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Guadecitabine alters DNA methylation of immune-related pathways and genes in recurrent platinum-resistant ovarian cancer

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BACKGROUND

Ovarian Cancer (OC)

- Most lethal gynecological malignancy[1].
- Majority of the patients initially respond to platinum-based drugs, but most relapse and acquire chemoresistance[1].
- Poor overall response rate (ORR) to immune checkpoint blockade (ICB)[1].
- Aberrant DNA methylome in immunoregulatory genes, leading to immunoediting[2,3] - potential cause of lower ORR to ICB.
- Further research required to identify immunoregulatory genes that are regulated by DNA methylation.

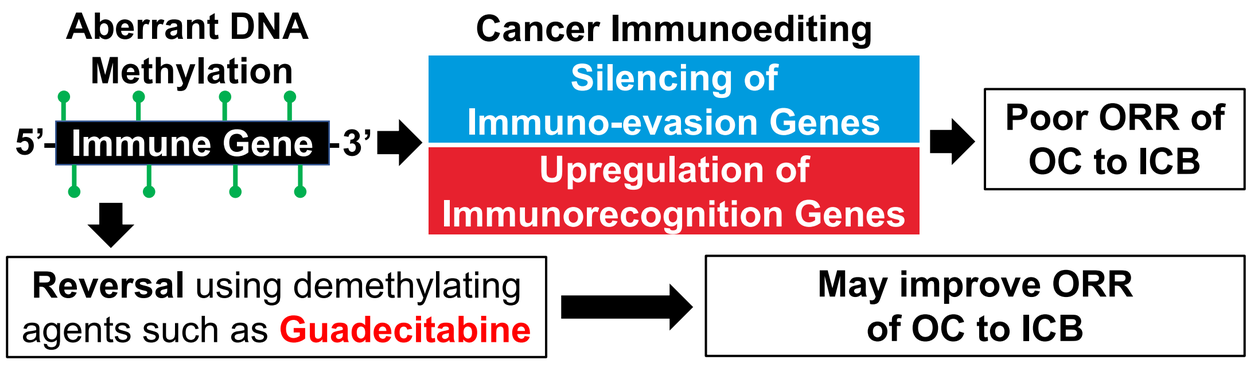

Figure 1: Schematics showing the acquisition of immunoediting via the aberrant DNA methylation of immune genes, leading to the poor overall response of ovarian cancer to immune checkpoint blockade. Reversal of abnormal methylome using guadecitabine may improve the effects of immune checkpoint inhibition.

- **Hypothesis**: Expression of immune-related genes are regulated by DNA methylation in platinum-resistant and relapsed OC and can be modified by guadecitabine treatment.
- **Aim**: Identify immunoregulatory genes that are controlled by DNA methylation in recurrent platinum-resistant OC.

MATERIALS AND METHODS

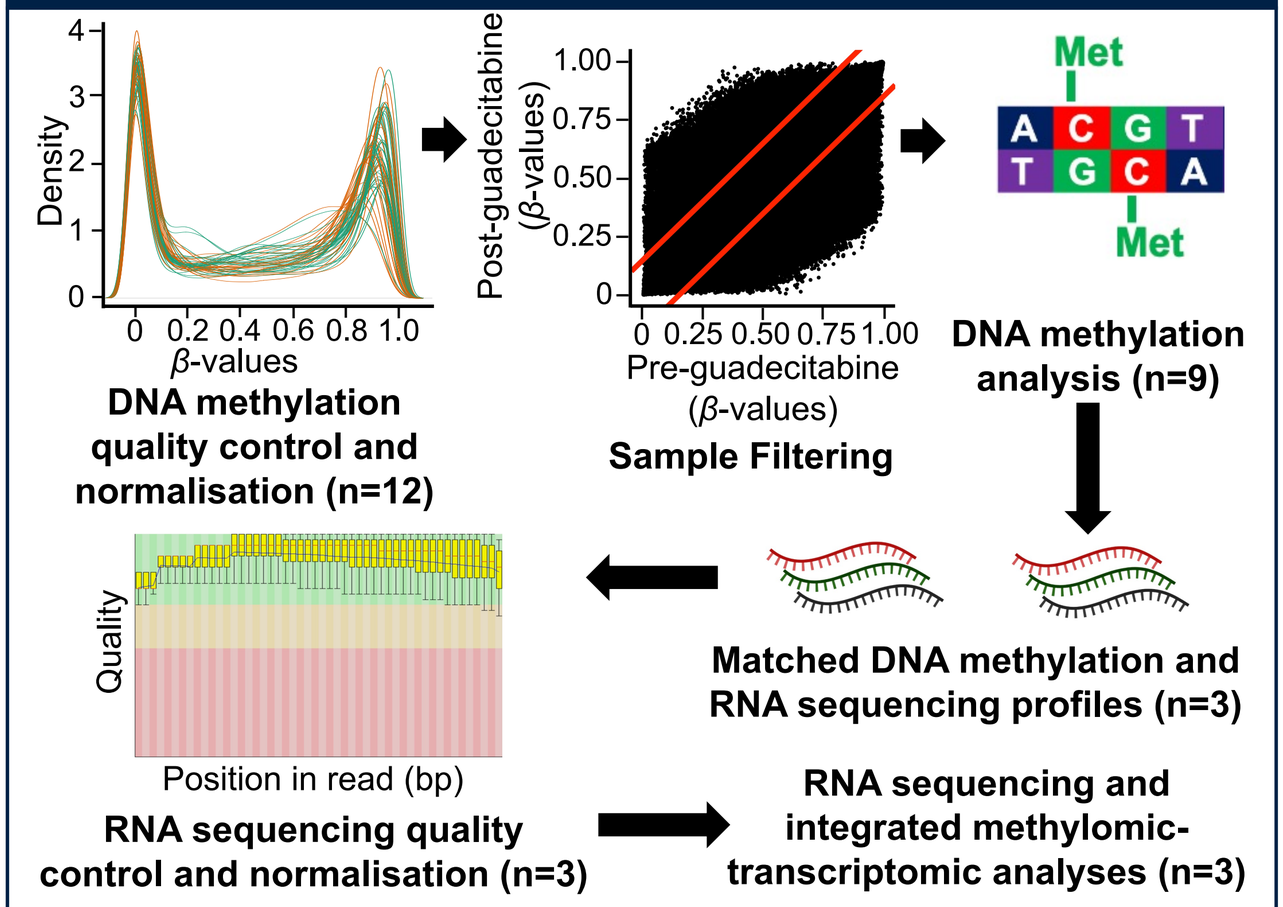

Figure 2: Data analysis workflow. DNA methylation (GSE102119) and RNA sequencing (GSE102118) data used for the analysis were from a clinical trial done by Fang *et al.*[4] and were downloaded from NCBI Gene Expression Omnibus.

RESULTS

DNA methylation heatmap and RNA expression volcano plot

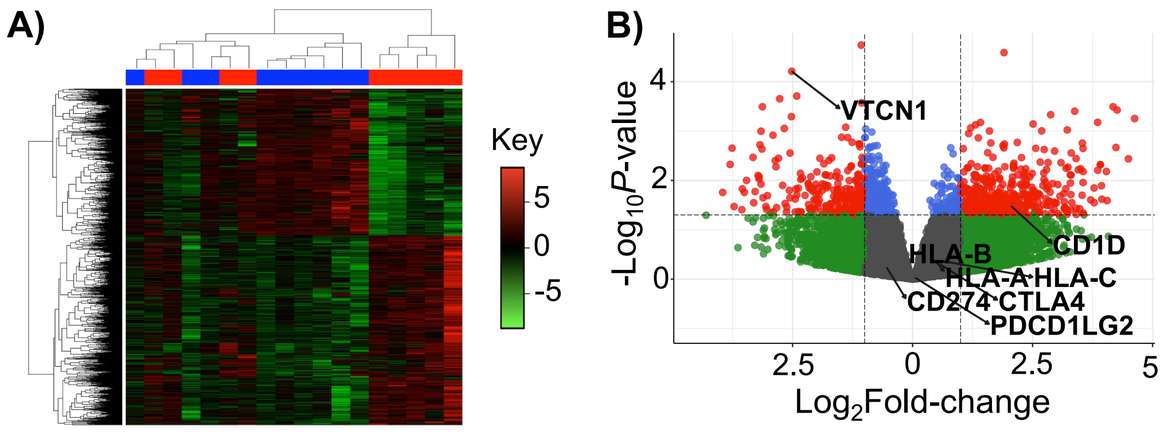

Figure 3: A) Heatmap showing hierarchical clustering of 9646 differentially methylated CpG sites (P<0.05; n=9) in pre-guadecitabine (blue column) vs post-guadecitabine (red column) using Euclidean distance. Red = high methylation; green = low methylation relative to CpG sites across all samples. **B)** Volcano plot of gene expression. Plot of $\log_2 P$-value against -$\log_{10}$fold-change of gene expression for patients post-guadecitabine. Number of genes = 36560 (n=3). Annotated key immune genes. Gray = no statistical significance ; green = $\log_2$fold-change ≥ 1.0; blue = P<0.05; red = $\log_2$fold-change ≥ 1.0 + P<0.05.

Guadecitabine altered immune-related pathways

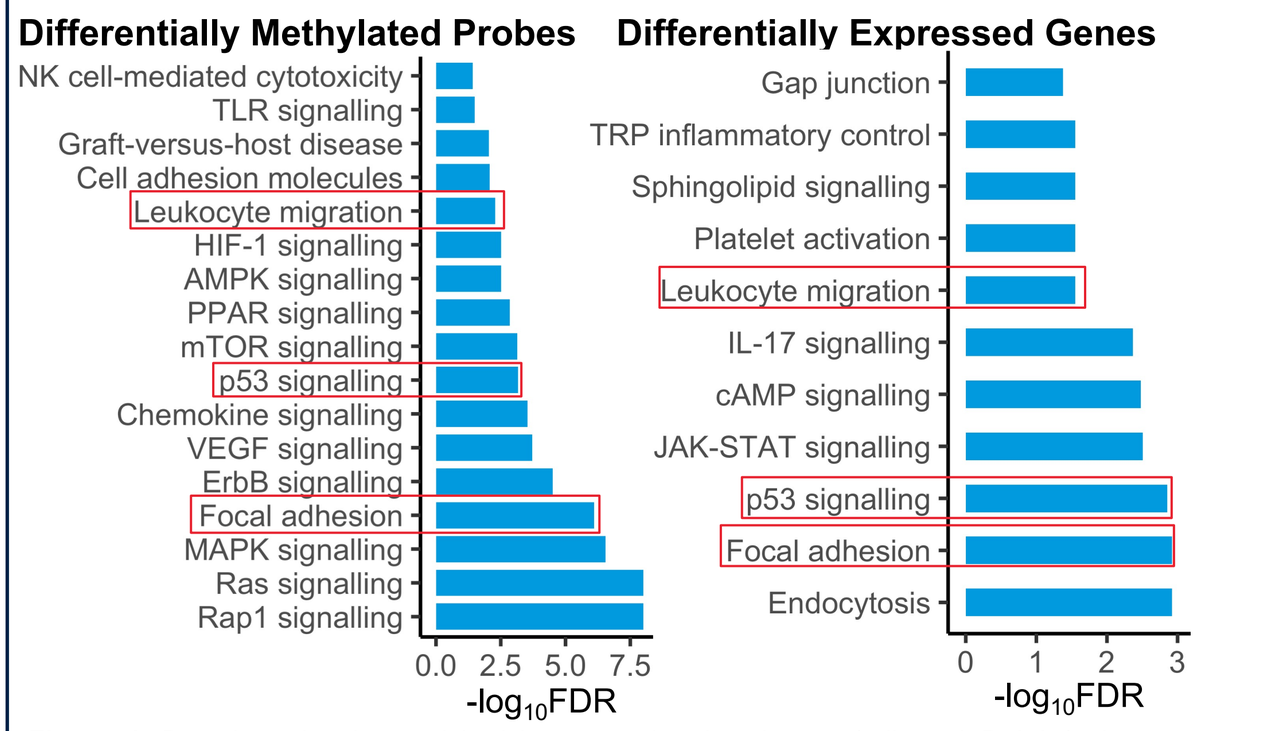

Figure 4: Bar plots showing enriched immune-related pathways (adjusted P<0.05) via gene set enrichment analysis using genes linked to differentially methylated CpG sites or probes (left; P<0.05; n=9) and differentially expressed genes (right; P<0.05; n=3) in recurrent platinum-resistant patients pre-guadecitabine vs. post-guadecitabine. Red box highlights the overlapping immune pathways enriched between DNA methylation and expression post-guadecitabine.

CD1D and VTCN1 expression might be regulated by DNA methylation

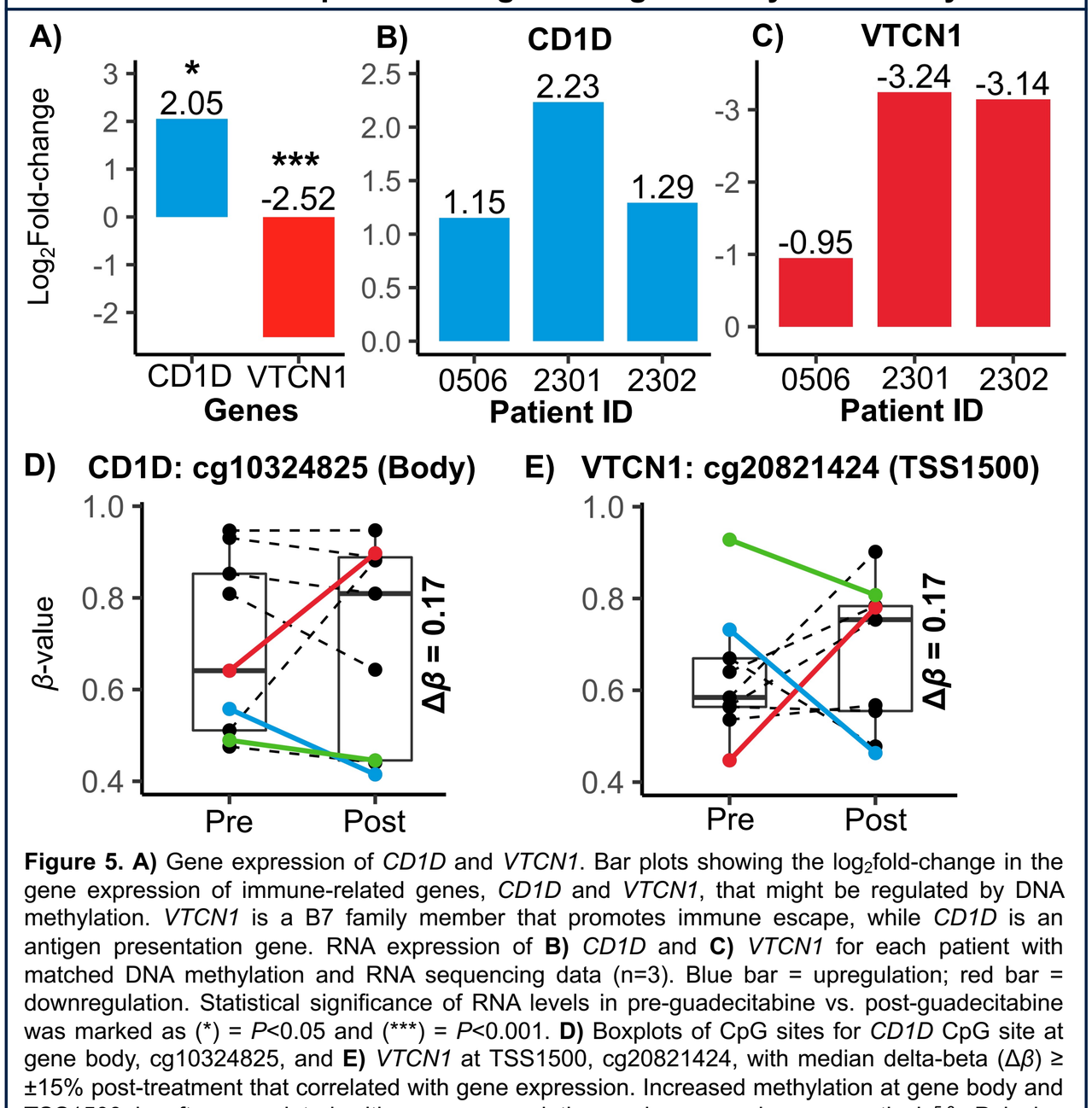

Figure 5. A) Gene expression of *CD1D* and *VTCN1*. Bar plots showing the $\log_2$fold-change in the gene expression of immune-related genes, *CD1D* and *VTCN1*, that might be regulated by DNA methylation. *VTCN1* is a B7 family member that promotes immune escape, while *CD1D* is an antigen presentation gene. RNA expression of **B)** *CD1D* and **C)** *VTCN1* for each patient with matched DNA methylation and RNA sequencing data (n=3). Blue bar = upregulation; red bar = downregulation. Statistical significance of RNA levels in pre-guadecitabine vs. post-guadecitabine was marked as (*) = P<0.05 and (***) = P<0.001. **D)** Boxplots of CpG sites for *CD1D* CpG site at gene body, cg10324825, and **E)** *VTCN1* at TSS1500, cg20821424, with median delta-beta (Δβ) ≥ ±15% post-treatment that correlated with gene expression. Increased methylation at gene body and TSS1500 is often correlated with gene upregulation and suppression, respectively[5,6]. Pairwise matching of β-value per patient pre- and post-guadecitabine as presented by connected lines. Black = patients without RNA sequencing profile; red = 0506; blue = 2301; green = 2302. Highlighted the heterogeneity in DNA methylation and response to guadecitabine per patient.

CONCLUSIONS AND FUTURE DIRECTIONS

- **Conclusions:**
  1. DNA methylation profiles and response to guadecitabine is heterogenous amongst patients.
  2. Guadecitabine induced immune-related pathways by DNA methylation and/or expression changes in recurrent platinum-resistant OC.
  3. *CD1D* and *VTCN1* might be controlled via DNA methylation (as observed in the cohort) and can be primed with guadecitabine to improve ICB strategies.
- **Future Direction:**
  1. Implication of methylome-transcriptome of *CD1D* and *VTCN1* in a larger cohort from a more common and aggressive type of OC.

References: **1)** Matulonis UA et al., 2020. *J Clin Oncol*, doi.org/10.1200/JCO.2020.38.15_suppl.6005. **2)** O'Donnell JS et al., 2019. *Nat Rev Clin Oncol*, doi.org/10.1038/s41571-018-0142-8. **3)** Campoli M et al., 2008. *Oncogene*, doi.org/10.1038/onc.2008.273. **4)** Fang F et al., 2018. *Cancer Res*, doi.org/10.1158/0008-5472.CAN-17-1492. **5)** Jjingo D et al., 2012. *Oncotarget*, doi.org/10.18632/oncotarget.497. **6)** Mishra NK et al., 2017. *Oncotarget*, doi.org/10.18632/oncotarget.15993.