and observed partial HSPC depletion in BM, spleen and peripheral blood. Notably, we did not observe histological lesions or increase in apoptosis (evaluated with Caspase 3 immunohistochemistry) due to organ toxicity in kidney, brain and BM of ADC-treated mice. To evaluate the efficacy of non-genotoxic conditioning in favouring HSPC engraftment early in life, we performed mismatched HSCT in WT ADC-conditioned mice, and compared it to transplantation after sublethal total body irradiation or without conditioning. Twenty weeks after transplant, we observed low but persistent donor chimerism in ADC-treated mice compared to irradiated controls in peripheral blood, BM, spleen and thymus.As reported in literature, very low level of donor cells engraftment is sufficient to restore bone phenotype in osteopetrotic mice. We hypothesized that ADC conditioning could successfully guarantee bone phenotype amelioration and reduction of the conditioning toxicity in the osteopetrotic mouse model. This approach may be even more advantageous in the GT setting, in which autologous HSCT avoids the risk of graft rejection. We plan to apply ADC conditioning on osteopetrotic mice before the transplant of lentiviral vector GT Lin- cells, to test the efficacy of our strategy on this severe bone disease model. *Acknowledgements.* This project has received funding from the European Calcified Tissue Society.

Novel AAV Capsids for Brain, Eye and Muscle Tissues

50. Endothelial-Tropic AAVs for Genetic Access to Whole-Brain Vasculature in Wild-Type Mouse Strains Following Non-Invasive Systemic Delivery

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The neurovascular unit (NVU) is a vital yet understudied component of the nervous system. Malfunction of non-neuronal cell types within the NVU, including endothelial cells, can facilitate the progression of neurological disorders (Yu et al, Frontiers in Neuroscience, 2020), but limited options for cell-type specific transgene delivery hamper its study. Adeno-associated virus (AAV) vectors for gene delivery to the brain are commonly administered via intra-cranial injections, resulting in tissue damage and limited, uneven spatial coverage. Systemic AAV delivery provides a non-invasive, brainwide alternative for genetic access. Having engineered vectors that efficiently cross the blood-brain-barrier (BBB) with broad tropism in rodents (e.g. AAV-PHP.eB), we turned our focus to engineering cell-type-specific vectors that could access vasculature without targeting other components of the NVU. Using M-CREATE directed evolution (Kumar et al, Nature Methods, 2020), we identified a family of endothelial-enriched capsid variants, including one named AAV-CAP.X1. Following intravenous (I.V.) injection, AAV-CAP.X1 targets vasculature with high cell-type specificity and efficiency throughout the body, including the brain. After injecting 3E11 vg total of AAV-CAP.X1 packaging CAG-GFP into adult C57BL/6J mice, 97% (+/- 0.8%) of the GFP+ area in the hippocampus are CD31+ (demonstrating specificity), and 73% (+/- 9.1%) of the CD31+ area in the hippocampus is GFP+ (proving efficiency; note that an increased dosage of 1E12 vg per mouse resulted in even greater CD31+ labeling without losing specificity). As AAV-CAP.X1 vascular infectivity in the periphery may complicate applications that focus on brain-specific endothelial transduction, we introduced point mutations on the AAV-CAP.X1 capsid and incorporated microRNA target sites into the cargo genome that successfully de-target AAV-CAP.X1 from the liver without impairing brain transduction. AAV-CAP.X1 can be used across multiple genetically diverse mouse strains, with efficient labeling of both capillaries and arteries in the brains of C57BL/6J, FVB/NJ, CBA/J, and BALB/cJ mice following I.V. administration. We also observed a significant increase in transduction compared to its parent capsid AAV9 on multiple human-derived cell lines *in vitro*. In its brain-targeted form, AAV-CAP.X1 could be paired with pre-clinical therapeutic cargo both to probe vascular contributions to neurological disease and to inform intervention strategies. More broadly, gene delivery via endothelial-tropic AAV capsids could, in principle, be applied to study diverse pathologies that may benefit from vascular remodeling. Our evolving knowledge regarding vascular pathology in COVID-19 that could underlie generalized organ dysfunction demonstrates the timeliness and potential importance of such vectors.

51. RNA-Driven Evolution of AAV Capsid Libraries Identifies Variants with High Transduction Efficiency in Non-Human Primate Central Nervous System

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Widespread transduction of the central nervous system (CNS) by viral or non-viral vectors still represents a considerable challenge in gene therapy. Local delivery of Adeno-Associated Virus (AAV) vectors to the CNS by intraparenchymal or intrathecal administration typically results in strong but heterogeneous transduction and is associated with potential risks related to invasive delivery. By contrast, intravascular delivery of engineered AAV vectors capable of crossing the blood-brain barrier should allow a broader CNS transduction, thanks to the high density of the brain vascular network. We applied our previously described RNA-driven biopanning TRACER platform to perform directed evolution of an AAV9 capsid library in cynomolgus monkeys (*Macaca fascicularis*). Following two rounds of intravenous dosing and neuron-specific library selection, a synthetic pool of variants was tested by multiplexed RNA enrichment analysis. This yielded a series of capsid variants with enhanced performance relative to AAV9. Five selected candidates were tested individually by low dose intravenous injection and their tropism for the CNS analyzed by measuring transgene RNA expression, viral DNA biodistribution and immunohistochemistry. All five variants were markedly improved over AAV9, with a subset showing 10-fold or more improvement of transduction in the brain. The highest performing variant displayed more than 1,000-fold higher RNA expression in the brain and 100-fold higher in the spinal cord. Immunohistochemical analysis indicated that this enhanced new