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Capsid Selection Strategy for the Development of Gene Therapies Based on Structural and Functional Analyses of a Panel of AAVHSCs

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Capsid selection, when developing a gene therapy, is a decisive step to increase success in the clinic. The capsid specific tissue, cellular pathway and cell-type tropism among different species and routes of administration all play critical roles in this strategy. First identifying the cell/tissue/organ or organ systems that need to be corrected based on the clinical presentation of the target disease and then overlapping this need with a capsid's characteristics can give rise to the best comprehensive outcome for the affected individual. We leverage AAVHSCs, a panel of 15 naturally occurring adeno-associated virus (AAVs) isolated from healthy human hematopoietic stem cells (HSC) which differ from Clade F member AAV9 and each other by one to four amino acids. The naturally occurring variations in the AAVHSCs are located in different functional regions of the capsid, including but not limited to, hypervariable regions, phospholipase domain, basic regions and glycan binding sites, which render each capsid with a unique set of features. By understanding the relationship of how each variation, based on structural and functional mapping, contributes to the unique characteristics of each capsid, we identify key insights to help guide our AAVHSC program capsid selection strategy across rare metabolic and neurological diseases. These capsid specific features are not limited to tropism, but also include overall immune responses, cellular or glycan binding and transduction kinetics. Herein we present, *in vivo* murine and non-human primate data, as well as *in vitro* data sets illustrating our AAVHSC capsid selection strategy for therapeutic applications. *In vivo*, we execute this strategy by comparing cellular vector genomes (vg), biodistribution, transgene output, translatable biomarkers and/or behavioral analyses across our intravenous programs or studies. Additional disease-specific evaluations, such as accessing the blood circulation or cerebrospinal fluid via secretion or crossing successfully into the nervous system via its wide range of blood-barriers, are included when necessary to guide the dose-selection rationale and achieve the most appropriate systemic distribution. *In vitro*, information on how the unique and naturally occurring capsid variations influence detailed functions such as trafficking, second strand synthesis or vg translation is also being utilized to further guide capsid selection. This vast amount of information and understanding of the AAVHSCs has been utilized in selecting capsids for therapies targeting lysosomal storage disorders. An example exemplifying this rationale is our gene therapy approach to Hunter Syndrome, which required not only widespread distribution to key tissues such as lung and kidney, but also benefited from a capsid targeting the choroid plexus for secretion and cross-correction in the central nervous system. These approaches in mind along with our panel of 15 naturally occurring AAVHSCs allows Homology Medicines to embrace a capsid selection rather than a "one-capsid-fits-all" limitation to best develop disease specific therapeutics.