RESISTANCE RELATIONSHIPS BETWEEN PLATINUM AND PARP-INHIBITORS IN OVARIAN CANCER.

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RCSI

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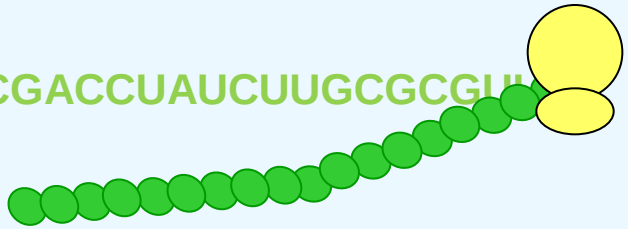
Kinds of BRCA1/2 Mutations

Wild Type

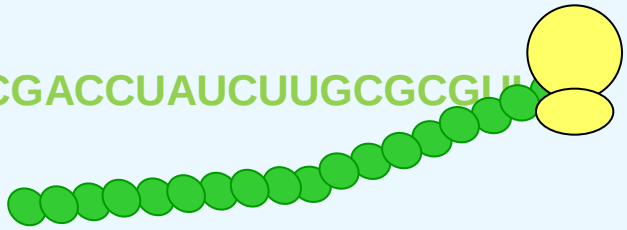
UCAGGGACUUCGACCACGACCUAUCUUGCGCGUU



Full Length Functional Protein

Polymorphism

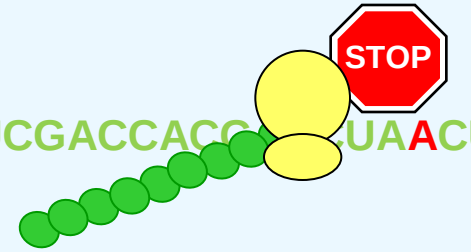
UCAGGG**G**CUUCGACCACGACCUAUCUUGCGCGUU



Full Length Functional Protein

Deleterious Mutation

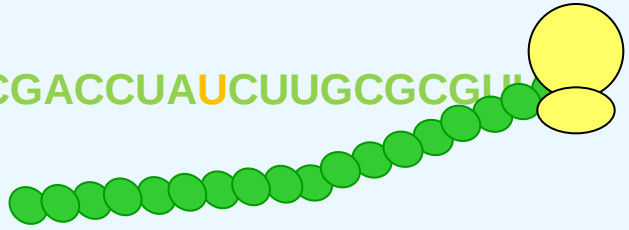
UCAGGGACUUCGACCACG UA**A**CUUGCGCGUUC



Truncated Non-Functional Protein

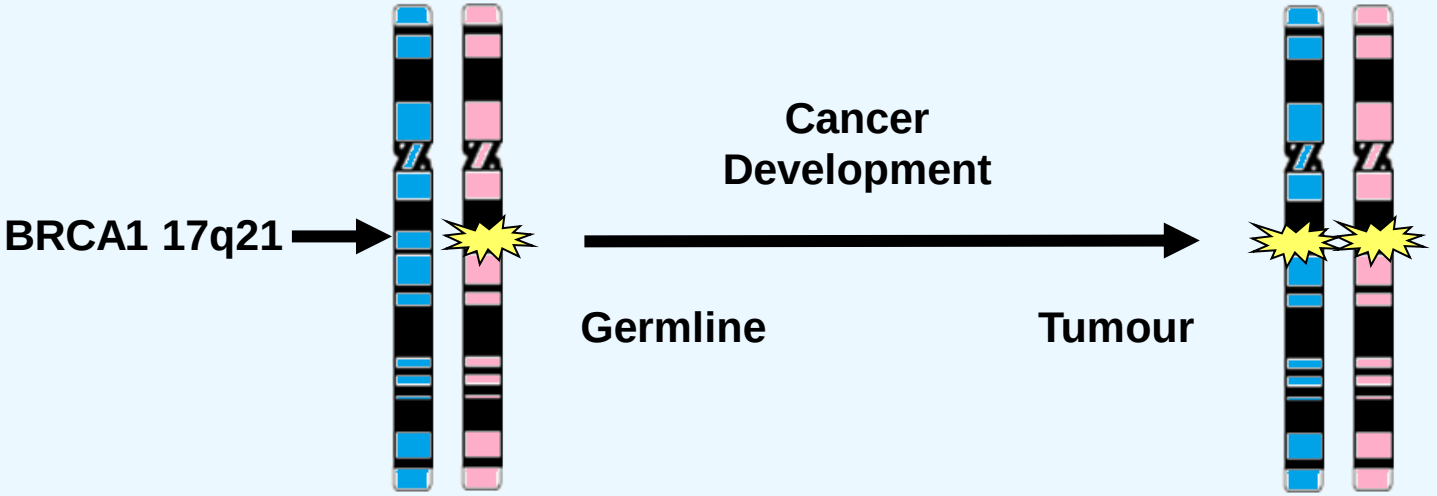
Reversion Mutation

UCAGGGACUUCGACCACGACCUA**U**CUUGCGCGUU



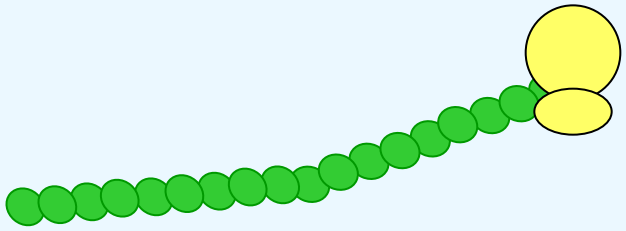
Full Length Functional Protein

Heterozygous or Homozygous?



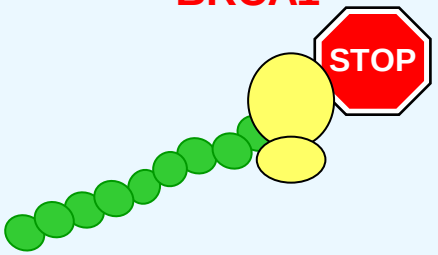
Heterozygous
Deleterious Mutation

Functional BRCA1

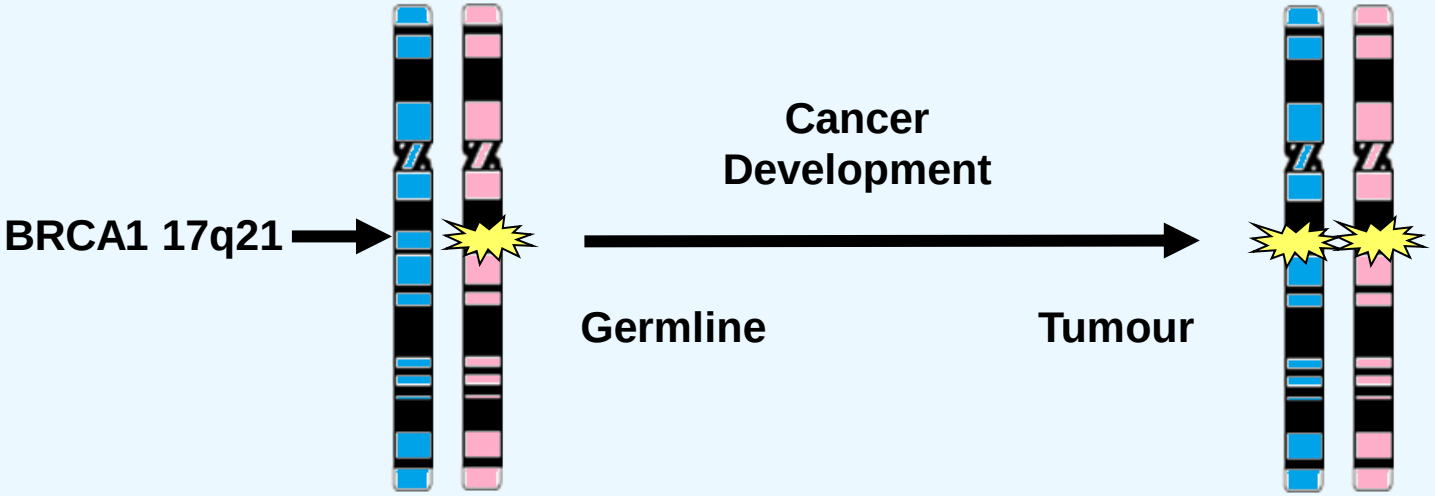


Homozygous
Deleterious Mutation

Non-Functional
BRCA1

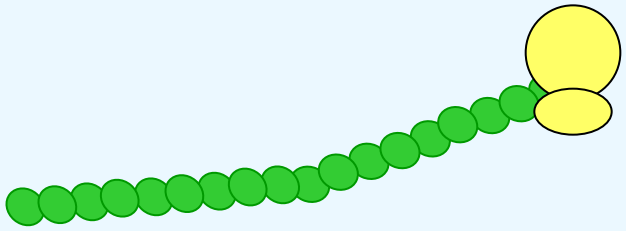


Germline or Somatic?



Heterozygous
Deleterious Mutation

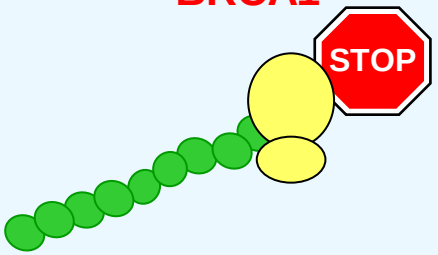
Functional BRCA1



- Test DNA from blood or other non-tumour tissue to assess germline

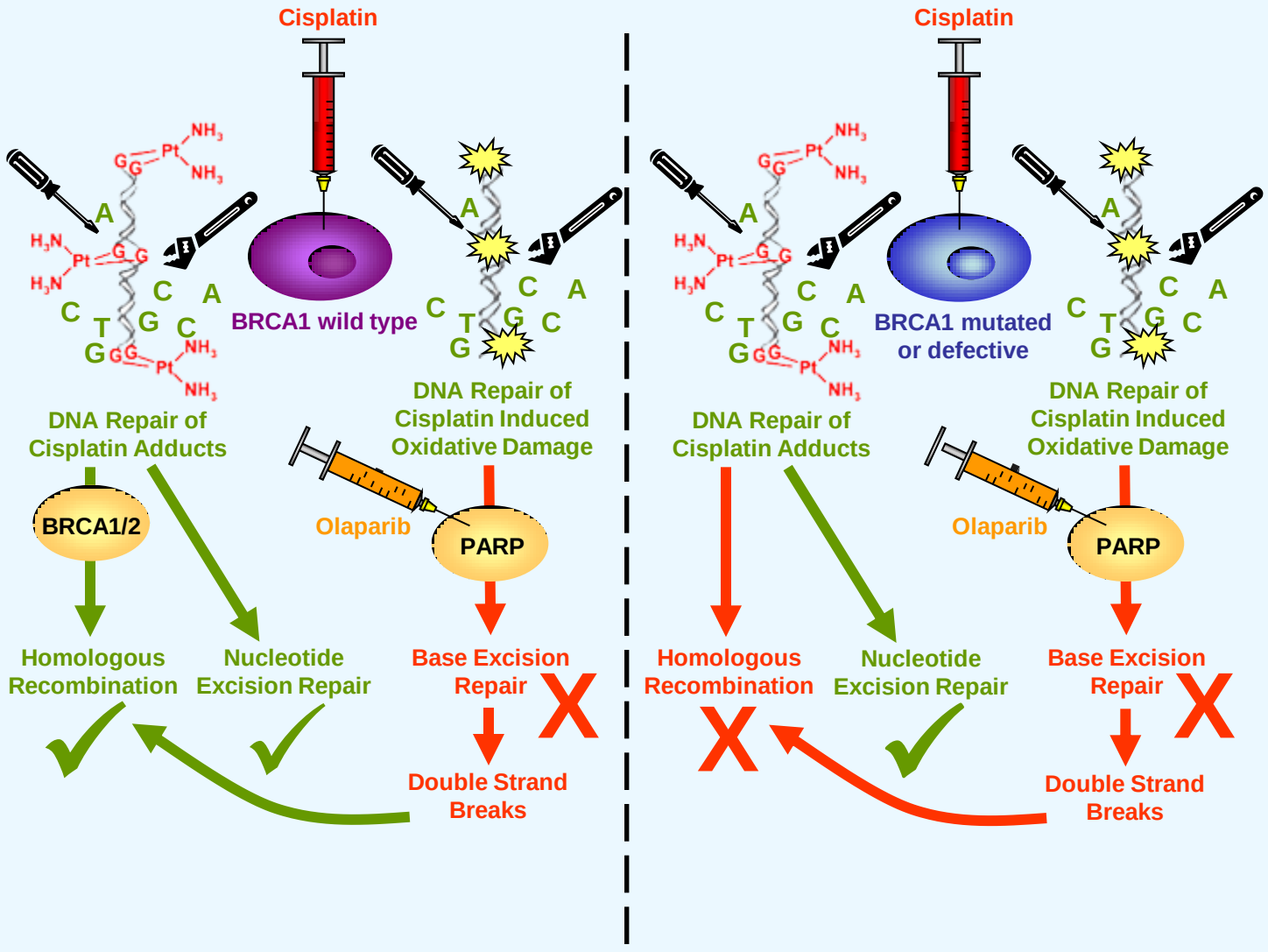
Homozygous
Deleterious Mutation

Non-Functional
BRCA1



- Testing the tumour or cell lines made from tumors is the combined result of germline and somatic mutations

BRCA1, Cisplatin and PARP Inhibition



Aims of Study

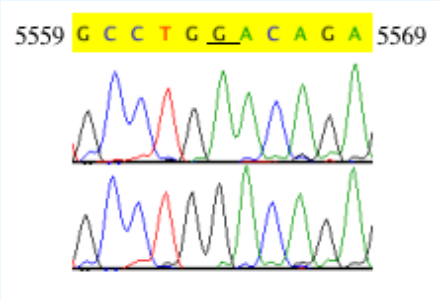
- To screen a panel of 41 ovarian cancer cell lines for BRCA1/2 deleterious mutations and BRCA1 gene methylation.
- To determine if BRCA1/2 deleterious mutated and methylated cell lines are more sensitive to platinum and parp inhibitor chemotherapy.
- To determine if any BRCA1/2 wild-type cells are highly sensitive or resistant to platinum and parp inhibitors.
- To discover gene expression profiles of platinum and parp inhibitor resistance and sensitivity and determine any overlap between these profiles

BRCA1/2 Gene Sequencing

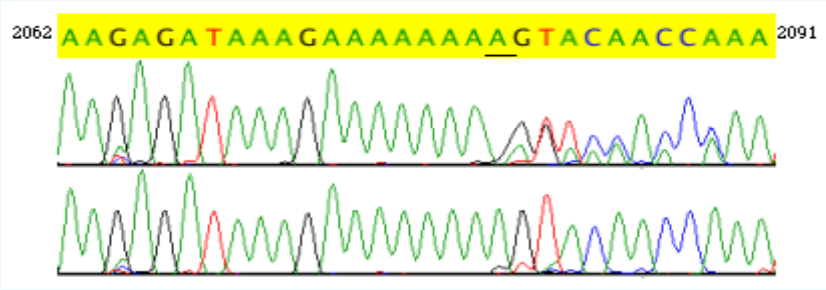
•The full length of BRCA1 and BRCA2 genes were sequenced in a panel of 41 ovarian cancer cell lines.

• Only one cell line had a functionally deleterious mutation in BRCA1 (SNU-251).

• Seven cell lines had heterozygous mutations in BRCA1 (IGROV-1) or BRCA2 but these have no functional impact on the protein.



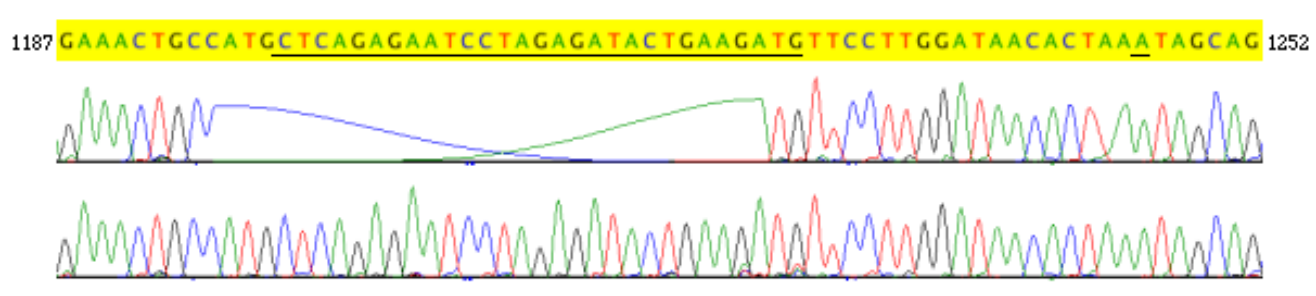
Top: SNU-251 showing homozygous deletion W1815X (5564G>A)
Bottom: BRCA1 Wild type sequence



Top: IGROV-1 showing heterozygous deletion - 2080delA
Bottom: BRCA1 Wild type sequence

BRCA1/2 Gene Sequencing

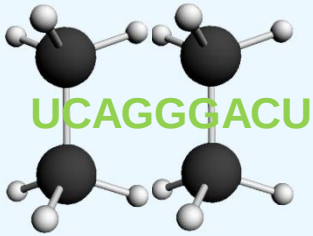
• Two cell lines had deleterious mutations as well as an additional reversion mutation which has restored the protein back to wild type BRCA1 (UPN-251) and BRCA2 (PEO1).



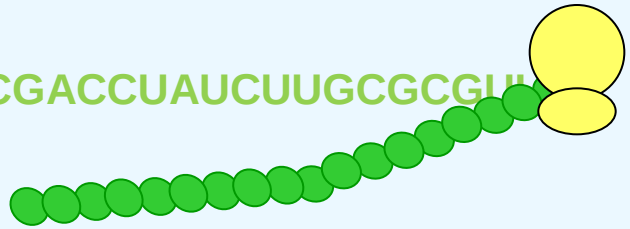
Top: UPN-251 showing homozygous deletions - 1199del29 + 1246delA Reversion Mutation
Bottom: BRCA1 Wild type sequence

BRCA1 Gene Methylation

Gene Methylation

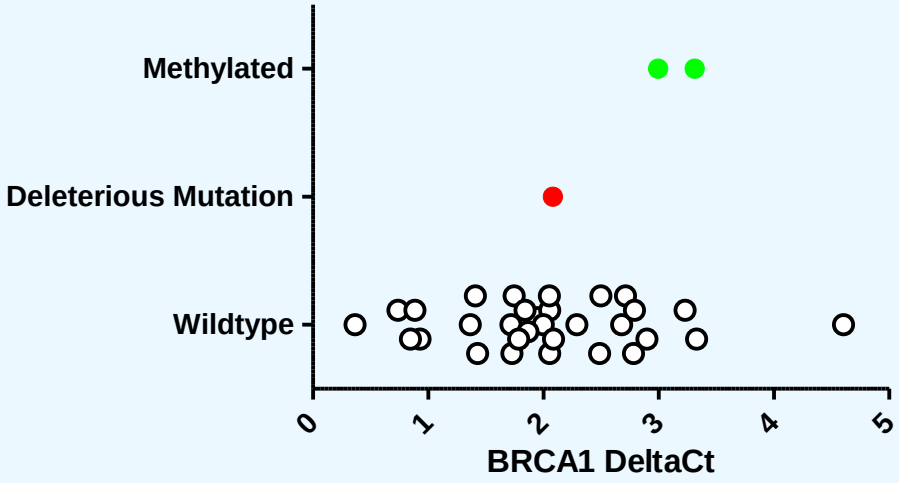


UCAGGGACUUCGACCACGACCUAUCUUGCGCGU



Decreased Expression of Full Length Functional Protein

• BRCA1 gene methylation was examined in the panel of 41 ovarian cancer cell lines. Two cell lines were found to be methylated A1847 and OVCAR8.

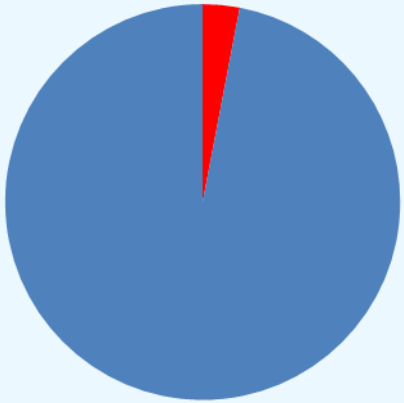


• The methylated cell lines have a corresponding decrease in BRCA1 mRNA expression.

• The SNU-251 cells have similar BRCA1 expression levels to wild type cells. This is due to the location of the QPCR primers. The SNU-251 cell line's deleterious mutation at the very tail end of the gene sequence.

Frequency of BRCA1/2 mutations

Unselected Ovarian Cancer Cell Lines from n=33 Patients

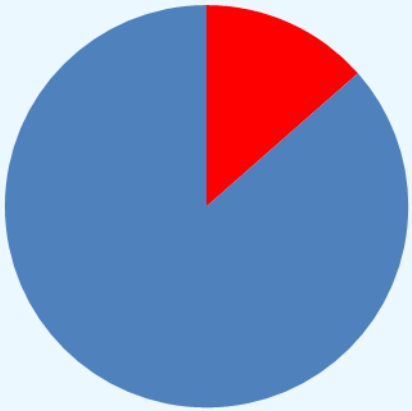


BRCA1/2 Deleterious Mutations 3%

Wild Type 97%

- Represents both germline and somatic mutations

Unselected Invasive Ovarian Tumours (Literature 1-3)



BRCA1/2 Deleterious Mutations 8.6-13.7%

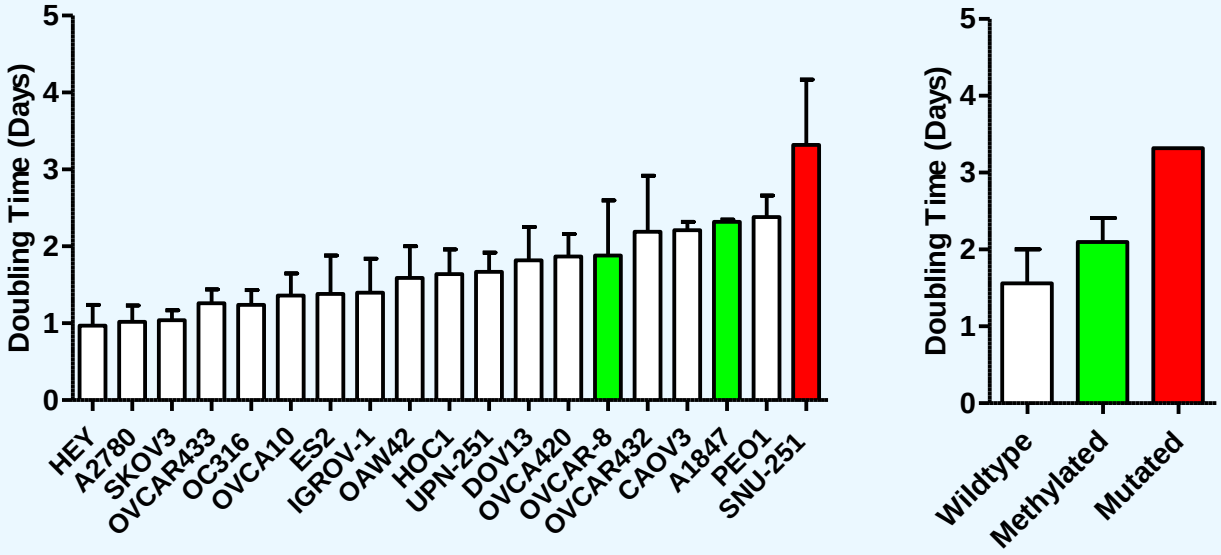
Wild Type 91.4-86.3%

- Represents germline mutations only. Studies including somatic have reported 18.3% mutated in ovarian tumours (4)

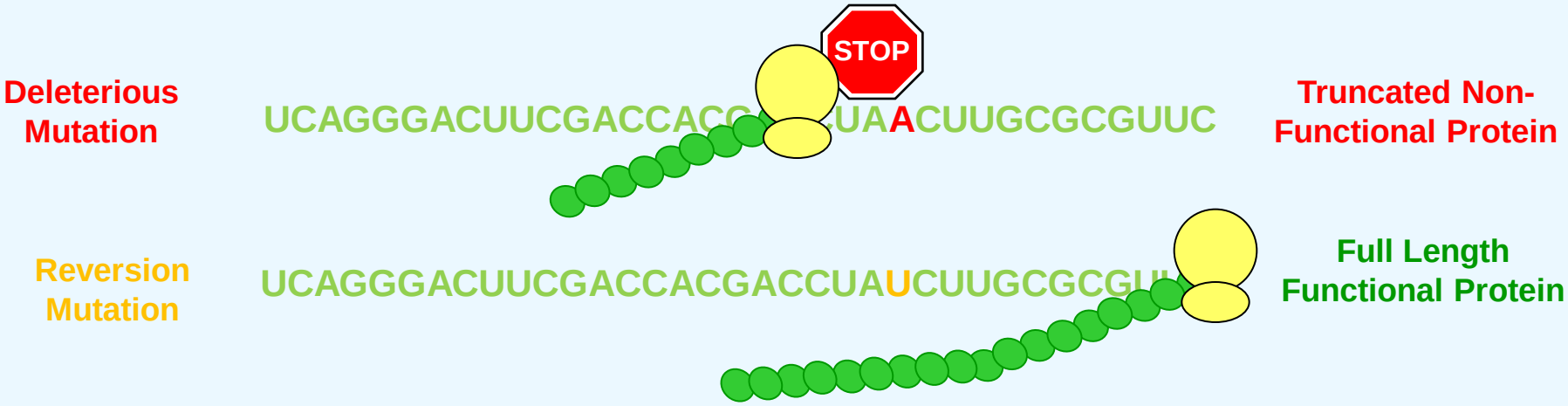
1) Pal et al 2005 Cancer; 104(12):2807-16 2) Rubin et al 1998 Am J Obstet Gynaecol; 178(4):670-7 3) Risch et al 2001 Am J Hum Genet 2001 68(3):700-10 4) Hennessy et al 2010 J Clin Oncol 28(22):3570-6.

Selective Pressure Against BRCA1/2 Mutations?

- Primary culture protocols usually involve physical disruption of the tissue, enzymatic digestion and selection of attached colonies in tissue culture.
- The more robust the cell the more likely it is to survive the process, cells without the full complement of DNA repair pathways, such as BRCA1/2 mutants are likely to be at a disadvantage.
- We examined the growth rate of 19 cell lines from the ovarian cell line panel and the deleterious mutant SNU-251 had the slowest growth rate.



Selective Pressure Against BRCA1/2 Mutations?



- Two cell lines in the cell panel had deleterious mutations and an additional reversion mutation (UPN-251) and (PEO1), also suggesting that there is a selective pressure against BRCA1/2 mutations in culture.
- This effect has been observed in drug resistant cell lines, as well as in cancer patients post chemotherapy.

Capan-1 Pancreatic Cancer BRCA2 mutated

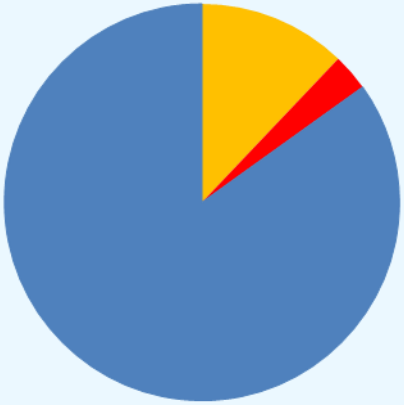
Resistance Developed with Cisplatin

7/14 Resistant Clones BRCA2 Restored

Sakai et al 2008 Nature 451(7182):1116-1120

Heterozygous Mutations

Unselected Ovarian Cancer Cell Lines from n=33 Patients

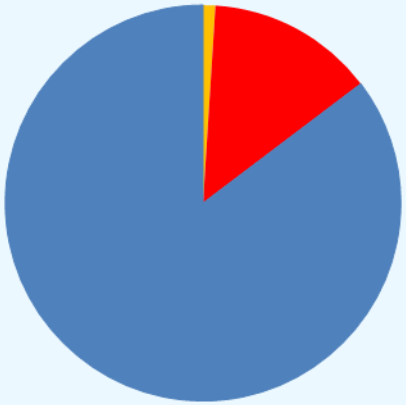


BRCA1/2 Heterozygous Deleterious Mutations 12.4%

BRCA1/2 Homozygous Deleterious Mutations 3.0%

Wildtype 84.8%

Unselected Invasive Ovarian Tumours



BRCA1/2 Heterozygous Deleterious Mutations 1%

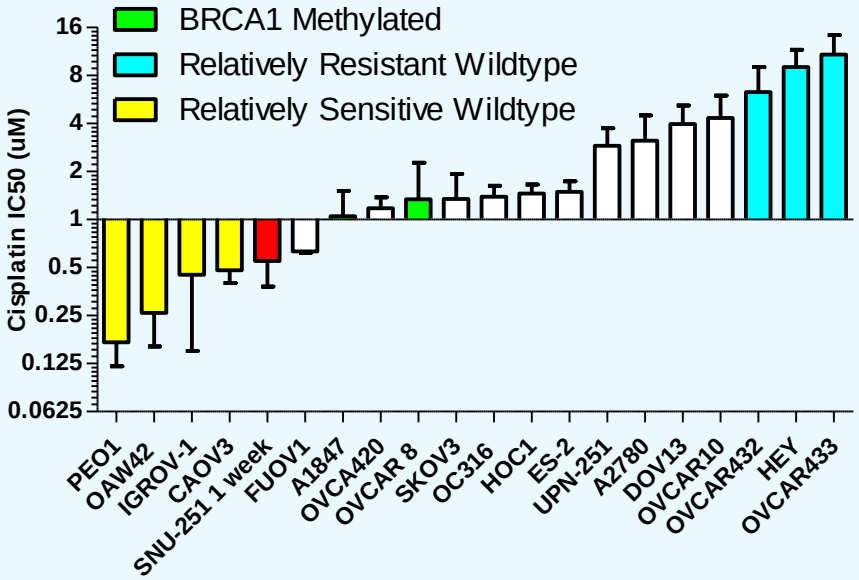
BRCA1/2 Homozygous Deleterious Mutations 8.6-13.7%

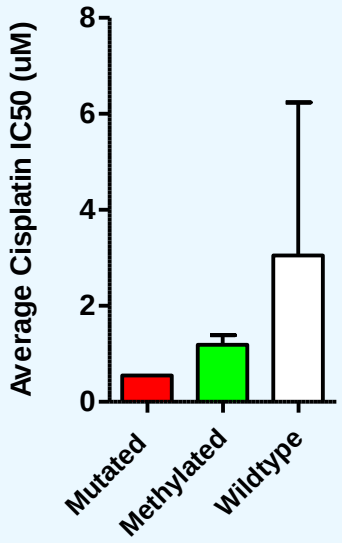
Wild Type 90.4-85.3%

- The high rate of heterozygous mutations was unexpected, these have no functional impact on the BRCA1/2 protein. No LOH was observed at the BRCA1/2 locus in these cells.
- This is a much higher rate than observed in clinical ovarian cancer. These may represent heterogeneity within a BRCA1/2 tumour with the heterozygous cells selected for in culture.

Cytotoxicity of Cisplatin

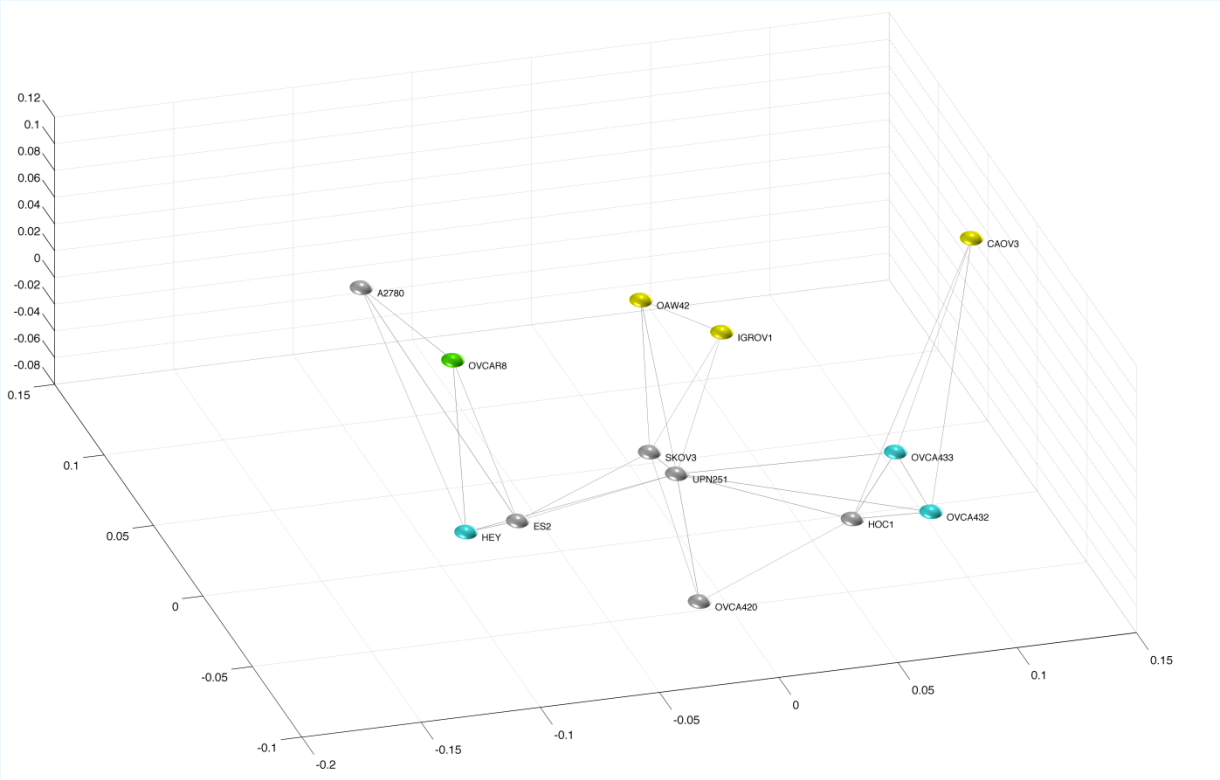
• A smaller panel of 20 cell lines were chosen to investigate the impact of BRCA1/2 dysfunction on sensitivity to platinum. Seventeen wild-type cell lines were compared to the methylated cell lines (A1847 and OVCAR8) and the deleterious mutant (SNU-251).





Biomarkers of Cisplatin Resistance

- Affymetrix whole genome arrays were performed on the panel of cell lines. Gene expression was compared between:-
  - Relatively Cisplatin Sensitive OAW42, CAOV3 and IGROV-1
  - Relatively Cisplatin Resistant HEY, OVCAR432 and OVCAR433



- When comparing the relatively cisplatin resistant or sensitive cell lines on a whole genome basis they do not cluster together distinct from the other cell lines.

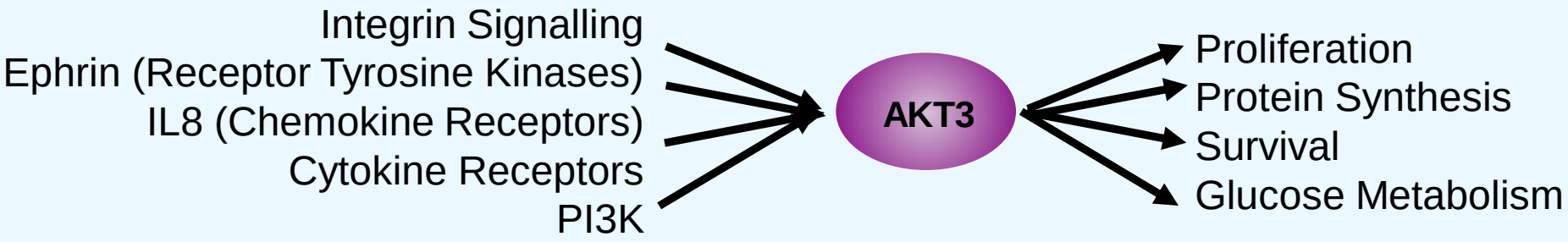
Biomarkers of Cisplatin Resistance

• 305 genes were significantly different between the two groups of cell lines. These were analysed by Ingenuity Pathway Analysis. Pathways with a significant number of altered genes were:-

- Integrin Signalling
- Ephrin Receptor Signalling
- IL8 Signalling
- Glutathione Pathway

Biomarkers of Cisplatin Resistance

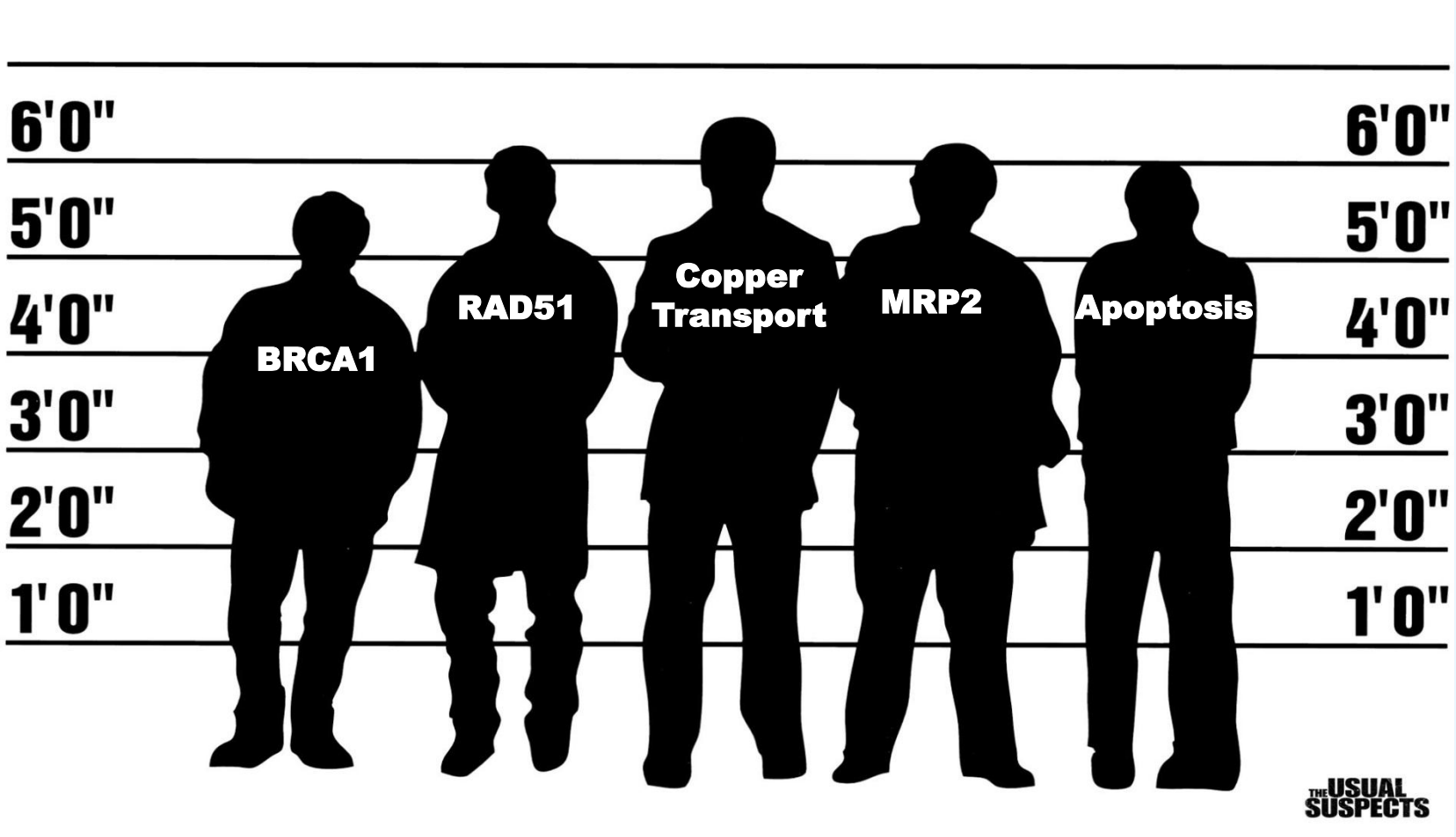
- 305 genes were significantly different between the two groups of cell lines. These were analysed by Ingenuity Pathway Analysis. Pathways with a significant number of altered genes were:-
  - Integrin Signalling - ↑ITGA6 (alpha integrin) → ↑AKT3
  - Ephrin Receptor Signalling - ↑ EPHA4 → ↑ AKT3
  - IL8 Signalling - ↑IL8 → ↑AKT3



- Activation of the Akt/mTOR pathway prevents cisplatin-induced apoptosis in ovarian cancer cells

Peng et al (2010) BBRC 394(3) 600-605.

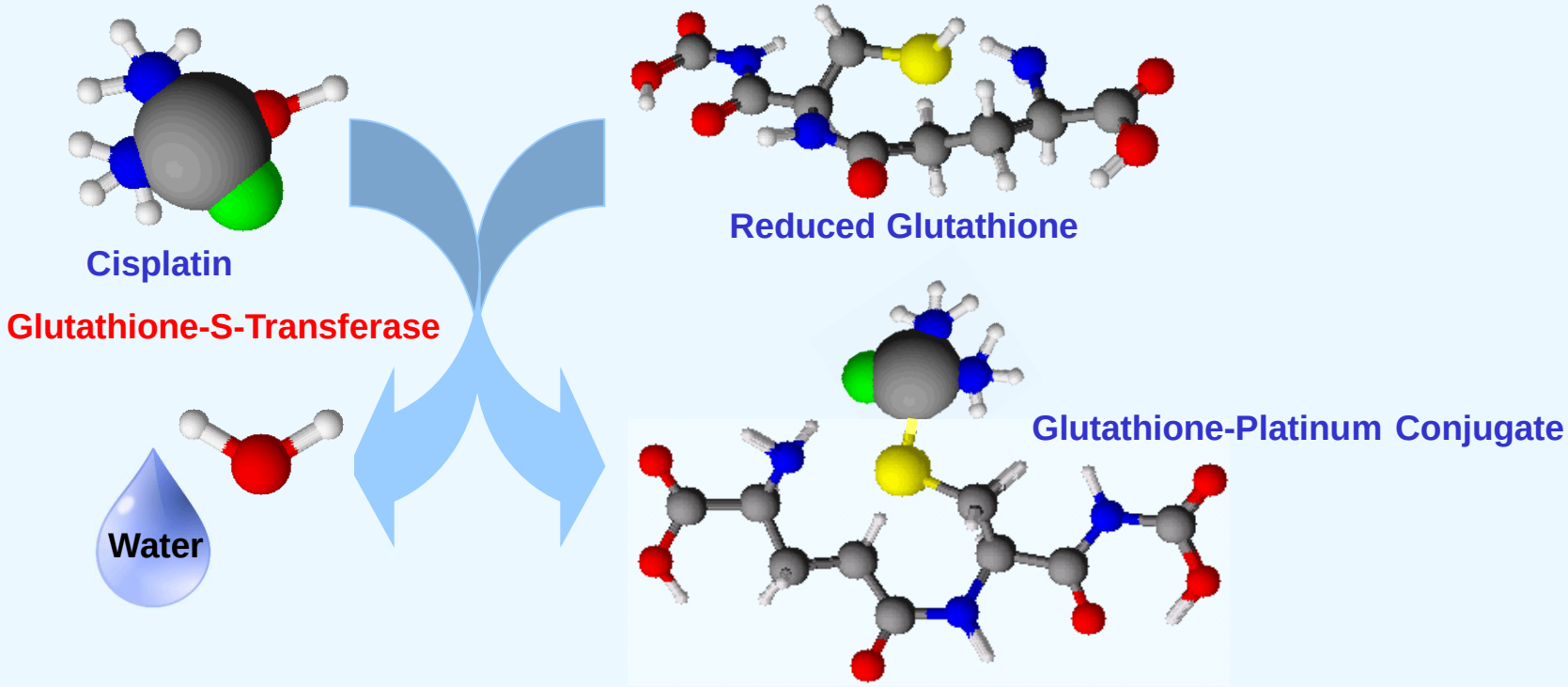
Absence of “Usual Suspects”



- However, this may be due to us comparing untreated cell lines rather than genes responsive to cisplatin treatment

Glutathione and Cisplatin Resistance

•The platinum-glutathione conjugate can no longer bind to DNA hence removing the toxic effect of the drug

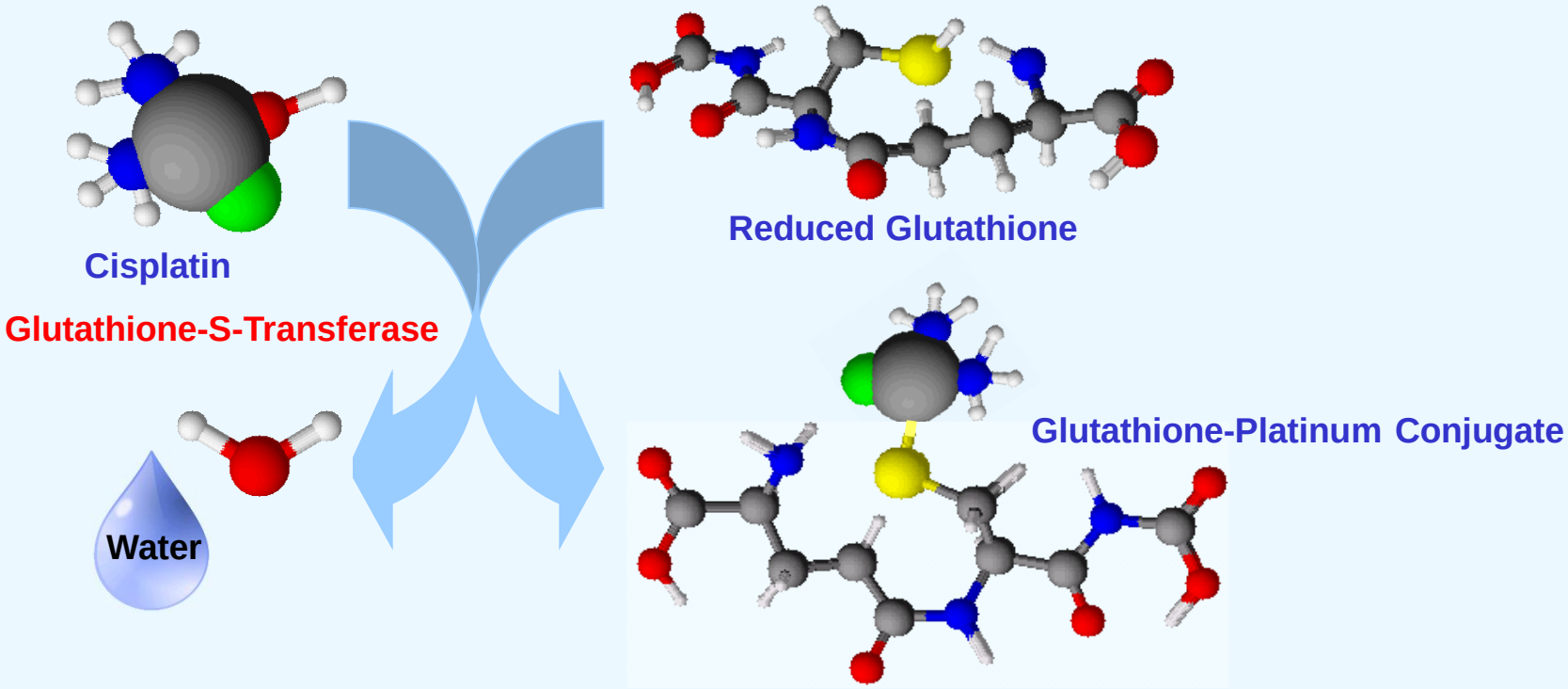


• Any increase in activity the glutathione pathway, synthesis, recycling or conjugation to cisplatin can potentially mediate cisplatin resistance.

• However, we see no change in glutathione reductase or synthetase

Glutathione and Cisplatin Resistance

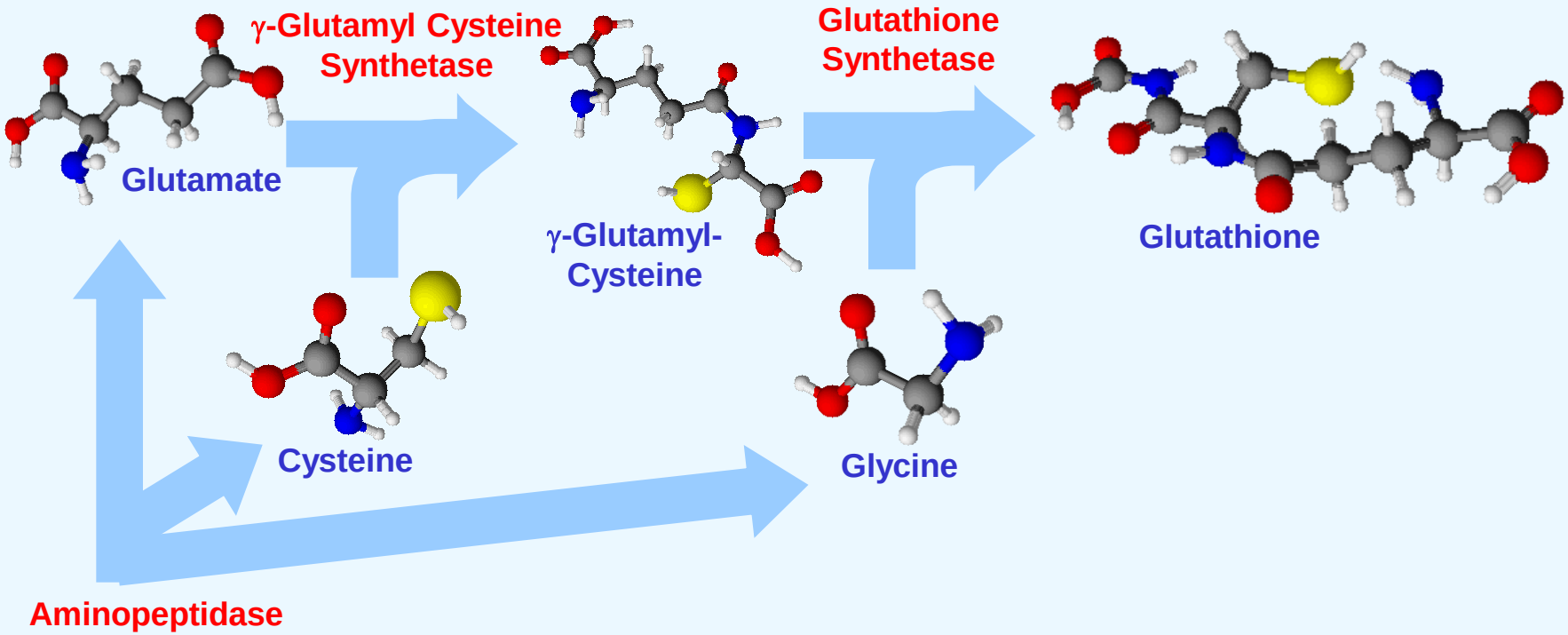
• Glutathione functions to detoxify xenobiotics such as cisplatin by a conjugation reaction. The platinum-glutathione conjugate can no longer bind to DNA hence removing the toxic effect of the drug



• In contrast we see a decrease in GSTM1, GSTM2 and GSTM3 associated with cisplatin resistance.

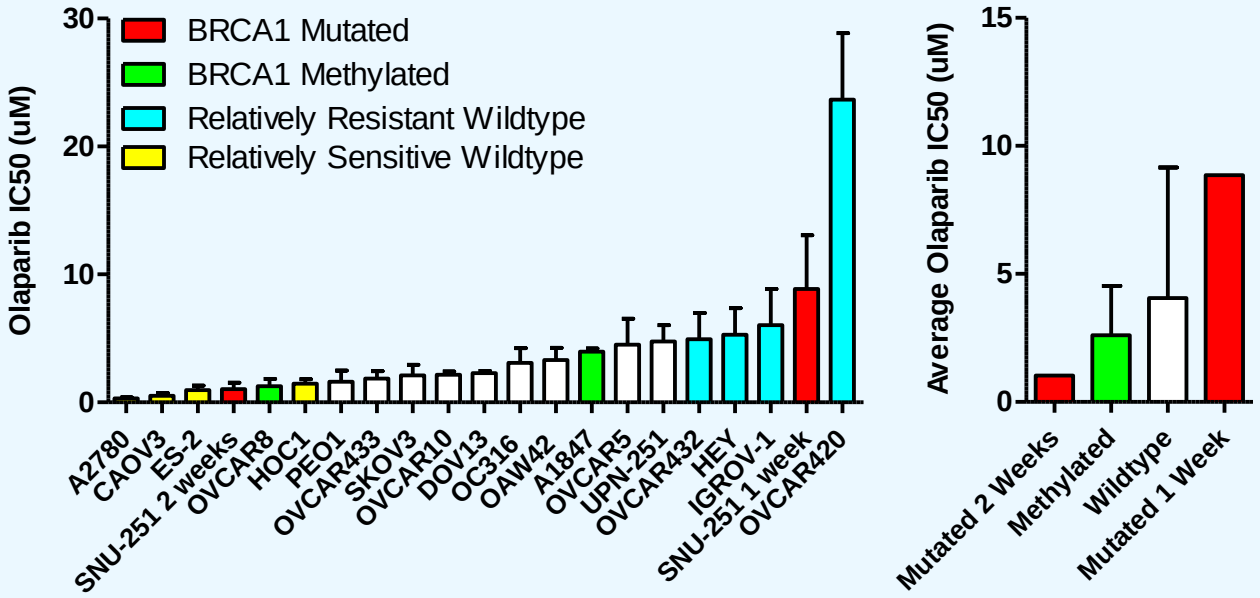
Glutathione and Cisplatin Resistance

• Glutathione is a tri-peptide which is synthesised within the cell from the amino acids glutamate, cysteine and glycine



• We see an increase in aminopeptidase associated with cisplatin resistance which may increase the available building blocks for glutathione synthesis.

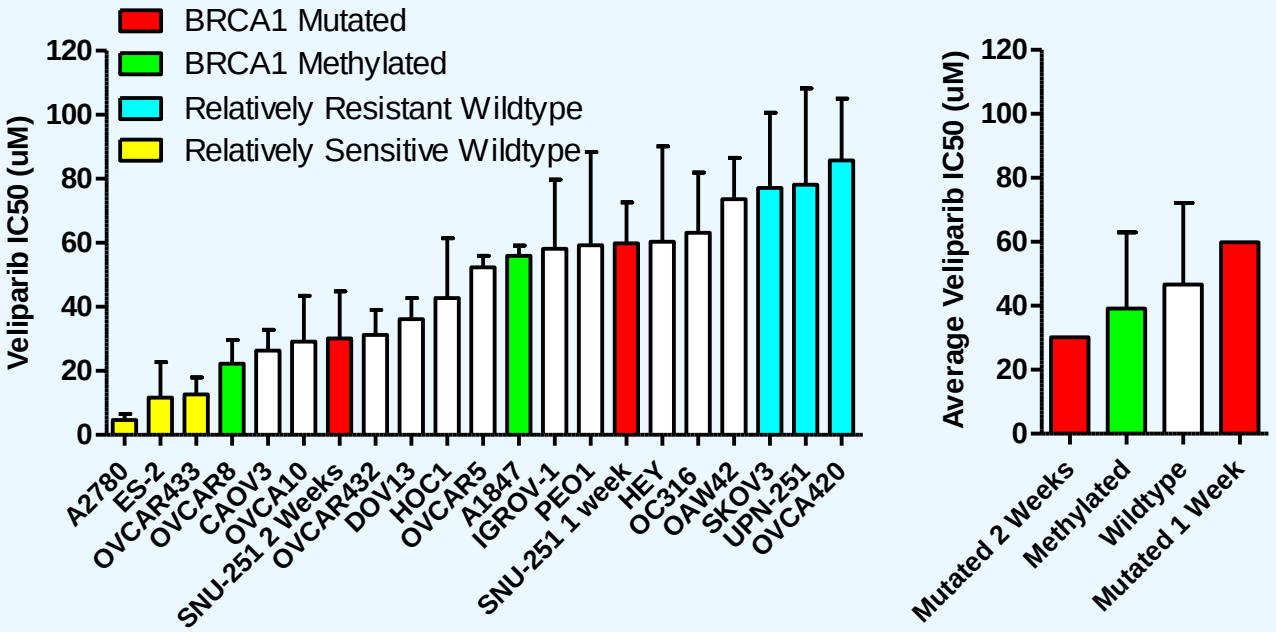
Cytotoxicity of Olaparib



- Unexpectedly, the SNU-251 cell line with the deleterious mutation in BRCA1 was one of the most resistant cell lines to olaparib of the panel.
- On average the BRCA1 methylated cell lines A1847 and OVCAR8 were relatively sensitive to Olaparib.

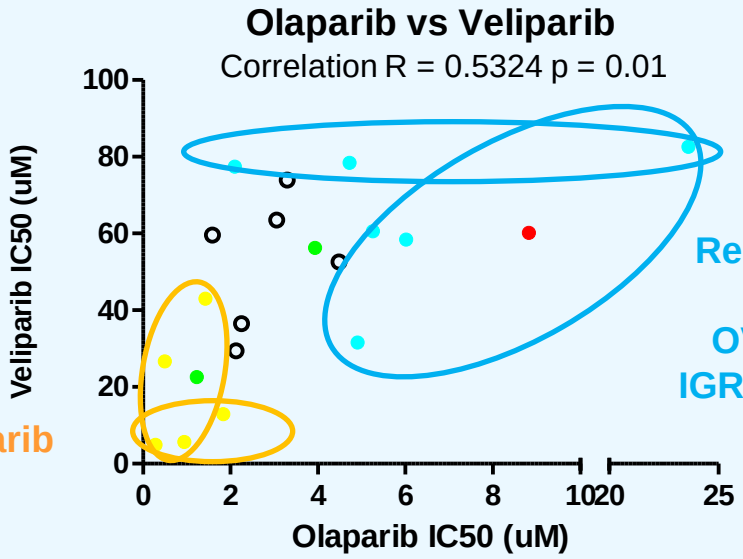
Cytotoxicity of Veliparib

- A similar trend was observed for Veliparib, SNU-251 was relatively resistant, and in a one week assay but not in a 2 week assay.
- The methylated cell lines tended to be sensitive to Veliparib.



Cytotoxicity of Olaparib vs Veliparib

Relatively Veliparib Resistant
SKOV3, UPN-251, OVCAR420



Relatively Olaparib Resistant
OVCAR432, HEY, IGROV-1, OVCAR420

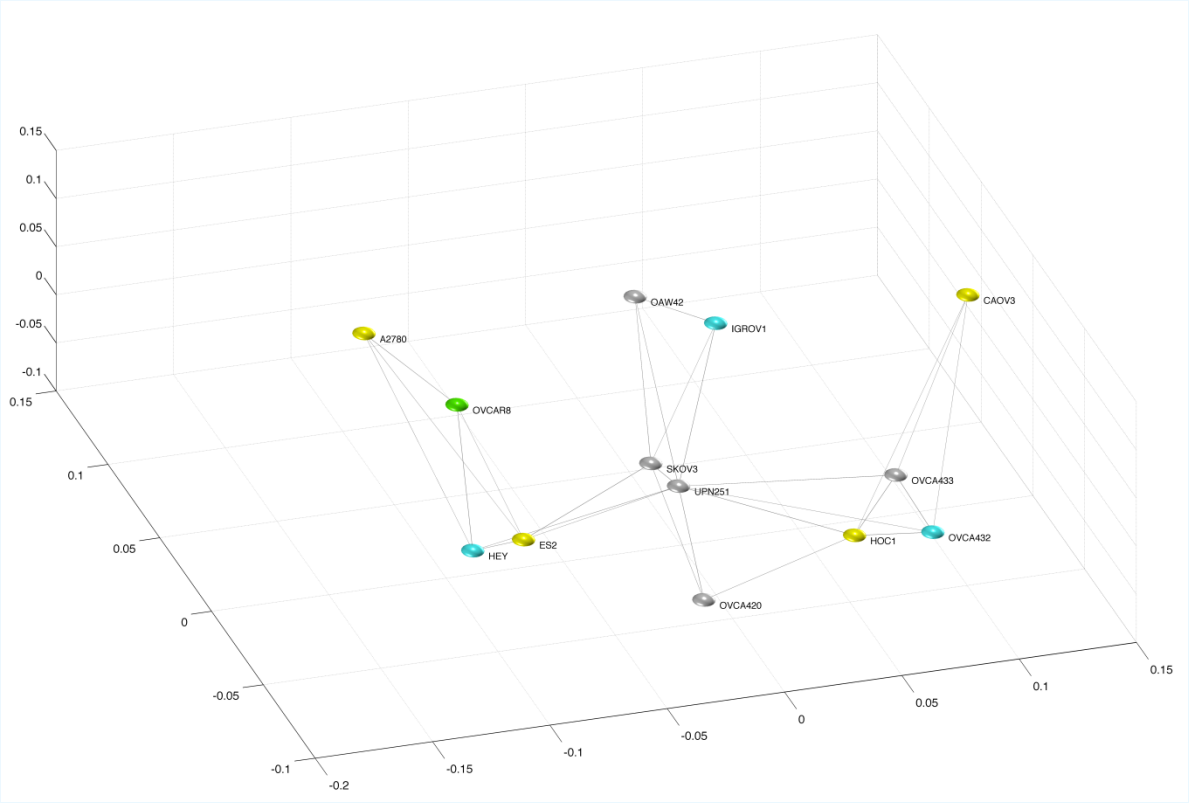
Relatively Veliparib Sensitive
A2780, ES-2, OVCA433

Relatively Olaparib Sensitive
A2780, CAOV3, ES-2, HOC1

- This suggests a similar but slightly divergent mechanism of resistance where a cell line specialises more in resistance to one agent over the other.

Biomarkers of Olaparib Resistance

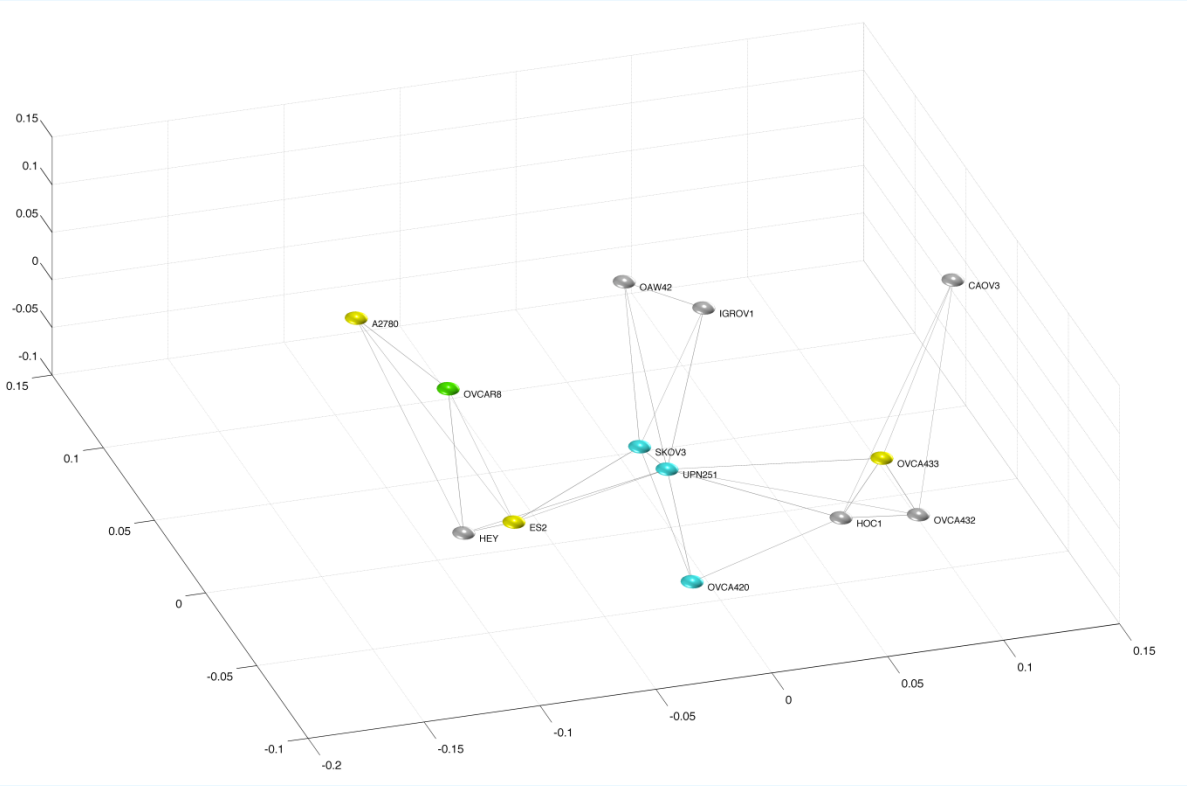
- Affymetrix whole genome arrays were performed on the panel of cell lines. Gene expression was compared between:-
  - Relatively Olaparib Sensitive A2780, CAOV3, ES-2, HOC-1
  - Relatively Olaparib Resistant HEY, IGROV-1, OVCAR420 OVCAR432



- When comparing the relatively olaparib resistant or sensitive cell lines on a whole genome basis they do not cluster together distinct from the other cell lines.

Biomarkers of Veliparib Resistance

- Affymetrix whole genome arrays were performed on the panel of cell lines. Gene expression was compared between:-
  - Relatively Veliparib Sensitive A2780, ES-2, OVCAR433
  - Relatively Veliparib Resistant OVCAR420, SKOV3, UPN-251



- When comparing the relatively veliparib resistant or sensitive cell lines on a whole genome basis they do not cluster together distinct from the other cell lines.

Biomarkers of Parp Inhibitor Cross Resistance

- 25 genes were significantly different between the olaparib resistant and sensitive cell lines.

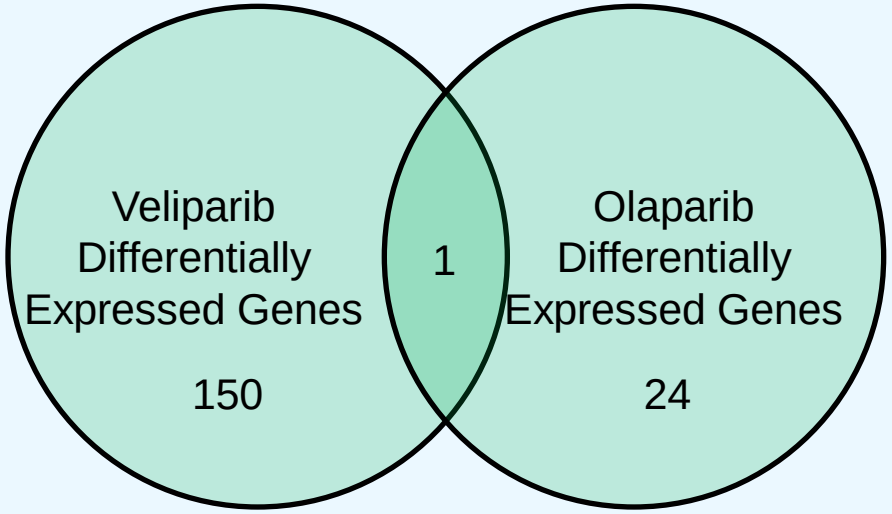
- 151 genes were significantly different between the veliparib resistant and sensitive cell lines.

- These were analysed by Ingenuity Pathway Analysis. The top pathways were different for Olaparib and Veliparib, most were general cancer pathways. Of interest:-

Olaparib – G2/M DNA Damage Checkpoint Regulation

Veliparib - Pyrimidine De Novo Biosynthesis

Biomarkers of Parp Inhibitor Cross Resistance

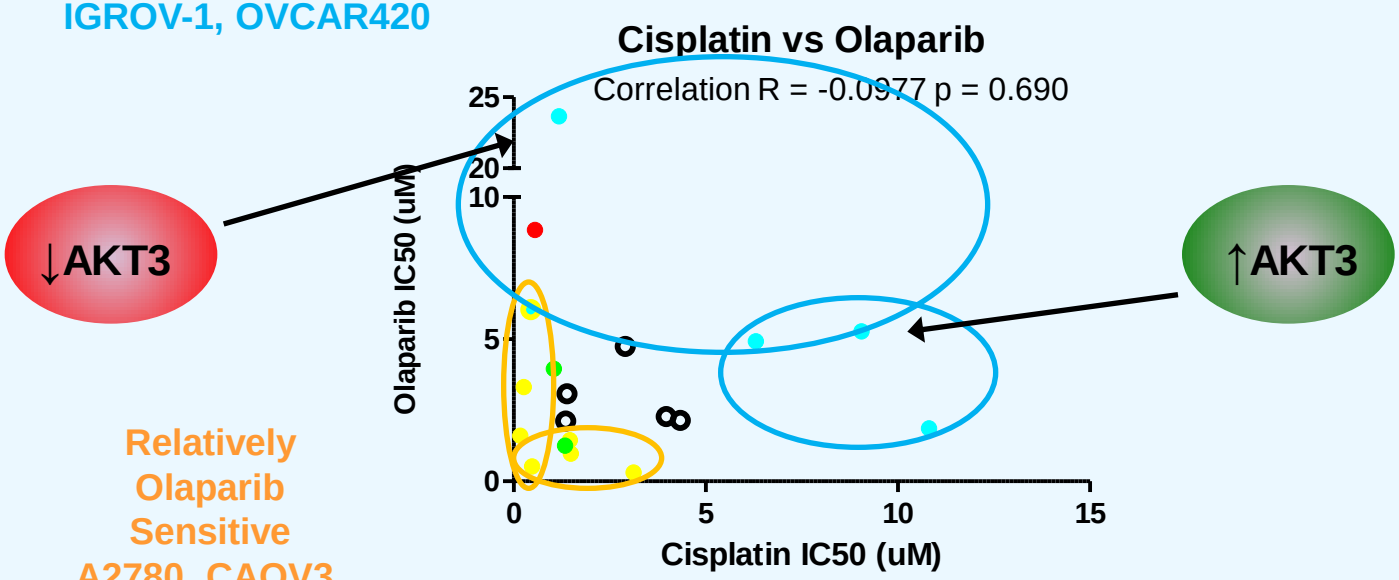


- Only 1 gene was significantly differentially expressed in both Olaparib and Veliparib resistant cell lines PLCL2 - phospholipase C-like 2
- Phosphodiesterases that cleave the polar head groups from inositol lipids
- Thought to activate the Src pathway.

- PLCL2 was increased in the sensitive cell lines. Also found in the NCI-60 panel as a general marker of drug potency.
- However, the overall gene expression profiles are similar suggesting a common mechanism of resistance between the two agents but some specificity in a different subset of genes.

Cytotoxicity of Cisplatin vs Olaparib

Relatively Olaparib Resistant
OVCAR432, HEY, IGROV-1, OVCAR420



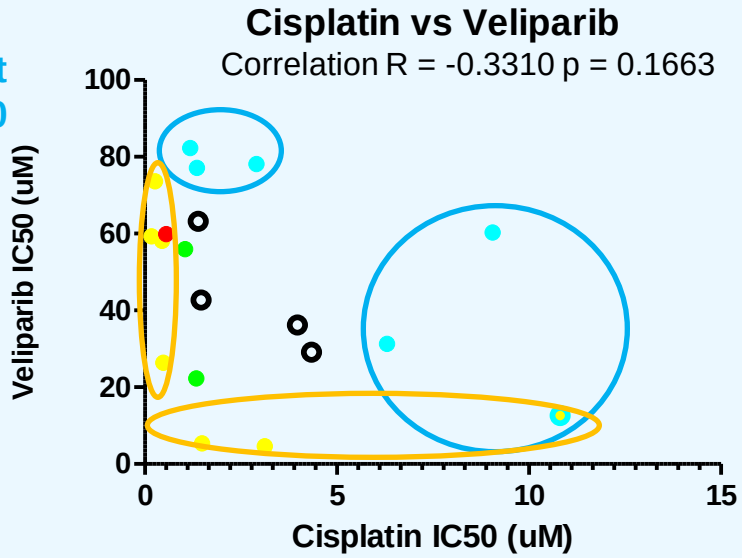
Relatively Olaparib Sensitive
A2780, CAOV3, ES-2, HOC1

Relatively Cisplatin Sensitive
PEO1, OAW42, CAOV3, IGROV-1

Relatively Cisplatin Resistant
OVCAR432, HEY, OVCAR433

Cytotoxicity of Cisplatin vs Veliparib

Relatively Veliparib Resistant
SKOV3, UPN-251, OVCAR420



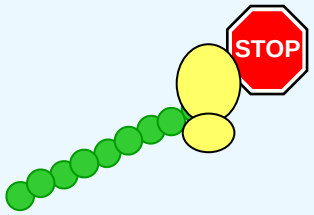
Relatively Veliparib Sensitive
A2780, ES-2, OVCA433

Relatively Cisplatin Sensitive
PEO1, OAW42, CAOV3, IGROV-1

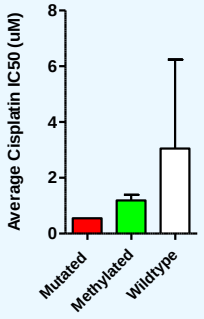
Relatively Cisplatin Resistant
OVCAR432, HEY, OVCAR433

- Cisplatin appears active in veliparib-resistant cell lines.
- Veliparib somewhat active in cisplatin-resistant cell lines.
- Potential for combination treatments of these agents for ovarian cancer (toxicity permitting).

Conclusions



• Deleterious BRCA1/2 mutations are rare in the panel of ovarian cancer cell lines we have studied 2.6%. There appears to be selective pressure against BRCA1/2 mutations in cell culture.



• BRCA1 mutated and methylated cell lines are sensitive to platinum and parp inhibitor chemotherapy. There are cohorts of BRCA1/2 wild-type cell lines that are sensitive to platinum and parp inhibitors.

• The AKT pathway may be a ‘global’ pathway suitable for use as a broad platinum resistance biomarker at the gene level. Rather than using the ‘usual suspects’

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Thanks To:-
Kirsten Timms

Please come and see poster presentations

- Developing platinum and taxane resistant ovarian cancer cell lines: Investigating the role of BRCA1.
- Collateral sensitivity to cisplatin in KB-8-5-11 is confluence dependant.