EBF

Spring Focus Workshop

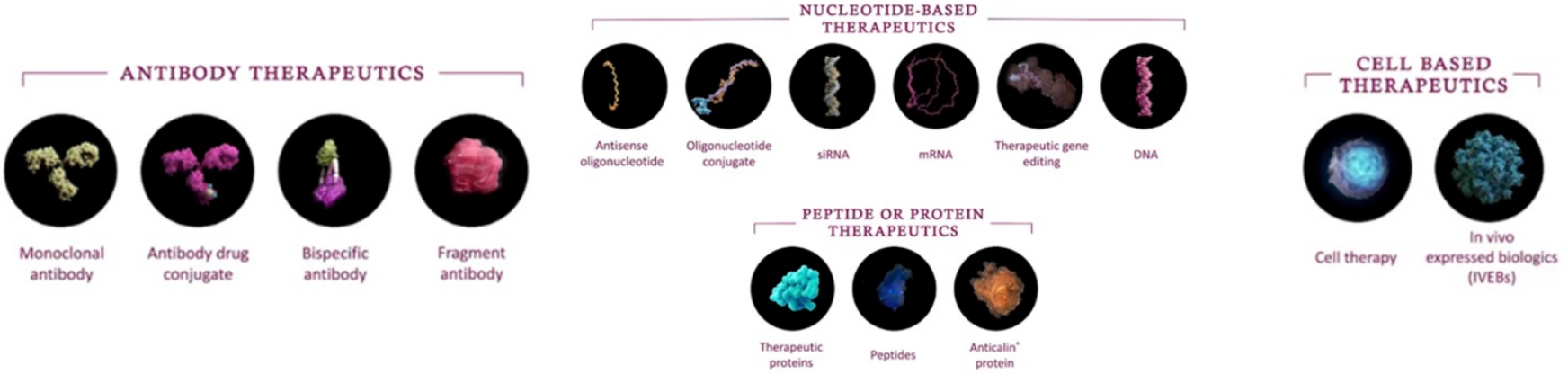
EBF Survey Feedback – Immunogenicity Related to Oligonucleotide and Peptide Drugs

Jo Goodman, On behalf of EBF

8-9 June 2023 – Malaga, Spain

Immunogenicity landscape is evolving as drug portfolios shift into new(er) modalities

UNWANTED
Immunogenicity



With modalities such as oligos and peptides we face new challenges and questions

- **How do we classify these therapeutics?**
  - May be chemically synthesized and have conjugation
  - Usually much smaller than traditional biologics
  - What is a peptide and what is a protein?
  - Small and large molecule considerations (metabolism, immunogenicity)

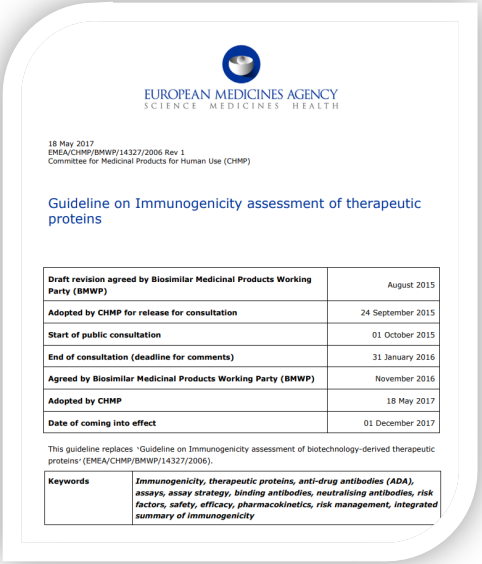
- **Should we follow traditional approaches for immunogenicity assessment and analysis?**
  - Risk assessment (molecule, patient, formulation, conjugation, dosing, route, likelihood and impact of consequence, $T_{1/2}$ etc.)
  - Often predictive PK for oligos
  - Assays and/or platforms, positive control generation, labelling
  - Size may mean that less likely to produce an immune response
  - Lower molecular weight – may require larger molar concentration of ADA to observe an effect
  - What is the same and what is different?

With modalities such as oligos and peptides we face new challenges and questions

- **Perspectives may differ**
  - One size does not fit all
  - Agency expectations can differ
    - Often need to demonstrate evidence of absence (rather than absence of evidence)
    - Regulatory experiences for similar molecules (e.g. incretin mimetics) have looked very different
  - So can we draw from experience with other molecules in the same class?
  - Risk can be viewed differently
  - Expectations for further characterization and/or neutralizing antibody assays (nAb)
  - But ultimately it should be driven by scientific and data approaches

So we look towards regulatory guidance and industry perspectives/experience

EMA 2017 – “proteins and polypeptides”



EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

18 May 2017
EMEA/CHMP/BMWP/14327/2006 Rev 1
Committee for Medicinal Products for Human Use (CHMP)

Guideline on Immunogenicity assessment of therapeutic proteins

Draft revision agreed by Biosimilar Medicinal Products Working Party (BMWP)	August 2015
Adopted by CHMP for release for consultation	24 September 2015
Start of public consultation	01 October 2015
End of consultation (deadline for comments)	31 January 2016
Agreed by Biosimilar Medicinal Products Working Party (BMWP)	November 2016
Adopted by CHMP	18 May 2017
Date of coming into effect	01 December 2017

This guideline replaces 'Guideline on Immunogenicity assessment of biotechnology-derived therapeutic proteins' (EMEA/CHMP/BMWP/14327/2006).

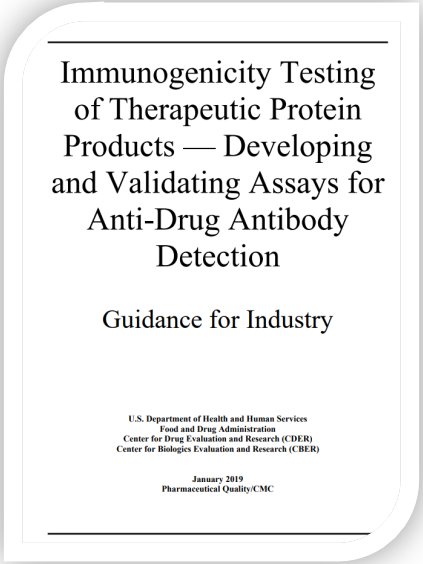
Keywords	***Immunogenicity, therapeutic proteins, anti-drug antibodies (ADA), assays, assay strategy, binding antibodies, neutralising antibodies, risk factors, safety, efficacy, pharmacokinetics, risk management, integrated summary of immunogenicity***

The general principles adopted and explained in this document mainly apply to the development of an unwanted immune response against a purified therapeutic protein in patients and to a systematic evaluation of it. The guideline applies to proteins and polypeptides, their derivatives, and products of which they are components, e.g. conjugates. These proteins and polypeptides are mainly produced by recombinant or non-recombinant expression systems. Throughout this guideline, the term “therapeutic protein” is used.

This guideline does not apply to coagulation factors, vaccines, or heterogenous immunoglobulin preparations, such as human immunoglobulins purified from plasma.

- NMPA 2021 guideline on immunogenicity states peptides are in scope but no mention of oilgonucleutides

FDA 2019 – “case by case basis” for some peptides and oligonucleotides



Immunogenicity Testing of Therapeutic Protein Products — Developing and Validating Assays for Anti-Drug Antibody Detection

Guidance for Industry

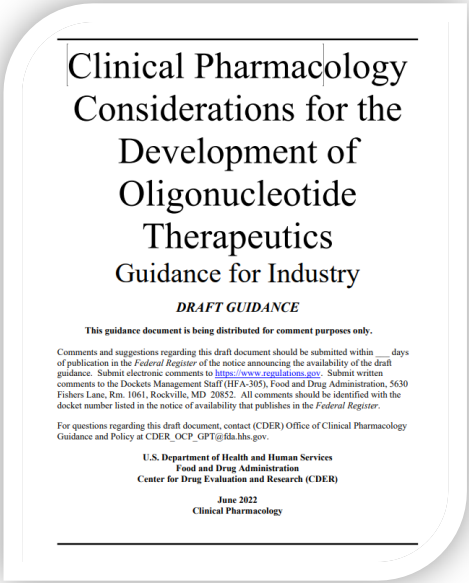
U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)
Center for Biologics Evaluation and Research (CBER)

January 2019
Pharmaceutical Quality/CMC

This guidance provides recommendations to facilitate industry’s development and validation of assays for assessment of the immunogenicity of therapeutic protein products during clinical trials. Specifically, this document includes guidance regarding the development and validation of screening assays, confirmatory assays, titration assays, and neutralization assays.[2,3] For the

drug antibodies (ADAs).[4] This guidance may also apply to some peptides, oligonucleotides, and combination products on a case-by-case basis.[5]

FDA 2022 – Focus on oligos



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

An unwanted immune response to an oligonucleotide therapeutic can be generated to the carrier, backbone, oligonucleotide sequence, or any novel epitopes created from the whole drug (carrier plus oligonucleotide). The development of oligonucleotide therapeutics is rapidly evolving, and new chemistries, modifications etc. can significantly affect the immunogenicity risk and clinical immunogenicity assessment of a particular product.

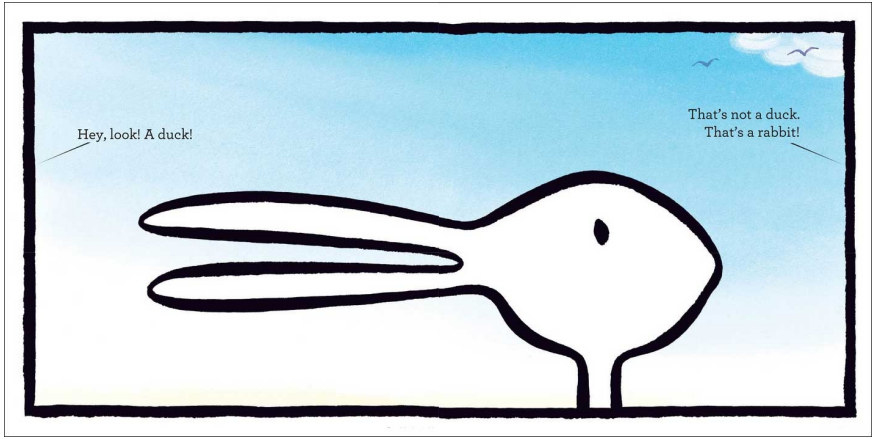
The clinical immunogenicity assessment for an oligonucleotide therapeutic should follow a risk-based approach and be included in a product-specific immunogenicity risk assessment as outlined in the FDA guidance entitled *Immunogenicity Assessment for Therapeutic Protein Products* (August 2014). Some considerations when determining the immunogenicity risk of an oligonucleotide therapeutic include, but are not limited to:

- **Product factors**: base sequence, base modification, backbone modification, strandedness, purity, modified nucleotides, secondary and tertiary structures, and carrier components (e.g., PEGylated lipid nanoparticles)
- **Pharmacology of the product**: mechanism of action, cell/tissue target, expression profile, route of administration, dosing regimen (chronic versus acute)
- **Patient characteristics**: immune activation status of the population (e.g., auto-immune or inflammatory conditions), concomitant medications (ability to influence the incidence or clinical impact of anti-drug antibodies (ADAs) (e.g., immunosuppressants such as chemotherapy)

In certain circumstances, the FDA could also recommend assessing for nucleotide sequence-specific antibodies and/or bioactivity (e.g., neutralization, enhancement). Any recommendations for these assays will be informed by clinical concerns, such as oligonucleotide sequence cross-reactivity, novel structures, or modifications and should be discussed with the relevant review Division on a case-by-case basis.

[4] See the FDA guidance *Immunogenicity Testing of Therapeutic Protein Products — Developing and Validating Assays for Anti-Drug Antibody Detection* (February 2019).

Engaging the EBF community

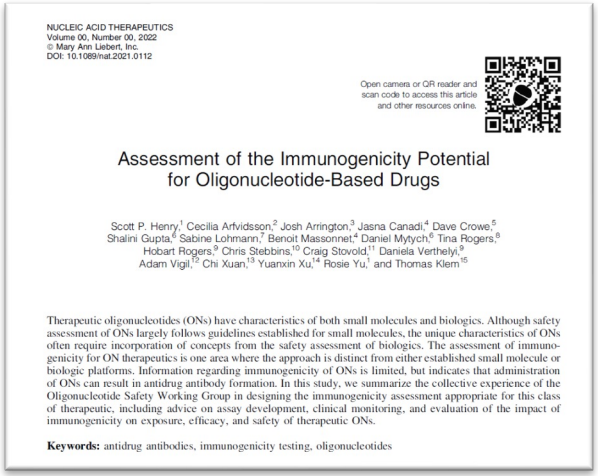


- **Survey consisted of 2 parts:**
  - Part 1: Oligos (n=8)
  - Part 2: Peptides (n=9)

Do we see things the same way?

Part 1: Oligonucleotide Immunogenicity

- Focussed on key recommendations from white papers



NUCLEIC ACID THERAPEUTICS
Volume 00, Number 00, 2022
© Mary Ann Liebert, Inc.
DOI: 10.1089/nat.2021.0112

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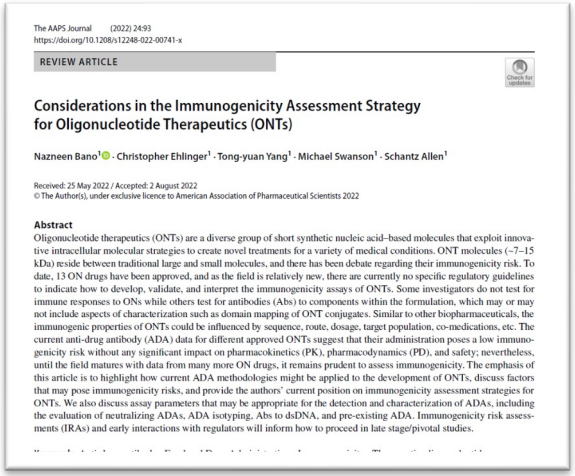
Assessment of the Immunogenicity Potential for Oligonucleotide-Based Drugs

Scott P. Henry,[1] Cecilia Arfvidsson,[2] Josh Arrington,[3] Jasna Canadi,[4] Dave Crowe,[5] Shalini Gupta,[6] Sabine Lohmann,[7] Benoit Massonnet,[4] Daniel Mytych,[6] Tina Rogers,[8] Hobart Rogers,[9] Chris Stebbins,[10] Craig Stovold,[11] Daniela Verthelyi,[9] Adam Vigil,[12] Chi Xuan,[13] Yuanxin Xu,[14] Rosie Yu,[1] and Thomas Klem[15]

Therapeutic oligonucleotides (ONs) have characteristics of both small molecules and biologics. Although safety assessment of ONs largely follows guidelines established for small molecules, the unique characteristics of ONs often require incorporation of concepts from the safety assessment of biologics. The assessment of immunogenicity for ON therapeutics is one area where the approach is distinct from either established small molecule or biologic platforms. Information regarding immunogenicity of ONs is limited, but indicates that administration of ONs can result in antidrug antibody formation. In this study, we summarize the collective experience of the Oligonucleotide Safety Working Group in designing the immunogenicity assessment appropriate for this class of therapeutic, including advice on assay development, clinical monitoring, and evaluation of the impact of immunogenicity on exposure, efficacy, and safety of therapeutic ONs.

Keywords: antidrug antibodies, immunogenicity testing, oligonucleotides



The AAPS Journal (2022) 24:93
https://doi.org/10.1208/s12248-022-00741-x

REVIEW ARTICLE

Considerations in the Immunogenicity Assessment Strategy for Oligonucleotide Therapeutics (ONTs)

Nazneen Bano[1] · Christopher Ehlinger[1] · Tong-yuan Yang[1] · Michael Swanson[1] · Schantz Allen[1]

Received: 25 May 2022 / Accepted: 2 August 2022
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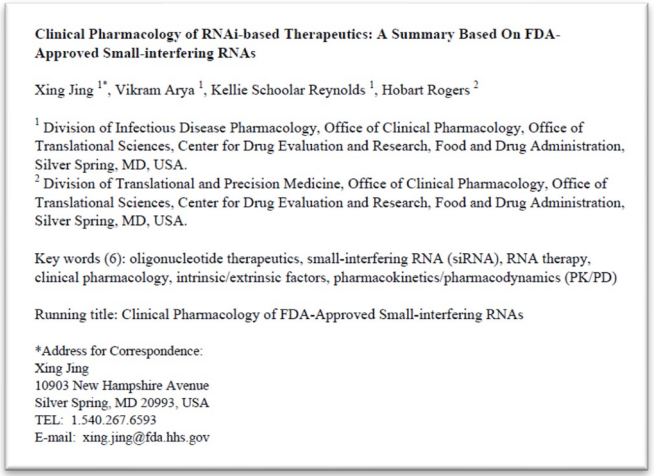
Abstract
Oligonucleotide therapeutics (ONTs) are a diverse group of short synthetic nucleic acid–based molecules that exploit innovative intracellular molecular strategies to create novel treatments for a variety of medical conditions. ONT molecules (~7–15 kDa) reside between traditional large and small molecules, and there has been debate regarding their immunogenicity risk. To date, 13 ON drugs have been approved, and as the field is relatively new, there are currently no specific regulatory guidelines to indicate how to develop, validate, and interpret the immunogenicity assays of ONTs. Some investigators do not test for immune responses to ONs while others test for antibodies (Abs) to components within the formulation, which may or may not include aspects of characterization such as domain mapping of ONT conjugates. Similar to other biopharmaceuticals, the immunogenic properties of ONTs could be influenced by sequence, route, dosage, target population, co-medications, etc. The current anti-drug antibody (ADA) data for different approved ONTs suggest that their administration poses a low immunogenicity risk without any significant impact on pharmacokinetics (PK), pharmacodynamics (PD), and safety; nevertheless, until the field matures with data from many more ON drugs, it remains prudent to assess immunogenicity. The emphasis of this article is to highlight how current ADA methodologies might be applied to the development of ONTs, discuss factors that may pose immunogenicity risks, and provide the authors' current position on immunogenicity assessment strategies for ONTs. We also discuss assay parameters that may be appropriate for the detection and characterization of ADAs, including the evaluation of neutralizing ADAs, ADA isotyping, Abs to dsDNA, and pre-existing ADA. Immunogenicity risk assessments (IRAs) and early interactions with regulators will inform how to proceed in late stage/pivotal studies.



Clinical Pharmacology of RNAi-based Therapeutics: A Summary Based On FDA-Approved Small-interfering RNAs

Xing Jing [1*], Vikram Arya [1], Kellie Schoolar Reynolds [1], Hobart Rogers [2]

[1] Division of Infectious Disease Pharmacology, Office of Clinical Pharmacology, Office of Translational Sciences, Center for Drug Evaluation and Research, Food and Drug Administration, Silver Spring, MD, USA.
[2] Division of Translational and Precision Medicine, Office of Clinical Pharmacology, Office of Translational Sciences, Center for Drug Evaluation and Research, Food and Drug Administration, Silver Spring, MD, USA.

Key words (6): oligonucleotide therapeutics, small-interfering RNA (siRNA), RNA therapy, clinical pharmacology, intrinsic/extrinsic factors, pharmacokinetics/pharmacodynamics (PK/PD)

Running title: Clinical Pharmacology of FDA-Approved Small-interfering RNAs

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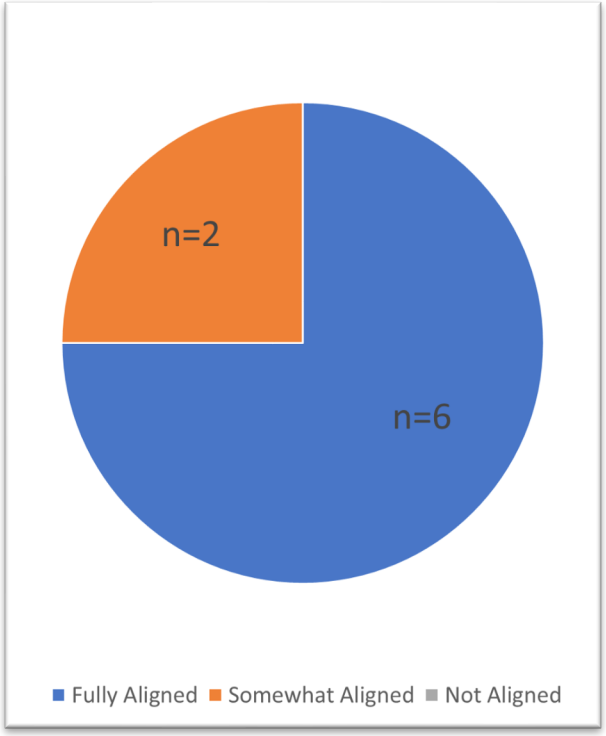
1. Fully aligned
2. Somewhat aligned
3. Not aligned

Q1. Risk assessment

- "A risk assessment is **performed early in program development**, taking some of the **unique aspects of oligonucleotides** that need to be evaluated (such as chemical composition, mechanism of action, innate immune response activation) in to consideration and **revise as new information becomes available**"

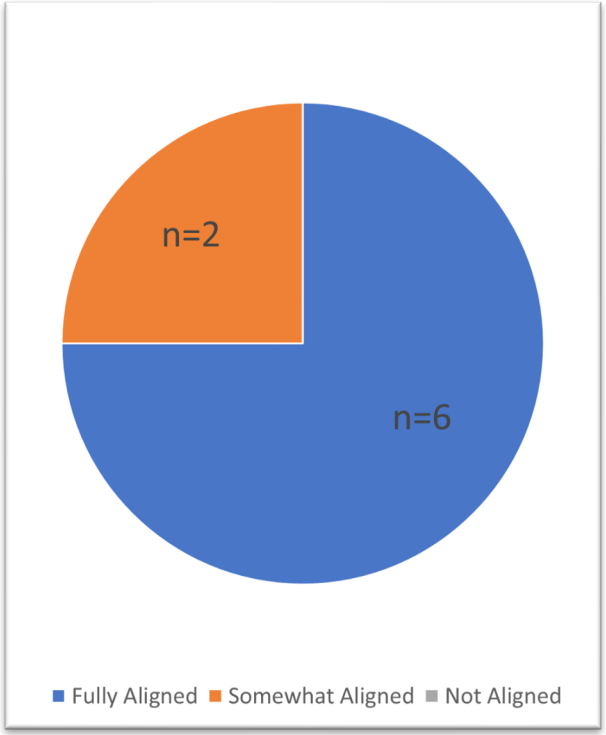
- *Comments:*
  - *Sponsor driven*



Q2. Timing of Assessments

- "**Timing** of the immunogenicity assessment is **driven by the observation of PK parameters, activity, and safety, as well as by established class experience, provided other risk factors are unchanged** (patient population, route, etc.), i.e. **a 'collect and bank' strategy is applied during the early development and analysis only triggered in case of an atypical PK/PD or safety event** "

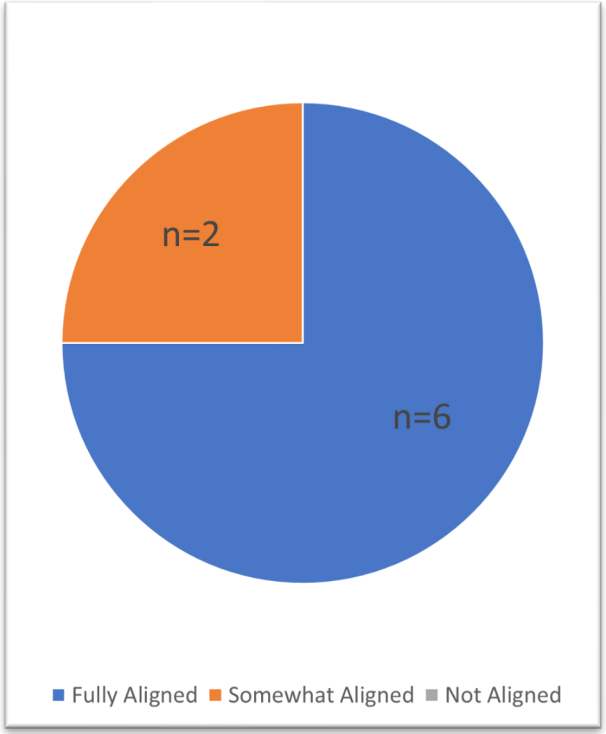
- *Comments:*
  - *Nonclinical yes but not for clinical (would test)*
  - *PK of oligos is short (therefore PK may not be a good criteria whether to test for ADA)*



Q3. Regulatory Guidance

- "**Current guidance** for immunogenicity method validation **is applied**. PC **anti-Oligonucleotide antibodies are likely to be class specific, not sequence specific**"

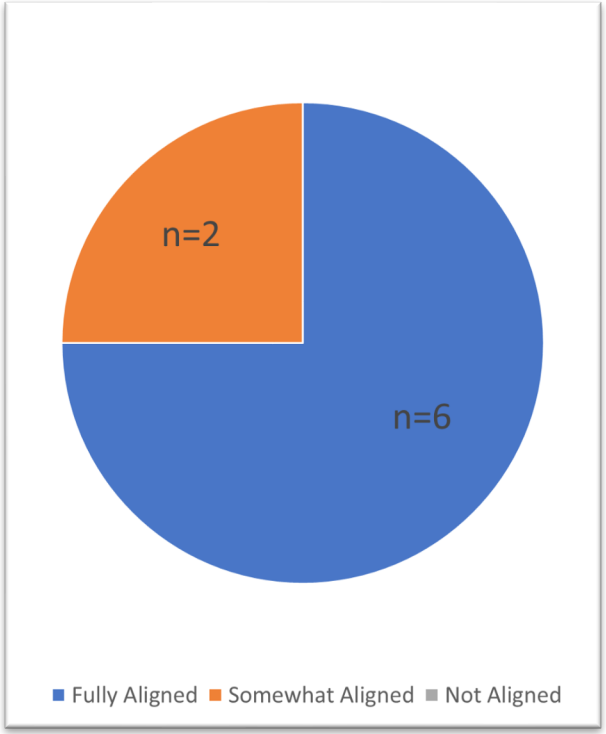
- *Comments:*
  - *Some of our pipeline molecules have PCs that are sequence specific while others do not*
  - *Not convinced that PCs are class specific*
  - *Class specific PC generation may not be possible in all cases*
  - *Have used PCs raised against other closely related ASOs*



Q4. Neutralising Antibody Assays (nAb)

- "**Neutralising antibody assays** for oligonucleotide therapeutics **are not generally considered**, instead **other ways of addressing potential changes to PK/PD are considered appropriate**"

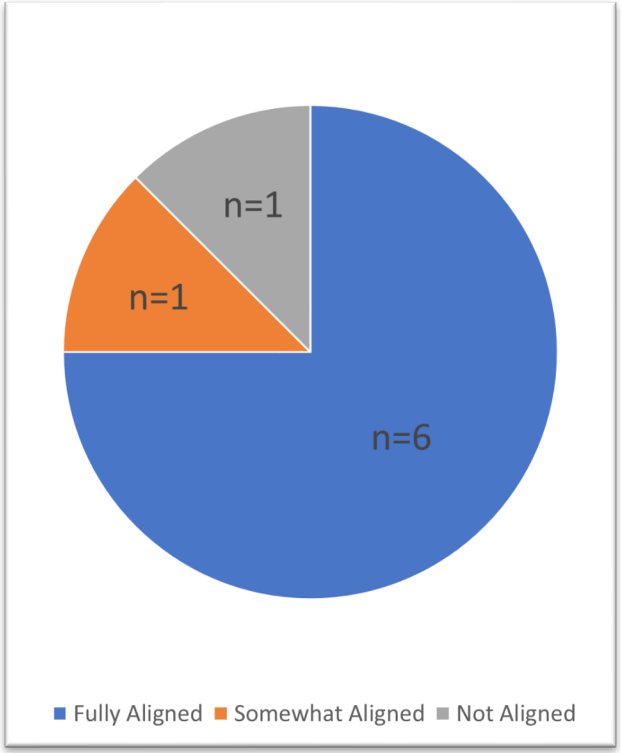
- *Comments:*
  - *If low immunogenicity risk then nAb assays and other approaches are justified*
  - *This approach is not specific to oligos but can also be used for other biotherapeutics*
  - *Have not performed a nAb for an oligo*



Q5. Nonclinical Studies

- "**Nonclinical immunogenicity studies** for oligonucleotide therapeutics **are not always required**"

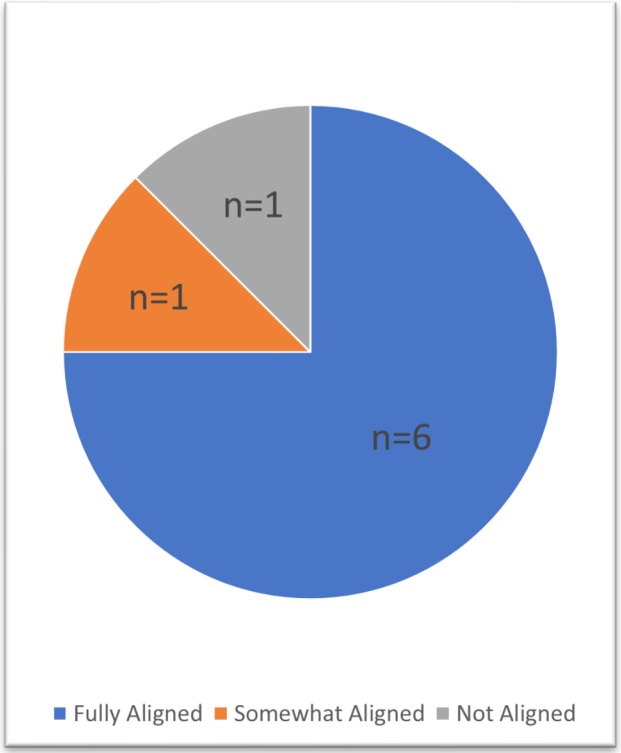
- *Comments:*
  - *Not performed for nonclinical studies*
  - *Driven by PK data only and only assessed in NHP studies*
  - *Don't think one can generalize this for oligos*



Q6. Clinical Studies

- "Immunogenicity samples are **always collected in clinical trials**. The timing and frequency of sample acquisition may be influenced by the nature of the study (eg, number of doses, early or pivotal trial), while **sample testing during the early clinical development is determined by clinical findings and risk assessment**. If the class of oligo is considered **low risk ADA samples will only be collected and banked**"

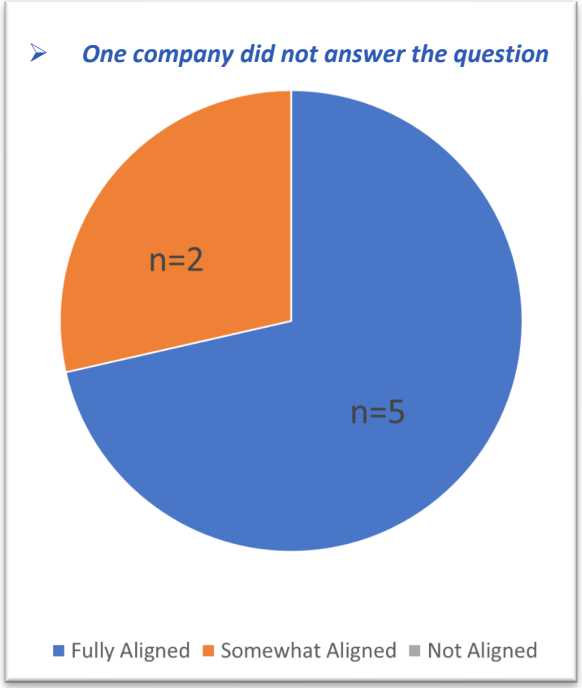
- *Comments:*
  - *Always collected but only tested following a clinical finding or impact to PK*
  - *We always take samples and analyze them*



Q7. Increases in Protein Expression or Alteration

- "If the Oligonucleotide **results in increases in protein expression or alteration of a protein, an assessment of the immunogenicity of the resulting protein may be needed**"

- *Comments:*
  - *Oligo peptide molecules in our portfolio have not had this effect, however other modalities have had this impact and immunogenicity to the resulting protein was assessed*
  - *Would consider if required, but haven't had to do this thus far*



Part 2: Peptide Immunogenicity

- **4 questions focussed on:**
  - Regulatory guidance
  - Nonclinical immunogenicity
    - Is the community applying https://pubmed.ncbi.nlm.nih.gov/33729007/
  - “Collect and Bank” strategy
  - Regulatory expectations (e.g. FDA 2019)

or negative), depending on immunogenicity risk assessment. Additional characterization assays, including isotyping, epitope mapping, and assessing cross-reactivity (for example, to endogenous counterparts or to other products), may be useful.

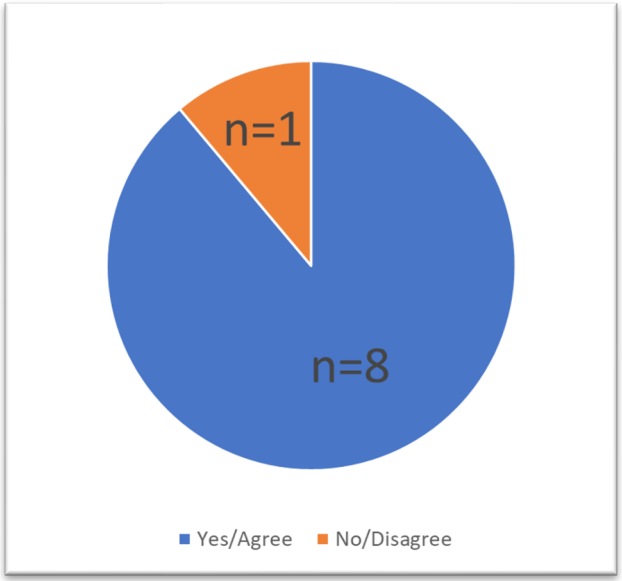
half-lives after last exposure. When there is a high risk of serious consequences from ADAs, sponsors should plan to collect samples from subjects until ADAs return to baseline levels.

1. Yes/Agree
2. No/Disagree

Q1. Regulatory Guidance

- "We utilize **current regulatory guidelines** for peptide therapeutics for immunogenicity assessment and immunogenicity method validation **for peptide therapeutics as we would for biologics**"

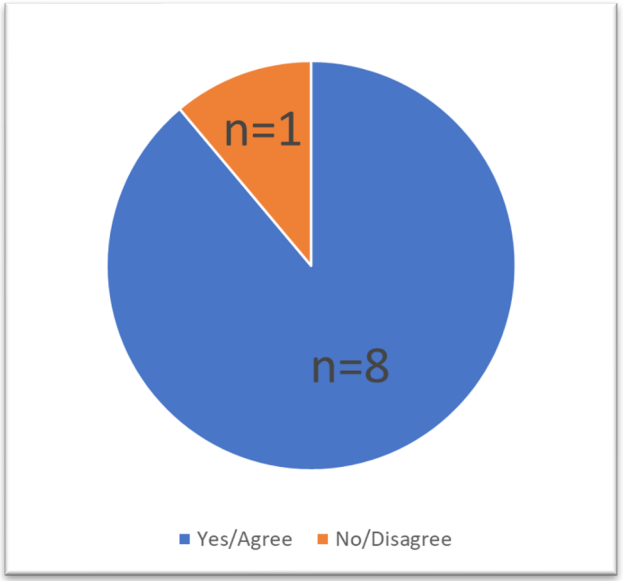
- *Comments:*
  - *Classification of a peptide >12-15 amino acids*
  - *Case by case approach taken*
  - *For smaller peptides, strategy is based on evaluation of in vitro immunotox data and ex vivo PK/PD safety findings in preclinical and clinical studies*



Q2. Nonclinical Assessments

- "**Nonclinical immunogenicity studies** for peptide therapeutics **are not always required**"

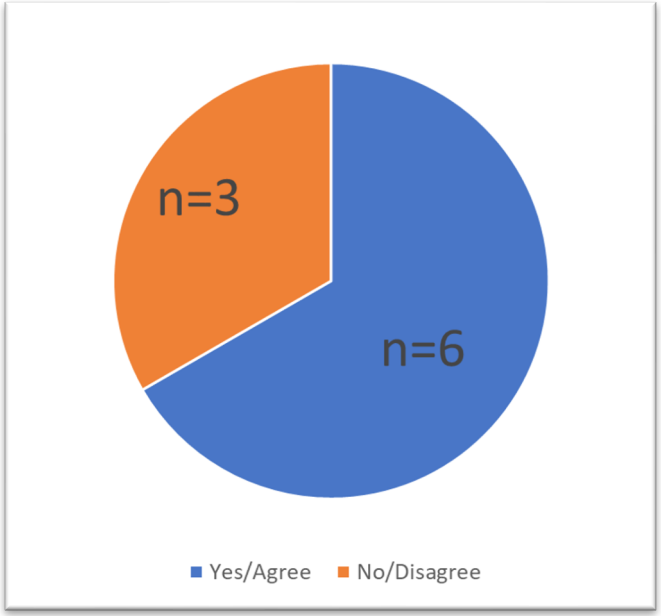
- *Comments:*
  - *Prove animals are exposed to drug, therefore we need to test if reduced PK and/or PD. 0.1% FPR used.*
  - *Driven by PK/PD, 0.1% FPR, no confirmatory but the approach is not specific to peptides*



Q3. ‘Collect and Bank’

- “A **‘collect and bank’ strategy can be applied during early development** (nonclinical and early stage clinical studies) and **analysis only triggered in case of atypical PK/PD or safety event**”

- *Comments:*
  - *Agree for preclinical but clinical would depend on risk assessment/prediction*
  - *Needs to be assessed in all clinical trials*
  - *Case by case but what will you learn from a single dose study??? – just shows that the assay is working and often tolerance is not broken until after end of study (consider COU and resources)*
  - *This CRO recommends ADA assessment especially in early stage studies*

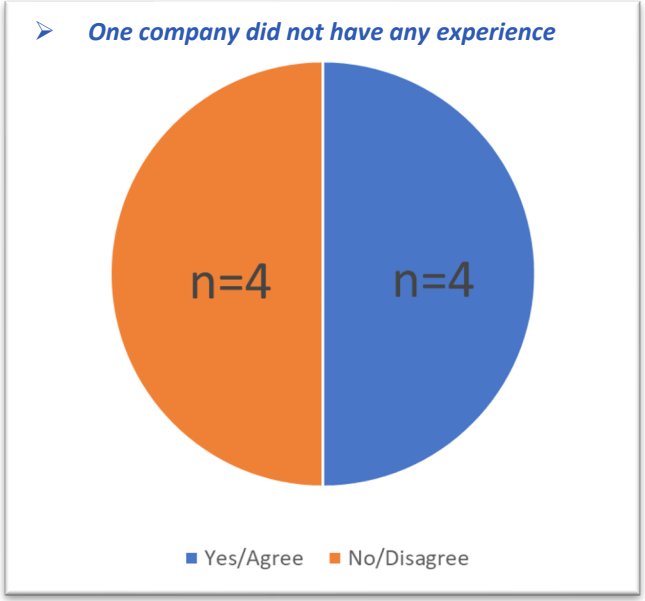


Q4. Regulatory Expectations

- "**Regulatory expectations** for peptide therapeutics **can be high even in the absence of clinical consequence**"

- *Comments:*
  - *Several companies had no recent peptide experience*
  - *Requirements are excessive in some cases but many aspects should remain such as follow up of ADA*
  - *Often lack statistical power in early studies to convince the agencies that immunogenicity is not a concern. Many peptides are similar to endogenous molecules; cross-neutralization can be high risk (have to prove otherwise)*
  - *Too much focus on nAbs during the treatment phase and leads to assays with poor sensitivity/DT. Prefer to correlate ADA titres to PD. No value in follow up to baseline when there are no clinical signs. ADA may continue to be present for years.*
  - *Regulatory expectations are high but extensive characterisation is not always necessary*
  - *Endogenous counterpart or cross-reactivity – full evaluation should be applied*
  - *Follow up of ADA looks at incidence and further safety endpoints are not usually collected – what value does this bring to the patient*

- ***One company did not have any experience***



Summary

- Oligos and peptides often require elements of both small and large molecule approaches
- Despite their smaller size, short(er) half life and often being chemically synthesized, immunogenicity assessment will form part of the development package
  - *Biologics guidance provides a starting point*
- Immunogenicity assessment for these molecules come with challenges
  - *Generation and selection of PC*
  - *Some of the in silico or in vitro tools are not available for oligos*
  - *Labelling of the molecules*
  - *Assays, sensitivity and drug tolerance*
- As with all modalities, specific risk assessments should be conducted early and refined as further data become available
- Continue the discussion in the round tables
  - *What should be the same and what should be different from biotherapeutics when defining immunogenicity strategy for oligos and/or peptides?*

Acknowledgements

- Survey responders
- EBF community and EBF teams
- EBF SC and Chair

Contact Information

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European Bioanalysis Forum vzw

www.e-b-f.eu