A tumour-DNA methylation biomarker to predict response to treatment in patients with ovarian cancer: a multi-centre study

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Methylation of **MYLK3** gene promoter region: a biomarker to stratify surgical care in ovarian cancer in a multi-centre study

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Accepted for publication 3rd March 2017
8.8% of TOTALLY debulked women progressed or died within 12 months, with median survival of 3 months.
Surgical Complications

Can we predict poor prognosis post surgery?
DNA methylation

- Addition of CH₃ methyl group to cytosine which is paired to guanine (CpG)
- Genetic sequence remains unaltered
- 5-methyl cytosine methylation, can down regulate gene transcription
- Measured easily with pyrosequencing and Illumina beadchip array.

- Clinically useable
- Potential cell free DNA
- Bed side testing in future

✓ It is very stable
✓ Easier to quantify than RNA
✓ Robust high throughput technology
Where to look?

- **FGF4**
- **FGF21**
- **MYLK2**
- **MYLK3**
- **MYL7**
- **ITGAE**

**27K Illumina methylation array**

- **65 probes**
- Δ methylation >20% adjusted for age, stage, grade, chip

- **27 probes**

**Validation**

- **Bisulphite pyrosequencing** (N=89 tumour DNA)
- **27K Illumina methylation array** (N=277 tumour DNA)

**65 probes**

- **Six with largest effect**
Results **MYLK3**

**Median Overall Survival**
- Hammersmith high methylation: 52.4m
- Hammersmith low methylation: 33.0m
- Charité high methylation: 68.0m
- Charité low methylation: 34.7m
- TCGA high methylation: 44.5m
- TCGA low methylation: 34.0m

Charité

Low methylation: 68.0m
High methylation: 34.7m

Logrank: $P=0.05$
Multivariable: $P=0.05$
$n=47$

TCGA

Low methylation: 44.5m
High methylation: 34.0m

Logrank: $P=0.009$
Multivariable: $P=0.021$
$n=201$

Hammersmith

Low methylation: 52.4m
High methylation: 33.0m

Logrank: $P=0.017$
Multivariable: $P=0.029$
$n=39$

Charité

Low methylation: 68.0m
High methylation: 34.7m

Logrank: $P=0.05$
Multivariable: $P=0.05$
$n=47$

TCGA

Low methylation: 44.5m
High methylation: 34.0m

Logrank: $P=0.009$
Multivariable: $P=0.021$
$n=201$
All datasets combined

Methylation at cg13247990 (MYLK3) and debulk status

- Total debulk, low methylation
- Total debulk, high methylation
- Any residual, low methylation
- Any residual, high methylation

Survival benefit from total debulk appears to be lost in the presence of low methylation.

High methylation improves prognosis for women with residual disease.
High, intermediate and low methylation

In totally debulked patients there is a continuous effect with increasing methylation

Should we be offering radical surgery to those with the lowest levels of methylation?
Correlation with gene expression

**Correlation of MYLK3 Methylation and Expression**

- $P = 0.550$
- $\text{Rho} = -0.042$

**TCGA: Overall Survival according to expression of MYLK3**

- Logrank $P = 0.143$
- Multivariable $P = 0.152$
Summary

• *MYLK3* methylation independently affects survival after surgery.

• Not fully understood why *MYLK3* has this effect.

• High *MYLK3* appears to be beneficial and potentially serves as a useful biomarker.

• 8.8% progressed or died within 12 months of total debulk – could *MYLK3* be useful in selecting patients for treatment options?

• *MYLK3* may be one of the first steps in tailoring surgery according to tumour biology.

• Is there potential to increase methylation of *MYLK3*?

• Possible to measure cell-free circulating tumour DNA in blood.
Acknowledgements & Questions

Imperial College
London

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Roberto Dina
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Hani Gabra
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