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Giacomo Vezzani (34, Scarlato / Frigimelica / Merola):
Use of B-cell platform to characterize antigens of interest in virus and Gram-negative bacteria

Human antibody responses to infections or vaccinations result in the elicitation of antibody repertoires with enormous sequence diversity that often target the same antigens or epitopes although with variable affinity and efficacy. B cell sequencing and antibody characterisation from individuals during the course of an immune response can provide crucial information to design optimized vaccines or therapeutics. Single B cell screening strategies have emerged as important technologies for efficiently sampling the natural antibody repertoire of infected or vaccinated subjects. Having access to a method to interrogate different B cell subsets provides an attractive option to produce large and diverse panels of high-quality antibody, with the possibility to find rare B cell clones producing IgG with unique and desirable characteristics.
Our in-house B-cell platform consists in the e recombinant production and functional evaluation of antibodies derived from single cell sorted B-cells derived from human peripheral blood. We have developed a method enabling contextual VH and VL cloning and expression, while keeping their endogenous VH:VL pairing. We are exploiting our B-cell platform to isolate and characterize human monoclonal antibodies (HumAbs) derived from Human Citomegalovirus (HCMV) chronically infected patients and from subjects immunized with Meningococcus B vaccine Bexsero.
The HCMV glycoprotein B (gB) is essential to viral entry and a potential vaccine candidate. To date, only post-fusion form of gB is available as pure protein while its pre-fusion conformation, thought to be the ideal antigen, has not been elucidated yet. The goal of this project is to identify and express anti-gB humAbs isolated from human plasmablasts of HCMV infected donors as tools for the identification of crucial gB epitopes and as a tool to gain structural information on a gB “native” pre-fusion and post-fusion conformational species. HumAbs were initially tested for their ability to neutralize HCMV infection in vitro. Luminex technology was then used to enrich anti-gB antibodies excluding all species recognizing other abundant HCMV envelope proteins and characterising for gB post-fusion binding. Best candidates will then be used in co-expression with soluble gB and further selected for their ability to produce a secreted stable gB/Ab complex to be crystallized. Determination of the tertiary structure is a prerequisite to generate an efficacious antigen via protein engineering structural stabilization.
BEXSERO® is a multicomponent vaccine against one of the major etiological agents of sepsis and invasive meningococcal disease: Neisseria meningitidis serogroup B (MenB). Detergent-extracted outer membrane vesicles (dOMVs) are one of the components of the vaccine, whose broad protection could be due to several antigens present on dOMV surface. To unravel the relative contribution of the different antigens in eliciting protective antibody responses, we isolated human monoclonal antibodies (HumAbs) from single-cell sorted Plasmablasts (PBs) of adult vaccinees peripheral blood. Recombinant HumAbs have been screened for binding to dOMVs and BEXSERO® recombinant proteins by Luminex bead-based assay. dOMVs positive mAbs are tested for functionality by serum bactericidal assay (SBA) and characterized in a tailor-made protein-microarray containing a panel of prioritized dOMV-specific proteins, with the aim to identify meningococcal antigens/epitopes that contribute to the protection induced by dOMVs. The bactericidal mAbs identified so far recognize the outer membrane protein PorB, confirming the high contribution of this protein in eliciting protective antibody response. Surface exposed PorB loops have been expressed and purified in a scaffold protein to be tested with bactericidal mAbs to investigate PorB immunoreactivity. Additional analyses are ongoing to further elucidate the contribution to BEXSERO® protection of different MenB antigens.

Viola Viviani (33, Scarlato / Bartolini):
Dissection of the protective response elicited by Detergent-extracted Outer Membrane Vesicles in the 4CMenB vaccine

Detergent-extracted Outer Membrane Vesicles (DOMV) are one of the four components of the 4CMenB vaccine currently licensed for use against *Neisseria meningitidis* serogroup B (MenB) in addition to 3 other recombinant protein components. Early clinical trials demonstrated DOMV safety, immunogenicity and beneficial effects in vaccine formulation, mainly attributed to the protection elicited by the immunodominant PorA protein. Nevertheless, DOMV offer a complex reservoir of surface-exposed proteins with potential immunogenic properties whose relative contribution in eliciting protective antibodies has not been fully elucidated.
We observed from clinical trials that the addition of DOMV in the vaccine formulation enhanced the breadth of coverage of the response with respect to the recombinant protein components alone. Testing antisera from vaccinees in serum bactericidal assay (SBA), formulations with DOMV were bactericidal against a panel of 12 distinct additional MenB strains. To unravel the DOMV components involved in such protection, 30 major DOMV-specific antigens were cloned and expressed in E. coli as recombinant proteins or in GMMAs (Generalized Modules for Membrane Antigens) in order to maintain their native conformation. Samples generated were both included in a tailor-made protein microarray to immunoprofile the antibody repertoire raised by DOMV/4CMenB formulations and were used for mouse immunization studies in order to assess their ability to induce functional bactericidal antibodies.
Protein microarray analysis revealed a subset of 8 DOMV antigens recognized by mouse DOMV/4CMenB antisera, suggesting these as highly immunogenic protein in the DOMV and potentially responsible for the additional protective responses. Moreover, specific antisera derived from mice immunization against each of these proteins, individually showed high levels of antibodies and were able to recognize the native antigens across different MenB strains. In particular, three DOMV-antigens among those immunoreactive in protein microarray (OpcA, NspA and PorB), induced antibodies able to kill 11 of the 12 genetically diverse meningococcal strains used in SBA. In addition, the specificity of the protective role of OpcA was also confirmed with 4CMenB vaccinee sera as the SBA activity was lost against knockout derivatives of individual strains of the panel of 12.
In conclusion, we identified PorA-independent antigen components within DOMV that contribute to the broad cross-protection induced by the 4CMenB vaccine, supporting the key role played by DOMV in this multivalent formulation.
This work was funded by GlaxoSmithKline Biologicals SA and Alma Mater Studiorum Università di Bologna

Luigia Cappelli (35, Maione / Cozzi / Scarlato):
Exploring different self-assembling nanoparticles in displaying structurally defined epitopes

To cope the growing need of medical interventions for many infectious diseases, novel vaccines containing isolated and highly purified antigenic protein have been developed. Compared to early vaccines, they are safer but unable to induce high level of protective immunity. To overcome this latter, the design of antigen nanoparticles results to be a promising strategy. (J. López-Sagaseta et al., 2016). In fact, virus-like particles (VLPs) and protein nanoparticles (NPs) thanks to their repetitive and highly ordered structure might present multiple copies of the desired antigens. So, the supra-molecular assemblages can potentially mimic the size and the shape of the natural host-pathogen surface interactions with potential to induce potent B- and T-cell responses (Y Hsia et al. Nature 1–3 (2016) doi:10.1038/nature18010).
The primary aim of this project is to explore the potential of self-assembling NPs as platform for the display of antigen in the vaccine research evaluating their heterologous expression with different systems, purification and characterization in terms of solubility, stability and immunogenicity. Antigens from different pathogenic bacteria have been genetically fused to several NPs of different size and geometry. All the fusion proteins have been successfully cloned, expressed and purified. The correct assembly of chimeras has been verified with size exclusion chromatography and TEM analysis.
The approach used for rational design of new chimera-NPs and the preliminary results obtained regarding the recombinant production of those NPs and their biochemical characterization will be presented.

Annapaola Petrosino (35, Danielli):
Phage-tron: developing a phage-based microprocessor

Bacteriophages are in the spotlight for their innovative application as biosensors. This project aims at developing an innovative phage-based biosensor based on a microprocessor with an AND gate logic. In this setting, the output C is obtained only in case condition A and condition B are simultaneously met. Such a

biosensor would improve on the classic ones by eliminating the need of time-consuming washing steps usually performed to remove signal background. To implement the microprocessor a triple split GFP system will be engineered, involving the tripartite association between two GFP tags, GFP10 and GFP11, each of them fused to the pVIII proteins of two distinct M13 bacteriophages, and the complementary GFP1-9 detector. When the two phages approach to each other, following the recognition of both analytes (A and B), GFP10 and GFP11 self-associate with GFP1-9 to reconstitute a functional fluorescent GFP.
To begin assessing the suitability of M13 phages with split GFP systems, we have carried out preliminary fluorescence assays based on the partition of GFP into two fragments (simple split GFP), GFP 1-10 and GFP 11. The latter tag was produced as a fusion protein with the pVIII phage proteins, while GFP 1-10 was purified from inclusion bodies in Escherichia coli. Using the two independently produced components, we characterized the sensor in vitro with encouraging results. The phage biosensor and GFP1-10 were individually non-fluorescent, but capable of assembling and reconstituting the intact and functional protein, reaching a easily detectable fluorescence signal.
These results set the basis for further engineering of the phage microprocessor using the triple split GFP system.

Jacopo Rossi (34, Trost / Zaffagnini):
Redox homeostasis and proteins modification in plants: the central role of glutathione

Redox post-translational modifications (PTMs) play a pivotal role in the control of proteins function, acting on their stability, structure, and catalysis. In plants, these redox PTMs are particularly important when facing various abiotic or biotic stresses, which cause an increase production of reactive oxygen and nitrogen species (ROS and RNS). In fact, various enzymes have evolved to sense oxidative bursts in order to adjust cellular metabolism and permit plant adaptation to environmental challenges. Cysteine thiols (-SH) are at the center of this interplay and glutathione (GSH), the most abundant non-protein thiol in the plant cell, acts as a buffer that heavily influences directly or indirectly the redox state of protein cysteines. The homeostasis of glutathione is safeguarded by many proteins, namely glutathione reductase (GR), thioredoxin (TRX), and S-nitrosoglutathione reductase (GSNOR), which participate in the maintenance of a reduced glutathione pool and avoid the accumulation of its oxidized forms (GSSG and GSNO). As part of my work as a PhD student, I first analyzed the structural and functional properties of plant GSNORs along with the interactions between these enzymatic systems and their specific reducing relevance using recombinant enzymes and plant protein extracts. Finally, in order to shed light on the effects linked to altered glutathione homeostasis, I analyzed the functionality of the metabolic enzyme glyceraldehyde-3-phosphate dehydrogenase (GAPDH) in wild-type *Arabidopsis* plants and knockout mutants lacking cytoplasmic GR and GSNOR.

Serena Jasmine Aleo (34, Ghelli / Rugolo):
Analysis of mitochondria involvement in Fabry Disease

Fabry disease (FD) is an X-linked lysosomal storage disorder (LSD) related to an inborn error of glycosphingolipid catabolism, resulting from deficient or absent activity of the lysosomal enzyme α-galactosidase A. This defect leads to the progressive accumulation of globotriaosylceramide and related glycosphingolipids in the plasma and lysosomes of endothelial, renal, cardiac and nerve cells (Desnick et al., 2001), ultimately resolving in compromised energy metabolism, oxidative stress, ionic dyshomeostasis and cell death (Germain, 2010). Mitochondrial impairment has been reported to occur in LSDs as a cause of cellular dysfunction (Plotegher and Duchen, 2017) though no clear evidence is yet available for FD, apart from reduced activity of OXPHOS complexes I, IV and V in human fibroblasts (Lücke et al., 2004). We therefore assayed the occurrence of a mitochondrial damage by microscale oxygraphy experiments, testing the oxygen consumption rate of four patient-derived fibroblast lines bearing classic mutations on GLA gene cause of a severe phenotype. Notably, only patient 2 and 4 showed a strong reduction of maximum respiratory capacity when in forced oxidative conditions, suggesting a respiratory impairment may show up depending on GLA mutation and solely when the respiratory chain is induced to work at high rates. Western blot experiments on the same fibroblasts reported no significant differences in the expression levels of representative subunits of OXPHOS complexes, indicating that the reduction of the oxygen consumption rate may be related to a decreased activity of respiratory complexes. No detectable alterations of mitochondrial mass were either observed. Since in the presence of mitochondrial impairment an oxidative stress may arise, levels of mitochondrial (i.e. manganese superoxide dismutase and peroxiredoxin 3) and cytosolic (i.e. catalase) antioxidant proteins were also assessed. Indeed, a significant increase of SOD2 was revealed in patient 2 and 3, and the same trend was observed for Prx3 and catalase in patient 1 and 4 respectively. This led us to hypothesize an oxidative imbalance may actually occur in FD fibroblasts. The extent of ROS production in the same cells is currently under investigation.

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Stefano Miglietta (33, Porcelli / Ghelli):

Unravelling the role of mitochondrial chaperone MCJ during the metabolic adaptation and chemoresistance in ovarian cancer

Ovarian cancer (OC) is the most lethal gynecological tumour due to its extremely silent invasive capacity. The high mortality of OC is often determined by a therapeutic failure caused by the development of pharmacological chemoresistance (1-2). In this context, recent studies have shown that the condition of therapeutic resistance is influenced by the cellular metabolic state and mitochondrial bioenergetic efficiency (3-4). Interestingly, it has been reported that in OC the chemoresistance acquirement is associated with the epigenetic silencing of DNAJC15 gene that codifies for MCJ mitochondrial co-chaperonine (5-7). The latter is reported as an endogenous negative regulator of electron transport chain (ETC), able to modify the structure and function of mitochondrial respiratory chain supercomplexes (8). It is known from the literature that OC growth and chemoresistance is often mediated by the activation of the oncogenic β-catenin pathway that can be finely regulated by the mitochondrial energy state (9). We have therefore hypothesized that MCJ, modulating the bioenergetic profile, may lead to re-establish pharmacological sensitivity and growth of OC by inhibiting β-catenin pathway. In this context, our preliminary data show an increased sensitivity to cisplatin and a low proliferation capacity in MCJ-overexpressing chemoresistant cell line. Further, the expression of MCJ leads to an increased expression of several mitochondrial proteins and activation of enzymes involved in lipids anabolism as well as to a decrease of multi-drug resistance proteins and β-catenin expression levels in OC cell lines. Based on these data, we speculate that MCJ may affect mitochondrial ETC efficiency that leads to an increased mitochondrial biogenesis and lipids synthesis. This metabolic modification would induce a degradation of β-catenin associated to a reduction of OC chemoresistance and growth. The dissection of this novel molecular and metabolic-related mechanism, possibly linked to tumorigenesis and pharmacological resistance of OC, set the bases for the discovery of new biomarkers for the development of adjuvant therapies for this silent killer disease.

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Ana Catarina da Silva Almeida (33, Porcelli):

Development of cancer cell models to test metabolic intervention as an anticancer strategy

In the recent years, it has become evident that the role of mitochondria in the metabolic rewiring that is essential for cancer development and progression. The metabolic profile during tumorigenesis has been performed mainly in traditional 2D cell models, including cell lines of various lineages and phenotypes. Although useful in many ways, their relevance can be often debatable (Kapałczyńska et al. 2018; Duvan et

al. 2017). Improved models are now being developed using 3D cell culture technology, contributing with increased physiological relevance (Rodrigues et al. 2018; Sachs and Clevers 2014). In this work, we have improved a technology for the generation of 3D models from healthy and tumor colon tissue, based on organoid technology, and perform their molecular and biochemical characterization and validation. Ultimately, cryopreservation technology was applied to these models, and optimal results were obtained in terms of cell viability and functionality of the cryopreserved models. The utilization of this technology allows the conversion of cell models into “plug and play” formats. Therefore, cryopreserved technology facilitates the accessibility of specialized cell models to the universe of cell-based research and application, in cases where otherwise such specialized models would be out of reach.

Houda Abla (33, Porcelli / Iommarini):

αKGlogues-induced pseudonormoxia to prevent metabolic adaptation in tumorigenesis

Solid tumours sustain their high growth rate through a metabolic and hypoxic adaptation mainly orchestrated by Hypoxia Inducible Factor-1α (HIF-1α), a transcription factor that controls the transcription of target genes implicated in glycolysis, angiogenesis and survival (Semenza et al., 2010). The stability of this protein is regulated by O2 and α-ketoglutarate (αKG)-dependent Prolyl Hydroxylases (PHDs), leading to its proteasomal degradation. We previously reported that the lack of respiratory Complex I causes the accumulation of αKG leading to HIF-1α degradation. This metabolic signalling renders cancer cells unable to adapt to hypoxia, a condition termed pseudonormoxia, with a strong inhibition of tumour growth in vivo (Kurelac et al., 2019). In this study, we tested the ability of seven different cell permeable αKG ester analogues (αKGlogues) to provoke PHDs-mediated degradation of HIF-1α and to prevent cancer cells adaptation. Such αKGlogues are chemically stable and two of them are shown to promote HIF-1α degradation in hypoxia in two different cell models (thyroid and kidney cancer cell lines). Metabolites profiling through LC/MS-based metabolomics confirmed the accumulation of αKG a few hours post-treatment. Furthermore, the two compounds selectively affected tumour cell proliferation and in vitro clonogenic properties, only exerting a mild effect on the non-cancer counterparts. Therefore, these data strongly suggest the ability of αKG to reactivate PHDs in thyroid and renal carcinomas cell lines while severely altering their growth.

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Ylenia Beniamino (34, Zambelli):

Structure, function and interactions of Ndrg1, a protein involved in nickel-dependent carcinogenesis

Nickel is a natural element of the earth’s crust and the exposure to this metal via inhalation, ingestion and dermal contact results in hazardous effects on human health [1]. In particular, many soluble nickel compounds are classified as human carcinogens by IARC [2] due to the correlation between their inhalation and the increased incidence of lung cancer [3].
The expression of the human protein N-myc downstream regulated gene 1 (NDRG1), member of the NDRG family, is up-regulated in lung cancer and is associated to higher cancer aggressiveness and worse prognosis [4]. Nickel induces its expression, by activating the hypoxia pathway [5][6]. NDRG1 itself shows Ni(II) binding activity in its intrinsically disordered C-terminal domain [7][8]. This part is also phosphorylated in specific serine and threonine residues, and this is related to the biological activity of the protein.
Despite the numerous studies demonstrating the role of NDRG1 in carcinogenesis, its structure, interactions and mechanisms of action are unclear. The understanding of these points is important, in order to design anticancer drugs specifically targeting this protein [9].
My research project focuses on the structural, biophysical and biochemical characterization of NDRG1. I improved the protocols, previously designed during the first year of PhD, for the heterologous *Escherichia coli* expression and purification of the full-length protein, the N-terminal and the C-terminal domains. The secondary structure content and the hydrodynamic properties of the proteins in solution were determined by circular dichroism and light scattering, respectively. In addition, the structural characterization of the C-terminal domain through NMR spectroscopy and its nickel binding activity via calorimetric experiments are currently underway. I am also developing a protocol for the expression and purification of O6-alkylguanine DNA alkyltransferase (MGMT), a possible NDRG1 interaction partner, to study their Ni(II)-dependent protein-protein interactions.

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Andrea Arleo (34, Capranico / Marinello):

Cell-type specific effects of Topoisomerase I poisons on immune gene activation in cancer cells

Campthotecin (CPT) and LMP-776 (LMP) belong to a class of molecules named Topoisomerase I (Top1) poisons. Top1 regulates DNA supercoiling by catalyzing a controlled rotation of a cut strand around the intact one of a DNA duplex. During the catalytic activity, Top1 forms the so-called DNA cleavage complex, which can be recognized and bound by Top1 poisons, preventing DNA re-ligation by the enzyme. The poison activity can eventually lead to irreversible DNA double-strand cuts (DSB) and cell apoptosis.
Previous findings of our lab have shown that Top1 poisons transiently enhance nuclear R-loops formation in cancer cells, the processing of which can result in irreversible DSB. A main aim of the present project is to understand the mechanisms linking DSB accumulation to the induction of an immune response by Top1 poisons in cancer cells of different histological origin.
The results show that CPT and LMP at non cytotoxic doses induce a similar increase in the number of micronuclei in human cancer cells of different histology. Overexpression of RNaseH1 (a nuclease that specifically degrades the RNA of a hybrid duplex) reduces the formation of micronuclei and DSBs by Top1 poisons, suggesting that R-loops and DNA damage can play a role in the production of micronuclei. As micronuclei can be a source of cytoplasmic DNA, which can activate the cGAS/STING pathway leading to an innate immune gene response, I have determined the expression levels of type I Interferons (IFNa/b) and other cytokines in different cancer cells. The results show that mRNA levels, analyzed under the same experimental conditions, are markedly increased in most cancer cell lines. Interestingly, STING silencing by siRNA in HeLa cells markedly reduced IFN and cytokine expression induced by Top1 poisons, showing that STING is required for the poison induction of an immune response in cancer cells. However, in the lung cancer DMS114 cell line Top1 poisons did not increase cytokine and IFN transcript levels, and a western blotting analysis showed that STING was not expressed in these lung cancer cells. A bioinformatic analyses of STING gene methylation and expression in the TCGA dataset revealed that STING expression is highly variable in human lung cancers and often the absence of STING expression is likely due to an hypermethylation of the gene. Our finding thus show that the STING pathway is activated by micronuclei induced by Top1 poisons leading to immune gene activation in cancer cells, and that STING gene methylation can be a cause of impairment of the STING pathway and therefore inability of Top1 poisons to induce an immune response in cancer cells.
This knowledge may have an impact for patient stratification and the choice of the most effective therapeutic regimen in patients lacking STING expression.

Simona Paglia (33, Pession):

Genetic and molecular analysis of the contribution of cell polarity disruption to brain cancer

Primary glioblastoma (GBM), the most common and aggressive adult brain tumour, is refractory to conventional therapies, leading to poor prognosis with most patients dying within 1 year from diagnosis. GBM displays striking cellular heterogeneity, with stem-like glioblastoma stem cells (GSCs) responsible for tumor recurrence and resistance to therapy. Alterations of the tumour suppressor gene PTEN are prevalent in primary GBM; cancer stem cells show a dysfunctional PTEN/aPKC/Lgl axis and an altered cell polarity. I deregu-

lated this axis in the fly's neural stem cells, causing neoplastic growth and formation of neurogenic tumour masses that kept growing in the adult, leading to premature death.
With the aim to understand the role of cell polarity disruption in this tumorigenic process I carried out a molecular characterisation and a RNAseq analysis of brain cancers from our fly model. The contribution of the most deregulated genes to the malignant phenotype are being analysed by functional tests in Drosophila and in patient-derived cell lines in collaboration with the Ottawa Hospital Research Institute.

Giulia Gobbi (33, Ciarrocchi / Ambrosetti / Sancisi):
Dissecting functional and molecular crosstalk between TAZ and its associated lncRNAs in lung cancer progression

Lung cancer represent the main cause of cancer-related death world-wide, with an overall five years survival of 16%. Thus, better understanding of the molecular mechanisms at the basis of lung cancer pathology is crucial to ensure the most appropriate management for patients. Long-non coding RNAs (lncRNAs) have become important players in the regulation of several physiological and cancerous processes. In our laboratory, we identified two lncRNAs, named TAZ-AS202 and TAZ-AS203. These transcripts share the promoter region and are transcribed in antisense orientation with the TAZ gene. TAZ and YAP are two transcriptional coactivators that, forming complexes with TEADs and SMADs transcription factors, enhance a gene expression program, promoting cancer cells proliferation, migration, invasion and drug-resistance. Similarly to TAZ downregulation, TAZ-AS202 silencing strongly impairs lung cancer cells proliferation and motility, while TAZ-AS203 has no effect on these features. TAZ-AS202 silencing does not affect TAZ and/or YAP mRNA, protein levels, phosphorylation status or nucleus-cytoplasmic localization, but decreases the expression of YAP/TAZ main target genes, implying an uncovered functional cooperation between TAZ-AS202 and YAP/TAZ in the regulation of downstream genes. Intriguingly, RNAseq analysis shows that EPH-Ephrin pathway is positively regulated by both TAZ and TAZ-AS202, suggesting that, at least in part, TAZ-AS202 and TAZ pro-oncogenic activity in lung cancer depends on EPH-Ephrin deregulation.
Overall, our data highlight a pro-oncogenic function for TAZ-AS202 in lung cancer through a previously uncovered molecular mechanism and might lay the basis for the future use of TAZ-AS202 as novel biomarker for lung cancer patients.

Eugenia Lorenzini (34, Ciarrocchi / Ambrosetti):
TRIM28 contribution to Malignant Pleural Mesothelioma

Malignant Pleural Mesothelioma (MPM) is a rare but aggressive cancer arising from pleura mesothelial cells. It is characterized by high mortality and dismal prognosis. Molecular bases of this disease remain unknown, representing a relevant limitation to the development of effective targeting strategies. To fill this gap, we performed a CRISPR-Cas9 genome-wide screening in MPM cells (MSTO-211H). We identified 233 new genetic vulnerabilities, most of which are genes involved in epigenetic regulation and chromatin organization. Among these, the epigenetic keeper TRIM28 showed a dramatic effect on MPM cell survival and a striking correlation with patients' survival probability. Using several knockdown strategies and multiple MPM cell lines we confirmed that TRIM28 KO reduced dramatically cell proliferation and colony formation. Cell cycle analysis showed modest alterations while apoptosis was significantly induced in TRIM28 KO cells. To get deep into this mechanism, we performed gene expression profile by RNA sequencing. Of the 448 genes significantly altered in MSTO-211H TRIM28-KO cells, 195 were down-regulated of which 35% were involved in chromosomal maintenance and mitosis. Noticeably, the TRIM28 target genes UBE2S, VRK1, TYMS and CKS1B were also found as essential genes in our screening. Analysis of the TCGA MPM-gene expression dataset demonstrated a strong correlation among TRIM28 and its targets and showed a significant association of these genes with a negative survival probability of patients, thus providing a strong in vivo validation of our analysis. Overall, we identified a new essential molecular program for MPM survival and progression.

Eleonora Poeta (33, Monti):
Altered epigenetic regulation affects brain cells proliferation and differentiation in in vitro models of the rare genetic demyelinating disease AGC1-deficiency

AGC-1 deficiency is a rare genetic demyelinating disease caused by mutations in the SLC25A12 gene coding for the mitochondrial aspartate-glutamate carrier 1 (AGC-1). In patients, AGC-1 reduced activity leads to profound developmental delay, epilepsy and hypomyelination due to the reduction in N-acetylaspartate (NAA), the myelin CNS precursor.

Our lab previously reported a deficit in proliferation and an early differentiation in two different AGC-1-deficiency in vitro models, i.e. siAGC-1 Oli-Neu cells (immortalized oligodendrocytes precursors cells, OPCs) and neurospheres derived from the sub-ventricular zone of AGC-1 heterozygous C57BL6N mice (Petralla et al., 2019). Here, we investigate whether an altered epigenetic regulation of genes involved in brain cells' proliferation/differentiation mechanisms could be responsible for the differences observed. Therefore, we analyzed expression and localization of transcription factors known to be involved in brain cells proliferation and differentiation, as well as histones post-translational modifications and HAT/HDACs activity/expression in both AGC-1-deficiency in vitro models (Oli-Neu cells and neurospheres). Then, by acting through pharmacological inhibitors (i.e. curcumin and SAHA), we studied whether modifications of HAT and HDACs activity could lead to changes in brain cells proliferation and differentiation. Interestingly, in addition to an altered epigenetic and expression profile in both models, we observed a different differentiation commitment of control and AGC1-deficiency neurospheres, when cultured in presence or absence of inhibitors, with different cellular phenotypic differentiation depending on the specific treatment. Taken together, these data supported our hypothesis that an altered epigenetic regulation of the proliferation/differentiation processes could be involved in the biological differences observed in in vitro models of AGC1-deficiency.

Reference: Petralla S, Peña-Altamira LE, Poeta E, et al. Deficiency of Mitochondrial Aspartate-Glutamate Carrier 1 Leads to Oligodendrocyte Precursor Cell Proliferation Defects Both In Vitro and In Vivo. Int J Mol Sci. 2019;20(18):4486. Published 2019 Sep 11. doi:10.3390/ijms20184486

Marta Viggiano (34, Maestrini):

An increased burden of rare exonic variants in NRXN1 microdeletion carriers is likely to enhance the penetrance for autism spectrum disorder

Autism Spectrum Disorder (ASD) is characterized by a complex polygenic background, but with the unique feature of a subset of cases (~15-30%) presenting a rare large-effect variant. However, clinical interpretation in these cases is often complicated by incomplete penetrance, variable expressivity and different neurodevelopmental trajectories. NRXN1 intragenic deletions represent the prototype of such ASD-associated susceptibility variants. From array-based comparative genomic hybridization (aCGH) analysis in 104 ASD individuals, we identified an inherited NRXN1 deletion in a trio family. We carried out whole-exome sequencing and deep sequencing of mitochondrial DNA in the ASD proband with the NRXN1 deletion (exons 7-23) and in the unaffected parents, in order to evaluate the contribution of the burden of rare variants towards the phenotypic outcome in NRXN1 deletion carriers. We identified a higher number of exonic rare variants in the ASD child compared to the unaffected NRXN1 deletion-transmitting mother, and this increased burden remains significant if we restrict the analysis to potentially deleterious rare variants only ($p= 6.07x10-5$). We also detected significant interaction enrichment among genes with damaging variants in the proband, suggesting that additional rare variants in interacting genes contribute collectively to cross the liability threshold for ASD. This study underlines the importance of a comprehensive assessment of the genomic background in carriers of NRXN1 deletions, or other "likely pathogenic" variants, as the contribution of additional interacting rare variants to modulate the phenotypic manifestation might represent a widespread mechanism in neurodevelopmental disorders.

Piergiuseppe De Rosa (35, Perini):

Characterization of a corepressor complex involved in MYCN-driven neuroblastoma tumorigenesis

Neuroblastoma is a malignant pediatric tumor derived from neural crest cells. Since MYCN amplified tumors show poor prognosis and therapy response, understanding the mechanisms which drive its oncogenesis could help to develop new therapeutic strategies. Preliminary data obtained through random mutagenesis on a Th-MYCN transgenic mouse model revealed that a single missense mutation in the sequence coding the NHR4 domain of ETO gene strongly reduces the severity of tumor phenotype, thus, suggesting a crucial role of this gene in neuroblastoma development.
To understand the role of ETO in neuroblastoma, we analyzed its interactome in a MYCN-amplified neuroblastoma cell line by performing an immunoprecipitation assay coupled with mass spectrometry. We found some of ETO well-known interactors, like TCF4, TCF12, HDAC1, HDAC2 and HDAC3 and, interestingly, we also found four novel partners which had never been previously identified: the histone demethylase LSD1 and the corepressor RCOR3, which are part of the Co-REST repressor complex, and the transcription factors HAND2 and TWIST1, involved in neural crest differentiation. To go deeper into the role of the ETO missense mutation, we performed Co-IP experiments for most of the protein partners that came out from mass spectrometry data. Notably, we found that, between the tested proteins, RCOR3 is the only factor that totally lose interaction with the ETO mutant protein. Moreover, the absence of ETO abolish RCOR3 association with chromatin in neuroblastoma cell line.

Overall, our data suggest that ETO may be involved in neuroblastoma tumorigenesis by interacting with RCOR3 through its NHR4 domain and that this complex could modulate the activity of transcription factors involved in neural crest cells differentiation.

Tuesday, September 15, 2020

Francesca De Chirico (35, Monti):
Understanding the role of microglial exosomes in neuroinflammation spreading: an in vitro study

Neuroinflammation is a crucial and common mechanism in neurodegenerative diseases and microglia, the immune cells of the brain, play a crucial role in this pathological process. In fact, in pathological conditions, microglia can acquire two different activated phenotypes: i) in early phases, an anti-inflammatory phenotype (M2) with the release of neurotrophic and neuroprotective factors, while later ii) a pro-inflammatory phenotype (M1) associated with neurotoxic properties, the secretion of pro-inflammatory cytokines and of nitric oxide (NO). The shift of microglial phenotype from the neuroprotective M2 to the neurotoxic M1 could be related to a change in the protein and/or miRNA content in exosomes, vesicles involved in intercellular communication. This suggests that the neuroinflammation spreading present in many neurodegenerative diseases could be mediated by the release of exosomes in the extracellular environment and therefore by the effect that these vesicles have on the non-activated or resting microglia. Therefore, we focused our attention on miRNAs, already identified as involved in microglial activation, trying to understand their role in the spreading of neuroinflammation through exosomes.
First, to evaluate whether activation could be transmitted among microglial cells indirectly through exosomes, activation has been pharmacologically induced in a microglial murine cell line (N9) by using LPS towards a M1 activation or ATP towards M2. Then, the media conditioned by differentially activated microglia or the exosomes extracted from these media has been given to non-activated cells to evaluate their activation though the analysis of specific commonly used markers in western blot. Furthermore, protein analysis has been performed through western blot on conditioned media or isolated exosomes or cell lysates to characterize possible changes in expression of proteins known to be involved in neuroinflammation or neurodegeneration, while miRNA analysis through qRT-PCR, in order to identify miRNAs differentially expressed or released through exosomes. In exosomes derived from activated microglia, the autocrine role of specific miRNAs, especially those regulating activated microglial phenotypic shift, could help to clarify the molecular mechanisms of microglial activation spreading and could provide new potential targets for immunomodulation.

Ottavia Tartagni (34, Zuccheri):
Drug cytotoxicity analysis of uniform 3D tumor spheroids, measurement of morphology and viability

Currently, in vitro models are constantly evolving to better mimic tissue structure, function and to reflect organism response to treatments. Three-dimensional (3D) multicellular tumor spheroids (MTS) are an emerging structure in biomedical research and remain the best characterized and most widely used 3D models; however, spheroid sizes tend to be heterogeneous, making them unsuitable for high-throughput drug testing. To avoid this challenge, we have developed defined size microwell arrays using nonadhesive silicon elastomer device. Our device has been applicable to a wide variety of cancer cell lines to fabricate sizecontrolled 3D spheroids.
In order to provide a robust and effective platform for in vitro drug evaluation, our prototype device has been implemented, containing more than 400 microwells. The results shown focus on spheroid size control, drug treatments and viability assays using a human colorectal carcinoma cell line (HCT 116). Moreover, to investigate the growth phenomenon over long-term cultivation we followed the growth of different sized spheroids and on the thirteenth day, the tumor spheroids stained with live/dead assay with FDA and Propidium Iodide was observed under a fluorescence microscope.
Specifically, to evaluate the potential role of this device prototype for anticancer drug screening, we tested drug response of MTS cultured in our device. We treated spheroids with different drug concentrations, performed growth and morphological aspect monitoring before and after drug exposure. Then, we adapted standard MTT assay to assess cell viability as analytical end point for drug testing. Devices were further incorporated with agarose low-melting point for in situ chemotherapeutic effects analysis with live/dead cell staining.

In addition, we have developed agarose-made devices with the same microwell design and array. We verified the potential of this material to produce tightly packed and uniform spheroids. In summary, a microwell-based spheroid culture model can provide a useful tool for drug screening, which is crucial for drug discovery and development. Future works will focus on testing other viability assays, apoptosis markers expression and immunofluorescence analysis.

Roberto Ciaccio (33, Perini):
MYCN-E2F3 axis: new insights on neuroblastoma biology through protein interactome profiling

Neuroblastoma (NB) is the third most common neurogenic-extracranial solid cancer of infancy and childhood. The most aggressive subtype of NB, which carries the worst overall prognosis, occurs where the MYCN gene is amplified. Many questions remain concerning what discriminates MYCN-amplified from non-amplified tumours. Our data provide new insights about how high MYCN can establish a dynamic regulatory axis with the E2F3 transcription factor, impacting the development of the high-risk cancer phenotype. High E2F3 expression is consistently associated with poor survival across different NB datasets regardless of MYCN expression, thus highlighting its crucial role in NB progression. To better understand how E2F3 works despite MYCN status and to assess the contribution of the two known E2F3 isoforms (E2F3a and E2F3b), we examined the complexity of their protein interactome by using the proximity-dependent biotin labelling (BioID) in both high and low MYCN expression conditions. These analyses revealed respectively 96 and 100 protein candidates belonging to the comprehensive proteomics map of both E2F3a and E2F3b proteins, underlining for the first time the mutual dependency of MYCN status and the proteomic profiling of these two transcription factors in NB disease. Our unbiased screen uncovered many potential candidate proteins that help to fill the knowledge gap in understanding what is the impact of MYCN on E2F3 biology, shedding light on the molecular principles that leads the MYCN/E2F3 axis to foster the oncogenic programme.

Renée Concetta Duardo (34, Capranico):
Genetic and molecular mechanisms of genomic instability induced by DNA Topoisomerase 1 poisons leading to innate immune response

Immunotherapy has progressed considerably in recent years and has become an important treatment for human cancers either alone or in combination with other therapeutic interventions. At the same time, the success of classical antitumor chemotherapy has recently been proposed to be due to a stimulation of innate and adaptive immunity by anticancer drugs. However, the molecular mechanisms are not yet fully clarified.
Topoisomerase I (Top1) poisons are among the clinically-effective drugs used in standard chemotherapeutic regimen for colon, ovary and lung cancers. Top1 is a key enzyme that resolves topological stress accumulated during transcription, replication and chromatin remodelling by allowing strand rotation through a transient single-strand break introduced by the enzyme into the DNA duplex substrate. Anticancer Top1 poisons trap DNA-enzyme intermediates that can lead to irreversible DNA double-strand breaks (DSB), cell death and genome instability.
In agreement with data from other laboratories, we have results showing that structurally different Top1 poisons, Camptothecin (CPT) and LMP76, induce the formation of micronuclei in human cancer cells. Micronuclei can be a source of cytoplasmic DNAs that can activate STING-dependent pathways leading to an innate immune response and production of type I interferons and other cytokines in cancer cells. However, as the mechanisms of micronuclei formation have not been established yet, the main aim of my PhD project is to establish the mechanism of micronuclei induction and immune response by Top1 poisons in human cancer cells.
Using immunofluorescence assays and EdU labelling of nascent DNAs, my results show that sub-cytotoxic doses of LMP76 and short treatment induce, after 24 hours from drug treatments, the formation of micronuclei that do not contain EdU labelled DNA in human cancer HeLa and U2OS cells. Under similar experimental conditions, LMP76 was able to produce significant levels of γH2AX, a marker of DSBs in replicating cells but much lower levels in non-replicating cells. Further results show that EdU-negative micronuclei formation is not dependent on transcription while being dependent on R-loops, as overexpression of RNaseH1 markedly reduces micronuclei formation.
Altogether, the data are consistent with the formation of micronuclei due to under-replicated DNA caused by Top1 poisons, thus highlighting previously unknown aspects of drug activity. In the second part of my PhD program, I will determine whether and how R-loops have a role in the mechanism of under-replication and which protein factors are key players in the mechanisms.
My results will eventually help in developing new strategies for effective personalized interventions using Top1-targeted compounds as immuno-modulators in cancer patients.

Annamaria Zannoni (33, Scarlato / Roncarati):
HP1043 mediated transcriptional regulation in *Helicobacter pylor*i

HP1043 is an OmpR-like dimeric orphan response regulator essential for the viability of the human pathogen Helicobacter pylori as it controls the expression of several genes involved in key cellular processes. Therefore, it represents an ideal target for the design of novel antimicrobial strategies. To this aim, the function of HP1043 mutants was studied in vitro to identify amino acid residues responsible for HP1043 dimerization and DNA recognition. Moreover, an in-silico protein-DNA docking was performed to generate a structural model of the interacting HP1043 dimer and its target DNA. Experimental validation of the model by EMSA analysis in the presence DNA groove binders and IC-box substitution shows that HP1043 recognizes target promoters by interacting with both major and minor groove. Taken together, this information will be used in the future to carry out a virtual screening of small molecule libraries in order to identify compounds able to specifically interfere with dimer formation or target DNA interaction and likely block H. pylori infection.
To further characterize the mechanism of regulation in vivo, a conditional mutant strain overexpressing a synthetic copy of the gene altered in nucleotide sequence yet encoding the wild type amino acid sequence was generated. A 2-fold induction of the synthetic protein does not determine an increase in wild type hp1043 or HP1043 target genes' transcript abundance, pinpointing a strict mechanism of regulation that has yet to be determined.
Finally, in collaboration with Prof. Giorgi's lab, we are building a DNAshapeR based algorithm to evaluate whether it is possible to predict ChIP-seq identified HP1043 targets on the basis of DNA shape features common to the few experimentally validated target promoters. In case of success of this proof of principle, an online tool to predict putative new targets of other transcriptional regulators will be implemented.

Paolo Emidio Costantini (33, Cappelletti / Vitali / Fedi):
Pathogen-derived extracellular vesicles inhibit HIV-1 infections

Vaginal microbiota dominated by lactobacilli generally reduce transmissibility and infectivity of several sexually transmitted infections, such as *N. gonorrhoeae*, HSV and HIV-1. Recently, it was reported that the protective effect of *Lactobacillus* against HIV-1 is, in part, mediated by released of extracellular vesicles (EVs) by these symbiotic bacteria. Here, we investigated whether EVs derived from 4 opportunistic human pathogens (*S. aureus*, *E. faecium*, *E. faecalis* and *G. vaginalis*) influence HIV-1 infections.
Purified EVs were characterised through nanoparticle tracking analysis (NTA) and then used to treat immortalized human T cell during HIV-1 infections. As a result, EVs-derived from *S. aureus*, *E. faecium*, *E. faecalis* and *G. vaginalis* were all able to inhibit HIV-1 infections in a dose-dependent manner, while they were not cytotoxic for human T cells. Furthermore, it was demonstrated that all the tested EVs specifically interact with the glycoprotein gp120 of the HIV-1 virion. Proteinase pre-treatment of bacterial EVs led to a complete loss of their antiviral properties as well as their ability to interact with gp120. Finally, it was observed that the Elongation Factor TU (EFTU) present in *L. gasseri* EVs inhibited HIV-1 in a dose-dependent manner. Interestingly, this moonlighting protein was also detected in EVs derived from other bacterial species including *S. aureus*, *E. faecium* and *G. vaginalis*.
In conclusion, our findings demonstrate that pathogen derived-EVs prevent HIV-1 infections in vitro through their proteic component and interact with the essential viral protein gp120. In addition, the moonlighting protein EFTU reduce HIV-1 infections, suggesting a common antiviral mechanism among bacteria that produce EVs equipped with EFTU.

Federico D'Agostino (35, Scarlato / Roncarati):
Identification of *Helicobacter pylori cncR1* targetome through MAPS technology

The importance of *Helicobacter pylori* as a human pathogen is underlined by the plethora of diseases it is responsible for, ranging from chronic gastritis to peptic ulcers and gastric carcinomas. The peculiar small genome size combined with its paucity of transcriptional regulators, highlights the relevance of post-transcriptional regulatory mechanisms. Among them, the cag- non-coding RNA1 (CncR1) is a trans-acting small non-coding RNA (sRNA) that appears to play a pivotal role in the programmed expression of important genes necessary for H. pylori infection and persistence in the gastric niche. Although cncR1 sequence is well conserved among all cag-pathogenicity island positive (cag-PAI+) strains, its targets have not been deeply characterized and its regulatory circuit remains poorly understood.
This project aims to uncover the CncR1 full targetome through a technology called MS2-Affinity Purification coupled with RNA Sequencing (MAPS). This method takes advantage of the strong affinity between MS2 viral protein and MS2 RNA aptamer used to tag in vivo the CncR1 sRNA. The implementation of the experi-

mental data with computational ones obtained from sRNA Target Prediction Organizing Tool (SPOT) computational pipeline will be helpful to deepen the analysis of CncR1 RNA:RNA interactions.
To this end, a set of plasmids in which the cncR1 sequence is embedded between two homology regions of *H. pylori* G27 genome has been constructed. To avoid possible interference with the intrinsic terminator of transcription, the MS2 aptamer sequence was inserted at 5'-end region in three different configurations of MS2::CncR1. These plasmids were then used to generate mutants of *H. pylori* G27 harbouring the tagged or untagged gene in a cncR1 null background. These strains are currently used to verify transcriptional regulation of CncR1 and of a few known gene targets.

Benedetta Manca (34, Scarlato / Giuliani):
Functional characterization of the gonococcal polyphosphate pseudo-capsule

Neisseria gonorrhoeae is an exclusively human pathogen able to evade the host immune system through multiple mechanisms. *Gonococci* accumulate a large portion of phosphate as polyphosphate (polyP) on the exterior of their cells, but its function has not been yet defined. Its polyanionic nature has suggested that it may form a protective shield on the cell surface, however its existence has remained controversial so far.
The goal of this project was to confirm the presence of a capsule-like material and investigate its role in immune evasion.
To detect polyP on the gonococcal surface, the C-terminal domain of the *E.coli* exopolyphosphatase, which has the ability to bind polyP, was used as a molecular probe in confocal microscopy and in FACS. Results indicated the presence of localized spots of polyP on bacteria and highlight the occurrence of a polyP matrix embedding bacterial aggregates. To evaluate the role of polyP in resistance to complement-mediated killing, the enzymes involved in polyP polymerization (two polyphosphate kinases, PPK1 and PPK2, one exopolyphosphatase, PPX) were genetically deleted generating mutant strains with altered polyP production. Single and double knockouts of the 2 kinases resulted in a strong reduction of polyP accumulation on the surface of cells grown in a chemically defined medium, instead strains devoid of exopolyphosphatase accumulated polyP. These phenotypes correlated with altered resistance to complement-mediated killing. Indeed, PPK mutants were more sensitive to killing compared to the parental strain whereas, the PPX mutant showed increased resistance when exposed to active human serum suggesting a direct impact of polyP in the modulation of the complement cascade.
Interestingly, polyP pseudo-capsule was found on the surface of serum resistant PorB1b strains but not on PorB1a strains for which other serum resistant mechanisms are extensively described in literature.
Data generated suggest a role of polyP pseudo-capsule in the interference with the complement cascade. Further studies will be performed to evaluate the importance of polyP in other host-pathogen biological interactions.
This work was sponsored by GlaxoSmithKline Biologicals SA. BM is a student at the University of Bologna and participates in a post graduate studentship program at GSK.

Abstracts with no presentations

Sonia Nicchi (34, Scarlato / Brettoni / Maione):
***Moraxella catarrhalis* actively paralyses neutrophils to evade the host innate immune response**

Chronic obstructive pulmonary disease (COPD) is a multidimensional disease, having complex pathogenesis with *Moraxella catarrhalis* (Mcat) and non-typeable *Haemophilus influenzae* (NTHi) being the most prevalent bacteria found in the sputum of individuals with exacerbation. Among the aspects that characterise COPD pathogenesis, the oxidative stress (caused by reactive oxygen species, ROS) is one of its most important trademarks. Neutrophils are the main producers of ROS, which can further boost the overall antimicrobial response by promoting Extracellular Traps (ETs) formation and autophagy. Bacteria have evolved several strategies to impair ROS-dependent killing, relying on both "intrinsic" resistance mechanisms, such as detoxification of radical species, and "extrinsic" resistance mechanisms, that directly suppress ROS production. Considering the growing evidences that ROS tolerance pathways are general determinants of virulence, we aim to investigate whether Mcat BBH18 strain, isolated from sputum of a COPD patient, is able to actively evade this first defense line of the innate immune response.
For this purpose, we infected the human cell line HL-60 (differentiated in neutrophils-like cells) with BBH18 and assessed the capability of bacteria to dampen the cellular immune response. We have shown that the BBH18 strain is readily phagocytosed by HL-60 in an IgG-independent manner and that bacterial internalization increases over time. Interestingly, we demonstrated that BBH18 barely induces ROS and ET-related responses in infected cells if compared to the co-infecting bacterium NTHi. Moreover, Mcat is able to

actively suppress PMA-induced ROS production in HL-60, in a contact-dependent but phagocytosis-independent manner. Further work is needed to shed light on the underlying mechanism. Lastly, preliminary data suggest that, once internalized, Mcat seems able to interfere with the autophagic pathway of the infected cells and survive for extended periods of time.
This work was funded by GlaxoSmithKline Biologicals SA and Alma Mater Studiorum Università di Bologna.

Paolo Cinelli (35, Scarlato / Rosini / Maione):
Deciphering the molecular mechanisms underlying N-linked glycosylation in Gram-negative bacteria

Glycans presented on the surface of bacteria mediate some of the early interactions between microbes and mammalian host. These polysaccharides are involved in a multitude of processes, including resistance to serum-mediated killing, bacterium-host interactions, and regulation of the host immune response.
Surface polysaccharides have also been used as target antigens for the development of vaccines. It is well known that most polysaccharides are not strong antigens because they act as T cell-independent antigens, therefore they're unable to induce immunoglobulin class switching and long-term B cell memory in children below the age of 2 years. For this reason, starting in the early 1980s, glycoconjugate vaccines were developed in order to obtain more effective and long-lasting immunogenic vaccine products. Traditionally, glycoconjugate vaccines are obtained through a semi- or a fully-synthetic approach, were the polysaccharide is extracted from the target bacterium or synthetized in vitro and linked to a carrier protein via a chemical or enzymatic process. Although these types of vaccines have seen immense commercial success, their manufacturing processes are not free from drawbacks, including batch to batch variation, heterogenous product formation and high manufacturing costs.
Recently, bacterial glycosylation systems have been developed to produce innovative glycoconjugates vaccines. In these systems, heterologous expression of polysaccharides can be associated with the glycosylation of selected protein targets by expressing an oligosaccharyltransferase (i.e. *C. jejuni* PglB) in an engineered *E. coli* strain. These systems have been shown to be efficient and promising tools to produce glyco-conjugated vaccines. A better understanding of the mechanisms underlying N-glycosylation could further improve these systems. In order to provide new insights into the N-glycosylation mechanism, we analyzed the contribution in the bioconjugation system of additional ancillary elements. Among them, we initially focused on periplasmic chaperones and analyzed their contribution by overexpression or deletion of the encoding genes in glycocompetent *E.coli*.
Another approach that will be tested is the reconstruction and modification of export machineries such us the SecYEG operon, in order to explore the key components required for glycosylation across different export pathways.