



**Project Acronym:** **GlycoModels**

**Project Number:** 748880

**Project title:** 3D glyco-engineered models to address the role of glycosylation in gastric cancer clinical management

Marie Skłodowska-Curie Individual Fellowship awarded to **Meritxell Balmaña**

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Glycobiology in Cancer Group

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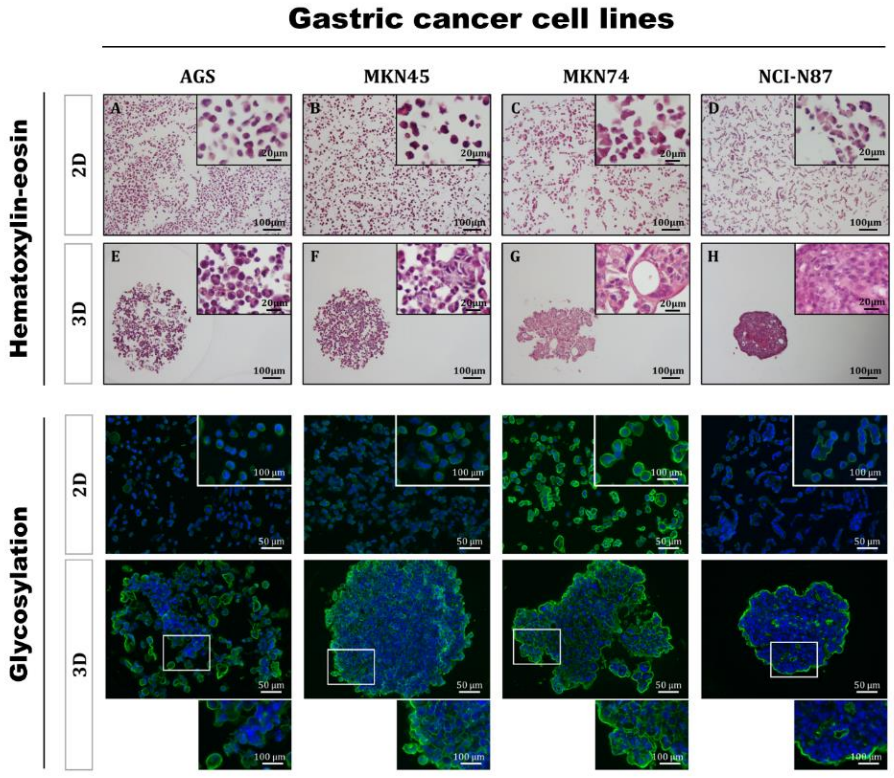
Period covered by the report: from 01/05/2017 to 30/04/2019

## *Summary of the context and overall objectives of the project*

Gastric cancer is an important health problem, being the fifth most common cancer and the third leading cause of cancer death. Its late diagnosis accompanied with a bad prognosis keeps this malignancy among the most deadly. After generations of cancer therapy based on chemical drugs causing severe side effects and limited efficiency, cancer treatment undergoes a paradigm shift towards targeted therapy, employing both monoclonal antibodies and small molecule inhibitors of receptor tyrosine kinase activity, as the most promising tool for the years to come. However, there are numerous patients that do not respond to these novel treatments due to mutations in downstream signalling pathways or to undefined molecular mechanisms. Protein glycosylation has been shown to modulate cellular receptor functions and alter antibody binding affinities and therefore foreshadows to play a major role in cancer therapy resistance.

The GlycoModels project aims to identify glycan alterations on cell receptors that lead to targeted therapy resistance of gastric tumours. Using cutting-edge gene editing techniques, gastric cancer cell lines will be glyco-engineered to express hallmark glycan epitopes and applied in 3D-culture systems as advanced models mimicking the tumour microenvironment. These 3D-GlycoModels will display differential response to treatment with the most promising targeted therapy agents for key cell receptors (HER2, VEGFR, MET and RON) involved in growth, progression, and spread of cancer. In-depth analysis of the GlycoModels, covering glycan characterisation of the cell receptors, disclosing activated signalling pathways, and transcriptomics will unveil our understanding on driving mechanisms in therapy resistance and the glycosylation patterns involved. The structural validation of these glycoforms in human samples will deliver new biomarkers to be used in personalised therapy selection and thus, improve the outcome of advanced gastric cancer patients.



## *Work performed from the beginning of the project to the end of the period covered by the report and main results achieved (including an overview of the results and their exploitation and dissemination)*

The GlycoModels project awarded to Dr. Balmaña aimed at unravelling the glycan modifications of gastric tumour cells using a 3D context to identify the glycan alterations on key cell receptors that lead to targeted therapy resistance in cancer patients.

Specifically, the researcher was involved in the development of gastric and colorectal cancer cell lines overexpressing/silencing of different genes (e.g. ST6Gal1). The researcher also contributed in the implementation of the CRISPR/Cas9 technology in the host group. Dr. Balmaña successfully implemented in the laboratory novel 3D cell culture systems that are expected to have a great impact on the field, since until recently, research regarding aberrant glycosylation in cancer has been performed using monolayer cultures only. Different 3D spheroid methodologies (ULA plates and microtissues) were implemented and optimised for different gastric cancer cell lines. Also, and automated imaging analysis system was developed to standardized the analysis of the 3D spheroids. Dr. Balmaña characterised using lectins and glycan-targeting antibodies the glycophenotype of the 3D GlycoModels by flow-cytometry and immunofluorescence, including the expression of mucins. The features displayed by the 3D GlycoModels allowed to address the response to targeted therapy. The increased in sialylation was associated with resistance to tyrosine kinase inhibitors, such as crizotinib, in the 3D spheroids of gastric cancer cells. The activation of a large panel of receptor tyrosine kinases as well as the downstream signalling were analysed using arrays. The validation of potential glycan biomarkers in clinical samples was performed to corroborate that *O*-glycans truncation promoted the colocalization of the CD44v6 cancer-related glycoform with the receptor tyrosine kinase RON, and concomitantly increased the receptor activation.

The work performed by Dr. Balmaña during the MSC fellowship lead to the publication of 8 scientific manuscripts and other 2 that are currently under revision. Besides, the work developed in the laboratory by the researched and the different collaborations of the project supervisor that involved Dr. Balmaña will also result in future publications.

***Balmaña M**, Mereiter S, Diniz F, Feijão T, Barrias CC, Reis CA.*
*Multicellular Human Gastric Cancer Spheroids Mimic the Glycosylation Phenotype of Gastric Carcinomas.*
*Molecules. 2018 Oct 30;23(11). pii: E2815. doi: 10.3390/molecules23112815.*

*Freitas D*, **Balmaña M***, Poças J, Campos D, Osório H, Konstantinidi A, Vakhrushev SY, Magalhães, Reis CA. *equally contribution*
*Different isolation approaches lead to diverse glycosylated extracellular vesicle populations.*
*Journal of Extracellular Vesicles, 2019, 8:1, 1621131, DOI: 10.1080/20013078.2019.1621131*

*Albuquerque APB, **Balmaña M**, Mereiter S, Pinto F, Reis CA, Beltrão EIC.*
*Hypoxia and serum deprivation induces glycan alterations in triple negative breast cancer cells.*
*Biol Chem. 2018 Jun 27;399(7):661-672. doi: 10.1515/hsz-2018-0121.*

*Albuquerque APB, **Balmaña M**, Reis CA, Beltrão EIC.*
*Identification of appropriate housekeeping genes for quantitative RT-PCR analysis in MDA-MB-231 and NCI-H460 human cancer cell lines under hypoxia and serum deprivation*
*Journal of Molecular and Clinical Medicine 2018, 1 (3): 127-134. doi: 10.31083/j.jmcm.2018.03.001*

*Duarte HO, **Balmaña M**, Mereiter S, Osório H, Gomes J, Reis CA.*
*Gastric Cancer Cell Glycosylation as a Modulator of the ErbB2 Oncogenic Receptor.*
*Int J Mol Sci. 2017 Oct 28;18(11). pii: E2262. doi: 10.3390/ijms18112262.*

*Rodrigues JG, **Balmaña M**, Macedo JA, Poças J, Fernandes Â, de-Freitas-Junior JCM, Pinho SS, Gomes J, Magalhães A, Gomes C, Mereiter S, Reis CA.*
*Glycosylation in cancer: Selected roles in tumour progression, immune modulation and metastasis.*
*Cell Immunol. 2018 Nov;333:46-57. doi: 10.1016/j.cellimm.2018.03.007.*

*Mereiter S, Martins ÁM, Gomes C, **Balmaña M**, Macedo JA, Polom K, Roviello F, Magalhães A, Reis CA.*
*O-glycan truncation enhances cancer-related functions of CD44 in gastric cancer.*
*FEBS Lett. 2019 May 11. doi: 10.1002/1873-3468.13432.*

*Mereiter S, **Balmaña M**, Campos D, Gomes J, Reis CA.*
*Glycosylation in the new era of cancer targeted therapy: where are we heading?*
*Cancer Cell. (Accepted).*

The scientific manuscripts published within the GlycoModels project have been also summarised and published in the Portuguese Association for Cancer Investigation both in Portuguese and English to explain the relevance of the work to non-specialists.

The researcher of the GlycoModels project has participated in several events to disseminate the results to a non-specialist audience (European Researchers Night | Noite Europeia dos Investigadores) and she has also engaged in activities to bring science closer to high-school students (Ciencia Viva no Laboratorio).

### *Progress beyond the state of the art, expected results until the end of the project and potential impacts (including the socio-economic impact and the wider societal implications)*

The GlycoModels project goes beyond the state-of-the art by producing original insights in the field of therapy resistance in cancer, adding a new mechanistical level of regulation based on the tumour glycosylation profile. On one hand, this work will unveil specific glycoforms involved in treatment response and on the other hand, provide the required models to improve our understanding of the role of glycosylation in carcinogenesis and cancer progression. The proposed methodology is innovative as it is the first time that glyco-engineered cell models will be used in 3D-cell culture to address the functions of cellular glycosylation in health and disease. This multidisciplinary approach will provide a unique collection of glyco-engineered gastric 3D-cell models becoming an unprecedented tool for the study of the role of glycosylation that will create a niche of research for the applicant, placing Dr. Balmaña in the forefront of research in the field of glycosylation in cancer. Furthermore, the strategy developed during this Marie Curie action will provide a novel approach that could afterwards be applied to different types of cancers, and even other diseases.

This proposal is intended to study the relation between the altered glycosylation of cell receptors and the sensitivity/resistance to targeted therapy in gastric cancer patients. Thus, it is expected that the results will provide clinicians with new tools to determine the best treatment for each patient. In this sense, the group is in close collaboration with the IPO-Porto Hospital and the clinicians will participate in the discussion of the results as well as contribute to design strategies that foresee clinical application of the identified glycomarkers.