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Posters abstracts

1 – Versatile Tethering System to Control Cell-specific Targeting of Bioengineered Extracellular Vesicles

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Keywords : extracellular vesicles, drug delivery, targeting, bioengineering, synthetic biology, therapy

Extracellular Vesicles (EVs) are natural vectors of communication involved in a broad range of physiological functions. Significant efforts were devoted to use EVs for therapeutic delivery. The main challenges have been controlling and enhancing the fundamental steps of EV-mediated delivery. We and others developed solutions to improve cargo/therapeutic loading into EVs and to enhance EV content delivery through the use of viral or non-viral fusogens. Recently, only a few solutions have been proposed to achieve targeting with limited flexibility. Here, we present a versatile system that enables precise EV targeting to specific cell types while allowing quantitative assessment of targeting efficiency using luminescence and fluorescence analysis. In this system, EVs are genetically engineered to express a chimeric adapter protein anchored to their surface via a glycosylphosphatidylinositol (GPI) anchor. This protein includes a fluorescent and/or luminescent domain for visualization/quantification and a streptavidin domain to recruit biotinylated antibodies or ligands specific to cell-surface antigens or receptors. We validated this platform with three different combinations of ligand/target cells, demonstrating up to 40-fold increase in EV uptake. Combined with previously developed modules for enhanced cargo loading and delivery, this adaptable system promises to provide a comprehensive solution for targeted therapeutic delivery using EV-based vectors.

2 – Establishment of production, isolation and purification of *F. duncaniae* extracellular vesicles for therapeutic application

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Keywords : probiotic, extracellular vesicles, bioproduction, biotherapy

Probiotics are Live Biotherapeutic Products (LBP) based on microorganisms. Since the exploitation of live anaerobic bacteria could bring additional challenges to bioproduction, we propose a novel approach based on extracellular vesicles (EVs) to treat inflammation. Faecalibacterium duncaniae A2-165 is a human commensal bacterium, very abundant in the human microbiota and showing very well characterized anti-inflammatory properties. EVs are cell-derived membrane-surrounded nano- and microvesicles that carry and deliver bioactive molecules. We adapted the culture and EVs production conditions of F. duncaniae A2-165 to Good Manufacturing Practice criteria using food-grade particle-free media and a semi-automated

Tangential Flow Filtration system. We found that A2-165 secrete small EVs in a production range of 180-210 particles/bacteria/h when cultured in vegan medium at 37°C for 4h. Remarkably 40,5% of the EV population is positive for MAM (Microbial Anti-Inflammatory Molecule), an effector of the anti-inflammatory properties of F. duncaniae. Finally, we demonstrate the SOP for EV isolation and purification using the TFF30kDa system with 70% EV recovery as a potential scale-up method. Our first EV batches were distributed among our partners to test the anti-inflammatory and neuromodulatory properties in vitro and in vivo in various preclinical models.

3 – Miniaturized Asymmetrical Flow Field Flow Fractionation (AF4) for size separation and purification of biotherapeutics

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Keywords : biotherapeutics, microfluidics, separation techniques, microfluidic chip, Asymmetrical Flow FIeld Flow Fractionation, SEC

In recent decades, nanoparticles have gained significant attention in biotherapeutics, due to their ability to enhance drug delivery by improving biodistribution, protecting payloads, and enabling targeted tissue delivery. To assess their efficacy and safety, in-depth physico-chemical characterisation is essential. Traditional size-based separation methods like size-exclusion chromatography face limitations in resolution and particle range. To overcome these criticisms, and to allow for efficient separation, an innovative analytical technique has been developed: microfluidic asymmetrical flow field-flow fractionation (µAF4) model. With a separation range from 10kDa up to 500nm, µAF4 enables high-resolution, sensitive and fast separation even for smaller particles.To further optimise performance, we introduce a patented thermoplastic micrometric version of AF4. It consists in a two-level device encapsulating a track-etched membrane to achieve separation of nanoparticles ranging from 10nm to 500nm. The fabrication and assembly are achieved in less than 10min and can easily be scaled up taking advantage of the pressure sensitive adhesion properties of the thermoplastic. We quantify the pressure and flow range for detection and cross flow, and demonstrate flow focusing on latex nanoparticles. This advancement enhances the accessibility and integration of AF4 into analytical workflows for nanomedicine development, offering a promising platform for membrane-based nanoparticle separation in biotherapeutics.

4 – Vésicules extracellulaires de cellules souches mésenchymateuses génétiquement modifiées : nouvelle thérapie topique pour la restauration de la transparence de la surface oculaire

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Keywords : Vésicules extracellulaires, bioproduction, thérapie cellulaire, biothérapie, translationnel

La cornée est recouverte d'un épithélium qui assure le maintien de sa transparence. Certaines pathologies, dont l'origine peut étre traumatique, inflammatoire ou génétique, entraïnent une opacification de la cornée conduisant à la cécité. Notre projet vise à formuler un collyre contenant des vésicules extracellulaires (VEs) issues de cellules souches mésenchymateuses (CSM) génétiquement modifiées. En apportant par voie topique des molécules thérapeutiques stratégiques, l'objectif est d'induire la réexpression des gènes déficients sur la surface oculaire en exploitant les propriétés de vectorisation des VEs ainsi que leurs propriétés pro-cicatrisantes et anti-fibrotiques. Une première étape de bio-ingénierie a consisté à valider et optimiser la transfection des CSM grâce à des stratégies virales et non virales. Les VEs issues de ces CSM modifiées génétiquement ont ensuite été produites, isolées et caractérisées. Nous avons pu mettre en évidence l'enrichissement en ARNm et protéines d'intérét au sein des VEs ainsi produites. Nous évaluons actuellement leurs propriétés thérapeutiques in vitro et in vivo. En définitive, la possibilité de stabiliser ou méme restaurer la transparence cornéenne par l'association de la modulation de l'expression de gènes déficients et des propriétés régénératives des VEs de CSM pourra permettre d'ouvrir de nouvelles possibilités en médecine réparatrice de la surface oculaire.

5 – On-chip tumor spheroid formation for high-throughput screening of chemo-immunotherapies

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Keywords : microwell, based microfluidic platform, spheroid, colorectal cancer, high, throughput, 3D models, tumor microenvironment

Colorectal cancer (CRC) is the second deadliest cancer and a major global health challenge with over 45,000 new cases in 2023 and 18,000 deaths. Immune evasion plays a central role in tumor progression and relapses. This creates a strong need for in-vitro models that better mimic the tumor microenvironment and drug penetration. Combining microfluidic and 3D tumor spheroids, present promising alternatives by reducing dependence on animal models, improving physiological relevance, and enabling high-throughput screening on uniform biological samples. We developed a microwell-based microfluidic platform for the high-throughput formation of spheroids in a simple, low-cost, and reproducible format. The microwell ensures uniform spheroid formation. The chip can be opened for retrieval, reused across experiments, and supports long-term culture. Its short working distance is compatible with high-resolution imaging. The platform, integrated into standard 6-well plates, is fabricated by assembling a styrenic block copolymer onto a micro-milled polystyrene substrate. Each chip contains 600 microwells (200µm diameter), arranged along a serpentine microchannel and two reservoirs. Filling is achieved by capillarity and fluid exchange by hydrostatic pressure. Structures formed with various cell lines, including CT26, are currently under characterization. We have validated recovery, and comparisons with ULA plates and hanging drop methods are ongoing.

6 – A human-in-the-loop Active-Deep Learning Framework for Automated Interictal Epileptiform Discharges (IED) Detection from EEG Data

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Keywords : Interictal Epileptiform Discharge (IED), electroencephalography (EEG), deep learning, active learning

Epilepsy is the second most burdensome neurological disorder globally. Interictal Epileptiform Discharges (IEDs) in electroencephalography (EEG) are critical biomarkers for diagnosis and treatment but are challenging to detect automatically due to their short duration, diverse morphologies, variable background activity, and unpredictable occurrence. We present a novel human-in-the-loop active deep learning framework for automated IED detection that significantly reduces dependence on expert annotation. We will present result from human patients as well as epileptic mice models, showing that the framework detected 80–90% of expert-identified IEDs while reducing manual effort by 90% compared to current state-of-the-art methods. This approach is being developed into a prototype software tool for interactive use in experimental neuroscience and clinical research.

7 – An optical mapping system to track arrhythmia mechanisms in heart diseases

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Keywords : cardiovascular disease, heart, arrhythmias, voltage, calcium

Over the course of a human lifetime, the heart beats an average of more than 2.5 billion times. The heartbeat originates from an electrical event known as the action potential, which begins in the sinoatrial node and spreads throughout the myocardium to trigger contraction. Any disturbance in the generation or propagation of the action potential can lead to cardiac arrhythmia, resulting in serious health consequences. The optical mapping system, acquired with support from DIM BIOCONVS, was installed in our laboratory in February 2025. This system allows the simultaneous recording of membrane potential and calcium using fluorescent probes in both intact cardiac tissue and cultured cells, including human induced pluripotent stem cells, providing a versatile platform for studying cardiac arrhythmias. The presentation will highlight the various possible

applications of the system and the initial results obtained from different research projects in our laboratory.

8 – Soft thermoplastic elastomer compartmentalized chip for neurofluidics: a study to assess axonal growth in extracellular vesicle-based assays

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Keywords : neurofluidics, axonal growth, extracellular vesicles, Schwann cells, biotherapies, peripheral nervous system

Compartmentalized microfluidic devices enable controlled studies of interactions between individual cell types. In neuroscience, they are commonly used to investigate axonal growth and drug responses. However, most platforms are limited to two cell types and a single assay per chip. To address these limitations, a soft thermoplastic elastomer (sTPE) device was previously developed to compartmentalize dorsal root ganglia (DRG) explants, guide axonal growth, and induce myelination. Building on this, we present a 3-plex microfluidic platform featuring a 95-microchannel-array per condition. Objectives of this study include: (i) characterizing the hydrodynamic properties of the 3-plex system, (ii) evaluating its utility for studying axonal responses to extracellular vesicles (EVs) from primary Schwann cells and the MSC80 cell line, and (iii) developing a workflow to assess EV effects on axonal networks. Methods and Results: Hydrodynamic behavior was validated via simulations and experiments. EVs (median size: 135±15nm) were isolated and applied every two days at three concentrations. Axonal length, surface coverage, nuclei count, and branching were quantified at days 2, 4, and 6. Conclusions: This multiplexed platform supports simultaneous testing of three conditions and reveals that EVs impact both axonal structure and length. Future work will explore EVs from varying Schwann cell maturation stages.

9 – 3D skeletal muscle constructs from human pluripotent stem cells for Myotonic Dystrophy Type 1 modeling

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Keywords : 3D skeletal muscle, disease modeling, Myotonic dystrophy type 1

Myotonic dystrophy type 1 (DM1) is the most common adult-onset muscular dystrophy. Although the causal mutation was identified decades ago, limited understanding of its pathogenesis

hampers therapeutic progress. Recent advances in 3D culture systems using human pluripotent stem cells (hPSCs) provide new opportunities to develop more predictive translational models. We implemented a transgene-free protocol in 2D culture to differentiate hPSC lines into myogenic progenitor cells that fused to form myotubes exhibiting a high fusion index, well-organized sarcomeric structures and spontaneous contractions. A subset of $Pax7^{+}$ cells adjacent to muscle fibers supported regeneration in a cardiotoxin (CTX) injury assay. Myogenic progenitors were then embedded in 3D hydrogel scaffolds with anchoring pillars to engineer skeletal muscle constructs that responded to acetylcholine and electrical stimulation within 7 days. We applied our 3D skeletal muscle model to hPSCs carrying mutations in the DMPK gene to model DM1. We validated the pathological relevance of the model by observing known molecular hallmarks of the disease, such as the presence of nuclear foci and splicing defects. We also evidenced functional defects with decreased contractile force at 7 and 14 days of differentiation, mirroring the muscle weakness seen in DM1 patients.

10 – Implementing a scalable natural product discovery pipeline in biosynthetically rich Pseudomonadota

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Keywords : Synthetic Biology, Secondary metabolites, Antibiotics, Burkholderia, Pseudomonas

Ever since the discovery of penicillin in 1928, secondary metabolites (SMs) synthesised by microorganisms have revolutionised medicine and become a pillar of modern therapeutics to treat infections, cancer, transplant rejection, metabolic disorders and parasitic diseases. However, the discovery rate of new microbial SMs using traditional approaches has plummeted, with efforts of the last 50 years often culminating in the rediscovery of known molecules. The host laboratory has set in place a synthetic biology framework to significantly increase the pace of SM discovery from Streptomyces spp that has already yielded several active candidates against multidrug-resistant bacteria. Following this success in mining genomes of a monoderm organism, we aim to implement the framework on diderm genera with the highest biosynthetic gene content outside of the Actinomycetota phylum: Burkholderia and Pseudomonas. Our new approach will focus on identifying novel bioactive molecules, particularly against clinically relevant diderms, contributing to the fight against the current microbial resistance crisis which claims over 1.14 million lives each year.

11 – Enrichment of genetically modified hematopoietic stem cells through epigenome editing and antisense oligonucleotides

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Keywords : β hemoglobinopathies, gene therapy, genome editing, base editing, epigenome editing, antisense oligonucleotides

β-hemoglobinopathies are caused by mutations disrupting the production of the adult β-globin. The clinical severity is mitigated by reactivation of the fetal γ-globin expression. We reported that base editing (BE) of the γ-globin promoters in hematopoietic stem/progenitor cells (HSPCs) leads to γ-globin reactivation in vitro. However, xenotransplantation experiments showed that BE efficiency in long-term HSCs is reduced. Here, we developed a strategy for ex vivo or in vivo selection and enrichment of genetically modified cells by downregulating CD33, a receptor expressed on HSCs but dispensable for hematopoietic cell activity. Enrichment is achieved by using an antibody–drug conjugate targeting CD33 to select CD33-negative cells, which are also more likely to be well-transfected with BE and highly edited at the γ-globin promoters. Targeting CD33 either by epigenome editing or gapmer antisense oligonucleotides resulted in substantial CD33 knockdown in a cell line and in HSPCs. We then combined each strategy with BE of the γ-globin promoters in HSPCs. Our results showed efficient γ-globin reactivation along with CD33 downregulation. Importantly, BE efficiency was significantly increased by selecting the edited cells. Collectively, these findings provide a method to improve the therapeutic potential of gene-editing treatments for β-hemoglobinopathies by maximizing the frequency of corrected cells.

12 – Intra-arterial injection of OTR4132, a novel neuroprotector in acute ischemic stroke: the MaTRISS trial

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Keywords : Matrix therapy, Glycosaminoglycans, RGTA®, Regenerative Medicine

Background and objectives. There is an important need for the development of neuroprotective therapeutic agents that could be combined with reperfusion strategies in acute ischemic stroke to improve patient prognosis. OTR4132 is a mimetic of heparan sulfates, which demonstrated neuroprotective effects in animal models. The aim of this study was to assess the safety and tolerability of single doses of OTR4132 in patients with acute ischemic stroke caused by large vessel occlusion who underwent endovascular thrombectomy (EVT). Methods. MaTRISS study (NCT04083001) is a multi-center, first-in-man, open-labeled, dose-escalation study. OTR4132 was administered intra-arterially, immediately after EVT recanalization (Thrombolysis in cerebral infarction (TICI) score of 2b - 3). The primary endpoint was the rate of investigational treatment-related severe adverse events occurring from baseline to 7 days after injection. All other safety and efficacy endpoints were exploratory. Result. Nineteen patients were recruited in the study and 6 increasing doses (0.2 to 2.5 mg) of OTR4132 were tested. Four patients presented at least one serious adverse event. None was considered linked to the treatment. One patient died

of intracranial hemorrhagic transformation after 24 hours, and the causality remained unknown. Conclusions. These results need to be confirmed in a larger multicenter randomized efficacy clinical trial.

13 – Integrin-targeted delivery of etoposide via RGD-functionalized solid lipid nanoparticles for high-risk neuroblastoma

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Keywords : Neuroblastoma, solid lipid nanoparticles, cyclic RGD, integrin, targeted therapy, etoposide, nanomedicine

High-risk neuroblastoma (NB) remains a significant therapeutic challenge due to tumor heterogeneity and the systemic toxicity of high-dose chemotherapy. Consequently, there is an urgent need for innovative therapies to improve patient outcomes. To address this, we developed solid lipid nanoparticles (SLNs) surface-functionalized with cyclic RGD peptides for integrin-targeted delivery of etoposide (ETP). Optimized targeted SLNs were prepared via hot homogenization and ultrasonication, followed by surface conjugation of cRGDfC using maleimide-thiol chemistry. This ligand was selected after evaluating three conjugation approaches: non-covalent, amide, and thioester, with maleimide-based thiol coupling achieving the highest decoration efficiency (~100%) and stable physicochemical properties (diameter In vitro cellular uptake of fluorescent SLNs was assessed in two NB cell lines: SH-SY5Y (integrin-high) and MYCN-amplified SK-N-BE(2) (integrin-low). RGD-functionalization significantly enhanced uptake in SH-SY5Y cells. Cell viability assays showed that RGD-ETP-SLNs reduced IC_{50} values compared to both free ETP and non-targeted SLNs. Flow cytometry confirmed apoptosis as the primary mode of cell death, with progressive late apoptosis and minimal necrosis (<5%) among treatments. These findings support RGD-SLNs as a promising platform for targeted NB therapy. Future studies will focus on validation in 3D tumoroid models to evaluate tumor penetration and translational potential.

14 – Development of a 3D polysaccharide porous membrane for the modelling of the outer blood-retina barrier

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Keywords : Tissue engineering, In vitro modelling, Outer blood, retinal barrier, Polysaccharide hydrogel, Freeze, drying, Pre, vascularization

The outer blood-retina barrier (oBRB), composed of the retinal pigment epithelium (RPE), on the Bruch's membrane (BM) and overlying the vascularized choroid, is disrupted in several retinal dystrophies, leading to vision loss. Our aim is to engineer an implant that mimics the entire oBRB, by developing a new biomaterial with both the RPE and a pre-vascularization. A polysaccharide hydrogel was synthesized and characterized in terms of porosity, permeability, mechanical and degradation properties. RPE cells derived from hiPSCs were seeded on top of the hydrogel. Human retinal microvascular endothelial cells (HRMVEC) were seeded on the opposite side, 10 days after the RPE cells, to form the pre-vascularization over 3 weeks. Fabrication process led to membranes with a smooth non-porous surface in which RPE cells formed a tight monolayer within a week. On the other side, a porous surface connected to the inner porosity hosted the pre-vascularization. Each cell type remained located on its dedicated side. Cellular survival and expression of typical markers increased in co-culture showing beneficial interactions between the two cell types. In conclusion, we designed a membrane mimicking the oBRB structure and suitable for co-culture with precise localization of each cell type and beneficial interactions between RPE and ECs.

15 – Microfluidic stimulation of extracellular vesicles bioproduction in a metastatic context

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Keywords : Microfluidics, bioproduction, extracellular vesicles, cancer

The cancer metastatic cascade includes a dissemination step in the blood circulation. We analyzed MCF-7 (epithelial) and MDA-MB-231 (metastatic) breast cancer cells in a microfluidic device mimicking blood circulation. The more aggressive MDA-MB-231 cells survived longer under repeated mechanical stress, linking malignancy to biomechanical resilience. Under shear stress and deformation similar to conditions faced by circulating tumor cells,both cell lines released more extracellular vesicles (EVs), explaining their presence in bloodstream. Surprisingly, suspended MCF-7 cells (losing both cell-substrate and cell-cell adhesion) produced more EVs than MDA-MB-231 (losing only substrate adhesion), contrasting with adherent-cell studies. Combined shear stress + deformation boosted EV production far more than shear alone, with a 34-fold increase in MDA-MB-231 (vs. 6-fold in MCF-7) after 30 minutes. These findings suggest distinct biogenesis pathways: one responsive to deformation, another to adhesion loss. By targeting these mechanisms, we aim to reduce metastatic spread by impairing circulating cancer cell survival. Additionally, analyzing EV surface proteins could help assess tumor aggressiveness and refine treatments. Finally, we validated a pressure-based flow control system, Omi, Fluigent SAS, to replace manual peristaltic pumps for cell recirculation. This upgrade ensures stable, reproducible flow conditions and precise shear stress regulation-critical for long-term experiments.

16 – Optimization of a scalable production process of Adeno-Associated Virus Vectors for research purposes and development of an AAV vector catalog to target subcellular compartments.

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Keywords : AAV production, AAV, Gene therapy, Fluorescent markers

Most in vivo studies developing AAV-based gene therapies for neuromuscular and neurological diseases require high-quality vectors in sufficient amounts. To meet this need, the MyoVector core facility was established in 2013 within the Centre for Research in Myology (Institut de Myologie). The platform produces recombinant AAV (rAAV) vectors for research and preclinical applications, using scalable methods aligned with clinical standards. We generate single-stranded and self-complementary rAAVs across multiple serotypes (AAV1–9, rh10, AAVmyo) carrying therapeutic genes (e.g., microdystrophin, SMN, CRISPR-Cas9) or reporters (GFP, luciferase). In collaboration with Genethon, we developed a two-step workflow: upstream transient triple transfection of HEK293 cells in serum-free suspension culture, followed by downstream PEG precipitation, iodixanol ultracentrifugation, and formulation. This process yields purified vectors within one week. Although production efficiency varies with serotype/transgene combinations, optimization of upstream parameters (cell density, DNA amount, DNA:reagent ratios) significantly improved reproducibility and yields. The process is scalable, cost-effective, and adaptable to diverse genetic payloads. In collaboration with MyoMolBiol and MyoImage platforms, we also provide rAAVs expressing fluorescent markers for subcellular compartments. Overall, MyoVector delivers robust, high-yield and high-quality rAAVs to support both academic and translational research.

17 – Maximum Entropy Models for N6-Methyladenosine recognition and evolution in Influenza A viruses

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Keywords : Influenza A virus, Methylation m6A, Machine Learning, Evolution

N6-methyladenosine(m6A) is a chemical modification, widely observed in RNA sequences, consisting of the addition of a methyl group(-CH_3) to the nitrogen atom in position 6 of adenosines. Many viruses use m6A to regulate key processes, such as gene expression and translation, and to control viral replication and infection. Improving our understanding of the role of m6A could enhance our ability to predict and prevent future pandemics involving high-risk viruses such as Influenza A(IAV). To achieve this, precise mapping of m6A sites in IAV is required, as well as comparing subtype maps and tracking their evolution. Although experimental methods can detect m6A, IAV data are limited and experimental maps are costly and time-consuming. Therefore, it is critical to develop theoretical models to predict m6A landscapes and study their dynamics.

Maximum-Entropy Models are a machine learning method which is simple and fast to infer. I will present a model trained on m6A from human cell lines(HEK293) and applied to IAV that infects human cells. Our predictions will be compared to experimental results and deep-learning models. Furthermore, the abundance of typical m6A will be quantified, constrained by the production of amino acid sequences, to infer selective pressures to trace their evolution over time.

18 – Expanded-DMPK allele targeting by a C(T/A)G-binding domain associated to a locus localizer reverses DM1-associated defects in patient muscle cells

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Keywords : DM1, Myotonic dystrophy type 1, gene therapy, genome engineering, allele specificity

Myotonic dystrophy type 1 (DM1) is a rare neuromuscular pathology and the most prevalent muscular dystrophy in adults. DM1 originates from an expansion of CTG microsatellites within the DMPK gene. The number of the repeats, from 50 to over several thousand, correlates with the severity of symptoms and the earlier age of onset. The pathophysiology is explained by a toxic RNA gain-of-function, whereby DMPK transcripts from the expanded allele form nuclear foci that sequester MBNL proteins, leading to their loss-of-function and widespread spliceopathy. Most of the therapeutic approaches aim to neutralize the toxic DMPK transcripts but are unable to distinguish the two DMPK alleles, raising concerns about the impact of DMPK protein deficiency. In this context, we sought to target the expanded DMPK locus with a DNA repeat-binding protein conjugated to different domains that relocates the locus into nuclear areas with low transcriptional activity. In a DM1 muscle cell model, our approach normalized mis-splicing markers and reduce the presence of foci. Furthermore, the high level of remaining total DMPK transcripts suggests that the wild-type allele is preserved. Altogether, our findings highlight that the nuclear relocation of the expanded DMPK-locus as additional therapeutic avenue for DM1.

19 – Systematic discovery of anti-viral molecules produced by anti-phage systems

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Keywords : defense systems, natural products, synthetic biology, antivirals

It was recently discovered that some compounds, mainly synthesized by Biosynthetic Gene Clusters (BGCs), can protect bacteria against phages. This represents a specific type of defense system named anti-phage «chemical defense». Among defense systems encoded by the bacterial pangenome, some have been found to be similar to known anti-viral systems in eukaryotes. This

raises the possibility that certain bacterial antiviral systems could also target eukaryotic viruses, making their associated compounds antiviral agents with potential clinical applications. However only 3% of predicted BGCs have been linked to a characterized compound, using random screening methods of known natural products. The discovery of lanthiphages demonstrated that BGCs encoding antiphage molecules can be predicted based on their colocalization with known defense systems. In this study, we aim to systematically discover anti-viral molecules produced by anti-phage systems using comparative genomics methods.

20 – Phage display for high throughput proteolytic profiling

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Keywords : Synthetic Biology, NGS, deep learning, peptide library, phage display

Evolution of antimicrobial resistance represents a rising global health crisis, potentially leading to 10 million annual deaths by 2050. Antimicrobial peptides (AMPs), those that are unmodified and ribosomally synthesized with more than 4000 natural examples across all domains of life, offer a credible solution due to their ease of development, broad-spectrum activity, and lower emergence of resistance. However, rapid digestion of existing AMPs by proteases under physiological conditions prevents their wide adoption. Here we address this bottleneck by designing relatively protease-resistant AMPs in given physiological conditions, e.g., body fluids. We aim to initially build a comprehensive profile of the proteolysis patterns through a phage display-mediated screening of a 10^7 randomized peptide library. The proteolytic profiles will be used to prioritize protease-resistant AMPs designed via generative deep learning, exploring a vast sequence space.

21 – COMPARATIVE ANALYSIS OF ANTI-FIBROTIC PROPERTIES OF PLASMA RICH IN GROWTH FACTORS AND AUTOLOGOUS SERUM IN VITRO ON CORNEAL FIBROBLASTS.

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Keywords : cornea, myofibroblast, fibrosis, autologous serum, plasma rich in growth factors, wound healing

Autologous serum (AS) eye drops are widely used as treatment of severe ocular surface defects because of their wound healing properties. However, they don't prevent fibrosis and haze formation. Plasma rich in growth factors (PRGF) has been proposed as an alternative. In our studies, we assay in vitro the anti-fibrotic properties of PRGF and AS in order to validate this proposal. Primary corneal fibroblasts were extracted from human corneas and treated with PRGF or AS prepared from blood of healthy donors. The anti-fibrotic properties of PRGF and AS were assessed on models of myofibroblast differentiation, scratch wound and gel contraction. In addition, the effect of both treatments on a corneal epithelial cell line on a scratch wound assay was studied. Our studies demonstrate that PRGF and AS show no differences in wound closure in

corneal fibroblasts and epithelial cells. Prevention of myofibroblast differentiation by PRGF is significantly higher than AS and PRGF prevents collagen gel contraction whereas AS does not. Our results show that PRGF has an enhanced anti-fibrotic effect based on the prevention of myofibroblast differentiation and contractility inhibition compared to AS. Furthermore, PRGF is as effective as AS in promoting migration in both corneal epithelial and stromal cells.

22 – Epigenetic and epitranscriptomic regulator ZNF217 rejuvenates T-cell therapy

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Keywords : T cell therapy, CAR T cells, TILs, immunotherapy, genome editing, CRISPR, Cas9

T cell exhaustion is a dysfunctional state marked by reduced polyfunctionality and memory potential, limiting antitumour immunity and adoptive T cell therapy. Genome-wide CRISPR screens identified SOCS1 as a major intrinsic checkpoint inhibiting CD4 CAR-T proliferation/persistence and CD8 CAR-T cytotoxicity. SOCS1-deficient CAR-T cells display effector-memory rather than exhausted phenotypes, but transcriptomic analysis reveals compensatory upregulation of other SOCS family members (SOCS3 and CISH) over time, which are also strong inhibitors of T cells. The epitranscriptomic regulator METTL3 promotes the decay of SOCS1, SOCS3, and CISH transcripts in T cells, offering a strategy to target multiple inhibitors simultaneously. We have demonstrated that METTL3 is inhibited by the transcriptional repressor ZNF217. Znf217 deletion in murine tumour-specific T cells induces Mettl3-dependent reprogramming toward a progenitor-like state. In human anti-CD19 CAR-T cells, ZNF217 knockout increases METTL3 and IL7R while reducing SOCS1 and CISH proteins. Functionally, ZNF217-deficient CAR-T cells show sustained expression of stem-associated markers (CD62L, IL7R, SLAMF6), enhanced persistence, proliferation, polyfunctionality, and potent antitumor activity in both leukaemia and solid tumour models. We further identified a molecular glue which degrades ZNF217 and phenocopies ZNF217-KO, in CAR-T and TILs from ovarian cancer patients, enabling transient reprogramming of TCT with resistance to exhaustion.

23 – Development of cellular models for the evaluation of lipid nanoparticle-based therapies in preeclampsia.

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Keywords : Lipid nanoparticles cellular models preeclampsia

Pregnancy-associated disorders represent a major public health concern. Preeclampsia especially, as it is the second leading cause of maternal mortality in France,driven by the overexpression of sFlt-1 protein in maternal circulation. The absence of therapies is largely attributed to the lack of models. Consequently,the development of reproducible cellular models that accurately recapitulate the pathological condition is essential for the evaluation of potential therapeutic

molecules. We demonstrated that the culture of villous cytotrophoblasts in the presence of cobalt chloride induces sFlt-1 overexpression,without compromising cell viability. This model enables the assessment of gene silencing strategies using oligonucleotides delivered via lipid nanoparticles. By microfluidic,we optimized DMAPAP-based lipid formulations and identified two lead nanoparticles,with a composition of DMAPAP:helper-lipid:cholesterol:PEG-lipid = 50:10:38.5:1.5,exhibiting sub-100 nm diameters,low polydispersity,and robust stability. Complexation with splice-switching oligonucleotides (SSO) and siRNA resulted in increased particle size and heterogeneity;these effects were mitigated by alginate coating. Fine-tuning the zeta potential to –10 mV enhanced colloidal stability. Both formulations demonstrated high SSO complexation,and studies on primary placental cells indicated that both lipid composition and alginate coating influence biocompatibility. These findings provide a rational framework for the design of DMAPAP-based formulations as safe and efficient nucleic acid delivery systems in in vitro preeclampsia models.

24 – Mechanical forces and topological morphogens drive 3D Self-Organization in nematic-like biological tissues

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Keywords : cell differentiation mechanobiology, mechanical constraints

Biological tissues attain their proper shape and organized structures during development through responses to internal and external signals, with mechanical cues playing a critical role. These forces guide cellular organization, leading to the formation of complex self-organizing structures that underpin embryonic patterning. Recent theories propose that "topological morphogens" drive these processes. However, the study of three-dimensional (3D) tissues remains challenging due to the limitations in available model systems and the complexities of modeling such systems. We address these challenges by utilizing self-organizing cellular aggregates, particularly spindle-shaped C2C12 myoblasts, subjected to controlled mechanical stretching. Our results demonstrate that these cells form a multilayered, actin-oriented tissue structure, where mechanical forces drive long-range 3D organization and muscle differentiation. Interestingly, the tissue surface emerges as a focal point for differentiation, correlating with directional order, as evidenced by single molecule fluorescent in situ hybridization.

25 – Human neuromuscular developmental characterization in congenital myotonic dystrophy

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Keywords : Congenital dystrophy myotonic, Neuromuscular organoids, human Induce Pluripotent Stem Cells (hIPSC)

Congenital myotonic dystrophy (CDM) is a rare genetic neuromuscular disorder characterized by myotonia, muscle weakness, and broader developmental impairments of the neuromuscular system. Although recent studies have highlighted the systemic nature of the disease (J. Tahraoui-Bories et al., 2023; M. Nakamori et al., 2022; Frison-Roche et al., 2025), the underlying developmental mechanisms remain poorly understood. This is largely due to the scarcity of human tissue samples and the lack of physiologically relevant in vitro models, which hampers the identification of therapeutic targets. To address this, we developed a human neuromuscular organoid model derived from induced pluripotent stem cells (hiPSCs). By directing hiPSCs toward a neuromesodermal lineage, we simultaneously generate key components of the neuromuscular system within a single 3D structure. Initial characterization across developmental stages revealed the presence of both myogenic and motor neuron progenitors by day 10. By day 50, the organoids displayed a spatially organized architecture with polarized muscle and neural domains, sarcomere formation in muscle fibers, and the establishment of neuromuscular contacts. Ongoing transcriptomic analyses aim to identify dysregulated cellular pathways in CDM, providing new insights into disease pathogenesis and paving the way for the development of targeted therapies.

26 – A Scalable Approach to Unlock Fungal Cryptic Secondary Metabolites

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Keywords : Fungi, Natural Product, Synthetic Biology, Cloning

In this project, our approach is to solve the technical levers for unearthing cryptic biosynthetic gene clusters (BGCs) two fungi genus, Podospora and Aspergillus, on a large scale with the ultimate aim of identifying new bioactive secondary metabolites (SMs) capable of meeting healthcare needs worldwide. A scalable genome screening approach will enable the identification of hundreds of novel BGCs simultaneously by leveraging high-throughput screening and heterologous expression techniques. We aim to unlock the therapeutic potential of these fungal SMs through a citizen science approach to soil sampling, engaging the public in fungal diversity exploration and including it into the research process.

27 – Development of a fully hydrolysable nutritive hydrogel for fuelling human mesenchymal stromal cells energetic needs in ischemia

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Keywords : Mesenchymal Stromal Cells, ischemia, hydrogel, glucose, cell survival, starch

Human mesenchymal stromal cells (hMSCs) show great promise in regenerative medicine, but their survival after transplantation is compromised in ischaemic environments. Nutrient-releasing hydrogels, particularly glucose polymer-based ones, have been suggested as a way of improving survival. However, many of these gels lack full hydrolysability, which can result in poor tissue integration. This study aimed to design a fully hydrolysable, fibrin-based hydrogel that

would support hMSC survival for over seven days. Five starch-derived glucose polymers were screened for enzymatic degradability using amyloglucosidase. Wheat starch, amylopectin and maltodextrin were found to be fully hydrolysable, with only maltodextrin having a loading capacity high enough to fuel hMSCs for 21 days. By adjusting the hydrogel composition, we achieved glucose release kinetics matching hMSC consumption rates under hypoxic conditions. In vitro assays confirmed the ability of selected compositions to sustain hMSC viability up to 21 days in hypoxic conditions. In vivo, subcutaneous implantation demonstrated enhanced hMSC survival at D7 compared to glucose-free controls, although the effect declined by D14. These results demonstrate that fibrin hydrogels loaded with maltodextrin can improve the post-transplantation survival of hMSCs while remaining fully degradable. Further optimisation is required to minimise leakage and maximise the therapeutic potential for clinical applications.

28 – Enabling rapid and flexible CAR engineering via a Modular Cloning strategy

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Keywords : CARTune, mammalian synthetic biology, Immunotherapy, Solid tumors

Chimeric antigen receptor (CAR) T cells have shown remarkable clinical responses in advanced malignancies, leading to the FDA approval of seven therapies for liquid tumors. Despite this success, their application to solid tumors remains limited. Our project focuses on improving a tunable CAR-T system, CARTune, developed by our team. CARTune enables control of CAR activity, thereby reducing side effects. In this system, the CAR is retained in the endoplasmic reticulum in a steady state and released to the cell surface in an active state upon biotin treatment. However, current designs do not achieve full retention, resulting in unwanted basal activity. To adress this, we are developing a modular cloning approach that enables flexible assembly and rapid testing of CAR and CARTune constructs. This strategy allows targeted modification of individual CAR domains and streamlines the optimization process. Ultimately, our goal is to enhance CARTune's precision and safety, advancing its potential for solid tumor applications.

29 – Modeling Fibrosis in Duchenne Muscular Dystrophy organoids

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Keywords : DMD, organoids, FAPs, Fibrosis

Duchenne muscular dystrophy (DMD) is a severe muscle wasting disorder, caused by genetic mutations of dystrophin, a protein essential to preserve muscle fiber integrity after mechanical stress. The progression of the disease is dictated by detrimental consequences of lack of dystrophin such as muscle degeneration, inflammation and fibrosis ultimately leading to loss of muscle function and impairment of gene therapy strategies. Fibrosis is primarily driven by aberrant activation of fibro-adipogenic progenitors (FAPs) orchestrated by intercellular communications with muscle cells through secreted cytokines and myokines. Our data obtained in DMD-iPSC-derived human muscle organoids, including fibroblasts, indicate that MDys, current

gold standard for DMD gene therapy, improves muscle function but only partially reduce profibrotic activity in fibroblasts. We then optimized our system to include FAPs to analyze their cellular plasticity and fibrotic potential in DMD context. We aim to elucidate signaling events leading to perturbation of muscle cells and FAPs secretome to identify new candidates enhancing gene therapy efficacy and more broadly anti-fibrotic targeted therapy. Comparative analyses between Control and dystrophic MYOrganoids including FAPs will be performed using imaging, single-nuclei transcriptomics, and functional muscle force assays to support the physiological relevance of our system and its suitability as platform for therapeutic investigation.

30 – Unlocking 3D tissue architecture via fast, non-toxic clearing and autofluorescence imaging

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Keywords : Label free, autofluorescence, image scanning microscopy, confocal detection, histology, whole organ clearing, morphological analysis

Tissue clearing combined with advanced optical imaging enables deep, high-resolution 3D visualization of complex biological systems-overcoming the depth and context limitations of traditional histological thin tissue slices. In the present study, we apply our patented UbiClear protocol to a broad range of specimens, including mouse brain, gut, testis, insects (e.g., spiders, woodlice, hornets), feathers, and algae, allowing intact structural imaging at cellular resolution. Using both confocal and light-sheet microscopy, we generate volumetric datasets that capture fine morphological detail across large tissue volumes. Notably, we harness tissue autofluorescence, often rather seen as a drawback, as a powerful source of intrinsic contrast. This enables label-free visualization of tissue architecture, acts as a built-in counterstain, and complements exogenous labeling. Our approach reveals spatial organization and biological connectivity without the need for extensive sectioning. Confocal microscopy offers fine axial resolution, while light-sheet imaging enables rapid imaging of large volumes. Together, they form a versatile workflow that bridges cellular detail with whole-organism scale, unlocking new potential for biological discovery across diverse samples.

31 – The therapeutic potential of targeting immune checkpoints in Idiopathic Pulmonary Fibrosis

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Keywords : Lung fibrosis, Immune Chechpoints, Immunomodulation

Idiopathic pulmonary fibrosis (IPF) is a chronic and fatal interstitial lung disease. The mechanisms underlying the pathophysiology of IPF remain poorly understood. Recent studies highlighted the potential regulatory roles of immune cells in IPF progression, mainly through the release of various growth factors and cytokines. Immune checkpoints (ICs), which are key regulators of immune responses, have been extensively studied in the context of cancer, where

their therapeutic targeting has shown considerable success. In this project, we aim to investigate the role of ICs in IPF. Using a transcriptomic approach, we identified the most relevant ICs expressed in the lungs of IPF patients compared to healthy donors. Multiparametric flow cytometry is employed to assess the expression of these receptors at the protein level and to correlate their expression with disease severity using a stratification approach. Ex-vivo experiments are conducted to evaluate how modulation of the identified ICs through inhibition or overexpression affects the profibrotic activity of immune cells. In parallel, transgenic mouse models have been developed to explore the functional role of these receptors in lung fibrosis development. Overall, this project could have the potential to reveal novel immune mechanisms leading to new diagnostic and therapeutic tools against this lethal disease.

32 – Rescue of lysosomal acid lipase deficiency in mice by AAV liver gene transfer

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Keywords : gene therapy, rAAV8, lysosomal acid lipase deficiency

Lysosomal acid lipase deficiency (LAL-D) is an autosomal recessive disorder caused by LIPA mutations, leading to fatal lipid accumulation in the liver and spleen. The most severe form causes early death without treatment. Weekly enzyme replacement therapy is the only current treatment, but it is not curative and may be limited by neutralizing antibodies. To develop a curative approach, we characterized a new lal-/- mouse model, confirming loss of LAL activity, lipid accumulation, hepatosplenomegaly, dyslipidaemia, and an inflammatory hematologic profile. We then evaluated in vivo gene therapy by systemically injecting 3-month-old male lal-/- mice with AAV8-hLIPA under the hepatocyte-specific hAAT promoter. Transduced hepatocytes would produce LAL to correct their own metabolic impairment and secrete the enzyme in bloodstream to cross-correct affected tissues. We conducted a 3-months follow-up study of 4 different AAV-8 doses and observe a dose-dependent restoration of enzymatic activity, correction of liver and hematologic parameters, and reduction of hepatosplenomegaly. With the minimal corrective dose, we assessed phenotype correction in lal-/- mice at 1-, 3-, and 8- months post-injection. Overall, our in vivo gene therapy strategy represents an effective approach to correct LAL-D disease opening the possibility of developing a curative treatment to improve patients' life expectancy and quality.

33 – From Cells to Extracellular Vesicles: Establishing a GMP-Compliant Process for Acellular Therapy

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Keywords : Cell therapy, acellular therapy, extracellular vesicle, mesenchymal stromal cell, biotherapy

Mesenchymal Stromal Cells (MSC) are of particular interest for preventing or repairing organ/tissue damage. They exert immunomodulatory activity and promote tissue repair. Their therapeutic effects are mainly mediated by the secretion of a wide range of soluble molecules and extracellular vesicles (EV). A new paradigm has emerged in the past years, which consists in shifting from cell therapy to a more flexible acellular therapy approach, thereby opening a new and promising field in nanomedicine. At the French Armed-forces Blood Transfusion Center (CTSA, Clamart), our advanced therapy medicinal product unit is dedicated to clinical-grade production of autologous MSC intended for injection in patients with severe burns (MTI-PP 007). In order to implement a GMP process for acellular therapy, we are adapting our manufacturing process to incorporate the harvest of conditioned media, concentration by tangential filtration, and the filling and finishing step, all within closed systems. A wide range of analytical methods is used to assess the quality of the final product (Sterility, particle concentration and size, phenotype, functionality activity, purity...). This work will allow us to propose a rapid and efficient manufacturing, in accordance with good manufacturing practices (GMP), of biological medicinal products to prevent tissue injury/promote tissue repair following trauma.

34 – Characterization of gamma delta T cells in Autoimmune Myasthenia Gravis

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Keywords : Myasthenia Gravis, Thymoma, γδ T cells, cytometry

γδT cells are of growing interest in cancer and autoimmunity. Our research focuses on myasthenia gravis (MG), an autoimmune disease characterized by muscle weakness due to autoantibodies against acetylcholine receptors at the neuromuscular junction. MG is a relevant model since its effector organ is the thymus, where T cells develop, and which may display follicular hyperplasia or thymoma. We first analyzed γδT cells by CyTOF in fresh blood from MG patients (n=8), corticosteroid-treated MG (n=7), and healthy controls (HC) (n=8). Several clusters were identified using CD27, CD45RA, CD161, CCR6, CCR7, CD28, and CD57. Among them, naïve γδT cells appeared increased in MG. To enlarge the cohort, a new flow cytometry (FC) panel was optimized and applied to HC (n=24), MG (n=16), treated MG (n=7), thymectomized MG (n=7), thymoma-associated MG (n=13), and thymoma without MG (n=15). Naïve γδT cells were increased in treated MG. Vδ2 γδT cells were consistently reduced in MG, regardless of thymus status, suggesting a role of the thymus in their peripheral dissemination or recruitment to the

myasthenic thymus. In thymoma, however, blood Vδ1 cells were increased. These results highlight distinct γδT cell alterations in MG and thymoma and support extending FC analysis to thymocytes.

35 – Refined cellular models to assess therapeutic options for extracellular matrix-related neuromuscular disorders

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Keywords : Neuromuscular disorders, Collagen VI, Fibrosis, Myomatrix, Therapy

Extracellular matrices (ECM) constitute a unique microenvironment for cells in tissues. In skeletal muscle, myofibers are surrounded by a thin basement membrane and a collagen-rich interstitial stroma. Collagen type VI (COLVI) ensures the architectural integrity of the ECM, anchors cells and mediates signalling pathways through transmembrane receptors. COLVI deficiency, due to mutations in the COL6A1-3 genes, leads to rare neuromuscular disorders, termed COL6-related dystrophies (COL6-RD) for which there is no treatment. A significant histological signature of COL6-RD is skeletal muscle fibrosis (increased ECM components in the interstitium). COLVI itself is a biomarker and a driver of fibrosis. We sought to optimize cellular models for COL6-RD research, based on the «scar-in-a-jar» system. Dermal fibroblasts and muscle fibro-adipogenic precursors cultured in these optimized conditions display accelerated and enhanced expression of fibrosis markers, including COLVI. Using complementary readouts we analyzed the deposited ECM, in control and patient-derived cells. In conclusion, these refined culture conditions provide novel pro-fibrotic cellular models for neuromuscular disorders, which will be valuable tools for the functional validation of therapeutic strategies. In particular, we focus on a therapeutic option using engineered tRNAs, designed to counteract nonsense mutations, thereby restoring the expression of functional COLVI in the ECM.

36 –Protein capture through Liquid-Liquid Phase Separation with a diverse group of sharp minds*

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Keywords : Peptide engineering, Synthetic Biology

37 – CRISPR activation of utrophin as a mutation-independent approach for Duchenne Muscular Dystrophy therapy

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Keywords : CRISPR, DMD, epigenetic

Duchenne muscular dystrophy (DMD) is a severe genetic disorder affecting 1 in 5.000 newborn males. It is caused by mutations in the DMD gene, leading to dystrophin deficiency and progressive muscle degeneration. Current dystrophin-based therapies aim to restore truncated dystrophin forms. However, these approaches can elicit immune responses and some are mutation-specific, limiting their therapeutic scope. An alternative approach, potentially applicable to all DMD patients regardless of their genetic mutation, involves upregulating utrophin (UTRN), a structural and functional paralogue of dystrophin. UTRN reactivation has shown therapeutic potential in preclinical models, improving the dystrophic phenotype without triggering immune responses. Here, we employed a CRISPR activation (CRISPRa) system with catalytically inactive Cas9 fused to a histone acetyltransferase, enabling targeted UTRN epigenetic activation without DNA cleavage. We first established a nucleofection-based RNA delivery protocol for immortalized myoblasts derived from DMD patients. We then co-delivered in vitro transcribed mRNA encoding CRISPRa components along with individual synthetic sgRNAs targeting the UTRN promoter. RT-qPCR and Western blot analyses revealed significant utrophin upregulation. We are currently evaluating combinations of sgRNAs and CRISPRa effectors to maximize UTRN expression. Our findings highlight CRISPRa-mediated histone acetylation as a novel mechanism for targeted UTRN overexpression in the context of DMD therapy.

38 – Should we re-use plastic vessel for adherent cell culture?

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Keywords : reduced plastic consumption, sustainable development, cell culture, change in experimental habits

How can we, in rigorous manner, reduce plastic consumption and guarantee the reliability of experimental results? Are the culture flasks reusable when flipping them up down under sterile conditions? Our lab started a multidisciplinary study at the interface between physics and biology to investigate this crucial question. First, we explore cell division time, cell size and morphology and oxidative stress as cell wellness indicators. Second, cytoskeleton and adhesion molecules, which are environmental mechanical sensors, will be observed. Last, using an atomic force microscope, we plan to study the substrate reuse impact on cell mechanical properties. I will present some of the preliminary results we obtained focusing on classical cell lines. This project is a feasibility demonstration and promote reflection on plastic savings in all cell biology laboratories. This twofold saving in consumables could be a significant achievement within our units. It may promote other testing initiatives in terms of indicators or cell lines. Compared to switching from plastic to glass equipment, this proposal is based on a net saving in plastic material and without cleaning devices and water costs.

39 – Humanized Bone Marrow Organoids to reveal novel spatial features of the leukemia microenvironment

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Keywords : Leukemia, Bone marrow organoid, Microenvironment, Vessels, Spatial transcriptomic

Mouse models have long been used to study the role of the bone marrow (BM) niche in leukemia, but translation to humans remains limited. We implemented a human BM organoid (BMO) model (Abarrategi et al., 2017; Passaro et al., 2017) to screen leukemia patient-derived samples. We have validated the capacity of this model to sustain human leukemic engraftment over time and observed several architectural and functional differences between healthy and leukemic BMO. To gain insight into their spatial organization, we have analyzed a panel of healthy vs leukemic BMO via spatial transcriptomic. The analysis shows high quality RNA yield from the samples, as well as good preservation of tissue morphology and architecture. We could identify regionally distinct clusters of lymphoid or myeloid differentiation in healthy BMO, associated with specialized vessels. More specifically, the vascular compartment could be classified in three subgroups, regionally and molecularly defined. We are currently analyzing how different leukemias impact on the vascular niche heterogeneity. This organoid platform holds great promise to enhance our ability to uncover novel vulnerabilities extending beyond cancer research to applications in infectious diseases and advanced therapeutics.

40 – Pathogen-induced mechanical forces regulate the release of small endothelial vesicles

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Keywords : Pathogen, mechanical forces, type IV pili, endothelial vesicles

Infection tolerance limits disease severity by improving tissue damage control that protect the host from essential dysfunction. Type IV pili (T4P), a multifunctional bacterial structures, exert mechanical forces upon retraction, signaling to both bacteria and host cells. For Neisseria

meningitis (Nm), responsible for meningitis and purpura fulminans, T4P retraction promotes bacteremia and lethal sepsis. Using both wild-type (WT) and a retraction-deficient strain (ΔpilT) we study the consequences of these mechanical tension on vascular cells. Nm infection mechanically activates signaling cascades that lead to endothelial cell junction destabilization and death in vitro, while monolayer integrity is preserved upon ΔpilT infection. Using a humanized mouse model, despite similar vascular colonization and inflammation, WT infection reduces coverage of the endothelial glycocalyx barrier and allow occlusive thrombi in vivo, unlike ΔpilT infection. Furthermore, T4P induces endothelial small extracellular vesicles release in vitro. Proteomic analysis of conditioned media strongly suggests that T4P retraction triggers the release mediators of cell stress, implicate in endothelial dysfunction and disease severity. Interestingly, ΔpilT infection promotes the release of proteins that allow the maintenance of vascular cell integrity and contribute to tolerance mechanism. Collectively, these results suggest a new understanding of how mechanical forces from bacterial pathogens influence infection tolerance.

41 – Novel homology-mediated end joining -IDLV precise integration for Therapeutic Genome Editing in Hematopoietic Stem Cells

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Keywords : Targeted gemone editing, IDLV, HSPCs

Ex vivo gene therapy of hematopoietic stem and progenitor cells (HSPCs) offers promise for treating inherited blood disorders. While CRISPR-Cas9 enables precise gene correction, delivering large DNA sequences efficiently remains challenging. AAV6 vectors, commonly used for homology-directed repair (HDR), are limited by toxicity and packaging constraints. Integrase-defective lentiviral vectors (IDLVs) are safer and larger but rely on HDR, which is less active in long-term HSPCs. We developed a novel class of IDLVs that harness the homology-mediated end joining (HMEJ) pathway, active throughout the cell cycle. These vectors include microhomology arms and sgRNA target site, allowing Cas9 to cut both the genome and vector, exposing homology arms for precise integration. Using this system, we achieved efficient, directional, and seamless integration of GFP under the HBA promoter in K562 cells and the AAVS1 locus. In human HSPCs, we demonstrated targeted integration of GFP and therepeutic trangenes (LIPA and F8) with confirmed protein expression and activity. Enhancing integration via NHEJ inhibition and longer homology arms doubled efficiency. Combining both strategies yielded up to 40% integration, matching AAV performance. In conclusion, we propose a platform for HSPCs gene editing, with strong potential for clinical translation in monogenic diseases, immune modulation, and regenerative medicine.

42 – Patient-derived myoblasts and FAPs: easy-to-use models for studying neuromuscular diseases

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Keywords : Cellular models, Neuromuscular diseases, FAPs, Myoblasts

The development of innovative and tailor-made therapies for neuromuscular diseases requires easy-to-use and suitable cellular models. Patient-derived muscle stem cells, known as myoblasts offer an ideal in vitro model that includes the genetic environment of each mutation. However, the limited proliferative capacity of primary myoblasts, especially in the context of degenerative conditions, hampers their broader application. To overcome this limitation, we have established a robust strategy to immortalize myoblasts derived from muscle biopsies of dystrophic patients. Immortalized myoblasts maintain their original behavior and retain their ability to differentiate and fuse into myotubes. To date, we have generated more than 187 human immortalized myoblast lines including 36 neuromuscular diseases such as DMD, DM1, LGMDR2, OPMD, SMA, FSHD and control subjects. In recent years, Fibro-Adipogenic Progenitors (FAPs), key players in muscle homeostasis, regeneration and fibrosis, have become a focus of interest. To support research in this field, we have already generated 19 immortalized FAPs derived from muscles of patients with diseases such as of DMD, FSHD, COL6 related myopathies or SMA as well as from healthy controls. These FAPS are currently being characterized. These cellular tools offer significant advantages: they are easy-to-use and allow rapid testing of therapeutic strategies.

43 – Unbiased CRISPR/Cas9 sreening to identify cis-regulatory elements associated with utrophin repression in Duchenne muscular dystrophy

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Keywords : Utrophin, DMD, CRISPR/Cas9

Duchenne muscular dystrophy (DMD) is a severe X-linked neuromuscular disorder caused by mutations leading to dystrophin loss. This results in progressive muscle degeneration, loss of ambulation, and premature death. Current therapies aim to restore dystrophin expression, either exogenously (via rAAV-mediated microdystrophin delivery), or endogenously (using exon-skipping oligonucleotides or genome editing). However, these strategies are limited by immune response issues, and their mutation-specific nature, reducing the therapeutic scope. An alternative strategy involves upregulating utrophin (UTRN), a dystrophin paralogue capable of functionally compensating for dystrophin loss without triggering immune response. Although UTRN is highly expressed during foetal development, its repression in adult muscle remains poorly understood. We aim to identify cis-regulatory elements involved in UTRN repression using a CRISPR/Cas9 saturation mutagenesis screen targeting its promoter, 5'UTR, and 3'UTR. To monitor expression changes, we developed and validated a GFP-based utrophin reporter cell line. A custom sgRNAs library will be used to mutate regulatory regions, and cells with increased GFP will be isolated via FACS. Sequencing will reveal perturbations linked to utrophin upregulation.

The identification of repressive elements should highlight new actionable therapeutic sites for endogenous utrophin upregulation, offering broader applicability and reduced immunogenicity compared to dystrophin-based approaches.

44 – Tricyclo-DNA antisense oligonucleotide compounds to tackle toxic CUGexp-RNA in a mouse model of Myotonic Dystrophy.

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Keywords : Antisens oligonucleotide, therapy, ASO, Tricyclo, DNA, gapmer, Myotonic Dystrophy

Antisense oligonucleotides (ASOs) represent a promising therapeutic approach for neuromuscular disorders such as Myotonic Dystrophy type I (DM1), a multisystemic disease caused by CTG repeats expansions in the DMPK gene. These expansions result in toxic CUG-expanded (CUGexp) RNA that accumulate in nuclei, forming foci and sequestering MBNL proteins, associated with RNA metabolism defects and toxicity. ASOs can degrade CUGexp transcripts or disrupt pathological RNA-binding protein interactions, but their effective delivery to skeletal muscles after systemic administration remains a major challenge. Here, we report the use of tricyclo-DNA (tcDNA), a potent ASO chemistry with enhanced RNA affinity, biostability and nuclease resistance, as a potential treatment for DM1. As a proof of concept, we tested a tcDNA-gapmer targeting CUGexp-containing transcripts in the skeletal muscle of DM1 transgenic mouse model with 220 CTG repeats. A single intravenous injection led to a complete, correction of the myotonia observed in this model, a global normalization of splicing defects and significantly reduced RNA foci through a 80% degradation of CUGexp-RNA. Therapeutic effects were sustained for up to six months and low repeated dosing also showed promising results, supporting the potency, durability and translational potential of tcDNA ASOs for systemic treatment of DM1.