Ardena Insight

Oligonucleotide therapeutics: How to measure plasma concentration, tissue distribution and immunogenicity?

Oligonucleotides are becoming increasingly important as therapeutic agents. Since the commercial launch of the first oligonucleotide therapeutic (ONT) in 1998 (Vitravene®), ten ONT products have received market approval, the majority of which were approved in the last four years. ONTs share properties with chemical (e.g. they are synthesized via chemical processes) and biological pharmaceuticals (e.g. in terms of selective tissue distribution), and therefore present unique development challenges.

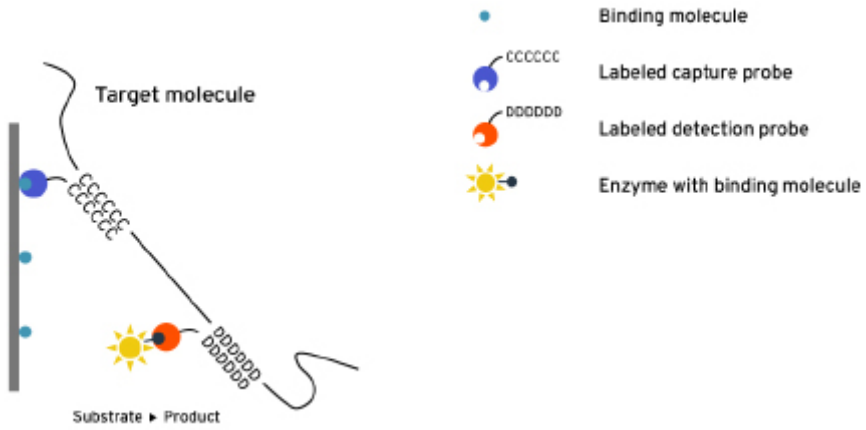
The preclinical safety evaluation for ONTs has generally followed the regulatory guidelines for small molecule pharmaceuticals. However, ONTs may elicit an immune response, just like biotechnology-derived products do, and therefore require the assessment of immunogenicity. Quantification of ONTs in biological matrices is also conducted using the techniques applied for biological drugs. This newsletter presents a short overview of the industry-standard techniques used for the measurement of plasma concentration, tissue distribution and immunogenicity of ONT products.

THERAPEUTIC	INTERNATIONAL NONPROPRIETARY NAME	CLASS	THERAPEUTIC AREA	ROUTE OF ADMINISTRATION	YEAR APPROVED
Vitravene®	Fomivirsen	Antisense	Cytomegalovirus Retinitis, HIV infections	Intravitreal	1998 (FDA), 1999 (EMA)
Macugen®	Pegaptanib sodium (FDA), Pegaptanib (EMA)	RNA aptamer	Wet macular Degeneration	Intravitreal	2004 (FDA), 2006 (EMA)
Kynamro®	Mipomersen sodium	Antisense	Hypercholesterolemia	Subcutaneous	2013 (FDA)
Defitelio®	Defibrotide sodium (FDA), Defibrotide (EMA)	Polydisperse mixture of predominantly single-stranded polydeoxyribonucleotide sodium salts	Hepatic Veno-Occlusive Disease	Intravenous	2016 (FDA), 2013 (EMA)
Spinraza®	Nusinersen	Antisense	Muscular Atrophy, Spinal	Intrathecal	2016 (FDA), 2017 (EMA)
Exondys® 51	Eteplirsen	Morpholino	Muscular Dystrophy, Duchenne	Intravenous	2016 (FDA)
Heplisav-B®	Hepatitis B surface antigen	Immunostimulatory	Vaccines	Intramuscular	2017 (FDA)
Onpattro®	Patisiran	Small Interfering RNA	Amyloidosis, Familial	Intravenous	2018 (FDA and EMA)
Tegsedi®	Inotersen	Gapmer antisense	Amyloidosis	Subcutaneous	2018 (FDA and EMA)
Waylivra®	Volanesorsen	Gapmer antisense	Hyperlipoproteinemia Type I	Subcutaneous	2019 (EMA)
Givlaari®	Givosiran	Small Interfering RNA	Acute Hepatic Porphyria	Subcutaneous	2019 (FDA), 2020 (EMA)

Commercially Approved Oligonucleotide Therapeutics

Quantification of ONTs in plasma is most often conducted via hybridisation enzyme-linked-immunosorbent-assay (ELISA) with colorimetric, fluorescent or chemiluminescent detection. This technique has been become the industry standard and is also widely utilised within Ardena. Depending on the sequence and structure of the ONT, hybridisation-ligation ELISA may be used, where binding of the ONT to the capture probe is enhanced by T4 DNA ligase-mediated ligation. Hybridisation ELISA is suitable for analysis of ONTs regardless of the nature of the compound, matrix or delivery mechanism and can be considered as the platform for plasma quantification.

A key component of any hybridisation ELISA method development is the design of ONT-specific capture and detection probes. These probes are designed in our labs to maximize affinity with the target ONT while minimising the potential for self-annealing. Other method development components include the minimisation of dilution volume, sensitivity and robustness (for instance by exploring different incubation temperatures and equipment settings) and materials and reagents (including lot-to-lot variation). At Ardena, typical validation parameters include response function, precision and accuracy, sensitivity, selectivity, dilution linearity (prozone effect) and stability, which are evaluated in at least six validation runs.



Schematic illustration of the hybridisation ELISA technique

Assessing ONT biodistribution is also a crucial part of any preclinical development program. To this end, our bioanalytical teams develop qualified methods for the analysis of the ONT in different tissues or even specific sites within the tissue. Extraction of the ONT from tissue is typically accomplished by homogenization followed by proteinase K treatment at higher temperatures. Extraction procedures are developed specifically for each tissue to ensure maximal recovery. Our teams qualify the method for ONT quantification in tissue homogenate in three runs to determine response function, precision and accuracy, dilution linearity and stability.

Like therapeutic antibodies, ONTs may trigger an immune response such that anti-drug-antibodies (ADAs) are formed. If this happens, the ONT is at risk of being cleared undesirably rapidly from the body. To measure potential ADA formation, bridging ELISA with chemiluminescence detection is the most widely used technique. At Ardena, we use a tiered approach consisting of screening, confirmation and titration. Validation parameters include screening and confirmation cut-point determination, sensitivity, end-point titer, selectivity, precision, drug interference, stability and prozone effect.

To date, we have worked on over 25 bioanalytical projects for ONTs and are actively expanding our analytical and development capabilities for this novel class of pharmaceuticals.

AREA	DESCRIPTION
BIOANALYTICAL	- Bioanalysis, biodistribution and immunogenicity assessment - Hybridisation ELISA as primary analytical tool - Expertise with antisense oligonucleotide, small interfering RNA and double-stranded RNA - Species: human, mouse, rat, cynomolgus monkey - Biodistribution in kidney, liver, muscle, brain, lung and others
DRUG PRODUCT	- Development and manufacture of parenteral solution formulations - Development and manufacture of parenteral controlled-release, nanomedicine products
CLINICAL SUPPLY	- Packaging - Just-in-timelabelling - Warehousing - Coordination of temperature-controlled shipments - Sample collection kit assembly and logistics
DOSSIER	- Synthesis process criticality analysis - Determination of critical process parameters (CPPs), critical material attributes (CMAs) and key process parameters (KPPs) - Development of control strategies - Drug substance and drug product IMPD writing