Minimally-invasive classification of pediatric solid tumors using reduced representation bisulfite sequencing of cell-free DNA: a proof-of-principle study

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1 Diagnosis of cancer in children is often difficult

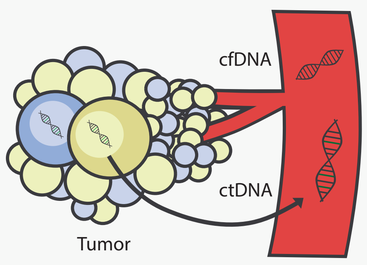
The **diagnostic workup** of pediatric cancer patients with solid tumors requires **a number of investigations** to be performed (different imaging modalities, detection of tumor markers,…).

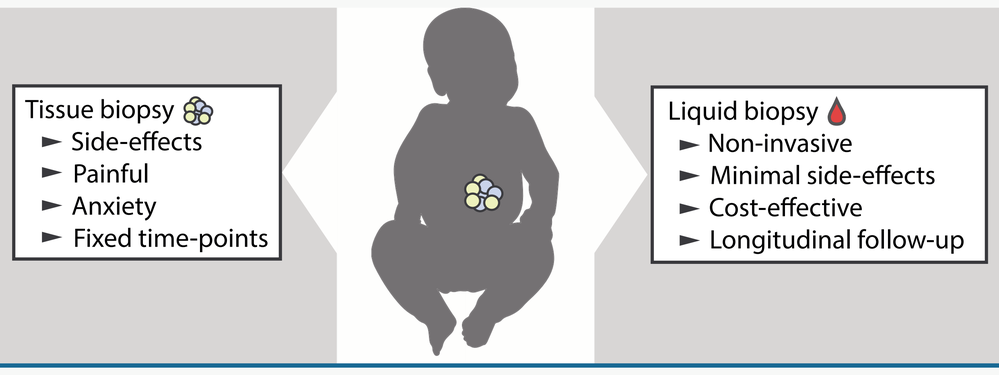
A **true diagnosis** can often only be made after tumor sampling via a surgical **biopsy**. Many of the pediatric tumors are of the "small blue" histology type making it **hard to distinguish** them from each other **based on histology alone**. **Immunohistochemical staining** of the tumors is required, but is an expensive, **time-consuming** and empirical process that sometimes requires multiple staining rounds to be fully conclusive.

For **some types** of pediatric tumors, like renal or brain stem tumors, **sampling of the tumor is not possible**, and treatment needs to be started empirically based on imaging results and clinical characteristics alone, **leading to misdiagnosis** and inadequate treatment.

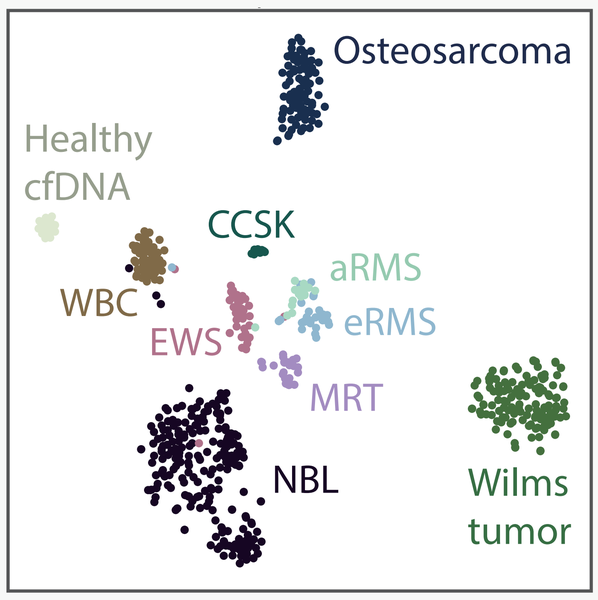
2 Liquid biopsies are a source of tumor DNA

Detection of copy number aberrations (CNAs) is feasible from cfDNA. A retrospective proof-of-principle study on 37 cases including both low- & high-risk neuroblastoma showed that **DNA copy number alterations** can be reliably detected from as little as **5 ng of cfDNA** from plasma, and that overall concordance is very high between arrayCGH on the primary tumor biopsy, and shallow whole genome sequencing on cfDNA (Van Roy, 2017). However, the **CNA profile is not specific enough** for the classification of pediatric solid tumors according to the histopathological diagnosis.





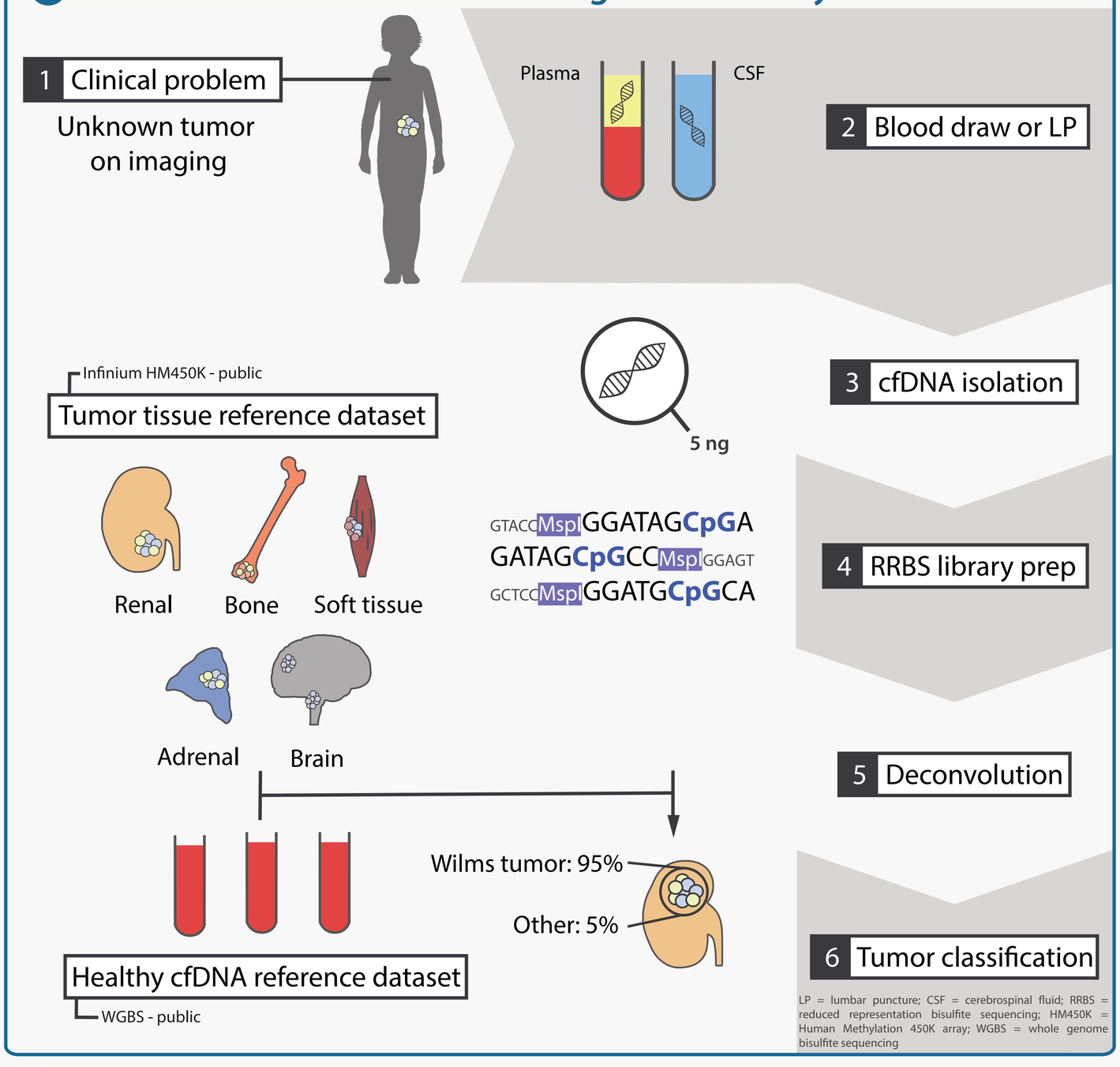
3 DNA methylation is tissue-specific



t-SNE dimensionality reduction plot (n = 634) of publicly available methylation profiles of pediatric primary tumors (Infinium Human Methylation 450K) shows that data of different entities clusters separately.

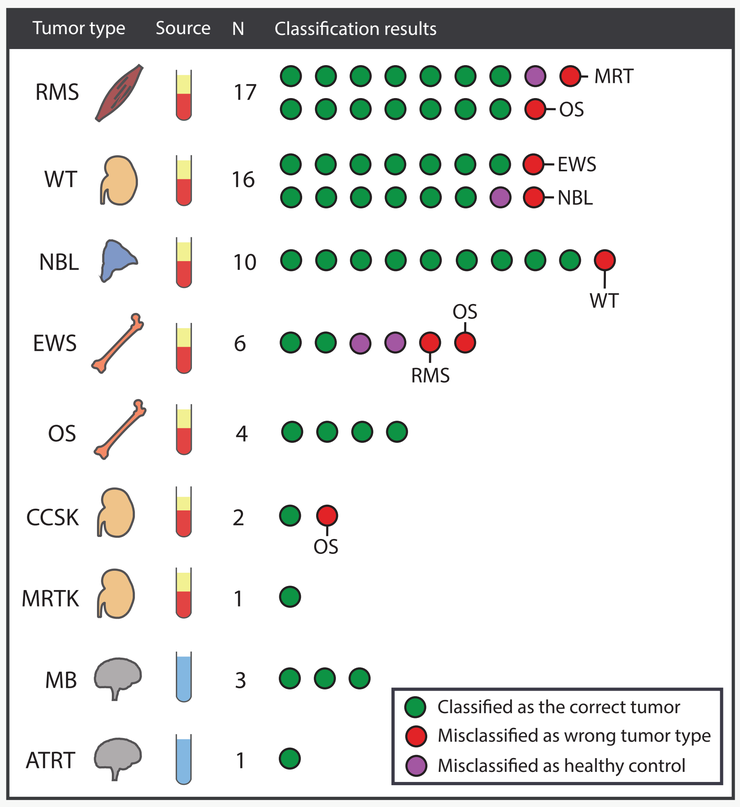
NBL = neuroblastoma; CCSK = clear cell sarcoma of the kidney; (a/e)RMS = alveolar/embryonal rhabdomyosarcoma; EWS = Ewing sarcoma; WBC = white blood cells; RCC = renal cell carcinoma; MRT = malignant rhabdoid tumor.

4 Procedure for classification using cfDNA methylation



LP = lumbar puncture; CSF = cerebrospinal fluid; RRBS = reduced representation bisulfite sequencing; HM450K = Human Methylation 450K array; WGBS = whole genome bisulfite sequencing

5 Pretreatment classification is possible in multiple cancer types



We profiled **60** diagnostic pediatric cancer cases, of which 56 plasma samples and 4 CSF samples and we were able to **classify 49 samples** (= 81.6%) according to their histopathologic diagnosis.

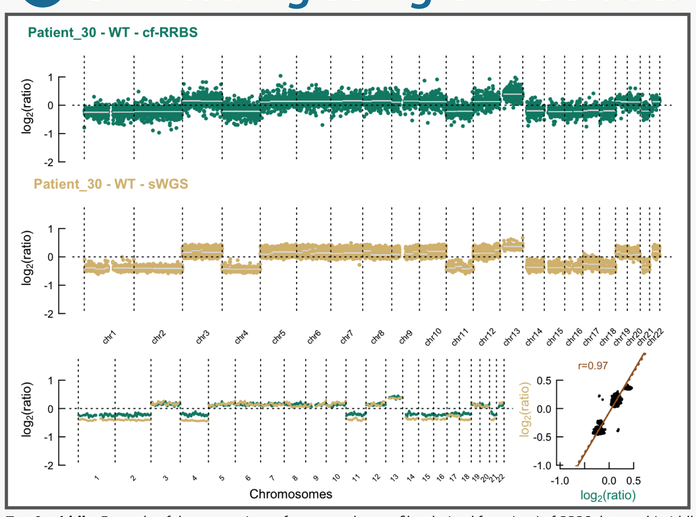
Importantly, with our methods we were able to **distinguish MRTK and CCSK** from Wilms tumor. This could have an important clinical application as these patients are not biopsied before starting chemotherapy.

Preliminary results hint at the possibility for classification of brain tumors from CSF cfDNA, with differentiation of ATRT from medulloblastoma.

In addition, we could correctly classify **9 samples** which had **a flat cfDNA CNV profile**, suggesting that methylation analysis might be more sensitive in case a low tumor fraction in cfDNA is present.

NBL = neuroblastoma; WT = Wilms tumor; RMS = rhabdomyosarcoma: OS = osteosarcoma: MRTK = malignant rhabdoid tumor of the kidney; EWS = Ewing sarcoma: MB = medulloblastoma; ATRT = atypical teratoid-rhabdoid tumor; CCSK = clear cell sarcoma of the kidney.

6 CNA calling using cfRRBS data is feasible



Top & middle: Example of the comparison of copy number profiles derived from (top) cf-RRBS data and (middle) sWGS data with 400 kb binsize of a low-quality sample. Lower left: sliding window of 10 Mb average log2 ratio. **Lower right:** scatterplot between cf-RRBS and sWGS with Pearson r. The dotted line equals least squares fit; the solid line equals the orthogonal regression fit. Cf-RRBS, cell-free reduced representation bisulfite sequencing; sWGS, shallow whole genome sequencing.

For all cfDNA samples, matched shallow whole genome sequencing data (sWGS) was available. Copy number aberrations could be detected with **sWGS in 42 out of 60** samples with WisecondorX.

If CNAs were present in cfRRBS (n = 32), the correlation of the CNA profile with sWGS was high (0.87 [0.82 - 0.94]). However, the reverse was not true; several samples (n = 10) showed CNAs according to sWGS but none using cf-RRBS.

None of the 18 (out of 60) samples that showed no CNA using sWGS had evidence for CNA based on cfRRBS data.

Future plans

- **Prospective** sample **collection** is currently **on-going** and **pre-analytical variables** are being tested in healthy volunteers.
- Inclusion of children with a benign condition mimicking cancer will allow the calculation of the specificity of the assay.
- Development of an RRBS-based, reference dataset from FFPE is currently ongoing.

Availability

cfRRBS method: "A versatile method for circulating cell-free DNA methylome profiling by reduced representation bisulfite sequencing" on biorxiv

cfRRBS proof-of-concept paper: "Minimally-invasive classification of pediatric solid tumors using reduced representation bisulfite sequencing of cell-free DNA: a proof-of-principle study" on biorxiv

Scripts, methods and supporting files on Github

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Illustrations partly modified from smart.servier.com

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