LIQUID BIOPSY, SOLID FINDINGS: New insights into pathogenesis and cisplatin resistance in testicular germ cell tumors

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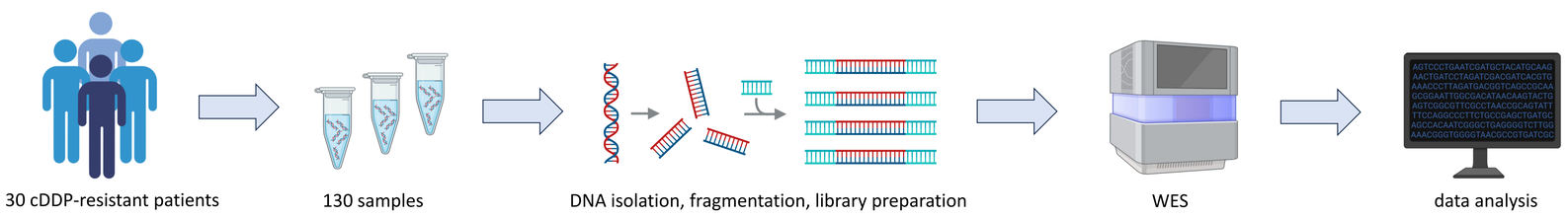
INTRODUCTION

Most patients with testicular germ cell tumors (TGCTs) respond well to **cisplatin (cDDP)-based chemotherapy**. However, ~**10-15%** develop **cDDP resistance**, resulting in treatment failure with **no effective alternative treatments** available. The **molecular mechanisms** underlying cDDP resistance and TGCT pathogenesis remain incompletely understood. To address this issue, we performed **whole-exome sequencing (WES)** of tumor and cell-free DNA (cfDNA) samples from **patients with cDDP-resistant TGCTs** to **identify genetic alterations that may contribute to disease development and therapy resistance.**

MATERIAL AND METHODS

- 30 cDDP-resistant TGCT patients
- 130 samples
  - *primary tumors*
  - *metastases* (in 8/30 pts)
  - *cfDNA from multiple timepoints*
  - *peripheral blood* (background control)
- DNA isolation (Qiagen)
- DNA fragmentation (Covaris S220)
- DNA quality control (Agilent Bioanalyzer)
- Library preparation (Agilent SureSelect XT HS2)
- WES (Illumina NextSeq 500, 2000)
- Data analysis
  - somatic nonsynonymous single nucleotide variants
  - variant read ≥ 3x, quality reads ≥ 5x, variant allele frequency (VAF) ≥ 5%



CONCLUSION

- Somatic variants in **primary tumors** — reflecting **TGCT pathogenesis** — were mainly identified in genes related to **spermatogenesis** or **testis development,** the **Hippo signaling pathway** and **genomic stability maintenance.** Variants detected only in **metastases** — associated with **cDDP resistance** — were enriched in genes involved in **DNA-damage response** and **epigenetic regulation.**
- **Liquid biopsy** detected on average **34%** of variants shared between primary tumors and metastases, supporting its value for **minimal residual disease monitoring.**
- Of the variants **exclusive for metastases,** on average 12% were captured in cfDNA.

RESULTS IA – TGCT PATHOGENESIS

Recurrent somatic variants detected in primary tumors

TPTE2 ***10/30 pts*** ***VAF: 5-84%***

DNAH12 ***8/30 pts*** ***VAF: 5-43%***

CATSPERB ***5/30 pts*** ***VAF: 7-14%***

Spermatogenesis and/or testis development

MST1 ***16/30 pts*** ***VAF: 5-22%***

TEAD4 ***3/30 pts*** ***VAF: 5-9%***

Hippo signaling pathway

RBMX ***10/30 pts*** ***VAF: 7-40%***

Genomic stability

RESULTS IB – cDDP RESISTANCE DEVELOPMENT

Recurrent somatic variants detected only in metastases

MDC1 ***5/8 pts*** ***VAF: 5-71%***

PARP4 ***4/8 pts*** ***VAF: 47-57%***

DNA-damage response

MAGEC1 ***5/8 pts*** ***VAF: 7-74%***

Cancer-testis antigen

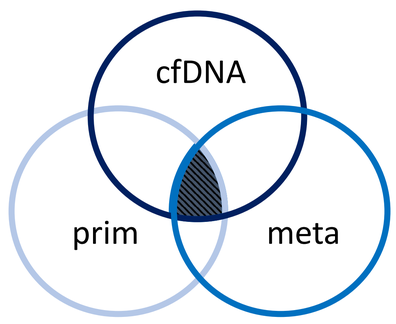
TET1 ***3/8 pts*** ***VAF: 43-54%***

PRDM9 ***3/8 pts*** ***VAF: 6-19%***

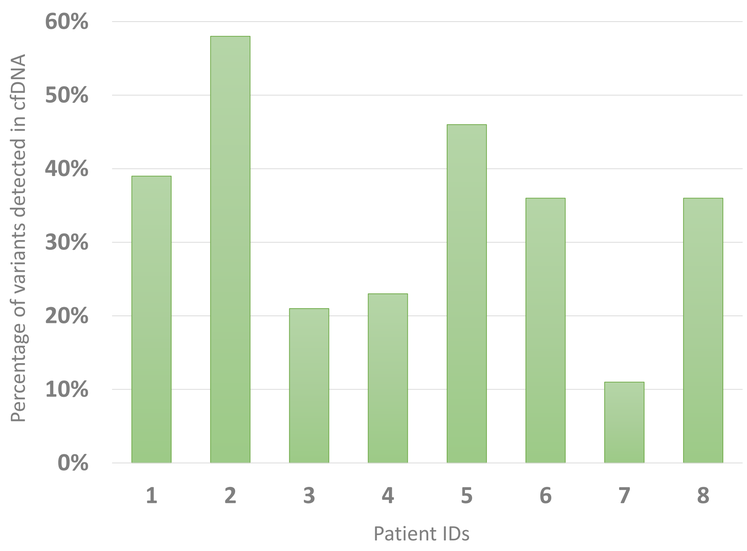
Epigenetic regulation

RESULTS II – DETECTION OF TUMOR SOMATIC VARIANTS IN cfDNA

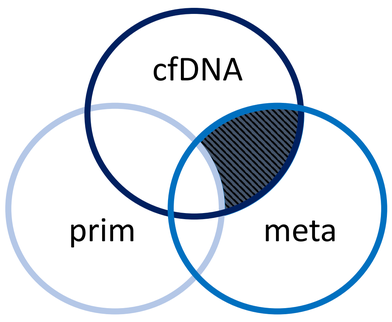
Detection of somatic variants shared between primary tumors and metastases



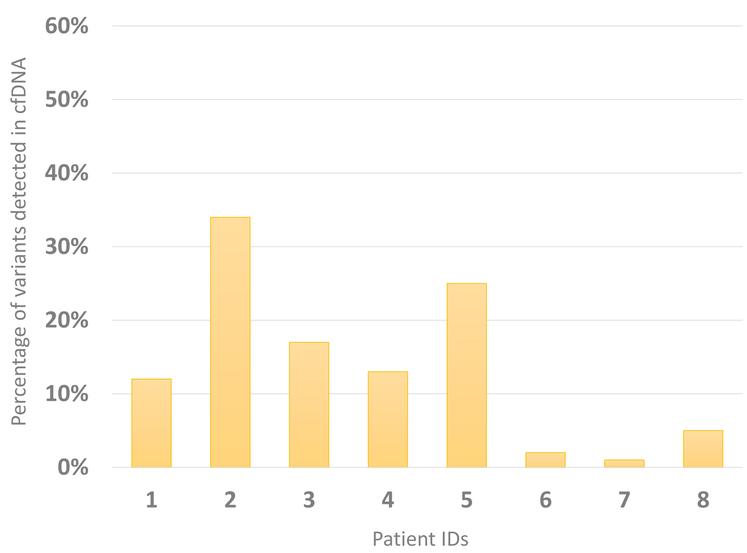
On average, 34% (11-58%) of variants shared between primary tumors and metastases were also detected in cfDNA.



Detection of somatic variants unique to metastases



On average, 12% (1-34%) of variants unique to metastases were also detected in cfDNA.



prim = primary tumor; meta = metastasis

DISCUSSION

- **Primary tumors** harbored variants associated with impaired **germ-cell development** and disrupted **Hippo signaling pathway**, in line with a **developmental origin** of TGCTs.
- In contrast, **metastases** exhibited alterations in **DNA-damage response** pathways and **epigenetic regulation**, indicating that resistant clones may arise through enhanced DNA-damage tolerance mechanisms and modified epigenetic regulation. Key candidate genes will be **functionally validated** in the next phase of the project.
- **Liquid biopsy** showed **value for minimal residual disease monitoring**, identifying an **average of 34%** of shared tumor variants. cfDNA detected **lower proportion** of **metastasis-exclusive variants** with an **average of 12%,** likely reflecting limited sampling in some patients during disease progression.

Acknowledgement

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