**Key element of the clinical scale-up manufacturing process used for Glybera® developed by the National Heart Lung and Blood Institute investigators at the National Institutes of Health**

The Dutch biotechnology company, uniQure, recently received European Medicines Agency approval for Glybera®, an adeno-associated virus vector to treat lipoprotein lipase deficiency (LPLD). LPLD is a rare genetic disease affecting one or two per million people. With a normal diet, patients lacking sufficient levels of lipoprotein lipase have abnormally high serum triglycerides and also high levels of very low-density lipoprotein (VLDL) resulting in acute severe pancreatitis and chronic conditions associated with high levels of VLDL, such as cardiovascular diseases. Dietary restrictions reduce the incidence of the pancreatitis, however, lapses in dietary compliance lead to hospitalization.

uniQure adapted a novel process for producing recombinant adeno-associated virus (rAAV) developed by Dr. Robert M. Kotin and colleagues at the National Heart, Lung, and Blood Institute of the National Institutes of Health. The conventional method for producing rAAV involves transiently co-transfecting cultured human cells. The cell number and the transfection efficiency determine the rAAV yield. Therefore, scaling-up production requires proportional larger surface area for adherent cell cultivation, intrinsically limiting rAAV production. Seeking an alternative to adherent cell rAAV production, Kotin and colleagues discovered that the linear single-stranded genome of recombinant adeno-associated virus (rAAV), could be “rescued” from a duplex form, e.g. in a plasmid, and replicate efficiently as AAV DNA in Sf9 cells, an insect cell line commonly used for protein expression. Based on this unexpected finding, Kotin’s lab

then designed baculovirus vectors that stoichiometrically express two AAV non-structural proteins required for AAV DNA replication and encapsidation, and the three structural proteins that comprise the non-enveloped capsid. A separate baculovirus expression vector carries the AAV vector genome. The baculovirus-infected insect cell release more infectious particles into the medium until all the cells are infected by the baculoviruses required for rAAV production. The first generation rAAV production process was developed into a scalable process using several bioreactor configurations for producing large quantities of rAAV. While uniQure's current production platform is based on a 50-liter bioreactor configuration, Kotin's lab routinely produces non-GMP vectors in 200 liter single-use bioreactors and the platform allows further expansion. These production levels are particularly relevant for the development of gene therapies to treat diseases that affect large patient populations or requiring larger doses. This technology platform can now be exploited to find solutions for many severe genetic and other disorders.