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Rho-kinase Involvement in an Animal Model of Subretinal Fibrosis

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Background: Subretinal fibrosis can evolve in the course of neovascular age-related macular degeneration (AMD). Until now, there is no successful treatment nor established animal model for subretinal fibrosis. Intravitreal anti-VEGF treatment can reduce the choroidal neovascularization (CNV), but not the subretinal fibrosis. Rho-associated protein kinases (ROCK) have multiple functions, including the regulation of smooth muscle cell contraction, cell migration, maintenance of cell viability and morphology, in part by regulating stress fibers and focal adhesions. ROCK has also been implicated in fibrosis formation. Rho-kinase inhibitors are compounds that target rho-kinase and inhibit the ROCK pathway.

Purpose: Investigate the impact of the ROCK inhibitor fasudil on subretinal fibrosis after CNV in AMD.

Methods: To induce CNV-related fibrosis. We used a 532-nm laser inserted in a slit-lamp delivery system. After development of fibrosis on day 35, the mice (C57BL/6J, 8-9w, mix gender) were treated intraperitoneally with fasudil for two weeks. The volume of fibrosis was quantified with optical coherence tomography (OCT) measurements every week. Additionally, we performed autofluorescence and fluorescence angiography at every time point to document fibrotic changes over time. Choroidal flat mounts were used for immunohistochemistry (Collagen 1, Isolectin B) in order to visualize development in vascularization and extracellular matrix. Western blot was performed to evaluate the protein level of fibrotic related markers.

Results: The leakage in the fluorescence angiography decreased after treatment and the subretinal fibrosis in the OCT images increased over the investigated time. Correspondingly, from day 21 to day 49 after laser injury, the expression of Collagen 1 increased, whereas isolectin B decreased. After treatment, we can see the volume of leakage decreased. The western blot indicate there are 2 peaks appeared during the whole 49 days after laser. The pan-ROCK inhibitor, Fasudil, substantially reduced subretinal fibrosis in vivo.

Conclusions: The current results indicate that the pan-ROCK inhibitor fasudil may has a therapeutic potential for the treatment of subretinal fibrosis in neovascular age-related macular degeneration.

ID1-expression in glioblastoma-associated microglia: Impact on anti-tumor immunity

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Background: Glioblastoma (GBM, WHO grade 4) is the most common malignant primary brain tumor without curative treatment options. The emergence and successful clinical implementation of immunotherapies for other cancer entities leads to the hope that immunomodulatory strategies can be adopted for brain tumors. The immune tumor microenvironment (iTME) of GBM consists mainly of brain resident microglia (MG), monocyte-derived macrophages (MdM), and to a much lesser extent of T-lymphocytes. In our previous work, MG-focused single-cell RNA sequencing (scRNAseq) analysis of five human GBM-biopsies identified a subcluster of a transcriptionally immunosuppressive MG phenotype that is characterized by *ID1* (inhibitor of DNA-binding protein 1) expression. ID1 is a helix-loop-helix (HLH) protein that heterodimerizes with transcription factors (TF) and by that abrogates their DNA binding and transcriptional capacity. Interestingly, *ID1* has been shown to be induced by tumor-secreted factors and subsequently plays a major role in controlling the lineage-commitment from dendritic cell (DC) differentiation to the expansion of highly protumorigenic immunosuppressive myeloid-derived suppressor cells (MDSC) in melanoma. Pathway analysis of differentially expressed genes in Id1-overexpressing bone-marrow-derived myeloid cells defined MHC-II -and I-associated proteins and costimulatory molecules such as CD86 and CD40 as Id1-downstream mediators that were significantly downregulated in an Id1-dependent manner. Gene set enrichment analysis of our scRNAseq data similarly demonstrated a strong downregulation of proteins of the antigen processing and presentation pathway via MHC-II and I and a downregulation of costimulatory molecules in *ID1*-high MG. This supports the relevance of our scRNAseq-finding and highly suggests a potential causative role of *ID1* in an immunosuppressive tumor-associated MG-phenotype.

Hypothesis and Research Aims: Based on the higher expression of *ID1* in a transcriptionally defined immunosuppressive MG population and the eminent evidence in the literature for its implication in conveying tumor-mediated immunosuppression, we hypothesize that *ID1* plays a key role in shaping the iTME in GBM and represents a promising treatment target that could increase the responsiveness to immunotherapy in GBM. The major aim of this project is to interrogate the role of *ID1* in MG and the effect of its modulation on MG phenotype and the iTME as a potential synergistic and potentiating target of immunotherapy in GBM. To accomplish this, we will (I) functionally characterize the phenotype of *ID1*$^{-/-}$ and *ID1*-overexpressing MG *in vitro*; (II) examine the antitumor effector function of *Id1*$^{-/-}$ MG and the iTME in a syngeneic GBM mouse model; (III) study the pharmacological targetability of *ID1* and intend to mechanistically elaborate ID1 regulation in tumor-associated MG. Furthermore, we will investigate mono and combinatorial ID1-augmented immunotherapies in an established ex-vivo patient-derived GBM organoid (GBO) model and subsequently scrutinize promising therapy combinations in an orthotopic GBO xenograft mouse model.

Methods: *ID1* will be deleted and overexpressed in human and mouse MG cell lines by CRISPR/Cas9- and lentivirus-based approaches. The MG phenotype and the phagocytic capacity will be assessed in flow cytometry and tumor cell co-culture experiments. To elaborate the role of *ID1* in the tumor-mediated inflammatory response of MG we will compare the transcriptome of $ID1^{-/-}$ and $ID1^{wt/wt}$ MG stimulated by GBM-conditioned medium (GCM). To identify upstream regulators of *ID1* in MG, we will correlate the proteome of the GCM to known *ID1*-regulating factors and confirm their mechanistic action by blocking antibodies, pathway inhibitors and recombinant protein stimulation. We will then proceed *in vivo* by generating $Id1^{fl/fl}$ x $Sall1^{CreERT}$ mice and investigate the effect of MG-specific *Id1*-deletion with and without combinatorial immunotherapies in a syngeneic GBM mouse model. Pharmacological targetability of ID1 will be assessed *in vitro* and *in vivo* with the ID-inhibitor AGX51 and the FDA-approved drug pimozide.

Significance: Deepening the understanding for the resident and recruited myeloid compartment in GBM can help extend the knowledge about gliomagenesis and help develop strategies that make GBM accessible to innate and adaptive immune cell targeted therapies.

The MD-PhD curriculum: the French perspective

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Throughout the world, MD-PhD curriculums share the common goal of combining clinical and scientific training, leading to the acquisition of both a medical doctorate (MD) and a doctorate in science (PhD). However, in practice, each country and each program have their own organization and singularities. With this presentation, we would like to introduce the French MD-PhD curriculum, and its similarities and differences with the Swiss MD-PhD curriculum. More specifically, we will discuss how initiating a PhD earlier in an academic career can both present advantages and pose specific challenges.

Studying the polypharmacy administered following acute spinal cord injury

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Objective: In addition to paralysis and sensory loss, acute spinal cord injury (SCI) is accompanied by a litany of secondary complications (e.g., neuropathic pain, spasticity) and adverse events (e.g., infections), which are managed with a variety of medications. Despite decades of clinical application, little is known about the impact of these medications on neurological recovery. The aim of the current study was to comprehensively evaluate pharmacological management practices in acute spinal cord injury and determine what impact, if any, they could have on neurological recovery when administered alone or in a combinatorial fashion.

Methods: We performed a secondary analysis of a completed clinical trial (Sygen) and an observational study (SCIRehab) to provide a comprehensive, structured overview of what constitutes “standards of acute pharmacological care” after SCI. From the concomitant drug files, which exist for each person in the Sygen trial and SCIRehab, we extracted generic drug name and information on dosing (i.e., start and end date, frequency). Information on drug indication (i.e., reasons for administering a drug) was only available for patients enrolled in the Sygen trial. We then conducted an in-depth literature review to gather biological evidence of concomitantly administered medications with disease-modifying properties. Lastly, we searched the existing literature to determine which of the identified concomitant medications may alter the effectiveness of emerging investigational treatment strategies due to overlapping mechanisms of action.

Results: We identified a total of 770 unique medications, which were administered during the first 2 months after injury among the 2040 patients studied (Sygen: n=797, SCIRehab: n=1243). The daily intake ranged from 0 to 43 different medications per day per patient, with an average of 16.6 medications per patient in the course of the first 60 days following injury (21.3 and 13.6 for the Sygen and SCIRehab studies, respectively). From our initial review of preclinical literature, 226 studies evaluating

113 unique drugs demonstrate evidence of disease modifying properties (i.e., beneficial or detrimental) on histological and/or functional outcomes in animal models. All our results can be visually and interactively explored on the RxSCI platform we developed (https://jutzelec.shinyapps.io/RxSCI/).

Conclusion: Our study revealed a high degree of polypharmacy in the acute stages of spinal cord injury, with potential to both positively and negatively impact neurological recovery. This raises the distinct possibility of drug repositioning, either by broadly introducing or reducing the administration of a drug, in order to enhance neurological outcomes after acute injury. Our systematic literature corroborated that the knowledge gap regarding the effect of commonly used drugs on disease progression and their potential to alter the effectiveness of disease modifying treatments is not unique to spinal cord injury.

An extended CRISPR-Cas9 Knock Out Screen to Dissect Mechanisms of T Cell Dysfunction in Human Tumors

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Cancer immunotherapies, in particular immune checkpoint inhibitors and adoptive T cell transfer, have demonstrated remarkable efficacy in a variety of oncological entities[1,2]. However, only a minority of patients responds to existing therapies. This resistance is in part attributed to the dysfunction of tumor-infiltrating T lymphocytes (TILs)[3]. While checkpoint inhibition transiently elevates TIL effector functions, it fails to achieve durable epigenetic reprogramming of dysfunctional T cells[4,5]. Many features of and pathways to dysfunction remain controversial[6].

We hypothesize that there are yet to be characterized genes involved in T cell dysfunction, which could be targeted to improve current immunotherapies. To identify such genes, we have conducted a pooled CRISPR/Cas9 knock out screen in an ex vivo T cell dysfunction model, in which healthy donor CD8+ T cells are repetitively stimulated with tumor cells.

T cells in this model resemble dysfunctional TILs on a phenotypic and transcriptomic level and were characterized with MS proteomics to compile a library of 1'637 genes to be screened. We have identified multiple genes associated with rescued T cell function. Currently, we are validating these findings by performing single gene knock outs in our ex vivo T cell dysfunction model and in T cells adoptively transferred to tumor-bearing mice.

We are also preparing to conduct a secondary, focused screen with a single cell sequencing read out. In a later stage, we aim to analyze the transcriptome and epigenome of knock out cells to elucidate the mechanism by which genes of interest contribute to T cell dysfunction.

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Endoscopic Surgery for Spontaneous Supratentorial Intracerebral Haemorrhage: A Systematic Review and Meta-Analysis

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Introduction: Treatment for spontaneous supratentorial intracerebral haemorrhage (SSICH) is limited and consist of either best medical treatment (BMT) or surgical hematoma evacuation. Treatment methods and choice of surgical technique are debated, and so far, no clear advantage of endoscopic surgery (ES) over conventional craniotomy (CC) or BMT was shown. The aim of this systematic review and meta-analysis was to investigate the differences in outcome, morbidity, and mortality between ES and CC or BMT.

Methods: We systematically searched Embase and PubMed databases for randomized controlled trials comparing ES to CC or BMT. The primary outcome was favourable clinical outcome after 6 months. Secondary outcomes were morbidity and mortality rates and duration of surgery.

Results: Seven articles were eligible for the outcome analysis with 312 subjects in the control (216 CC, 96 BMT) and 279 in the treatment group (ES). Compared to BMT, ES showed significantly improved favourable outcome (*RR 1.93[1.12;3.33], p=0.02*) and mortality rates (*RR 0.63[0.44;0.90], p=0.01*). No significant difference in favourable outcome and mortality was seen in ES compared to CC (*RR 2.13[0.01;737], p=0.35*; *RR 0.42[0.17;1.05], p=0.06*). ES showed significantly lower morbidity (*RR 0.41[0.29;0.58], p<0.01*), and infection rates (*RR 0.33[0.20;0.54], p<0.01*) compared to CC. Duration of surgery was significantly shorter for ES compared to CC (*SMD -3.17[-4.35;-2.00], p<0.01*).

Conclusion: ES showed significantly improved functional outcome and mortality rates compared to BMT while showing reduced length of surgery and lower complication rates compared to CC. Therefore, ES seems to be a promising approach for treatment of SSICH justifying further prospective trials.

Structural analysis of bone marrow stromal networks during chemotherapy

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Cytoreductive treatments such as chemotherapy and irradiation are widely employed as conditioning regimens in bone marrow (BM) transplantation and treatment of hematologic malignancies. Besides the rapid elimination of highly proliferative hematopoietic progenitors that populate the BM and constantly replenish mature hematopoietic cell compartments, conditioning regimens cause the partial destruction of the BM stromal microenvironment, including endothelial and mesenchymal cells. Thus, understanding the scope and dynamics of the injuries imposed on BM post-myeloablation, and the regenerative process occurring in BM tissues, remain of high clinical and basic interest. While the responses of the hematopoietic compartment to irradiation and chemotherapy have been widely assessed, the alterations induced by these treatments on specific stromal components are less well characterized.

By using 3D microscopy and computational image-based analytical pipelines, we provide a high-resolution visualization and quantification of the dynamics of endothelial cells and mesenchymal components after treatment with the antimetabolite 5-fluorouracil (5-FU).

We report that chemoablation i) elicits major structural injuries to vascular and mesenchymal networks, which include vasodilation, rupture of vessel walls and fragmentation of the interconnectivity of the otherwise highly structured CARc mesh and ii) despite these effects on structural integrity, sinusoidal endothelial cells (SECs) and CARc show a remarkable regenerative potential that drives the complete restoration of hematopoietic function and BM parenchyma after highly destructive myeloablative stress.

Dynamics of T Cell Repertoire Renewal Following Autologous Hematopoietic Stem Cell Transplantation in Multiple Sclerosis

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Autologous hematopoietic stem cell transplantation (aHSCT) is a highly effective treatment of multiple sclerosis (MS). It involves mobilizing hematopoietic stem and progenitor cells (HSPCs), destroying the immune system by high-dose chemotherapy, and subsequently reinfusing autologous HSPCs for immune reconstitution. Prior studies showed that this procedure depletes autoreactive cells and leads to subsequent renewal of adaptive immune cells. However, surviving T cells have been described, but their dynamics and possible proinflammatory potential early (≤ 6 months) after aHSCT have not been studied. Here, we aim to understand the dynamics and extent of new and surviving T cells after aHSCT in MS.

In 27 patients, we applied multidimensional flow cytometry, T cell receptor (TCR) sequencing, telomere length profiling, and HLA-genotyping to address this question. Early post-aHSCT, naïve T cells are barely detectable, while effector memory (EM) T cells quickly reconstitute to pre-aHSCT levels. EM CD4+ T cells early post-aHSCT show shorter telomeres and higher expression of senescence- (CD57+, CD27-, CD28-) and exhaustion markers (PD1+, CD39+, TIM3+) compared to pre-aHSCT. We find a median TCR repertoire overlap of 26 % between the early post-aHSCT EM CD4+ T cells and pre-aHSCT, indicating broad persistence of EM CD4+ T cells early after transplantation. This EM CD4+ TCR repertoire overlap declines to a median of 15% at 12 months post-aHSCT, while the naïve TCR repertoire entirely renews, showing a median overlap of only 0.1%.

Our data support the complete renewal of the T cell repertoire by nascent T cells late after aHSCT, while substantial survival of pre-aHSCT EM CD4+ T cells is evident in the early phase.

Identifying key regulators of the Segmentation Clock in the Mouse Presomitic Mesoderm

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Presomitic Mesoderm (PSM) is an embryonic tissue primarily organized along the main anterior-posterior(A/P) axis of vertebrates and periodically forms somites at its anterior end. The tissue is characterized by the existence of gene transcription waves sweeping through it from its posterior to the anterior end. Beyond the directionality of the waves, the polarity of the PSM along the A/P axis is visible at the level of gene expression gradients (Wnt3a, Fgf8) and cell behavior (cell motility), which peak at the posterior end. The periodicity of wave generation and propagation is characteristic for the different vertebrae species and is called the segmentation clock.

In this system, we sought to apply chemical perturbations on other signaling pathways associated with cell motility, directionality, polarity and mechanosensitivity. The analysis of the effect on oscillation dynamics is done by incorporating a two-dimensional explant culture model derived from the posterior most part of the mouse PSM (posterior tip).

Imaging and time series analysis revealed that AZA1 (a chemical inhibitor of Rac1 and Cdc42 Rho GTPases) can increase the periodicity of oscillations almost two-fold compared to control samples. Alongside with the continuation of the screen, we started investigating the mechanism through which AZA1 exerts its actions on the segmentation clock by performing titration experiments first, together with experiments of compound addition with a time delay. Lastly, we employed in situ hybridization and quantitative PCR for downstream Wnt, Notch and FGF targets in order to reveal any potential interplay between Rac1, Cdc42 and these central regulators of somitogenesis.

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