Evaluation of New Molecules and Nano-Delivery Systems in 2D and 3D Tumor Cell Models for Cancer Therapy

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INTRODUCTION

Cancer remains one of the leading causes of mortality worldwide, representing a major global health challenge. According to the International Agency for Research on Cancer (IARC), in 2024, an estimated 20 million new cancer cases and 9.7 million cancer-related deaths were reported globally, with breast, lung, and colorectal cancers among the most prevalent [1]. Traditional cancer treatments, including surgical resection, chemotherapy, and radiotherapy, are associated with non-specific targeting, drug resistance, and significant systemic toxicity. In recent years, targeted therapy and immunotherapy have emerged as innovative strategies by specifically modulating molecular pathways and immune responses involved in tumor development and non-response to therapy. These therapeutic strategies still have several limitations that prevent them from being fully effective and lead to poor prognoses, especially for therapy-resistant and more lethal tumors. Among solid tumors, Pancreatic Ductal Adenocarcinoma (PDAC) is one of oncologic disease with the worst prognosis, with a 5-year survival rate of less than 12% [2] . It is the twelfth most common cancer and the sixth leading cause of death worldwide. Despite the low incidence, PDAC is a huge challenge for oncologists due to the late diagnosis, limited sensitive to currently used therapies, and acquired drug resistance that increase the lethality of this disease [3]. The molecular mechanisms, sustaining the aggressiveness of PDAC, are multifaceted and involved in inhibiting drug transport, altering target-associated signaling, promoting immune evasion, and suppressing programmed cell death pathways. Consequently, the project investigates the urgent need for innovative, more effective and low-toxicity therapeutic approaches that function independently of intrinsic and/or acquired resistance (MDR) mechanisms in PDAC. Such advancements are essential to improving patient's outcome, enhancing quality of life, and mitigating the side effects associated with conventional treatments.

A hallmark feature of PDAC is its highly complex and dynamic tumor microenvironment (TME), which plays a central role in tumor progression, metastasis, and therapeutic resistance. The TME is composed of a heterogeneous network of malignant cells, stromal components, immune cells, and extracellular matrix (ECM) elements that interact to create a protective niche for tumor survival. In particular, cancer-associated fibroblasts (CAFs), tumor-associated macrophages (TAMs), and other immunosuppressive cell populations actively contribute to the establishment of a pro-tumorigenic environment. These cells promote immune evasion, sustain inflammation, and modulate signaling pathways that enhance tumor growth and resistance to therapy.

In PDAC, the TME is further characterized by pronounced desmoplasia, a dense, fibrotic accumulation of stromal connective tissue. The PDAC desmoplastic stroma is a complex of heterogeneous cellular and non-cellular members, such as extracellular matrix (EMC) proteins, like collagen and fibronectin, pancreatic myofibroblastic stellate cells (PaSCs), lymphatic and vascular endothelial cells, immune cells that metabolize the matrix. This creates a physical and biological barrier, hindering drug penetration into the fibrous tissue and protecting tumor cells from the immune surveillance [4].

To overcome this barrier, the research project proposes the implementation of combination therapies with novel synthetic compounds. Integrating new precursors of the HIV-1 protease inhibitor Darunavir with conventional chemotherapeutics aims to sensitize tumor cells to cytotoxic agents, enhance effective drug concentrations, and achieve synergistic potentiation, targeting also the tumor microenvironment. By hitting multiple signaling pathways simultaneously, this approach seeks to mitigate the emergence of multi-drug resistance (MDR) and/or increase the sensitivity of cells to standard therapies, allowing *metronomic* or low-dose chemotherapy [5]. Furthermore, replacing a portion of traditional chemotherapy doses with targeted formulations may reduce the systemic burden on the patient, thereby minimizing adverse side effects while improving clinical outcomes.

In this context, nano-delivery systems, such as liposomes and niosomes, represent a promising strategy for enhancing therapeutic efficacy. Despite their potential, many compounds exhibit poor bioavailability, poor absorption, rapid metabolism, and limited biodistribution, significantly reducing their therapeutic efficacy in vivo. Nanocarriers can significantly improve the bioavailability, stability, and cellular uptake of these molecules, offering promising strategies for cancer therapy. Several nano systems, such as polymeric nanoparticles (NPs), solid lipid nanoparticles (SLNs), nanostructured lipid carriers (NLCs), liposomes, niosomes, and nanoemulsions (NEs), have demonstrated the potential to optimize drug delivery [6]. For example, PEG/folic acid-modified liposomes loaded with cisplatin and gemcitabine have shown an increased capacity to induce apoptosis in tumor cells while maintaining low toxicity in healthy cells [7]. Ultimately, these systems enable the co-delivery of synthetic molecules and chemotherapeutic agents, potentially increasing drug efficacy and minimizing adverse effects.

METHODS

During the first year, the research project will employ the following methodologies:

- **Cell Culture Maintenance:** growth and maintenance of pancreatic cancer and healthy cell lines under standard culture conditions;
- **Cytotoxicity:** assess standard chemioterapic (gemcitabine) and Darunavir precursors, in monotherapy and/or in combination, in pancreatic cancer lines and healthy cell lines using the MTS assay;
- **Combination Studies:** perform drug combination studies and synergy analysis using, for example, Compusyn software.
- **Real-Time Cell Proliferation Monitoring:** evaluate treatment response with xCELLigence RTCA and perform migration/invasion assays;
- **Cell cycle Analysis and apoptosis by flow cytometry (FACS).**

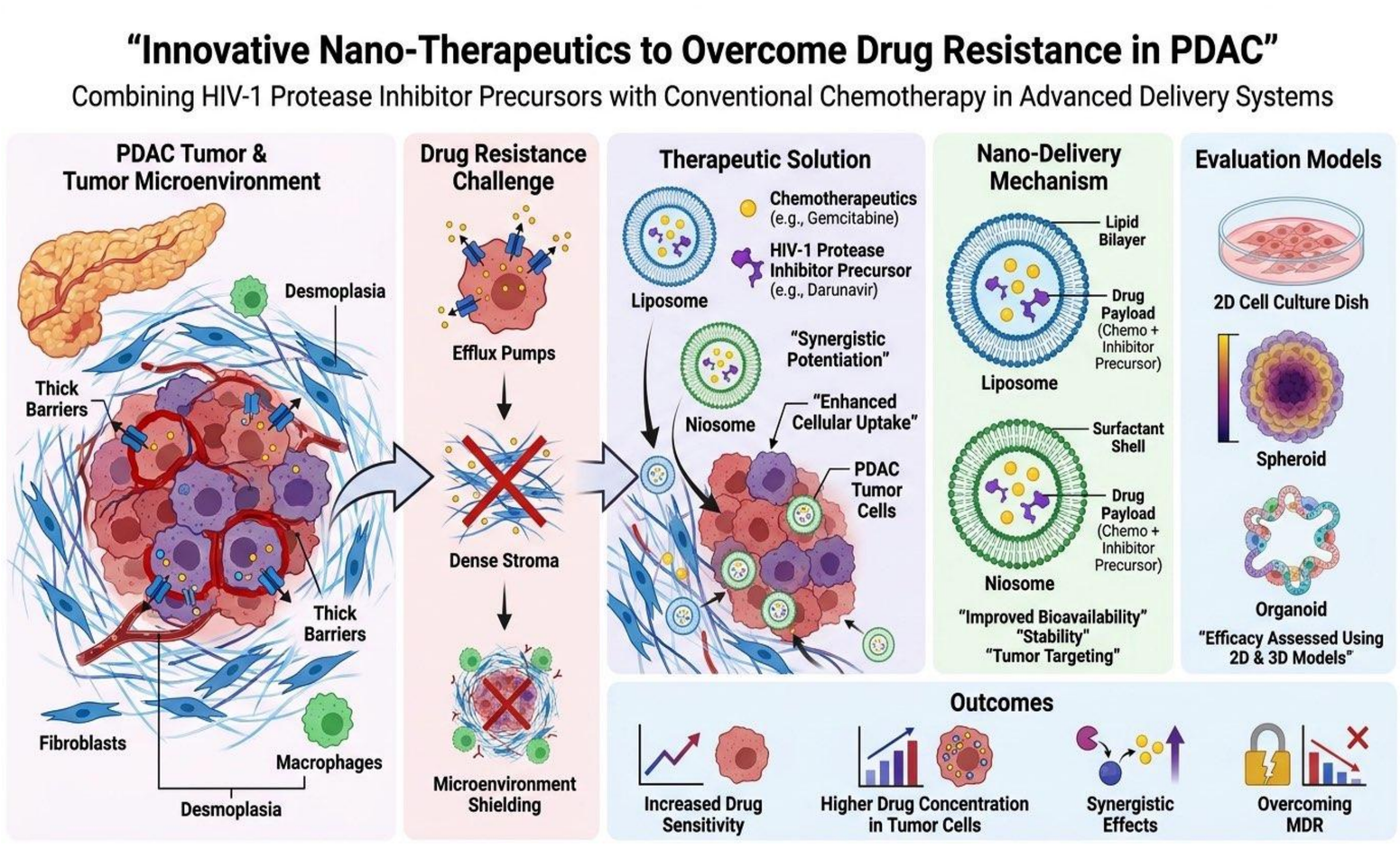


Figure 1. Research Workflow.

EXPECTED RESULTS

In terms of expected outcomes, we look forward that the tested compounds will exhibit selective cytotoxicity toward pancreatic cancer cell lines, with lower IC_{50} values compared to healthy cells.

Real-time monitoring is expected to reveal significant inhibition of tumor cell proliferation and alterations in cell morphology following treatment, especially when compounds are used in combination or delivered via nanoformulations.

Functional assays should show reduced migratory and invasive capabilities of treated cancer cells, suggesting an impact on their metastatic potential.

Flow cytometric analysis are expected to indicate cell cycle arrest and increased apoptosis in tumor cells, findings that should be supported by corresponding alterations in the expression of key proteins involved in cell survival, proliferation, and intracellular signaling pathways.

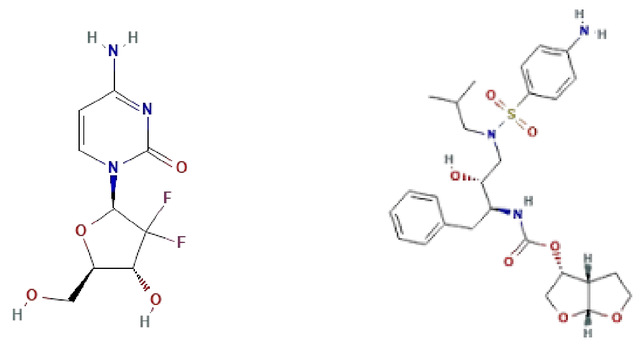
In 3D spheroid and organoid models, we expect to observe decreased growth, viability, and structural integrity upon treatment, confirming the efficacy of the agents in more physiologically relevant systems. Nano-delivery systems are anticipated to enhance cellular uptake of bioactive molecules, resulting in improved therapeutic outcomes compared to free drugs.

Overall, these results will provide strong evidence for the antitumor potential of selected molecules and nanocarrier strategies as innovative and anticancer therapies.

CONCLUSIONS

The research project seeks to assess the antitumor activity of several molecules, precursors of HIV-1 protease inhibitors, in pancreatic cancer cell lines, both as monotherapy and in combination, and to validate their efficacy in 3D models (spheroids and organoids), with the aim of overcoming drug resistance in PDAC and developing more effective, translational therapeutic strategies (**Figure1, Figure2**).

A.



B.

Phenotype and Genotype of Pancreatic Cancer Cell Lines

CELL LINE	KRAS	TP53	CDKN2A/p16	SMAD4	Differentiation	Morphology (2D)	3D Spheroid Structure
BxPC-3	WT	Mutated (220 Cys)	WT	Mutated (HD)	Well/Moderate	Epithelial, Cobblestone	Tight, Compact, Smooth
Capan-1	Mutated (12 Val)	Mutated (159 Val)	Mutated (HD)	Mutated (577 Leu)	Well	Small Epithelial	Compact, Glandular-like
MIA Paca-2	Mutated (12 Cys)	Mutated (248 Trp)	Mutated (HD)	WT	Poor/Undifferentiated	Spindle/Fibroblast-like	Loose, Irregular/Aggregate
PANC-1	Mutated (12 Asp)	Mutated (273 His)	Mutated (HD)	WT	Poor	Spindle/Epithelial (Mixed)	Large, Irregular, "Grape-like"

HD—homozygous deletion

Figure 2. A. Structure of Gemcitabine and Darunavir, one of HIV protease inhibitors **B.** Phenotype and genotype of Pancreatic cancer cells.

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