Talk Abstracts

Viral suppressive capacity: assessing CD8 T cell responses in HIV-infected individuals under antiretroviral therapy

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Background

Potent HIV-specific immune responses and a small latent viral reservoir are likely required to control viral rebound after treatment interruption. Here we investigate the associations between the viral suppressive capacity of CD8+ T-cells, their immune phenotype and the HIV reservoir in a cohort of antiretroviral therapy-treated (ART) patients.

Methods

Thirty-six patients under ART with suppressed viremia and six healthy donors were recruited. Total HIV-1 DNA and unspliced mRNA (usRNA) levels were measured in PBMCs using digital droplet PCR. Inducible viral transcriptional activity was quantified with the TILDA assay. Viral suppressive capacity of CD8+ T cells was assessed by p24 ELISA running the viral inhibition assay (VIA): a co-culture system of HIV-superinfected CD4 and autologous CD8 T cells. Immunophenotyping of T cells was executed using 18 color flow cytometry. Cytometry data was clustered using viSNE and SPADE in the Cytobank environment. Linear regression and student t-test were used for statistical analysis.

Results

CD8+ T-cell suppressive capacity was significantly ($p < 0.01$) increased after HIV peptide stimulation. Viral suppressive capacity correlated with HLA-DR expression in CD8+ T cells during co-culture ($p < 0.005$). At baseline, the suppressive capacity correlated with CD160/PD-1 co-expression in CD8 TEMRA ($p < 0.001$).

Conclusions

Despite heterogeneity in terms of immune responses, phenotype and reservoir size, T-cell exhaustion markers CD160 and PD-1 were predictive for the suppressive capacity of CD8+ T cells. This opens future prospects for therapeutic interventions restoring immune responses in HIV-1 infected patients.

A multi omic view of phage-host dynamics within a mixed microbial community

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Microbial communities are omnipresent and play important roles within a variety of environments and processes. Two examples that illustrate the diversity of those communities are the human gut microbiome and the microbial consortia found in biological wastewater treatment systems. Viruses, in particular bacteriophages (or phages), are some of the most abundant and diverse components within microbial communities and are believed to influence their structure and dynamics. However, the roles of bacteriophages within those communities are not well understood due to limitations in experimental and analytical methodologies. Fortunately, high-throughput omic data derived from microbial communities, i.e. metagenomics and metatranscriptomics, enable access to genomes and transcriptomes of both phages and their associated hosts. Here, we study phage-host dynamics within an extensively sampled (weekly, over one and a half year) model system of lipid accumulating microbial populations, derived from foaming sludge at a biological wastewater treatment plant. The generated metagenomics and metatranscriptomics datasets were analyzed using an integrated omics workflow. Approximately 130 host and 15,000 putative phage populations were associated via information from the CRISPR-Cas system(s); a memory-based prokaryotic anti-viral defence mechanism. Furthermore, the phage-host abundance information was used to infer a phage-host co-ocurrence network. Subsequently, the associated phages and hosts were used to describe bacteriophage dynamics and activity. In addition, we identified certain dominant bacterial populations that actively utilize the CRISPR-Cas system to target invasive genetic elements. Finally, the associative information will be used for the formulation of a model to elucidate bacteriophage and host dynamics.

Automated high-throughput high-content autophagy and mitophagy phenotyping in Parkinson's disease

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Autophagy and mitophagy are cellular processes essential for homeostasis. Multiple diseases converge in autophagy or mitophagy impairments as a common pathological mechanism. The autophagy field has the need to classify autophagic and mitophagic structures in a high-throughput manner. Here we developed a novel high-throughput phenotyping platform with automated high-content image analysis to assess autophagy and mitophagy alterations in a polygenetic Parkinson's disease cohort. As a proof of concept we outlined the autophagy and mitophagy alterations in cell lines carrying the Parkinson's disease associated mutations LRRK2(p.G2019S), VPS35(p.D620N), and PINK1(p.I368N).

A 3-gene panel improves the prediction of left ventricular dysfunction after acute myocardial infarction

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Background - Identification of patients at risk of poor outcome after acute myocardial infarction (AMI) would allow tailoring healthcare to each individual. However, lack of prognostication tools renders this task challenging. Previous investigations suggest that gene expression of blood cells may inform about prognosis after AMI.
Objective -To address the added value of gene expression of blood cells to predict left ventricular (LV) dysfunction after AMI.
Methods - Five genes (LMNB1, MMP9, TGFBR1, LTBP4 and TNXB) selected from past studies were measured in peripheral blood samples obtained at reperfusion in 449 MI patients. 79 patients had LV dysfunction as attested by an ejection fraction (EF) $\leq$ 40% at 4-month follow-up and 370 patients had a preserved LV function (EF >40%).
Results - LMNB1, MMP9 and TGFBR1 were up-regulated in patients with LV dysfunction and LTBP4 was down-regulated, as compared with patients with preserved LV function. The 5 genes were significant univariate predictors of LV dysfunction. In multivariable analyses adjusted with traditional risk factors and corrected for model overfitting, a panel of 3 genes - TNXB, TGFBR1 and LTBP4 - improved the prediction of a clinical model (p=0.00008) and provided a net reclassification index of 0.45 [0.23-0.69], p=0.0002 and an integrated discrimination improvement of 0.05 [0.02-0.09], p=0.001. Bootstrap internal validation confirmed the incremental predictive value of the 3-gene panel.
Conclusion - A 3-gene panel can aid to predict LV dysfunction after MI. Further independent validation is required before considering these findings for molecular diagnostic assay development.

Breast cancer cell escape from natural killer cell lysis is mediated by actin cytoskeleton remodelling

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Natural killer cells (NKs) are lymphocytes of the innate immune system that target and kill cancer cells through the secretion of lytic granules containing granzyme B and perforin. This process requires the formation of a specialized cell-cell interaction region termed the immune synapse (IS). Previous reports have established that the formation of the IS largely rely on sequential rearrangement of actin filaments within NKs. In contrast to the well-characterized configurations of actin filaments in NKs during the lytic process, there is a gap of knowledge regarding the organization and role of the tumor cell actin cytoskeleton during NK attack. In this project, we showed that actin cytoskeleton of NK-resistant tumor cells accumulate at the IS. Using the high-throughput ImageStreamX Mark II Imaging Flow Cytometer, we found that the majority of cells belonging to the resistant cell lines respond to NK cell attack by local and fast accumulation of actin near the IS. Furthermore, we show that actin accumulation is associated with autophagosome clustering at the IS which drives the degradation of granzyme B inside the target cell. Moreover, our data suggest that the ability of cancer cells to respond to NK attack by fast actin remodelling is associated with their EMT status. We extended these data by analysing a large panel of breast cancer cells and showing that mesenchymal cells are significantly more resistant to NK-mediated cell death as compared to epithelial cells. Together our data demonstrate that resistant breast cancer cell lines respond to NK cell attack by a tumor actin remodelling and accumulation at the region of the IS. Targeting actin signalling pathways and regulators involved in these processes allowed to increase the susceptibility of resistant cells to NK cell-mediated cell death.

How pharmacokinetics influence the link between exposure to pesticides and their accumulation in hair

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Originally used for illicit drug detection, hair analysis is increasingly considered for the assessment of human exposure to pollutants. Although the relationship between intake and resulting concentration in hair remains incompletely elucidated, incorporation from blood is considered the major pathway. Pharmacokinetics are reasonably suspected to modulate the transfer of chemicals from blood to hair. The present work investigated the influence of pharmacokinetic parameters of pesticides in blood on the resulting correlation between exposure level and concentration in hair.

In the first rats experiment, females were force-fed with pesticides over a 90 days-period at 7 different doses, in order to observe the relationship between exposure level and concentration in hair. The second experiment provided blood pharmacokinetic parameters by analysing blood sampled at different time?s points after a single administration.

Pharmacokinetic parameters were independently investigated in order to understand their influence on incorporation into hair. Secondly, we studied how adjustment with the pharmacokinetic parameters could improve the association between the concentration in hair and the exposure level.

The best correlation with the concentration of pesticides in hair was observed when the exposure level was adjusted with the parameter mean residence time (MRT) of pesticides in blood (Rpearson of 0.77; $p<0.001$). The results also confirmed the correlation between plasma and hair concentration by adjusting plasma concentration with area under the curve, Cmax and MRT (Rpearson of 0.87; $p<0.001$).

Our results support the hypothesis that concentration of pesticides in hair is proportionally associated with level of exposure after adjustment with some blood pharmacokinetic parameters.

A study of the molecular mechanisms underlying the response of human colorectal adenocarcinoma enterocytes to prebiotics and probiotics

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Dysbiosis, a microbial imbalance in the gut, is associated with developing colorectal cancer (CRC). On the other hand, high fiber diets, supplemented with prebiotics and/or probiotics, are thought to have a major effect on the gut microbiota and are inversely correlated with the risk of developing CRC. The combinatorial effect of a probiotic bacterial strain Lactobacillus rhamnosus GG with a dietary high-fiber simulated ileal environment medium on Caco-2 cells was assessed using the microfluidics-based GIT co-culture model called HuMiX. Generated multiomics data uncover that the combination of dietary fiber together with probiotics, is necessary to increase the expression of anti-proliferative genes and tumor suppressors in our CRC model. We provide a novel, mechanistic understanding of the interplay between dietary components, bacterial metabolism and human physiology required to understand the potential of pre-and probiotics in prevention and combinatorial therapies of CRC.

GBA mutation as a modifier of familial Parkinson's Disease

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Causative mutations for Parkinson's Disease (PD) are heterogeneous in term of penetrance, phenotype and age of onset in patients carrying the same mutations. This led to the search of modifying factors that could influence disease expressivity. Mutations in GBA1, encoding the glucocerebrosidase (GCase), are an important and common risk factor for both familial and sporadic PD. Patients carrying a GBA mutation (5-10%) are more likely to progress to dementia, develop earlier axial motor symptoms and have a slightly earlier age of onset. To decipher whether GBA could act as a modifier of familial PD, fibroblasts from a PD patient harbouring homozygous mutation in PARK2 and a point mutation in GBA1 have been reprogrammed into induced pluripotent stem cells and differentiated into small neuronal precursor cells and midbrain-specific dopaminergic neurons. Characterisation of the patient-derived cells revealed an impairment in GCase activity and a loss of parkin protein. The mutant cells displayed a higher sensitivity to oxidative stress leading to a decrease in mitochondrial membrane potential, to the accumulation of reactive oxygen species and to an increase in number of apoptotic cells. Also, the study of alpha-synuclein metabolism revealed a decreased level of intracellular alpha-synuclein concomitant with an increase of alpha-synuclein release in the media. To investigate GBA mutation specific effect on these phenotypes, the GBA molecular chaperone Ambroxol was used to rescue GCase activity. Interestingly, the pharmacological rescue of GBA reverses the alpha-synuclein phenotype. To further understand the modifying role of GBA mutation, mitochondrial function will be studied under Ambroxol treatment.

Influence of proteins on carotenoid digestion and aspects of bioavailability

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BACKGROUND: Dietary intake and plasma levels of carotenoids have been associated with many health benefits such as reduced risk of age-related macular degeneration, cardiovascular disease, type 2 diabetes and certain types of cancer. However, carotenoid absorption depends on several host and dietary factors, including lipids and divalent minerals. One factor that has never been systematically studied is the influence of co-digested proteins on carotenoid solubility/bioaccessibility. OBJECTIVE: To elucidate the current knowledge regarding protein-carotenoid interactions, by studying effects of co-digested proteins on carotenoids in-vitro, then their impact on carotenoid bioavailability in-vivo. DESIGN: A literature survey is carried out on the influence of proteins on the bioaccessibility of lipophilic dietary constituents. For studying protein-carotenoid interactions during simulated gastro-intestinal (GI) digestion, several proteins are evaluated. Bioaccessibility of pure carotenoids and from solid and liquid food matrices will be studied, as well as colonic recovery following fermentation to investigate potential carotenoid degradation products/metabolites. 24 healthy adults will be recruited and served a protein/carotenoid-rich meal, with plasma concentrations and triacylglycerol-rich lipoprotein fractions as the observed outcomes. EXPECTED RESULTS: Proteins may be involved in stabilizing lipid droplets in oil-in-water emulsions in the GI tract, and may prevent their aggregation. Proteins are expected to enhance the solubilization of lipid droplets, reduce their size, and foster their transition to mixed micelles, resulting in improved carotenoid bioaccessibility/bioavailability. CONCLUSION: This would have consequences also for other liposoluble molecules such as fat-soluble vitamins, and would be relevant for the public health sector, as well as food and pharmaceutical industries.

Novel therapeutic strategies to prevent cognitive impairment in pharmacoresistant epilepsy

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Dravet syndrome (DS) is one of the most frequent form of pharmacoresistant epilepsy with an incidence of 1/30,000. More than 40 anti-epileptic drugs (AEDs) have been approved, but still about 30% of epilepsy patients do not respond adequately to any of the available AEDs, including Dravet syndrome patients. Approximately 80% of DS patients have de novo mutations in SCN1A, which encodes the Nav1.1 sodium channel alpha subunit. The mortality rate is 15-20%. An important hallmark of DS is that most patients have to cope not only with the disease itself but also with many additional medical problems that may be associated with epilepsy. Indeed, apart from psychiatric disorders, many patients suffer from impaired cognitive performance caused either by the disease itself and/or by the side effects of the medications prescribed. Recent studies on zebrafish have demonstrated its ability to be a promising in vivo model for biomedical research. The zebrafish and human genomes share high homology - for example, 85% of the known epilepsy genes in humans are found in the zebrafish genome. In this view, the zebrafish is becoming a relevant genetic model organism for human DS. This project focus on the cognitive functions of zebrafish DS models in order to find similarities between cognitive impairments in human patients and our zebrafish model. Then, we are exposing zebrafish and mouse DS models to AEDs and novel anti-epileptic compounds known to suppress seizures in these models in the hope that we can find a new AED effective for DS patients.

Long Non-coding RNAs And Heart Failure

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Background - A significant proportion of patients develop left ventricular (LV) remodeling leading to heart failure after acute myocardial infarction (AMI). Being able to identify these patients would represent a step forward towards personalized medicine. Since men and women with AMI have different clinical profiles, risk factors, pathophysiology and outcome, it is important to find ways to risk stratify patients in a gender-specific manner. Objectives - To determine the ability of cyclin dependent kinase inhibitor 1C (CDKN1C) to risk stratify AMI patients, in a gender-specific manner. Methods - CDKN1C expression was measured in blood samples obtained at admission in a test cohort of 447 AMI patients and a validation cohort of 294 patients. The study end-point was LV function assessed by the ejection fraction (EF) at follow-up. Results - In the test cohort, CDKN1C was downregulated in patients with a reduced EF (<40%). This observation was specific to women. CDKN1C was a significant univariate predictor of LV function in women only. In multivariable analysis including demographic and clinical parameters, CDKN1C predicted LV function in women (odds ratio [95% confidence interval] 0.44 [0.23-0.82]) but not in men (0.90 [0.70-1.16]). Addition of CDKN1C to a multivariable clinical model reduced the Akaike information criterion, attesting for an incremental predictive value, in women (p=0.006) but not in men (p=0.41). Bootstrap internal validation confirmed the added value of CDKN1C in women. The female-specific predictive value of CDKN1C was validated in the independent cohort. Conclusion - CDKN1C is a novel female-specific biomarker of LV function after AMI.

Impact of the human papillomavirus vaccination in Luxembourg

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Background: Cervical cancer is caused by persistent human papillomavirus infection (HPV). Many countries have introduced vaccination against HPV in the last decade, but efficacy and effectiveness outside of clinical trial settings remains to be determined. In Luxembourg, vaccination program was introduced in 2008 offering Cervarix and Gardasil vaccines. Since, infection with oncogenic HPV types is a necessary requirement for progression to cervical cancer, one way to assess early vaccination impact is to measure the prevalence of HPV infections in the population and compare prevalence rates and genotype diversity in vaccinated and unvaccinated young women in Luxembourg.

Methods: We are conducting large cross-sectional prevalence study in young women aged 18-29, who have a medical visit at Family Planning or private gynaecologist. Gynaecologists collect cervical swabs for routine screening and participants fill in a sexual behaviour and risk factor questionnaire and inform consent. Cervical swabs are processed by Hologic imagine system and HPV test performed using AnyplexII HPV28 genotyping kit. Data analysis is performed using Stata 14.

Preliminary results: The mean age of 628 women was 22.5 years. 112 women were vaccinated with Cervarix and 182 with Gardasil. 50% of women were HPV positive and the most frequently detected HPV genotypes were HPV42(12.0%), HPV53(11.7%), and HPV51(5.9%). Unadjusted vaccine effectiveness against HPV16 and HPV18 was 95.6%(95%CI 66.9-99.4, p¡0.001), however against HPV16, HPV18, HPV6 and HPV11 was 86.5%(95%CI 64.9-94.8, p=0.002).

Conclusions: HPV vaccination is highly effective against HPV genotypes 16,18,6 and 11, however further investigation is required to evaluate vaccination effectiveness against HPV related cancers.

Towards Small-Molecule Therapies For a Juvenile Form of Batten Disease

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Several mutations of ATP13A2/PARK9/CLN12 are known to cause an autosomal recessive form of early-onset Parkinsonism, called Kufor-Rakeb syndrome (KRS). Recently, different mutations in this same gene were identified to lead to neuronal ceroid lipofuscinosis (NCL, also called Batten disease) and to spastic paraplegia 78. The link between these neurodegenerative diseases is further supported by studies in animal models, but the molecular mechanism(s) underlying the connection between ATP13A2 deficiency and disease pathogenesis is not understood. Nevertheless, all this indicates that ATP13A2 plays a crucial role in neuronal cells and that its deficiency leads to neurodegeneration. In order to elucidate the molecular mechanisms involved in the pathogenesis, we recently initiated functional studies of the ATP13A2 protein, mainly in the context of the juvenile form of Batten disease. Taking advantage of the fact that ATP13A2 is highly conserved from yeast to humans, we have developed disease models for the Batten disease and Kufor-Rakeb syndrome variant of ATP13A2 in budding yeast. The results obtained so far with these models point to an implication of ATP13A2 in heavy metal resistance, with potentially different pathways involved for different types of metal. In parallel, we are developing a knock-out model for ATP13A2 in zebrafish which we seek to use for further validation of the phenotypes observed in the yeast model. Additionally, we have developed a screening strategy to exploit the combination of both models for a more rapid identification of bioactive compounds with therapeutic potential as orphan drug candidates for Batten disease.

Predicting the cell death responsiveness and sensitization of melanoma cells to MEK inhibitor trametinib

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Malignant melanoma is highly aggressive form of skin cancer responsible for vast majority of skin cancer related deaths. Treatment of advanced stages with small molecules kinase inhibitors is currently limited to patients with BRAF mutations. Recent insight into the heterogeneous nature of melanoma suggests a more personalized approach to treatment may be necessary to overcome cancer drug resistance and improve patient care. For this approach to work, reliable molecular signatures that can accurately predict treatment responsiveness need to be identified. In this study we apply multiplex phosphoproteomic profiling across a panel of 24 melanoma cell lines with different mutations, to predict responsiveness to MEK inhibitor trametinib. Supported with multivariate statistical analysis and multi-dimensional pattern recognition algorithms, the responsiveness of individual cell lines to trametinib could be predicted with high accuracy (83% correct predictions), independent of mutation status. We also successfully employed this approach to predict whether individual melanoma cell lines could be sensitized to trametinib. We identified that the combination of MEK inhibition with selective targeting of c-JUN and FAK proteins with siRNA-based depletion or chemical inhibitors sensitize resistant cell lines and can potentially increase the efficacy of MEK treatment. Our findings suggest that such multiplex proteomic analysis coupled with systems biology-based approaches could assist in personalizing treatment decisions for melanoma patients.

Functional Analysis of LncRNAs in Glioblastoma and Chemoresistance

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Background: Radio-chemotherapy represents the standard treatment against Glioblastoma (GBM), yet, cancer cells evolve resistance mechanisms leading to inevitable tumor relapse. Over the last years a new family of RNA molecules with fundamental importance in controlling gene expression has been uncovered. Long non-coding RNAs (lncRNAs) are epigenetic regulators involved in tumorigenesis and chemoresistance. Using RNA-sequencing we isolated a number of novel lncRNAs and predicted their potential role in GBM drug-response. Here we characterize one of these unknown molecules, 'UBR5-AS1', including its gene structure, isoform expression and function in GBM. Results: The UBR5-AS1 gene is located on the human chromosome 8q22.3 in antisense orientation of UBR5 gene (ubiquiting-protein ligase E3 component n-recognin 5). The UBR5-AS1 gene structure is predicted to have three exons, however recent annotations from LNCipedia database suggest the presence of two additional isoforms. Using RACE-PCR (rapid amplification of cDNA ends) from GBM cell RNAs, we identified 10 linear isoforms and one circular RNA. Subcellular fractionation of nuclear and cytosolic RNA shows that UBR5-AS1 is predominantly nuclear in GBM cells. RT-qPCR revealed that this gene is expressed at the basal level in normal brain and reduced in GBM. We further show that UBR5-AS1 is induced along with other DNA-damage response genes upon temozolomide treatment, especially in chemo-sensitive GBM cells. Finally, overexpression of UBR5-AS1 significantly decreased GBM cell proliferation upon low dose of temozolomide treatment. Conclusion: We identified a novel lncRNA 'UBR5-AS1' that sensitizes GBM to chemotherapy. Further experiments will focus on the mechanistic role of UBR5-AS1 in chemoresistance of GBM.

Analysis of the signal transduction network upstream of L-plastin Ser5 phosphorylation in breast cancer cells and tissues

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Ser5 phosphorylated L-plastin has been previously identified as a molecular indicator for deregulated ERK/MAPK signalling in invasive breast carcinomas. As cancer progression is often the result of not only one, but several interconnected deregulated signalling pathways, the "Plastin" project is aimed at further dissecting signal transduction upstream of L-plastin Ser5 phosphorylation. In an experimental approach, activation of ERK/MAPK and PI3K/AKT pathways will be performed through receptor tyrosine kinase stimulation, and key signaling molecules will be chemically inhibited. The resulting effect on downstream target molecules (output nodes) such as L-plastin, ERK, AKT, FAK or Src will be assessed by performing immunoblot-based quantification of the activity level of the different output nodes, and this data will then be evaluated by taking a computational modelling approach. Moreover, the project aims at investigating whether L-plastin expression and/or its Ser5 phosphorylation have an impact on breast cancer cell migration and invasion capacities, which is to be achieved by lentiviral transduction techniques used to silence L-plastin or knock-out individual L-plastin domains with nanobodies, or to introduce L-plastin variants (wild type, Ser5 phosphorylation-defective or Ser5 phosphorylation wild type-mimicking). Finally, to investigate whether there is a link between our main findings and the molecular subtype and clinicopathological parameters in breast cancer tissue, immunohistochemistry (IHC) will be performed on tissue samples. First results obtained from the beginning of the project will be presented and discussed.

Constraint-based modelling highlights metabolic variability of iPSC-derived dopaminergic neurons during differentiation and stress conditions

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Over the past decade, induced pluripotent stem cells (iPSCs) techniques have been giving a better insight in the patient cell types and enable the development of a human disease models. The main limitation of the compelling way to develop cellular models is a cellular heterogeneity between iPSC lines.
In order to tackle this problem and verify findings in the Parkinson's disease (PD) cellular model, we evaluated gene expression and metabolic profiles between induced dopaminergic neurons, generated from iPSCs, throughout the neuronal differentiation process and treated with different rotenone concentrations.
Differential gene expression was analysed in neurons derived from different neural progenitor cells (NPC) passages (passage number 8, 23, 32) and neurons treated with different rotenone concentrations (0.06, 0.25, 0.5 and 1 M of rotenone). Furthermore, we generated a context-specific, genome-scale metabolic models by mapping the transcriptomic data onto a global reconstruction of the human metabolism (Recon 3D).
The neuroepithelial stem cell (NPC) passage number and rotenone had a significant effect on the gene expression. Some metabolic systems, as central carbon metabolism, are represented differently across the models of the untreated neurons. Across the models of neurons treated with different rotenone concentrations, taurine metabolism is most affected, as well as inositol and glutathione metabolism, all being neuronal markers of response to oxidative stress.
The primary parameter in determining the metabolic signature of neurons derived from the iPSCs is the passage number of the NPCs.
