Setting the scene: metabolites & oligonucleotide and peptide drugs = a different story

Anna Laurén, on behalf of the EBF

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M10 started the discussions on a need to discuss "neither small nor large" drug modalities

- How about metabolites for Peptides and Oligo therapeutics?

When?

- For Oligo therapeutics there was also the considerations around tissue exposure vs PD

Scope

The guideline is applicable to the bioanalytical methods used to measure concentrations of chemical and biological drug(s) and their metabolite(s) in biological samples (e.g., blood, plasma, serum, other body fluids or tissues) obtained in nonclinical toxicokinetic (TK) studies conducted according to the principles of GLP, nonclinical pharmacokinetic (PK) studies conducted as surrogates for clinical studies, and all phases of clinical trials, including comparative bioavailability/bioequivalence (BA/BE) studies, in regulatory submissions. Full method validation is expected for the primary matrix intended to support regulatory submissions. Additional matrices should be validated as necessary.

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Metabolite oligos, peptides and proteins

- “ICH M3 (R2)”; and “FDA Safety Testing of Drug Metabolites (2020)”.
  - Applies to small molecule “new chemical entity (NCE)” pharmaceuticals.
  - ICH M3 (R2): Metabolism in test species and *in vitro should be* available generally before Phase 3.
  - The nonclinical characterization of metabolites with an identified concern should be considered case by case.

- ICH S6 (R1): Section 4.2.3 Metabolism:
  - Understanding the behavior of the biopharmaceutical in the biologic matrix, (e.g., plasma, serum, cerebral spinal fluid) and the possible influence of binding proteins is <u>important for understanding the pharmacodynamic effect</u>.

- FDA Clinical Pharmacology Considerations for the Development of Oligonucleotide Therapeutics. Draft June 2022
  - Appropriate bioanalytical methods should be used to <u>characterize the parent oligonucleotide and any relevant metabolites</u>, including chain-shortened metabolites. Refer to FDA BMV guidance.

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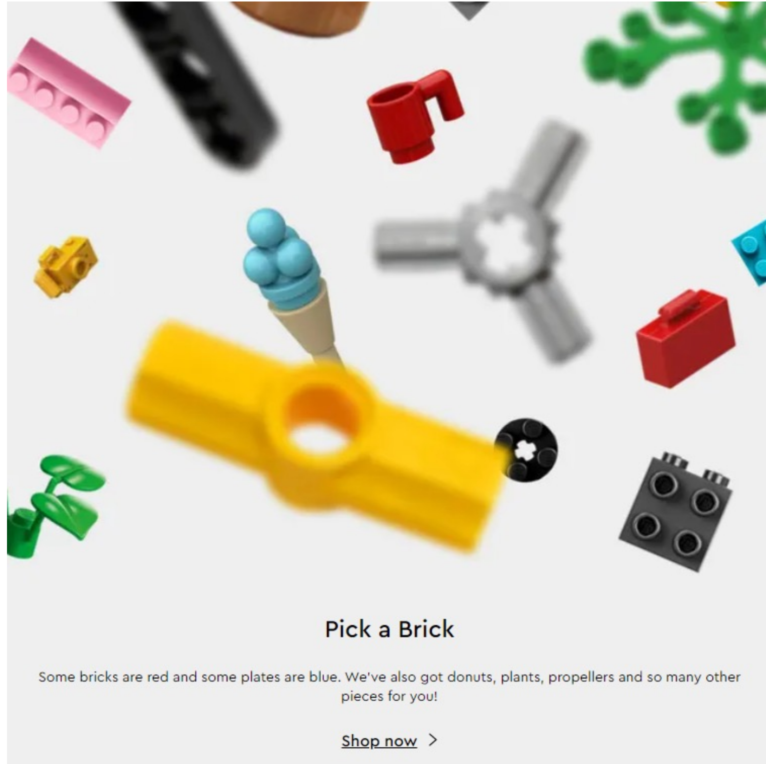
The building blocks



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- To what detail do we need to characterise when individual building blocks are already well known?
- When the ”building blocks” are natural?
- When is there a risk for new toxicity?
- Changed PD? Do we need to know details of metabolites?
- Use a scientific fit for purpose strategy!

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Is the strategy for peptide and oligo drugs the same as for NCE?

Not that many metabolites require a validated method as per BMV

Pre-phase 1:

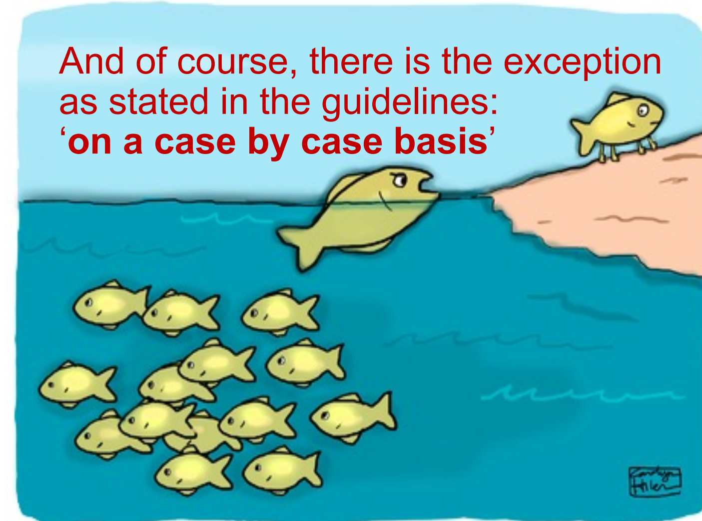
- None, except pro-drugs?
- Metabolites dosed as test article should not be considered as metabolites

Phase 1-2

- None, except pro-drugs?
- Sponsors may want to consider to already include metabolites with documented activity > 25% (albeit, how to decide on '> 25%'?

> Phase 2, and considering metabolite profiling info

- UMM
- > 10% of drug exposure and not covered during tox studies
- Active metabolites as per FDA/EMA guidelines



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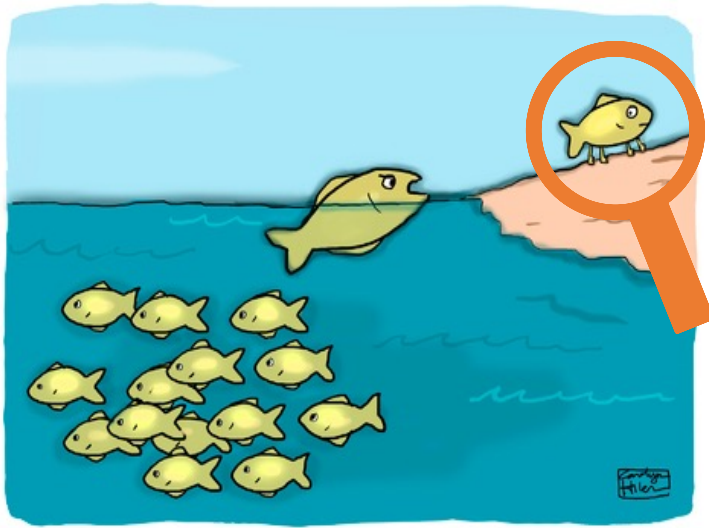
Peptide and oligoe drugs and the exceptions?



- When is the exception the right exception?
- What do we use for early internal decision making and what is for a regulatory requirement?

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Co-measuring metabolites in the peptide PK assay

- A peptide case:
  - Original PK Assay strategy: LBA using specific antibody reagents for the peptide.
    - ➡ Nonclinical TK studies showed unexpected $T_{1/2}$ (longer than expected).
      - ➡ Change to LC-MS/MS indicated unexpected T1/2 was due to metabolite (natural deamidation).
        - ➡ LBA co-measured metabolite and parent.
          - ➡ LC-MS/MS assay needed >30 minutes per sample for separation between parent and metabolite.
- Deamidated metabolites for peptides are not toxic and often with identical PD effect.
- Peptide metabolites due to aminoacid shortening are not toxic and often with a decreased PD effect.
- PD effect part of early dose setting models.
- Is it practical to use a 30 minute/sample LC-MS/MS assay for large phase 3 studies? (eg Type2 Diabetes 9000 subject study)?

Consider separate Phase 3 PK cohort/Late PK study for metabolite quantification to confirm early PK/PD model.

Oligonucleotides, analytes and metabolites

- *Oligonucleotide therapeutics generally are cleared rapidly from systemic circulation. However, these drugs have longer tissue and pharmacodynamic half-lives.*
- *Sponsors should characterize plasma pharmacokinetics of an oligonucleotide therapeutic following single and multiple doses early in drug development.*
- *Plasma pharmacokinetics might not reflect the target tissue distribution, pharmacodynamics, safety, or efficacy.*
- *In multiple-dose studies, sponsors should include an assessment of appropriate pharmacodynamic biomarkers (e.g., target mRNA, target protein, or a downstream biomarker that reflects modulation of the target protein) or consider other response measures.*
- *Additionally, appropriate bioanalytical methods should be used to characterize the parent oligonucleotide and any relevant metabolites, including chain-shortened metabolites. Refer to the FDA guidance entitled Bioanalytical Method Validation (May 2018) for additional details.*

Clinical Pharmacology Considerations for the Development of Oligonucleotide Therapeutics

Guidance for Industry

DRAFT GUIDANCE

This guidance document is being distributed for comment purposes only.

Comments and suggestions regarding this draft document should be submitted within ___ days of publication in the *Federal Register* of the notice announcing the availability of the draft guidance. Submit electronic comments to https://www.regulations.gov. Submit written comments to the Dockets Management Staff (HFA-305), Food and Drug Administration, 5630 Fishers Lane, Rm. 1061, Rockville, MD 20852. All comments should be identified with the docket number listed in the notice of availability that publishes in the *Federal Register*.

For questions regarding this draft document, contact (CDER) Office of Clinical Pharmacology Guidance and Policy at CDER_OCP_GPT@fda.hhs.gov.

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)

June 2022
Clinical Pharmacology

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Oligonucleotides timing of investigations

- *Specific considerations should be given to the chemistry (e.g., backbone modification, conjugation), drug target, plasma protein binding, and route of administration as these factors determine the distribution of the oligonucleotide therapeutic to the liver, kidneys, and other tissues as well as determine the exposure (local or systemic) to the drug.*
- *To determine the appropriate approach for characterizing the impact of organ function on the pharmacokinetics, pharmacodynamics, and safety of the oligonucleotide therapeutic, the sponsor should identify the role of the liver and kidney in the disposition and elimination of the oligonucleotide therapeutic by considering in vitro, preclinical, and early Phase 1 clinical data.*
- *These early assessments should be used to inform the enrollment of subjects with a full range of hepatic and/or renal function in the late-phase trials.*

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Discussions needed

- Justifications for historic knowledge within platform is warranted.
- Bioanalytical methods be used to characterize the parent oligonucleotide and any relevant metabolites, should not per default refer to Bioanalytical Method Validation Guidelines.
- A risk-based assessment is recommended to justify when tissue & urine PK analysis is needed.

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PK assay considerations - sensitivity vs. specificity/selectivity

- Do you ask your stakeholder what they need and their justification?
- Or do you use your favorite method?
- Oligonucleotides accumulate in tissues (to high concentrations).
- At later timepoints and for quantification in plasma it is often necessary to use more sensitive methods (HPLC-FL or HELISA/HECL).

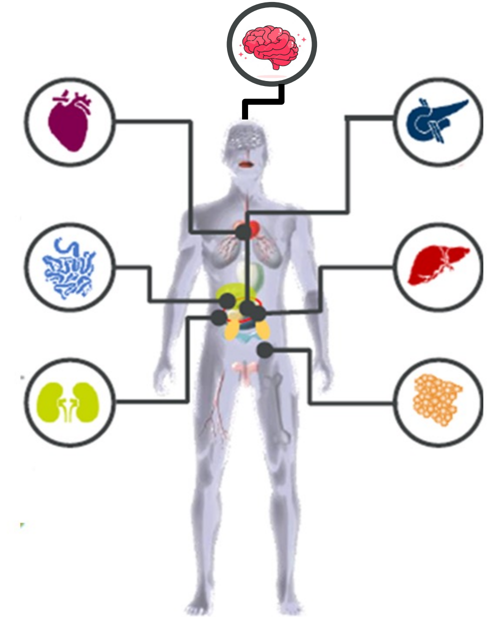
	Specific/Selective to parent	Sensitivity* plasma	Sensitivity* tissue
LC-UV	Yes	10-50 ng/mL	100 ng/mL
HPLC-FL	Yes	1-2 ng/mL	20 ng/mL
HELISA	May co-measure metabolite	0.1-2 ng/mL	<10 ng/mL
HECL	May co-measure metabolite	0.1 ng/mL*	<1 ng/mL*
SI-qRT-PCR	May co-measure metabolite	pg/mL	10-80 ng/mL

Multiple bioanalytical approaches often required

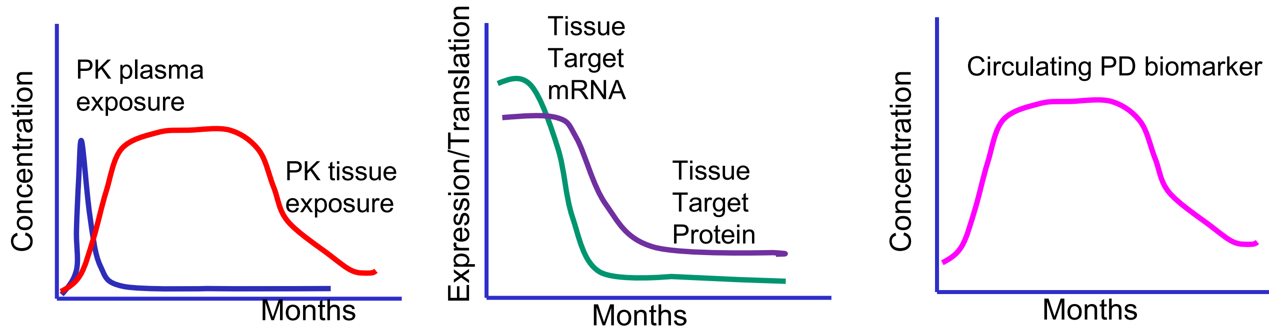
** General number. Better sensitivies has been reported.*

Oligo Exposure in plasma vs tissue

- Oligos are designed to have a short half life in circulation.
- Main exposure is expected in tissue and main metabolites in tissue.
- Different target mRNA/proteins have different halflife.
- Ethical considerations to collect biopsies in humans:
  - PD and biomarkers are used as surrogate of tissue exposure.

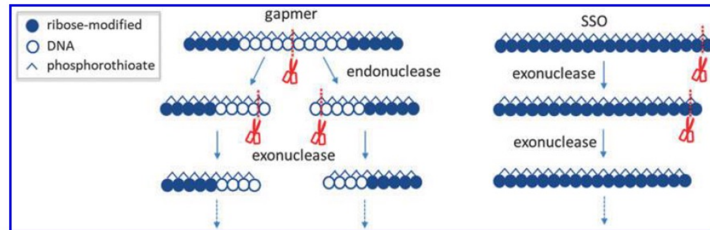


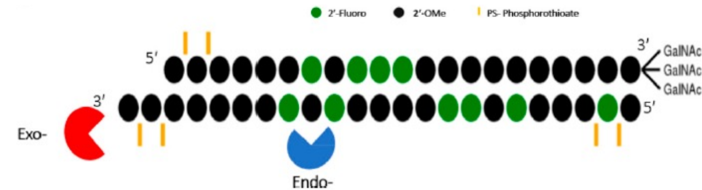
Example of siRNA knockdown



Metabolites for ASO/siRNA: Biotransformation in plasma vs tissue

- Hydrolysed by endonucleases and/or exonucleases present in the plasma and tissues throughout the body.
- Biotransformation products of ASO/siRNAs are generally shortmers formed via backbone hydrolysis.
- Shortening is frequently initiated by endonuclease cleavage, followed by exonuclease trimming.
- Metabolites are generally not a safety concern.





Weidolf *et al.*, Distribution and biotransformation of therapeutic antisense oligonucleotides and conjugates. Drug Discov Today 2021

Takakusa *et al.,* Drug metabolism and pharmacokinetics of antisense oligonucleotide therapeutics typical profiles, evaluation approaches, and points to consider compared with small molecule drugs. Nucleic Acid Ther 2023

Andersson *et al.*, Drug metabolism and pharmacokinetic strategies for oligonucleotide- and mRNA-based drug development. Drug Discov Today 2018

The ADME of siRNA GalNAc conjugates, Chris MacLauchlin, Alnylam, 16th Oligonucleotide Therapeutics Society, 202

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Biotransformation of GalNac siRNA investigated *in vitro*

- Highly predictable
- “Natural breakdown products”
- Similar between human and monkey cells

In vitro GalNAc and linker metabolism for siRNA8 in cultured hepatocytes

Analyte	Total AUC siRNA8 Sense Strand Metabolite Profile		
	Rat	Monkey	Human
		%	
−3 GalNAc	Not detected	48.7	45.0
−3 GalNAc, −1linker1	Not detected	20.3	27.5
−3 GalNAc, −1linker1, −1linker2	Not detected	5.2	Not detected
−3 GalNAc, −2linker1	Not detected	12.2	14.9
−3 GalNAc, −2linker1, −1linker2	Not detected	3.6	Not detected
−3 GalNAc, −3linker1,	99.5	8.5	11.0
−3 GalNAc, −3linker1, −1linker2	Not detected	1.6	Not detected
Full-length siRNA8	66.6	71.0	74.9
AS(N-1)3′ siRNA8	29.7	21.2	15.9

McDougall *et al.,* The nonclinical disposition and pharmacokinetic/pharmacodynamic properties of N-acetylgalactosamine–conjugated small interfering RNA are highly predictable and build confidence in translation to humans, DMD 2022 (781-797)

Main metabolites of oligos are often active *in vitro*

- 3'N-1(AS) is an often-observed metabolite and may be pharmacologically active.
- *In vitro* potency testing reveal several truncated metabolites might be active.

TABLE 5

Pharmacological activity assessment for siRNA2 metabolites (transfected) in plated HEP3B

Designation (AS)	Target mRNA Baseline Remaining
	%
Parent	16.4
3′N-1	10.3
3′N-2	11.4
3′N-3	13.5
3′N-4	15.9
3′N-5	12.2
3′N-6	47.7
3′N-7	81.1
3′N-8	95.7
5′N-1	78.6
5′N-2	20.7
5′N-3	89.0
5′N-4	78.5
5′N-5	95.2
5′N-6	100
5′N-7	100
5′N-8	100

Ramsden *et al*., Leveraging microphysiological systems to address challenges encountered during development of oligonucleotide therapeutics. ALTEX 2022, 273-296.
McDougall *et al*., The Non-clinical Disposition and PK/PD Properties of GalNAc-siRNA are highly predictable and build confidence in translation to human. Drug Metabolism and Disposition 2022, 781-797.

Low concern for toxic metabolites of oligos

- siRNA toxicity is believed to correlate with chain length.
- Catabolism of the siRNA is expected to reduce rather than increase the level of toxicity compared to the parent compound.
- Oligonucleotide Safety Working Group (OSWG) has concluded: often a low need for generating metabolism data at an early stage in development.
- No reports of toxic oligo metabolites in literature.

Humphreys *et al*., Emerging siRNA Design Principles and Consequences for Biotransformation and Disposition in Drug Development. J Med Chem. 2020.
Marlowe *et al*., Recommendations of the Oligonucleotide Safety Working Group's Formulated Oligonucleotide Subcommittee for the Safety Assessment of Formulated Oligonucleotide-Based Therapeutics. Nucleic Acid Ther. 2017.

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Consider holistic assay strategy on Oligo platform

- Example when **historic data is available within oligo platform (eg GalNac in liver)**.
- Circulating biomarkers and PD is available.

Study type	Assay and matrix	Validation?
PKPD/DRF – non-GLP	PK assay plasma	Scientifically valid assays for CoU
	PK assay target tissue* §	Scientifically valid assays for CoU
	qPCR mRNA target tissue*	Scientifically valid assays for CoU
	Downstream PD and biomarkers	Scientifically valid assays for CoU
Tox - GLP	PK assay plasma	ICH M10 validation
	PK assay target tissue* §	ICH M10 validation consider using surrogate matrix
	Downstream PD and biomarkers	Biomarker CoU validation
Human - GCP	PK assay in plasma	ICH M10 validation
	Separate metabolite identification initiated during phase 2. Only in-vitro and in plasma.	ICH M10 validation: only those metabolites contributing to >25% activity (ie low likelihood)
	Downstream PD and biomarkers	Biomarker CoU validation

**Target tissue PK/qPCR/protein KD is not needed when historic data from platform is available*
§ Consider collect and bank for biotransformation and metabolite investigations.

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Consider holistic assay strategy on Oligo platform

- Example on **high complexicity** when **limited data is available within oligo platform**.
- New target tissue.
- No circulating biomarkers or PD is available.

Consider collect and bank for biotransformation and metabolite investigations.

Study type	Assay and matrix	Validation?
PKPD/DRF – non-GLP	PK assay plasma*	Scientifically valid assays for CoU
	PK assay target tissue*	Assay technology based on CoU
	qPCR target mRNA target tissue (+ off target tissues).	Assay technology based on CoU
	Target protein in target tissue	Assay technology based on CoU
Tox - GLP	PK assay plasma*	ICH M10 validation
	PK assay target tissue*	ICH M10 validation consider using surrogate matrix
	qPCR target mRNA target tissue (+ off target tissues).	PCR CoU validation
	Target protein in target tissue may be needed.	Biomarker CoU validation
Human - GCP	PK assay in plasma*	ICH M10 validation
	PK assay target tissue	ICH M10 validation consider using surrogate matrix
	Separate metabolite identification initiated during phase 2. Only in-vitro and in plasma.	ICH M10 validation: only those metabolites contributing to >25% activity (ie low likelihood).
	qPCR target mRNA target tissue may be needed.	PCR CoU validation. Consider ethical aspects.
	Target protein in target tissue may be needed.	Biomarker CoU validation. Consider ethical aspects.

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For further consideration

- Multiple approaches to understand exposure is important for oligos and peptides
  - When do we mainly trust PK assay exposure?
  - When do our stakeholders need additional data (eg PD) for modelling?
  - Should we always measure just because we can?
  - What are the BioA lab's responsibilities and possible role to challenge what endpoints that are assessed?
  - What platform strategies and historical data may be considered?

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Acknowledgements

EBF community and EBF teams for discussion on the topic since 2010

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Questions

Contact Information

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Preparing the session round table metabolites & tissues

Anna Laurén, on behalf of the EBF

Round Table discussions Oligos and Peptides

- How quantification for peptides and oligos is either similar or differs from the processes applied for small molecules (aka NCEs or New chemical entities).
- How do metabolite profiling impact the PK assay exposure?
- Stage of drug development in which the metabolite profiling are done or makes sense?
- Specific scientific challenges and regulatory expectations may be (real or perceived) different from previous recommendations?

Bioanalysis (2016) 8(12), 1297–1305

Best practices for metabolite quantification in drug development: updated recommendation from the European Bioanalysis Forum

Philip Timmerman*[,1], Stefan Blech[2], Stephen White[3], Martha Green[4], Claude Delatour[5], Stuart McDougall[‡,6], Geert Mannens[1], John Smeraglia[5], Stephen Williams[4] & Graeme Young[3]

Round Table discussions Oligos and Peptides

- Need for setting up an 'ICH M10' compliant assay to quantify metabolites?
- Need for a 'ICH M10' compliant assay to specifically measure parent drug?
- And when?

Scope

The guideline is applicable to the bioanalytical methods used to measure concentrations of chemical and biological drug(s) and their metabolite(s) in biological samples (e.g., blood, plasma, serum, other body fluids or tissues) obtained in nonclinical toxicokinetic (TK) studies conducted according to the principles of GLP, nonclinical pharmacokinetic (PK) studies conducted as surrogates for clinical studies, and all phases of clinical trials, including comparative bioavailability/bioequivalence (BA/BE) studies, in regulatory submissions. Full method validation is expected for the primary matrix intended to support regulatory submissions. Additional matrices should be validated as necessary.

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Round Table Discussions Peptides

- What exposure do you measure for peptides?
  - Compare to NCE or a therapeutic protein?
  - Do you per default select your favorite assay?
  - Do you consider the specificity for metabolites during method validation?
  - When the ”building blocks” are natural?
  - When is there a risk for new toxicity?
  - Changed PD? Do we need to know details of metabolites?

Chromatographic methods: Specific/selective assay.
Ligand binding methods: May co-measure metabolite.

ICH M3 (R2):

- Metabolism in test species and in vitro should be available generally before Phase 3.
- The nonclinical characterization of metabolites with an identified concern should be considered case by case.

ICH S6 (R1): Section 4.2.3 Metabolism:

Understanding the behavior of the biopharmaceutical in the biologic matrix, (e.g., plasma, serum, cerebral spinal fluid) and the possible influence of binding proteins is important for understanding the pharmacodynamic effect.

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Round Table Discussions Oligoes

- Are your stakeholders aware on what you measure?
  - Chromatographic methods: 15/20%RE/%CV and specific/selective assay.
  - Ligand binding methods: 20/25%RE/%CV and may co-measure metabolite.
  - PCR for PK: ??/??%RE/%CV and may co-measure metabolite.

- Do you per default select your favorite assay?
- Do you include specificy/selectivy for potential metabolites in method validation?

- When can we use assay technology (eg PCR for oligoe quantification) not included in M10 guidelines?

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Round Table discussions Tissue

Scope

The guideline is applicable to the bioanalytical methods used to measure concentrations of chemical and biological drug(s) and their metabolite(s) in biological samples (e.g., blood, plasma, serum, other body fluids or tissues) obtained in nonclinical toxicokinetic (TK) studies conducted according to the principles of GLP, nonclinical pharmacokinetic (PK) studies conducted as surrogates for clinical studies, and all phases of clinical trials, including comparative bioavailability/bioequivalence (BA/BE) studies, in regulatory submissions. Full method validation is expected for the primary matrix intended to support regulatory submissions. Additional matrices should be validated as necessary.

- What is the target matrix for oligos and/or metabolite quantification?
- And when?

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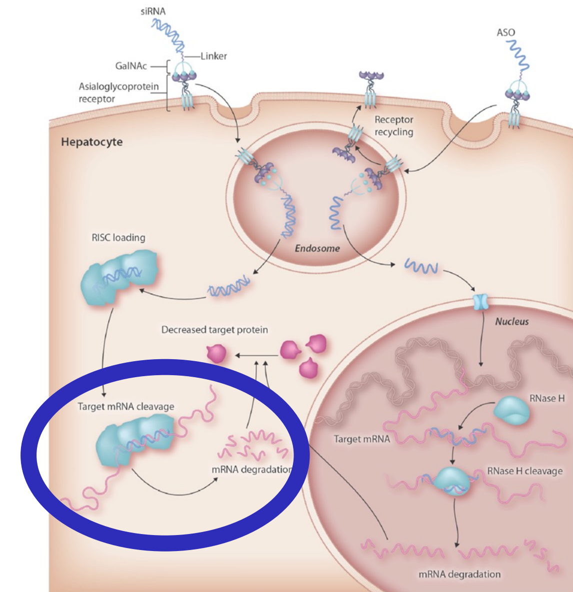
Round Table Discussions Oligoes and Tissue

- Are your stakeholders aware on where you measure?
- Oligoes: Exposure and Metabolites mainly in tissue.
  - Can we use surrogate matrix for tissue quantification?
  - Is understanding of the non-clinical metabolite quantity important?
  - How often can we obtain tissue in clinical studies?

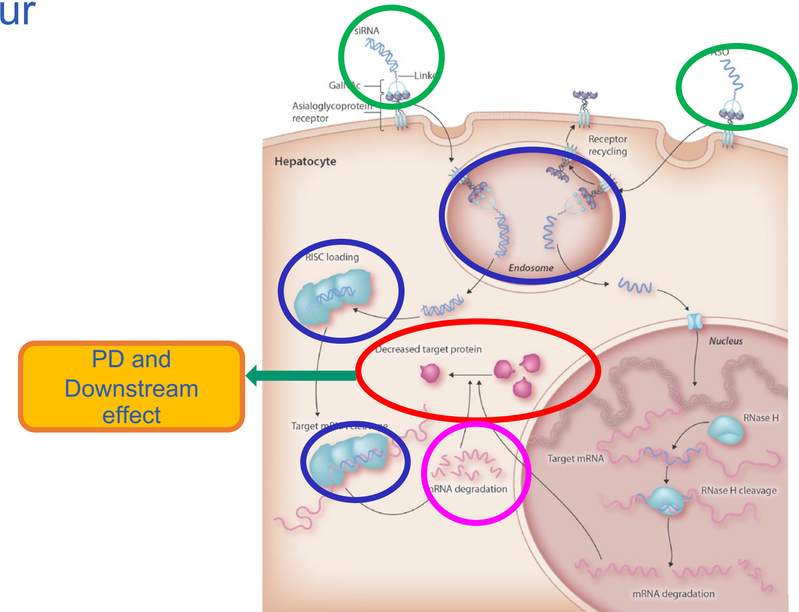
- Oligoes in the cell: true effect of exposure is in the RISC loaded complex.
  - RISC immunoprecipitation assays only practical in early research nonclinical pharmacology studies.

- Is there a risk we would like to do more just because we can?



Round Table Discussions Oligoes, plasma and tissue

- Do you discuss the overall assay strategy for your stakeholders modelling?
- What is most important to understand for dose setting?
  - Concentration in plasma?
  - Concentration in tissue?
  - mRNA expression?
  - Tissue target protein?
  - PD and Downstream circulating proteins?
- When can you use historic data for platform?
- Is there a risk we would like to do more just because we can?



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Round Table discussions

- Joint questions for exposure bioanalysis and metabolite quantification.

- Consider if the response would be different in peptides from oligos as well as technology (Chrom/LBA/PCR).

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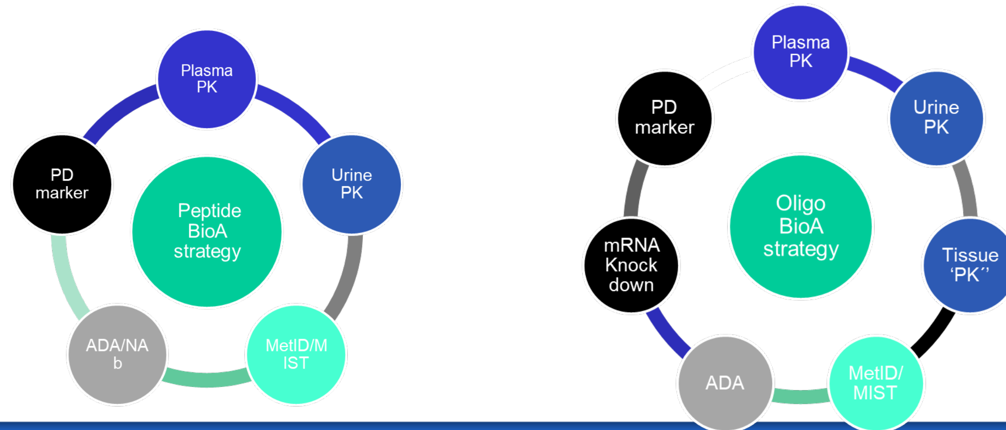
Q1 Historic data and Analytical strategy

- Do you consider in using historic data on platforms for setting the analytucal strategy?

- Do you engage with your stakeholders to discuss analytical strategy?

- Does the analytical strategy include all aspects of exposure, PD and efficasy?



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Q2. When have you been shared information about possible metabolites in a peptide/oligo drug project? Ie when was metabolite profiling done?

1. Never communicated from my stakeholders
2. Pre-phase 1 from *in vitro*/nonclinical studies on plasma/serum and target tissue
3. Pre-phase 2 from *in vitro studies* and using residual plasma/serum samples from phase 1
4. Pre-phase 3 from *in vitro studies* and using residual plasma/serum samples from phase 2/3

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Q3. Do you include possible metabolites in your bioanalytical validation for PK assay specificity/interference (Chrom/LBA/PCR)?

1. Yes
2. No never
3. Not until after metabolite profiling in clinical studies and only when metabolites are expected contributing to 25% expected *in vivo* activity
4. Never communicated from my stakeholders

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Q4 When will your company perform validation according to M10 on the metabolite profiling and metabolite quantification assays?

1. I do not know since my department only focus on PK exposure assays
2. Before sample analysis in nonclinical GLP studies
3. Before clinical sample analysis in phase 1 studies
4. Before clinical sample analysis in phase 2/3 studies
5. Before clinical sample analysis in phase 2/3 studies and only when metabolites are expected contributing to 25% expected in vivo activity

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Q5 PK tissue sample analysis

- 1. Do your company use surrogate matrix for oligo PK tissue exposure assays?
- 2. Do you company have experience with investigation oligo PK exposure in tissue patient samples?

Q6 Oligo questions on metabolites and tissue

1. Do you company have experience metabolite profiling from tissue patient samples?
2. Did your company in any given oligo project identify metabolites contributing to 25% expected in vivo activity?

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Q7 Bonus: Collect and bank strategies

1. Do you collect and bank in nonclinical studies for biotransformation investigations?
    1. Do you include ethical and 3R aspect?
2. Do you collect and bank in clinical studies for biotransformation investigations?
    1. Do you include details in clinical study protocol and informed consent?