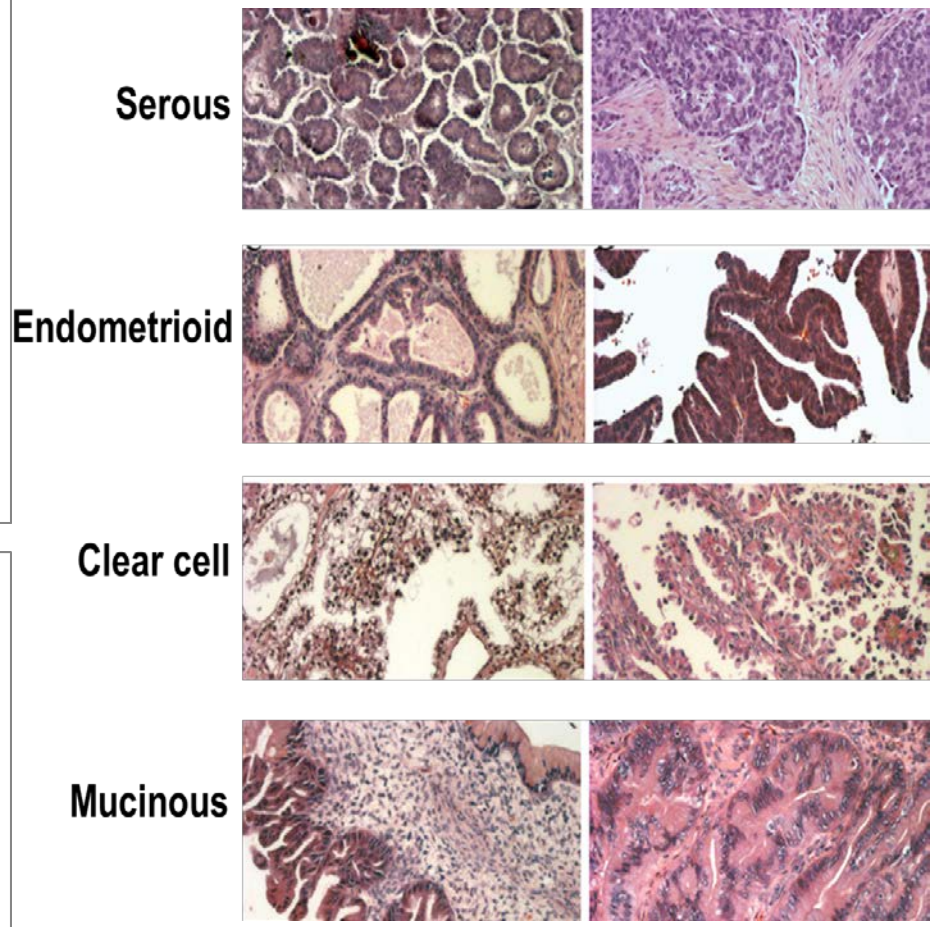
Honoraria	Astra Zeneca, Novartis, Roche, MSD, Bayer

Histological Subtypes of Epithelial Ovarian Cancer

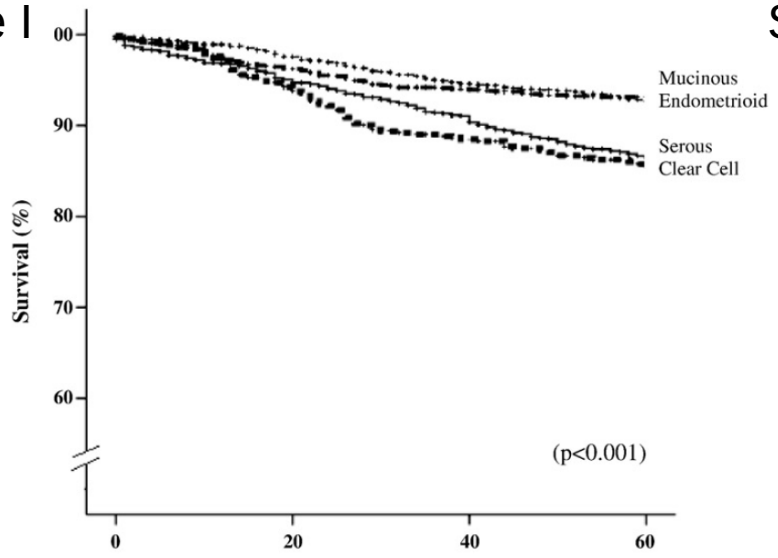




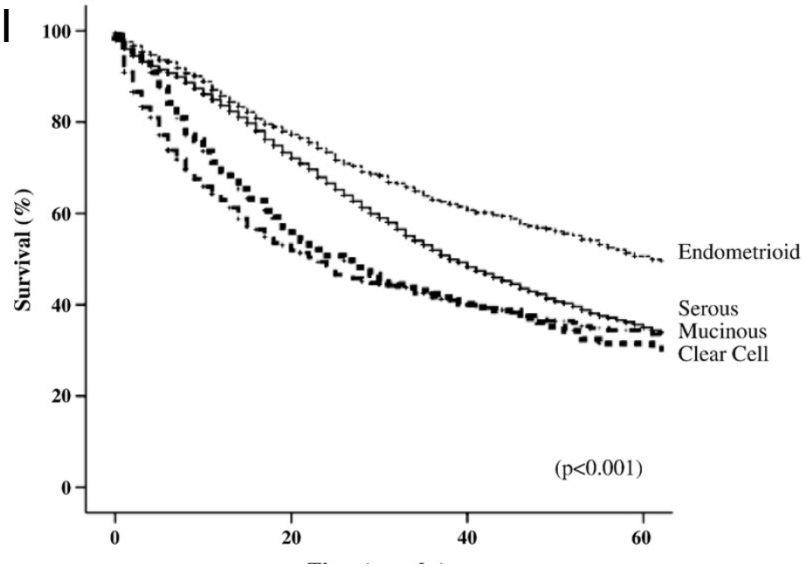


Ovarian Cancer:
Different Subtypes = Different Outcomes

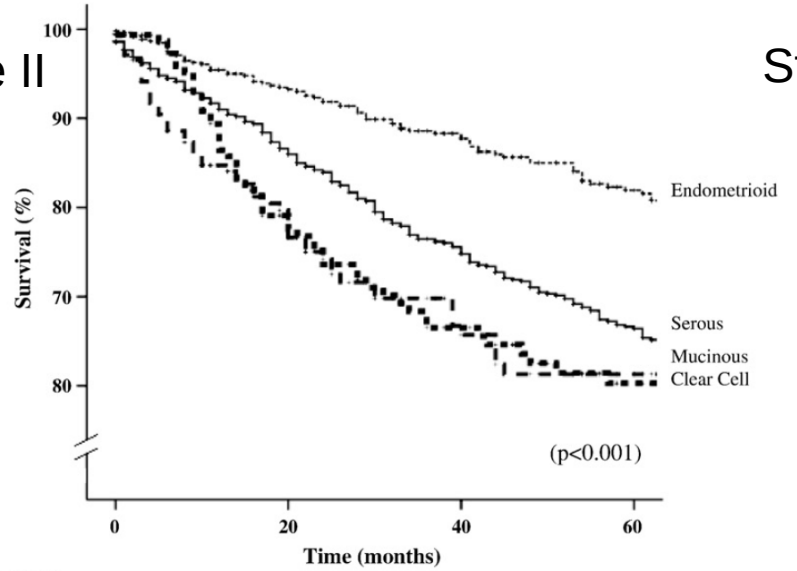
Stage I



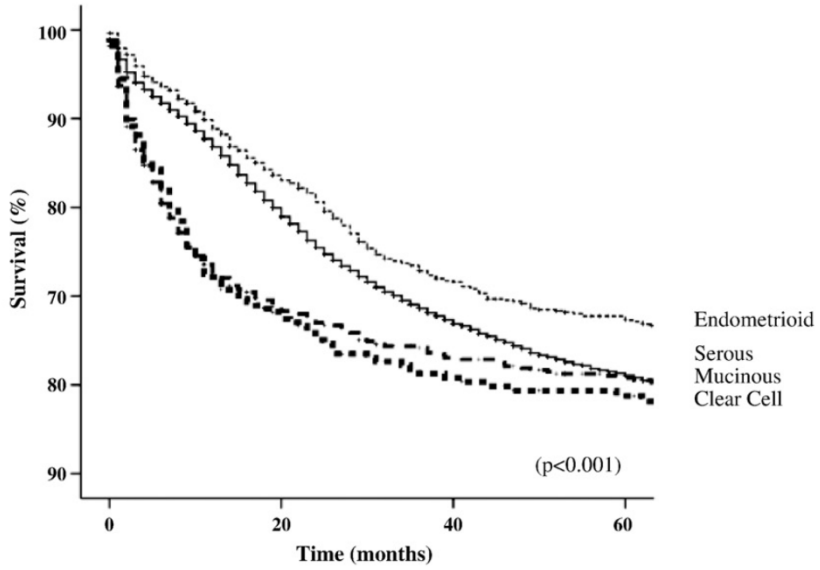
Stage III



Stage II



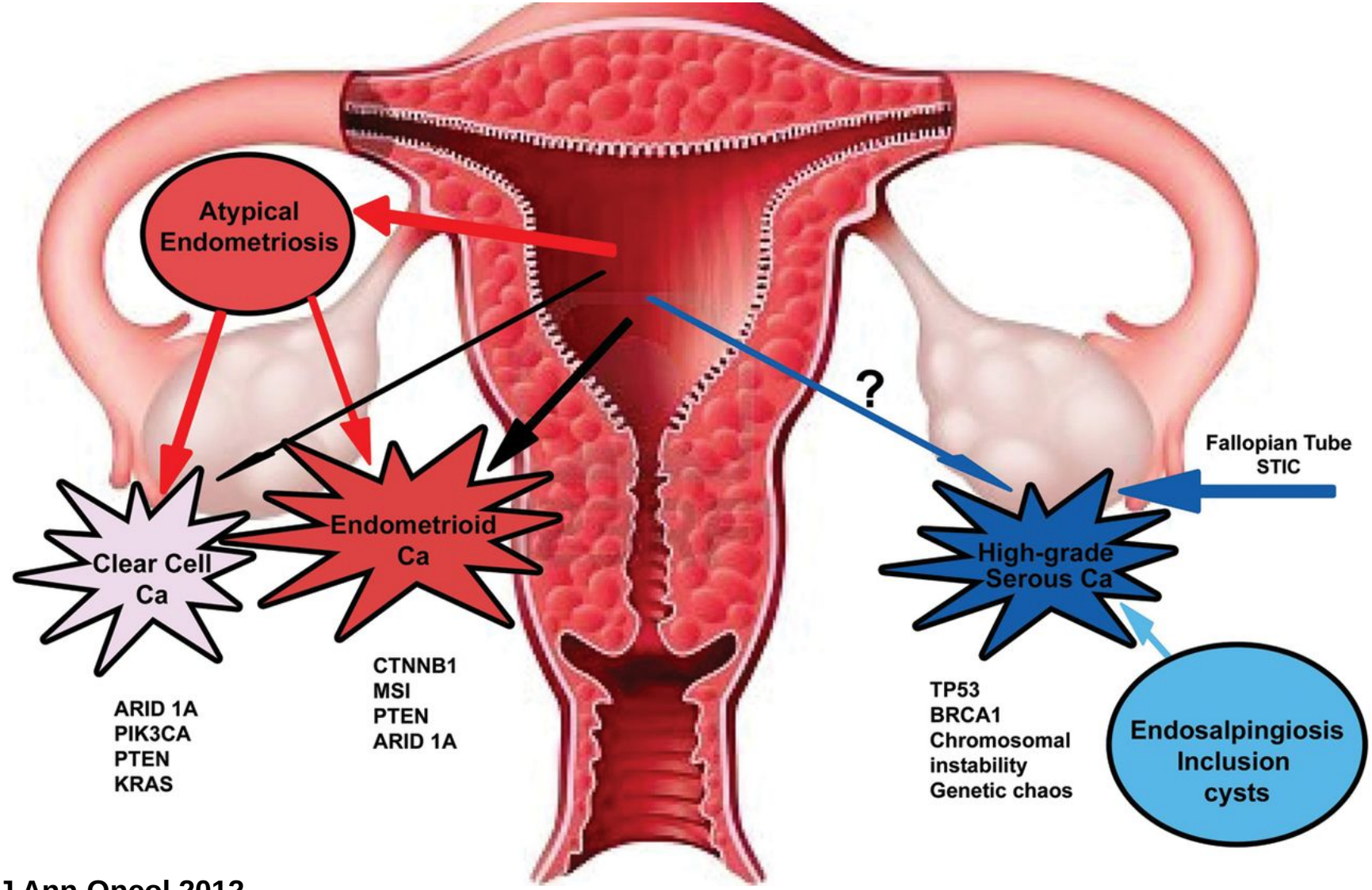
Stage IV



Chan et al Gynae Onc 2008

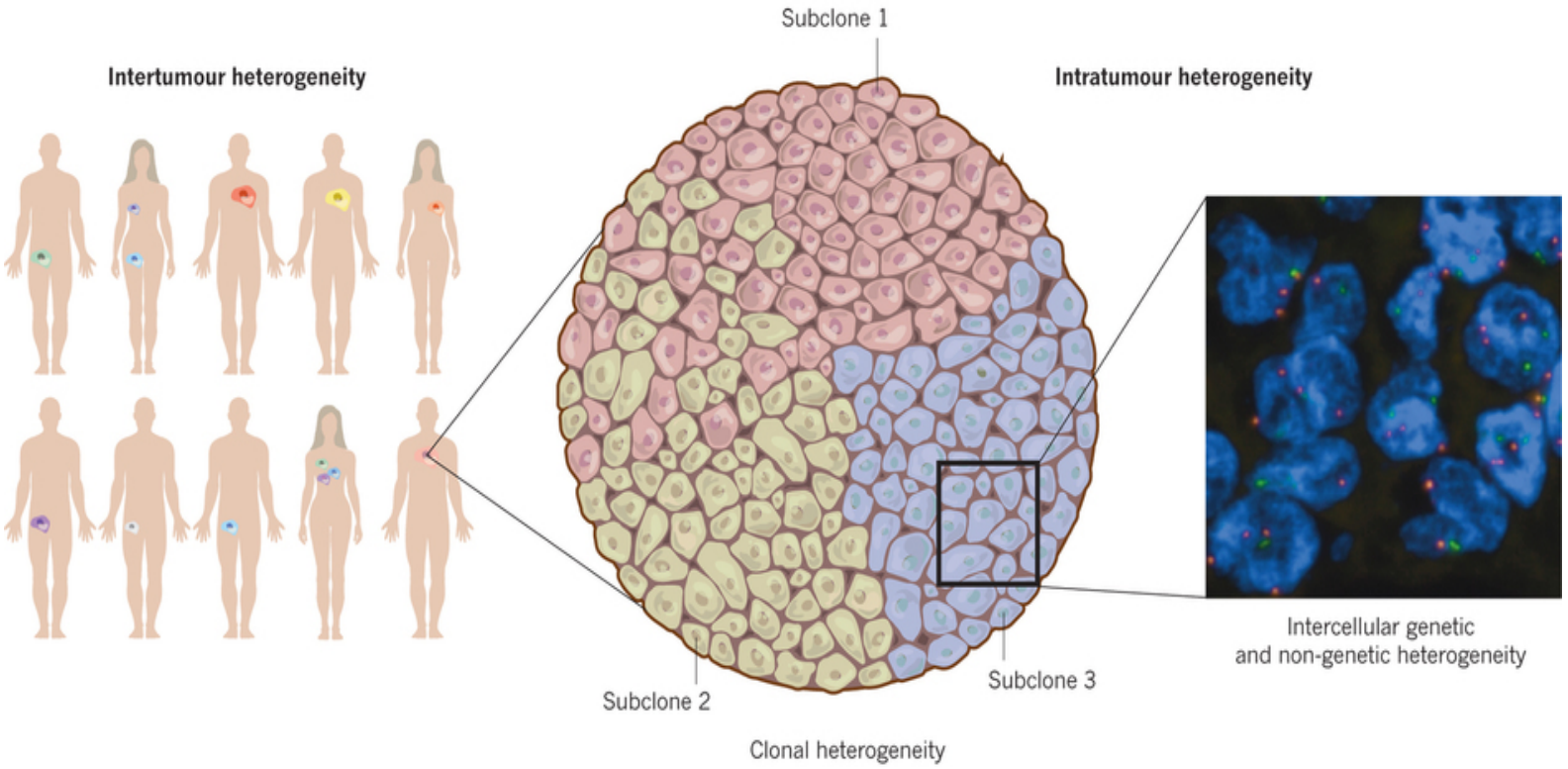
Ovarian Cancer:

Different subtypes = Different Origins = Different Molecular Abnormalities



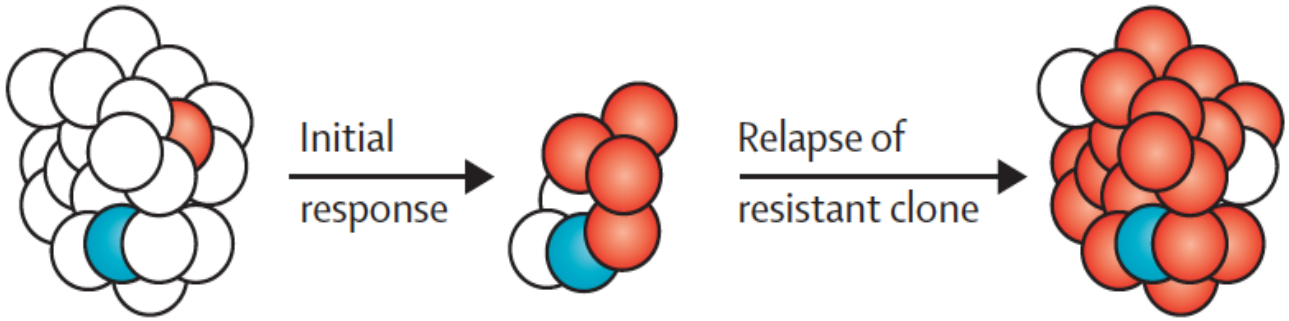
Prat J Ann Oncol 2012

Intertumour and intratumour heterogeneity



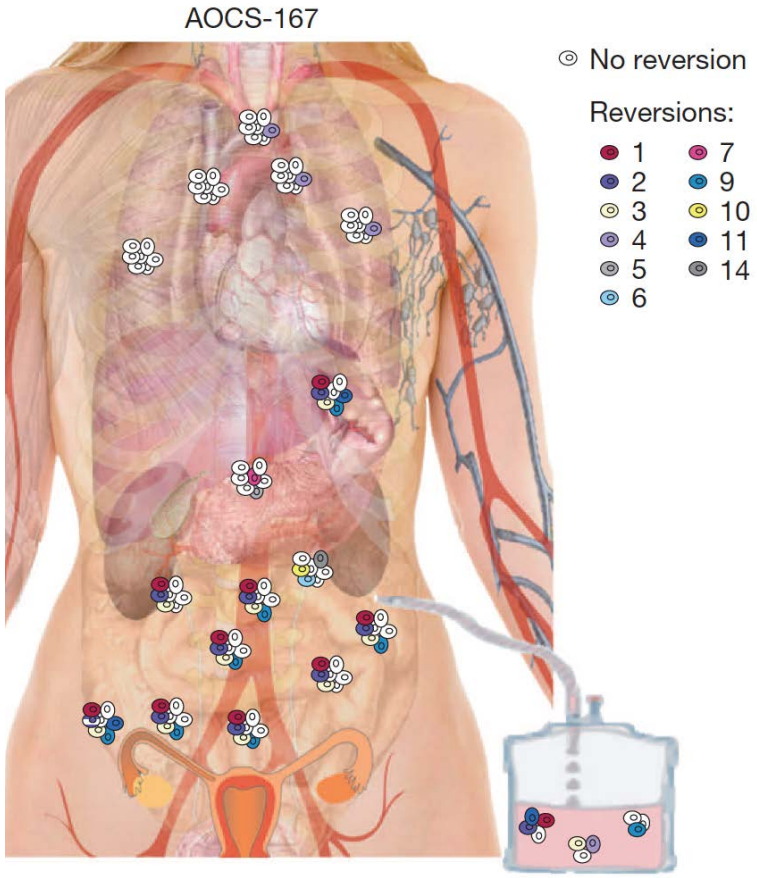
Burrell et al Nature 2013

Tumour Evolution and Drug Resistance

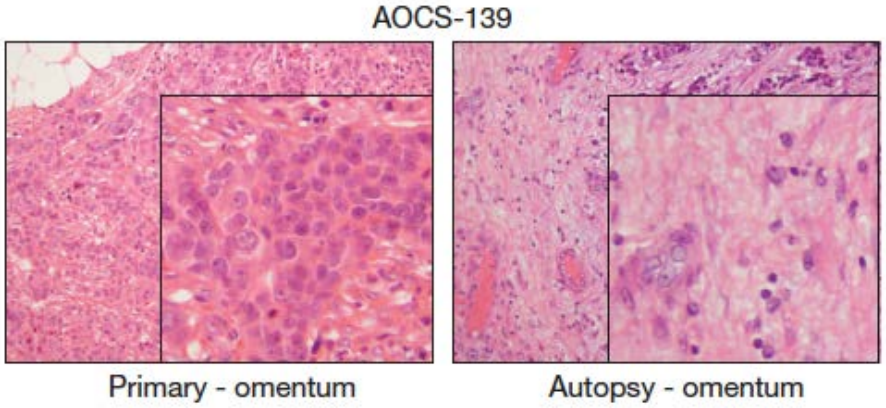


Turner and Reis-Filho Lancet Oncol 2012

PARPi resistance: heterogeneity in recurrent tumours



PARP inhibitor resistant BRCA2 germline mutant patient with independent reversion events detected at various metastatic sites (autopsy biopsy)



Extensive stromal reaction in omental lesion obtained at biopsy

Patch et al Nature 2015

OVARIAN CANCERS ARE

HISTOLOGICALLY,

MOLECULARLY,

INTRATUMORALLY

HETEROGENEOUS:

<u>MULTIPLE DISEASE ENTITIES</u>

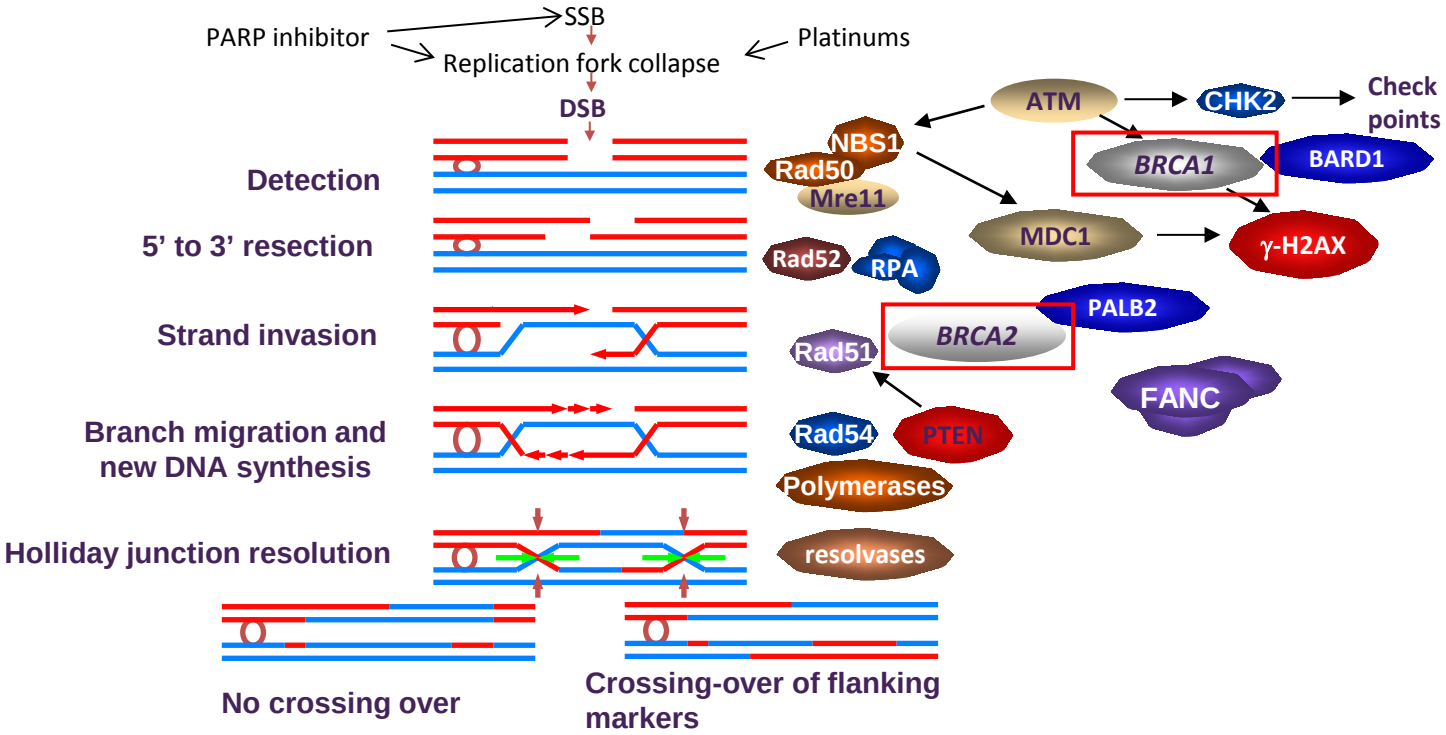
→ Therapeutic approaches need to start taking these issues into consideration

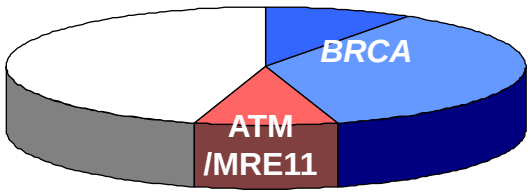
Precision therapy in gynaecological cancers:

1. Targeting Homologous Recombination deficiency (HRD)

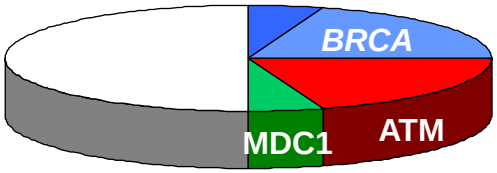
1. Gene expression signatures

Homologous recombination deficiency (HRD) and cancer

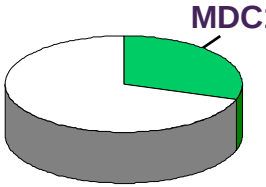




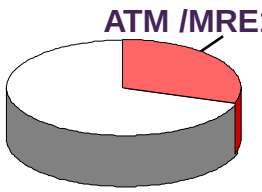
Serous ovarian



TN breast



NSCLC



Gastric/CRC

CRC, colorectal cancer; DSB, double-strand break;
NSCLC, non-small cell lung cancer; SSB, single-strand break

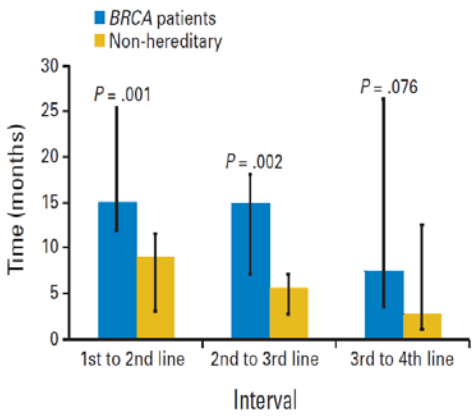
Chemotherapy response in BRCAmut EOC (BMOC)

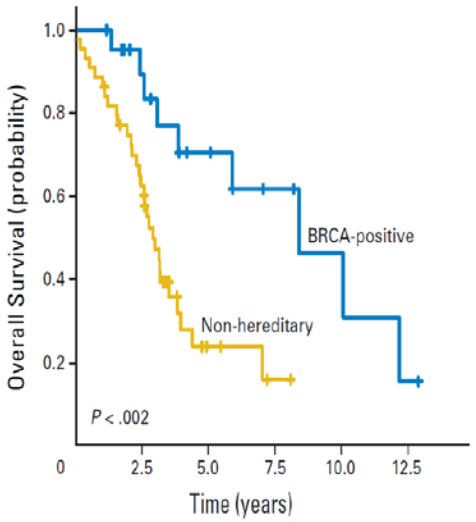
TABLE 1. Overall Response Rates following Chemotherapy in Patients with BMOC

Chemotherapy Regimen	ORR in Platinum-Sensitive BMOC	ORR in Platinum-Resistant BMOC	References
Platinum-Based Chemotherapy	First Line		
	87%[a] *BRCA1* (83 patients)	-	Vencken et al[9]
	92%[a] *BRCA2* (13 patients)	-	Vencken et al[9]
	96%[b] (21/22 patients)	-	Tan et al[8]
	Recurrent		
	65%[c] (48 patients)	80%[c] (8/10 patients)	Alsop et al[5]
	92%[b] (11/12 patients, second line)	-	Tan et al[8]
	100%[b] (7/7 patients, third line)	-	Tan et al[8]
	100%[d] (6/6 patients)	-	Leunen et al[20]
Paclitaxel Monotherapy	60%[b] (9/15 patients)	27%[b] (3/11 patients)	Tan et al[21]
Pegylated Liposomal Doxorubicin	57%[d] (13/23 patients)	77%[d] (10/14 patients)	Adams et al[22]
	39%[d] (13/33 patients) in relapsing disease < 12 months after most recent platinum-based chemotherapy	-	Kaye et al[23]
Trabectedin	41%[b] (36/88 patients)	-	Lorusso et al[24]
Topotecan	-	0% (0/9 patients)	Hyman et al[25]
Mitomycin C	33%[e] (2/6 patients)	66%[e] (4/6 patients)	Moiseyenko et al[26]
Melphalan	-	CR in 1 patient	Osher et al[27]

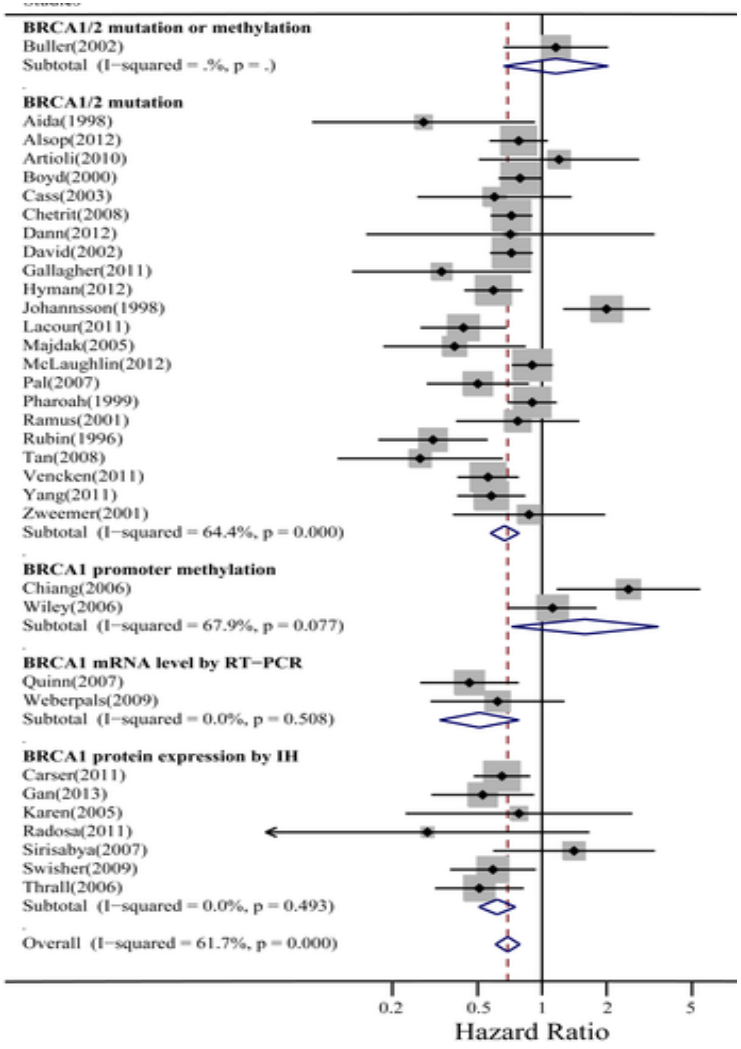
Tan and Kaye 2015 ASCO Educational Book

BRCAness Syndrome: Improved responses and increased survival for BRCAmut vs non- hereditary EOC





BRCA+ vs Non-hereditary:
Median OS 8.4 vs 2.9 years
HR = 0.3



BRCA mutants with Ovarian Cancer

Excellent responses to chemotherapy

Improved survival compared to non-BRCA mutants

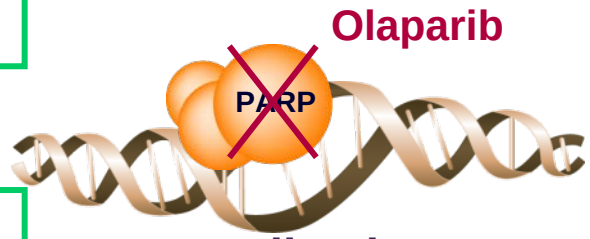
1 Tan et al JCO 2008
2 Bolton et al JAMA 2012
3 Sun et al PLoS One 2014

Olaparib – Poly (ADP-ribose) polymerase (PARP) inhibitor: Synthetic lethality in DNA repair defective backgrounds

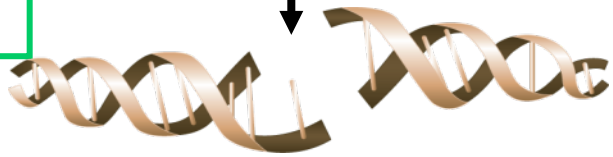
10,000–20,000 DNA SSBs occur each day in cells

Olaparib traps PARP on the DNA SSB preventing repair and effectively generating a protein-DNA adduct

During replication unrepaired SSBs bound by trapped PARP result in fork collapse and DNA DSBs



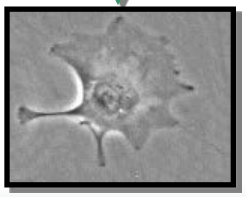
Replicating cells



Normal cell

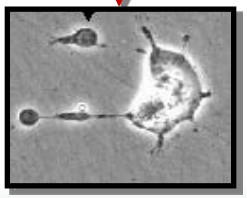
Repair by homologous recombination (HR)

Survival



Cancer cell with HRD, eg *BRCA*m

No effective repair (No HRR pathway)



Cell death

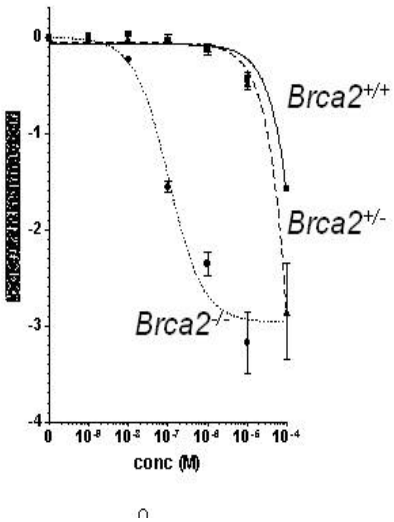
Tumour-specific killing by single agent olaparib (synthetic lethality)

PARP inhibitors for BRCA1/2 mutant patients

2005 — Pre-clinical — Early Clinical Trials (Phase I, incl. IB) — Randomised Clinical Trials (Phase II and III) — 2015

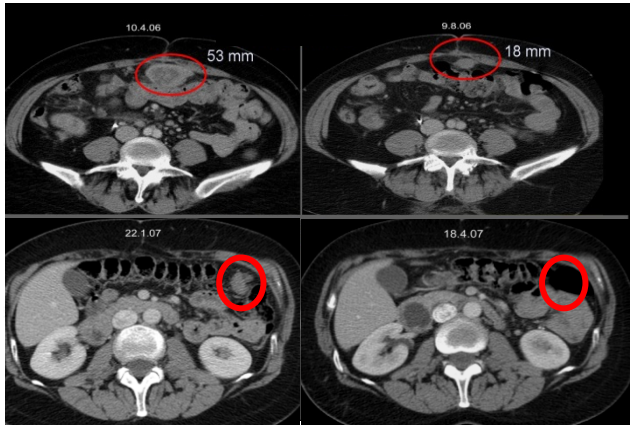
PARP : poly(ADP) ribose polymerase

Exquisite preclinical efficacy of PARPi



Farmer et al, Nature 2005

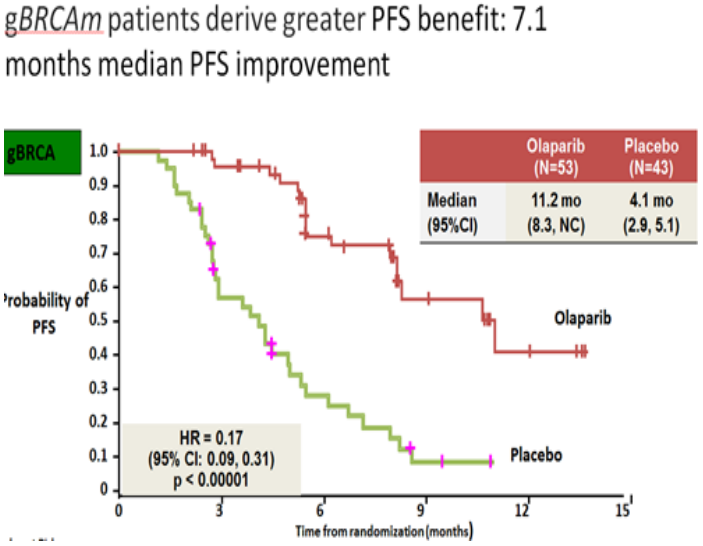
Phase I trial confirms excellent tolerance and expansion in 50 BRCA patients showed 46% response.



"this is nothing like chemotherapy

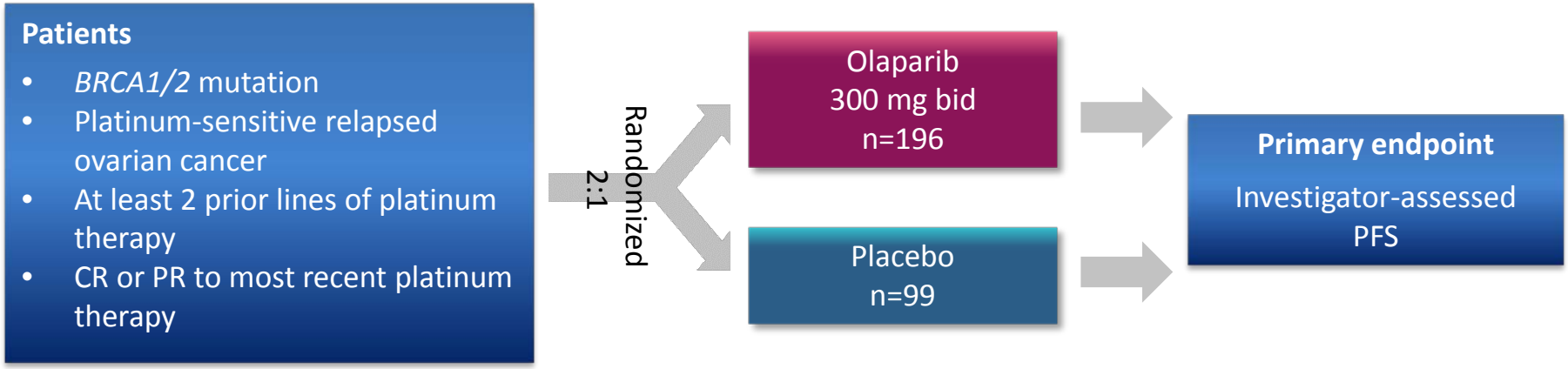
Fong P et al. N Engl J Med, 2009; **361**, 123-134;
Fong P et al. J Clin Oncol, 2010; **28**, 2512-2519

Randomised trial (maintenance therapy) showed marked PFS benefit particularly in BMOC



Ledermann et al, NEJM 2012 366 1382-92
Ledermann et al Lancet Oncology 2014

SOLO2/ENGOT-Ov21: study design



Sensitivity analysis: PFS by blinded independent central review (BICR)

- Key secondary endpoints:
  - Time to first subsequent therapy or death (TFST), time to second progression (PFS2), time to second subsequent therapy or death (TSST), overall survival (OS)
  - Safety, health-related quality of life (HRQoL*)

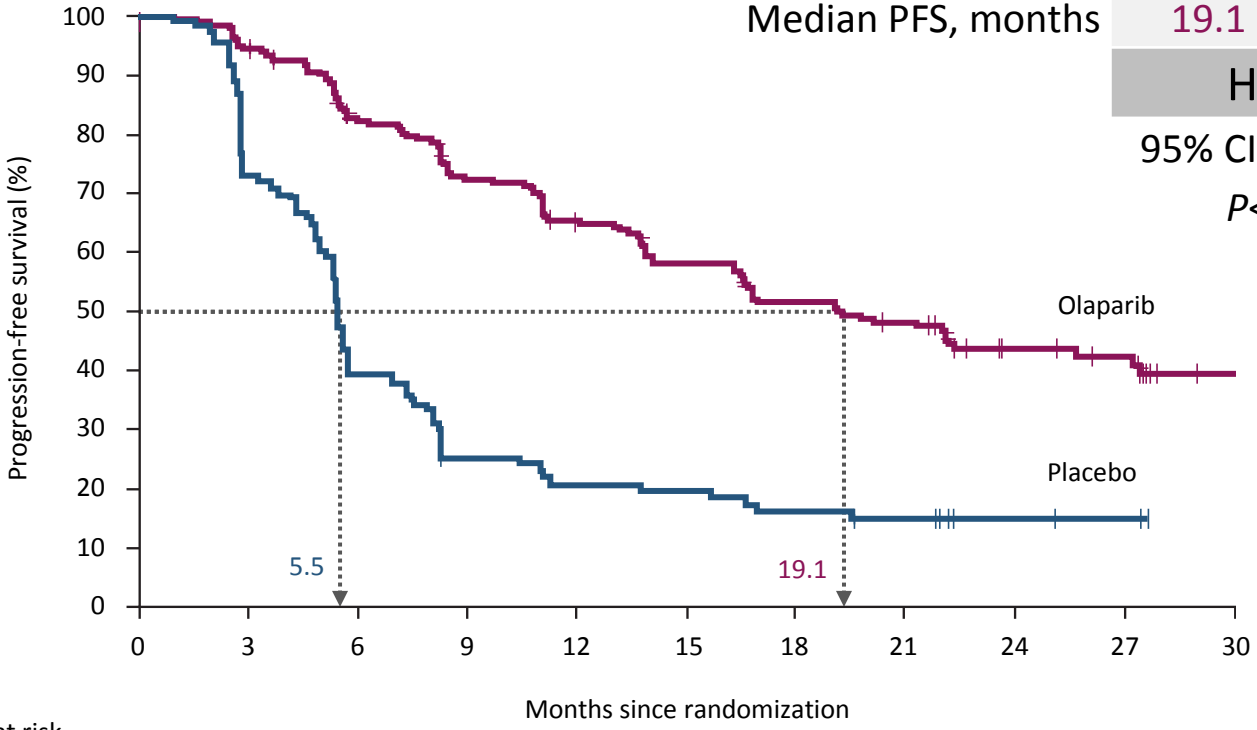
solo2 BRCAm Ovarian Cancer

*Primary endpoint for HRQoL was trial outcome index (TOI) of the FACT-O (Functional Assessment of Cancer Therapy – Ovarian)

Pujade-Lauraine E et al SGO 2017

SOLO2: PFS by investigator assessment

	Olaparib (n=196)	Placebo (n=99)
Events (%)	107 (54.6)	80 (80.8)
Median PFS, months	19.1	5.5
HR 0.30		
95% CI 0.22 to 0.41		
P<0.0001		



No. at risk

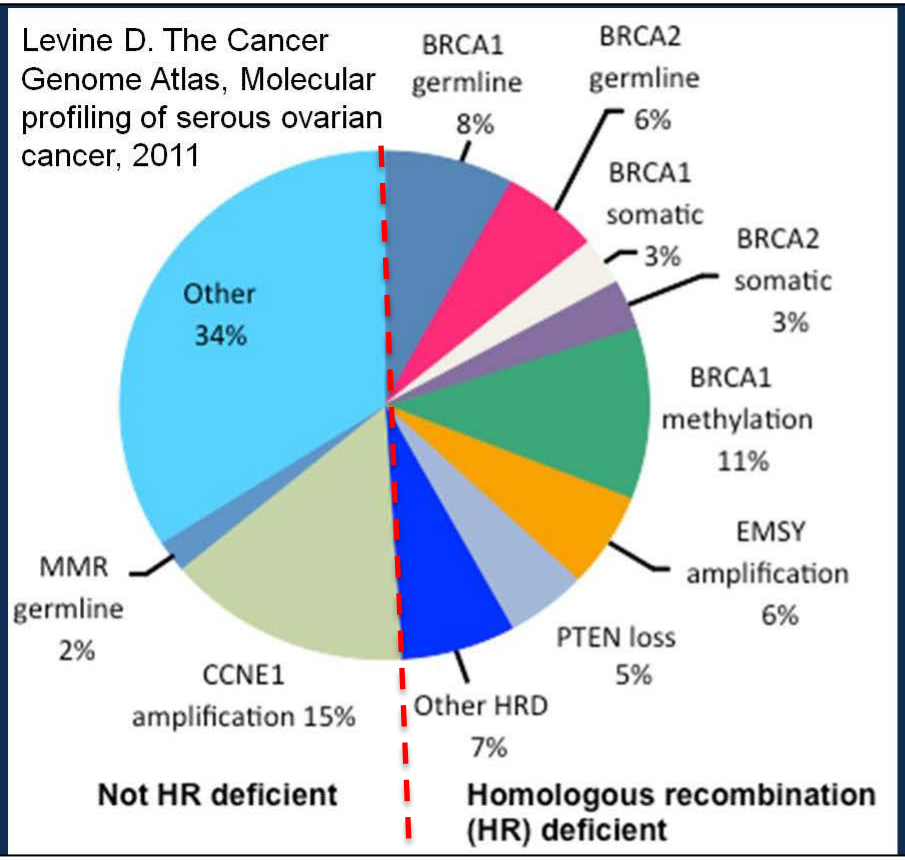
Olaparib	196	182	156	134	118	104	89	82	32	29	3
Placebo	99	70	37	22	18	17	14	12	7	6	0

solo2 BRCAm Ovarian Cancer

Median follow-up was 22.1 months in the olaparib group and 22.2 months for placebo

Pujade-Lauraine E et al SGO 2017

Other HR-related aberrations in high grade serous EOC



HR-pathway gene	Observed Frequency All Epithelial Ovarian Cancer (%)	Observed Frequency High Grade Ovarian Cancer (%)
RAD51C	0.41 – 2.9	1.9
RAD51D	0.35 – 1.1	0.95
RAD51B	0.06	0.95
RAD50	0.2 - 1.0	-
RAD54L	-	0.5
ATM	0.8 - 0.86	0.32 - 1.0
BRIP1	0.9 – 4.0	0.32 - 1.0
CHEK2	0.4 – 5.0	0.32 - 1.0
FANCA	-	0.5
FANCI	-	0.5
NBN	0.2 – 1.0	0.63 - 1.0
PALB2	0.2 – 2.0	0.63

Kristeleit, Miller, Kohn ASCO Ed Book 2016

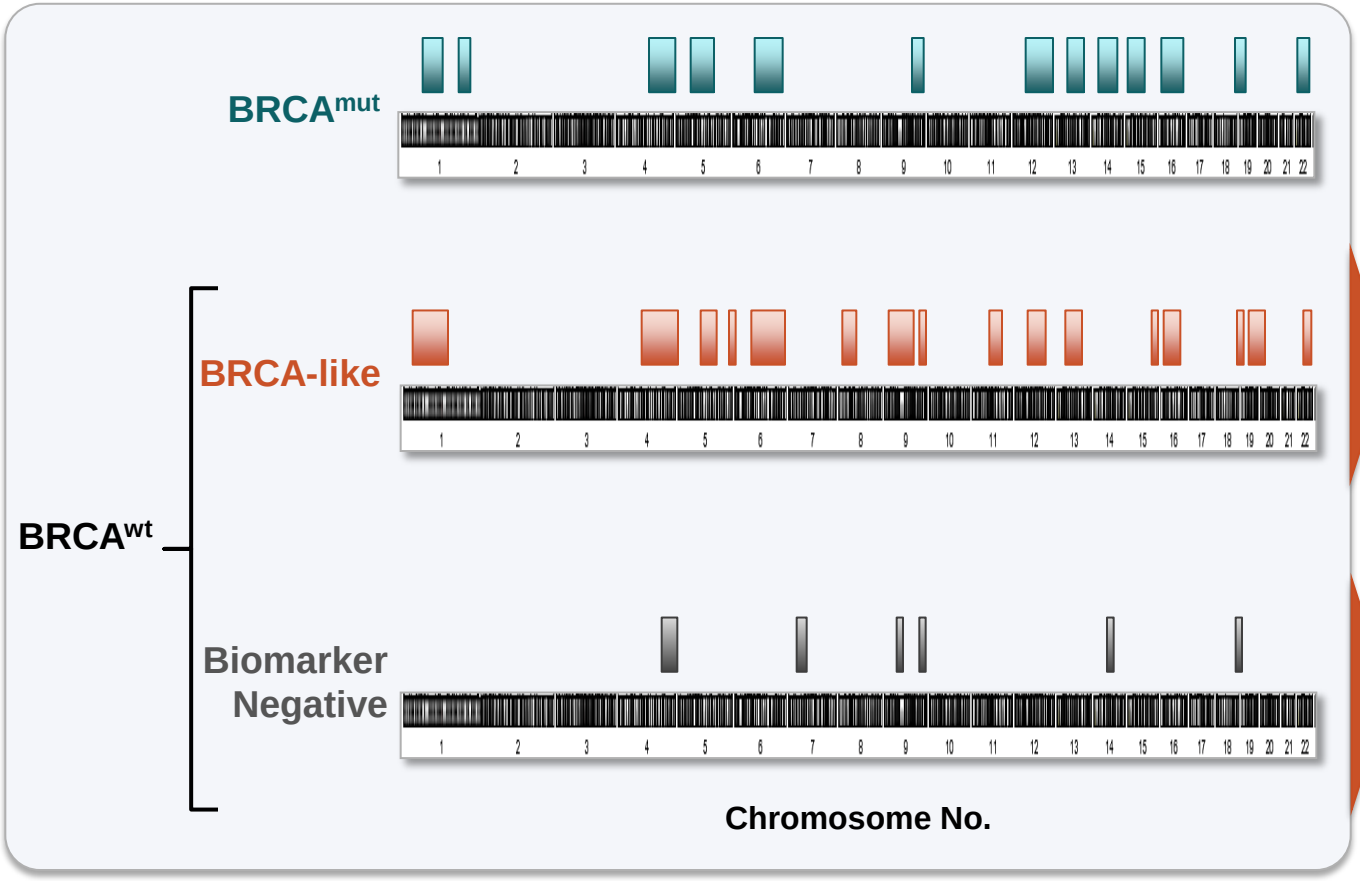
≈50% of HGOC patients may have alterations in the HR pathway per TCGA

Can we move beyond single candidate gene assay approaches?

Biomarkers for utility of PARPi beyond candidate gene mutations – what's cooking?

- **Functional test for loss of HR (RAD 51 foci-formation)**
  - Mukhopadhay et al, Clin Cancer Res, 2010, Graeser et al, Clin Cancer Res, 2010
- **Molecular signature (gene array)**
  - Konstantinopoulos et al, J Clin Oncol, 2010
- **Immunohistochemistry for BRCA 1 protein**
  - Garg K et al. Am J Surg Pathol 2013
- **Whole genome assays**
  – LOH scar assay: Myriad and Foundation Medicine

ARIEL 2 study:
HRD causes genome-wide loss of heterozygosity (LOH)



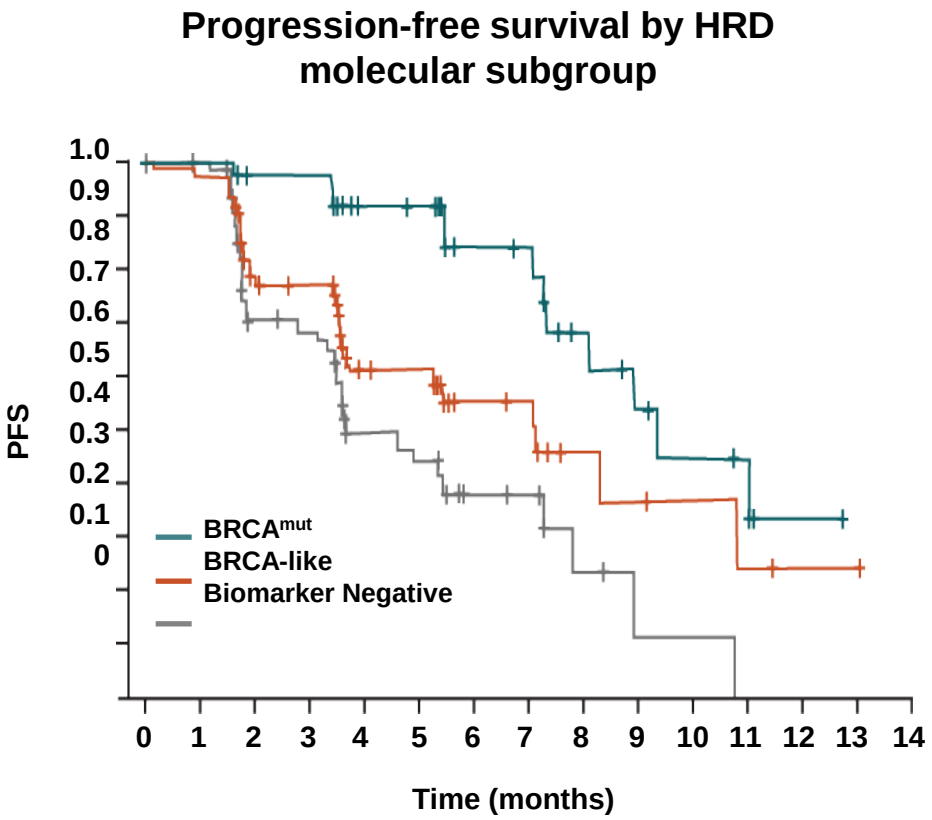
Hypothesis 1:
Ovarian cancer patients with high genomic LOH suggesting BRCA-like signature will respond to rucaparib.

Hypothesis 2:
Ovarian cancer patients who are "Biomarker Negative" (ie, with low genomic LOH) will not respond to rucaparib.

NGS=next-generation sequencing; mut=mutation; wt=wild type.

McNeish et al ASCO 2015

ARIEL 2: Primary efficacy analysis - PFS in BRCA^{mut} and BRCA-like versus Biomarker Negative



McNeish et al ASCO 2015

HRD Subgroup	Median PFS, mo (90% CI)
BRCA^{mut}	9.4 (7.3, Not Reached)
BRCA-like	7.1 (3.7, 10.8)
Biomarker Negative	3.7 (3.5, 5.5)

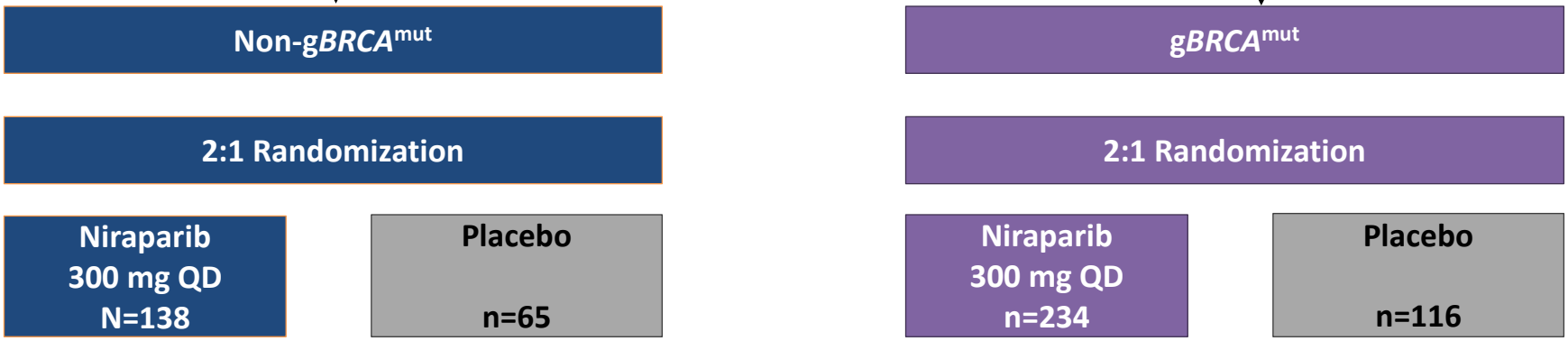
Subgroup Comparison	Hazard Ratio (90% CI)
BRCA^{mut} vs Biomarker Negative	0.47 (0.35, 0.64)
BRCA-like vs Biomarker Negative	0.61 (0.41, 0.92)

NOVA: Niraparib Maintenance in Patients with Recurrent Ovarian Cancer

Phase III, multicenter, randomized, double-blind, placebo controlled study

- Platinum-sensitive recurrent high grade serous ovarian cancer
- ≥2 prior regimens of platinum-based chemotherapy
- Received at least 4 cycles platinum-based therapy and, following treatment, have an investigator-defined CR or PR with no observable residual disease of <2cm and CA-125 WNL or a decrease of >90% that was stable for at least 7 days

N=553



Primary Endpoint: PFS by central, blinded review

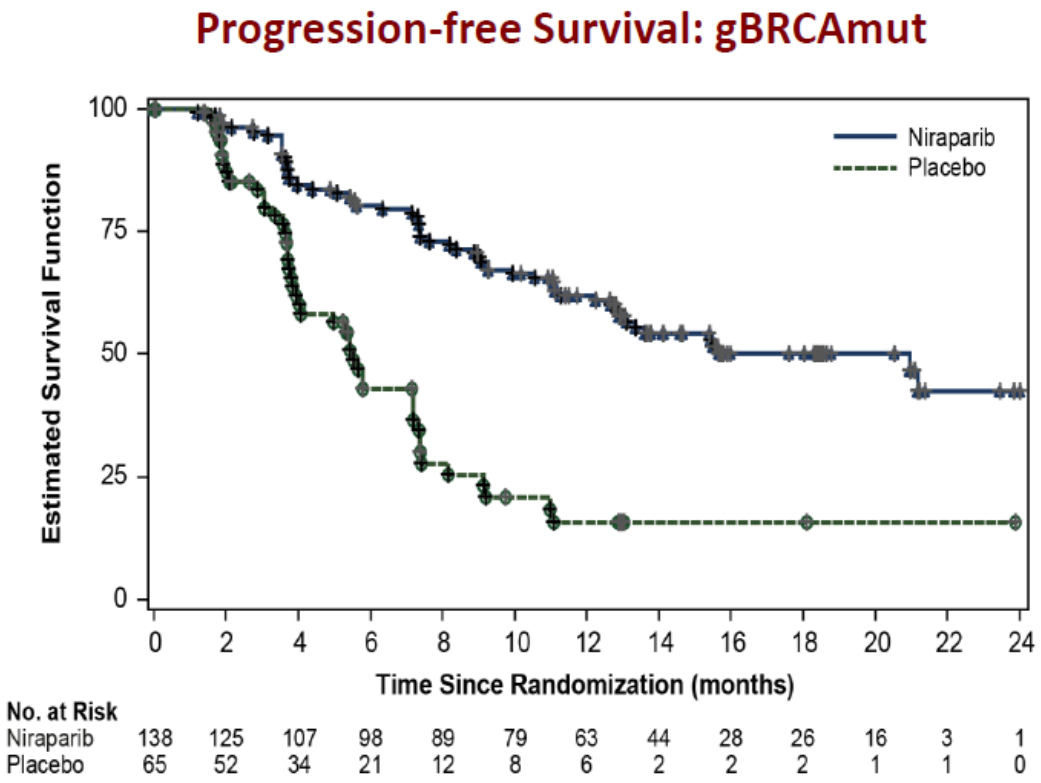
Tested at 100 events to achieve p<0.05

- HRDpos population
- Tested at 100 events to achieve p<0.05
- If test was positive then:
- Test overall non-gBRCAmut cohort (p<0.05

CA-125, cancer antigen 125; CR, complete response; HRDpos, homologous recombination deficiency positive; PR, partial response; WNL, within normal limits; QD, every day.

Mirza et al. NEJM. 2016; October 8

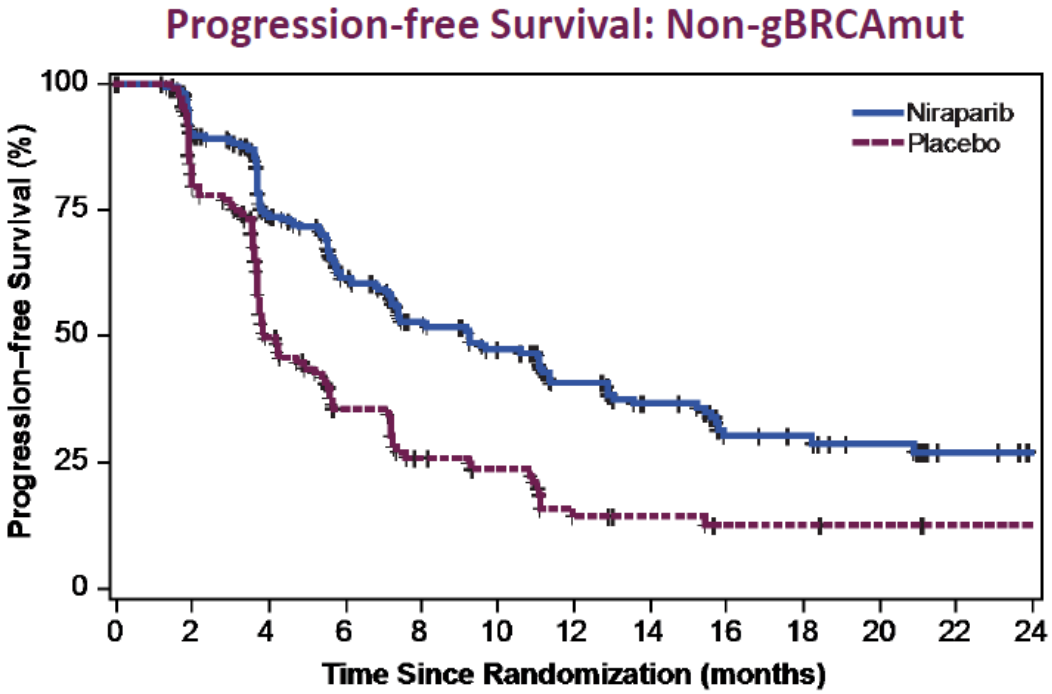
NOVA: Niraparib Maintenance – germline BRCAmut



Treatment	PFS Median (95% CI) (Months)	Hazard Ratio (95% CI) p-value	% of Patients without Progression or Death	
			12 mo	18 mo
Niraparib (N=138)	**21.0** (12.9, NE)	**0.27** (0.173, 0.410) p<0.0001	62%	**50%**
Placebo (N=65)	**5.5** (3.8, 7.2)		16%	16%

Mirza et al ESMO 2016

NOVA: Niraparib Maintenance – non-germline BRCAmut



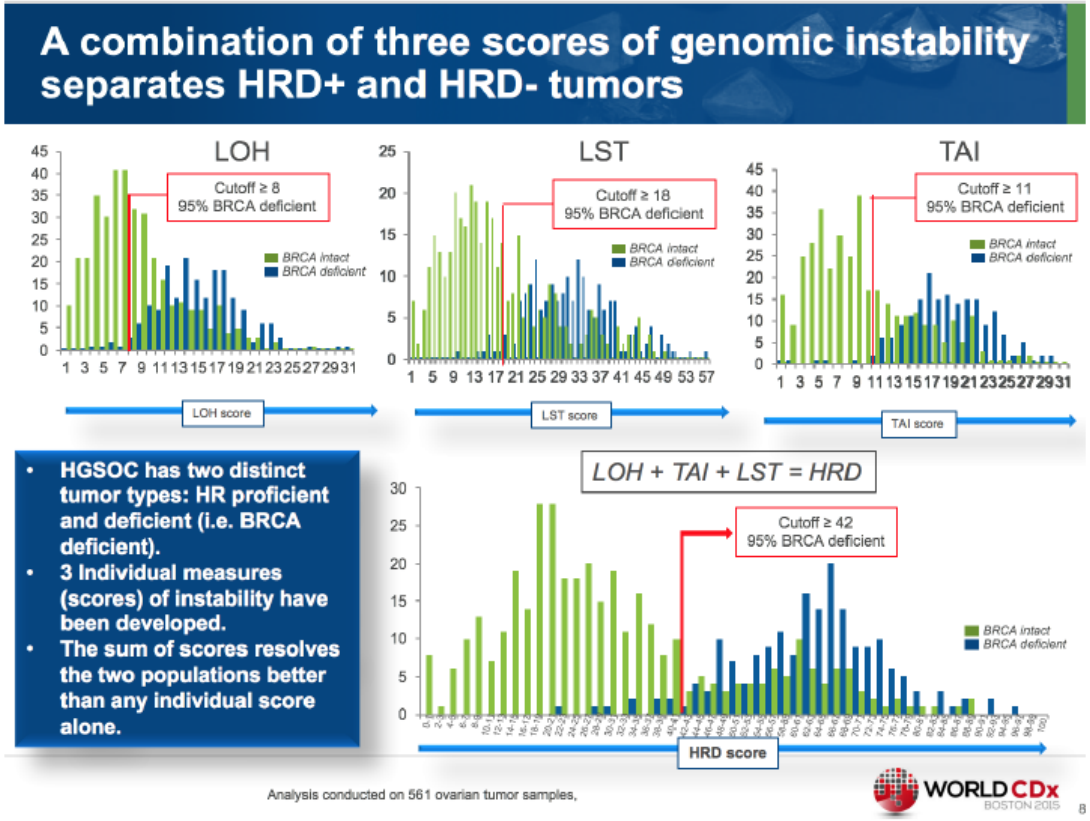
Treatment	PFS Median (95% CI) (Months)	Hazard Ratio (95% CI) p-value	% of Patients without Progression or Death	
			12 mo	18 mo
Niraparib (N=234)	9.3 (7.2, 11.2)	0.45 (0.338, 0.607) p<0.0001	41%	30%
Placebo (N=116)	3.9 (3.7, 5.5)		14%	12%

Mirza et al ESMO 2016

The Myriad HRD test

Testing for Homologous Recombination Deficiency (HRD)

- Loss of Heterozgosity,
- Large-scale State Transitions,
- Telomeric Imbalance



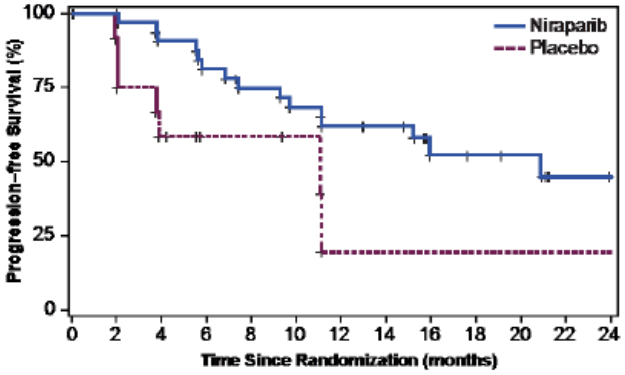
Mirza et al ESMO 2016

NOVA: Exploratory analysis using HRD biomarker – non-germline BRCAmut

HRD-positive

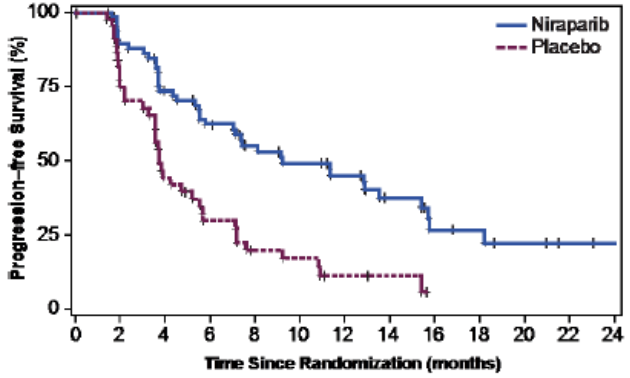
sBRCAmut

Treatment	PFS Median (95% CI) (Months)	Hazard Ratio (95% CI) p-value	% of Patients without Progression or Death	
			12 mo	18 mo
Niraparib (N=35)	20.9 (9.7, NR)	0.27 (0.081, 0.903) p=0.0248	62%	52%
Placebo (N=12)	11.0 (2.0, NR)		19%	19%



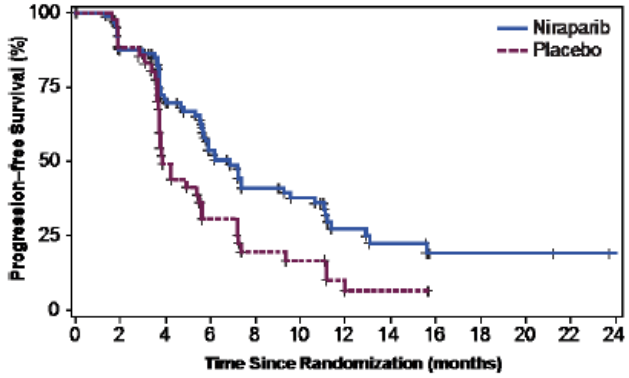
BRCAwt

Treatment	PFS Median (95% CI) (Months)	Hazard Ratio (95% CI) p-value	% of Patients without Progression or Death	
			12 mo	18 mo
Niraparib (N=71)	9.3 (5.8, 15.4)	0.38 (0.231, 0.628) p=0.0001	45%	27%
Placebo (N=44)	3.7 (3.3, 5.6)		11%	6%



HRD-negative

Treatment	PFS Median (95% CI) (Months)	Hazard Ratio (95% CI) p-value	% of Patients without Progression or Death	
			12 mo	18 mo
Niraparib (N=92)	6.9 (5.6, 9.6)	0.58 (0.361, 0.922) p=0.0226	27%	19%
Placebo (N=42)	3.8 (3.7, 5.6)		7%	7%



Mirza et al ESMO 2016

Does route of chemo administration matter in *BRCA1/2* mutant EOC?

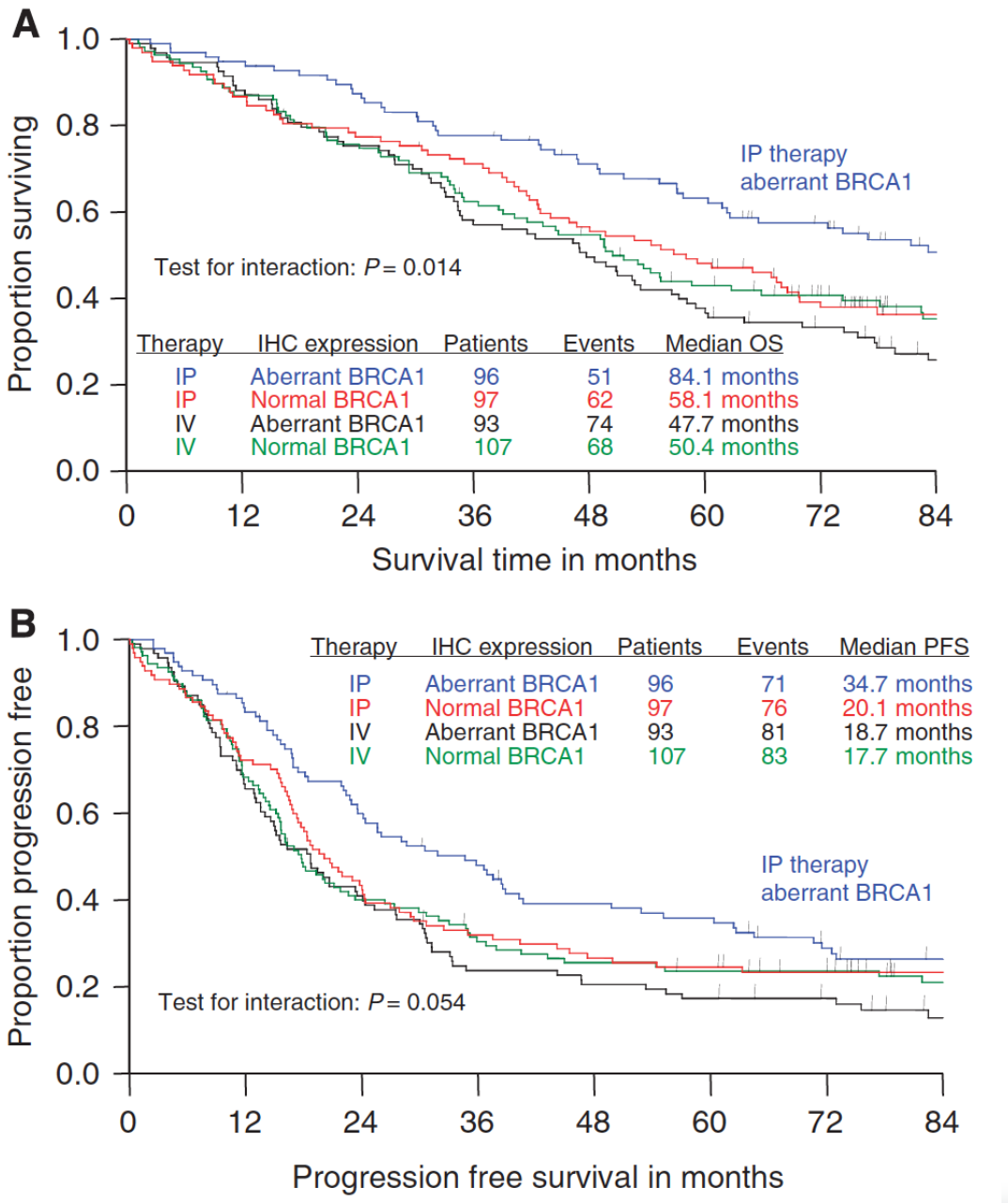
Aberrant vs normal BRCA1 expression in IP chemo pts:

ΔPFS = 14.6 mths
ΔOS = 26mths

Aberrant BRCA1 expression for very low to no staining (**<**10% staining; 0 or 1 score)

Normal BRCA1 expression for **>**10% BRCA1 staining (2–4 score).

Lesnock et al BJC 2013



Knowledge of BRCA1/2 or other HRD status in patients with ovarian cancer will have crucial implications for choice of therapy in the primary and recurrent disease setting.

Mdm C

July 2010 - diagnosed Stage IIIC ovarian cancer – optimal debulking surgery

July - Nov 2010 - has 6 cycles adjuvant Carboplatin & Paclitaxel

March 2013 - ca125 @ 900.

April 2013 - started 4 cycles of chemotherapy Carboplatin and Caelyx → interval surgery in **July 2013** - completed 2 more cycles of carbo/caelyx by **Sept 2013**

July 2014 - 6 Nov 2014: PD → started 6 cycles of chemotherapy (Carboplatin + Weekly Taxol for first 4 cycles; then got allergy to Carbo G2 → Cisplatin + weekly Taxol for last 2 cycles) → CR

- **April 2015** PD on CT scan with disease over vaginal vault → Started KPT330 study --> **PD on 4/6/15**

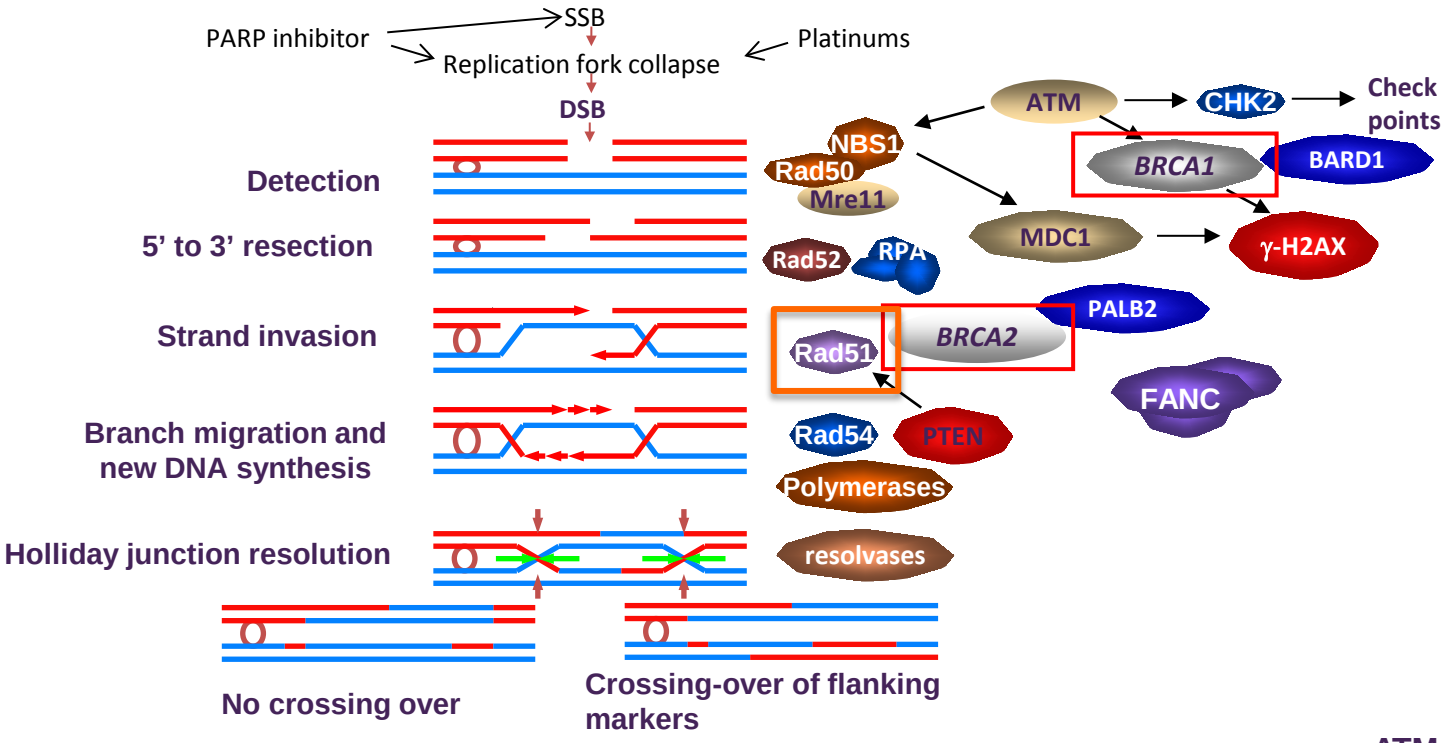
- **9/6/15-12/11/15:** Completed cycle 8 carbo/gem (desen regimen for carbo) chemo with good response.

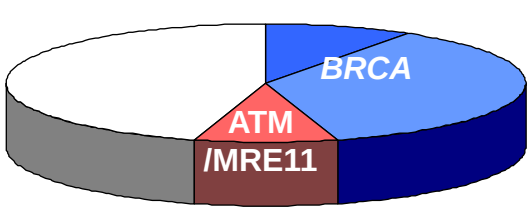
- **15/2/16: Disease progression - germline testing – No *BRCA1/2* mutation but *RAD51C* pathogenic mutation**

- **7/3/16-14/6/16:** Low-dose abdominal radiotherapy with weekly paclitaxel study

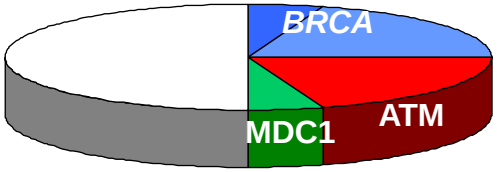
- **PD on 30/6/16 → CA125 17256, in bowel obstruction** → what next?

BRCA1/2 loss leads to homologous recombination deficiency (HRD) and cancer

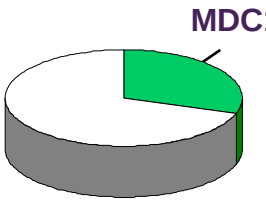




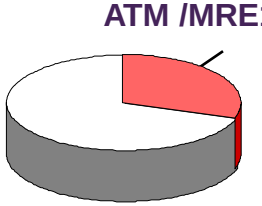
Serous ovarian



TN breast



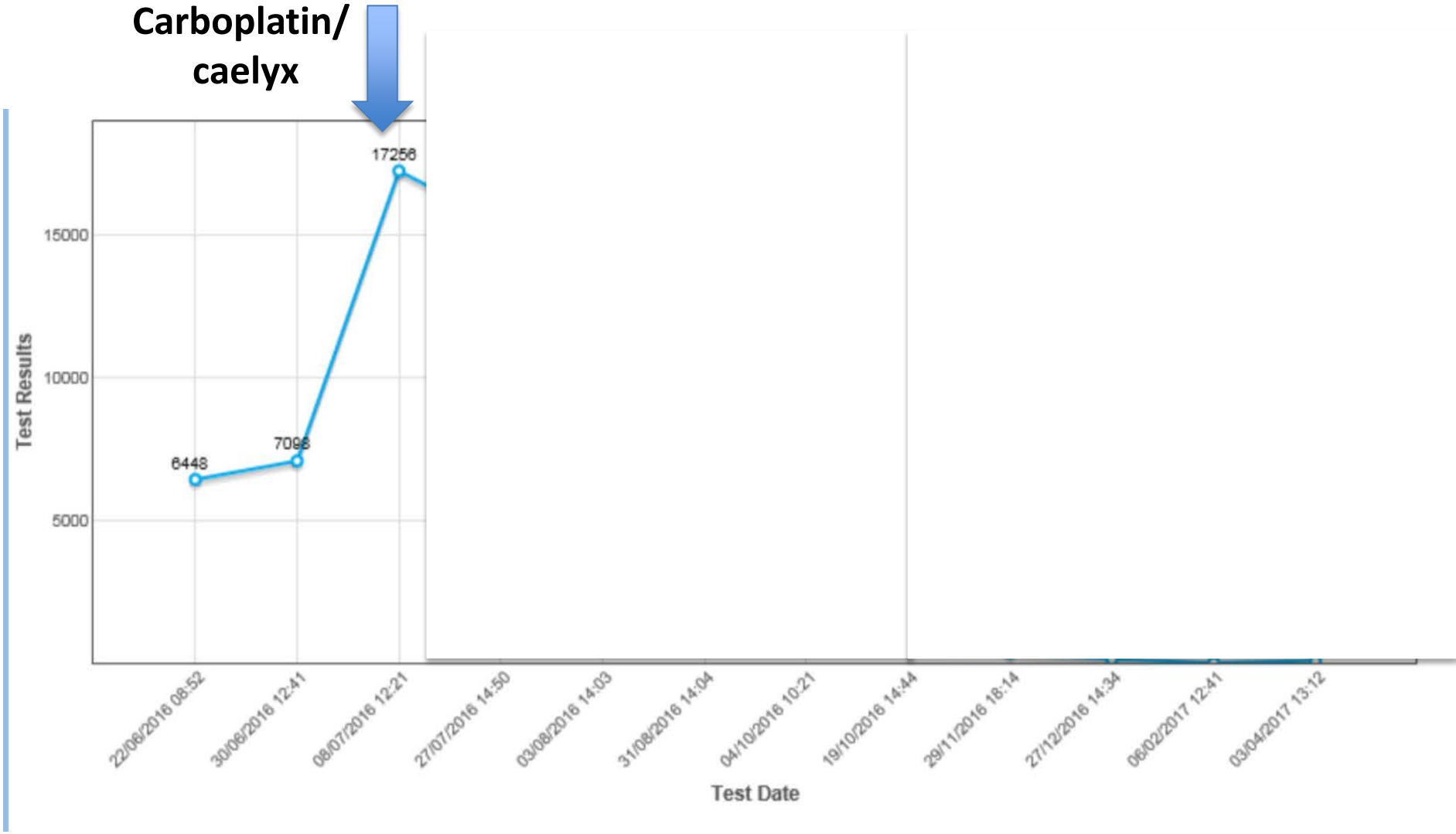
NSCLC

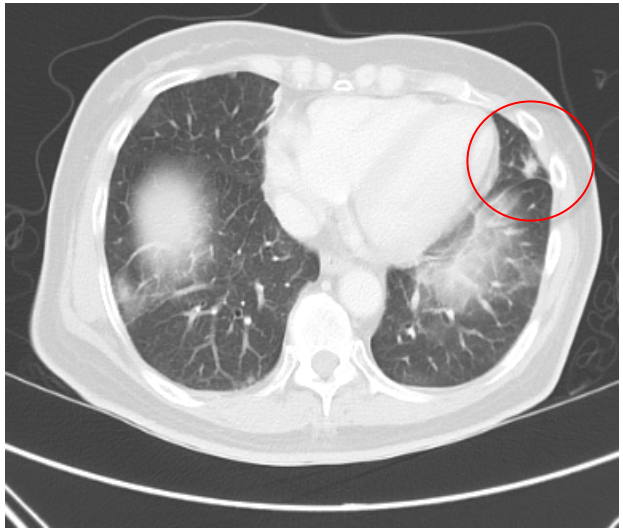


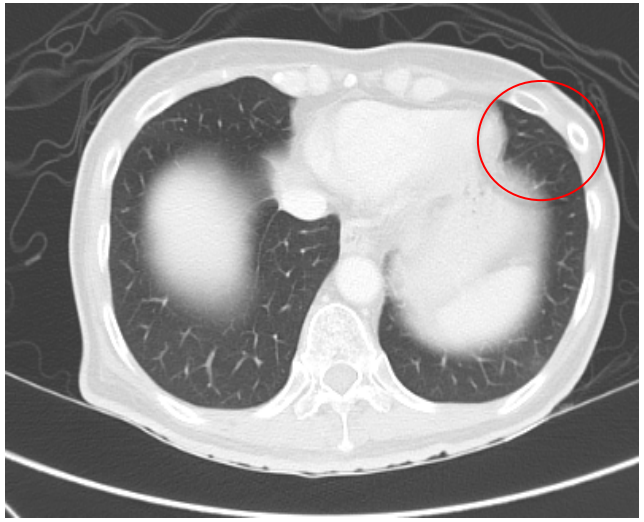
Gastric/CRC

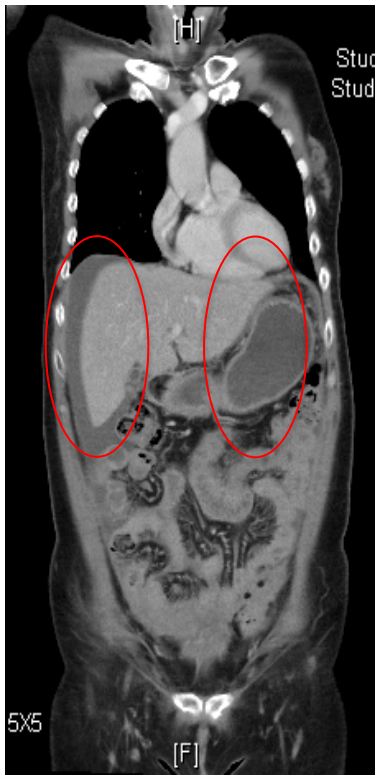
CRC, colorectal cancer; DSB, double-strand break;
NSCLC, non-small cell lung cancer; SSB, single-strand break

CA125 from July 2016 to April 2017

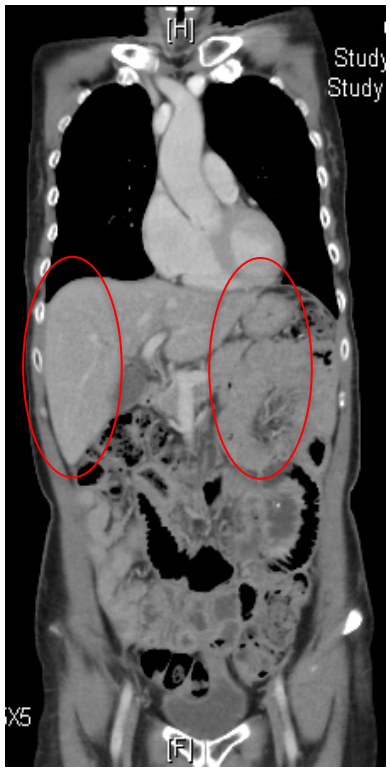








July 2016

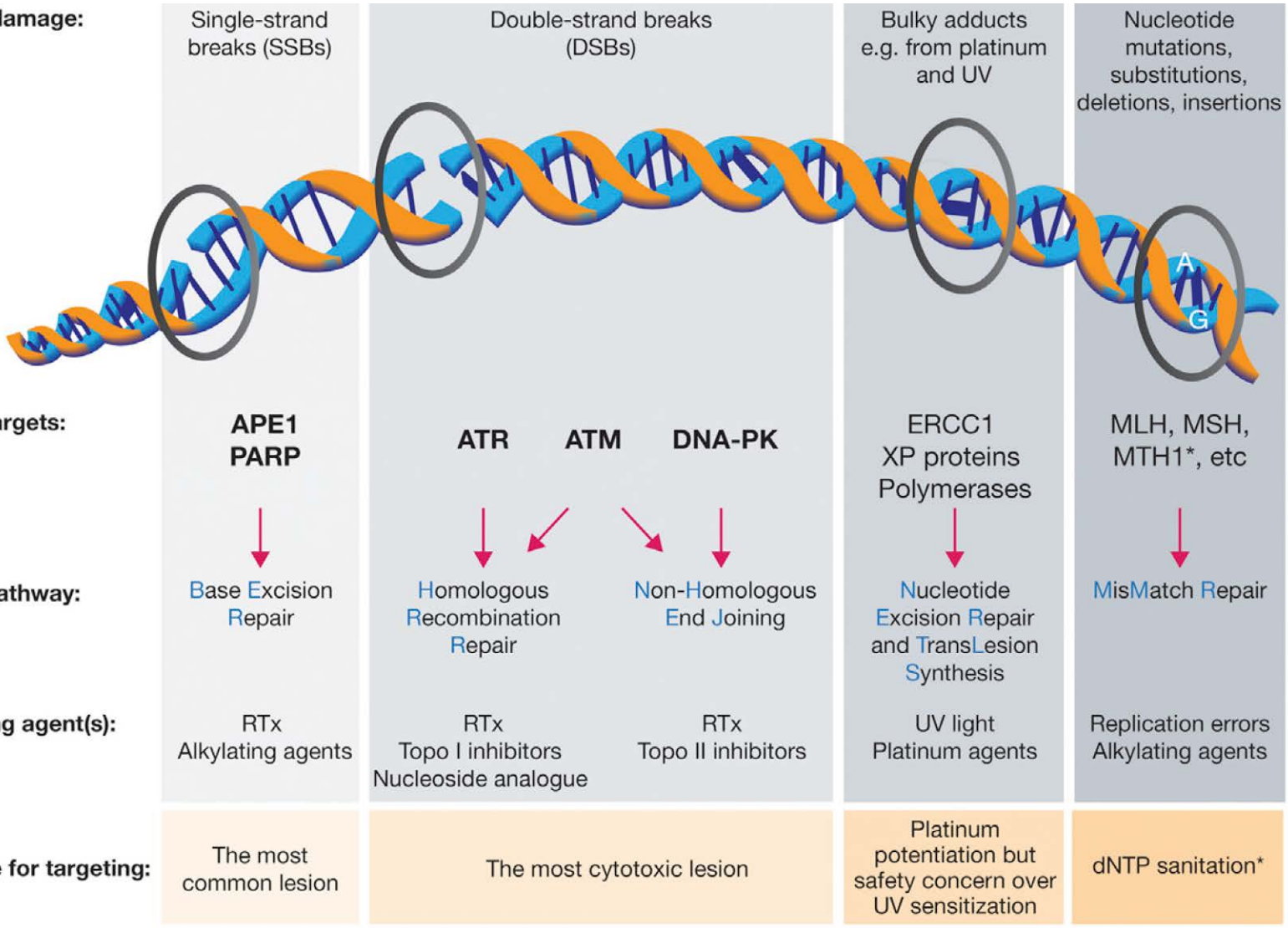


April 2017

July - Sept 2016:
Completed 3 cycles carbo/caelyx chemotherapy → bowel obstruction improved
Able to eat and drink

Sept 2016 to April 2017:
olaparib maintenance

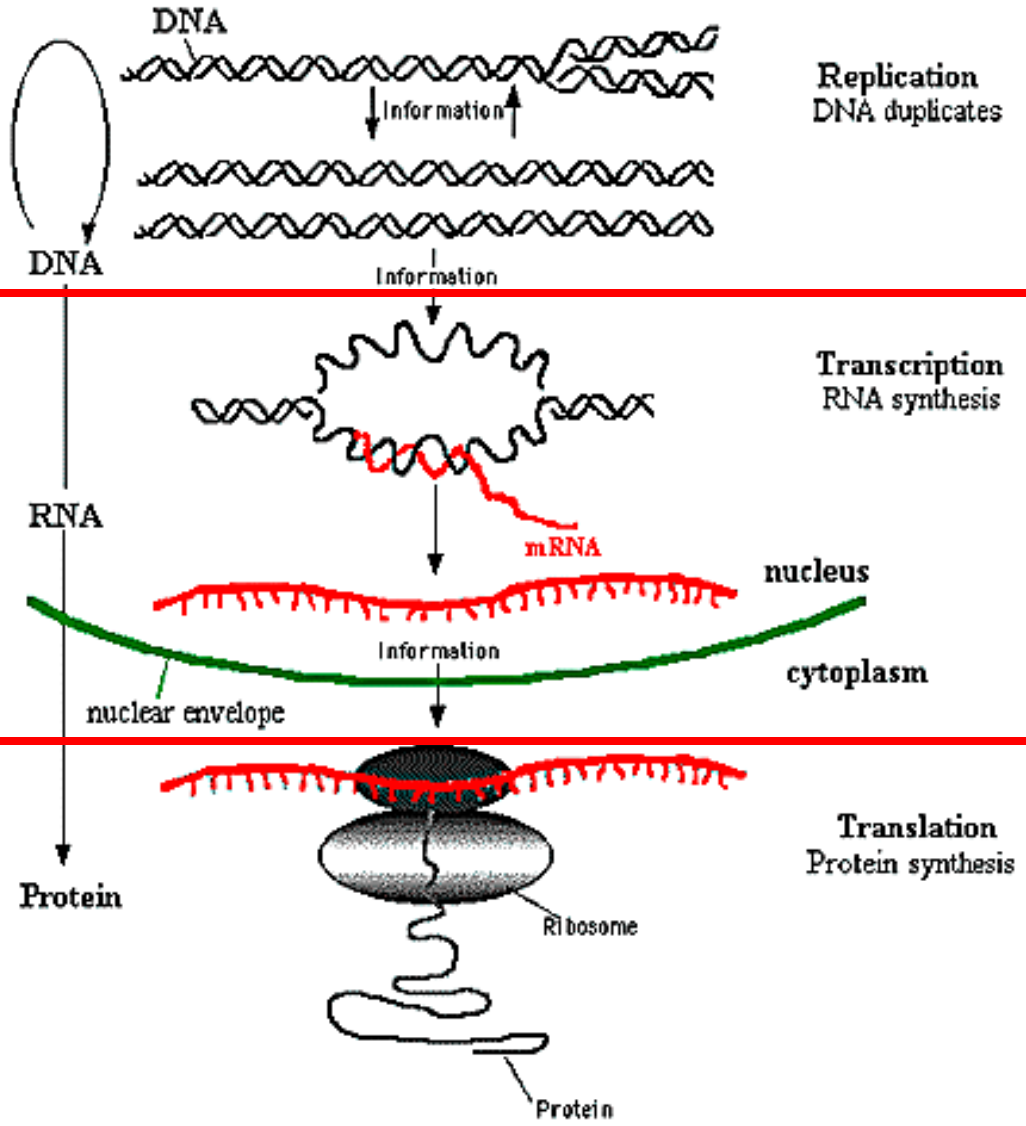
DNA Damage Response Pathways: More than just Homologous Recombination



*MTH1/dNTP sanitation proposed as an opportunity but emerging data have not been able to provide validation
Shown in bold are SSB and DSB repair targets that are currently being evaluated in clinical trials

Gene Expression Subtypes in Ovarian Cancer

Central Dogma of Molecular Biology



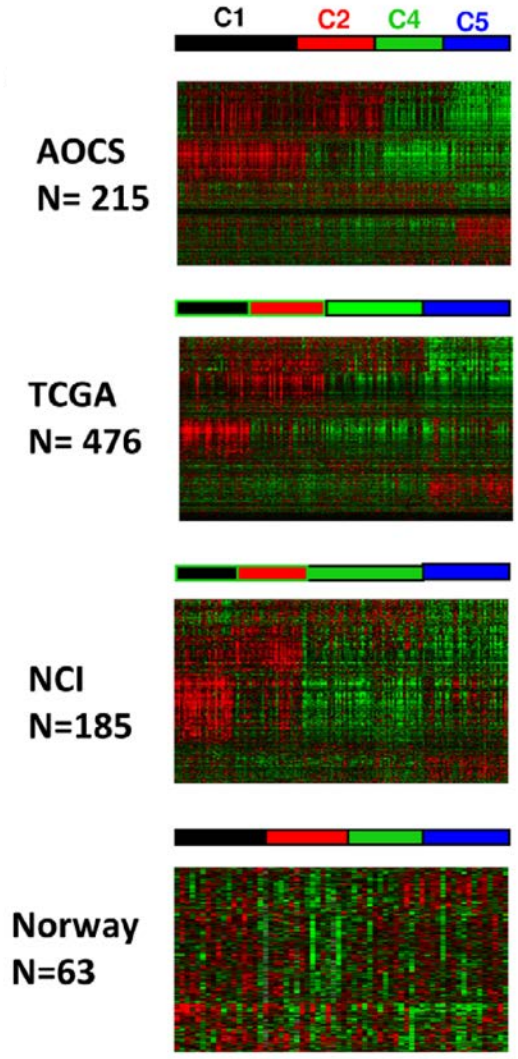
GENOME
- DNA sequencing: mutations
- FISH/CISH: amplification/ translocations

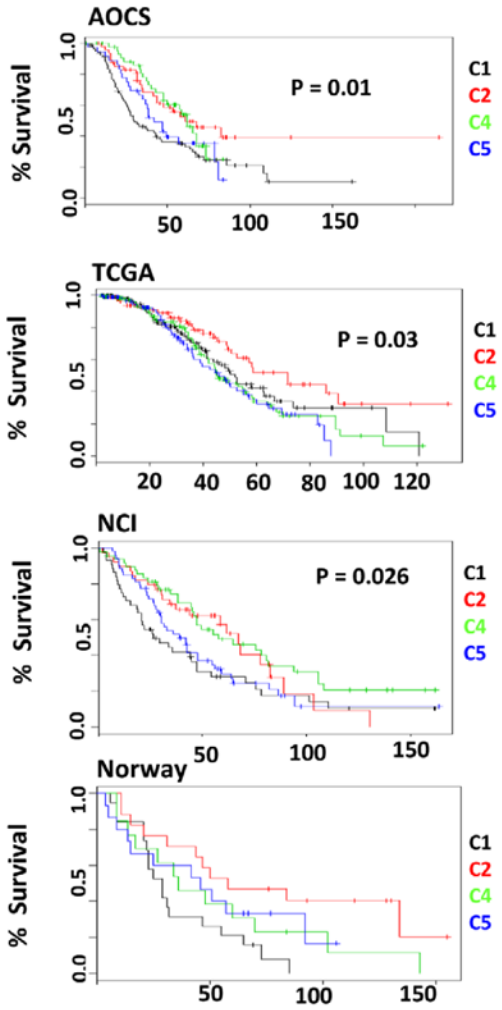
TRANSCRIPTOME:
- mRNA expression: measure mRNA levels
- RNA sequencing: alternative gene spliced transcripts, post-transcriptional changes, gene fusion, mutations/SNPs and changes in gene expression

PROTEOME:
- Immunohistochemistry
- Western blots
- Mass spectrometry
- ELISA

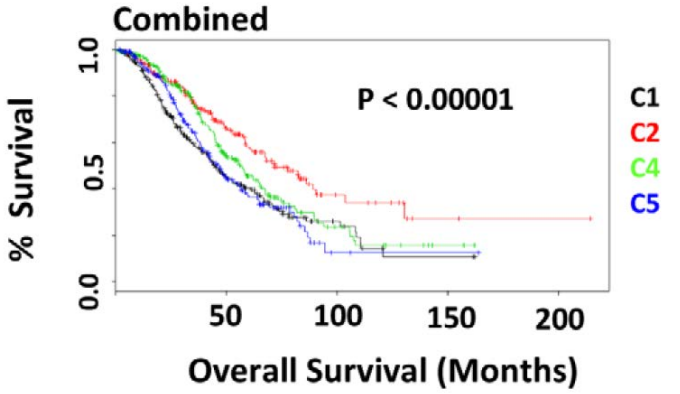
→ Measure protein expression

Molecular Subtypes of High-Grade Serous Ovarian Cancers and Survival Outcome



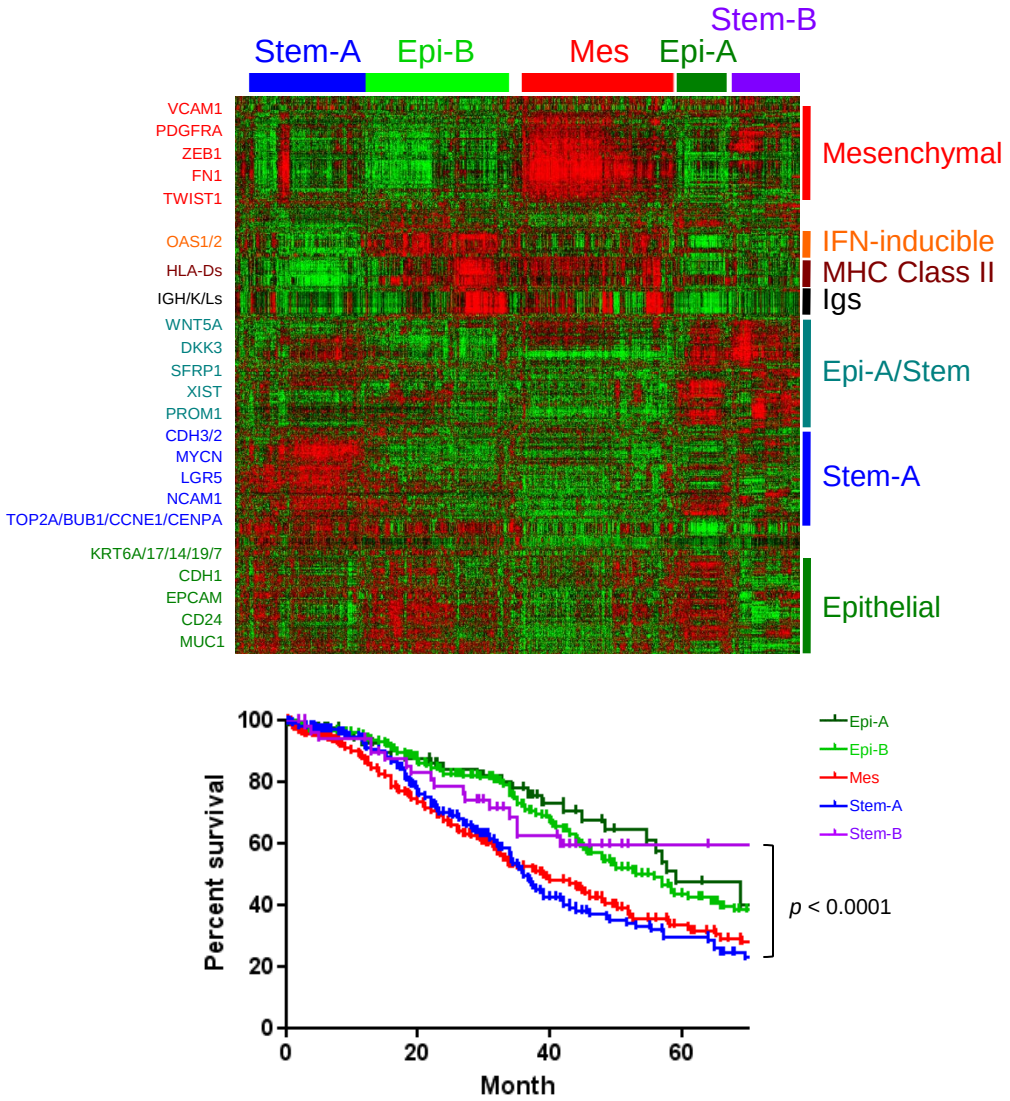


Groups Compared	hazard ratio	*P-value*
C5 vs C1	0.96	0.81
C5 vs C2	1.79	0.0001
C5 vs C4	1.31	0.045



Helland et al Plos One 2011

EOC Gene expression molecular subtypes



- Meta-analysis (***Tan, Miow, & Huang et al., EMBO MM, 2013***) of 1,538 EOCs including all histological types.

4 subtypes showing survival differences

Proposed	Tothill *et al.*, 2008	TCGA, 2012
Epi-A	C3	Differentiated
Epi-B	C4	
	C2	Immunoreactive
Mes	C1	Mesenchymal
Stem-A	C5	Proliferative
Stem-B	C6	

Tan, Miow & Huang et al., EMBO Mol Med, 2013

Molecular subgroups of EOC e.g. Epithelial/ C2/ Immunoreactive, Mesenchymal/C1 , or Stem-like/ C5/ Proliferative with distinct survival outcomes.

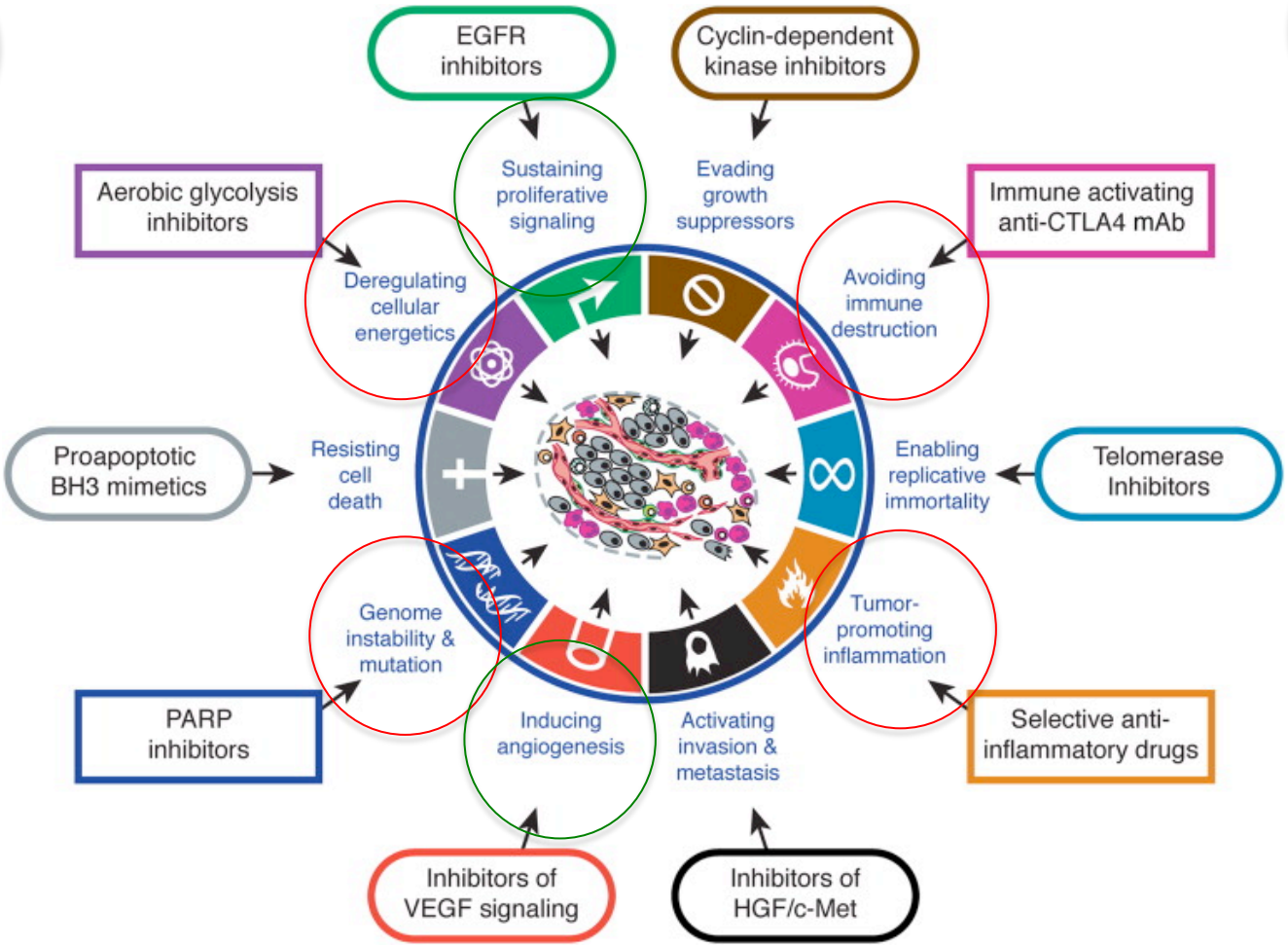
Targeting the Hallmarks of Cancer: ?relevance of gene expression signatures

Patient A

C2

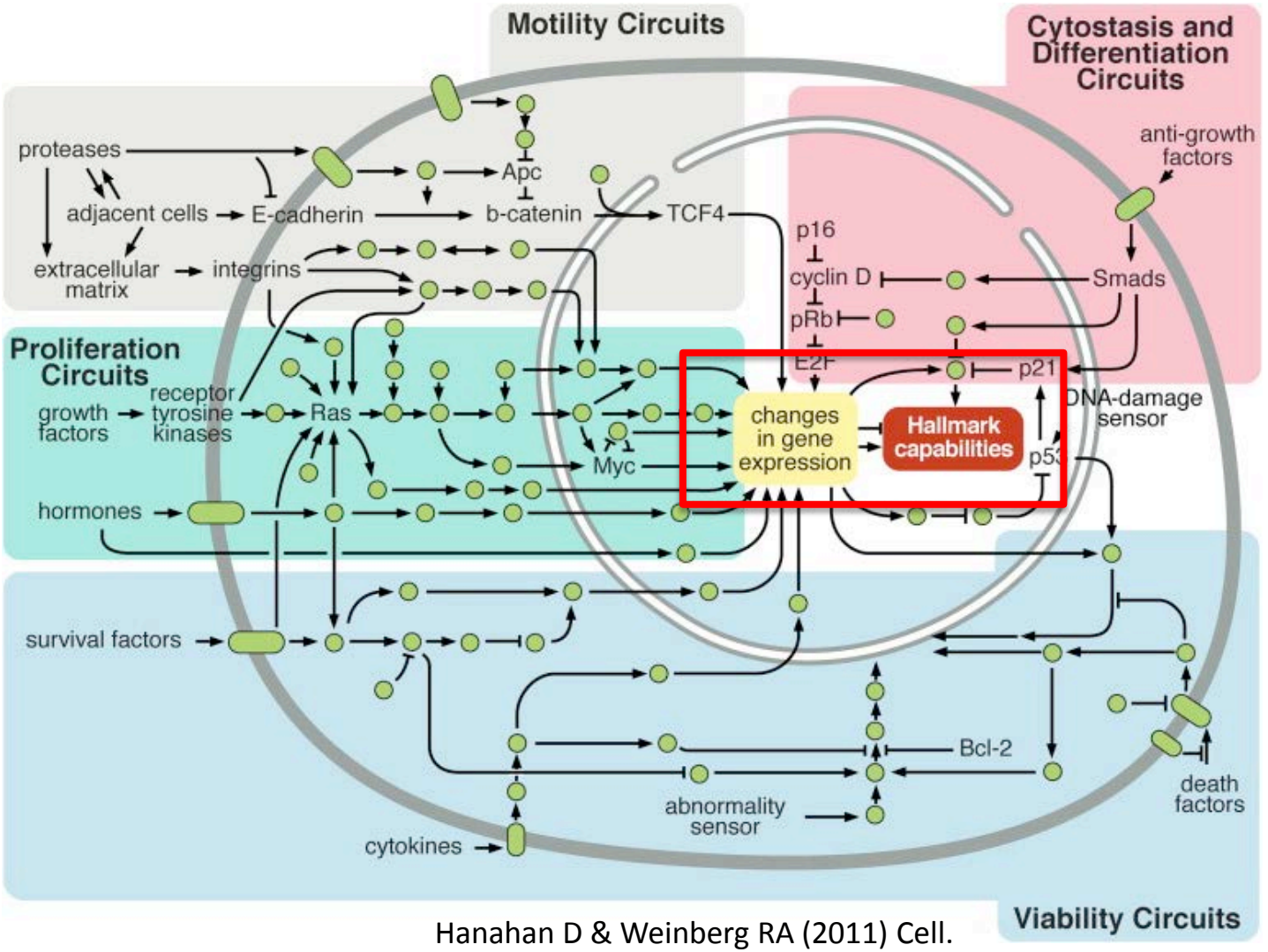
Patient B

C5



Hanahan D & Weinberg RA (2011) Cell.

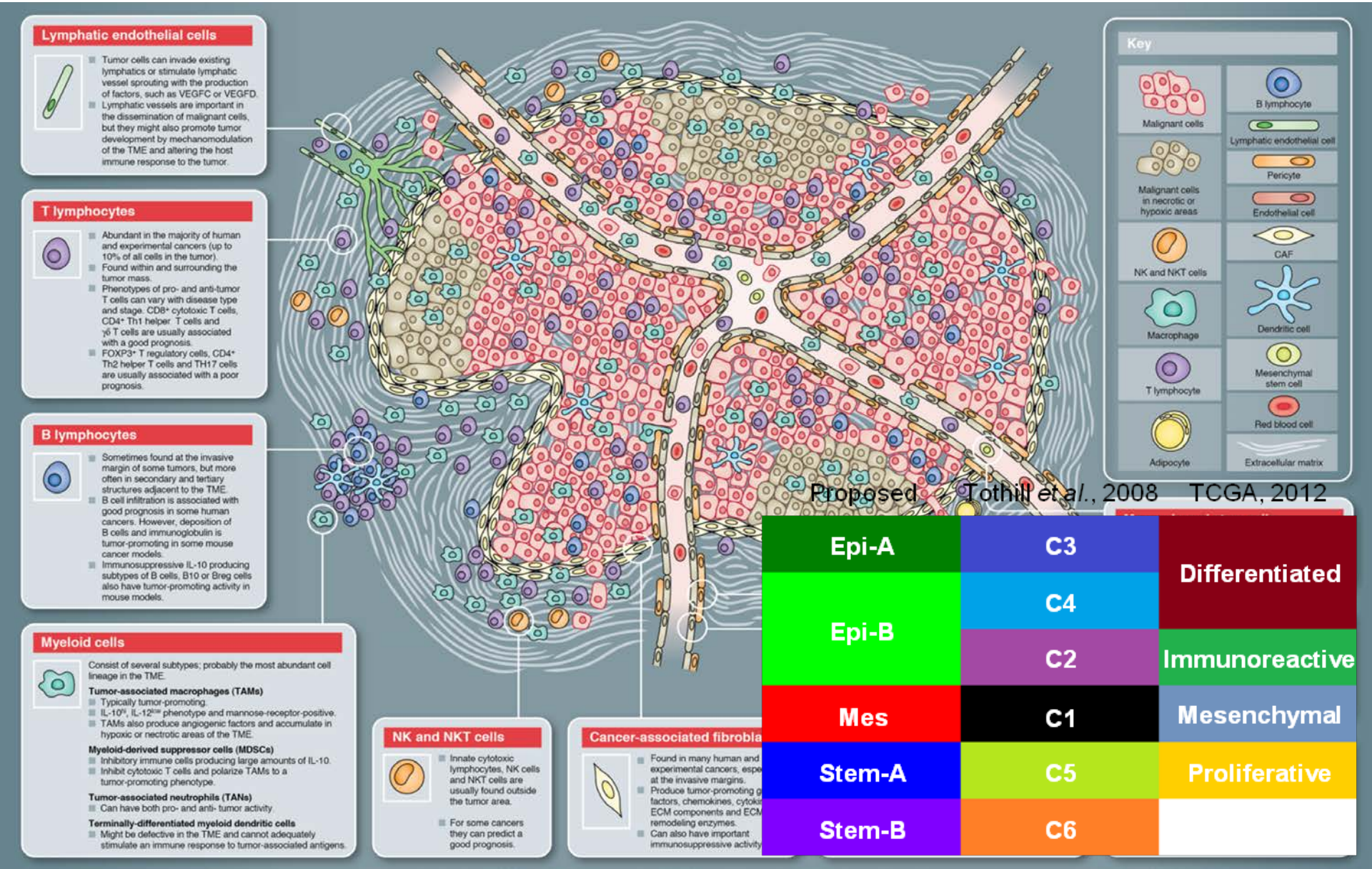
Deciphering the network: can we target the complex molecular circuitry of cancer for precision therapy?



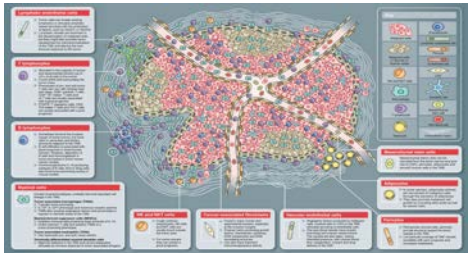
Hanahan D & Weinberg RA (2011) Cell.

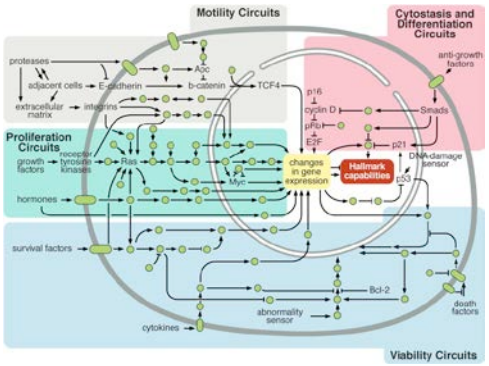
Molecular subtypes likely to reflect aggregate phenotype of tumour cells & microenvironmental factors

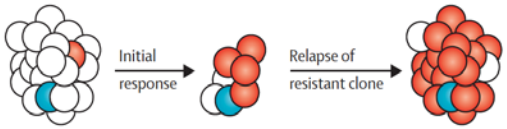
Figure from Balkwill et al Journal of Cell Science, 2012

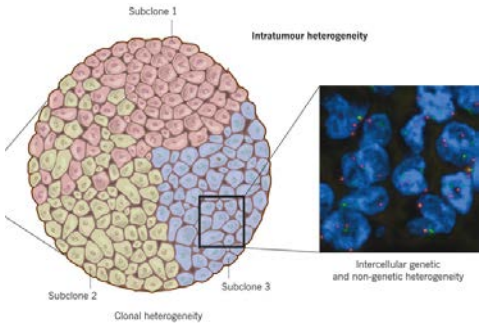


Gene expression signatures to guide therapy?

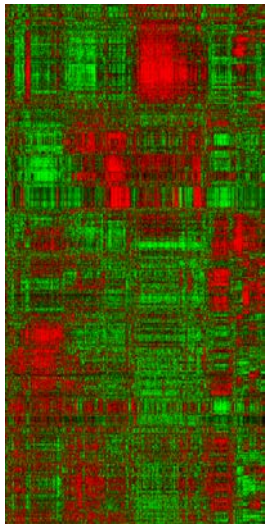






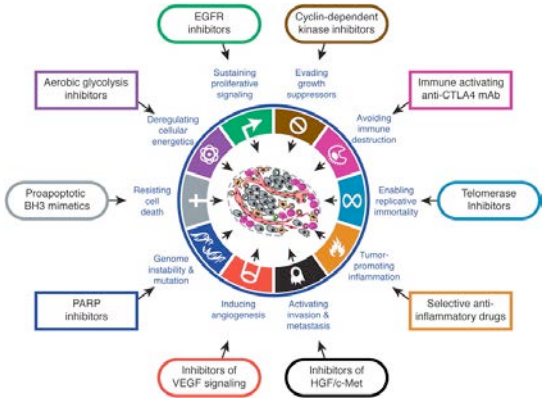


Tumor gene expression signature



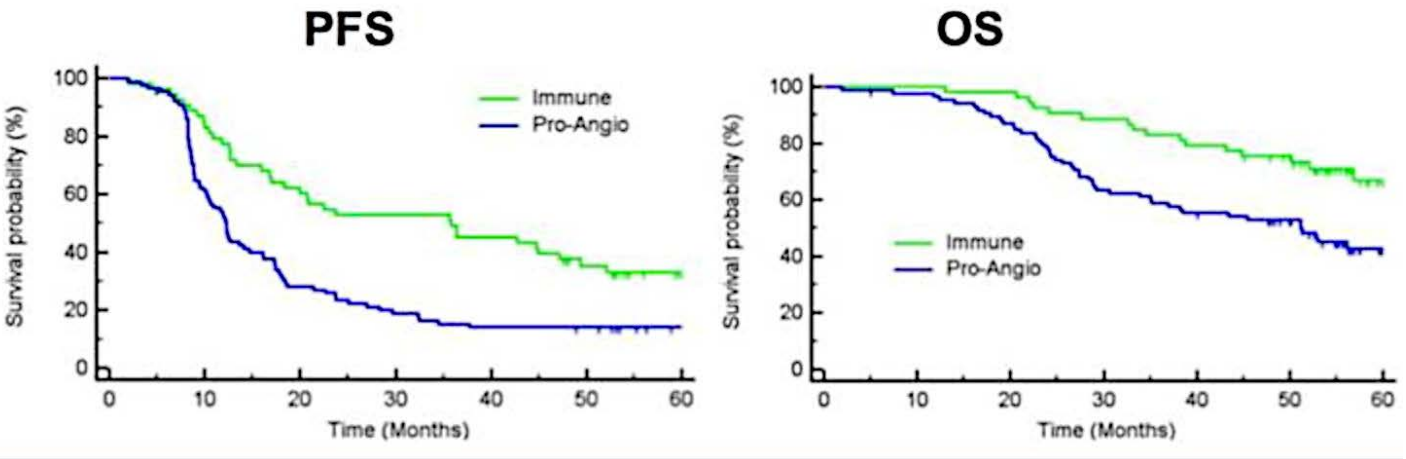
Epi-A	C3	Differentiated
Epi-B	C4	
	C2	Immunoreactive
Mes	C1	Mesenchymal
Stem-A	C5	Proliferative
Stem-B	C6	

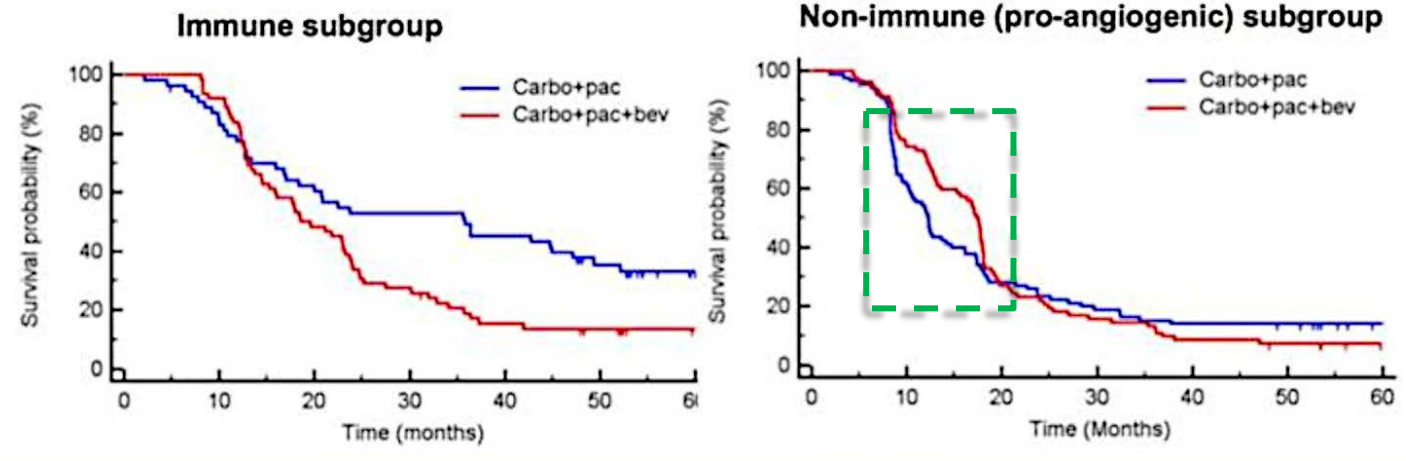
Hallmark capabilities



Therapeutic Tool Kit:
Chemotherapy, Biologics
TKIs, Immunotherapy

Outcome of 'immune' and 'proangiogenic' groups of ovarian cancer in ICON 7





<u>Control arm ICON7</u>
Immune and proangiogenic groups

Bev had adverse effect on PFS in immune subgroup

Benefit in pro-angiogenic subgroup

Gourley et al ASCO 2014

Clinical Cancer Research

Bevacizumab may differentially improve ovarian cancer outcome in patients with proliferative and mesenchymal molecular subtypes

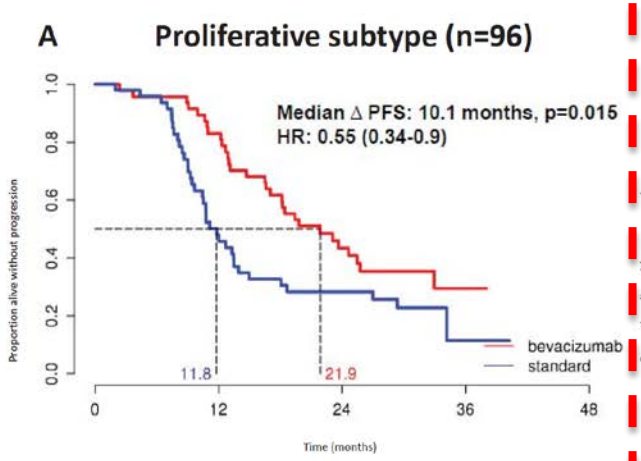
Stefan Kommoss, Boris Winterhoff, Ann Oberg, et al.

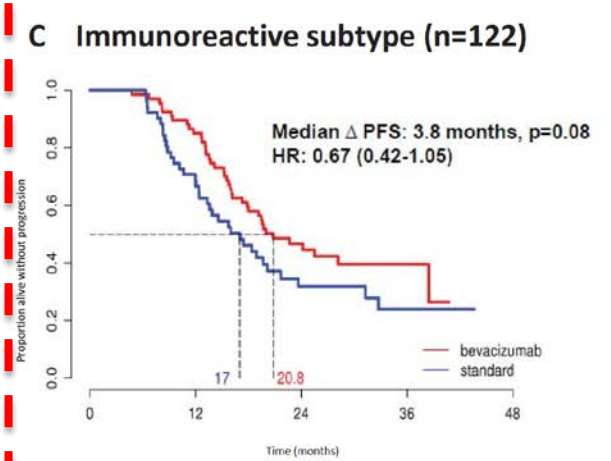
Clin Cancer Res Published OnlineFirst February 3, 2017.

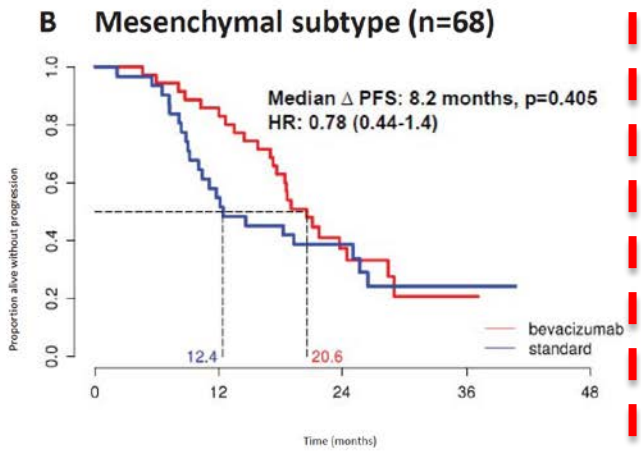
Mesenchymal and proliferative subtypes share an angiogenic gene expression profile (GEP)

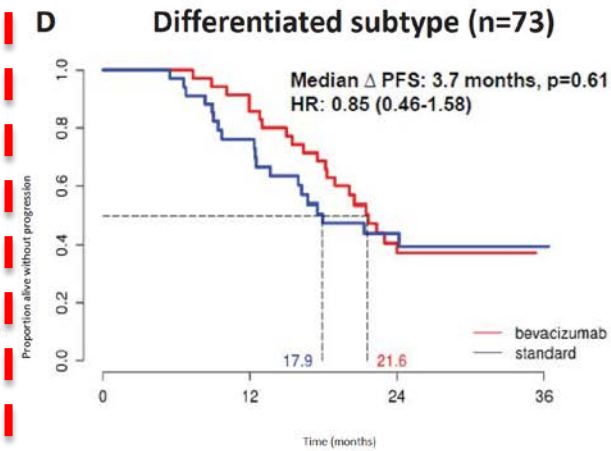
Angiogenic GEP = 8.2 to 10.1 mth increased PFS

Non-angio GEP = 3.7 to 3.8 mth increased PFS

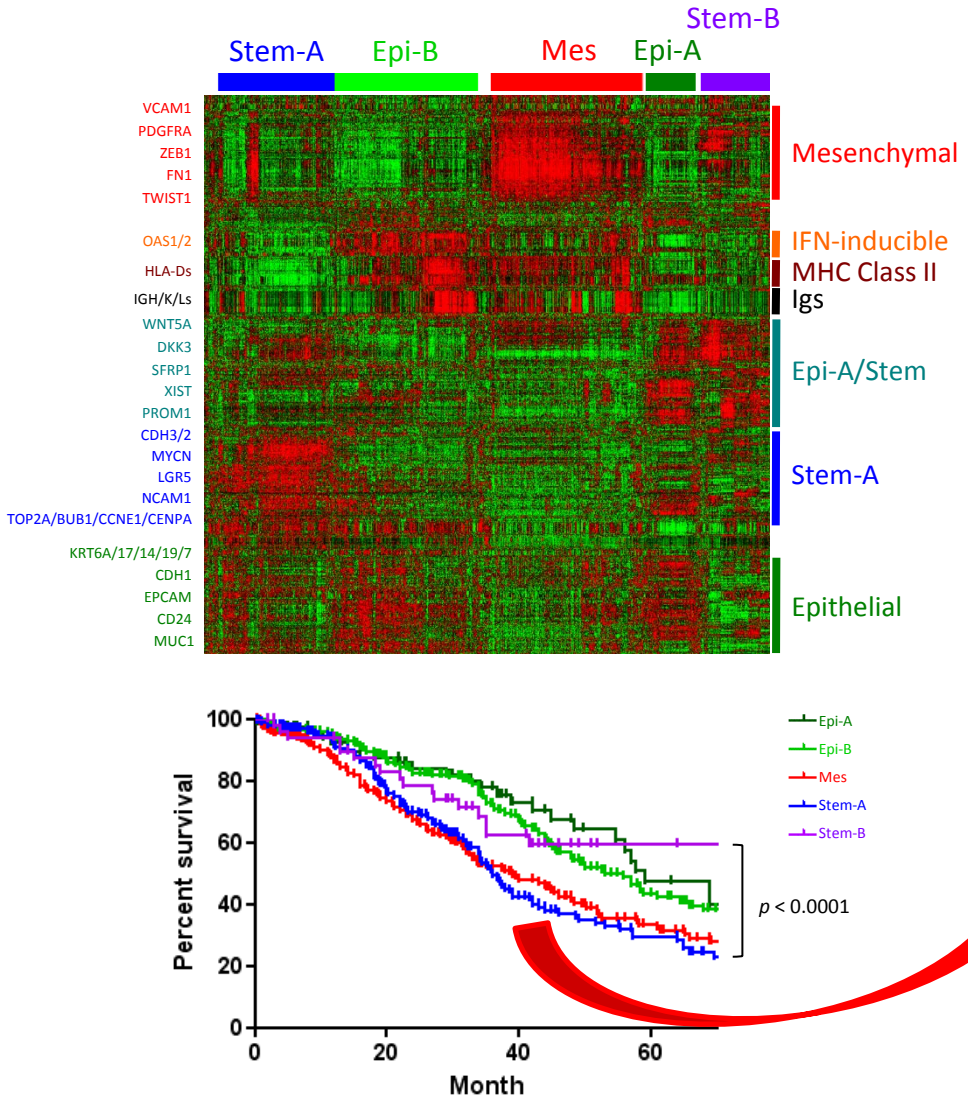








C1/Mes and C5/Stem A subtypes have worse outcomes



- Meta-analysis (***Tan, Miow, & Huang et al., EMBO MM, 2013***) of 1,538 EOCs including all histological types.
  - 5 subtypes
  - showing survival differences

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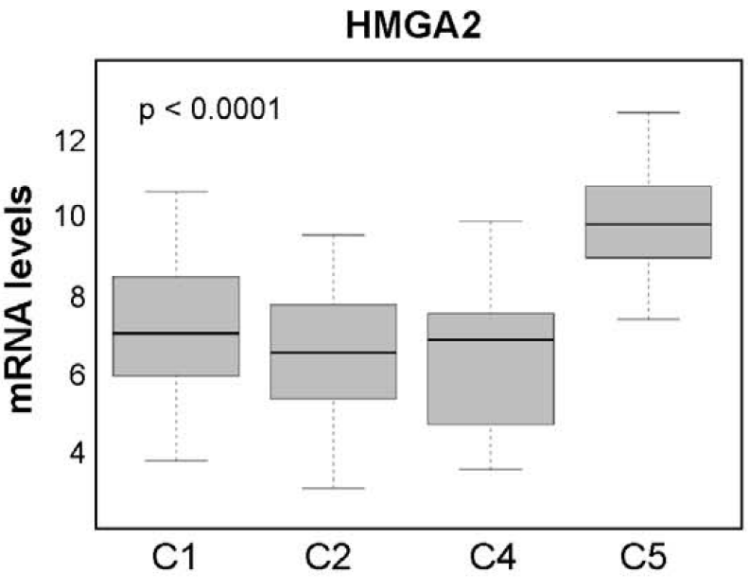
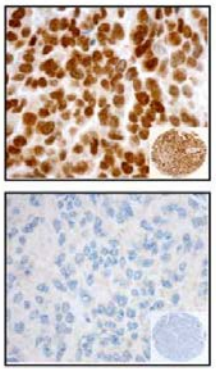
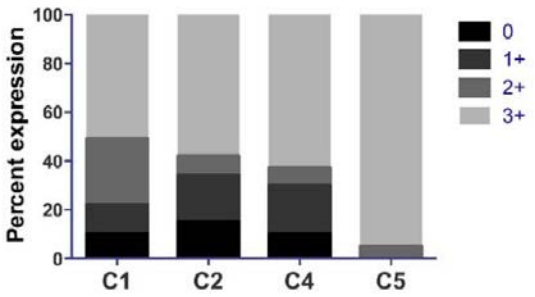
	C2	Immunoreactive
Mes	C1	Mesenchymal
Stem-A	C5	Proliferative
Stem-B	C6	

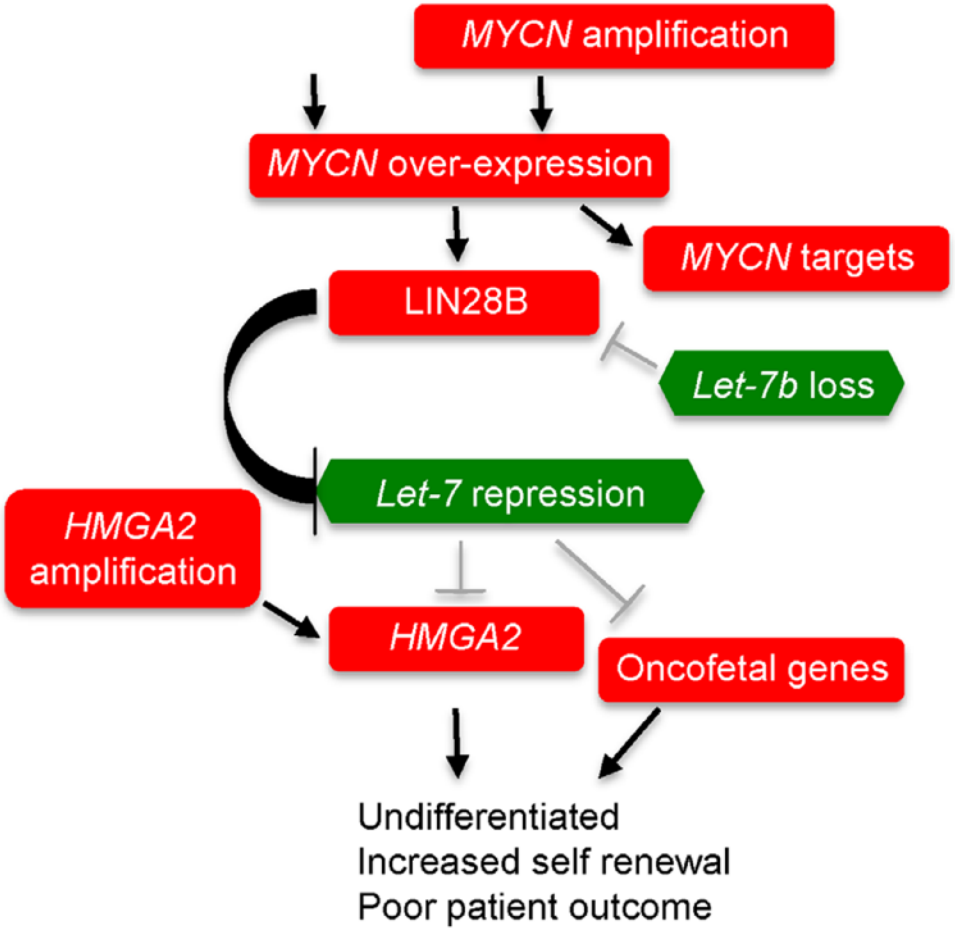
Tan, Miow & Huang et al., EMBO Mol Med, 2013

Gene dysregulation in StemA/ C5 HGSOC



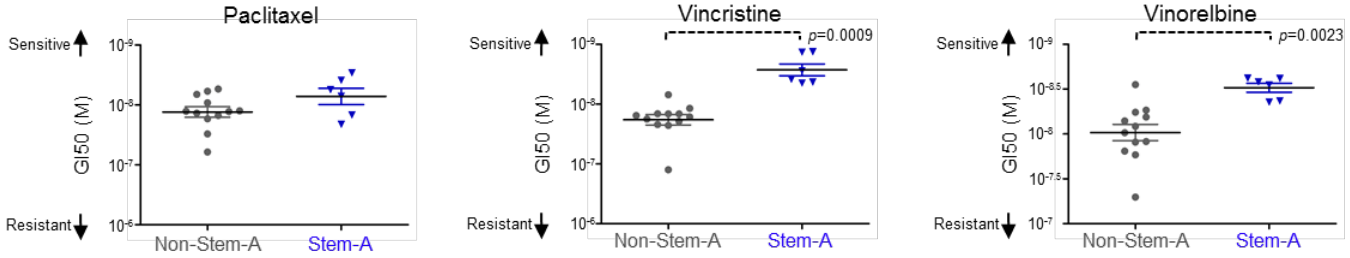






Helland et al Plos One 2011

Anti-Microtubule Agents for Stem-A/C5 Subtype



Dr Ruby Huang
CSI/ NCIS

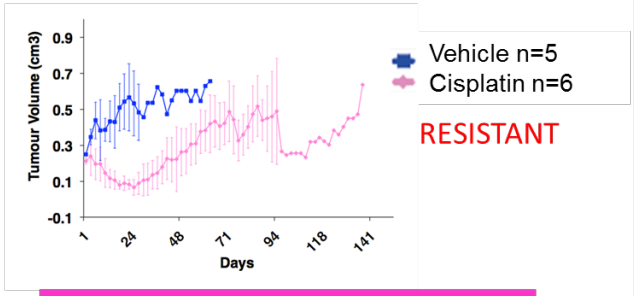
Tan, Miow & Huang et al., EMBO Mol Med, 2013

Stem-A/C5-like PDX

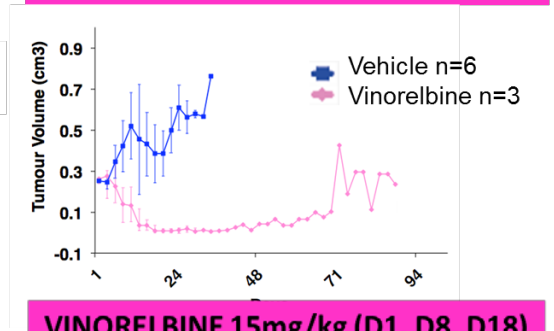
C5 signature
HMGA2 high
Lin28 high
MYCN mRNA high

PDX PH018

CISPLATIN 4mg/kg (D1, D8, D18)



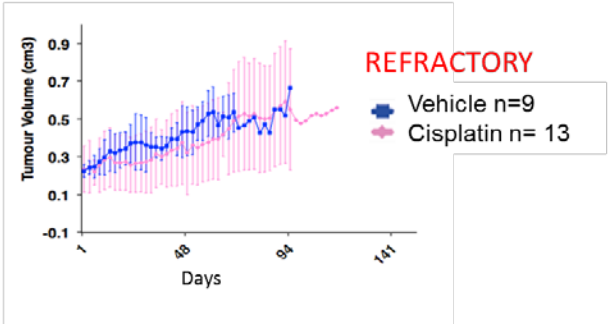
VINORELBINE 15mg/kg (D1, D8, D18)



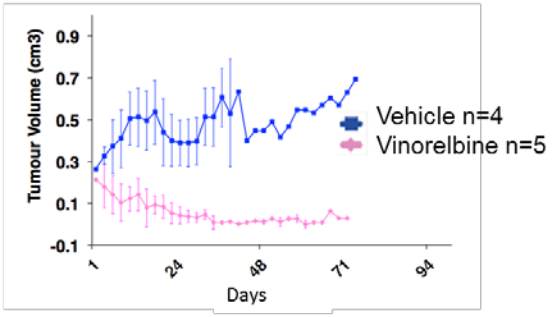
C5 signature
HMGA2 high
MYCN high

PDX PH041

CISPLATIN 4mg/kg (D1, D8, D18)



VINORELBINE 15mg/kg (D1, D8, D18)



Dr. Clare Scott WEHI, PDXs from Mayo Clinic (Paul Haluska)

Vinorelbine in Stem-A/C5-like Subtype Platinum Resistant Ovarian Cancer Phase II Study (VIP trial)

KK Women's and Children's Hospital
SingHealth

National University Cancer Institute, Singapore

Peter Mac

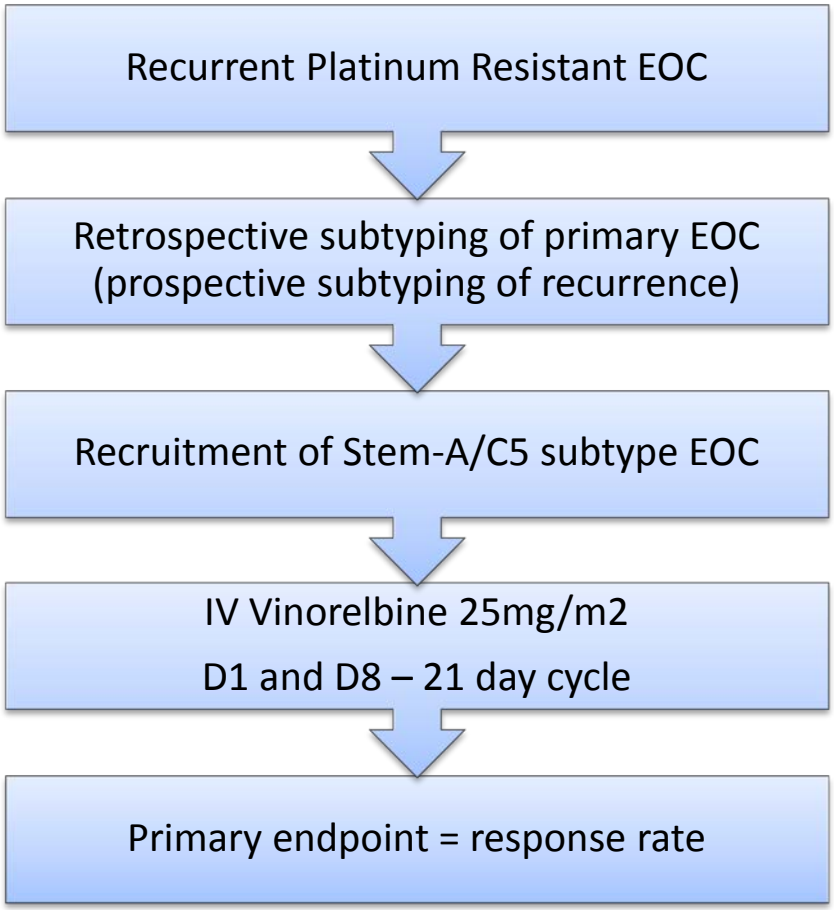
National Cancer Centre Singapore
SingHealth

Singapore PI:
David Tan

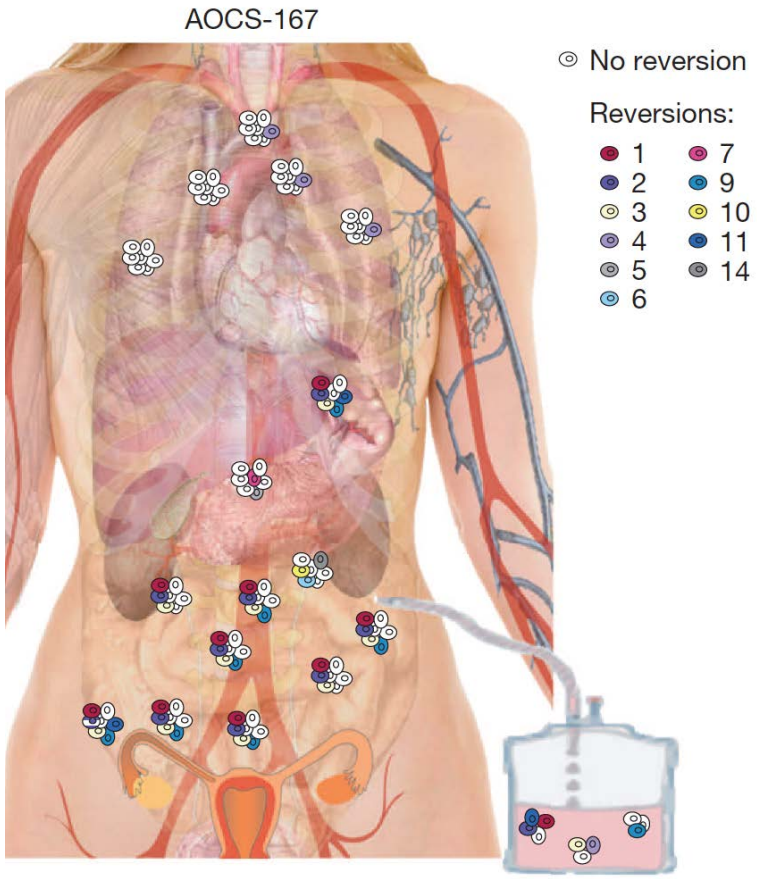
Australia PI:
Linda Mileshkin

Funded by NMRC CTG grant and OASIS (ANZGOG) grant

Phase II, Single-arm

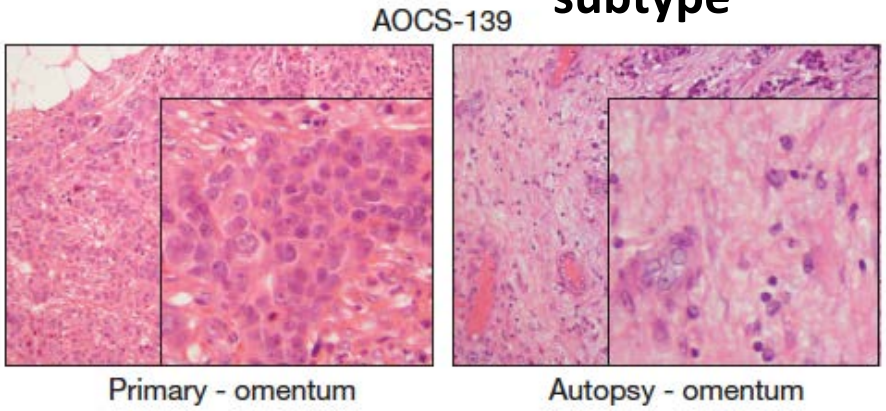


PARPi resistance: Mes (C1) subtype switch



PARP inhibitor resistant BRCA2 germline mutant patient with independent reversion events detected at various metastatic sites (autopsy biopsy)

C1/Mes subtype

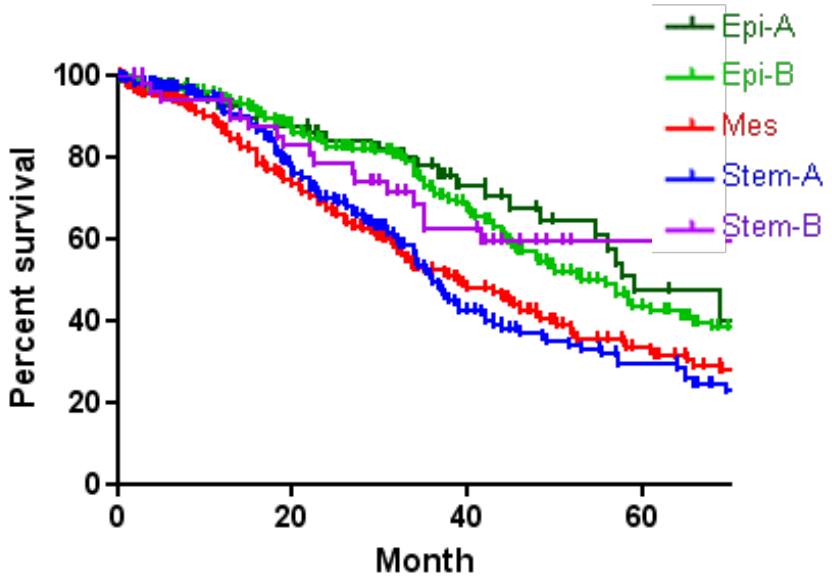


Extensive stromal reaction in omental lesion obtained at biopsy

Patch et al Nature 2015

Mes (C1) switch in platinum-resistant relapsed OC

Proposed	Tothill *et al.*, 2008	TCGA, 2012
Epi-A	C3	Differentiated
Epi-B	C4	
	C2	Immunoreactive
Mes	C1	Mesenchymal
Stem-A	C5	Proliferative
Stem-B	C6	



Sample	Same Subtype	Platinum Sensitive Primary Primary.Subtype	Platinum Resistance Relapse (Distant Metastasis) Relapse.Subtype
1019 T	0	EpiB	Mes
144 T	0	StemA	Mes
152 T	1	EpiB	EpiB
174 T	0	StemA	Mes
282 T	1	Mes	Mes
357 T	0	EpiB	Mes
367 T	0	EpiB	Mes
379 T	1	StemA	StemA
384 T	0	EpiA	Mes
417 T	1	Mes	Mes
418 T	0	EpiB	Mes
560 T	1	EpiB	EpiB
614 T	1	Mes	Mes
654 T	1	Mes	Mes
681 T	0	EpiB	Mes
683 T	0	Mes	EpiB
724 T	0	StemB	EpiB
738 T	0	EpiA	Mes
783 T	1	StemB	StemB
788 T	0	EpiA	Mes
800 T	1	Mes	Mes
821 T	1	EpiA	EpiA
924 T	0	EpiB	Mes

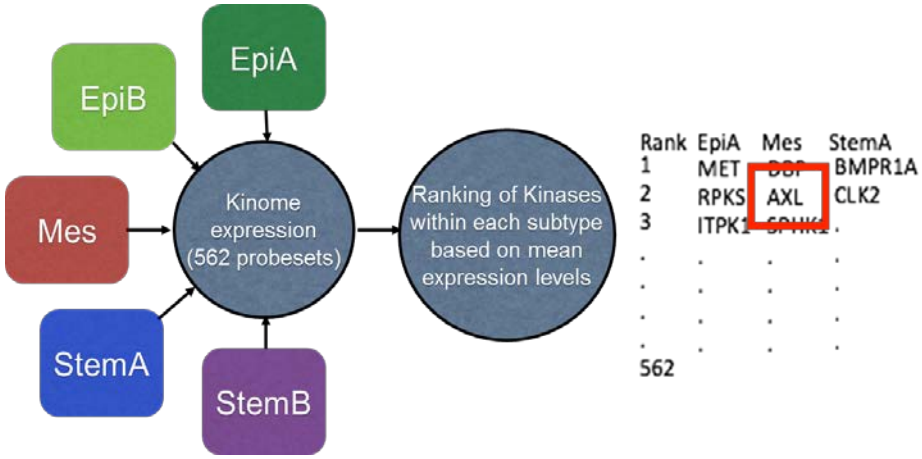
Marchini et al. 2013:
23 paired tumor samples – reanalysis by gene exp subtypes

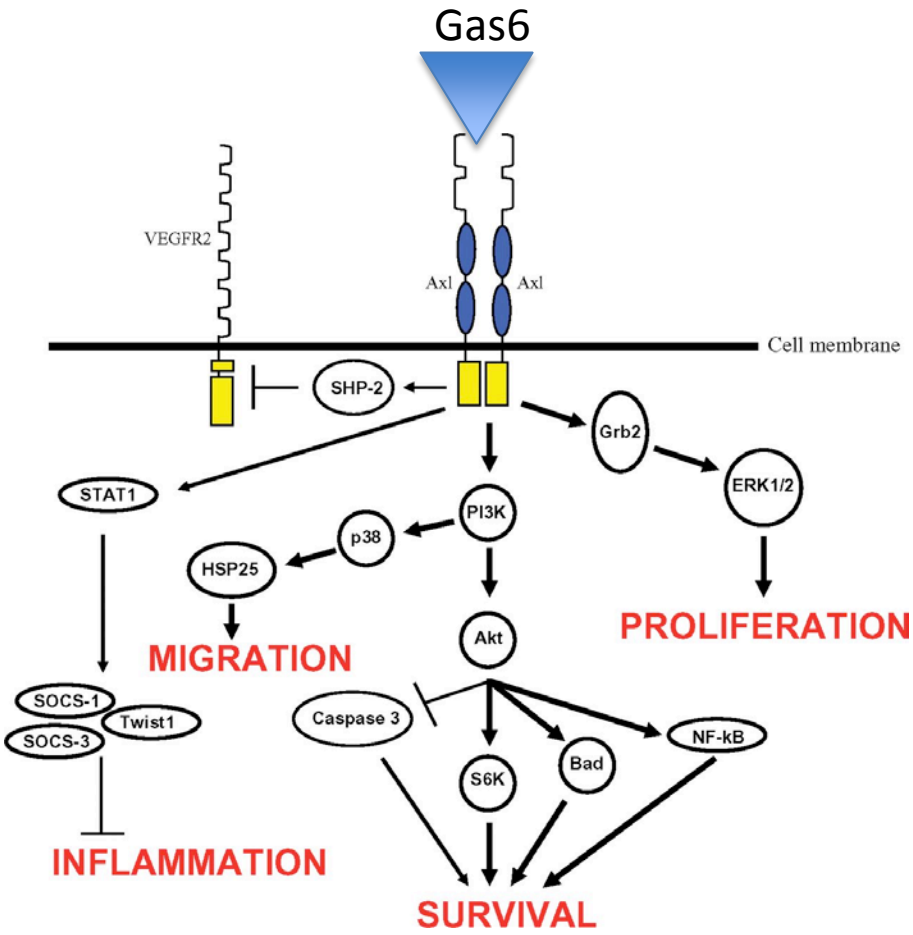
Mes (C1) subtype switch required for disease progression and chemoresistance

	Malek et al., 2012	Brodsky et al., 2014	Marchini et al., 2013
	Primary vs Peritoneum	**Primary vs Omentum**	**Primary vs Relapse**
	11 pairs	9 pairs	23 pairs
Same Subtype	6 (54.5 %)	4 (44.4 %)	10 (43.4 %)
Subtype Switch	5 (45.5 %)	5 (55.6 %)	13 (56.5 %)
Epi to Mes	1 (9.1 %)	3 (33.3 %)	9 (39.1 %)
Epi to Stem-A	1 (9.1 %)	0 (0.0 %)	0 (0.0 %)
Stem-A to Mes	0 (0.0 %)	2 (22.2 %)	2 (8.7 %)
Others	3 (27.2 %)	0 (0.0 %)	2 (8.7 %)

Targeting Gas6/AXL in Mes (C1) EOC Subtype

AXL Highly Ranked in Mes Subtype

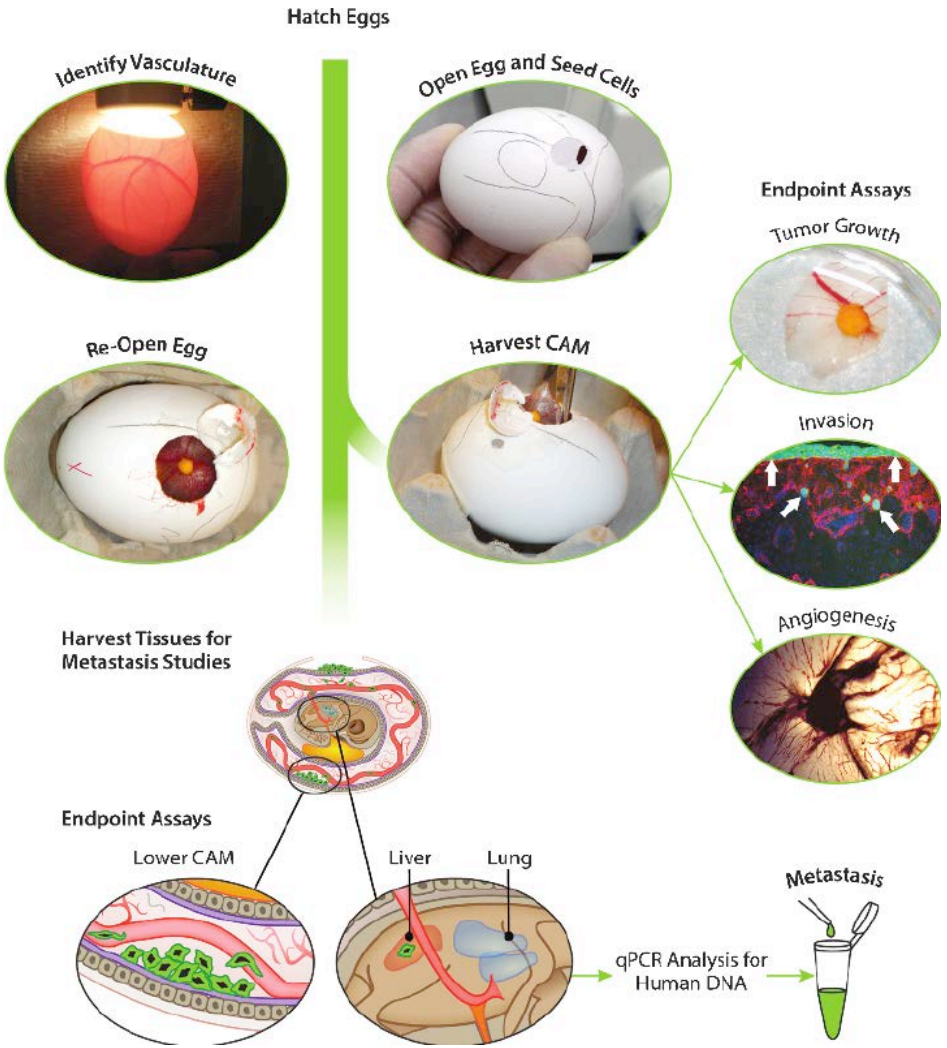




Antony et al Science Signaling 2016
Huang et al Mol Cell Oncol. 2016

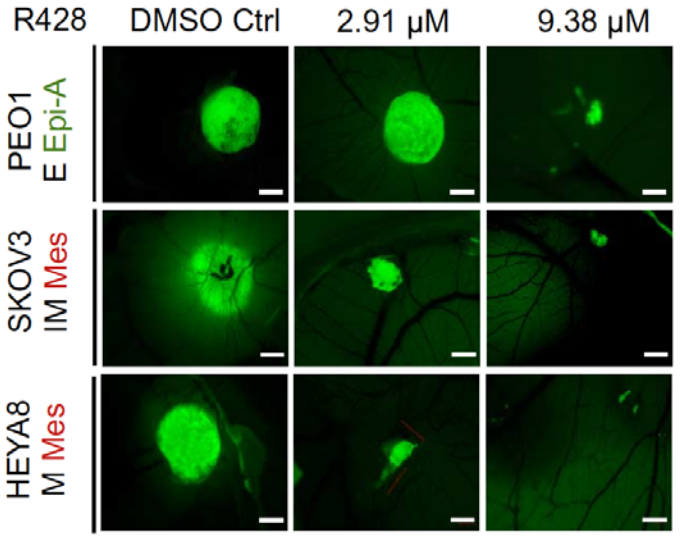
Chorioallantoic membrane (CAM) assay:

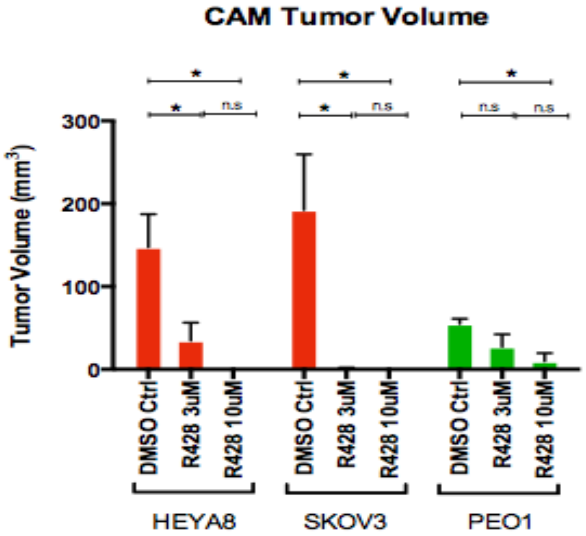
Fertilized chicken eggs hatched at day 0. D7-10, a window is opened on the egg to seed human cancer cells.



AXL inhibitior:R428

Chick ChorioAllantoic Membrane (CAM)





Antony et al., Sci Signaling

Ovarian Clear Cell Carcinomas

- 10-25% of all epithelial ovarian cancers (EOC) – 25% in oriental population
- Only 2-5% of ovarian cancer patients recruited into ovarian cancer clinical trials
- Associated with a poorer prognosis and resistance to conventional platinum-based chemotherapy → need better treatments!

Tan et al BJC 2013

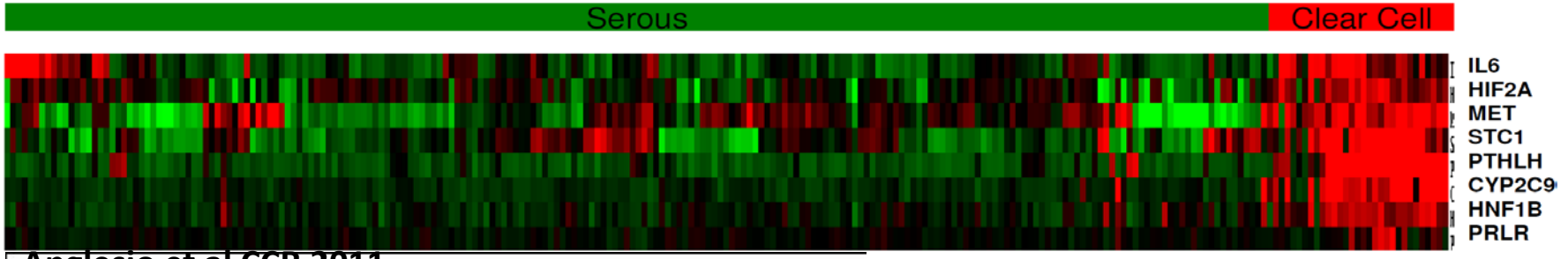
Response to Chemotherapy in Recurrent OCCC

RECIST Response Rates to individual treatments						
Treatment	**N**	**No. Evaluable**	**Platinum-Sensitive**	**CR/PR (%)**	**CBR (%)**	**Median PFS (weeks)**
Plt-based	63	38	Yes (46)	18	39	17
		14	No (17)	14	36	11
Paclitaxel	8	7		0	14	8
Gemcitabine	7	7		14	14	4
Doxil/ Doxorubicin	29	25		4	16	10
Anti-angiogenic agents	15	13		8	46	14
Topoisomerase inhibitors	30	27		4	19	8
Hormonal therapy	8	6		0	17	12
Others	6	5		0	0	11

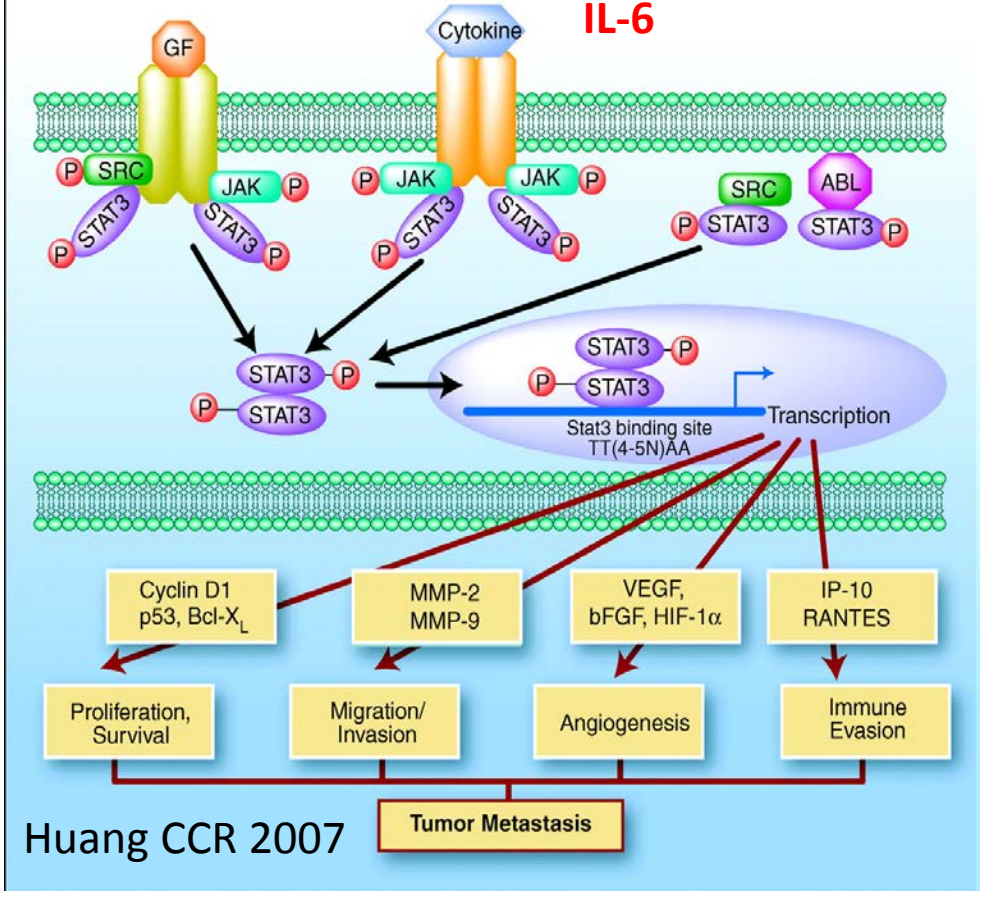
Response rate across all lines of treatment:
Overall response rate (ORR) = 13/142 (9%)
Median PFS = 11 wks (95% CI: 8,14)

Tan et al ASCO 2014

Angiogenesis & OCCC: Upregulation of IL6-STAT3-HIF1α Pathway

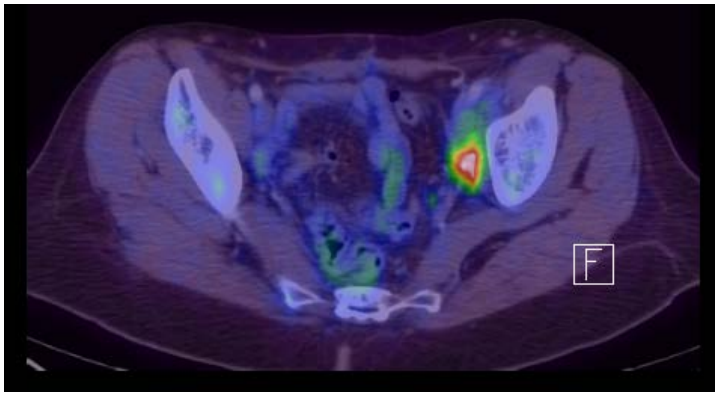


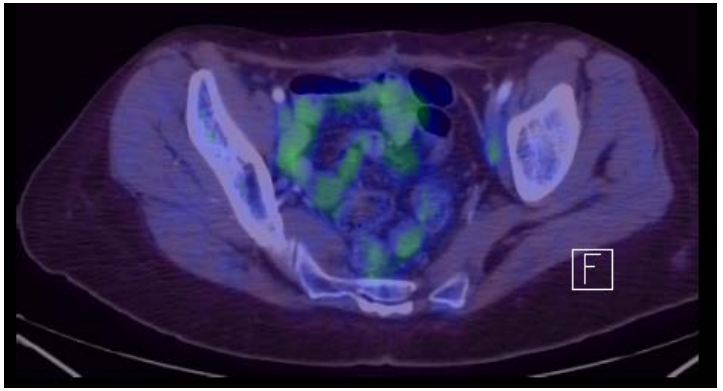
Anglesio et al CCR 2011

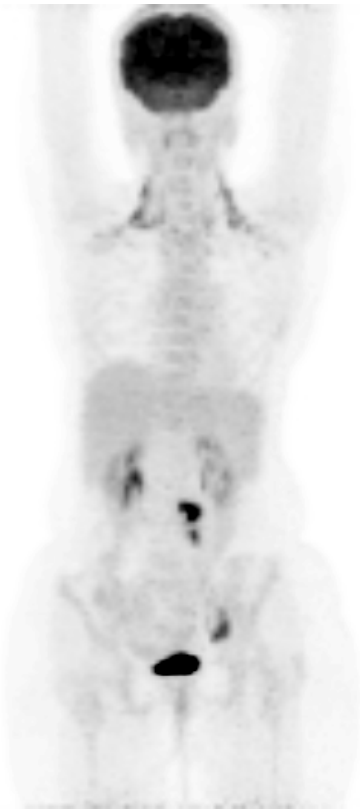


Huang CCR 2007

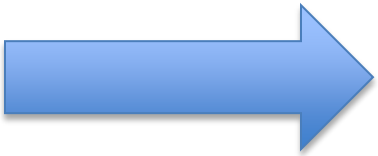
- **Increased IL-6 associated with increased DVT risk**
- **IL-6 signals via STAT3 and activates expression of downstream genes including *PTHLH* (hypercalcaemia) and *HIF1A* (angiogenesis)**

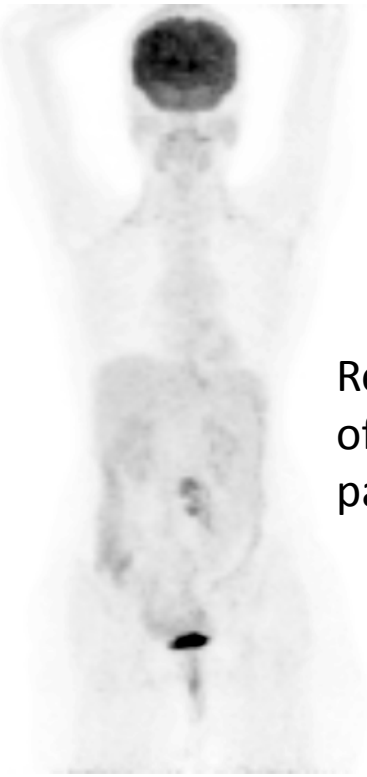






Sunitinib 50mg: 2 weeks on 1 week off 3-weekly schedule



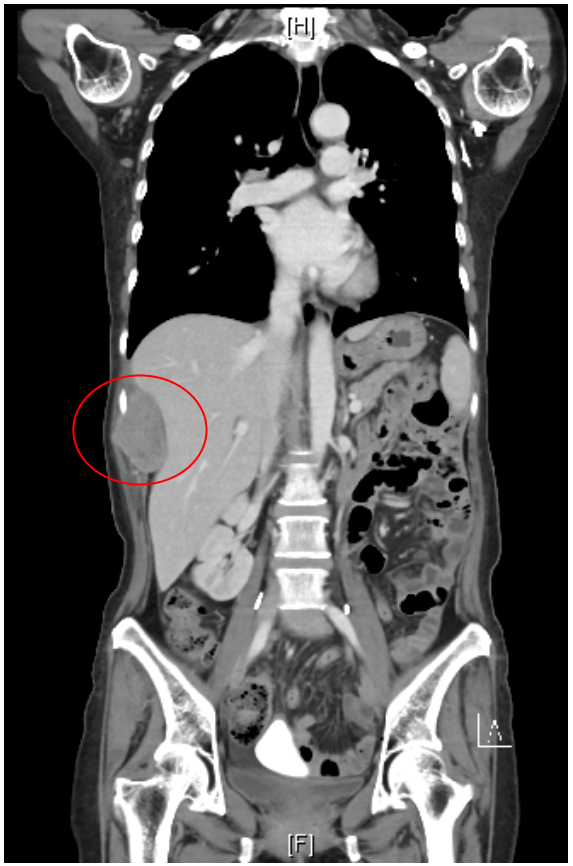


Resolution of back pain

Nov 2014

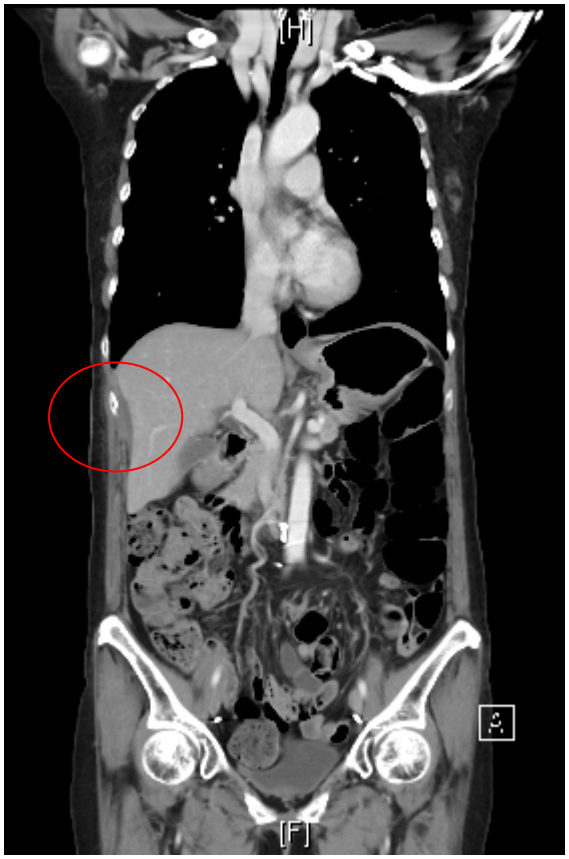
March 2015

OCCC patient progressing through 2 prior lines of platinum chemo



Sept 2015
CA125 368

Weekly paclitaxel + Bevacizumab x 10 cycles



Oct 2016
CA125 12.5

May 2017 → CA125 13.5

Immune Checkpoint Inhibitors in Ovarian Cancer

Immune checkpoint inhibitors in gynecological cancers

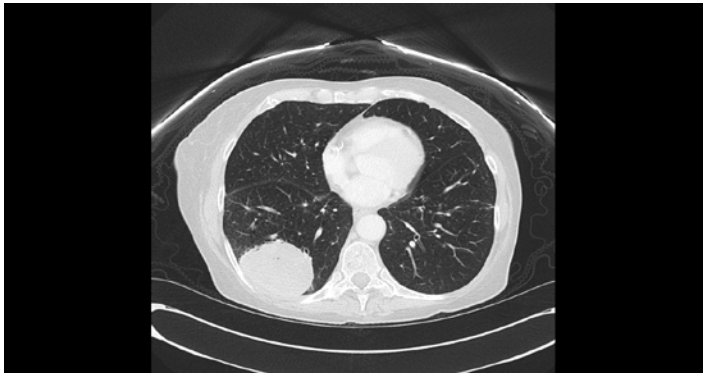
Table 1. Selected trials of PD-1/PD-L1 and CTLA-4 immune checkpoint blockade in ovarian cancer

Target	Antibody	IgG subclass	Study setting	Phase	No.	CR	PR	SD	ORR (%)	DCR (%)	Median PFS (wk)
PD-1	Nivolumab	Human IgG4	Relapsed platinum resistant EOC	II	20	2	1	6	15.0	45.0	14.0
	Pembrolizumab	Humanized IgG4	Advanced EOC	I	26	1	2	6	11.5	34.6	NA
PD-L1	BMS-936559	Human IgG4	Advanced EOC	I	17	0	1	3	6.0	23.5	NA
	Avelumab	Human IgG1	Relapsed platinum resistant EOC	I	124	0	12	55	9.7	54.0	11.3
CTLA-4	Ipilimumab+GM-CSF	Human IgG1	Advanced EOC	I	9	0	1	3	11.1	44.4	NA

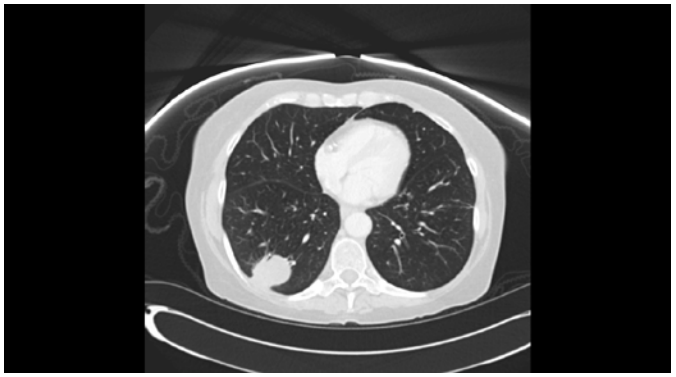
Heong et al J Gynecol Oncol. 2017

3/3 responses to PD1/PDL1 inhibitor in ovarian clear cell cancer (OCCC)

Avelumab 2/2 OCCC responses

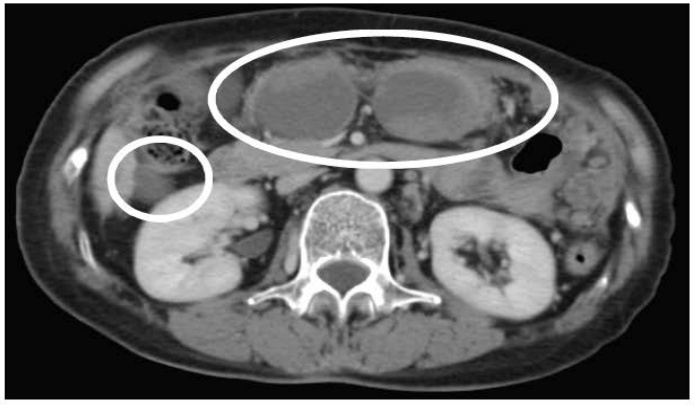


Baseline: 69 mm RLL lesion

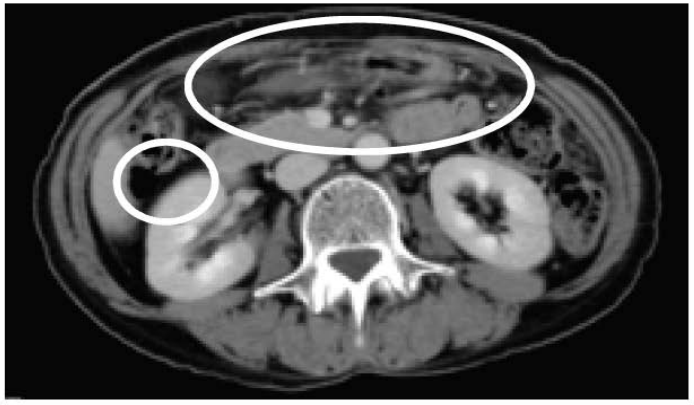


Week 25: 41 mm (-40.6%)

Nivolumab 1/1 OCCC response - CR



Baseline



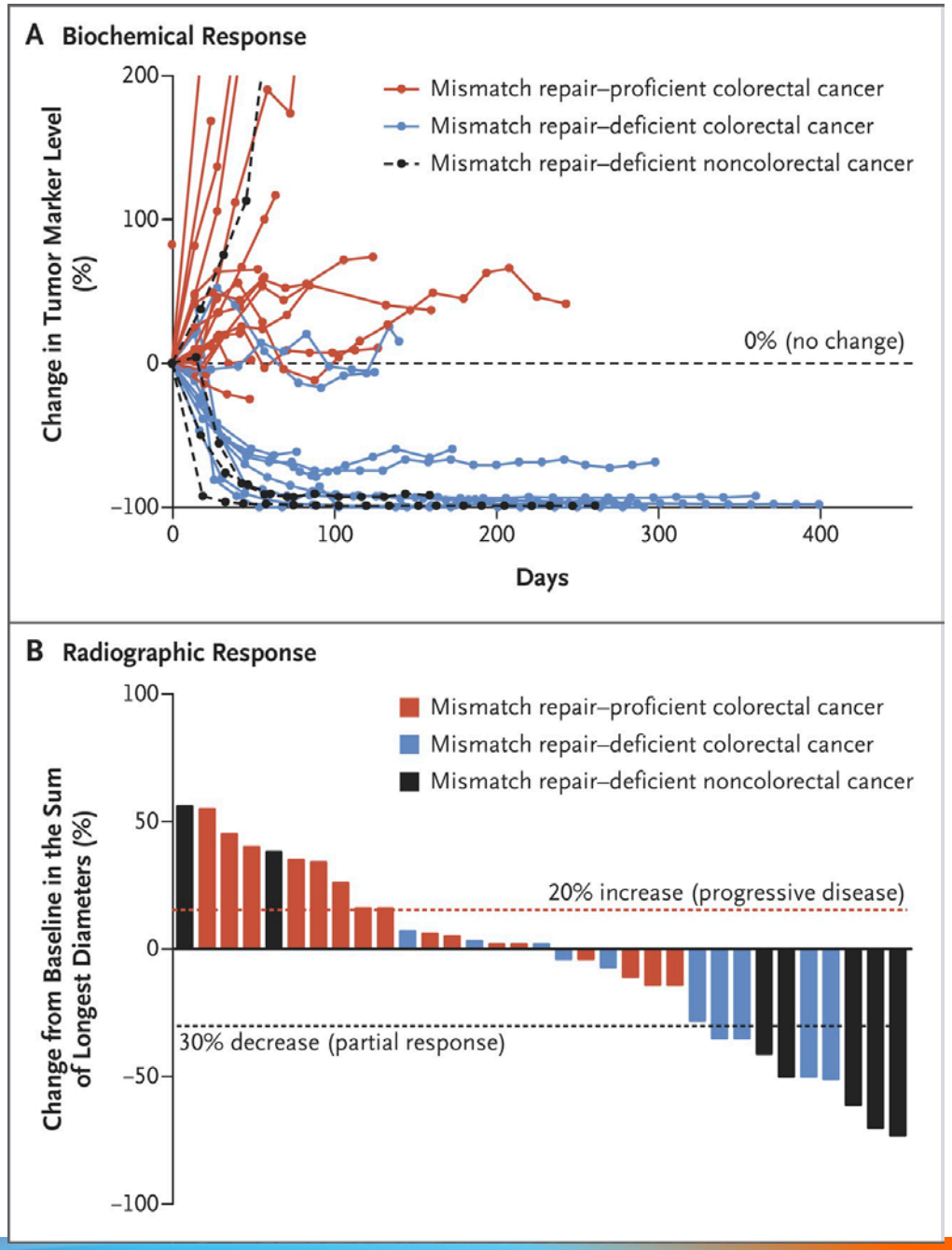
4 Months

Are PDL1/PD1 inhibitors more likely to work in OCCCs?

Disis et ASCO 2015
Hamanishi et al JC 2015

Clinical responses to pembrolizumab treatment in MMR deficient vs proficient cancers

MMR-deficient tumors:
Increased immune infitrates and increased mutations → increased neo-antigens. (Schumacher Science 2015)



Le DT et al. N Engl J Med 2015;372:2509-2520

Contents lists available at ScienceDirect

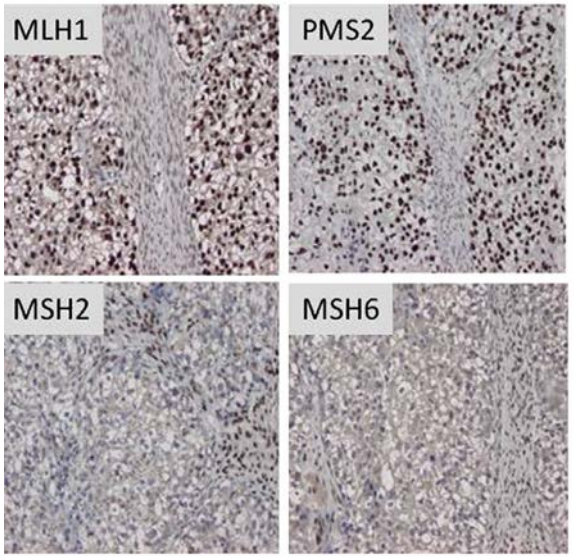
Gynecologic Oncology

journal homepage: www.elsevier.com/locate/ygyno

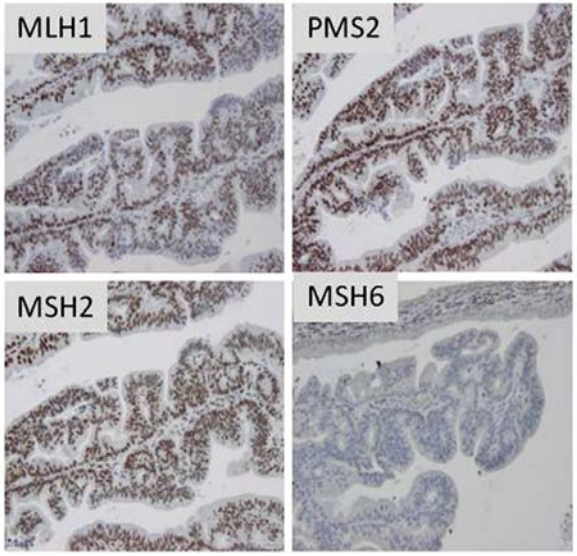
Ovarian cancer linked to lynch syndrome typically presents as early-onset, non-serous epithelial tumors

Zohreh Ketabi [a], Katarina Bartuma [b], Inge Bernstein [a], Susanne Malander [b], Henrik Grönberg [c], Erik Björck [d], Susanne Holck [e], Mef Nilbert [a,b,*]

[a] HNPCC-register, Department of Gastroenterology, Hvidovre University Hospital, Faculty of Health Sciences, Copenhagen University, Denmark
[b] Department of Oncology, Institute of Clinical Sciences, Lund University, Lund, Sweden
[c] Department of Medical Epidemiology and Biostatistics, Karolinska Institute, Stockholm, Sweden
[d] Clinical Genetics Unit, Department of Molecular Medicine and Surgery, Karolinska University Hospital, Karolinska Institutet, Stockholm, Sweden
[e] Department of Pathology, Hvidovre University Hospital, Faculty of Health Sciences Copenhagen University, Denmark

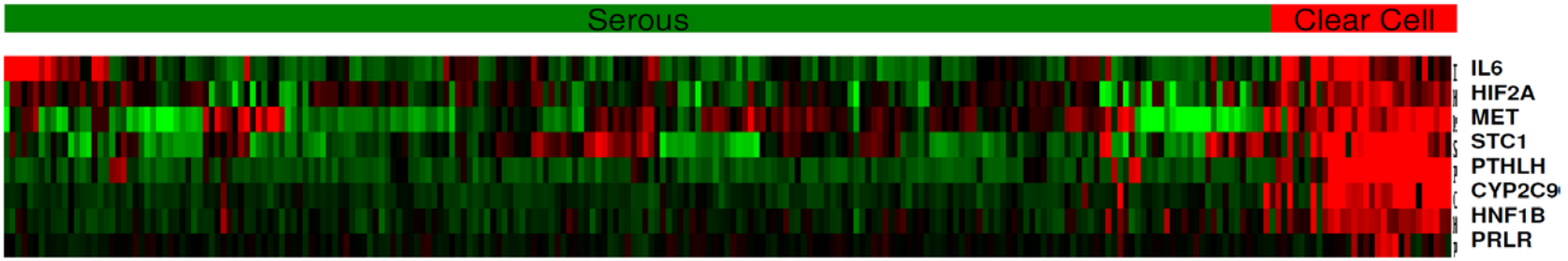


- Ovarian cancers developed at mean 48 years of age
- Histologically, endometrioid (35%) and clear cell (17%) tumors were overrepresented.
- The underlying MMR gene mutations in these families affected MSH2 in 49%, MSH6 in 33% and MLH1 in 17%.
- Immunohistochemical loss of the corresponding MMR protein was demonstrated in 92% of tumors



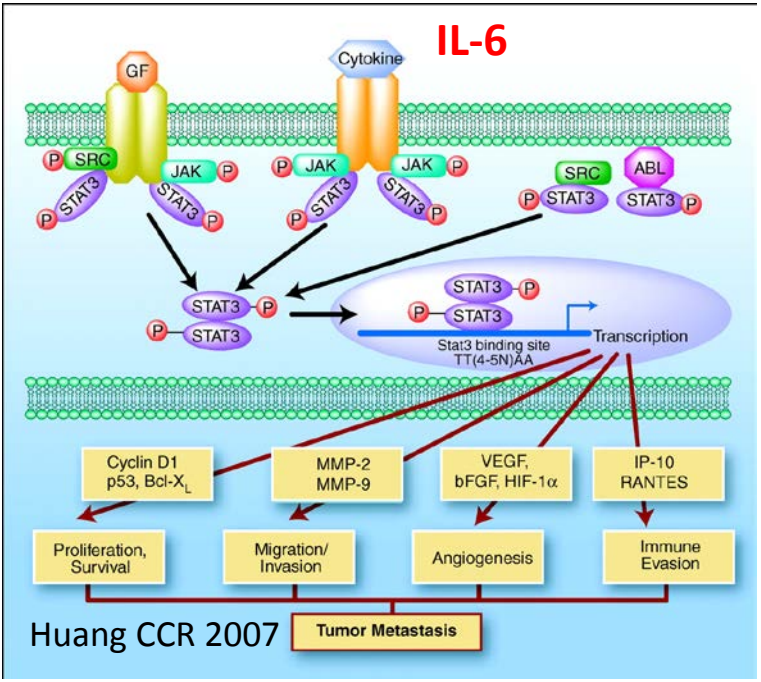
Gynecologic Oncology 121 (2011) 462–465

Upregulation of IL6 in OCCC: a marker of tumour inflammation

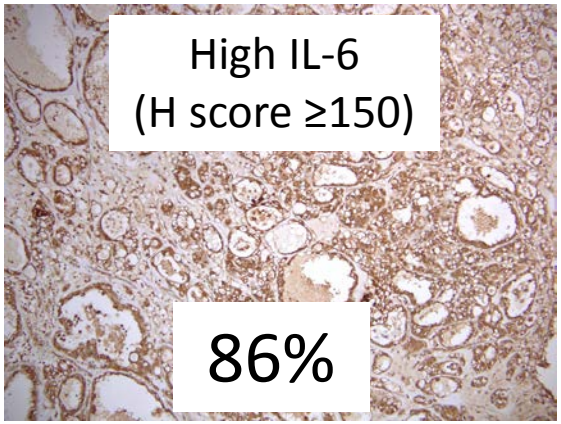


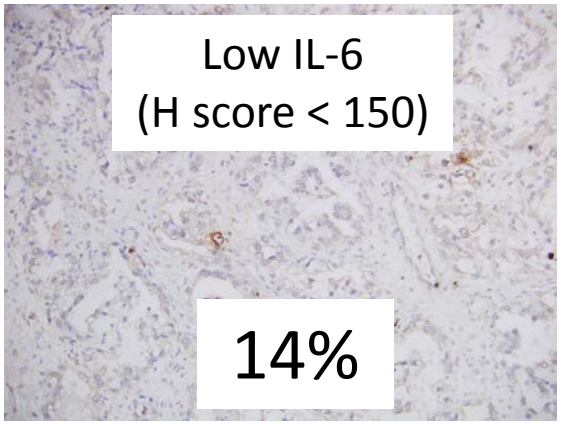
Anglesio et al CCR 2011

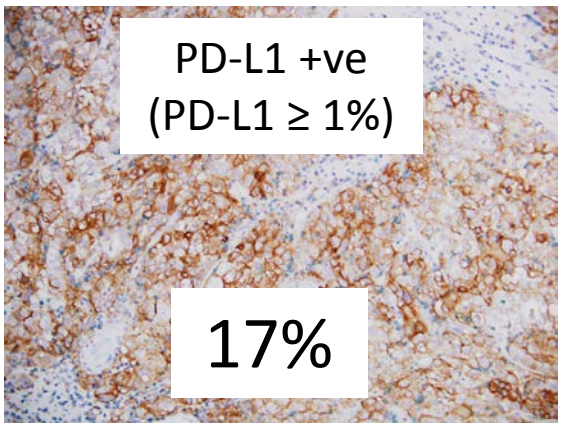
OCCC IL6 and PD-L1 expression, n=103: Courtesy of Dr D. Lim

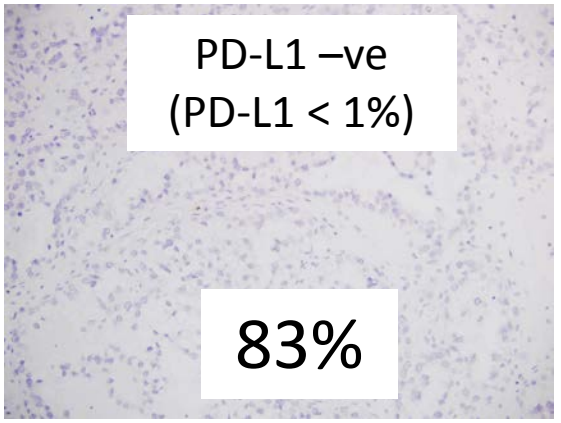


Huang CCR 2007







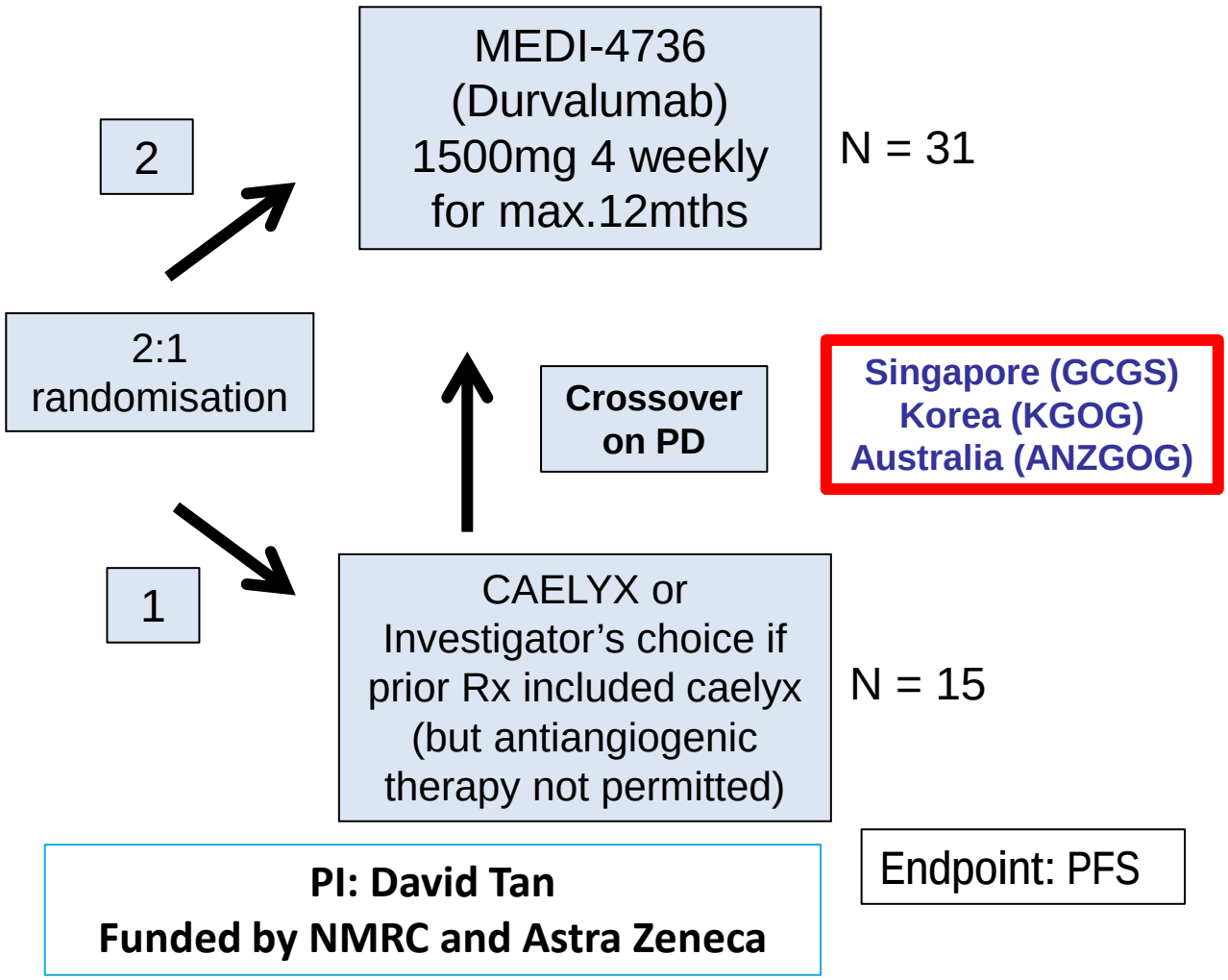


A Multicentre Phase II randomised trial of MEDI4736 (DURVALUMAB) versus physician's choice chemotherapy in recurrent ovarian clear cell adenocarcinomas (MOCCA)

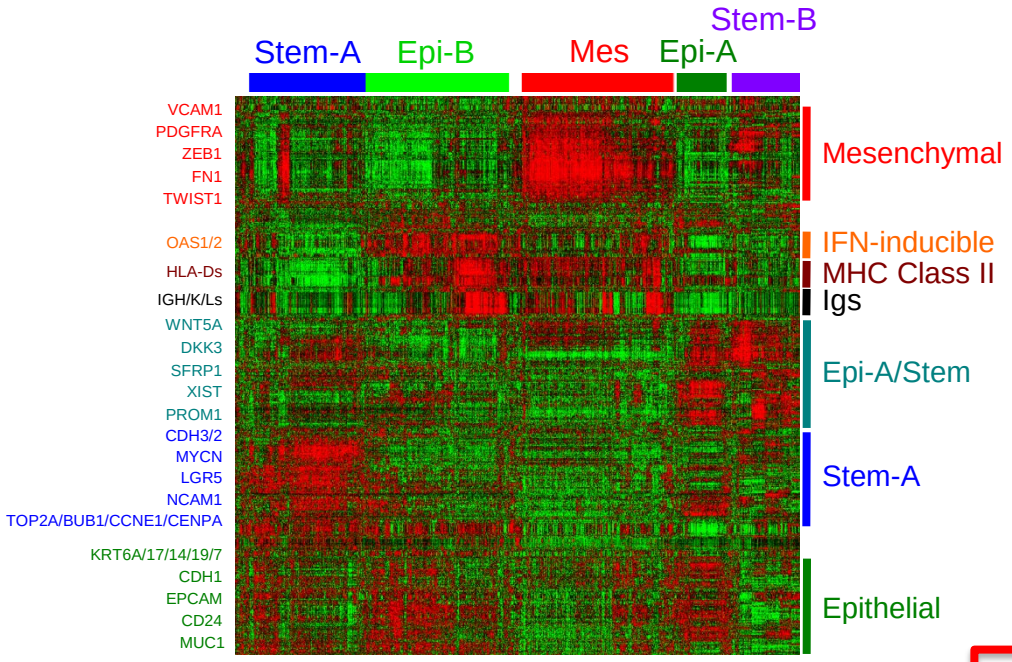
Relapsed Clear Cell Cancer Ovarian Cancer (>70% clear cell)

Inclusion
-Histologically confirmed
- WT1 negative
-Relapsed after at least 1 line of platinum-based chemotherapy
-Measurable disease by RECIST 1.1
-ECOG 0 / 1

Exclusion
-Concurrent use of experimental anti-cancer agent
-Untreated brain mets



EOC Gene expression molecular subtypes



❖ Meta-analysis (***Tan, Miow, & Huang et al., EMBO MM, 2013***) of 1,538 EOCs including all histological types.

4 subtypes showing survival differences

Proposed	Tothill *et al.*, 2008	TCGA, 2012
Epi-A	C3	Differentiated
	C4	
Epi-B	C2	Immunoreactive
Mes	C1	Mesenchymal
Stem-A	C5	Proliferative
Stem-B	C6	

Immunotherapy?

Tan, Miow & Huang et al., EMBO Mol Med, 2013

Ovarian Cancer Clinical Trials: Selecting the Right Tumours

Inclusion Criteria

Disease Related

- Subjects must have histologically or cytologically documented epithelial ovarian (FIGO Stage II-IV), fallopian tube or primary peritoneal cancer.
    - Subjects with pseudomyxoma [illegible] mesothelioma are excluded
- Radiographically documented progres[illegible]on per RECIST criteria with modifications or

What type of EOC?

- **Histological subtype? Grade?**
    - **Serous or Clear cell (OCCC) or Endometrioid or Mucinous?**
- **Molecular subtype? → Archival or recurrent tumour?**
    - ***PIK3CA* or *BRCA1/2* mutant? C1/2/4/5 gene expression subtype?**
- **Molecular subtype of a histological subtype?**
    - ***PIK3CA/ mTOR/ TSC* mutant OCCC/endometrioid EOC**
    - **C1/C5 high grade serous EOC**
- **Genotype of a molecular subtype of a histological subtype?**
    - **e.g. H1047R mutations of *PIK3CA/* E17K mutations of *AKT1* mutant OCCC**

Precision Therapy for Gynaecological Cancers: Why is molecular profiling useful?

- One size does not fit all

- Identifying the right therapy for the right patient will
  - improve outcomes
  - increase the benefit:risk ratio
  - Accelerate new therapeutic developments in cancer

- Evolving process – new targets = new biomarkers → efficient development of validated companion diagnostic markers essential

- Translational studies important to understand reasons for success and failure and to gain new insights in tumour biology that may provide new therapeutic opportunities

NCIS Gynaecological Cancer Research Group

Gynae Onc Clinical Trials Team

Gynae Med Onc
Lim Siew Eng
Lim Yi Wan
Valerie Heong

Gynae Surg Onc
Jeffrey Low
A. Cheran
Joseph Ng
Ruby Huang

Gynae Rad Onc
Johann Tang
Bok Ai Choo
Vicky Koh

Radiology
Thian Yee Liang
Bertrand Ang
Bernard Wee

Tissue Repository
Eng Chon Boon
Mahesh Choolani
Fong Chui Yee

Pathology Team
Diana Lim
Brendan Pang
Evelyn Koay
Benedict Yan

Preclinical/ Molecular Team
Ruby Huang
Richie Soong
Anand Jeyasekharan
Hyunh The Hung

Bioinformatics Core
Tony Tan

Data Processing

Tissue Processing (DNA, RNA)

Histopathology
Molecular Pathology
Biomarker Validation

Pathway directed therapy/ Precision Medicine

Expression Profiling (Molecular Subtype), Deep Sequencing (Somatic Mutation)

HR Biomarker/ Targeting DNA repair

PDX, Animal Model

NUS National University of Singapore

National University Cancer Institute, Singapore

CSI SINGAPORE
Cancer Science Institute of Singapore
an institute of NUS

National University Cancer Institute, Singapore

Committed To The Research of Cancers in Asia

HAEMATOLOGY ONCOLOGY RESEARCH GROUP

THANK YOU

NUHS National University Health System

david_sp_tan@nuhs.edu.sg