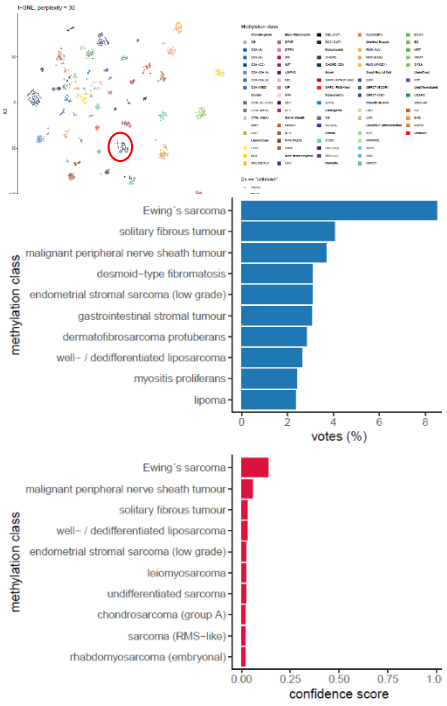
The Samuel Waxman Cancer Research Foundation (SWCRF), Alan B. Slifka Foundation (ABSF) and Israel Cancer Research Fund (ICRF) joint Request for Applications (RFA) collaborative applications focused on fusion-positive pediatric sarcomas.

Title: Combining whole genome sequencing, transcriptomics and methylation patterns for high-resolution classification, choosing treatment and monitoring response of pediatric sarcoma

Andrew L. Kung, Department of Pediatrics, Memorial Sloan Kettering Cancer Center, New York, New York.
Gal Goldstein & Dror Raviv, The Dyna and Fala Weinstock Department of Pediatric Hematology & Oncology, Aviad Zick, Department of Oncology, Sharett institute of Oncology, Hadassah Medical Center, Ein Karem, Jerusalem, Israel
Amir Eden, PhD, Dep. of Genetics, The Hebrew University, Jerusalem, Israel

Background: In up to 15-20% of patients, sarcoma cannot be classified using current pathological methods [1]. Identifying translocations and methylation patterns [2] can identify specific sarcoma sub-types facilitating treatment. Plasma derived cell free DNA (cfDNA) mutations and methylation pattern are widely utilized as a tumor marker. Tumor markers, used to monitor disease progression and response to treatment, are lacking for Sarcomas. Nanopore sequencing (NS) is a rapid, economic, point of care method to identify DNA mutations and methylation pattern in DNA from tissue and plasma.

Key preliminary data: Work by NY group prospectively examined using whole genome and transcriptome sequencing (cWGTS) to capture all clinically reported mutations in a single workflow that reports comprehensive cWGTS results in 9 days from tissue and plasma in 114 pediatric, adolescent, and young adult with solid tumors. This approach identified additional treatment options in 57% of patients [3]. The Jerusalem group developed NS as a rapid, economic, point of care method to identify DNA copy number alterations (CNA) and methylation pattern from tissue and plasma. We adapted the methylation-based sarcoma classifier [2] to NS. Using this approach, we successfully classified 11 of 11 sarcoma samples including two Ewing sarcomas (see Figure). In parallel, NS was applied to cfDNA allowing methylation and CNA based detection and quantification of tumor DNA in blood as well as detection of actionable mutations (*ERBB2* amplification) [4].



We hypothesize that comprehensive molecular profiling (CMP) that combines cWGTS and NS to identify chromosomal aberrations, the mutational landscape, methylation patterns and transcriptional patterns can accurately identify different types of sarcomas, predict treatment response (patient-specific, or sarcoma type associated) and provide blood-based treatment monitoring.

Premise and rigor of underlying research: Our publications [3, 4] along with the figure and other data we obtained, show that the techniques we use are solid and that combining them can lead to rapid diagnosis, informed treatment choice and a tool to monitor treatment. Moreover the rich data obtained by CMP can lead to novel insights of pediatric tumor biology

Specific Aims:

1) Integrate whole genome and transcriptome sequencing with nanopore-based methylation for CMP.

2) Compare CMP profiling and treatment recommendations of 20 pediatric sarcomas (PS) to current pathology and standard care.

3) Compare cfDNA based monitoring of 20 PS patients before, during and after completing treatment to current physical examination and imaging practice.

Novelty: The CMP parts are highly novel and have yet to be used together or compared to current practice.

Potential translational impact to cancer: Our goal is a tissue and blood test that can rapidly diagnose, suggest treatment and monitor outcome. The tests can transform PS care and lead to new insight of PS biology.

[1] Gounder, Mrinal M et al. “Clinical genomic profiling in the management of patients with soft tissue and bone sarcoma.” *Nature communications* vol. 13,1 3406. 15 Jun. 2022

[2] Koelsche, Christian et al. “Sarcoma classification by DNA methylation profiling.” *Nature communications* vol. 12,1 498. 21 Jan. 2021

[3] Shukla, N et al. “Feasibility of whole genome and transcriptome profiling in pediatric and young adult cancers.” *Nature communications* vol. 13,1 2485. 18 May. 2022

[4] Katsman, Efrat et al. “Detecting cell-of-origin and cancer-specific methylation features of cell-free DNA from Nanopore sequencing.” *Genome biology* vol. 23,1 158. 15 Jul. 2022