Tackling Pancreatic Ductal Adenocarcinoma with an Innovative Covalent Targeting of Glycolysis

Giulia Antonini[1], Alessio Mainetti[1], Alessandra Fiore[2], Antonio Scarano[3], Lea Sicuro[4], Serena Faggiano[3], Adriana Celesia[2], Chiara Tesoriero[5], Raffaella Pacchiana[2], Andrea Vettori[5], Luca Mollica[4], Lucia Tamborini[1], Massimo Donadelli[2], Paola Conti[1], Stefano Bruno[3], Chiara Borsari[1,*]

[1]Department of Pharmaceutical Sciences, University of Milan, Via Mangiagalli 25, 20133 Milan, Italy
[2]Department of Neurosciences, Biomedicine and Movement Sciences, Section of Biochemistry, University of Verona, 37134 Verona, Italy
[3]Department of Food and Drug, University of Parma, Parco Area delle Scienze 23/a, 43124, Parma, Italy
[4]Department of Medical Biotechnologies and Translational Medicine c/o L.I.T.A / University of Milan, Via F.lli Cervi 93, 20090 Segrate (MI), Italy
[5]Department of Biotechnology, University of Verona, 37134 Verona, Italy

Pancreatic ductal adenocarcinoma (PDAC) is a highly aggressive cancer with poor prognosis. Metabolic reprogramming drives its malignancy, making cancer metabolism an attractive therapeutic target. However, anti-glycolytic therapies face clinical limitations due to toxicity. Inhibition of 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase (PFKFB), particularly the PFKFB3 isoform, may offer a safer therapeutic window.[1] Herein, we report the first covalent PFKFB3 inhibitor featuring moderately reactive, drug-like warheads targeting a previously unexplored cysteine within the active site. Biochemical assays confirmed irreversible enzyme inhibition and covalent binding. Our lead compound selectively reduced viability across multiple PDAC cell lines and, in vivo, effectively suppressed tumor growth in zebrafish xenograft models, highlighting its potent and specific antitumor activity. Moreover, combination of compound **6** with standard chemotherapeutics (Gemcitabine, FOLFIRINOX) enhanced efficacy, revealing synergistic effects. This work introduces a novel covalent PFKFB3 inhibitor and supports anti-glycolytic therapy as a promising strategy for PDAC treatment.

[1] Boyd S, Brookfield JL, Critchlow SE, Cumming IA, Curtis NJ, Debreczeni J, Degorce SL, Donald C, Evans NJ, Groombridge S, Hopcroft P, Jones NP, Kettle JG, Lamont S, Lewis HJ, MacFaull P, McLoughlin SB, Rigoreau LJ, Smith JM, St-Gallay S, Stock JK, Turnbull AP, Wheatley ER, Winter J, Wingfield J. Structure-Based Design of Potent and Selective Inhibitors of the Metabolic Kinase PFKFB3. J Med Chem. 2015; 58 (8): 3611-25.

Acknowledgements: This work was supported by the Guido Berlucchi Foundation Mini Grant CANCERCOPE, number G43C23002440001, the Umberto Veronesi Foundation, My first SEED Grant DM 737/2021 from MUR, Proof of Concept funding from the Seed4Innovation acceleration program and Fondo Italiano per la Scienza FIS2 (grant number, FIS-2023-02269) from Italian Ministry of University and Research (to C.B.). In addition, this study was supported by funds from University of Milan - Piano di sostegno alla Ricerca 2025 - LINEA 2.