

## **Liquid biopsy for monitoring chemotherapy response in Wilms Tumor patients: Somatic tumor mutations in urine DNA before and after treatment.**

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**BACKGROUND:** Detection of circulating tumor DNA in urine has been shown as a viable method of cancer screening not only for urinary tract cancers but also for other tumor types. Applications of such method resemble those based on plasma DNA and include therapy response, like chemotherapy or radiation therapy, and also for monitoring tumor recurrence. As far as we know, there is a lack of studies for detecting tumor DNA in urine of patients diagnosed with Wilms tumor (WT), an embryonal kidney cancer type. Here we bring evidences for detection of two new somatic mutations in the urine of one WT patient.

**HYPOTHESIS:** Urine can be used as a tool for diagnostic and neoadjuvant chemotherapy response by detection of circulating tumor DNA (ctDNA).

**METHODS:** Whole exome sequencing (WES) of both tumor and leukocyte from one WT patient were carried out in Ion Proton platform with the purpose of detecting somatic mutations. Somatic mutations were then validated by Sanger sequencing in tumor and leukocyte. Those mutations were used as tumor markers for screening in urine samples collected during one year and four months of follow up, including samples collected before the WT patient treatment. A total of seven urine samples were collected. The somatic mutations found and validated were verified in DNA extracted from these urine samples by target sequencing in Ion Proton platform.

**RESULTS:** By using WES, two somatic mutations in genes *INTS1* (c.2257G>A) and *TNRC18* (c.3499delG) were found and confirmed by sanger sequencing. Both mutations were used as tumor markers for screening in ctDNA. Target sequencing of both alterations on the DNA from the patient's urine revealed the presence of these two somatic mutations in urine before neoadjuvant chemotherapy and surgery for removal of the kidney tumor. Interestingly, these variants could not be found in the urine sample after treatment. This patient presented metastasis in the lung, and both somatic mutations found in the primary tumor could be detected in the metastasis by WES. Subsequent urine samples showed a small percentage of these two somatic mutations (<1%), but due to the very low frequency of these variants, these results have to be confirmed by using other methodology such as digital PCR.

**CONCLUSION:** Altogether, this study showed new somatic mutations in two genes - *INTS1* and *TNRC18* – not associated with WT before, and also revealed the potential of urine as liquid biopsy for monitoring treatment response in WT patients.