and negative sub-populations in cervical cancer cells. Following the sorting, cell proliferation and migration assay were performed. In order to investigate the effect on the blockade of CD109, SiHa, Caski and C4-1 cells were transfected with CD109 siRNAs. XTT assay, migration and invasion assay and spheroid formation assay were performed. Immunohistochemistry (IHC) was performed for the detection of CD109 expression in cervical cancer tissue microarray (TMA).

Results and discussions The post-sorted CD109 (+) cells grew remarkably faster and have stronger migration capability than CD109(-) cells in Caski and C4-1. The CD109 knockdown cells with siRNA exhibited a slower cell growth, decreasing migration and self-renewal ability, as compared with the control group in SiHa, Caski and C4-1 cells. IHC of TMA indicated that CD109 was highly expressed in cancer cases than that in normal/benign cases.

Conclusion CD109 increased cell proliferation rate and migration ability in post-sorted cervical cancer cell lines Caski and C4-1. On the contrary, CD109 knockdown reduced cell growth, migration and self-renewal capability in cervical cancer SiHa, Caski and C4-1 cells. Cervical carcinoma showed high expression of CD109 protein by IHC. Further *in vivo* and *in vitro* functional assays are essential to characterise the CD109-positive sub-population in cervical cancer which may provide more information of cervical CSCs-related properties and resistance of radiation therapy, with the underlying molecular mechanism involved.

PO-297 ONCOGENIC ACTIVITY OF SOX1 IN GASTRIC CANCER

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Introduction Gastric cancer (GC) remains one of the leading causes of global cancer mortality due to therapy resistance. The infection with *Helicobacter pylori* is the major risk factor, particularly in patients carrying *H. pylori* highly virulent strains. GC contains a sub-population of gastric cancer stem cells (gCSC) with capacity of self-renewal that are critical drivers of tumour initiation, recurrence and therapy resistance. SOX transcription factors play a key role in the regulation and maintenance of stem cells during the embryonic development and are also involved in cancer. SOX1 is a well-established tumour suppressor in several types of cancer and has an oncogenic role in glioblastoma, but its impact in GC remains largely unknown. Therefore, we aimed to elucidate its function in GC and its putative role in the action of *H. pylori*.

Material and methods For this, we analysed GC patient samples, we co-cultured human GC cell lines with different *H. pylori* strains derived from patients of Donostia Hospital and we also performed functional studies of gain and loss of SOX1 function. Also, we performed transcriptomic analysis in SOX1-silenced cells and we carried out computational analysis using the ACRG datasets.

Results and discussions Our results revealed that SOX1 is highly expressed in human GC samples. Moreover, among a subset of SOX genes, SOX1 was the most significantly upregulated in gCSC derived from GC cell lines and also in cisplatin resistant cells, as well as in response to *H. pylori* exposure, in a virulence-dependent manner. In GC cells, SOX1-silencing impaired self-renewal capacity *in vitro* and reduced tumorigenicity and tumour growth *in vivo*. The up-regulation of SOX1 showed the opposite phenotype, indicating that SOX1 exerts an oncogenic role in GC. Notably, we found that in GC cells SOX1 was required for *H. pylori*-induced proliferation, acquisition of stem cell-like properties and induction of β-catenin. Furthermore, the transcriptomic analysis revealed a significant alteration of the E2F signalling pathway in SOX1 knockdown cells. Consistently, E2F1 silencing phenocopied SOX1 knockdown. Moreover, the inhibition of SOX1 downregulated E2F1, suggesting that E2F1 could be a downstream effector of SOX1 in GC.

Conclusion We identify for the first time an oncogenic role of SOX1 in GC. Our findings establish that SOX1-E2F-b-catenin is a critical axis for gCSC maintenance and for *H. pylori* action, postulating that its inhibition could constitute a promising strategy to combat therapy resistance in GC.

PO-298 MYC FAVOURS THE ONSET OF TUMOUR INITIATING CELLS BY INDUCING EPIGENETIC REPROGRAMMING OF MAMMARY EPITHELIAL CELLS TOWARDS A STEM CELL-LIKE STATE

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Introduction Breast cancer consists of highly heterogenous tumours whose cell of origin resulted difficult to be defined. Recent findings highlighted the possibility that tumor-initiating cells (TICs) may arise from dedifferentiation of lineage-committed cells, by reactivation of multipotency in response to oncogenic insults. MYC is the most frequently amplified oncogene in breast cancer and the activation of MYC pathway has been associated with the basal-like subtype, which is characterised by poor survival and lack of a specific therapeutic strategy. Although MYC has been considered a driver oncogene in breast cancer, its mechanism of action in tumour initiation has been poorly addressed.

Material and methods To evaluate the role of MYC in perturbing cell identity of somatic cells, we transduced hTERT-immortalised human mammary epithelial cells (IMEC) with a retroviral vector expressing low levels of the exogenous c-Myc. The effect of MYC overexpression was evaluated by performing morphological analysis and gene expression profiling. To verify whether MYC overexpression could enrich for cells with functional stem cell-like properties, we performed mammospheres assay. ChIP-seq analyses were performed to profile chromatin modifications and MYC binding in IMEC WT, -MYC and mammospheres. To determine whether MYC-reprogrammed IMEC were enriched for TICs, we performed *in vivo* injection in NOD/SCID mice and assessed long-term tumorigenic potential by performing serial transplantation assay. To assess the clinical relevance of our findings, we investigated the expression of MYC-dependent oncogenic signature in a database of breast cancer patients.

Results and discussions Overexpression of MYC induces transcriptional repression of lineage-specifying transcription factors, causing decommissioning of luminal-specific enhancers. Of note, MYC-driven dedifferentiation supports the onset of a

basal/stem cell-like state by inducing the activation of *de novo* enhancers, which drive the transcriptional activation of oncogenic pathways. MYC-driven epigenetic reprogramming favours the formation and maintenance of TICs endowed with metastatic capacity. Moreover, oncogenic pathways activated by MYC-modulated enhancers are associated with basal-like breast cancer in patients with a poor prognosis.

Conclusion MYC-driven tumour initiation relies on a cell reprogramming process, which is mediated by activation of MYC-dependent oncogenic enhancers, thus establishing a therapeutic rational for treating basal-like breast cancers.

PO-299 *IN VIVO* SHRNA SCREENING TO IDENTIFY QUIESCENCE-RELATED GENES REQUIRED FOR AML GROWTH

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Introduction AML is hierarchically organised with at the apex Leukaemia Stem Cells (LSCs), a rare cell population able to initiate and sustain the tumour growth. LSCs share many functional properties with normal Hematopoietic Stem Cells (HSCs) including self-renewal capacity and quiescence. Quiescent LSCs can survive to radiation and chemotherapy acting as a reservoir for leukaemia relapse, the major cause of death for AML patients. Therefore, LSCs quiescence is critical for leukaemia maintenance and few evidences suggest that quiescence regulation in pre-leukemic phase plays a pivotal role for leukemogenic process as well.

Material and methods We analysed the transcriptional deregulations induced by the expression of different leukemic oncogenes in HSCs and we examined the contribution of representative quiescence related genes in AML growth by *in vivo* RNA interference screening.

Results and discussions The transcriptional profile of oncogene-expressing HSCs is enriched in a quiescent stem cell gene signature, compared to normal HSCs. Therefore, we hypothesised that enhancement of the quiescent phenotype in HSCs could be a shared mechanism for leukaemia development and maintenance. The *in vivo* shRNA screening allowed the identification of genes whose silencing in AML blasts was sufficient to significantly decrease *in vitro* self-renewal and delay leukaemia growth *in vivo*.

Conclusion We identified quiescence-related genes, commonly deregulated by leukemic oncogenes at pre-leukemic level, which may offer new therapeutic targets in a wide group of AML patients.

PO-300 UNVEILING AND EXPLOITING CANCER STEM CELL EDITING AND IMMUNOGENICITY FOR PRECISION MEDICINE

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Introduction Immunogenic chemotherapy (IC) induces immunogenic cell death (ICD), which, similar to viral infection, leads to a cancer-cell autonomous Type-I-Interferon (IFN-I) signalling. This immunological signature is crucial for effective antitumor responses but may paradoxically promote the emergence of a rare population of cancer stem cells (CSCs) acting as a chemoresistant niche within the tumour and roots for metastasis and relapse. In this study, we have investigated the role of IFN-I during IC in inducing a cancer editing program resulting in the appearance of poor immunogenic CSCs.

Material and methods Human and murine tumour cell lines were treated *in vitro* with ICD-inducers or IFN-I as control and the induction of CSC were analysed by cytofluorometry, quantitative real time (qRT)-PCR, 3D culture and functional assays. Free and vesicle-mediated nucleic acid transfer during ICD has been characterised by co-culture experiments. IC-induced CSC immunogenicity has been studied through cytofluorometry, microfluidic devices and *in vivo* experiments. All experiments have been done in triplicate and statistical significance evaluated by two-tailed Student's *t* test and two-way ANOVA.

Results and discussions The transient/acute induction of IFN-I during ICD is followed by the appearance of a rare population of CSCs. Both free nucleic acids and extracellular vesicles are released during tumour ICD constituting the upstream inducers of IFN-I-mediated reprogramming of neighbouring cells. IC-induced CSCs display epithelial-to-mesenchymal transition traits, multidrug resistance and regenerative properties, and a significant tumorigenic potential when inoculated in immunodeficient and immunocompetent mice. As expected, tumour growth and size are reduced in the presence of an intact immune system. Experiments on microfluidic devices reveal a poor immunogenic potential of CSCs, further confirmed by the expression of immune checkpoint blockers.

Conclusion Our results pinpoint a surprising link between ICD, IFN-I and CSCs. Elucidating the mechanisms of CSC editing together with a deep characterisation of CSC (immune) properties could be crucial to prevent tumour relapse. This could undoubtedly have dramatic implications for the clinical management of cancer in an era of terrific development of precision combined chemo-immune therapy.

Poster Presentation: Cancer Genomics, Epigenetics and Genomic Instability

Genomic Alterations in Cancer

PO-301 DIAGNOSIS OF GLIOMA TUMOURS USING CIRCULATING CELL-FREE DNA

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Introduction Gliomas are the most frequent tumours of brain, and they make up about 30% of all brain and central nervous system tumours, and 80% of all malignant brain tumours. Diagnosis of different glioma tumour types and their tumour grade is an essential step to suggest a right treatment for the glioma patients. Existing standard diagnostic technique for glioma tumour includes tissue biopsy, which is a highly invasive and hence a risky technique for the patient's health. 'Liquid biopsy'