Vectorized Antibodies for In Vivo Expression by DNA and mRNA:

a landscape analysis of stakeholders, technologies, targets, business and financing from an industry perspective

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Key corporate characteristics of the 18 vectorized antibody technology companies are summarizedin Table 3. The vast majority (13/18) of them are US-based companies. Three of the five mRNA technology companies with in vivo expression of antibodies are located in Germany, AAV DNA company is based The Netherlands and one plasmid DNA company is headquartered in France.

Overall, the vectorized antibody technology companies are relatively mature as evidenced by the year of foundation, number of employees and financial situation. Eleven of the 17 companies (excluded U`Penn) were founded before 2016 and most of them are well financed. The three mRNA companies BioNTech, Curevac and Moderna Therapeutics are in a privileged financial situation due to their eminent role in COVID-19 vaccine development, manufacturing and commercialization.

The clinically most advanced vectorized antibody programs are based on virally delivered DNA: Regenxbio and Adverum Biotechnologies have clinical phase III and II programs, respectively. Plasmid DNA technology with electrotransfection and electroporation, respectively, has enabled clinical stage II and I programs (Eyevensys and Inovio Pharmaceuticals). Only one mRNA encoded antibody for in vivo expression has reached clinical stage I (Moderna Therapeutics).

The three DNA vectorized antibody programs with AAV delivery of direct injection are in large ophthalmic disease indications such as wet age-related macular degeneration (AMD), diabetic retinopathy or diabetic macular edema. The two clinical stage in vivo expressed antibody programs from Inovio and Moderna are for treatment of infectious diseases: Zika virus infection and Chikungunya virus infection. Both programs were funded by governmental and non-governmental funds such as the National Institutes of Health (NIH), the US Defense Advanced Research Projects (DARPA) and the Bill & Melinda Gates Foundation and served the companies to develop the technology. More recently, Homology Medicines announced that it is looking "forward to naming a development candidate in a new therapeutic area this year, demonstrating the broader capability of our AAVHSC platform, which may allow us to tackle diseases with **larger patient populations** in the future"

alleviating the need for chronic dosing and its associated risks. As a proof of concept, Sharma et al. (2021) focused on complement-related disorders and used anti-complement protein 5 antibody as payload. Antibody expression was demonstrated *in vitro* in transformed hepatoma cell lines, primary hepatocytes, and *in vivo* in mouse and human hepatocytes. In both *in vitro* and *in vivo* studies, the fully assembled antibodies were highly expressed (human IgG ELISAs and WB) and were functional (competitive ELISAs and *ex vivo* hemolysis assay). Stable and robust IgG expression *in vivo* was successfully demonstrated in NOD-SCID mice for the duration of the study (up to 26 weeks). *In vivo* expression was dose-dependent and steadily increased during the first 5 weeks, reaching a plateau by ~7 weeks post dose. Analysis of the cellular lysates at the end of study demonstrated the antibody was efficiently secreted and that there were no protein aggregates in the liver even at the highest doses examined, with serum IgG levels >20 mg/mL. IgG levels were dependent on vector design and capsid, and capsid-ranking differed in mouse vs human hepatocytes.

4.1.4 MeiraGTx

MeiraGTx Holdings plc was formed on May 1, 2018 under the laws of the Cayman Islands. Its predecessor, MeiraGTx Limited, a limited company under the laws of England and Wales, was formed on March 20, 2015. In connection with its IPO, the company reorganized whereby MeiraGTx Limited became a wholly owned subsidiary of MeiraGTx Holdings plc. End of December of 2020, MeiraGTx had 219 employees (Annual Report 2020 SEC Form 10-K). The company's principal office is located in New York, NY, USA, with office and laboratory space. MeiraGTx also owns a 29,000 square foot manufacturing facility is located, in London, United Kingdom

MeiraGTx is a clinical stage gene therapy company with six programs in clinical development and a broad pipeline of preclinical and research programs. The company has core capabilities in adeno-associated viral (AAV) vector design and optimization and gene therapy manufacturing, as well as a gene regulation technology. Though initially focusing on the eye, salivary gland and central nervous system, MeiraGTx intends to expand its focus in the future to develop additional gene therapy treatments for patients suffering from a range of serious diseases.

As a proof of concept, they focused on complement-related disorders and used anti-complement protein 5 antibody as payload.

Antibody expression was demonstrated *in vitro* in transformed hepatoma cell lines (Hepa1-6, Alexander, HuH7, HepG2), primary hepatocytes (murine and human), and *in vivo* in mouse and human hepatocytes. In both *in vitro* and *in vivo* studies, the fully assembled antibodies were highly expressed (human IgG ELISAs and WB) and were functional (competitive ELISAs and *ex vivo* hemolysis assay). Stable and robust IgG expression *in vivo* was successfully demonstrated in NOD-SCID mice for the duration of the study (up to 26 weeks). *In vivo* expression was dose-dependent and steadily increased during the first 5 weeks, reaching a plateau by ~7 weeks post dose. Analysis of the cellular lysates at the end of study demonstrated the antibody was efficiently secreted and that there were no protein aggregates in the liver even at the highest doses examined, with serum IgG levels >20mg/mL. IgG levels were dependent on vector design and capsid, and capsid-ranking differed in mouse vs human hepatocytes.

Thus, by utilizing AAVHSCs, which have broad tissue tropism across different cell types and species, they may be able to deliver an appropriate antibody payload to the liver and maintain durable levels of IgG production and therapeutic expression to meet the needs of patients with a variety of diseases currently managed with chronic antibody dosing.

Manufacturing:

Homology Medicines utilizes proprietary **Clade F AAV capsids** derived from hematopoietic stem cells (AAVHSCs). As their programs have progressed through development and into the clinic, they have increased focus on the Drug Product sciences which includes the thoughtful development of stabilizing formulations enabling high titers, easing of clinical storage and supply chains, and enhanced long-term stability (Karpes, 2021).

AAV preparations have long held a reputation as challenging not only for production, but for long-term stability at even low concentrations. Kapres et al. (2021) demonstrated not only the stability of AAVHSCs in the liquid state, but also the impact of novel formulations on capsid stability. Their AAV preparations achieve titers in excess of 1E14 vg/mL and demonstrate stability for a minimum of one year at 2-8°C and more than six months at room temperature. Benefits of vector stability in the liquid state are a reduction in the need for -80°C-storage

Francis et al. (2021) assessed the in vivo efficacy of R100.anti-VEGF gene therapy (a 4D-150 prototype) in the validated non-human primate (NHP) laser-induced choroidal neovascularization (CNV) model of wet age-related macular degeneration (wAMD) across a broad range of therapeutically relevant doses. NHPs were dosed IVT with the 4D-150 prototype and six weeks post-administration, underwent retinal laser photocoagulation to induce CNV lesions. After two and four weeks, the numbers of clinically relevant grade IV CNV lesions were assessed by fluorescein angiography. Anti-VEGF protein was assessed by ELISA over 12 months by serial in vivo aqueous fluid samples.

They subsequently performed a study in nonhuman primates (NHP) to assess the safety of 4D-150 and to measure expression of the aflibercept protein (retina and ocular fluids) and expression of VEGFC RNAi in the retina.

In the NHP CNV model, the 4D-150 prototype resulted in complete suppression of grade IV CNV lesions (0/72) in treated eyes compared to vehicle (19/72) at doses as low as 1E11vg/eye ($p<0.0001$). Measurable and dose-dependent anti-VEGF protein was detected in aqueous fluid as early as 14 days post dosing and sustained through 12 months. There were no ocular or systemic toxicities at any dose tested. Specifically, there was no evidence of chronic intraocular inflammation (uveitis). Single IVT administration of 4D-150 resulted in high levels of ocular and retinal aflibercept protein together with highly robust VEGF C RNAi expression, without evidence of toxicity.

In summary, 4D-150 was designed with the goal of improved efficacy over single mechanism anti-angiogenic approaches by inhibiting multiple VEGF isoforms, as well as PlGF, within the retina. An intravitreal anti-VEGF prototype of 4D-150 resulted in efficacy and safety through 12 months in the NHP CNV model. Intravitreal 4D-150 resulted in sustained and high levels of functional aflibercept and anti-VEGF C RNAi.

The company plans to initiate clinical evaluation of 4D-150 in the second half of 2021.

8.1.2 ADVM-022; AAV.7m8-aflibercept

ADVM-022 is a recombinant, replication-deficient adeno-associated virus (AAV.7m8) gene therapy. ADVM-022 utilizes a propriety vector capsid, AAV.7m8, carrying an aflibercept coding sequence under the control of a proprietary expression cassette (AAV.7m8-aflibercept).