AstraZeneca

Integrating Bioanalysis and Biotransformation Strategies for Oligonucleotide Therapeutics

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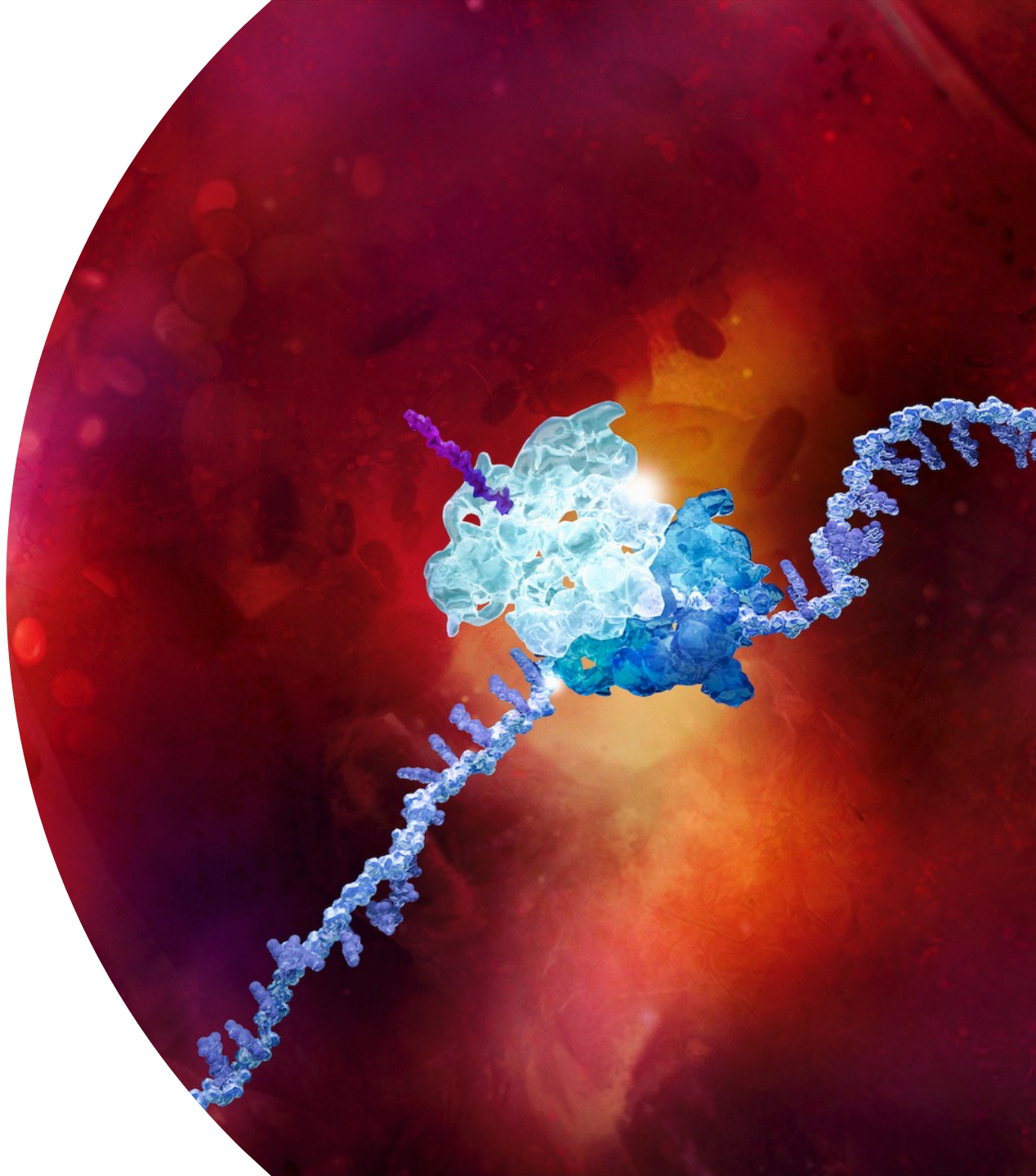
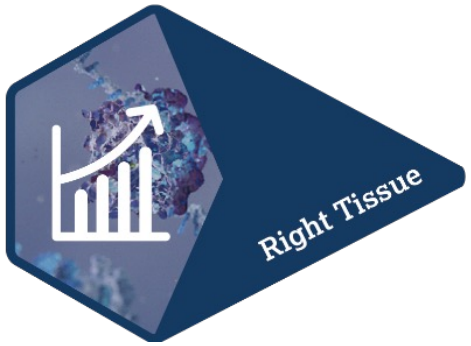
Integrated Bioanalysis / CVRM DMPK

09 June 2023



Why Oligonucleotide Therapeutics?

Identifying the **right target**



Making sure our molecule gets to the **right tissue** where it is needed

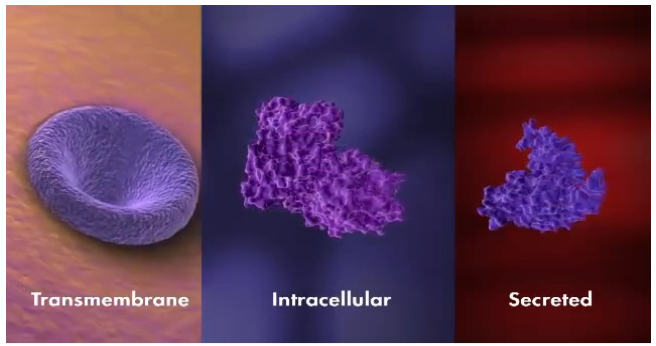
Ensuring **right safety** with minimal side effects

Selecting the **right patients** that will benefit

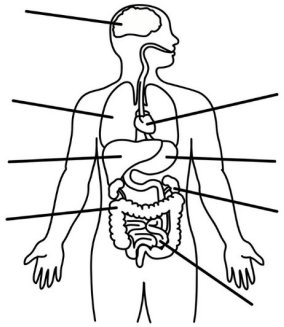
Defining the **right commercial** value and future viability

Underpinned by the **right culture** of truth-seeking behaviours and scientific rigor

Potential to target any type of protein



Potential to target any region of body



Potential to target any disease area

Oligonucleotide Therapeutics

ON ≠ small molecules

Size

Distribution

Species selectivity

Selectivity

Charge

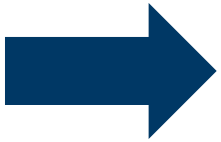
Immunogenicity

Mechanism of action

Metabolism

t1/2

Uptake mechanism



Same development principles but…

- Different screening cascades
- Different focus areas
- Different approaches

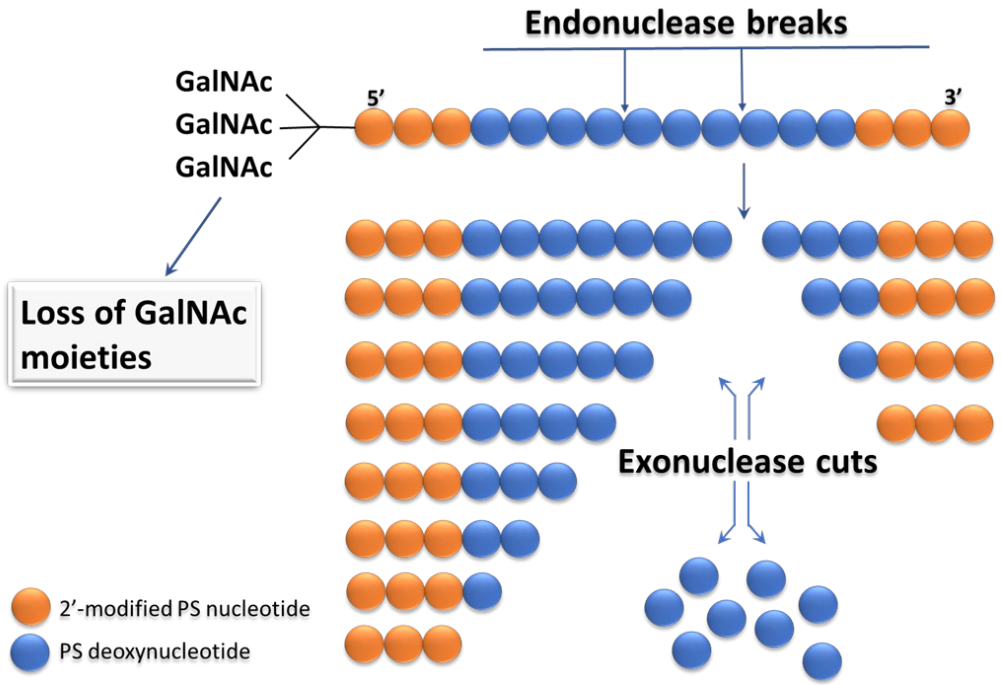
ON ≠ Biologics

- Regulatory landscape states “case by case” approach for oligonucleotide therapeutics with a focus on clinical
- Limited guidance for nonclinical studies and Biotransformation

Enzyme-mediated biotransformation of ASOs

Metabolism of gapmer ASO

- BT of ASOs mainly consists of hydrolysis of the phosphodiester linkages by nucleases (endo- or exo-, DNase or RNase)
- Nucleases are widely distributed in vivo and significant species differences are not expected
  - In vitro X-species study not needed
- Potential active metabolites (N-1, N-2)
  - should be identified early in project
- Conjugated ASOs: Intact conjugate will be cleaved off and metabolism of the conjugate will fall into its respective category for safety assessment.



Slide courtesy of Mette Lund Pedersen and Xue-qing Li

Illustration by Xue-qing Li

Yu et al. 2006 (doi:10.1124/dmd.106.012401.) and Weidolf et al 2021. (doi.org/10.1016/j.drudis.2021.04.002)

Biotransformation strategy

Phase	Study/Activity	ASOs
Prior to FTIH	In vivo metabolism (tox species, ss) - Metabolite identification and profiling	Yes Relevant tissues (liver and target tissues) in non-clinical tox species
	In vitro-X species	No
Ph1 – EoPh2	In vivo metabolism in human (SAD/MAD) and non-clinical species at SS (incl. additional species to cover tox/repro/carc) - Metabolite identification and profiling To be available at EoPh2 meeting, BT strategy confirmed at HA interaction	Yes Plasma and urine in human and non-clinical species Safety assessment of human metabolites
	Rodent/non-rodent radiolabelled ADME	No
	Human radiolabel ADME	No

*For conjugated ON with the incorporation of a novel conjugating moiety, BT/ADME studies may need to be conducted to describe the fate of the novel conjugating moiety and identify its related metabolites.

FTIH – First in human, EoPh2 – End of Phase 2; HA – Health Authorities

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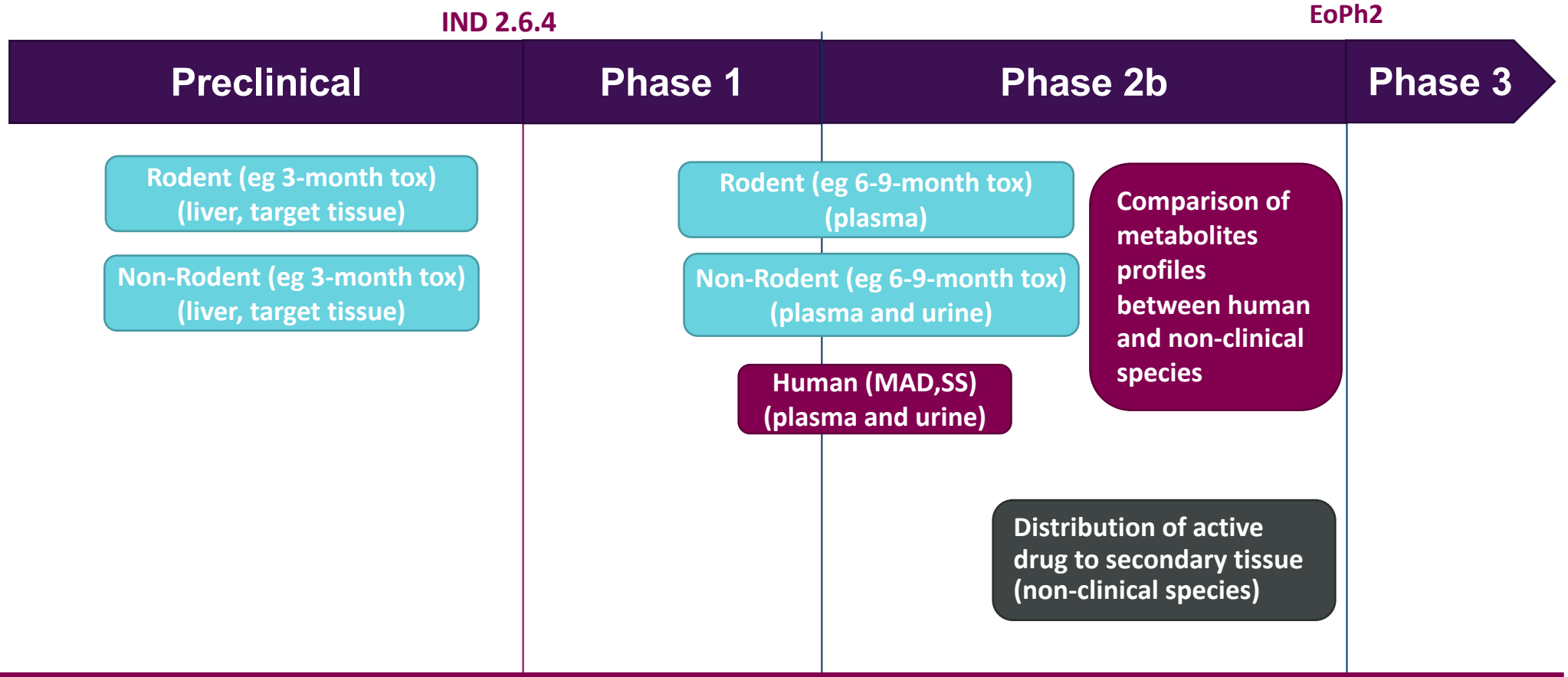
Biotransformation studies during development



- All samples are obtained from Tox and Clinical studies
- Recovery samples (non-clinical) are analysed to assess risk of accumulation
- Consider support to reprotox and carc (tissue, plasma)
- Distribution of the active compound to secondary tissues is further investigated before EoPh2

Slide courtesy of Mette Lund Pedersen and Xue-qing Li

Case Study: Biotransformation of a GalNAc conjugated ASO

Available BT samples from tox/safety studies

Steady state	Human	Monkey	Mouse	Rat	Rabbit
Plasma	X	X	X	X	X
Urine	X	X			
Liver		X	X	X	X
Kidney		X	X		

- Metabolite ID and profiling were investigated in different species and matrices
- Metabolite ID and profiling in tissue from monkey and mouse were already available from ph1
  - Kidney was included to gain knowledge since this was one of the early ASO programs at AZ
  - Additional tissues may be included for alternative targeting conjugates
- Rat and rabbit are included to support carcinogenicity and repro studies

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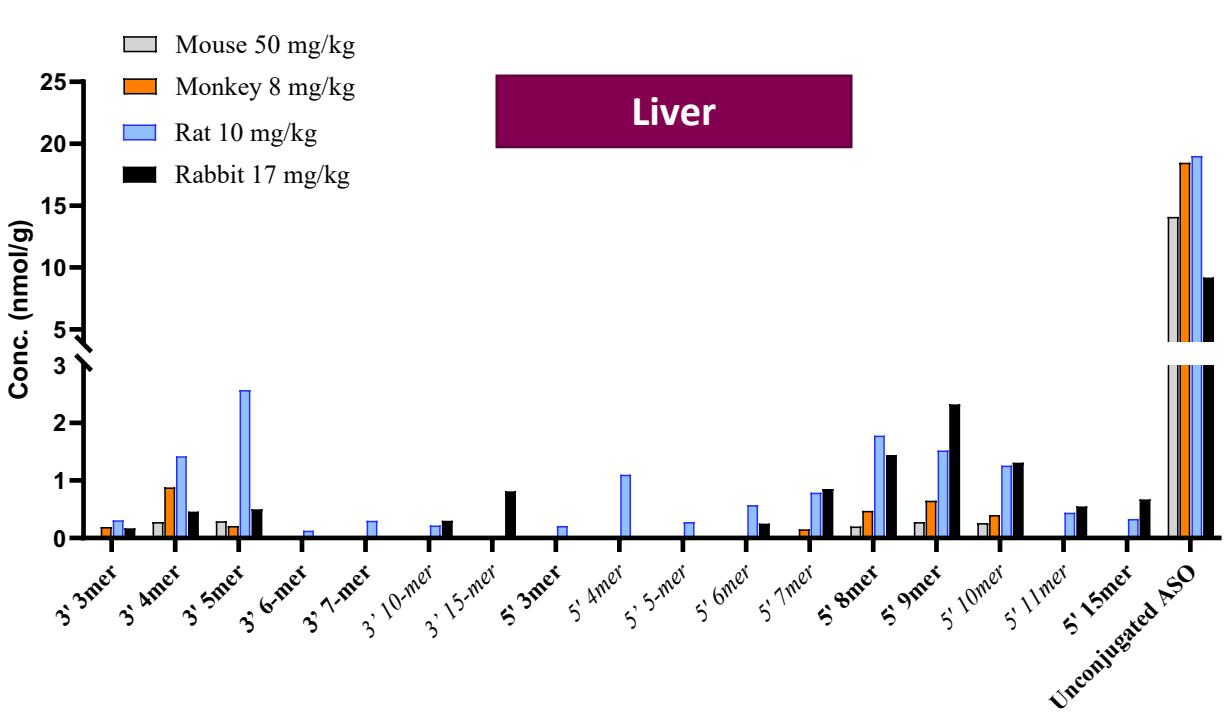
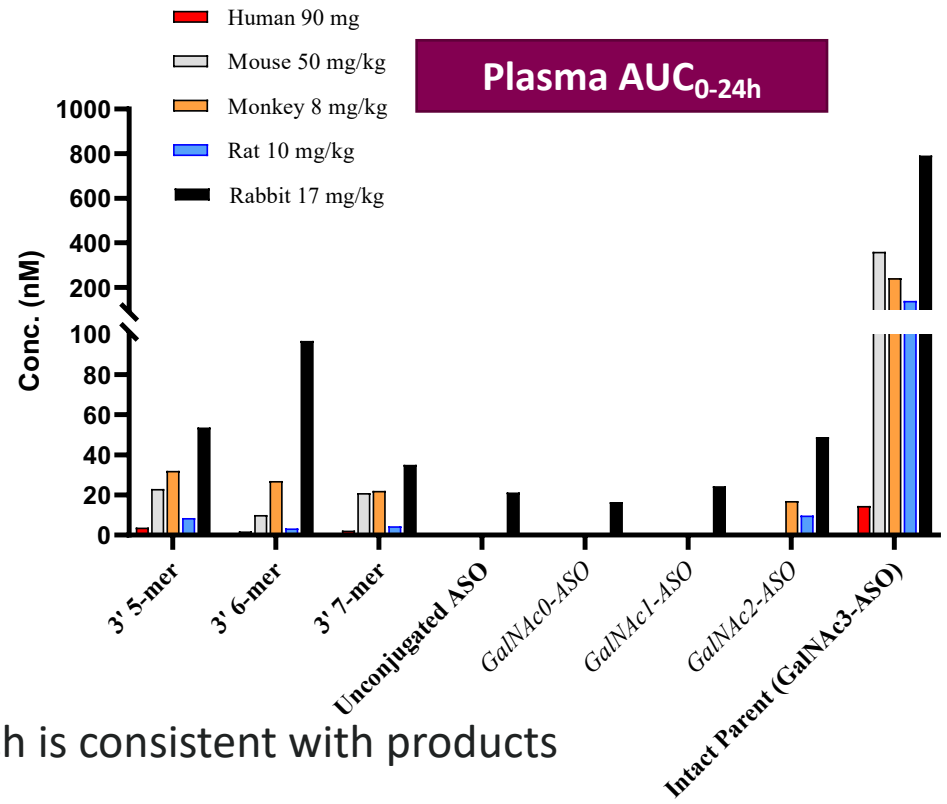
Case Study: Biotransformation of a GalNAc conjugated ASO

Comparison across species and safety assessment





- Biotransformation was primarily hydrolysis to form short-mers, which is consistent with products formed following cleavage by endonuclease and exonucleases
- Only low levels of the potential active metabolites were observed in tissue (N-1 or N-2) - they were not expected to contribute to the pharmacology
- All metabolites observed in human plasma were present in the non-clinical species at higher exposure (2-50 fold)
- Health Authorities endorsed this strategy at the EoPh2, to support ph3 and NDA/MAA

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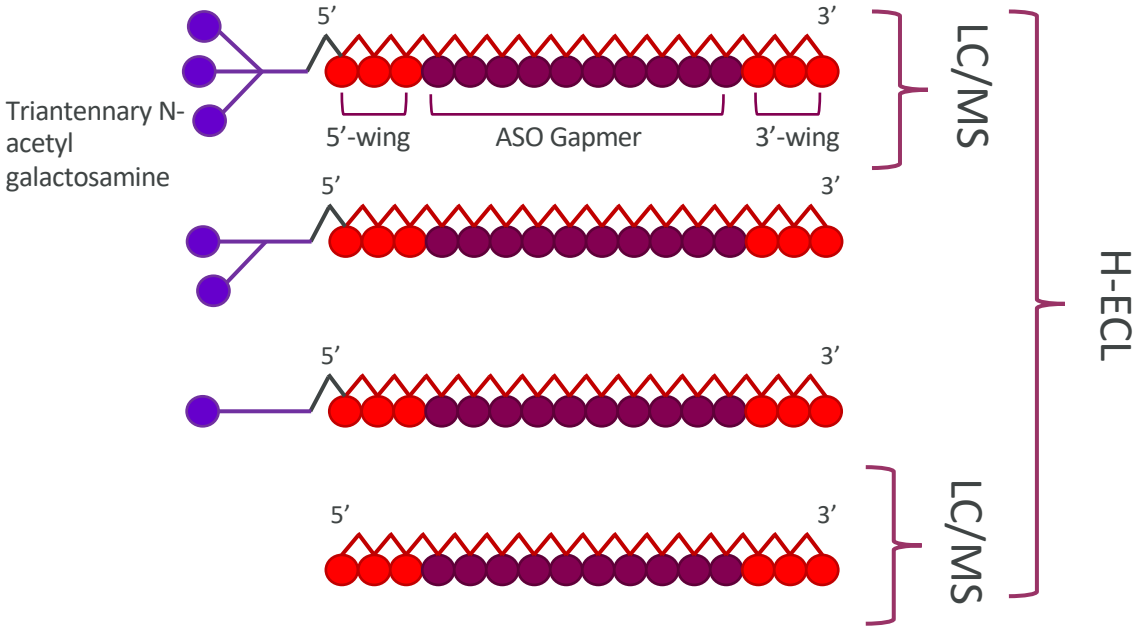
Data and figure by Xue-qing Li

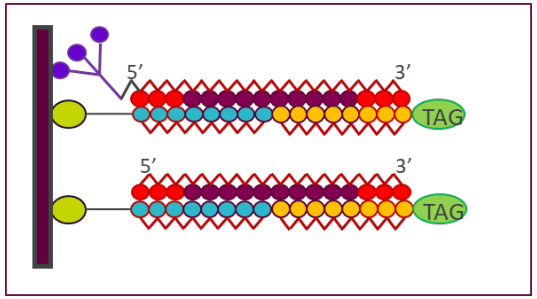
Biotransformation informs analyte selection and methodology for Bioanalysis

What do different bioanalysis assays measure?

Ion-Pair LC-MS/MS



H-ECL



- Hybridisation assays measure all potentially pharmacologically active forms of conjugated ASO
  - Need to verify equivalent measurement of conjugated and unconjugated ASO
- LC-MS assays need a reference standard for each species
  - Partially deglycosylated forms are difficult to synthesize and purify
- Consider Consistency of measured endpoint between non-clinical and clinical studies

Figure prepared by Cecilia Arfvidsson

Nonclinical Bioanalysis Strategy: GalNAc-ASO

Species	Matrix	Analyte	Technique	Validation Level	Rationale
Mouse & NHP	Plasma	Full length ASO species	Hybridisation Assay	Validated	Primary Measure of Exposure
Mouse	Liver and Kidney	Unconjugated ASO	LC-MS/MS	Tissue Assay	Tissue Accumulation
Mouse	Liver and Kidney	Unconjugated Surrogate ASO	LC-MS/MS	Tissue Assay	Benchmark exposure to clinical candidate
NHP	Liver and Kidney Cortex	Unconjugated ASO	LC-MS/MS	Tissue Assay	Determine Tissue half life and tissue to plasma ratio
NHP	Urine	Conjugated & unconjugated ASO	Hybridisation Assay LC-MS/MS	Validated	Determine renal clearance
NHP	Plasma	ADA (screening)	LBA	Trigger based on Tox findings	Informing immune-mediated Tox evaluation
Mouse	Liver***	Murine Target mRNA	RT-qPCR	Tissue Assay	Confirm evaluation of on on-target Tox
NHP	Liver***	NHP target mRNA	RT-qPCR	Tissue Assay	Confirm evaluation of on on-target Tox
Mouse	Tissues	Unconjugated ASO	LC-MS/MS	Tissue Assay**	Biodistribution
NHP	Tissues	Unconjugated ASO	LC-MS/MS	Tissue Assay**	Biodistribution

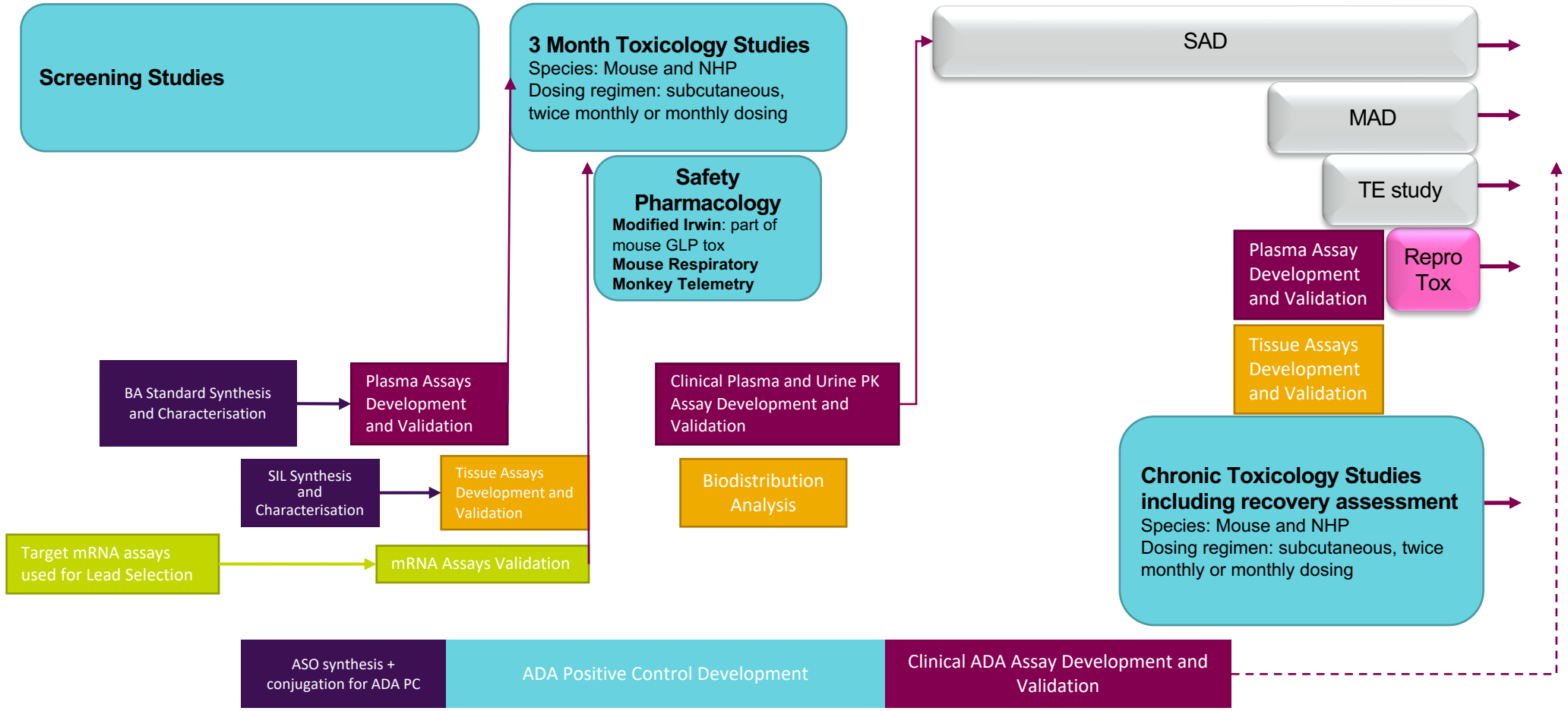
No plasma assay for murine surrogate ASO, tissue exposure only is used to benchmark exposure
** Liver assay used as basis for biodistribution study using tissues from GLP tox (Up to 8 tissues).
*** Target mRNA assay in NHP only run when *in vitro* activity is above a threshold level. Tissue selection may be driven by expression of target.

Early Clinical Bioanalysis Strategy: GalNAc-ASO

Species	Matrix	Analyte	Technique	Date Required	Rationale
Human	Plasma	Full length ASO species	Hybridisation Assay	PhI Start	Data used in dose escalation
Human	Plasma	Conjugated & unconjugated ASO	LC-MS/MS	End of PhI	May be used inform exposure:response relationship between Conjugated and Unconjugated ASO
Human	Urine	Conjugated & unconjugated ASO	LC-MS/MS	End of PhI	Confirmation of Urine exposure
Human	Plasma	ADA (3 Tier)	Immunoassay	Phase I End of Study	GLP Tox

- Push back on early inclusion of Immunogenicity Testing
  - ASO immunogenicity is typically low titer
  - HV immunogenicity may not be reflective of the target population
  - ASOs have an intracellular MoA – if they get into the cell they can be active
    - Limited relevance of Nab assays
- May include opportunistic analysis of tissue samples when biopsy samples available

Illustrative Development Plan



Bioanalysis Key Considerations

- Some major analytes are not Test Item
  - Ensure appropriate characterisation of Bioanalytical Standards
    - Unconjugated ASO
    - Chain shortened metabolites
- Evaluation of cross-reactivity & selectivity
  - Equivalence of conjugated and unconjugated ASO
  - Cross-reactivity for chain shortened metabolites
  - Interference from chain shortened metabolites
- Long lead time for ADA positive control
  - Bank samples from Tox and early clinical studies
  - There may be limited relevance of HV immunogenicity to target indication

H-ECL Metabolite Cross Reactivity

Metabolite Standard	AZD7503 Measured Concentration (nM)	CV (%)	Cross-Reactivity (%)
Metabolite 1 (N-1 from 3'-end [15-mer])	0.624	2.7	41.6
Metabolite 2 (N-8 from 3'-end [8-mer])	<LLOQ	NC	<0.7%
Metabolite 3 (N-8 from 5'-end [8-mer])	<LLOQ	NC	<0.7%
Metabolite 4 (N-13 from 3'-end [3-mer])	<LLOQ	NC	<0.7%
Metabolite 5 (N-13 from 5'-end [3-mer])	<LLOQ	NC	<0.7%

- Each metabolite spiked as molar equivalent concentration to HQC
  - Concentration reported relative to AZD7503 standard curve
- Some cross-reactivity observed with theoretical n-1 metabolite but this is only present in very limited amounts *in-vivo*

H-ECL - Metabolite Interference

Metabolite	AZDXXXX Conc. (nM)	Metabolite Conc. (nM)	Mean Conc. (nM)	CV (%)	Bias (%)
Metabolite 1 3’ N-1	ULOQ - 2.00	20% - 0.400	2.00	2.3	0.0
	ULOQ - 2.00	5% - 0.100	1.90	2.3	-5.0
	LLOQ - 0.0100	20% - 0.00200	0.0110	4.2	10.0
	LLOQ - 0.0100	5% - 0.000500	0.0104	4.8	4.0
Metabolite 2 3’ N-8	ULOQ - 2.00	20% - 0.400	1.85	2.8	-7.5
	ULOQ - 2.00	5% - 0.100	1.80	1.8	-10.0
	LLOQ - 0.0100	20% - 0.00200	0.0106	8.5	6.0
	LLOQ - 0.0100	5% - 0.000500	0.00891	5.1	-10.9
Metabolite 3 5’ N-8	ULOQ - 2.00	20% - 0.400	1.82	2.0	-9.0
	ULOQ - 2.00	5% - 0.100	1.80	3.7	-10.0
	LLOQ - 0.0100	20% - 0.00200	0.00977	12.1	-2.3
	LLOQ - 0.0100	5% - 0.000500	0.00960	4.1	-4.0
Metabolite 4 N-13 from 3'-end	ULOQ - 2.00	20% - 0.400	1.84	5.7	-8.0
	ULOQ - 2.00	5% - 0.100	1.75	4.0	-12.5
	LLOQ - 0.0100	20% - 0.00200	0.00993	3.5	-0.7
	LLOQ - 0.0100	5% - 0.000500	0.00940	5.0	-6.0
Metabolite 5 N-13 from 5'-end	ULOQ - 2.00	20% - 0.400	1.90	7.3	-5.0
	ULOQ - 2.00	5% - 0.100	1.90	7.1	-5.0
	LLOQ - 0.0100	20% - 0.00200	0.00930	5.1	-7.0
	LLOQ - 0.0100	5% - 0.000500	0.00868	4.6	-13.2

- 3’ N-1 represents the “worst case scenario”
- Although cross-reactivity is observed there is no functional impact on the assay performance for full length oligonucleotides

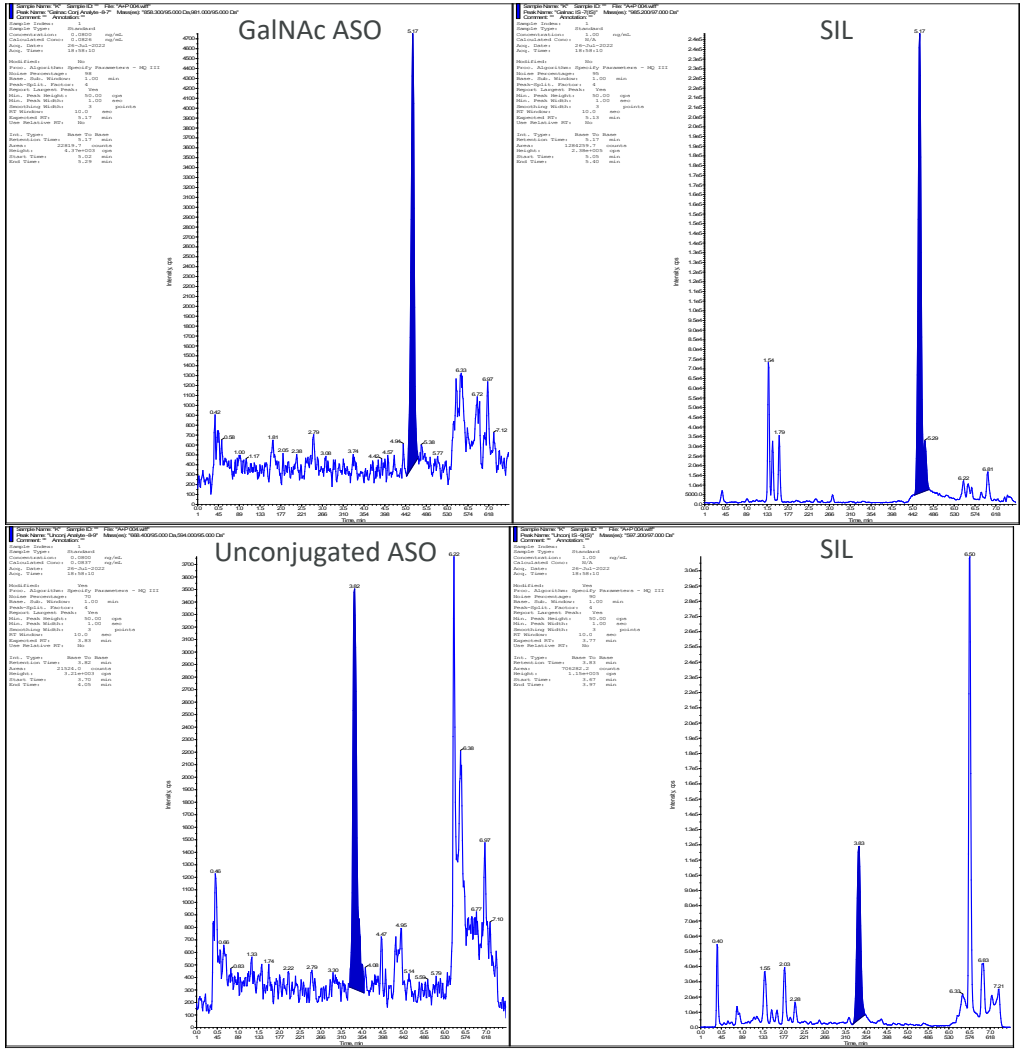
H-ECL Assay Unconjugated + Conjugated ASO

QC Level (nmol/L)	Mean Concentration (nmol/L)	CV (%)	Bias (%)	TE (%)
LLOQ 50/50 Mix	0.0419	6.3	4.8	11.1
LQC 50/50 Mix	0.107	1.1	7.0	8.1
MQC 50/50 Mix	0.634	2.4	5.7	8.1
HQC 50/50 Mix	3.45	3.2	15.0	18.2
ULOQ 50/50 Mix	4.68	5.2	17.0	22.2

- Accuracy and Precision tested with both the unconjugated and conjugated ASO to ensure equivalent response per molar unit
- Assay may perform better in one orientation compared to the other

LC-MS/MS Assay Considerations

- Use of 34-S ASO as Internal Standard facilitates improved sensitivity and assay robustness
- Assay development needs to evaluate for charge state overlap
- If needed ensure chromatographic separation
- Murine ASO often analysed using human ASO SIL
- No inclusion of GalNAc-ASO in tissue assays



Bioanalysis Endpoints

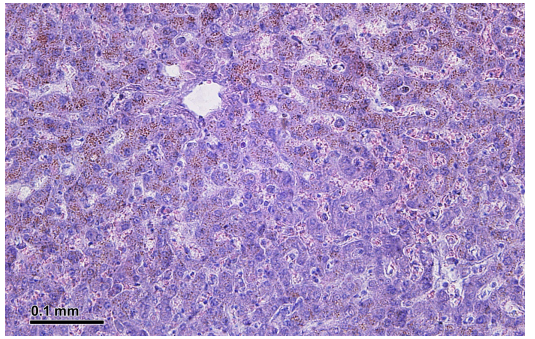
Plasma

- Initial rapid plasma clearance due to fast distribution to tissues
- Long terminal half-life

Urine

- Low amount excreted in urine
- Indicates low renal clearance

Tissue



- Highest exposures in target organ
- Long tissue half life
- Tissue accumulation

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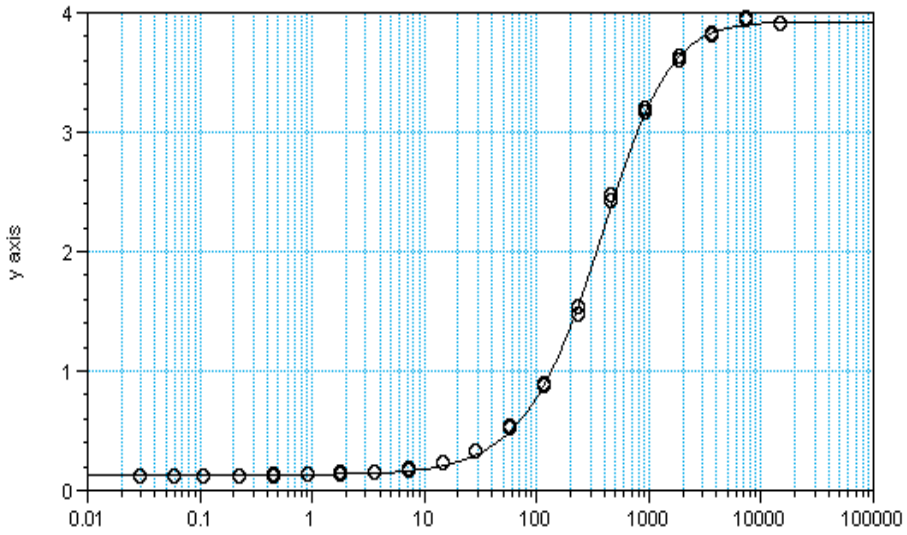
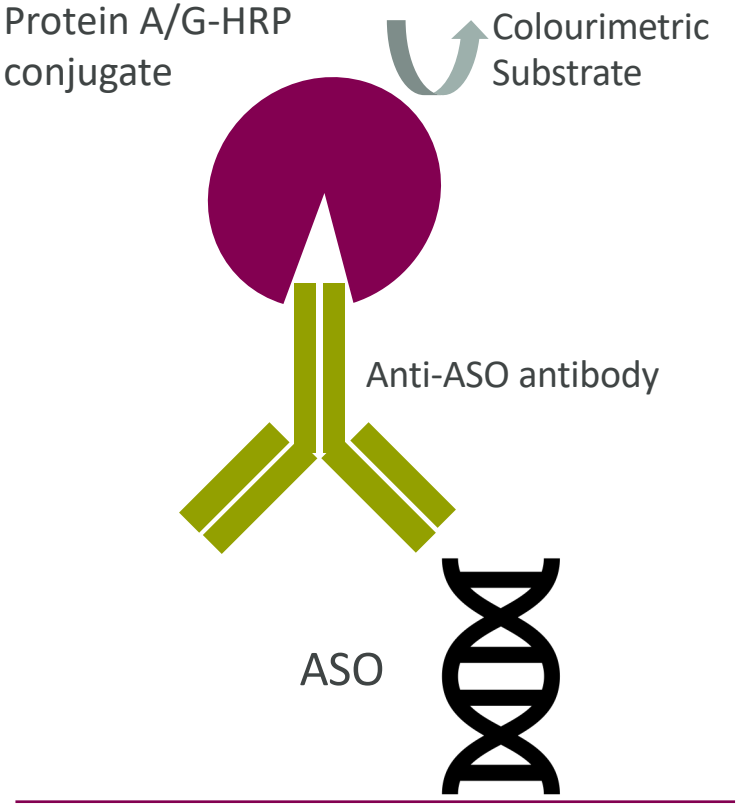
Bioanalysis informs concentration ranges and target tissues for Biotransformation studies

Anti-ASO Immunogenicity Assay

- Sequential format direct assay
- Anti-ASO antibodies are captured to ASO immobilised on a microtitre plate and detected using Protein A/G-HRP conjugate
- No evidence of prozone effect
- Very sensitive assays with good drug tolerance
- Consider the clinical relevance of low ADA titers relative to drug concentration



Assay Format



Working closely together, Biotransformation and Bioanalysis informs a comprehensive data package delivering PK, immunogenicity and ADME data required for regulatory interactions

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Questions?