CD44 glycoproteogenomics towards bladder cancer precision medicine and glycovaccines design

José Alexandre FERREIRA [1], Cristiana GAITEIRO [1], Janine SOARES [1], M RELVAS-SANTOS [1,2], A PEIXOTO [1], D FERREIRA [1], P PAULO [1], A BRANDÃO [1], H OSORIO [3], AMN SILVA [2], LL SANTOS [1]

[1] Research Center of IPO-Porto / RISE@CI-IPOP (Health Research Network), IPO-Porto / Porto Comprehensive Cancer Center, Porto, Portugal, [2]REQUIMTE-LAQV, Department of Chemistry and Biochemistry, Faculty of Sciences of the University of Porto, Portugal, [3] Institute for Research and Innovation in Health (i3S), University of Porto, Portugal.

jose.a.ferreira@ipoporto.min-saude.pt

Bladder cancer (BC) demands novel molecular targets for precision medicine. CD44 is a transmembrane glycoprotein linked to most cancer hallmarks. However, significant alternative splicing and multiple glycosylation generate a myriad of glycoproteoforms with potentially distinct functional roles, which remain mostly uncharacterized due to the lack of appropriate analytical tools. Transcriptome analysis of a large patient cohort showed remarkable CD44 isoforms heterogeneity and association between short CD44 standard splicing isoform (CD44s), invasion and poor prognosis. CD44 was also found carrying abnormal short-chain *O*-glycosylation not observed in healthy tissues. Glycoproteogenomics allowed, for the first time, the identification of clinically relevant glycoproteoforms by mass spectrometry. The link between abnormal CD44s glycosylation and invasion was confirmed in vitro, supporting findings from BC tissues. Building on glycoproteogenomics. we also enzymatically synthesized cancer specific CD44s-Tn glycopeptides that were covalently linked to immunogenic proteins generating an anti-cancer glycovaccine. Our glycovaccine was well tolerated *in vivo*, inducing both humoral and cellular immunity, including immunological memory. Generated antibodies exhibited specific reactivity against synthetic CD44s-Tn glycopeptides, CD44s-Tn glycoengineered cells and tumours. In summary, CD44s emerged as a biomarker of poor prognosis and CD44 carrying truncated *O*-glycans as promising molecular signatures for targeted interventions. A glycovaccine was prototyped for pre-clinical validation.

Acknowledgements: Portuguese Foundation for Science and Technology for assistant researcher position CEECIND/03186/2017 and projects REVERENT (2022.03621.PTDC) and RESOLVE (PTDC/MED-OUT/2512/2021)