



# Transcriptome Analysis and Theranostic Profiling of Ewing Sarcoma (ES) and Desmoplastic Small Round Cell Tumors (DSRCT)

Wenhsiang Wen, Anatole Ghazalpour, Wangjuh (Sting) Chen, Brian Rhees, Matthew J. McGinniss, and Zoran Gatalica  
Caris Life Sciences, Phoenix, AZ / Dallas, TX

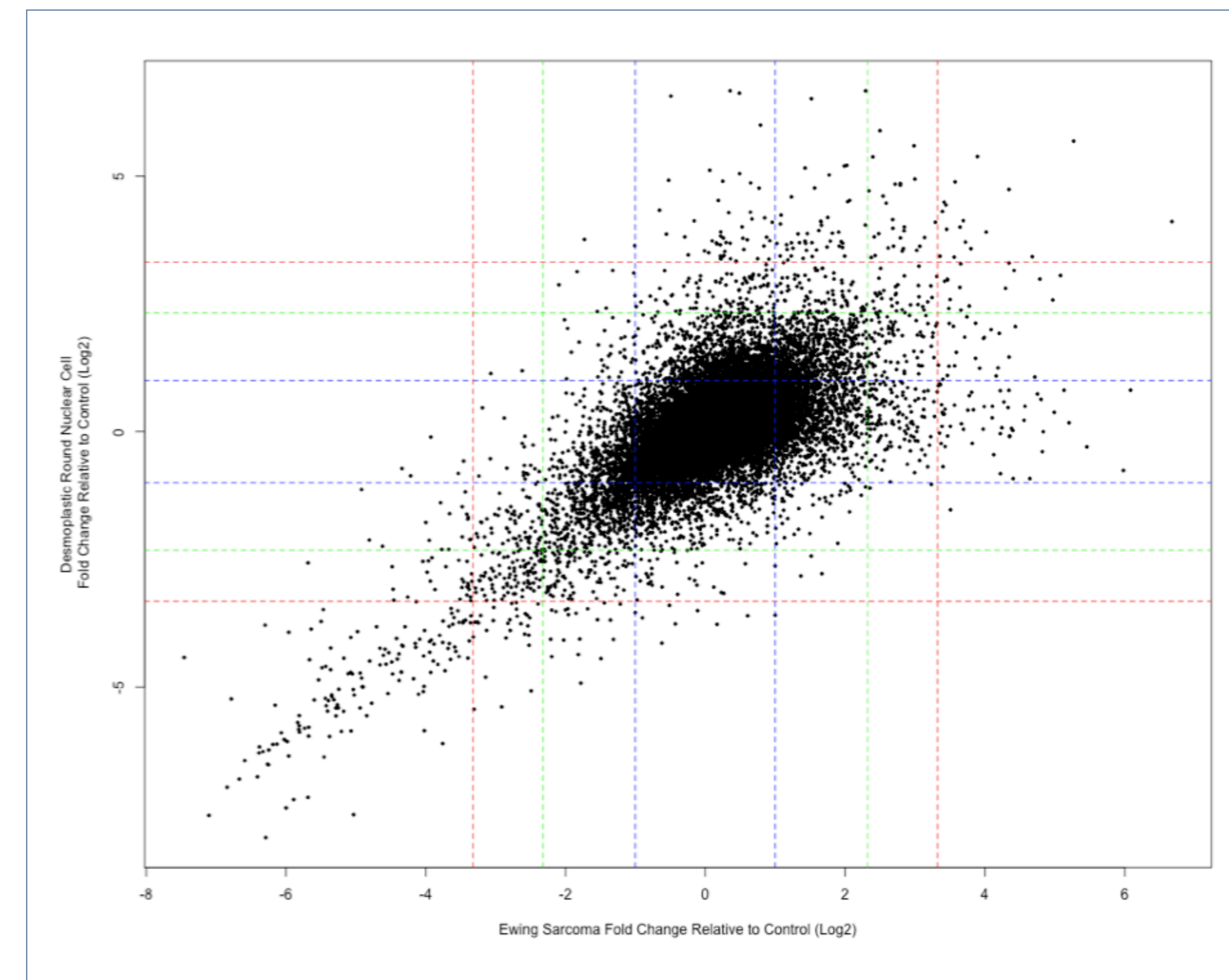


## Background

Ewing Sarcoma (ES)/PNET and Desmoplastic Small Round Cell Tumor (DSRCT) are sarcomas with distinct chromosomal translocations involving the EWS gene (predominately EWS-FLI1 and EWS-WT1; respectively). Their diagnosis and treatment have been difficult due to the rarity, diverse clinical presentation, overlapping histologic features and genetic complexity. Identification of therapeutically targetable genes or pathways in distinct tumor group and individual patient might provide more effective therapeutic strategies.

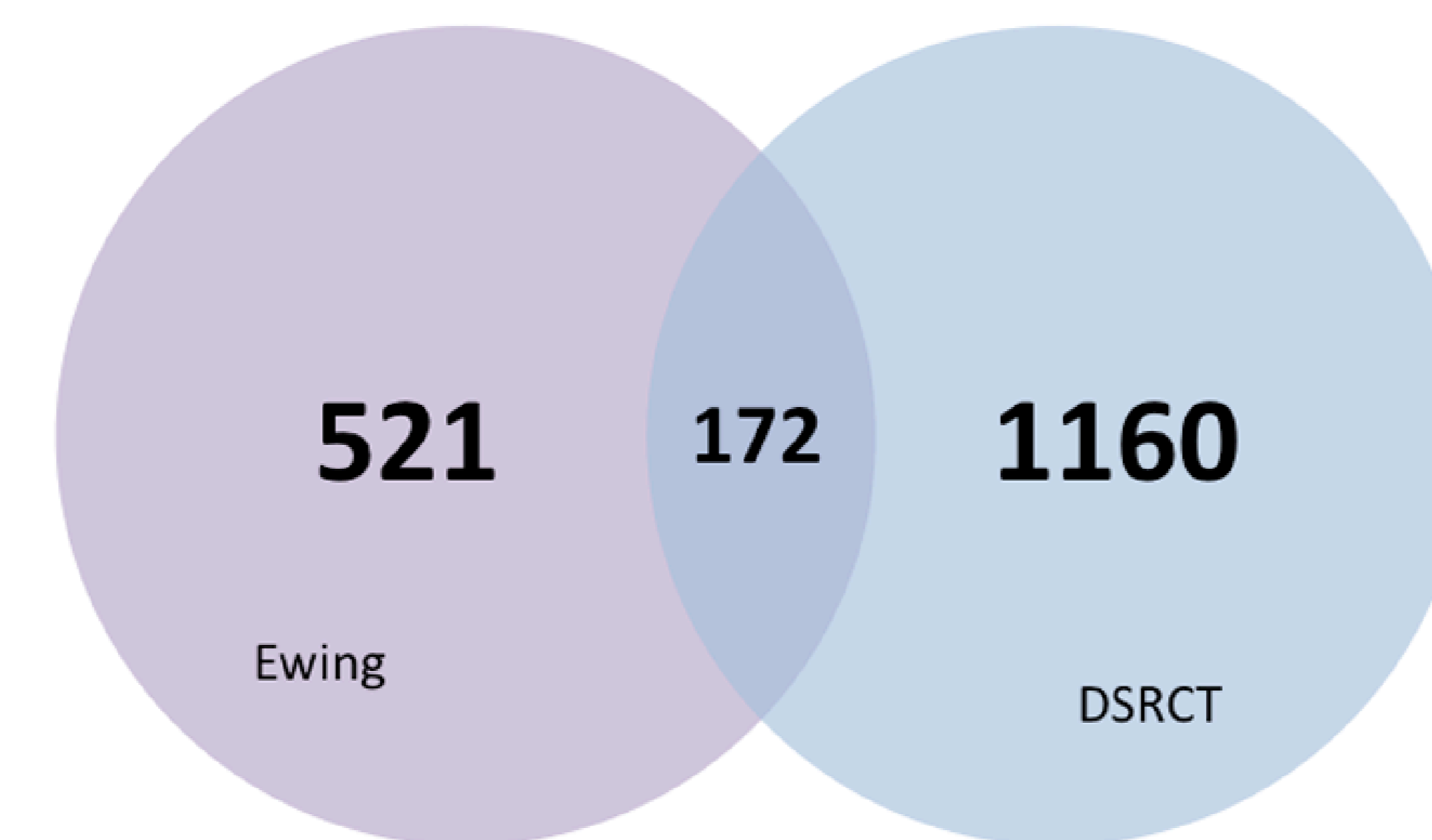
## Materials and methods

Twenty three cases(13 ES and 10 DSRCT) were analyzed using a molecular profiling service (CarisTargetNow™, Caris Life Sciences, Phoenix, AZ). The whole genome transcriptome analysis (29285 transcripts) was performed using HumanHT-12 beadChip (Illumina Inc, San Diego, CA) and comparison was made to pooled normal soft tissue (Fig 1). Pathway analysis of commonly up/down regulated genes in ES and DSRCT was performed using "Reactome"(www.reactome.org). Differences between ES and DSRCT were assessed by GSEA (Gene Set Enrichment Analysis <http://www.broadinstitute.org/gsea>). In addition, a selected number of chemotherapy-predictive (theranostic) biomarkers were evaluated using immunohistochemistry, FISH, and DNA sequencing.



**Fig. 1 – Comparison of differentially expressed genes (relative to normal soft tissue reference) between Ewing's sarcoma and DSRCT FFPE samples. Blue, green, and red horizontal and vertical lines depict 2 fold, 5 fold, and 10 fold over and under expressed genes respectively compared to control tissue.**

### Comparison of up regulated probes between Ewing's sarcoma and DSRCT (2 fold)



Total number of up regulated probes (by two fold) in each cancer type:  
Ewing = 693 probes  
DSRCT = 1332 probes

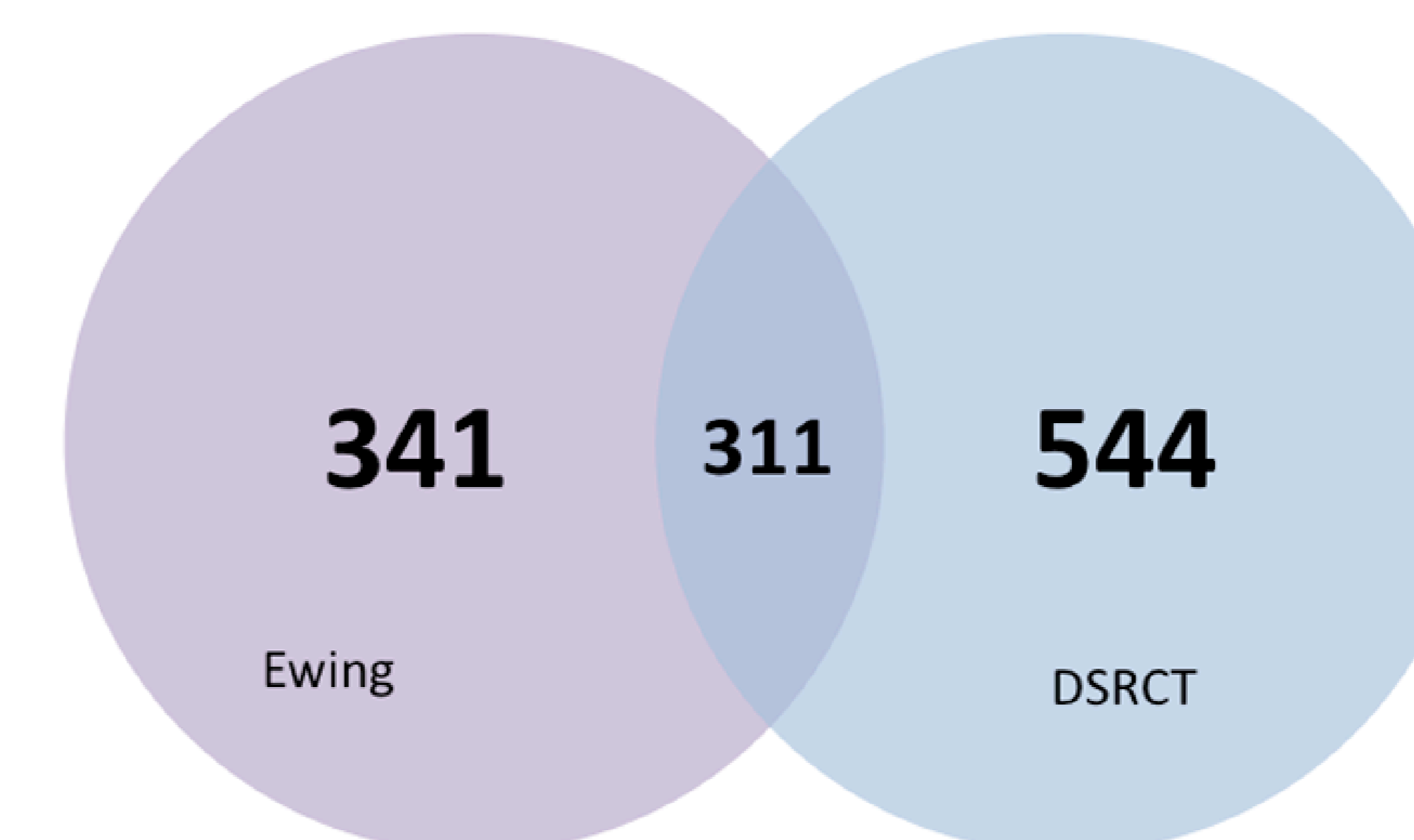
**Fig. 2 – Comparison of up regulated probes between Ewing's sarcoma and DSRCT (2 fold)**

## Results

We observed 172 commonly up (Fig 2) and 311 commonly down (Fig 3) regulated genes in ES and DSRCT (using 2 fold cut off). Cell cycle signaling, DNA replication and E2F mediated pathway genes were most commonly up regulated. GSEA analysis of differences between ES and DSRCT identified groups of genes associated with previously published in vivo and in vitro EWS-FLI1 models(Fig 4 (a)-(c)). These observations support the current knowledge that ES development is the result of mesenchymal progenitor cell transformation.

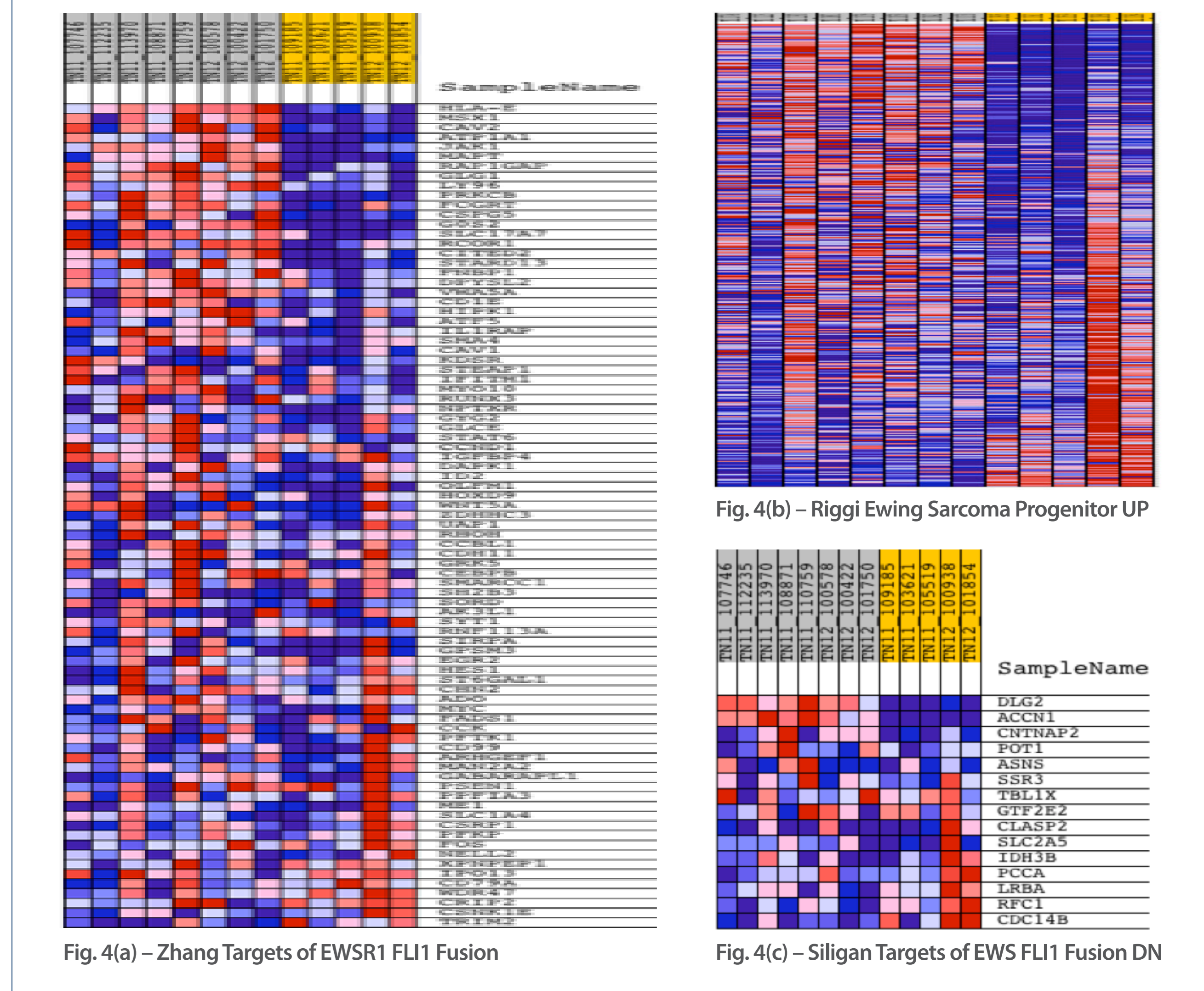
In addition, we observed overexpression of TOP2A and TOPO1 in approximately 50% of all cases, while ERCC1, TS, SPARC and MGMT showed significant inter-individual variations. No KRAS mutations or EGFR gene amplification were observed in any case.

### Comparison of down regulated probes between Ewing's sarcoma and DSRCT(2 fold)



Total number of down regulated probes (by two fold) in each cancer type:  
Ewing = 652 probes  
DSRCT = 855 probes

**Fig 3. – Comparison of down regulated probes between Ewing's sarcoma and DSRCT (2 fold)**



- EWS-FLI1 fusion protein up-regulates critical genes in neural crest development and is responsible for the observed phenotype of Ewing's family of tumors. Zhang J et al. *Cancer Research* 2005;65(11):4633-44.
- EWS-FLI-1 expression triggers a Ewing's sarcoma initiation program in primary human mesenchymal stem cells. Riggi et al, *Cancer Research* 2008;68(7):2176-85.
- EWS-FLI1 target genes recovered from Ewing's sarcoma chromatin. Siligan et al, *Oncogene* 2005;24(15) 2512-24

## Conclusion

- Transcriptome analyses identified common and differentially expressed genes/pathways between ES and DSRCT.
- Theranostic profiling provided individualized therapeutic directions regarding predicted susceptibilities to anthracycline, irinotecan, platinum analogs, fluorouracil and temozolomide.
- Alternative therapeutic targets in cell cycle regulation, DNA replication, and receptor TKI pathways might be effective in both ES and DSRCT.