



Fondazione
**ISTITUTO DI RICERCA
PEDIATRICA**

Annual Retreat 2018

Abstract book



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The task of the new Board of Directors, in agreement with the scientific director and the managing director, and on the basis of what has been done up to now, will be to make the Istituto di Ricerca Pediatrica Città della Speranza an attractive and facilitating environment for researchers, both for those who already work there and for those who will arrive in the future. This means creating a competitive work environment, made of synergy and dialogue among the researchers.

The IRP has excellent researchers. The goal will be to make them become a great orchestra in order to play a common score, but an increasingly complicated and competitive score, and activate a fruitful process through all the universally recognized research products: scientific publications, ability to attract public funding and, finally, the ability to provide products that meet the needs of the community in terms of health and productivity. All this will make the IRP a true Institute, a center of attraction for research.

Prof. Giuseppe Basso
President Istituto di Ricerca Pediatrica Città della Speranza



The first scientific retreat of the Istituto di Ricerca Pediatrica Città della Speranza represents another step towards the realization of the challenge started in the summer of 2017 with the transition to a new organizational model.

The goals are ambitious but attainable: strengthen existing research groups, allow good ideas to be implemented, and recruit new researchers who can contribute to their development, mutualize equipment and skills to optimize research times and costs, and dialogue constantly with the University and its multidisciplinary research, because innovation is born only where there is a sharing of knowledge.

Significant progress has been made in a few months. We have set up the Scientific Council and the Scientific Advisory Board, and constituted three working groups on essential themes to enhance human capital and existing infrastructures: culture, communication and facilities. We have also published the first funding announcement for internal research projects. The outcome has been very satisfactory.

Finally, the retreat represented an important training opportunity and an occasion for encounter, providing all the researchers with a clear picture of what is being done. There was a climate of deep scientific sharing. I hope it is the first of many occasions for discussion, exchange and convivial moments.

Prof. Antonella Viola

Scientific Director Istituto di Ricerca Pediatrica Città della Speranza



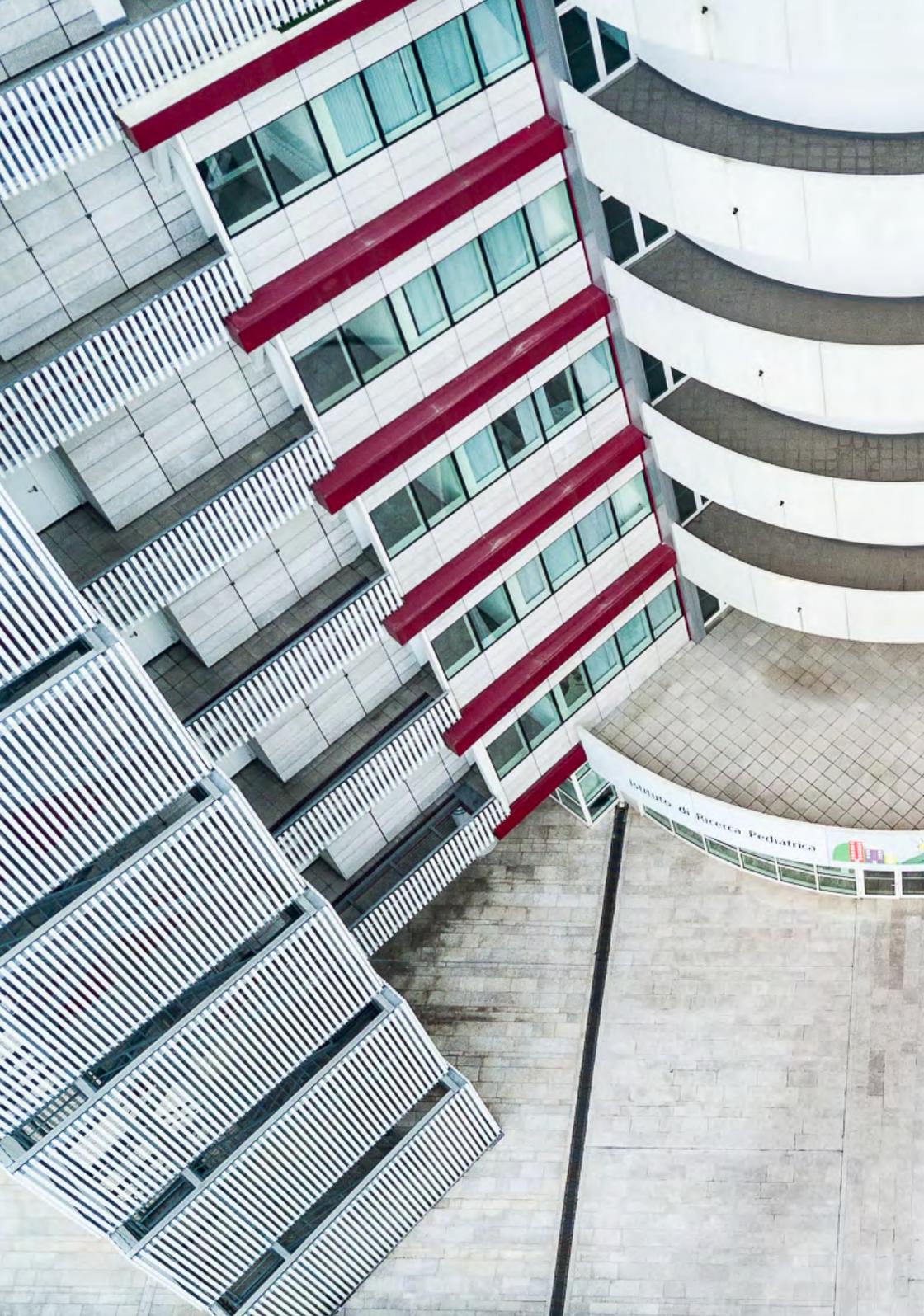
In 2017 we completed the restructuring of the Institute's governance model and the consequent adaptation of the charter. All the participants of the technical board have made an exceptional and collective effort, always having as their aim to bring the research from bench to the patient's bedside. To do so, we have decided to attract and keep the best scientists here, providing them not only with a fine building, but also with a functional structure.

I am convinced that the continuous and reasoned adaptation to the challenges of a world that is changing very quickly is a responsibility that we all have the duty to exercise, operating all the while in a context with multiple stakeholders that embrace the demands of scientific research, society, health and the economic world.

In order to be more competitive and become an international reference point, the Istituto di Ricerca Pediatrica has chosen to streamline the bureaucratic machine and entrust the researchers with decision power concerning the lines of research, the choice of which instrumentation to purchase and which funding to attract. So as to increase the quality of the results, the IRP wants to attract the attention of the world as an incubator for young talents, reach the creation of patents and involve companies in investments.

Our goal is to make a great research institute and Padua is unique of its kind thanks to the presence of a university and a hospital that are real motors. We need the territory to believe in this project and everyone to do their part based on their skills.

Dr. Andrea Camporese
CEO Istituto di Ricerca Pediatrica Città della Speranza



Istituto di Ricerca Pediatrica

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CEO: **Dr. Andrea Camporese**

Scientific Director: **Prof. Antonella Viola**

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Research Areas

PEDIATRIC ONCOLOGY

Giuseppe Basso
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Gianni Bisogno
Elisa Cimetta
Lara Mussolin
Luca Persano
Martina Pigazzi
Geertuy Te Kronnie
Gian Paolo Tonini
Giampietro Viola

PREDICTIVE MEDICINE

Eugenio Baraldi
Paola Cogo
Giuseppe Giordano

REGENERATIVE MEDICINE

Piergiorgio Gamba
Maurizio Muraca
Martina Piccoli
Michela Pozzobon

GENETICS AND RARE DISEASES

Leonardo Salviati
Maurizio Scarpa
Luisa Murer
Alessandra Murgia
Rosella Tomanin
Eva Trevisson

NANO MEDICINE

Marco Agostini
Lucia Delogu
Filippo Romanato

IMMUNOLOGY

Antonella Viola
Marcella Canton
Bruno Giometto



Press review

Città della Speranza, la nuova era
«Fondazione Veronesi è con noi»

CORRIERE DEL VENETO



INTERVISTA. Andina Camporese ha da poco terminato il suo incarico di presidente dell'Istituto di ricerca pediatrica

Città della Speranza
«La provincia berica è prima per donazioni»

«C'è una base di volontariato prezioso e una grande generosità nelle persone comate»



laVoce
DEL BERICO

TREVISOTODAY

Neuroblastoma, tecnologie e ricerca di eccellenza entrano in IRP con il progetto di Elisa Cimetta

La dottoressa trevigiana a settembre ha vinto il prestigioso starting grant dell'European Research Council per un progetto di ricerca basato su un tumore pediatrico



Maurizio Scarpa
Istituto di Ricerca Pediatrica Università di Pielova

dintorni
le eccellenze del territorio

Economia e Risparmio

Premio professione impresa: alla Torre della ricerca le eccellenze venete premiate da Raffaele Zanon ed Elena Donazzan

Padova24ore.it



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Press review

Nanoparticelle, sviluppi promettenti per il trattamento delle malattie infiammatorie



Donne e ricerca, un binomio vincente in IRP

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Vescicole per il trasporto di farmaci antitumorali:
da IRP arriva il brevetto

di Padova
il mattino

**Basso: "Quarant'anni di guerra
contro i tumori per salvare i nostri
bambini"**

*Sta per andare in pensione il direttore di Oncematologia pediatrica di Padova. Una vita per combattere malattie terribili come le leucemie infantili: "Che oggi si possono guarire"
di Elena Livieri*

CITTÀ DELLA SPERANZA Fondazione, il oda nomina Giuseppe Basso presidente e Antonella Viola direttore scientifico
**Ricerca, un milione di euro in più:
scendono in campo gli imprenditori**



Innovabiomed
MAGAZINE

INNOVABIOMED: UN NUOVO CAPITOLO NELLA STORIA DEL BIOMEDICALE
Due giorni dedicati agli stakeholder del settore dove le idee innovative verranno condivise

IL GAZZETTINO

Press review

PHARMASTAR
il Giornale on-line sui Farmaci

Leucemia linfoblastica acuta-T, studio italiano dimostra come superare la resistenza alla normale terapia

Nuove possibilità di curare la leucemia linfoblastica acuta di tipo T potrebbero aprirsi grazie ai risultati dello studio condotto dai ricercatori del Laboratorio di Oncoematologia Pediatrica dell'Istituto di Ricerca

IL GAZZETTINO



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IRP finanzia cinque nuovi progetti

Sono cinque i progetti di ricerca che la direzione scientifica di IRP ha finanziato nei giorni scorsi, per un ammontare complessivo di quasi 400mila euro.

“Vogliamo favorire il dialogo tra scienza e società” – Intervista alla prof.ssa Antonella Viola

Drepanocitosi, due studi fanno luce sul trapianto da genitore e il deficit linguistico

SCHIO – Un corto sulla Città della Speranza



**Falsa-scienza, gli esperti:
«Basta, adesso parliamo noi»**

► Relatori delle conferenze Garattini, Andreoli, Barbujani, Ragazzoni e Palazzi



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Press review



IL GIORNALE DI VICENZA

La Torre della ricerca ha cambiato i vertici

Due le figure all'ovale: il direttore scientifico Antonio Vio, specialista in biologia molecolare, e l'amministratore delegato Andrea Campese

CORRIERE DEL VENETO

Appuntamenti

Città della speranza e Cicap, lotta ai «fake»

Un maggio fitto di incontri per fare chiarezza su medicina e scienza



La giornata della scienza per 240 studenti

► Lezioni, discussioni, filmati e laboratori per i ragazzi dei licei

SCUOLA

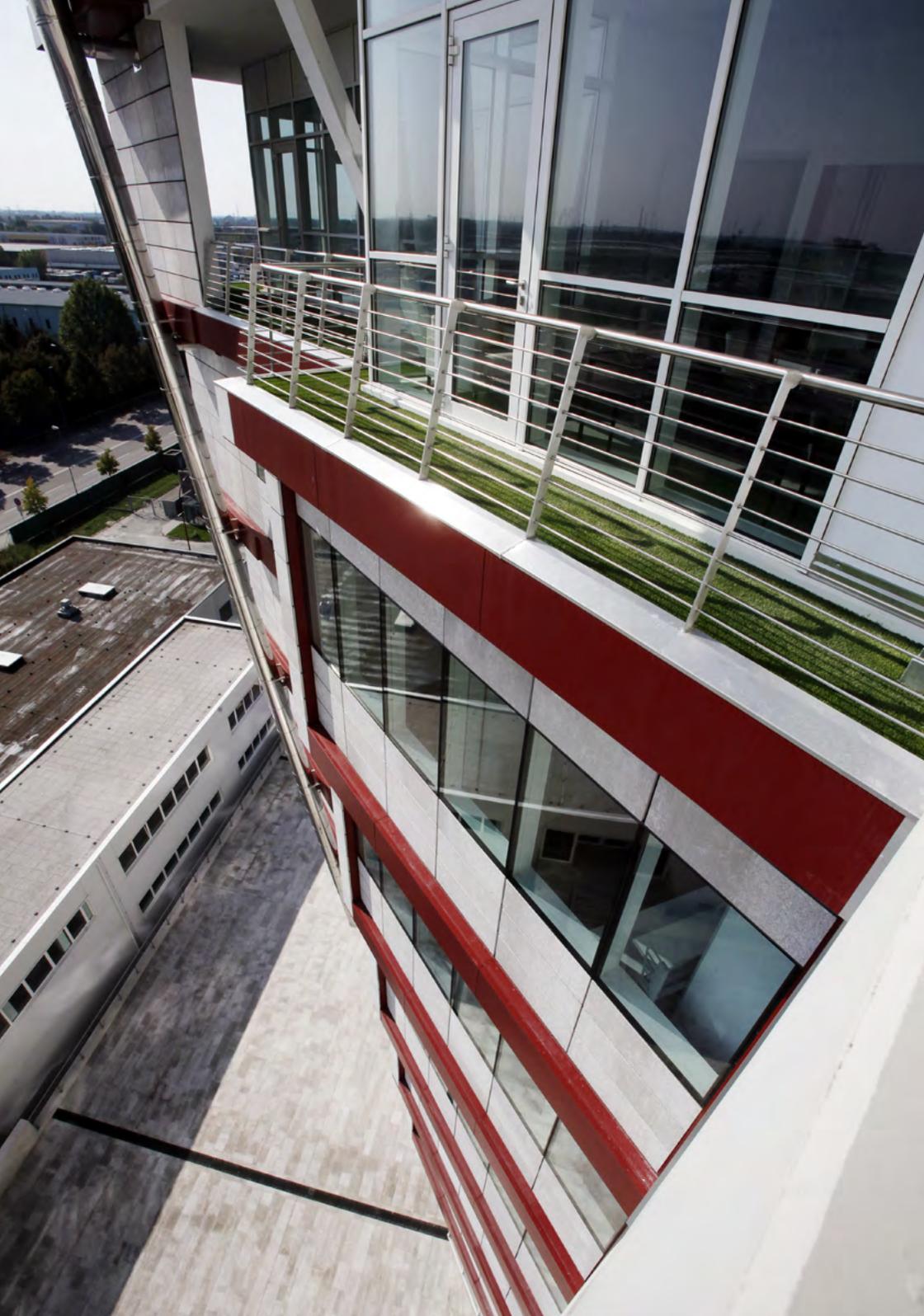
Lezioni, discussioni, filmati, attività di laboratorio e persino una canzone: si annuncia ricca di proposte la settima edizione della "Giornata nazionale della scienza" che l'Università di Padova, con il patrocinio di Euro-



secondo grado. L'iniziativa pone in primo piano i temi e i problemi affrontati dalla ricerca sulle cellule staminali e rientra nel grande appuntamento europeo dell'Unistem Day che vedrà coinvolti contemporaneamente 74 Atenei e Centri di ricerca in Austria, Francia, Germania, Polonia, Serbia, Spagna, Svezia, Gran Bretagna, Ungheria e Italia. La giornata padovana, inserita nel progetto "Continuità Scuola-Università", vedrà coinvolti 240 studenti delle classi terze, quarte e quinte dei licei scientifici Biondi e Pontal di Bi-

posso sempre oltre il proprio limite: ecco perché le scuole e l'Università ne sono custodi e maestri», afferma Daniela Lucangeli, professoressa alla Continuità formativa scuola, università e lavoro. «La giornata della scienza vuole essere un'occasione per l'apprendimento, la scoperta e il confronto nell'ambito della ricerca sulle cellule staminali», afferma Michela Pozzobon, responsabile del Laboratorio di Cellule staminali e medicina rigenerativa all'interno di Istituto di ricerca pediatrica.

IL GAZZETTINO



Abstracts



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Pediatric Oncology

SYK TARGETING AS A POTENTIAL THERAPEUTIC STRATEGY FOR PEDIATRIC HIGH RISK TEL-AML1 B-ALL PATIENTS

Serafin V, Porcù E, Cortese G, Mariotto E, Bresolin S, Basso G, Accordi B

The most frequent chromosomal rearrangement in childhood B acute lymphoblastic leukemia (B-ALL) is the reciprocal translocation t(12;21)(TEL-AML1). Although its presence is a strong independent predictor factor of a favorable prognosis, relapses still occur in 10% of patients. A distinctive marker able to distinguish patients at risk of relapse is still missing, thus we aimed to identify new potential biomarkers and/or therapeutic targets that could predict and/or prevent TEL-AML1 patients' relapse.

By Reverse Phase Protein Arrays, we performed a phosphoproteomic profiling of 62 pediatric TEL-AML1 patients.

We identified SYK Y525 as hyperactivated at diagnosis in patients who will experience relapse and at relapse occurrence. To validate SYK as a therapeutic target, we treated 3 t(12;21) cell lines and primary cells from TEL-AML1 patients

at diagnosis with the SYK inhibitors Entospletinib, Fostamatinib and PRT-060318. After treatment, all compounds were able to decrease cell proliferation. We tested inhibitors in combination with Vincristine, Dexamethasone and Ara-C, and Entospletinib resulted the one with the best synergistic effect. When we treated primary cells with Entospletinib and the three drugs together (VDA), we observed an augmented response to Entospletinib and an increased cell death with Entospletinib+VDA compared to VDA alone in patients who will experience relapse. We will now evaluate response to Entospletinib+VDA in primary samples of relapse. Finally, we are setting up the analysis of activated SYK by phosphoflow to validate it as a prognostic marker.

Our research aims to demonstrate that SYK could be considered as a new potential marker of relapse occurrence and a new therapeutic target for t(12;21) patients.

SCREENING AND TARGETING OF THE NUP214-ABL1 FUSION IN PEDIATRIC T-ALL

Serafin V, Campeggio M, Bresolin S, Rebora P, Conter V, Valsecchi MG, Basso G, Accordi B

In the 6% of adult T-Acute Lymphoblastic Leukemia (T-ALL) patients is expressed the constitutively active NUP214-ABL1 fusion kinase, which consists of the N-terminal region of NUP214 fused with the C-terminal part of ABL1. NUP214-ABL1 adult patients present high-risk features and survival data are indicative of an aggressive disease. No data are available on incidence and prognostic value of NUP214-ABL1 in pediatric T-ALL.

To answer these questions, a screening will be performed in the cohort of pediatric T-ALL patients enrolled in AIEOP-BFM ALL2000/R2006 protocol. The first step consists in a multiplex RT-PCR with a mix of forward primers for exons 22-26-29-31-34 of NUP214 and the reverse primer for exon 3 of ABL1. Positive patients will be next analyzed by single PCR followed by sequencing to identify the exact fusion transcript. We set up screening method

using ALL-SIL and PEER human T-ALL cell lines as positive controls. Of the 249 available patients, we already screened 109 cases and found 2 positive patients (incidence 1.8%), one with ex26NUP214-ex2ABL1 and the other with ex31NUP214-ex3ABL1. Once completed, results will be statistically analyzed together with clinical data to assess prognostic significance of this fusion.

NUP214-ABL1 cells are predicted to be highly sensitive to Dasatinib, thus we treated 7 T-ALL cell lines and found that NUP214-ABL1 cells are about ten times more sensitive to Dasatinib than the others. We plan to test efficacy of Dasatinib in NUP214-ABL1 patients' primary cultures.

Results obtained from these studies could be easily translated into clinic to identify and efficiently treat this pediatric T-ALL subgroup.



A HIF-1 /WNT SIGNALING CROSSTALK PROMOTES NEURONAL DIFFERENTIATION IN GLIOBLASTOMA

Rampazzo E, Boso D, Zanon C, Bresolin S, Maule F, Porcù E, Cani A, Della Puppa A, Trentin L, Basso G, Persano L

The Hypoxia Inducible Factor (HIF)-1 is the major sensor of microenvironmental oxygen, reported to sustain progression and therapy resistance in several tumors, including glioblastoma (GBM), the most aggressive and deadly brain cancer. Oppositely, HIF-1 have been also demonstrated to promote neuronal differentiation of neural cells during normal neurogenesis by interplaying with Wnt signaling. In this context, we previously demonstrated that Wnt signaling activation under hypoxic conditions is sufficient to induce a dramatic neuronal differentiation of GBM cells, thus weakening their aggressiveness.

Here, we investigated the mechanisms underlying this process and characterized the molecular players involved in the HIF-1 /Wnt signaling crosstalk. In particular, we show that a β -catenin/TCF1/HIF-1 complex directly controls the transcription of neuronal differentiation genes under

hypoxia. Moreover, we show that, at higher oxygen levels, another β -catenin co-factor, TCF4, is able to compete with TCF1 for the binding to these regulatory regions, but rather inhibits differentiation. To test the clinical relevance of our results, we stained by immunohistochemistry 142 glioma samples for HIF-1, TCF1 and β -III tubulin, a well known neuronal differentiation marker, showing that a strict positive correlation between these factors does occur in human samples. Moreover, the gene expression profile of these neuronal-differentiated areas clearly showed the activation of a Wnt signaling-related gene signature in these tissues. In conclusion, our results unveil a tightly controlled mechanism by which TCF1 and HIF-1 induce a reminiscent neuronal differentiation of GBM cells under hypoxia, which, in turn, is hampered in normoxia by TCF4, whose inhibitory function might sustain their aggressive phenotype.

BMP9 COUNTERACTS THE TUMORIGENIC AND PRO-ANGIOGENIC POTENTIAL OF GLIOBLASTOMA

Porcù E, Maule F, Boso D, Rampazzo E, Barbieri V, Zuccolotto G, Rosato A, Frasson C, Viola G, Della Puppa A, Basso G and Persano

Glioblastoma multiforme (GBM) is a highly vascularized and aggressive brain tumor, with a strong ability to disseminate and invade the surrounding parenchyma. In addition, a subpopulation of GBM stem cells has been reported to possess the ability to transdifferentiate into tumor-derived endothelial cells (TDECs), supporting the resistance to anti-angiogenic treatments of newly formed blood vessels. Bone Morphogenetic Protein 9 (BMP9) is critically involved in the processes of cancer cell differentiation, invasion and metastasis, representing a potential tool in order to impair the intrinsic GBM aggressiveness.

Here we demonstrate that BMP9 is able to trigger the activation of SMADs in patient-derived GBM cells, and to strongly inhibit proliferation and invasion by reducing both AKT/MAPK signaling and FAK/ RhoA/ Cofilin activation, together with a negative

modulation of MMP9. Intriguingly, BMP9 treatment is sufficient to induce a dramatic switch of the GBM stem-like cells (positive for CD133, Nestin, Nanog, Sox2) towards a more differentiated phenotype, as confirmed by the increased expression of GFAP and S100. Furthermore, BMP9 significantly counteracts the already reported process of GBM cell transdifferentiation into TDECs not only in in vitro mimicked TDECs models, but also in vivo in orthotopic xenografts in mice. Additionally, we describe a strong BMP9-mediated inhibition of the whole angiogenic process engaged during GBM tumor formation.

Based on these results, we believe that BMP9, by acting at multiple levels against GBM cell aggressiveness, can be considered a promising candidate, to be further developed, for the future therapeutic management of GBM.



LNCRNA BALR-2-CDK6 AXIS INFLUENCES METABOLIC STATUS AND CELL DIFFERENTIATION OF ACUTE MYELOID LEUKEMIA

Bisio V, Tregnago C, Borella G, Benetton M, Basso G, Pigazzi M

Background. Non-coding RNA genes are at least as frequent as protein-coding genes in the human genome; however, our knowledge on their function in cancer is still preliminary.

Purpose. We aim to we investigated BALR-2 involvement in pediatric acute myeloid leukemia (AML).

Methods. We retrospectively analyzed by RQ-PCR bone marrow samples of 132 children with AML. We compared patients' gene expression signatures (n=58, HTA affymetrix 2.0) with either high (4th quartile) or low expression (1st+2nd+3rd quartiles) of BALR-2. AML cell lines with different BALR-2 expression were used for in vitro experiments.

Results. The expression of BALR-2 was found higher in all AML samples as compared with those collected from healthy volunteers. The 4th quartile was enriched for patients who did not reach complete remission after therapy (28% vs 12%,

p=0.03). Supervised clustering analyses showed that patients with high BALR-2 had 57 coding and 12 non-coding RNAs significantly differentially expressed (FC[2], p<0.01), and an upregulation of processes regulating mitochondria (p<0.05). We silenced BALR-2 and revealed a decrease of mitochondrial mass (p<0.001), an enhanced depolarization, and an higher sensibility to FCCP after BALR-2 knockdown. We further investigated CDK6, being chromosomally adjacent to BALR-2, and demonstrated a positive correlation of these genes expression levels (p<0.05). Silencing of BALR-2 reduced CDK6 mRNA and protein levels, as well as phospho-RB its direct target, and increased myelomonocytic differentiation.

Conclusions. Taken together, our data suggest that pediatric AML may have a broad heterogeneity in metabolic requirements and capacities as well as mitochondrial energetic through BALR-2 expression.

NEW THERAPEUTIC OPPORTUNITIES FOR PEDIATRIC PATIENTS WITH T(6;11)-REARRANGED ACUTE MYELOID LEUKEMIA

Tregnago C, Bisio V, Benetton M, Borella G, Basso G and Pigazzi M.

The t(6;11)(q27;q23) MLL-AF6 translocation is associated with the worst prognosis among cases of pediatric MLL-rearranged acute myeloid leukemia (AML); discovery of pathways specifically targetable with drugs is, therefore, highly desirable. In this work, we performed a high throughput drug-screening assay testing a library of 1,280 pharmacologically active compounds in vitro on a series of different myeloid leukemia subtypes to specifically target the oncogenic MLL-AF6 chimera. We identified 10 compounds selectively decreasing cell viability of t(6;11)-rearranged cells. We prioritized fluspirilene and thioridazine, two antipsychotics drugs acting as dopamine-receptor (DR) antagonists. We found that all five DRs were highly expressed on both cell lines and AML primary blasts harboring the t(6;11); increased apoptosis and reduced clonogenic potential was observed when these cells were treated with one of the

selected drugs, thioridazine resulting the most powerful. Evaluation of the pathway affected by DRs inhibition showed decreased ERK phosphorylation, indicating that the RAS pathway depends on DRs activity. Targeting the RAS pathway by simultaneously using two non-overlapping drugs, a MEK inhibitor and thioridazine, produced increased reduction of phospho-ERK levels in vitro. Thioridazine, when used as single agent and even more when coupled with low-dose cytosine arabinoside, showed high efficacy in t(6;11)-AML xenografted mice, confirming that use of thioridazine may represent a novel approach to treat MLL-AF6-rearranged patients. These results confirm the unique biology of MLL-AF6 gene fusion, and suggest that innovative strategies targeting the RAS-driving transforming pathway may lead to improved cure rate of these children.



DEVELOPMENT OF INNOVATIVE PRECLINICAL IN VITRO AND IN VIVO TOOLS FOR AN EFFECTIVE THERAPEUTIC STRATEGY IN PEDIATRIC ACUTE MYELOID LEUKEMIA

Borella G and Bisio V, Da Ros A, Tregnago C, Basso G, Pigazzi M

Background. Pediatric patients with acute myeloid leukemia (AML) improved their outcome over these last twenty but 30% relapsed, and 5% of patients die from treatment-related complications.

Purpose. We aim to set up an innovative in vitro and in vivo three dimensional (3D) model to perform high throughput drug screening to be finalized into clinic trials of pediatric AML.

Material and methods: To mimic the BM niche we cultured blasts together with mesenchymal stem cells (MSCs) in an engineered hydroxyapatite scaffold up to 21 days. We performed gene expression analysis of MSCs derived from patients to uncover their properties with respect to healthy-MSCs. This 3D-AML model was used for a preliminary screening of 160 drugs from a commercial library of FDA-approved compounds, to target AML-MSCs. We transplanted 3D-scaffold+AML in immunodeficient mice to generate an in vivo

model.

Results. 3D-cultures sustained AML blast proliferation in vitro, and cell immunophenotype was comparable to those of the leukemia at diagnosis. The unsupervised hierarchical clustering showed that AML-MSCs clustered separately from the h-MSCs. Drug screening performed on AML-MSCs identified Lercanidipine active on them, both in 2D and 3D, without toxicity over h-MSCs. The combination of Lercanidipine with chemotherapics resulted highly synergic in 3D-cultures. The in-vivo implants of the 3D-AML are under evaluation.

Conclusion. We successfully created a new in vitro 3D-AML model by mimicking the human leukemic BM niche. We support the use of AML-MSCs with the blasts as a new innovative tool to permit a long-term culture of primary leukemia cells and predict more effective drug effects.

GENERATION OF AN IN VITRO 3D MODEL THAT SUSTAINS A RESERVOIR OF PATIENT DERIVED (PD) CELLS FOR STUDIES OF JUVENILE MYELOMONOCYTIC LEUKEMIA

Cani A, Tretti C, Rampazzo E, Frasson C, Persano L, Masetti R, Basso G, te Kronnie G and Bresolin S

Juvenile myelomonocytic leukemia (JMML) is a rare early childhood clonal hematopoietic disease characterized by hyper-proliferation of mature granulocytic and monocytic cells. Hematopoietic stem cells transplantation with a high risk of failure in about 50% of patients is the only curative treatment for this devastating disease. Ninety-five percent of JMML patients harbor mutations in genes related to RAS-pathway and a spread of secondary mutations were also identified in these patients related to disease outcome. Due to the nature of JMML bone marrow cells the generation of in vitro cell line models failed hitherto and has been considered unrealistic all together. The lack of an in vitro model has evidently hampered studies of JMML and the development of new treatment strategies. To better characterize the role of the JMML somatogenetic architecture and its relationship with the tumor microenvironment as crucial factors for therapeutic outcome, we established an optimized a serum-free in vitro culture 3D model. Aiming to reproduce the JMML in vitro niche a semisolid matrix was co-

cultured with PD-JMML cells and BM-derived mesenchymal stem cells (MSCs) The model efficiently supports the proliferation of PD-JMML cells for more than 60 days in culture under hypoxic conditions and recapitulates disease features. In particular, we observed a selection of an immature cell population characterized by activation of specific transcriptional circuits involved in the maintenance of an immature and proliferative status of JMML cells mediated by hypoxia. Moreover, we established CD34+ cells as the JMML initiating cells that support the growth of JMML cells. In conclusion, we present here an in vitro system that efficiently initiates and sustains proliferation of immature JMML cells. In this 3D culture system we observed cross-talk between stromal and hematopoietic cells through mechanisms that can not be reproduced in the canonical 2D culture. This functional model strongly contributes to unravel mechanisms related to therapy resistance of JMML cells and treatment failure in JMML related to genomic and transcriptomic alterations of the malignant cells.

LEUKEMIA AND LYMPHOMA PREDISPOSING GENETIC LESIONS IN PEDIATRIC PATIENTS

Bresolin S, te Kronnie G

The prevalence and spectrum of predisposing mutations among children and adolescents with cancer are largely unknown. It is estimated that at least 5-10% of cases diagnosed with cancer harbor constitutional genetic mutations that increase their lifetime cancer risk. Predisposing genetic lesions consist of constitutional chromosomal anomalies and sequence variants located within or near cancer predisposing genes. Genetic leukemia /lymphoma predisposition is a relatively new field of research. Recent WES-based studies found that a number of rare variants give rise to distinct leukemia/lymphoma prone syndromes. For some of these syndromes ALL represents the main cancer type e.g. rob(15;21)(q10;q10) c, PAX5 and ETV6 deficiency, whereas for other conditions leukemia is part of a more broad spectrum e.g. Li-Fraumeni syndrome, constitutional mismatch repair deficiency, ataxia telangiectasia and primary immune deficiencies. In addition some developmental syndromes only lead to a mild to moderate increase of leukemia risk (e.g. Noonan, Sotos, Weaver, Rubinstein Taybi syndromes). Leukemia/lymphoma predisposition genes can be broadly categorized into two groups: i) DNA repair genes (BLM, ATM, NBS1, MLH1, MSH2, MSH6 and PMS2) and ii)

genes involved in somatogenetic aberrations associated with leukemia/lymphoma (PAX5, RUNX1, ETV6, PTPN11, SH2B3 and NF1). The LFS associated gene TP53 can be assigned to both groups.

IKZF1 a B-cell development associated gene, often deleted in BCP-ALL, was one of the ALL risk alleles identified through genome wide association studies. In addition we recently published amplicon deep sequencing data on the mutational status of IKZF1 in pediatric Ph+ leukemia patients reporting 14 variants with predicted deleterious effects in the IKZF1 coding sequence corresponding to 12 distinct mutations in 12% of 98 patients screened (Lana et al. Leukemia, 2015). Three of the identified variants had a particular high (>50%) variant allele frequency pointing to a potential germline origin of the mutation. Follow-up studies of one of these mutations confirmed the germline nature of the mutation and also revealed the inherited origin of the mutation. Subsequent analyses of several cohorts of BCP-ALL (in total 4963 patients) revealed that 1% carried IKZF1 germline mutations, clearly identifying IKZF1 as one of the focuses of genetic predisposition research in ALL.

CIRCULAR RNA EXPRESSION, A 'NOT TO BE MISSED' COMPONENT IN LEUKEMIA RESEARCH

Gaffo E, Boldrin E, Dal Molin A, Bresolin S, Frasson C, Guglielmelli P, Vannucchi A, Debatin KM, Basso G, Meyer L, te Kronnie G and Bortoluzzi S

The human genome is sprinkled with circular RNA loci. By regular transcription these loci produce RNAs, but the two ends of the (in most cases) exonic transcripts are covalently closed by noncanonical splice reactions. This special class of RNAs has incited both basic and translational research and during the last years hundreds of studies have been published supporting the view that circular RNAs have functions that can diverge from those of canonical messenger RNAs or long noncoding RNAs. CircRNAs are expressed in all human tissues, including the hematopoietic compartment (Bonizzato et al. BCJ 2016) and the first results of leukemia research points at deregulation of circRNA expression and oncogenic potential of circRNAs transcribed from leukemia-specific chromosomal fusion loci.

We aim to identify specificities of the circRNAome of MLL rearranged (MLLre) acute lymphoblastic leukemia and order to establish the role of circRNAs in this devastating malignancy.

Analysis of high depth RNA-seq data sets of normal hematopoietic cell populations and three MLLre specimens using the CirComPara pipeline (Gaffo et al. ncRNA J. 2017) detected abundant circular RNA expression in both normal and malignant cells and circularity and backsplice junctions were confirmed by RNase R treatment and Sanger sequencing. Of the identified circular RNAs 34% was novel and circRNA and linear gene expression were in general poorly correlated.

Comparison of circRNAs expressed in MLLre ALL and normal cells of mature and stemcell populations identified specificities of leukemic cells. Of 12,200 highly expressed circRNAs, 15% had MLLre-characteristic expression. These circRNAs belong to known leukemia-associated loci, non-coding RNA genes, and newly detected circRNA genes. After a first validation round, a set of candidate circRNAs will be taken to in depth studies of molecular mechanisms and malignancy related functions.



RIBOCICLIB, A CDK4/CDK6 KINASE INHIBITOR, ENHANCES GLUCOCORTICOID SENSITIVITY IN B-ACUTE LYMPHOBLASTIC LEUKEMIA (B-ALL)

Bortolozzi R, Mattiuzzo E, Trentin L, Accordi B, Basso G, Viola G

Dysregulation of the cyclin D1-CDK4/CDK6 complex is frequently observed in almost all human cancer and contributes to aberrant cell proliferation and consequent tumorigenesis. Although many reports described the importance of CDK4/CDK6 in different set of human tumors, only few studies have been performed on leukemia. By gene expression analysis performed in a cohort of childhood patients affected by B-acute lymphoblastic leukemia (B-ALL) we found that both CDK4 and CDK6 are highly expressed. Moreover, Reverse Phase Protein Array (RPPA) analysis showed that cyclin D1 levels are higher in patients undergoing relapse. Starting from these considerations, we evaluated the effect of dual inhibition of CDK4/CDK6 in B-ALL and if this inhibition could enhance cytotoxic killing of leukemia cells after combination treatment with dexamethasone. We treated B-ALL cell lines with ribociclib, a highly specific CDK4/6 inhibitor. Treatment with ribociclib induced

growth inhibition of B-ALL cell lines, accompanied by strong cell cycle arrest in G1 phase, along with a dose-dependent decrease in phosphorylated retinoblastoma protein. Ribociclib exposure strongly synergize with dexamethasone in SEM and RCH-ACV, two dexamethasone-resistant cell lines, along with a strong decrease of proliferation and a significant increase of apoptotic cell death. These results were also confirmed on primary cultures derived from bone marrow of B-ALL pediatric patients. Immunoblot analysis showed a significant increase in glucocorticoid receptor (GR) along with some of its target genes, after combined treatment. Altogether our findings support the concept that pharmacologic inhibition of CDK4/CDK6 may represent a useful therapeutic strategy to control cell proliferation in B-ALL and provide new insight in understanding potential mechanism of glucocorticoid resistance.

THE NOVEL CHOLINE KINASE ALPHA INHIBITOR EB-3D INDUCES CELLULAR SENESCENCE IN TRIPLE-NEGATIVE BREAST CANCER AND REDUCES TUMOR GROWTH AND METASTATIC DISSEMINATION

Mariotto E, Bortolozzi R, Ronca R, Carta D, Accordi B, Serafin V, Basso G, López-Cara LC, Viola G

Choline kinase (ChoK) is the first enzyme of the Kennedy's pathway leading to the biosynthesis of phosphatidylcholine (PtdCho), the most abundant phospholipid in eukaryotic cell membranes. Compound EB-3D is a novel ChoK 1 inhibitor with a potent antiproliferative activity against a panel of several cancer cell lines. ChoK 1 is particularly overexpressed and hyperactivated in aggressive breast cancer. Here we report that EB-3D strongly impairs triple-negative proliferation, migration and invasion and the effect is irreversible. Reverse-phase protein array (RPPA) data revealed the activation of the metabolic sensor AMPK causing the dephosphorylation of mTORC1 downstream targets such as 4E-BP1, p70S6K and S6K suggesting that EB-3D may affect protein synthesis. Moreover, we demonstrate that EB-3D strongly synergizes with drugs used for triple-negative breast cancer treatment, in

particular with cisplatin. The absence of cell death previously reported in MDA-MB-231 following EB-3D treatment is essentially due to the induction of cellular senescence. Moreover EB-3D-induced senescence significantly sensitizes MDA-MB-231 cells to the apoptotic effect of cisplatin. The antitumor potential of EB-3D was evaluated also in vivo in syngeneic orthotopic EO771-C57BL/6 mouse model of breast cancer. The compound induces a significant reduction of the tumor mass at low doses. In addition, we also tested the possible anti-metastatic effect of EB-3D in both syngeneic EO771-C57BL/6 and xenogeneic NOD/SCID model engrafted with MDA-MB-231. In both model we observed a significant reduction of lung metastasis. Altogether, our results indicate that the novel ChoK 1 inhibitor EB-3D could be a promising new anticancer agent to improve aggressive triple negative breast cancer treatment protocols.



BIOLOGICAL EVALUATION OF NOVEL NOTCH1 LIGANDS IDENTIFIED BY COMPUTER-AIDED DESIGN

Rruga F, Bortolozzi R, Dal Pra M, Brancale A, Viola G

The Notch receptor is part of a core signalling pathway that has a central role in regulating cell proliferation, differentiation, development and homeostasis. Dysregulation of NOTCH signalling has been related to many human diseases, and it is one of the most commonly deregulated signalling pathway in cancer. Notch is a transmembrane protein that can interact with different ligands present on the extracellular surface of the cell membrane. In humans, there are numerous ligands identified. When a ligand and the receptor interact, two proteolytic events occur. One by ADAMs (S2) which liberates the extracellular EGF-like repeats. And secondly by -secretase (S3) which releases the NOTCH intracellular domain (NICD) that is responsible for transcriptional activity. We identify two potential binding sites that can

be explored in order to find small molecules able to activate or to block the Notch pathway: the binding site of Jagged1 in the Notch-1 surface and the negative regulatory region (NRR) of Notch-1. Performing a virtual screening of a library of 3×10^6 of small molecules, using a Structure-based, a Shape-based and a cross-combination of these two approaches, we identified 19 compounds that potentially target ligand binding site and 43 compounds potentially able to target NRR. The activity of these 62 compounds has been biologically evaluated in vitro in a panel of T-ALL cell lines, identifying 29 compounds able to inhibit cell proliferation with $GC50 < 100 \mu M$. Among them we identified, by immunoblot analysis and q-PCR, 11 best candidates able to inhibit Notch1 cleavage and the transcription of its target genes Hes1 and Hey1.

THE PRESENCE OF MUTATED AND DELETED PTEN IS ASSOCIATED WITH AN INCREASED RISK OF RELAPSE IN CHILDHOOD T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA TREATED WITH AIEOP-BFM ALL PROTOCOLS

Paganin M, Grillo MF, Silvestri D, Scapinello G, Buldini B, Cazzaniga G, Biondi A, Valsecchi MG, Conter V, te Kronnie G and Basso G

MP and MFG contributed equally to this work; GtK and GB contributed equally to this work

In T acute lymphoblastic leukemia cells showed loss of the PTEN tumor suppressor gene as a result of mutations or deletions.

Several leukemia study groups tried to understand if PTEN status could be a biomarker for refining early patient stratification at diagnosis and the question is still open.

We analyzed PTEN by sequencing the exon 7, in a cohort of 213 AIEOP T-ALL paediatric finding PTEN mutated in 12.1% (31/257) of the patients. The presence of the mutation is associated with a 5-year CIR of 38.7% (SE 8.7) while in the not mutated patients the 5-year CIR is 20.2% (SE 2.8); the difference between the two groups is statistically significant with a p-value=0.004.

PTEN status remain a useful genetic characteristic that predicts a worse outcome also in the group of patients with NOTCH1 not mutated.

In a cohort of 213 patients we analyzed also the presence of the deletion in PTEN. We detected in 14 out of 213 (6.6%) patients both mutation and deletion. Patients with co-presence of the two aberrations had a 5-year CIR of 42.9% (SE 13.2) significantly different from the CIR of 20.2% (SE 3.1) of the remaining patients (p-value=0.01).

In this AIEOP study on T-ALL patients we provide relevance to PTEN aberrations as a prognostic marker. It would be really important to perform a meta analysis of patients from several T-ALL cohorts so that a conclusion can be achieved on the utility of analyse PTEN status in the diagnostic for risk classification improvement.



LIQUID BIOPSIES IN PEDIATRIC RHABDOMYOSARCOMA: POTENTIAL TO IDENTIFY CIRCULATING TUMOR CELLS AND CELL-FREE DNA

Tombolan L, Zin A, Rossi E, Binatti A, Bortoluzzi S, Zamarchi R, Bonvini P and Bisogno G

Rhabdomyosarcoma (RMS) is the most common Soft Tissue Sarcoma in childhood accounting for 4% of all childhood malignancies. RMS develops from immature mesenchymal cells committed to skeletal muscle differentiation. Distant metastasis occurs at diagnosis in 20-25% of cases and their presence is the most negative prognostic factor. Chances of an effective cure in childhood RMS rely on the capacity to make an early and accurate diagnosis, detect metastatic disease and predict the response to treatment. Liquid biopsies, in particular the analysis of circulating tumor cells (CTCs) and cell-free circulating DNA (ctDNA), represent a powerful and non-invasive tool to discover novel biomarkers in pediatric solid tumor.

Thus, we evaluated EpCAM positive (CellSearch CS) and EpCAM low/negative (Autoprep-Sample-Collection-Device, ASCD)

Circulating Cells in peripheral blood and bone marrow of a cohort of pediatric RMS patients implementing the standard assay with a specific RMS marker (desmin). We detected CTCs in 67% of patients (4/6 pts) with both CS and ASCD device.

Parallel, we isolated ctDNA from plasma of RMS patients and performed a NGS analysis on ctDNA (targeted sequencing) and paired tumor samples collected at diagnosis (whole exome sequencing, WES). Analysis of sequencing data identified variants already associated to RMS (BRAF, FGFR4) as well as novel genomic alterations. Notably, a mutation in an important cancer gene was detected by WES in primary tumor and it was confirmed by ddPCR, in matched ctDNA and CTC, providing relevant new data on RMS dissemination mechanisms and a proof of principle of the proposed methodology.

HIGH-THROUGHPUT SCREENING OF CANCER AUTOANTIBODIES IN PLASMA OF CHILDREN WITH RHABDOMYOSARCOMA: A NOVEL APPROACH TO IMPROVE DISEASE DETECTION AND RISK ASSESSMENT

Poli E, Cattelan M, Zin A, Bisogno G, Bonvini P

Blood is the most accessible and less invasive biological material for biomarker discovery in cancer patients. Biomarker detection in blood is likely to become important for monitoring disease response to treatment and recurrence, either when biomarkers are produced by the tumor or represent a specific host response. It is well established that cancer elicits both cell-mediated and humoral immune response, the latter of which results in the production of tumour-associated autoantibodies (TAABs) against aberrantly expressed tumour-associated antigens (TAAs). Cancer autoantibodies are easily accessible in blood, have a longer half-life than antigens and because they reflect cancer immunologic reactivity are useful plasma-based biomarkers.

Herein, we performed a comprehensive characterization of the immune response biomarker profile of plasma samples from PAX3-FOXO1A-positive alveolar

rhabdomyosarcoma (ARMS) patients with localized and metastatic disease at diagnosis, employing a high-throughput protein microarray technology comprised of >9,000 GST-purified recombinant proteins. Antigens exhibiting reactivity to circulating autoantibodies were validated by indirect ELISA assay and used to generate panels suitable to improve disease detection, likewise to provide insights about tumor growth and evolution.

By performing proteome-scale analysis of RMS plasma samples we were able (1) to demonstrate the presence of antigens exhibiting stronger immunoreactivity in RMS patients compared to healthy donors, (2) to generate antigens cancer panels capable to improve disease detection and progression, (3) to identify circulating autoantibodies correlated with metastatic condition at diagnosis, (4) to reveal specific tumor antigens involved in RMS onset and progression.



COMPREHENSIVE CHARACTERIZATION OF HUMAN PLASMA-DERIVED EXOSOMES IN ANAPLASTIC LARGE CELL LYMPHOMA OF CHILDHOOD

Lovisa F, Gaffo E, Bortoluzzi S, Garbin A, Di Battista P, Carraro E, Faruggia P, Sala A, Piglione M, Vinti L, S.G. D'Amore E, Basso G, Pillon M, Mussolin L

Background. Anaplastic Large-Cell Lymphoma (ALCL) represents approximately 20% of paediatric non-Hodgkin's lymphomas of childhood. Modern therapeutic strategies lead to cure >70% of patients, whereas refractory/relapse diseases convey a dismal prognosis. Liquid biopsy has the potential to help clinicians screen for disease, stratify patients to the best treatment and monitor therapy response. In this field, evidences are accumulating on the role of plasmatic exosomes as cancer biomarkers and inter-cellular messengers actively contributing to the disease process.

Objectives. To analyze the role of plasmatic exosomes on tumorigenesis and disease progression in paediatric ALCL.

Patients and Methods. Plasmatic exosomes were obtained from plasma samples at diagnosis of 20 ALCL patients. Exosomal RNA was extracted and processed for small RNA-seq on an Illumina platform. Data were analyzed by using the software miRmore and DESeq2. In parallel, exosomal proteins were investigated by

Matrix-Assisted Laser Desorption/Ionization and Liquid Chromatography-tandem mass spectrometry.

Results. RNA-seq data analysis revealed different sRNA expression profiles among exosomes of healthy donors, remission and relapsed patients. miR-144-3p was found downregulated in both tumors' vs healthy donors'exosomes and in remission vs relapsed patients'exosomes, suggesting its role as oncosuppressor. Functional studies are in progress to validate putative oncomiRs.

Preliminary proteomic profiles of plasmatic exosomes showed 19 proteins specifically expressed in tumour exosomes, including integrins 1 and 6.

Conclusions. The identification of exosome-associated biomarkers is expected to provide important information about the molecular characteristics of pediatric ALCL, which might be used to improve diagnosis, treatment and prognosis of the affected children.

RNA-SEQ ANALYSIS OF EXOSOME SMALL RNA CONTENT IN PAEDIATRIC ALCL PATIENTS: A NEW ANALYSIS PIPELINE AND PRELIMINARY RESULTS

Di Battista P, Gaffo E, Lovisa F, Bortoluzzi S, Mussolin L

Background. Anaplastic Large Cell Lymphoma (ALCL) is a non-Hodgkin lymphoma that involves T cells. Emerging evidence shows that vesicular transport plays a critical role in cancer behavior. Microvesicles, in particular exosomes, carry proteins, transcripts and small non-coding RNAs (sRNA), known to actively contribute to the disease process.

Objectives. The main goals of our study were 1) Exosome transcriptional characterization; 2) Paired exosomes and primitive tumor cells comparison; 3) Complete remission (CR) and relapsing (REC) samples comparison.

Methods. RNA-seq, an high-throughput transcriptome analysis technique, was used to collect data. To identify and quantify the sRNAs including those not belonging the miRNA class, we implemented a new computational pipeline to extend the miR&moRe software capabilities. It included third party libraries and programs, used in a custom-conceived architecture. The pipeline

flow considered 1) quality and length sequence filtering on the reads; 2) mapping to human reference sequences without mismatches of the good quality reads; 3) integration with genome annotation of the alignments to identify the expressed genes and to quantify their abundance.

Results. Preliminary results show that miRNAs represent only a fraction of exosomes' content. Indeed, a considerable amount of non-miRNA sRNAs was identified, the most abundant class derived from Y-RNA genes and pseudogenes. Y-RNAs are non-coding sequences implicated in RNA processing, quality control and initiation of DNA replication.

Conclusions. In addition to miRNA profile, other sRNAs in tumor plasmatic exosomes could provide important information about the molecular mechanisms that characterize this aggressive lymphoma, and that could be used to improve treatment of affected children.



NPM-ALK EXPRESSION LEVELS ARE ASSOCIATED TO DIFFERENT MIRNA PROFILES IN PAEDIATRIC ANAPLASTIC LARGE CELL LYMPHOMA

Garbin A, Lovisa F, Di Battista P, Carraro E, Pizzi M, Garaventa A, Tondo A, Mura R, Buffardi S, Basso G, S.G. D'Amore E, Pillon M, Mussolin L

Background. Anaplastic large cell lymphoma (ALCL) is characterized by CD30 overexpression and frequently NPM-ALK chimeric protein expression. We previously identified two distinct subgroups of ALCL patients based on different gene expression signatures related to NPM-ALK transcript levels (ALK-low and -high).

Methods. miRNA expression profiling was conducted on 16 ALCLs (5 ALK-low and 11 -high) by microarray. Selected differentially expressed miRNAs were validated in 40 cases by qRT-PCR. Survival analyses were conducted using median value as a cut-off for patients' stratification. MiRNA putative targets were identified by miRanda tool. To assess the impact of specific miRNAs deregulation, we transfected SUPM2 cells with miRNA mimic and evaluated target protein expression by immunoblotting.

Results. Microarray analysis identified 19 miRNAs upregulated in ALK-low patients. We confirmed by qRT-PCR the differential

expression of miR-155, miR-100, miR-146a and miR-939. MiR-939 and miR-155 expression below the median value seems to be associated to a worse prognosis (EFS% 63 vs. 80 for both miR-939 and miR-155, p-values n.s.). In silico analyses identified JUNB as a putative target of miR-939. Overexpression of miR-939 in SUPM2 cell resulted in JUNB and AKT1 downregulation. In addition, the expression of CD30 (a known JUNB target) was reduced in patients expressing high miR-939 levels.

Conclusions. miR-939 prognostic significance should be confirmed in an extended cohort of patients. Luciferase assay will be conducted to confirm miR-939/JUNB 3'UTR interaction. Subsequently, to investigate the heterogeneous levels of aggressiveness observed in ALCL tumour, we will assess the effects of miR-939 overexpression/CD30 downregulation on response to anti-CD30 therapies.

MICROBIOREACTOR PLATFORMS AS IN VIVO-LIKE SYSTEMS TO PROBE THE ROLE OF NEUROBLASTOMA-DERIVED EXOSOMES IN CANCER DISSEMINATION

Elisa Cimetta

Most of the complexity of the microenvironment (μ Env) surrounding cells in our bodies is lacking in standard laboratory in vitro models, leading to readouts poorly predicting the actual in vivo situation. Engineers possess the knowledge and tools to develop technological solutions that, when coupled with biomedical expertise, can help surpass these limitations. The need to more faithfully recreate the in vivo μ Env is particularly felt in the field of tumor biology, as tumors are extremely heterogeneous, can condition the μ Env and are strongly affected by it. Exosomes, small vesicles secreted by the tumor, are a fundamental form of communication between cancer and the surrounding environment, influencing a host of target cells locally and at a distance. In one type of cancer, Neuroblastoma (NB), exosomes seem to be particularly important; yet, current approaches used to study their role in NB communication with local and distant μ Env fall short of providing clear answers. In particular, standard in vitro approaches lack: i. the possibility to generate exosome gradients, crucial to test their local and long-range effects; ii. the throughput required to test the limited exosome material on downstream processes; iii. the possibility to test gradients on 3D cultures

mimicking faithfully what happens in vivo; iv. the ability to explore long-range effects of NB-derived exosomes on in vitro replicas of target tissues.

Given these limitations, and based on our previous experiments, we hypothesize that the use of microbioreactors (μ BRs) and microscale technologies exploiting classical engineering principles would solve the limitations of the existing in vitro models used to study NB-derived exosomes. Our μ BRs generate time and space-resolved concentration gradients, support fast dynamic changes and reconstruct complex interactions between cells and tissues while performing multifactorial and parallelized experiments.

Key to all our platforms will be their capability to enable highthroughput screening of environmental effectors of tumor behavior in a more realistic setting. Our technologies have the potential to bridge the gap between standard in vitro techniques and in vivo biological phenomena, and will provide an unprecedented tool to understand certain aspects of cancer biology and ultimately advancing our understanding of NB.



TYROSINE KINASES AND TYROSINE KINASE RECEPTORS IN NEUROBLASTOMA: THERAPEUTIC TARGETING AND CORRELATION WITH AUTOPHAGY INDUCTION

Aveic S, Pantile M, Corallo D, Polo P, Sidarovic V, De Mariano M, Quattrone A, Longo L, Tonini GP

A growing field of evidence suggests the involvement of receptor tyrosine kinases (RTKs) in the cell transformation. Hyperactivation of RTKs, as the essential mediators of several cell signaling mechanisms, can occur due to an overexpression, dysregulation, or mutations in the RTK coding genes. At the clinical level the RTK-modifications can determine prognosis and therapeutic responses in several types of cancer, including neuroblastoma (NB). Therefore, successful targeting of RTKs remains a challenge. During the last three decades numerous drugs have been developed for a direct inhibition of RTKs or their downstream signaling pathways including MAPK, AKT, PI3K and mTOR tyrosine kinases. Nevertheless, it became evident that the acquired resistance to RTK inhibitors (RTKi) is a serious clinical problem. Autophagy activation is among the possible obstacles

for an adequate efficacy of RTKi. Autophagy is an evolutionarily conserved cellular mechanism that allows a recycling of the intracellular, metabolic-related, molecules. It is a complex, multi-regulatory process that helps the cells to survive stressful situations, or triggers the death signals in case the stress is prolonged and overwhelming. Since two years we are studying the autophagy activation in NB cells upon treatment with different RTKi. Our goal is to define whether autophagy might be a limiting factor for the cytotoxicity of selected RTKi, and whether we could increase their therapeutic efficiency by combining RTKi with autophagy blocking agents. Our current results sustain the combined use of RTKi and autophagy inhibitors as a possible new approach that increases the efficacy of RTKi in NB.

The work has been supported by Italian neuroblastoma Foundation.

ROLE OF LIN28B IN NEURAL CREST DEVELOPMENT, DIFFERENTIATION AND NEUROBLASTOMA PATHOGENESIS

Corallo D, Candiani S, Ori M, Monticelli S, De Sarlo M, Aveic S, Tonini G

The RNA-binding protein LIN28 was initially identified as an important regulator of developmental timing. Besides its physiological roles during development, LIN28 is a negative regulator of miRNAs biogenesis leading to the upregulation of key oncogenes such as MYCN.

Neuroblastoma is a solid tumor of the peripheral sympathetic nervous system (PSNS) that originates from neuroblasts of the migratory neural crest (NCC) and it is responsible for about 15% of childhood cancer-related mortality. Genomic amplification with elevated expression of LIN28B was observed in patients with MYCN gene amplification and it is associated with neuroblastoma dissemination and poor prognosis. Notably, aberrant overexpression of LIN28 remarkably induces the epithelial to mesenchymal transition (EMT) and promotes metastasis in several cancers.

However, a precise mechanism by which LIN28B participates in modulation of NCCs behavior and EMT in NB has not been still clarified. Here we show that transient overexpression of LIN28B in zebrafish and *Xenopus* model systems accelerates the migratory pattern of trunk NCCs and induces the expression of mesenchymal markers in the whole embryos, suggesting an activation of the EMT program. Moreover, we show that the overexpression of LIN28B facilitates the malignant phenotype in neuroblastoma by blocking neuroblast differentiation, as observed in the transgenic MYCN zebrafish model of NB. Together, our studies show that aberrant programmed expression of LIN28B in PSNS precursors is an effective driver of neuroblastomagenesis.

The work has been partially supported by Italian neuroblastoma Foundation.



NEUROBLASTOMA-PATIENT DERIVED ORGANIDS RETAIN GENOTYPE AND MORPHOLOGIC PATTERN OF TUMOR ORIGIN

Fusco P, Parisatto B, Rampazzo E, Persano L, Frasson C, Di Meglio A, Leszl A, Santoro L, Cafferata B, Zin A, Basso G, Tonini G, Esposito MR

Background. Neuroblastoma (NB) is a pediatric tumor of the sympathetic nervous system and half of all cases are High Risk (HR) with an overall survival less than 40% at 5 years from diagnosis. Recently, a new 3D organoids model able to recapitulate tumor growth in vivo and mimicking the morphology, genome and microenvironment of the tissue of origin developed from various tumors. To date this model is useful for pre-clinical drug screening and study of biological pathways.

Aim. The main aim is to establish organoids model from NB patients biopsies, capable of self-organizing in a structure with composition and genome reflecting the tissue of origin.

Results. We established 6 organoids derived from 4 HR-NB patients' primary cells provided from Molenaar's group and 2 derived from biopsies of 2 HR-NB patients at the diagnosis. The organoids retained all main features of the NB tumor of origin:

expression of specific marker such as NB84, and synaptophysin; specific chromosomal aberrations such as 1p36 loss, 17q gain, 11q loss and MYCN gene amplification; positivity to p75, DBH, TH proteins specific for adrenergic lineage. We confirmed the high positivity to the neuroblast markers CD56, the heterogeneous expression of stemness marker CD133 and the presence of cell subpopulations, like CD15-/CD29-/CD24+ representing the subpopulation neural cell differentiating towards neuroblasts and CD44-/CD24+ indicating high aggressive phenotype.

Conclusions. We were able to realize a novel NB preclinical model exhibiting self-renewal property providing a reservoir of NB patients' biological samples to study NB pathogenesis and to perform personalized drug screening.

The work has been supported by Italian neuroblastoma Foundation.



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Genetics and Rare Diseases

ISOLATION AND CHARACTERIZATION OF URINARY EXTRACELLULAR VESICLES IN THE FIELD OF NOVEL KIDNEY DAMAGE BIOMARKERS

Carraro A, Negrisolò S, Fregonese G, Murer L

Extracellular vesicles are lipid membrane-bound nanoparticles released from various cells type. They carry different types of cargo (e.g. miRNA, proteins) reflecting the physio pathological status of the cells and organs they originated from. Specifically, urinary extracellular vesicles (UEVs) and their miRNA content, could be helpful as novel biomarkers for kidney allograft injury. Due to their low amount, UEVs concentration and characterization remain a challenge. In this study we aim to identify the most efficient method to isolate UEVs and evaluate their miRNA content.

UEVs were isolated from urine samples using four different commercial kits. Urine and the isolated samples were purified by Izon qEVsingle SEC. Pre and Post purification of UEVs samples were quantified showing different concentrations, size and protein contamination. miRNA fraction of these

samples were extracted by commercial kit and quantified by Bioanalyzer 2100.

The UEVs isolated by different methods showed a high variability in concentration and size. Pre purified UEVs samples show a raw concentration between 1.27×10^8 – 4.38×10^9 with a protein concentration between 0.42 – 8.17 mg/ml. Post purified UEVs samples showed a concentration between 1.53×10^7 – 6.47×10^8 and a protein contamination of 0 mg/ml. The particle size of UEVs samples were found to be in the range of the microvesicles. miRNA analyses show a range of concentration between 37 – 231 pg/ μ l.

Based on these results the most efficient method to isolate UEVs is the Izon qEVsingle SEC column while the high concentration of miRNAs is shown by QIAGEN.

INTRARENAL VIRAL INFECTION AND HUMORAL REJECTION: A RETROSPECTIVE STUDY IN PEDIATRIC RENAL TRANSPLANT RECIPIENTS

Fregonese G, Bertazza Partigiani N, Carraro A, Negrisolò S, Murer L

Humoral rejection represents one of the risk factors for reduced transplanted kidney survival. Different studies in literature have linked CMV and BK virus infections to humoral rejections. We led a retrospective study in pediatric recipients (m. age 11.1 6.9 y), transplanted at our Center from 2011 to 2016, to evaluate the possible correlation between acute/chronic humoral rejection and viral infections. Humoral rejection incidence has been calculated based on protocol biopsies performed at 6, 12 and 24 months after transplantation (Banff '15) in association with anti-HLA antibodies (positive DSA: MFI>3000). Viral positivity (CMV, EBV, BKV, Parvovirus B19) was defined in two conditions: a) systemic with a persistent viremia for the 3 months preceding control biopsy; b) intrarenal with viral DNA isolation in bioptic tissue. The clinical samples considered were 175

protocol biopsies, 42 diagnosed as acute rejection and 6 as chronic rejection (75% cellular vs 25% humoral). The study has shown that there is no correlation between systemic viral infection and humoral or cellular rejection. Furthermore, our analysis emphasized that the localization of at least one graft-level virus is more correlated to humoral rejection than to cellular rejection (75%vs29%, $p=0.04$). In particular, the presence of Parvovirus B19 is highly associated with humoral rejection compared to the cellular one (50%vs13%, $p=0.04$). Our outcomes suggest a major risk of humoral rejection in patients with intrarenal infections. In particular, in transplanted patients Parvovirus B19 could increase the risk of humoral rejection, amplifying the exposition of MCH II antigens through INF pathway.



EFFECTIVE DIAGNOSIS OF NEURODEVELOPMENTAL DISORDERS (NDDs) BY COMPUTATIONAL ANALYSIS AND NEXT GENERATION SEQUENCING

Leonardi E, Aspromonte MC, Polli R, Bettella E, Bellini M, Cesca F, Murgia A

Neurodevelopmental disorders (NDDs) are clinically and etiologically heterogeneous conditions often characterized by comorbidities, such as Intellectual Disability (ID), Autism Spectrum Disorders (ASD), and epilepsy. Whole exome sequencing (WES) studies of family trios with NDDs have revealed a significant excess of de novo mutations in probands compared to the normal population, yielding a rich source of novel candidate genes, converging on a few biological key processes. However, more genome and candidate re-sequencing studies are needed to identify subsets of genes playing prominent pathogenic roles and allowing to discriminate among overlapping phenotypes.

Using the Ion Torrent platform, we developed a diagnostic low-cost next generation sequencing gene panel already transferred into clinical practice, replacing

single disease gene analyses for early ID/ASD diagnosis. To date, 190 patients have been sequenced with the gene panel and a confident diagnosis has been reached in 33 cases (17%). Likely pathogenic mutations have been identified in another 28 patients, reaching a total diagnostic yield of 30%, which is very good for an NGS gene panel. Among patients negative on the gene panel, we selected a patient cohort with clinical features of Rett syndrome, a well-known genetic condition characterized by ID and ASD. These have been enrolled for WES trio analysis, currently under way for a set of 11 family trios. Our approach has the potential to discover novel candidates, expanding the genetic landscape of the Rett spectrum, providing novel connections between known Rett genes, and improving our understanding of the underlying molecular mechanisms of pathology.

MOLECULAR MECHANISMS AND PHENOTYPES IN FRAGILE X DISORDERS

Polli R, Cesca F, Bettella E, Leonardi E, Aspromonte MC, Bellini M, Murgia A

Fragile X Disorders (FXDs) define a family of distinct clinical conditions based on complex mutations of the Fragile X Mental Retardation 1 (FMR1) gene. The different clinical expression and age of onset of these disorders depend on opposite pathogenic mechanisms associated with different classes of FMR1 mutations, represented by expansions of triplet repeats (CGG)_n in the 5' UTR sequence of the gene. Three are the known Fragile X phenotypes: Fragile X Syndrome (FXS) a neurodevelopmental condition leading form of inherited intellectual disability and autism spectrum disorder (ASD) is caused by an expansion of the repeated region exceeding 200 CGG elements and typically coupled with epigenetic silencing of the gene function (full mutation). The other two conditions with adult onset: Fragile X-associated Primary Ovarian Insufficiency (FXPOI) and Fragile X

associated Tremor Ataxia Syndrome are due to typically unmethylated CGG expansions ranging from 55 and 200 (premutations), characterized by transcriptional overexpression of FMR1 resulting in the translation of an altered cytotoxic protein. 120 Fragile X Families have been identified, with a total of 395 mutated individuals of which 158 males and 237 females. We have characterized 172 FMR1 full mutations (57 females and 115 males) of which 32 cases of size or methylation mosaicism (18.6%) and three very rare cases of unmethylated full mutations. Of 222 detected premutations 6.3% resulted heterogeneous in size, demonstrating possible high somatic instability even in this mutational category. The Multidisciplinary Fragile X Padua Network is a national referral center for molecular and clinical evaluation of FXDs.



DISCORDANT PHENOTYPES IN MONOZYGOTIC TWINS WITH MUCOPOLYSACCHARIDOSIS TYPE VI: A GENETIC AND EPIGENETIC MULTI-OMICS ANALYSIS

D'Avanzo F, Campagna D, Zanetti A, Vitulo N, Marchioretto L, Önenli-Mungan HN, Quartel A, Scarpa M, Valle G and Tomanin R

Mucopolysaccharidosis type VI (MPSVI) is an inherited lysosomal storage disorder due to the deficit of arylsulfatase B enzyme. The deficit leads to a progressive multisystemic disease with high genetic and phenotypic heterogeneity, still poorly understood.

In this study we evaluated two female MPSVI monozygotic twins showing a discordant phenotype, with some symptoms exclusive of one child and other symptoms shared with different severity. To verify if these clinical differences can be somehow explained by genetic and epigenetic factors, a multi-omics analysis on leukocytes and fibroblasts has been performed.

The available associations between the discordant traits and genes/variants has been retrieved from different databases and crossed with data obtained from genome, exome, transcriptome and methylome sequencing. A low coverage genome sequencing confirmed twins' monozygosity and evidenced no structural differences; also exome sequencing revealed no unshared somatic variants in genes known to be associated with the discordant symptoms. RNA-Seq on skin fibroblasts evidenced 164 differentially expressed genes, 53 of which

are known to be associated with at least one discordant symptom. Among them, MMP3 and GRIK2 have previously been associated with mitral valve regurgitation severity and intellectual disability, respectively.

Methylome sequencing showed more than 180K differentially methylated CpG sites, located in promoters but also in exons, introns and intergenic regions. The functional enrichment analysis of differentially methylated genes revealed, among others, the over-representation of categories related to nervous system development and functions which can be associated with the discordant trait of intellectual disability. A good correlation between methylation and gene expression has been found.

Beyond giving some insights into the possible mechanisms influencing the clinical outcome of MPSVI, this study also underlines the important role of epigenetics in the pathophysiology of this disease.

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CHARACTERIZATION OF THE NEURAL PATHOGENESIS IN THE ANIMAL MODEL FOR MUCOPOLYSACCHARIDOSIS TYPE II

De Filippis C, Rigon L, Salvalaio M, D'Avanzo F, Marin O, Scarpa M and Tomanin R

Mucopolysaccharidosis type II (MPSII) is a rare, X-linked genetic disorder, characterized by glycosaminoglycans (GAG) accumulation in several organs, due to the deficit of the lysosomal enzyme iduronate 2-sulfatase (IDS).

We analyzed GAG levels in urine, liver and brain of wild-type (WT) and knock-out (IDS-KO) mice aged 6 to 22 weeks, aiming at evaluating the time trend of these markers.

We also analyzed several cerebral markers of pathogenesis to establish whether they can be used as biomarkers of pathology and potentially as index of therapeutic efficacy.

Urine and liver analyses showed significantly elevated GAG levels in IDS-KO compared to WT mice, increasing with time from 10 to 18 and 22 weeks of age, respectively; no significant differences were noticed in liver between 6 and 10 weeks of age.

Brain analysis showed significantly elevated GAG levels in IDS-KO compared to WT mice at all ages, although not increasing with age.

Western blot and immunohistochemistry analyses conducted for GFAP (Glial Fibrillary Acidic Protein) and immunofluorescence analysis for LAMP2 (Lysosomal Associated Membrane Protein 2) showed increased levels of proteins in IDS-KO compared to WT mice at all ages and with time.

Protein TUBB3 (Tubulin isotype III), which stimulates neurons maturation, was found expressed at lower levels in IDS-KO vs WT mice and this could represent an index of reduced neuronal differentiation.

Levels of SYNI (Synapsin I), a synaptic efficiency marker, and NF-H (Neurofilament Heavy Polypeptide), a marker of mature neurons, did not significantly differ in the two mice, however further analyses are at the moment in progress for these 2 biomarkers.

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ENZYME-LOADED NANOPARTICLES FOR CNS DRUG DELIVERY IN MUCOPOLYSACCHARIDOSIS TYPE II

Rigon L, Salvalaio M, Pederzoli F, Legnini E, Belletti D, D'Avanzo F, De Filippis C, Ruozi B, Marin O, Vandelli MA, Forni F, Scarpa M, Tosi G and Tomanin R

Mucopolysaccharidosis type II (MPSII) is a lysosomal storage disorder due to the deficit of the enzyme iduronate 2-sulfatase (IDS), leading to the accumulation of the glycosaminoglycans (GAG) heparan- and dermatan-sulfate in most organs, including brain in about two-thirds of the patients. Main treatment is the weekly infusion of the functional enzyme, which, however, cannot cross the blood-brain barrier (BBB) and act on the neurological involvement.

In this study, untargeted and brain-targeted polymeric nanoparticles (g7-NPs), already tested with a high molecular weight model drug [Salvalaio et al, PLoS One 2016;11(5):e0156452], were loaded with IDS and evaluated both *in vitro* and *in vivo*.

Fibroblasts from MPSII patients were treated for 7 days with untargeted IDS-loaded NPs, this restoring physiological IDS levels and normalizing GAG content.

For the *in vivo* study, a weekly *i.v.* administration of g7-NPs-IDS was performed in the MPSII mouse model for a total of 6 weeks. Biochemical, histological and immune-histochemical/

fluorescence evaluations of liver and brain were conducted, highlighting a significant reduction of GAG deposits and a general improvement of the markers tested (GFAP, LAMP2) in brain, although still insufficient to achieve a normalization to non-pathological levels.

To overcome this limitation, new NPs formulations are now under production and testing, with the aim to obtain higher levels of encapsulation and stability of the enzyme, while maintaining, or further improving, a good transport efficiency to the central nervous system.

To this aim, we are now optimizing the reaction volumes to obtain a greater encapsulation of the enzyme and a lower burst release. In addition, we are evaluating BSA and Tween20 as additives/stabilizers, which, tested with model enzymes, helped to increase encapsulation efficiency and maintain enzyme activity, respectively.

This project is financed by Fondazione CaRiPaRo [Bando Ricerca Pediatrica, grant #13/09 (2012-2014) and #17/06 (2016-2018)].

COX16 IS REQUIRED FOR ASSEMBLY OF CYTOCHROME C OXIDASE IN HUMAN CELLS AND IS INVOLVED IN COPPER DELIVERY TO COX2

Cerqua C, Morbidoni V, Desbats MA, Doimo M, Frasson C, Sacconi S, Baldoin MC, Sartori G, Basso G, Salviati L, Trevisson E

Cytochrome c oxidase (COX), complex IV of the mitochondrial respiratory chain, is comprised of 14 structural subunits, several prosthetic groups and metal cofactors, among which copper. Its biosynthesis involves a number of ancillary proteins, encoded by the COX-assembly genes that are required for the stabilization and membrane insertion of the nascent polypeptides, the synthesis of the prosthetic groups, and the delivery of the metal cofactors, in particular of copper. Recently, a modular model for COX assembly has been proposed, based on the sequential incorporation of different assembly modules formed by specific subunits. We have cloned and characterized the human homologue of yeast COX16. We show that human COX16 encodes a small mitochondrial transmembrane protein that faces the intermembrane space and is highly

expressed in skeletal and cardiac muscle. Its knockdown in *C. elegans* produces COX deficiency, and its ablation in HEK293 cells impairs COX assembly. Interestingly, COX16 knockout cells retain significant COX activity, suggesting that the function of COX16 is partially redundant. Analysis of steady-state levels of COX subunits and of assembly intermediates by Blue-Native gels shows a pattern similar to that reported in cells lacking COX18, suggesting that COX16 is required for the formation of the COX2 subassembly module. Moreover, COX16 co-immunoprecipitates with COX2. Finally, we found that copper supplementation increases COX activity and restores normal steady state levels of COX subunits in COX16 knockout cells, indicating that, even in the absence of a canonical copper binding motif, COX16 could be involved in copper delivery to COX2.

C. ELEGANS AS A MODEL TO INVESTIGATE NOVEL GENES INVOLVED IN HUMAN GENETIC DISORDERS

Morbidoni V, Cerqua C, Desbats MA, Pannone L, Martinelli S, Sandri M, Salviati L, Trevisson E

Caenorhabditis elegans is a transparent nematode existing prevalently as an hermaphrodite. Around 60-80% of human genes have a corresponding homologue in worm and many disease pathways are conserved between *C. elegans* and higher organisms, making it an effective *in vivo* model.

Genetic interference mediated by double-stranded RNA (RNAi) has been a valuable tool in the analysis of gene function in *C. elegans*. Using this approach we silenced some COX assembly genes required for COX biogenesis (*cox-16*, *cox-19*, *cox-17*, *cox-11*, *sco-1*) and performed an extensive phenotypic characterization of silenced animals.

A COX-specific histochemical staining in worms allowed us to demonstrate that these genes are essential for COX activity, thus establishing multicellular models of COX deficiency that can be employed to test

novel therapies.

With the same approach we investigated TO1G9.2, a protein whose function is still unknown but is potentially involved in autophagy. However, since we couldn't detect a clearcut phenotype in silenced worms and considering that RNAi efficiency is extremely variable, we are developing a knock-out model using CRISPR/Cas9 technology. With this tool, which shows high efficiency in worms, we also aim to generate knock-in models to test the phenotypic effects of novel variants identified in humans. We are modeling novel mutations in POLR3C and TOGARAM1 genes in the worm orthologues. Neither of these genes has been associated so far with a human disorder. We stress the importance to set up models to validate new variants of uncertain significance that are identified with NGS technologies in patients.

CHARACTERIZATION OF HUMAN RECOMBINANT COQ4 AND ITS PUTATIVE ZN BINDING SITE MUTANTS

Morbiato L, Tonello F, Berto P, Zanotti G, Desbats MA, Polverino De Laureto P, Palazzi L, Pierrel F, Salviati L

Defects in genes involved in coenzyme Q (CoQ) biosynthesis cause primary CoQ deficiency, a clinically heterogeneous disorder with clinical manifestation ranging from fatal neonatal multisystem disorders to adult-onset isolated encephalopathy or nephropathy.

COQ4 codes for an ubiquitously expressed 265 amino acid protein that is peripherally associated with the mitochondrial inner membrane on the matrix side; the precise function of human COQ4 is not known, but the yeast ortholog Coq4p seems to play a structural role crucial in the stabilization of a multiheteromeric complex including several, if not all, of the CoQ biosynthetic enzymes. It has also been proposed that yeast Coq4p could be a zinc protease, based on the

presence of the zinc binding motif HDxxH. In this view the protein of interest could also play a role in the maturation of other COQ polypeptides. To investigate the role of COQ4, our idea is to produce the recombinant protein and to make a biochemical characterization using different approaches like analysis of zinc content, circular dichroism, limited proteolysis and mass spectrometry. To study the effective role of the putative Zn binding motif, we decided to purify and study 4 Coq4 motif mutants and some pathological mutations. Altogether our data provide new insights on CoQ4 features and its role in CoQ biogenesis. Further work will be aimed at obtaining crystals in order to solve the three-dimensional structure of the protein.



ACTA2 MUTATIONS DETECTED IN PATIENTS WITH TAAD AFFECT MITOCHONDRIAL DISTRIBUTION IN YEAST

Calderan C, Zordan R, Sartori G, Salviati L

Thoracic aortic aneurysm and dissection (TAAD) is a genetically heterogeneous condition. Mutations in the gene encoding smooth muscle alpha-actin (ACTA2) are the most common genetic cause of familial non-syndromic TAAD; to date, more than 30 heterozygous TAAD-associated ACTA2 mutations have been identified. We have identified 5 new heterozygous ACTA2 missense mutations in TAAD patients studied in our diagnostic laboratory. We have characterized their pathogenicity using yeast *Saccharomyces cerevisiae*.

Yeast expressing the different mutants displays only mild growth defects, but when we visualized mitochondria we observed a

significant reduction of percentage of cells with normal mitochondrial distribution, as a consequence of the disorganization of the cellular cytoskeleton. All mutations have shown to act with a dominant negative mechanism: the mutant allele affects the activity of the wildtype one when co-expressed in yeast. Ongoing experiments on actin staining aimed at visualizing yeast mutants cytoskeleton directly could confirm this dominant effect.

The relatively mild phenotype observed in yeast is consistent with the clinical features of the patients. In fact, the human disease has an adult onset and incomplete penetrance.

BIOCHEMICAL AND PHYSIOLOGICAL CHARACTERIZATION OF THE EFFECT OF VANILLIC ACID IN A COQ6 K.O. MAMMALIAN CELL LINE

Acosta MJ, Canton M, Salviati L

Coenzyme Q (CoQ), a polyisoprenylated benzoquinone lipid, is an essential component of the respiratory chain, where it acts as an electron transporter between complexes I and II and complex III. Human COQ6 encodes a flavin-dependent monooxygenase involved in the biosynthesis of CoQ and mutations in this gene cause primary CoQ deficiency. Our previous data, using a *Saccharomyces cerevisiae* Δ Coq6 model, showed that mutations identified in human COQ6 decrease CoQ synthesis and cause an impairment in the respiratory chain. Furthermore, we showed that the treatment with Vanillic Acid restored the respiratory growth of yeast Δ coq6 cells expressing the mutant hCOQ6 proteins.

In this work we present a biochemical and physiological characterization of a HEK 293 COQ6 K.O. cell line, generated by CRISPR technology. We show that the absence of COQ6 completely blocks CoQ10 biosynthesis, solving some aspects of COQ6 role in the CoQ10 synthesis process in mammal cells. In addition, the treatment with Vanillic Acid restores the level of CoQ10 close to the physiologic levels and consequently restores ATP and ROS production. Interestingly supplementation with exogenous CoQ increases ROS production in cells. These data support our hypothesis that Vanillic Acid could represent a real therapeutic option for COQ6 patients.



EXTRACELLULAR VESICLES DERIVED FROM LICENSED MESENCHYMAL STEM CELLS: A TUNABLE APPROACH TO REGULATE ANGIOGENESIS

Angioni R, Herkenne S, Liboni C, Sanchez-Rodriguez R, Cali B, D'Ambrogio C, Borile C, Romanato F, Muraca M, Viola A

Angiogenesis is the process that leads to the formation of new blood vessels from a pre-existing vascular network, playing a key role in many physiological and pathological processes. Consequently, targeting angiogenesis represents a very interesting therapeutic approach.

We have already shown that mesenchymal stem cells (MSCs) stimulated with pro-inflammatory cytokines (st-MSCs) block angiogenesis through the release of soluble factors. Thus, we highlighted the endothelium as a novel target during the MSC immunosuppressive effect. However, the therapeutic employment of MSCs to control inflammation is far to be clinically translated. The development of a cell-free therapeutic approach could represent a better cost-effective and safer procedure.

Here, we demonstrate that extracellular vesicles derived from stimulated MSC conditioned medium (EV stMSC-CM), but not from their unstimulated counterparts (EV unstMSC-CM), affect angiogenesis, thus recapitulating the MSC effect. EV stMSC-CM, expressing high levels of the ecto-5'-nucleotidase CD73, generate extracellular adenosine. We demonstrated that the EV stMSC-CM generated adenosine inhibits the endothelial cell migration, by inducing an excessive intracellular ROS accumulation, both in vitro and in vivo.

These results indicate that EVs derived from st-MSCs display anti-angiogenic properties and could be exploited for cell-free therapeutic strategies. Additionally, they pave the way for a better understanding of the MSC physiological role in vivo.

CXCL12 ALTERNATIVE SIGNALLING PATHWAYS MODULATE NEUTROPHIL NUCLEAR DEFORMABILITY

Calì B, Marcuzzi E, Cassarà A, Lefkimmiatis K, Kunkl M, Vetralla M, Pozzobon T, Gagliano O, Tuosto L, Elvassore N, Thelen M, Molon B, Vargas P, Viola A

Neutrophil migration is fundamental for immune responses. Upon inflammation, neutrophils leave the bone marrow and migrate towards inflamed tissue following chemotactic gradients. Although the cytoplasm can quickly change consistence and form to allow cells to penetrate the endothelium, the deformation of the nucleus, the largest and stiffest cellular organelle, seems to be an awkward process. Since immune cell migration is driven by chemokines, it is likely that chemokines may also modulate nuclear deformability. The homeostatic chemokine CXCL12 has been demonstrated to regulate neutrophil

homing and chemotaxis by binding its canonical receptor CXCR4. Exploiting live cell imaging and original micro-fabricated devices, we investigated the signaling pathways connecting chemokine signals to nuclear biomechanical properties modifications. Here we show that CXCL12 induces neutrophil nuclear deformation and sustains transendothelial migration towards inflammatory stimuli. Intriguingly, we found that atypical receptors and unexpected protein kinases are involved in the modulation of neutrophil nuclear deformability.



MONOAMINE OXIDASES IN INNATE IMMUNITY

Solazzo I, Brun P, Agnellini A, Sánchez-Rodríguez R, Castegna A, Blaauw B, Munari F, Zumerle S, Castagliuolo I, Viola A, Canton M

Reactive oxygen species (ROS) are well known to be fundamental for macrophages to kill invasive microorganisms. Moreover, they have an important role in regulating signal transduction pathways, gene expression and differentiation. Besides NADPH oxidase, mitochondria are gaining increasing relevance as a source of ROS in immune cells, although the exact sites of formation are only partially elucidated. Monoamine oxidase (MAO) is a relevant source of H₂O₂ in mitochondria, generated by oxidative deamination of biogenic amines. Since this enzyme has been

scarcely characterized in phagocytic cells, we aimed at clarifying whether it plays a role in the differentiation and activation of macrophages. Indeed, on the basis of our preliminary data, we hypothesize that oxidative stress induced by MAO activity may play a crucial role in excessive inflammation and tissue damage in sepsis, the leading cause of death in intensive care units in high-income countries. Thus, we are currently investigating whether clinical-grade monoamine oxidase inhibitors can be viable candidates in the treatment of sepsis.

THE IMPORTANCE OF IMMUNOHISTOCHEMISTRY AND CELL-BASED ASSAYS AS SECOND LEVEL DIAGNOSTIC TOOL IN AUTOIMMUNE ENCEPHALITIS

De Gaspari P, Camporese G, Zoccarato M, Zuliani L, Rosellini I, Flemming J, Barberio G, Caberlotto L, Dalmau J, Graus F, Giometto B

Introduction. Encephalitis is a severe inflammatory brain disorder that can have infectious or autoimmune etiology. A substantial number of neural and glial autoantibodies have recently been characterized in autoimmune encephalitis (AE). However, a sizable group of patients have syndromes suggestive of AE but is negative for all of the known antibodies. In this scenario, it is fundamental to be able to clearly characterize the presence of autoantibodies that can help the clinician to recognize and promptly treat these conditions.

Methods. 1622 serum and CSF samples were collected between 2014-2016 and tested for antineuronal antibodies using commercial kits. The samples were tested for onconeuronals, GAD, AQP4 and surface antibodies (e.g. NMDAR, CASPR2). Among all, 30 samples were negative for neuronal and glial antibodies, although following the proposed AE clinical criteria

(Graus et al, 2016). Therefore, these were further investigated by using in house immunohistochemistry both in brain and cerebellum frozen sections and CBA-assay.

Results. The IHC results highlighted 3 samples with a specific pattern in rat cerebellum frozen sections. These samples were further analyzed by cell-based assay (CBA). One serum belonging to a patient with AE symptoms was negative for mGLUR1 antibodies. The serum and CSF belonging to another patient resulted positive for AK5 antibodies in accordance to his clinic. However, no specific antibodies were identified in one patient clearly affected by paraneoplastic disease.

Conclusions. Antibody screening using IHC techniques and CBA assay are fundamental tools that should be used in routinely manner to ensure accurate and trustful AE clinical diagnosis.



Nano medicine

FROM NANOMEDICINE TO BIO-INSPIRED NANOMEDICINE: THE ROLE OF MICROFLUIDICS

Roberto Molinaro

In the past decades, biomimicry (biologically inspired design of materials) arose in the nanotechnology field as the cutting-edge strategy to simultaneously confer nano- and micro-particles multi-functionalities in order to negotiate biological barriers. To this purpose, drug delivery carriers mimicking leukocytes, red blood cells, platelets, and cancer cells have been developed using different approaches. These hybrid biomimetic carriers showed advantageous pharmaceutical properties, like defined size and shape, physical stability, ability to load and release chemically-different therapeutics, deriving from the synthetic backbone material (nano-porous silicon, liposomes, PLGA nanoparticles), combined with innate features (long circulation, selective targeting towards specific biological compartments, and intrinsic functionalities) typical of the biological source they derive from. However, the new biological functionalities transferred to these hybrid nanomaterials increased their degree of complexity from a regulatory standpoint, which implicates the need of a standardizable, batch-to-batch consistent, scalable, GMP-suitable, and high throughput assembly method. As a matter of fact, the difficulty of producing nanoparticles in a standardized and reproducible way and in sufficient quantity hindered their

successful translation to clinical applications. In response to this need, microfluidics – the science of manipulating fluids at the micrometer or smaller scale in a controlled fashion – emerged as a promising technique to allow for the well-controlled synthesis of nanoparticles, thus providing a versatile method to accelerate their transition to the clinic. By controlling the fluid mixing time, rate, and temperature it is possible to tune the final size and size distribution of resulting nanoparticles, as well as their loading capacity and batch-to-batch reproducibility. Recently, a microfluidic-based platform, called NanoAssemblr® (NA), has been developed for the manufacture of nanomedicines in a controlled, tuned, low-cost, and fully scalable fashion. Herein, we show for the first time a continuous-based process to incorporate membrane proteins deriving from leukocytes within the lipid bilayer of a liposome-like nanovesicle using NA technology. NA-made Leukosomes (NA-Leuko) have been fully characterized for their physical, pharmaceutical, and biological properties. Under microfluidic assembly, leukosome formulations incorporated 14 times more membrane proteins compared to their previous assembly method and retained their ability to target inflamed endothelium.

ACELLULAR PATIENT-DERIVED 3D COLORECTAL CANCER MODEL AS A TOOL FOR PRE-CLINICAL CANCER RESEARCH

D'Angelo E, Sensi F, Daronco S, Crotti S, Mammano E, Urbani L, Pucciarelli S, Piccoli M and Agostini M

Introduction. Colorectal cancer (CRC) tumour microenvironment (TME) is a dynamic compartment that provides biological, biochemical, and biomechanical cues influencing cell behaviour and drug response. New insights on cancer research can arise from a deeper understanding of the TME and cancer cell crosstalk. In particular, we focused our attention on the study of extracellular matrix, the most abundant component of TME in CRC.

Material and Methods. Surgically resected healthy colon mucosa and matched CRC were decellularized with a detergent-enzymatic treatment (DET). Analysis of biological properties of DET scaffolds was performed using: proteomic analysis using MS/MS, Chicken Chorioallantoic Membrane Assay (CAM), and migration assay. Finally, after DET scaffold recellularization with HT-29 and HCT-116 cell lines we performed a chemosensitivity test with 5-Fluorouracil and evaluated IL-8 gene expression.

Results. Acellular scaffolds recapitulate the ultrastructural environment of native tissue

as demonstrated by immunofluorescence. Proteomic analysis confirmed a different stromal composition between DET healthy mucosa and CRC in terms of secreted proteins. The 3D acellular model retained its biological properties: using CAM assay, we observed a decreased angiogenic potential in DET CRC compared with healthy tissue, caused by the direct effect of DEFA3. After 5 days of recellularization with HT-29 cell line, the CRC 3D acellular scaffold induced an over-expression of IL-8, DEFA3-mediated compared to healthy colon. Finally, HT-29 and HCT-116 exhibited reduced sensitivity to 5-Fluorouracil treatments when cultured in 3D acellular scaffolds compared with conventional 2D cultures.

Conclusions. These results reveal that normal and tumour acellular 3D models could be a useful tools to study colorectal cancer pathogenesis, proliferation, dissemination and drug response, mimicking the complex network of cell-cell and cell-matrix interactions.

NANOSTRUCTURED LAB-ON-CHIP FOR SURFACE PLASMON RESONANCE BIOSENSING AND IMAGING

Borile G, Rossi S, Capaldo P, Sonato A, Filippi A, Tregnago C, Ruffato G, Pigazzi M, Romanato F

Surface Plasmon Resonance (SPR) refers to the collective oscillation of conduction electrons at the interface between a conductor material (i.e. gold) and a dielectric (i.e. nucleic acids or proteins) upon light interaction. SPR-based sensors, with the advantage of being label-free, enzyme-free and real-time, are of growing interest in biomedical research. This kind of sensors are widely recognized as trustworthy tools for detecting biological specimens but are conventionally limited to single marker analysis and usually applied to molecular interactions. Nanostructured plasmonic biosensors have been developed to reach higher sensitivity and specificity, while offering a cost-effective instrumentation and a possible development to multiplex

analysis. Here we propose a sensing platform based on an innovative phase-interrogation method, which entails the exploitation of a nanostructured sensor. This technique is particularly suitable for integration of the plasmonic sensor in a lab-on-chip platform and can be used in a microfluidic circuit to ease the sensing procedures and limit the injected volume. As a proof-of-concept, specific recognition in liquid biopsy-mimicking medium and cell-drug interaction experiments were performed. This biosensing system is currently being developed as an innovative technology to profile a large number of genes in a single assay, with high accuracy and enhanced sensitivity, for the diagnosis of leukemia.

MULTI-MODAL LABEL-FREE EX-VIVO IMAGING USING A DUAL-WAVELENGTH MICROSCOPE WITH AXIAL CHROMATIC ABERRATION COMPENSATION

Filippi A, Dal Sasso E, Iop L, Armani A, Gintoli M, Sandri M, Gerosa G, Romanato F, Borile G

Label-Free Microscopy is a very powerful technique that can be applied to study samples with no need for exogenous fluorescent probes, keeping the main benefits of Multiphoton Microscopy, like longer penetration depths and intrinsic optical sectioning, while enabling serial multi-techniques examinations on the same specimen. Among the many Label-Free Microscopy methods, Harmonic Generation (HG) is one of the most intriguing due to its generally low photo-toxicity and relative ease of implementation. HG and common Two-Photon Microscopy (TPM) are well-established techniques, today routinely used in several research fields. However, they require a significant amount of fine-

tuning in order to be fully exploited, making them quite difficult to perform in parallel. Here, we present our designed multi-modal microscope, capable of performing simultaneously TPM and HG without any kind of compromise thanks to two, separate, individually optimized laser sources with axial chromatic aberration compensation. We also apply our setup to the examination of a plethora of ex vivo samples in order to prove its capabilities and the significant advantages of a multi-modal approach.

Keywords: Label-Free, microscopy, multiphoton, dual-wavelength, chromatic aberration, ex-vivo.



EXOSOME TRACKING WITH MULTI-PHOTON MICROSCOPY FOR QUANTIFICATION AND SIZE DETERMINATION

Borile G, Filippi A, Mafakheri E, d'Avanzo F, Tomanin R, Mussolin L, Romanato F

Extracellular vesicles (EVs) carry key information for intercellular communication via their surface molecules, proteins and nucleic acids content. The growing interest in the study of extracellular vesicles (EVs) is accompanied by the need to develop methodologies capable of responding to biomedical challenges. The nanometric size of exosomes (30-100 nm) and the sparse dimension of microvesicles (100-1000nm) make their quantification and characterization difficult and based on dedicated instruments. Thus, an accessible and user-friendly way to visualize and make a first characterization is required. Here, we describe methods to label and

track brownian motion of vesicles with a two-photon microscope (TPM). EVs were labelled with a fluorescent lipid dye or with a fluorescent protein upon genetic engineering. This method is analogue to Nanoparticle Tracking Assay (NTA), while the use of a TPM ensures the exclusion of out-of-focus signal. Vesicle concentration and size were evaluated and compared to a standard reference and TEM analysis. Furthermore, this same method was applied to track particle motion inside living cells and estimate particle uptake. The main advantage of this approach resides in the capability to monitor EVs motion inside *ex vivo* preparations.



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Predictive Medicine

ESTIMATING THE CONTRIBUTION OF SURFACTANT REPLACEMENT THERAPY TO THE ALVEOLAR POOL: AN IN VIVO STUDY BASED ON ¹³C NATURAL ABUNDANCE IN RABBITS

Giambelluca S, Ricci F, Simonato M, Vedovelli L, Correani A, Casiraghi C, Storti M, Cogo P, Salomone F, Carnielli VP

Respiratory Distress Syndrome (RDS) is a breathing disorder affecting preterm newborns due to lung immaturity and lack of lung surfactant, a lipoprotein layer which allows lung expansion and prevents alveolar collapse during expiration. Treatment with exogenous surfactant reduces the need for mechanical ventilation and the incidence of chronic lung disease. The aim of this study was to assess the contribution of exogenous surfactant phospholipids to the endogenous alveolar pool in vivo by stable isotopes in a rabbit model of RDS after exogenous surfactant therapy.

A feasibility study consisted in measuring the ¹³C/¹²C ratio of disaturated-phosphatidylcholine palmitate (DSPC-PA) in commercial surfactant (poractant alfa) and in bronchoalveolar lavages of 20 rabbits. The contribution of exogenous surfactant was then estimated in a rabbit model of lavage-induced surfactant deficiency

including 7 controls and 15 rabbits treated with different doses (5 rabbits per dose) of poractant alfa: 50, 100, 200 mg/kg. The ¹³C content of DSPC-PA was measured by Isotope Ratio Mass Spectrometry.

DSPC-PA in rabbits had a significant lower ¹³C abundance than poractant alfa, with a mean difference of 10.0 ‰, wide enough to distinguish the endogenous from the exogenous compound. The contribution of exogenous surfactant to the total alveolar surfactant in treated rabbits ranged from 42.9 to 90.2%, with a significant dose-effect.

This study describes a novel method to measure the contribution of the exogenous surfactant to the alveolar pool. Since no use of chemically synthesized tracers is required, it could be useful in human research and in surfactant replacement studies in preterm newborns.

ALVEOLAR SURFACTANT COMPOSITION IN PRETERM INFANTS WITH RESPIRATORY DISTRESS SYNDROME BEFORE EXOGENOUS SURFACTANT ADMINISTRATION: EFFECT OF GESTATIONAL AGE AND INFLAMMATION

Simonato M, Verlato G, Bassi E, Fantinato M, Correani A, Giambelluca S, Baraldi E, Cogo P, Carnielli VP

Background. Surfactant deficiency is a major cause of respiratory distress syndrome (RDS) in premature newborns but studies about its composition before exogenous surfactant replacement therapy are scarce.

Objectives. To examine the composition of the alveolar surfactant in preterm newborns with RDS at different gestational ages (GA) and with chorioamnionitis (chorio).

Methods. Ninety-three newborns with $GA \leq 32$ wks with RDS and 12 term infants with no lung disease were enrolled. Tracheal aspirate (TA) and blood samples were collected at birth before exogenous surfactant administration. TA Disaturated-phosphatidylcholine (DSPC) was measured by gas-chromatography, surfactant protein (SP)-A and SP-B by ELISA and myeloperoxidase (MPO) by EIA. The alveolar-capillary permeability was expressed as the ratio between plasma and TA albumin concentrations. The epithelial lining fluid (ELF) concentrations of the TA components were normalized by the urea dilution

method.

Results. Preterm infants had significantly lower ELF DSPC concentration than term infants and the alveolar-capillary permeability decreased significantly with increasing GA. The extremely premature infants ($GA \leq 28$ wks) showed no differences in surfactant composition but had the higher incidence of chorio (38% vs 9%) with the highest MPO activity compared to the group $28 < GA \leq 32$ wks. Preterms with chorio had higher DSPC and SP-B concentrations compared to a group of RDS infants matched by GA. Plasma albumin concentration along with GA were good predictors of mechanical ventilation length ($R^2=0.512$; $p < 0.001$).

Conclusions. Preterm infants at birth, before surfactant administration, had measurable amount of DSPC, SP-A, and SP-B, especially those with chorio. An increased alveolar-capillary permeability is the major effect of GA.



GLIAL FIBRILLARY ACIDIC PROTEIN PLASMA LEVELS DURING CONGENITAL HEART DISEASE SURGERY INVERSELY CORRELATE WITH MID-TERM, COMMUNICATION NEUROCOGNITIVE SKILLS

Vedovelli L, Padalino M, Cainelli E, Suppiej A, Sartori S, Falasco G, Simonato M, Carnielli VP, Stellin G and Cogo P

Background. Neuro-cognitive deficits at the beginning of school age may affect as high as 50% of children who underwent cardiac surgery for complex congenital heart diseases (CHD). The aim of the study was to identify which phases of cardiopulmonary bypass (CPB) are associated with an increased risk of impaired neurodevelopmental skills in children with complex CHD. This was done by means of glial fibrillary acidic protein (GFAP) plasma levels during CPB for CHD surgery, as a marker of neurological insult. We correlated GFAP amounts with clinical parameters and neurodevelopmental outcome.

Methods. We studied 45 children undergoing surgery for complex CHD. We measured plasma GFAP levels by ELISA at the following steps: anesthesia induction, CPB start, end of hypothermia, end of rewarming, end of CPB. Neurological assessment and Vineland Adaptive Behavior Scales (VABS-I)

were administered to patients after at least 18 months from surgery.

Results. GFAP was undetectable before surgery and it peaked at the end of hypothermia or rewarming. Multiple regression analysis showed that GFAP peak level and preoperative neurological comorbidity were significant independent predictors of neurological impairment, as showed by VABS-I communication domain IQ. Receiver operating characteristic curve showed that the model was highly significant.

Conclusions. Impaired neurodevelopment was associated with increase of GFAP plasma levels during cardiac surgery in infants. The identification of the neurological high-risk phases of CPB run could support the application of new neuroprotective strategies for CHD repair.

METABOLOMIC PROFILING WITH MASS SPECTROMETRY OF CHILDHOOD DIABETES

Pirillo P, Galderisi A, Stocchero M, Gucciardi A, Naturale M, Moret V, Monciotti C, Giordano G, Baraldi E

Type 1 diabetes mellitus (T1D), characterized by defects in insulin secretion or action, is one of the most common chronic diseases in childhood, that influences the entire life of the patient. Despite the good glycemic control that is possible to obtain with insulin replacement, T1D is still associated with an excess of mortality in adulthood population, suggesting that several metabolic disorders persist in T1D patients in insulin treatment.

The aim of our study is to investigate, if and which different metabolic alterations are already present in T1D childhood population, not far from diabetes onset.

The analysis was conducted on the urine specimens through high-definition mass spectrometry (HDMS). The samples were collected from 56 children with the diagnosis of T1D and in insulin replacement therapy and from 32 healthy volunteers comparable

for age, sex and puberty.

Applying the HMDS to the urine samples, we were able, after processing data with specific statistical analysis, to clearly separate the peers in two groups and to reveal alteration in different metabolic pathways. In particular, steroid hormones pathway seems to be altered in diabetic subjects, even if they are all in good glycemic control.

To confirm the data obtained, we are currently developing a method for the specific analysis of steroids, using labeled and unlabelled standards to build the calibration curve. The assay is performed through the analysis in UPLC-MS, with a Quadrupole XEVO TQ-S, operating in multi reaction monitoring. The method will be validated and applied to plasma and urine samples of diabetic children.



Regenerative Medicine

EXTRACELLULAR VESICLES (EVs) SECRETED BY MESENCHYMAL STEM CELLS (MSCs) EXERT OPPOSITE EFFECTS WITH RESPECT TO THEIR CELLS OF ORIGIN IN MICE WITH DSS-INDUCED COLITIS.

Grassi M, Tolomeo AM, Piccoli M, Franzin C, Jurga M, Van Wemmel K, Scarpa M, Marchiori C, Porzionato A, Castagliuolo I, Viola A, Pozzobon M, Muraca M

Several reports have described a beneficial effect of MSC administration in mice with experimental colitis. However, worsening of colitis following MSC treatment was also reported.

We compared the effects of murine MSC or MSC-EV administration in a dextran sulfate sodium (DSS)-induced colitis model. Since cytokine conditioning was reported to enhance MSC immune modulatory activity, the cells were kept either under standard culture conditions (naïve, nMSCs) or primed by IL1b, IL6 and TNFalpha (induced, iMSCs).

nMSCs and iMSCs administration was associated with clinical and histological worsening with respect to controls. However, mice treated with both nMSC-EVs

and iMSC-EVs showed clinical improvement, even if no significant difference was found in histological/morphometric score with respect to controls. These opposite effects were particularly evident with iMSCs. Cytokine expression in colon mucosa showed reduced TNFalpha and increased IL-10 in mice treated with iMSC-EVs.

In conclusion, both nMSCs and iMSCs worsened DSS-induced colitis, confirming that these cells can behave as pro-inflammatory agents depending on the environment. In contrast, both nMSC-EVs and iMSC-EVs showed a partially beneficial effect, suggesting a more predictable behavior and a safer therapeutic profile with respect to their cells of origin.

MODELLING FRAGILE X SYNDROME WITH iPSCS

Tolomeo AM, Laterza C, Murgia A, Muraca M, Elvassore N

Fragile X syndrome (FXS) is the major monogenetic cause for autism and mental retardation and is caused by a trinucleotide repeat expansion, methylation and silencing of Fragile X Mental Retardation 1 (FMR1) gene promoter. The molecular mechanism and timing leading to FMR1 silencing and protein loss are still unknown.

The generation of naïve induced pluripotent stem cells (iPSCs) showing a broader unmethylated genome (including in FMR1) opened a new hope for disease modeling of FXS.

In this project we aim at understanding the molecular mechanism of FXS taking place during the early phase of neural development, using FXS-patient specific naïve iPSCs and micro-technologies.

While collecting the patients' samples, we set up protocols for neural differentiation and mRNA based direct conversion of iPSCs into neurons. We are now optimizing these protocols using microfluidics, in order to create a scalable platform instrumental for our studies on FXS molecular mechanisms.



DIAPHRAGMATIC-DERIVED EXTRACELLULAR MATRIX PATCH EXERTS A POSITIVE LONG LASTING EFFECT ON MYOGENIC REGENERATION AND NERVE ATTRACTION WHEN USED IN A SURGICAL MOUSE MODEL OF DIAPHRAGMATIC HERNIA

Trevisan C, Dedja A, Maghin E, Pavan P, Caccin P, Franzin C, Urbani L, De Coppi P, Pozzobon M, Piccoli M

In congenital diaphragmatic hernia (CDH) many different approaches have been described for the treatment of large defects. Recently we characterized the extracellular matrix (ECM) obtained from decellularized mouse diaphragmatic muscle. This scaffold displayed important characteristics such as biomechanical features, immunomodulatory properties and ability to recruit specific myogenic progenitors. For these reasons, we focused our attention on using decellularized ECM as biologic tool for the treatment of diaphragm defects.

A surgical CDH mouse model with large defect was set up: Balb/c mice were used as recipients and C57Bl6/J mice as diaphragm ECM donors. Expanded polytetrafluoroethylene (ePTFE) patches were used as treatment controls. Mice were analysed after 30 and 90 days of defect closure through histology and molecular analysis.

We compared the efficacy of defect closure of decellularized diaphragm ECM and ePTFE. Survival was comparable between the groups (over 65%), but only in ePTFE treated mice we found cases of hernia recurrence. Moreover, in ECM transplanted mice there was no fibrosis, the scaffold promoted a good vascularization and exerted a strong activation of tissue remodelling and myogenesis that gave rise to a significant overexpression of both myogenic genes and proteins in respect to ePTFE treated controls. Importantly, neuronal progenitors were attracted by implanted ECM, indicating a beginning of nerves regeneration.

ECM derived scaffolds tested in a newly developed mouse model, exerted a positive effect when applied in a large diaphragm defect, influencing local remodelling, myogenic and nervous tissue regeneration in a more physiological manner in respect to standard of care prosthetic patches.

GENERATION OF A SELF-RENEWING SKELETAL MUSCLE CONSTRUCT USING CRYOPRESERVED DECELLULARISED DIAPHRAGMATIC EXTRACELLULAR MATRIX AND HUMAN MUSCLE PRECURSOR CELLS

Maghin E, Trevisan C, Alvarez-Fallas ME, Franzin C, Urbani L, Milan A, Biz C, Lazzari L, De Coppi P, Pozzobon M, Piccoli M

Naturally derived matrices are biocompatible materials obtained by tissue decellularization for tissue engineering purposes. Despite some promising applications described in literature, the use of acellular matrices to repair large defects has been only partially successful highlighting the need of a more efficient construct. Scaffold recellularisation may improve not only the structure of the matrix, but also its ability to functionally interact with the host. The development of such a complex construct is challenging, due to the complexity of the native organ and the difficulties in maintaining the cellular niche with intact both proliferation and differentiation potential, after damage or during growth.

In this study we used a mouse decellularized

extracellular matrix previously developed in our laboratory, testing two different (short and long term) storage conditions, mimicking the use of an off-the shelf product. We then isolated paediatric human muscle precursors and demonstrated that they can proliferate and differentiate when seeded on a decellularized matrix to give rise to a physiologically active 3D skeletal muscle structure. Furthermore, we exposed the engineered xenogeneic structure to an injury and demonstrated its ability to activate a regeneration response promoting a remodelling process. Functional reconstruction of an engineered skeletal muscle with maintenance of a stem cell niche makes this a promising tool towards future clinical applications.

DESIGN OF A BIOREACTOR FOR DYNAMIC MECHANICAL STIMULATION OF IN VITRO RECELLULARIZED DIAPHRAGMATIC SCAFFOLDS

De Cesare N, Maghin E, Trevisan C, Carraro E, Piccoli M, Pavan PG

The congenital diaphragmatic hernia is a serious pathology which implies the incorrect closure of the diaphragmatic muscle during the prenatal development. An innovative approach for the repair of this congenital malformation is the use of a portion of decellularized diaphragm extracellular matrix (ECM) recellularized with human muscle progenitor cells to close the defect, considering this as an alternative solution to the gold standard treatments (i.e. Gore-Tex patches). For this purpose, there is the need of a system able to reproduce a biologically active in vitro environment and mechanically stimulate the recellularized ECM, in order to have a functionalized tissue before in vivo implantation.

The present study aims at building a bioreactor that hosts recellularized diaphragm ECM and reproduces the dynamic biological condition as similar as possible to the in vivo situation.

The bioreactor system is composed by the incubator and the motor bench. The first is structured for host the muscle ECM, the second block is built to host the engine and drives the bench through a system based on Arduino. A custom software developed in MATLAB was ideated to control the experimental parameters.

A Finite Element Method approach was used to characterize the mechanical behaviour of the PDMS membrane on which the diaphragm is placed and to deduce the construction parameters used to design the motor bench.

The first experimental tests provided preliminary results about muscle morphology recovery in fresh diaphragm injured with cardiotoxin and stimulated for 48h in the bioreactor. Several tissue parameters were analysed with MATLAB scripts specifically developed.

TOWARD RHABDOMYOSARCOMA MICROENVIRONMENT DISSECTION: PRELIMINARY RESULTS

D'Agostino S, Frasson C, Tombolan L, Rampazzo E, Persano L, Bisogno G, Gamba P, Pozzobon M

Malignant cells do not act alone in cancer progression but need enduring interactions and cross-talk with supporting cell types (such as associated fibroblasts –CAFs–, myofibroblasts...) and extracellular matrix components (ECM) that form the tumor microenvironment (TME). Approximately 20-25% of cases of rhabdomyosarcoma (RMS) present metastasis at diagnosis suggesting that growth and metastatic potential likely depends on the interactions with the TME. We start dissecting the unexplored RMS microenvironment, taking into consideration CAFs. Generally, CAFs are responsible for the production and deposition of soluble and insoluble ECM molecules (collagens, structural proteoglycans) actively involved in cancer development, progression. The aim of our study is to analyze the involvement of CAFs in dynamic ECM RMS remodeling.

We evaluated (1) ECM protein expression in cancer cell lines, we studied (2) the

interaction between fibroblasts and RMS cell line using transwells and a scratch test. We used (3) spheroids to set fibroblasts-cancer cells interaction experiments (in vitro 3D model). Finally, we injected(4) in vivo sarcoma cell lines using as control colon and breast cancer cell lines, to check CAFs recruitment from the host.

RMS cell line did not attract fibroblasts both in 2D and in 3D culture system. In vivo, by cytofluorimetric analysis of the CAF marker PDGFR-A, we found that RMS cell line gave rise to a xenogenic tumor with a small percentage of CAF recruitment. On the contrary colon and breast cancer cell lines attracted much more fibroblasts from the host.

Our data suggest that in RMS tumor the role of CAF is negligible and other elements play a fundamental part to shape RMS TME.



EFFECTS OF EXTRACELLULAR MATRIX FROM DIFFERENT TISSUES TO REPAIR VOLUME MUSCLE LOSS DEFECT

Saggiaro M, Maghin E, Sgrò A, Franzin C, Grassi M, Gamba P, Muraca M, Piccoli M, Pozzobon M

The need of new biomaterials to replenish the loss of muscle mass is currently a challenge. Indeed, after congenital malformations, trauma or tumor surgery the volume mass loss can be filled with synthetic materials already used in the clinical practice but the regain of function is still very difficult to reach. Nowadays the decellularization of tissues allows the obtainment of the highest biocompatible scaffold without the genetic material, such as the extracellular matrix (ECM). This biomaterial retains the biomechanical properties, proteins and biochemical factors that characterized the native tissue.

In order to chose the best schaffold for volume muscle replenishment, the aim of this work was to analyze the effect of decellularized tissues from different origin, murine skin, intestine and muscle quadriceps, in a murine model of volume muscle loss.

ECM samples were obtained using a

detergent-enzymatic protocol and wild type immunocompetent mice were used as animal model. After 4,7,15 and 30 days samples were analysed by histology, Immunofluorescence, Real-time PCR.

Although inflammation was visible in all the implanted ECM, already after 15 days, muscle quadriceps scaffold showed newly formed and centre nucleated myofibers. No significant regeneration was observed with skin and intestine derived scaffolds. Specifically, in muscle implants, macrophagic response seemed directed toward tissue rebuilding.

Pro-regenerative results achieved only with implantation of muscle-derived scaffold underlined the importance of tissue-specificity in order to obtain the ideal material to repair muscle defects. To ameliorate angiogenesis and reduce fibrosis, extracellular vesicles (EV) are injected in implanted quadriceps ECM and the experiments are ongoing.



Annual Retreat

April 6th - 7th 2018
Villa Pace Park Hotel Bolognese,
Preganziol - TV

The first Annual Retreat of the Istituto di Ricerca Pediatrica Città della Speranza was held on April, 6-7, 2018 at Villa Pace Park Hotel Bolognese in Preganziol (Treviso). This was a unique opportunity for colleagues to get to know each other better (in total there were around 100 scientists, PhD and students present), to talk about the topics of the research programs, share experiences, exchange information and advice, and build networks for future collaborations.

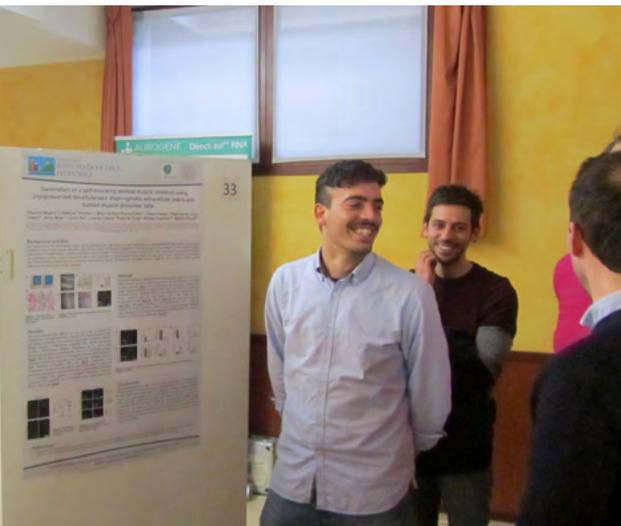
This event especially provided the occasion for young researchers to present their work through talks and poster presentations, and to interact on a personal and professional level.

The Special Guest was the Rector of the University of Padua, Rosario Rizzuto, who gave an interesting lecture about "Mitochondrial calcium signaling in cell life and death".

A particular thanks to Leika, Nikon and Zeiss, who led a stimulating workshop on new microscopy methods, and to Aurogene and Salix who kindly offered the "Poster Walking".

In conclusion, the dinner all together and the evening dance made the atmosphere even more inclusive and friendly.





Scientific Prizes

“Famiglia Masello in memoria di Rita Masello e Massimo Zilio” for the two best oral communications

Committee: Prof. Antonella Viola, Dr. Martina Pigazzi, Dr. Michela Pozzobon, Dr. Rosella Tomanin



Dr. Caterina Trevisan

(Dr. Piccoli Lab/ Dr. Pozzobon Lab)



Dr. Sonia Giambelluca

(Prof. Cogo Lab)

**“Elisa Camporese” and “Matteo Fochesato”
for the three best posters**

*Committee: Dr. Marco Agostini, Dr. Lara Mussolin, Dr. Martina Piccoli,
Dr. Luca Persano, Dr. Giampietro Viola, Dr. Eva Trevisson*



Dr. Elena Mariotto

(Prof. GP Viola Lab)



Dr. Diana Corallo

(Prof. Tonini Lab)



Dr. Cristina Calderan

(Prof. Salviati Lab)

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