in Vitro DDI Considerations for New Modalities

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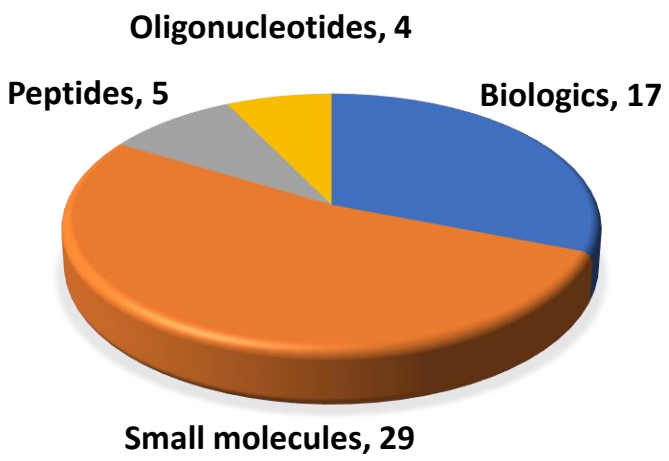
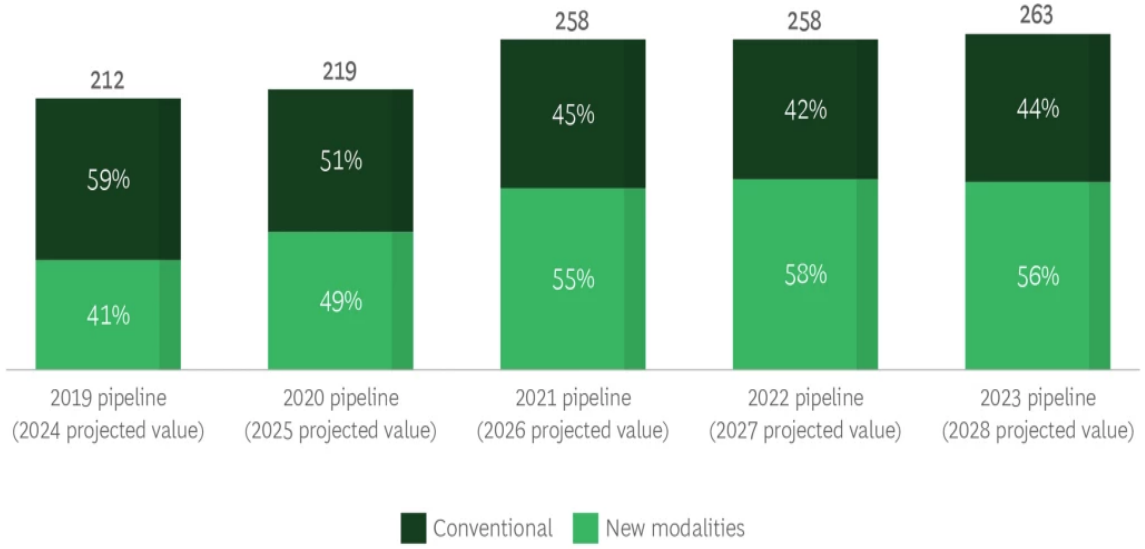
Emerging New Modality Drugs

- **Novel Chemical Modalities**
  - Therapeutic proteins
  - Oligonucleotides and peptides
  - Antibody drug conjugates
  - Protein degraders
  - Cell and gene therapy



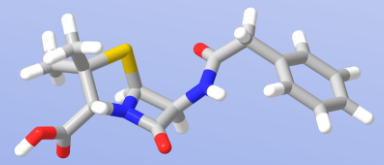
US FDA New Drug Approvals in 2023

Five-year forward projected pipeline value
($ Billion, Source: BCG 2023)

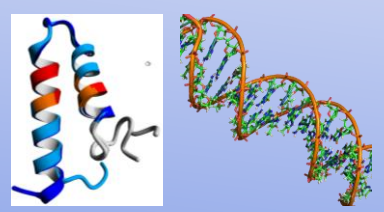


Beatriz G. de la Torre and Fernando Albericio, Molecules. 2024, 29(3): 585.
Advancing Health Through Innovation: New Drug Therapy Approvals 2023, FDA Center for Drug Evaluation and Research

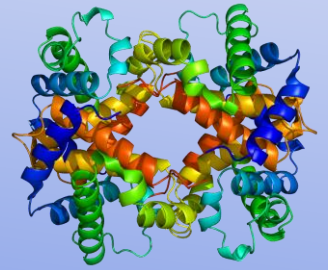
Regulatory Guidance on DDI Studies



- *In Vitro* Drug Interaction Studies — Cytochrome P450 Enzyme- and Transporter-Mediated Drug Interactions Guidance for Industry (FDA 2020)
- Clinical Drug Interaction Studies — Cytochrome P450 Enzyme- and Transporter-Mediated Drug Interactions Guidance for Industry (FDA 2020)
- Other regional guidance – EMA, 2012; PMDA 2018; NMPA 2021 (draft)
- M12 Drug Interaction Studies (ICH, May 2024)

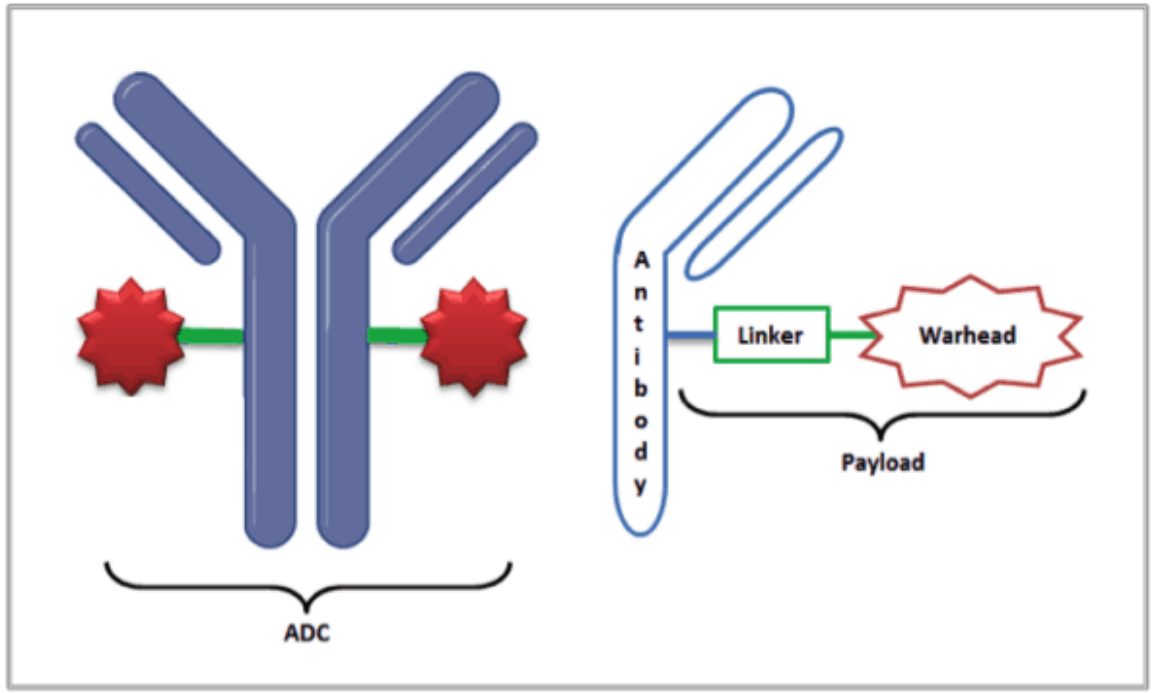


- Clinical Pharmacology Considerations for the Development of Oligonucleotide Therapeutics (FDA, draft 2022, final June 2024)
- *Clinical Pharmacology Considerations for Peptide Drug Products (FDA, draft Dec. 2023)*



- Drug-Drug Interaction Assessment for Therapeutic Proteins Guidance for Industry (FDA, June 2023)
- Clinical Pharmacology Considerations for Antibody-Drug Conjugates Guidance for Industry (FDA, draft 2022, final March 2024)

In Vitro DDI Guidance for ADC



From Sikorski, S.R.I.Q.R. The Clinical Landscape of Antibody-drug Conjugates. 2014

- **ADC development program should include *in vitro* DDI risk assessment of unconjugated payload and pharmacologically active metabolites**
  - Effect on CYP enzyme and transporter as perpetrator and victim
  - Characterizing systemic exposure of unconjugated payload is important for determining its DDI potential as an inhibitor and inducer
  - May need to conduct in *vivo* DDI as a victim based on *in vitro* result, payload toxicity and potential contribution of efficacy
  - Encourage use of PBPK modeling to assess the need for in vivo DDI
- **Antibody component: consider known or suspected mechanisms for DDI**

Clinical Pharmacology Considerations for Antibody-Drug Conjugates Guidance for Industry (FDA, March 2024)
Drug-Drug Interaction Assessment for Therapeutic Proteins Guidance for Industry (FDA, June 2023)

In Vitro DDI Guidance for Oligonucleotides

- **Oligonucleotides as substrates of CYP enzymes and transporters**
  - Oligonucleotides are not typically metabolized by CYP enzymes
  - They are not expected to be substrates of the 9 major transporters of concern
  - Evaluate potential as substrate of renal transporters if substantial renal active secretion (in draft but removed from final guidance)
- **Oligonucleotides as modulators of CYP enzymes and transporters**
  - Refer to *in vitro* DDI guidance as a general framework but an overall recommendation for specific types of oligonucleotide therapeutics (e.g., based on chemistry or delivery strategies) was not provided
  - Choose appropriate in vitro test systems
  - Based on current experience, oligonucleotide therapeutics do not modulate or minimally modulate CYP enzymes and drug transporters
  - Sponsor to provide adequate justification if *in vitro* assessments of oligonucleotide therapeutics as perpetrators of drug interactions are not conducted
- **Other possible mechanisms of DDI** – hybridization with CYP enzymes or transporter mRNA, interfering with heme metabolism or modulating cytokine expression

Clinical Pharmacology Considerations for the Development of Oligonucleotide Therapeutics (FDA, June 2024)

Oligonucleotide Safety Working Group (OSWG) Recommendations

- **Metabolism-mediated DDIs**
  - Substrate evaluation: not typically warranted, except conjugated oligonucleotide or novel components of LNP formulations.
  - Enzyme inhibition: DDI risk is generally low
    - May inhibit CYPs and UGTs via non-specific binding
    - Human hepatocytes is considered to be more relevant test system
  - CYP induction: recommends for all new platforms because of several positive induction results.
- **Transporters-mediated DDIs**
  - Substrate evaluation: specific transporters that are involved in tissue distribution and/or clearance pathway
    - Determine substrate potential of renal transporters for oligonucleotide with substantial renal excretion
    - Focus on hepatic efflux transporters for liver targeting oligonucleotides
    - Platform based approach is appropriate once substrate potential ruled out
  - Inhibition: recommend test a standard panel of transporters
    - Oligonucleotides, conjugates or LNP formulation components could inhibit transporters via non-specific binding
    - In addition to the standard panel, consider transporters that are specific to the distribution and/or clearance.
- **Timing: typically conducted before Phase 2, definitely required before starting Phase 3.**

OSWG Recommended Approaches to the Nonclinical Pharmacokinetic (ADME) Characterization of Therapeutic Oligonucleotides, Nuc. Acid Ther. 2023, 33, 287

In Vitro DDI Guidance for Peptides

- **Peptide drug product as substrates for CYP enzymes and transporters**
  - Generally not substrates of CYP enzymes and major drug transporters
  - In vitro experiments related to CYPs and transporters may be scientifically appropriate when hepatic and/or biliary excretion accounts for ≥20% of the overall elimination and/or the drug's target organ is the liver
  - In vitro experiments to evaluate a drug as a substrate of renal transporters may be appropriate for drugs with renal active secretion that accounts for ≥ 25% of systemic clearance of the drug and/or a drug that has renal toxicity
- **Peptide drug products as inhibitors and inducers of CYP enzymes and transporters**
  - In general, peptide drug products are not expected to significantly modulate CYP enzymes and drug transporters
  - Certain structural modifications may lead to modulation of CYP enzyme and transporters
  - For each development program, sponsors should provide their plans to evaluate the drug interaction liability of peptide drug products and refer to in vitro DDI guidance for small molecule

Clinical Pharmacology Considerations for Peptide Drug Products (FDA, draft Dec. 2023)

Review of In Vitro DDI for Peptides

- **Survey of companies involved in peptide drug development**
  - Unclear expectations from regulatory authorities on timing and value of *in vitro* DDI studies
  - Some general trends include:
    - Phenotyping was rarely performed
    - CYP inhibition and induction were most often performed
    - *In vitro* DDI studies tend to follow small molecule study design
    - Majority of studies reported negative DDI result
- **Review of approved peptide drugs**
  - CYP inhibition conducted on 26/31 drugs
  - CYP induction conducted on 22/31 drugs
  - Transporter interactions conducted on 24/31 drugs; more inhibition than substrate evaluation
  - The likelihood for DDI appears low for peptides > 2k Da, risk-based approach is reasonable for high MW peptide drugs
  - “Small molecule like” peptides and those contain nonpeptide motifs follow small molecule DDI

Sall et al, Clinical Pharmacology & Therapeutics, 2023, 113 (6), 1199-1216

Case Study 1

- **Test article is ADC, currently in phase 2/3 clinical trial for breast cancer**
- ***In vitro/in vivo* MetID studies identified linker-payload as the only circulating metabolite**
- **DDI assays conducted**
  - Direct and time-dependent CYP inhibition in HLM
    - No inhibition effect at up to 100 μM
  - CYP induction
    - No induction effect at physiologically relevant concentration
  - CYP phenotyping
    - HLM and recombinant enzymes
    - Not a substrate of the major CYP enzymes
  - Transporter substrate and inhibition
    - Tested on 9 transporters including MDR1, BCRP, MATE1, MATE2k, OAT1, OAT3, OATP1B1, OATP1B3, and OCT2;
    - Substrate of OATP1B1 and 1B3; Weak inhibition on OATP1B1/3, IC_{50} 0.8 – 2 μM
- **Conclusion: low *in vivo* DDI potential**

Case Study 2

- **Test article is ASO, MW ~ 4500**
- **Currently in phase 1 clinical trial for kidney disease.**
- **DDI assays recommended**
  - Direct and time-dependent CYP inhibition (conducted at another facility)
  - CYP induction
    - Tested at 6 concentrations 1 – 300 μM based on pre-study viability
    - Measured both mRNA and enzyme activity of CYP1A2, 2B6 and 3A4
    - Results - No induction observed, and concentration of TA in the incubation samples remains close to nominal
  - Transporter inhibition
    - Tested on 13 transporters including MDR1, BCRP, MRP3, MRP4, BSEP, MATE1, MATE2k, OAT1, OAT3, OATP1B1, OATP1B3, OCT2 and NTCP
    - Initial screening done at 10 and 100 μM, only MRP4 showed >50% at 100 μM
    - Followed up to determine MRP4 IC50 (73 μM)
- **Conclusion: low *in vivo* DDI potential**

Case Study 3

- **Test article is spherical nucleic acid of two ASO, MW ca. 7000 and 8000**
- **DDI assays conducted**
  - Direct and time-dependent CYP inhibition
    - Strong inhibition on 5 CYP isoforms in HLM (IC_{50}<1.5 μM)
    - Separately evaluated ASO1 and ASO2, also strong inhibition
    - Evaluated 4 select CYP isoforms in hepatocyte, much less inhibition effect (IC_{50} ~100 μM)
  - CYP induction
    - Tested at 3 concentrations 1, 10 and 100 μM based pre-study viability
    - Dose dependent increase of CYP1A2, 2B6 and 3A4 mRNA level
    - Dose dependent increase of CYP1A2 and 3A4 enzyme activity, but not 2B6 activity
- **Conclusion: potential high *in vivo* DDI potential if therapeutic concentration falls in the range tested**

Case Study 4

- **Test article is peptide, MW ca. 4800**
- **DDI assays conducted**
  - Direct and time-dependent CYP inhibition in HLM
    - Tested concentrations up to 100 μM
    - No inhibition observed
  - Transporter inhibition
    - Tested at 2 concentrations for MDR1 and BCRP, no inhibition at 10 μM
    - No inhibition on OAT1, OAT3, OATP1B1, OATP1B3, OCT2 at up to 30 μM
    - Weak inhibition of MATE1 and MATE2-k; IC_{50} 11 -30 μM
- **Conclusion: low *in vivo* DDI potential**

Summary

- *In Vitro* DDI evaluation for new modality molecules are expected by regulatory agency
- Which assays to conduct is case-by-case, and may change as science and regulatory guidelines continue to evolve
- Important factors to consider:
  - The property of the drug molecules and route of administration
  - Therapeutic concentration or anticipated systemic exposure
  - Understand clearance mechanism and excretion pathway will determine what types to *in vitro* DDI assays to conduct
  - When to conduct *in vitro* DDI is determined by overall program strategy
- *In Vitro* DDI evaluation at IND stage provides key information for planning clinical development strategy to mitigate potential clinical DDI risks
- Working with experienced CRO may help to avoid unnecessary work/time/$$ and generating potentially misleading data/results

Back up slides

DDI Summary Data of Oligonucleotide Drugs

- Reviewed DDI data on 14 IND and 7 NDA drugs (2012-2018)
- The DDI potential of oligonucleotide therapies was evaluated primarily via *in vitro* studies.
- Oligonucleotides have a limited potential to be victims of CYP-mediated interactions
- Oligonucleotides can act as a perpetrator (4/52 positive finding)
- CYP-mediated DDIs may be related to mechanisms of interaction (e.g., involvement in the heme-synthesis pathway or immune-related upregulation)
- The assessment of DDI potential was largely consistent with the systematic and risk-based recommendations of current DDI-relevant guidance.

Table 4 Summary of CYP-mediated and transporter-mediated DDI evaluation by test system

	DDI evaluation type (*n*)	Evaluation system (*n*)
CYP (*n* = 40)	CYP induction (15)	Human hepatocytes (13), other microsomes (1), not specified (1)
	CYP inhibition (19)	Human hepatocytes (5), human microsomes (11) other microsomes (1) multispecies S9 (1), and not specified (1)
	CYP substrate (6)	Human microsomes (3), multispecies microsomes (2), multispecies S9 (1)
Transporter (*n* = 12)	Transporter substrate (2)	Mammalian cell lines (1), Sf9 vesicles (1)
	Transporter inhibition (2)	Mammalian cell lines and Sf9 vesicles (1)
	Transporter substrate and inhibition (8)	Mammalian cell lines (2), mammalian cell lines and Sf9 vesicles (1), not specified (5)

Multispecies microsome = microsomes from animal species including human microsome, other microsome = non-human species microsome. DDI, drug-drug interaction.

Rogers et al, Clinical and Translational Science, 2021, 14, 468-475

Case Study 5

- **Test article is peptide, MW ca. 2100**
- **DDI assays conducted**
  - Direct and time-dependent CYP inhibition in HLM
    - Inhibited all CYP isoforms in HLM with IC50 of 9 -30 μM
    - No time dependent inhibition in HLM
  - Follow up CYP inhibition in human hepatocytes
    - Selected the 3 CYP isoforms on which TA demonstrated most potent inhibition effect
    - <25% inhibition observed at the highest test concentration
- **Conclusion: Low *in vivo* CYP inhibition potential**