These research projects were undertaken in partial fulfillment of the requirements for the MD degree at Sackler Faculty of Medicine, Tel Aviv University in 2020–2021. They were considered the most outstanding of the graduating class.

High-throughput analysis of CLIC5 interactants using a thermal-stability assay

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Background: The chloride intracellular channel (CLIC) protein family consists of six members in humans. CLICs are unique due to their metamorphic property, displaying both soluble and integral membrane forms. The transmembrane conformation was shown to give rise to ion-channel activity in vitro. In recent years, CLICs were implicated in a growing number of physiological processes in various organ systems and associated with distinct disease states. Indeed, the founding member of the family, CLIC5, was shown to be involved in hereditary deafness and various types of cancer. Nevertheless, the natural interactants and endogenous ligands of CLIC5 have not been discovered yet.
Objectives: To find ligands that affect the biochemical properties and activity of CLIC5. We hypothesized that such ligands could serve as important tools for resolving the long-sought cellular roles of CLICs and may offer novel therapeutic avenues for CLIC-associated conditions.
Methods: Using molecular biology and biochemical methods, CLIC5 was overexpressed in *Escherichia coli* and purified. Next, a high-throughput differential scanning fluorimetry thermal shift assay (TSA) was established and the interaction of approximately 500 natural compounds was examined.
Results: The TSA-based screening approach developed here allows to evaluate the effect of approximately 100 compounds in parallel within approximately 1 hour. Our proof-of-concept screening yielded 11 potential hits, significantly affecting the thermal stability of CLIC5. By examining the dose-dependence of this effect, we identified a specific interaction of CLIC5 with curcumin.
Conclusions: Using the approach we developed, large libraries of small molecules can be screened efficiently to identify novel CLIC5 interactants. Considering the participation of CLIC5 in various physiological and pathological processes, uncovering ligands that inhibit or activate CLIC5 may provide tools to modulate its activity and possibly to ameliorate CLIC5-related pathologies in the future.

Ultra-fine particles in induced sputum as a biomarker for lung inflammatory and functional state: a cross sectional study

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Background: The exposure to ambient particulate matter (PM) is associated with increased morbidity and mortality from respiratory, cardiovascular, and other causes. A major contribution to this adverse effect is attributed to particles at the nanoscale range (ultrafine particles [UFP] particles < 100 nm). Most of the information about human exposure to PM has been collected by environmental monitoring of inhaled particles.
Objectives: To evaluate the use of direct measuring of UFP in the sputum as a biomarker for lung inflammation and functional impairment.
Methods: The study population included 121 patients who underwent an induced sputum (IS) test as a part of a clinical evaluation for respiratory symptoms. Cell differential count was performed, and the UFP content was measured in each IS sample. The UFP content in the sputum was compared among patients with different inflammatory phenotypes based on IS granulocytes levels: eosinophilic inflammation (EI) IS eosinophils > 2.7%, neutrophilic inflammation (NI) IS neutrophils > 65%, and mixed granulocytic inflammation (MGI) including both IS eosinophils > 2.7% and IS neutrophils > 65%. The association between the IS-UFP content and pulmonary function test (PFT) parameters was also tested.
Results: Patients with MGI had a distinct profile of particles in IS, which was characterized by the highest percentage of UFP (relative to larger particles) compared to patients with EI, NI, or normal IS cell count. Furthermore, EI and NI were found to have an interaction effect regarding the IS–UFP profile, as demonstrated by the significantly different IS–UFP profile of patients with MGI compared to the profile associated with EI and NI independently. Last, the profile of UFP in the IS samples was also correlated with patient PFT. Reduced forced mid-expiratory flow (FEF) 25–75 or FEV1 were correlated with a higher IS–UFP

mean size. Reduced FEF25–75 was correlated with a lower IS–UFP concentration and percentage relative to larger particles.

Conclusions: To the best of our knowledge, this study is the first to report a distinct IS–UFP profile in patients with MGI, which suggests an interaction effect of EI and NI on the IS–UFP content. This finding may further support the consideration of MGI as a distinct inflammatory phenotype, beyond the simple combination of EI and NI independently. In addition, reduced PFT parameters were associated with a specific change in the IS–UFP profile. The results of this study shed light on the use of IS–UFP content as a biomarker for lungs inflammation and functional impairment. Further prospective studies are needed to establish a cause and effect relationship between lung inflammation and functional impairment and the IS–UFP content.

In-thrombus thrombin secretion: a new diagnostic marker of atrial fibrillation in cryptogenic stroke

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Endovascularly retrieved clots may be a potential resource for diagnosing stroke etiology. This method may influence secondary prevention treatment. We measure thrombin activity eluted by serially washing clots. We concluded that an assay measuring the change in thrombin in clots retrieved during acute stroke endovascular thrombectomy procedures may serve as a diagnostic marker of the origin of the clot. The suggested mechanism for these differences may be the clot location before its retrieval, with high blood flow causing thrombin washout in atherosclerotic clots, in contrast to atrium appendage low blood flow retaining high thrombin levels.

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