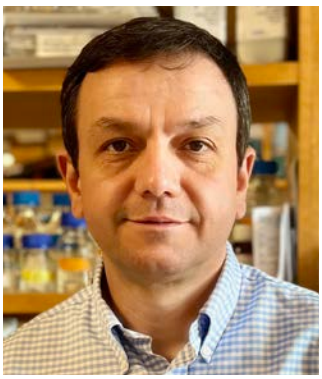
Ioannis Sanidas, PhD



Sanidas Laboratory

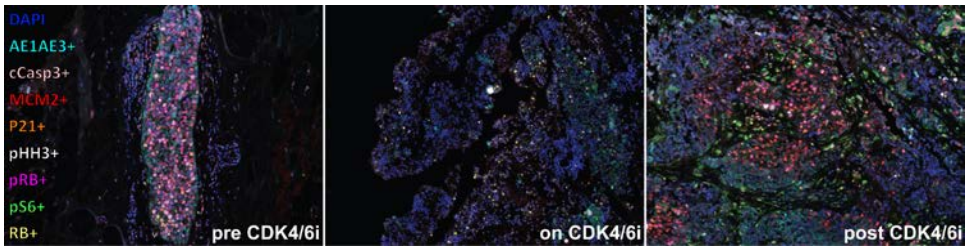
Ioanna Gkotinakou, PhD
Izabela Panova
Ioannis Sanidas, PhD
Alice Zheng

Cell cycle deregulation is a hallmark of cancer. **The Sanidas laboratory** examines the cell cycle in normal and cancer cells to discover vulnerabilities that can lead to novel therapeutic approaches. Our research primarily centers on the retinoblastoma tumor suppressor protein (RB), a key regulator of the cell cycle. RB is highly expressed in normal and cancer cells and prevents cells from dividing. Cyclin-dependent kinases (CDKs) phosphorylate and inactivate RB to enable cell proliferation. However, this description explains only a part of RB's activity; many additional functions have been attributed to RB, which are context-specific and mostly uncoupled from cell cycle regulation. This is part of the reason that although RB is genetically or functionally inactivated in most human cancers, its tumor suppressor activity is highly tissue type-specific. Understanding the molecular complexity of RB will allow us to identify the context-specific implications of its inactivation in human malignancies and optimize the advantages of the recently developed CDK inhibitors, which target the pharmacological activation of RB.

Over the last decade, a substantial amount of research has been devoted to molecular therapeutics targeting RB's activation, leading to the development of highly selective CDK inhibitors. These efforts have resulted in advanced cancer therapy methods, significantly prolonging the survival rate in Breast Cancer (BrCa) patients. Despite the widespread deregulation of the RB pathway in cancer cells, the effectiveness of these drugs remains limited to specific tumor types. At the Sanidas laboratory, we aim to address this conundrum through two lines of investigation: 1) Understanding the molecular complexity of RB and deciphering the context-specific implications of RB inactivation in cancer cells. 2) Investigating how CDK inhibitors work in various tumor types, with the goal of enhancing drug efficacy and determining the group of patients that will primarily benefit from this treatment.

Investigation of RB's mechanism of action

RB has often been described as a highly conserved cell cycle regulator with a universal mechanism of action. According to this conventional model, RB targets the E2F promoters to suppress the expression of cell cycle genes. This interaction is dependent on the cell cycle and inhibited by CDKs. However, this description explains only a part of RB's activity. RB is essential for the control of multiple transcriptional programs, the maintenance of chromosome stability, the commitment to cell lineage, and the emergence of drug resistance in cancer cells. These RB functions are context specific and largely independent of RB/E2F regulation. It is acknowledged that additional investigations are required to decipher the mechanisms governing this "non-canonical" RB activity. A significant obstacle hindering progress in this area has been that the RB research community has never really figured out how to deal with the molecular complexity of RB. Many studies have focused on the consequences of RB loss without being able to capture the details of RB in action. In the Sanidas laboratory, we have successfully developed sophisticated molecular tools to unravel the complexity of



The expression of the cell cycle marker phosphorylated RB (in pink) and the DNA replication marker MCM2 (in red) showed significant inhibition of active cell proliferation during treatment with the CDK4/6 inhibitor. However, upon developing resistance to CDK4/6 inhibition therapy, both markers were observed to be re-expressed. Multiplex imaging on human ER-positive Breast Cancer tumor biopsies pre-, on-, and post-treatment with CDK4/6 inhibitor. Tumor sections were stained for cytokeratins AE1/AE3 (epithelial cells marker), cleaved Caspase-3 (cCasp3), DAPI (DNA), MCM2, p21, phospho-Histone 3 Ser10 (pHH3), phospho-RB Ser807/Ser811 (pRB), phospho-S6 (pS6), total RB (RB), and DAPI, each represented by distinct colors.

RB's action. Precisely, we can now dissect RB into its distinct functional forms (Sanidas et al., 2019), separate the different pools of the chromatin-associated RB (Sanidas et al., 2022), and identify, using Micro-C analysis, the RB-mediated regulation of chromatin organization. These groundbreaking tools can finally provide the information needed to study RB. We aim to i) define the cell type-specific functions of RB, ii) elucidate why RB's tumor suppressor activity varies among different tumor types, and iii) determine the factors contributing to the tumor type-specific efficacy of drugs targeting RB activation. With the aid of these innovative tools, we can look into RB's mechanism of action with a significantly improved resolution, shedding light on previously uncharted aspects of RB's activity in cancer biology.

Targeting the cell cycle machinery in cancer therapy

The activation of RB's tumor suppressor activity represents a pivotal approach in molecular cancer therapeutics. Current strategies for recurrent, adjuvant, and de novo metastatic therapy in Estrogen Receptor-positive BrCa involve CDK4/6 inhibitors combined with hormonal therapy. Phase I clinical trials are underway for CDK2-specific inhibitors, targeting Cyclin E-amplified tumors, as well as tumors that progressed after CDK4/6 inhibition therapy. The Sanidas laboratory collaborates with the Termeer Center for Investigational Cancer Therapeutics at Mass General to study the mechanism of action of novel investigational drugs that target the cell cycle machinery. The efficacy of these drugs relies on the tumor type, genetic background, and treatment history. Our goals are to: i) optimize the cell cycle drugs' efficacy by defining their synergistic activity with other agents, and ii) identify biomarkers that predict response to these drugs.

Selected Publications:

Krishnan B, **Sanidas I***, Dyson NJ*. Seeing is believing: the impact of RB on nuclear organization. *Cell Cycle*, 2023 May 3;1-10.

Sanidas I, Lee H, Rumde PH, Boulay G, Morris R, Golczer G, Stanzione M, Hajizadeh S, Zhong J, Ryan MB, Corcoran RB, Drapkin BJ, Rivera MN, Dyson NJ, Lawrence MS. Chromatin-bound RB targets promoters, enhancers, and CTCF-bound loci and is redistributed by cell-cycle progression. *Mol Cell.* 2022 Aug 12:S1097-2765(22)00710-9.

Witkiewicz AK, Kumarasamy V, **Sanidas I**, Knudsen ES. Cancer cell cycle dystopia: heterogeneity, plasticity, and therapy. *Trends Cancer*. 2022 May 19:S2405-8033(22)00093-0.

Krishnan B, Yasuhara T, Rumde P, Stanzione M, Lu C, Lee H, Lawrence MS, Zou L, Nieman LT, **Sanidas I***, Dyson NJ*. Active RB causes visible changes in nuclear organization. *J Cell Biol*. 2022 Mar 7;221(3):e202102144.

Sanidas I, Morris R, Fella KA, Boukhali M, Tai EC, Ting DT, Lawrence MS, Hass W and Dyson NJ. A code of mono-phosphorylation modulates the function of RB. *Mol Cell* 2019, Mar 7;73(5):985-1000.

* Denotes equal contribution