Synthon

Generic Oligonucleotides

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Oligonucleotides - introduction

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- Chemically synthesized strands of nucleic acids
- Short, ~20 nucleotides long
- Act through complementary base-pairing with target RNA, influencing transcription, splicing, or translation and modulating gene expression or protein production

- Two main types:
  - ASOs:
    Single-stranded antisense oligonucleotides
  - siRNAs:
    Double-stranded RNA:
    Antisense strand = pharmacologically active moiety
    Sense strand = drug delivery device

siRNA and ASO - mode of action

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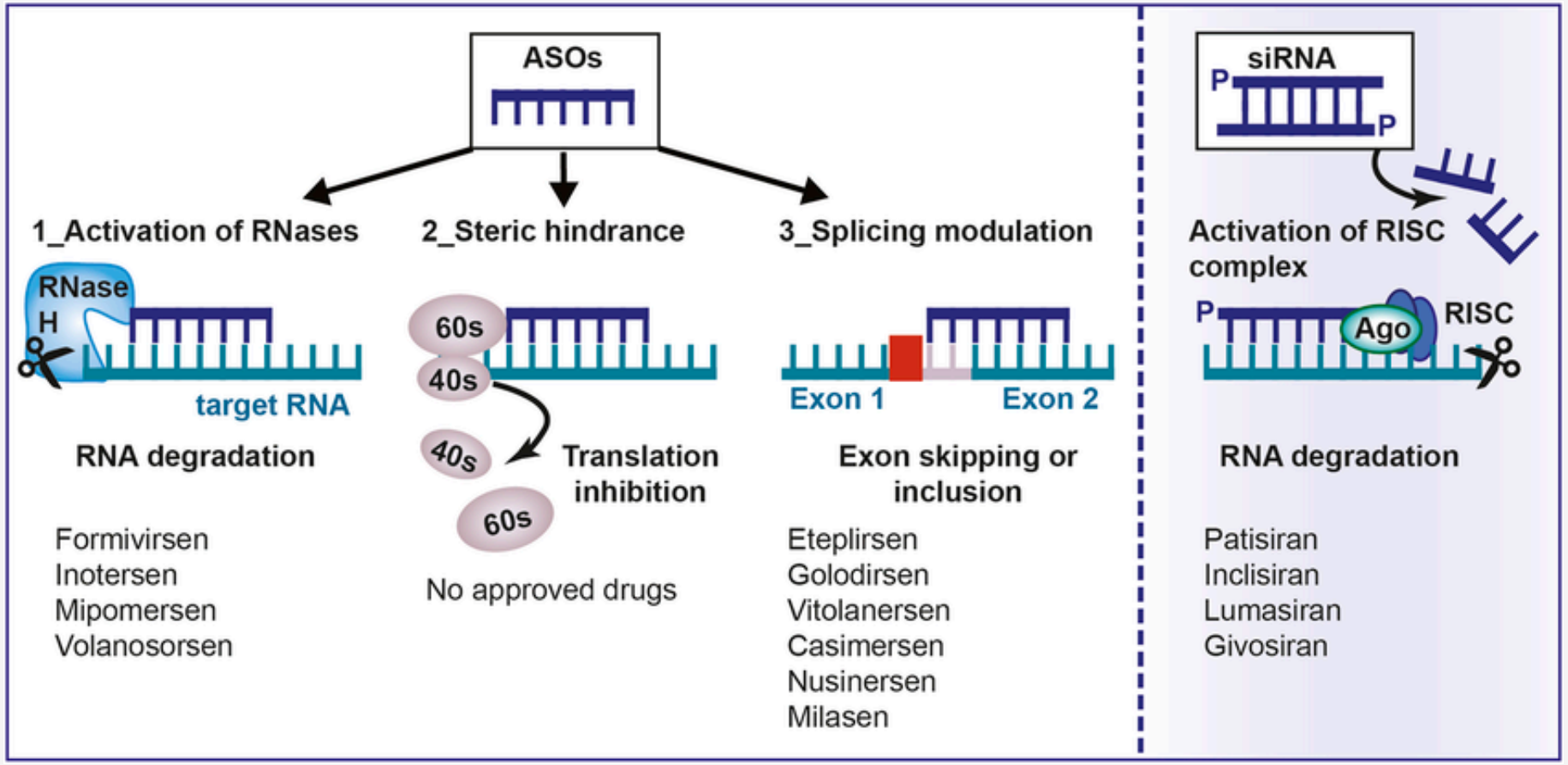


Collotta et al, Frontiers in Pharmacology, 2023

Oligonucleotides - modifications

Modifications needed to make them suitable therapeutic medicines:

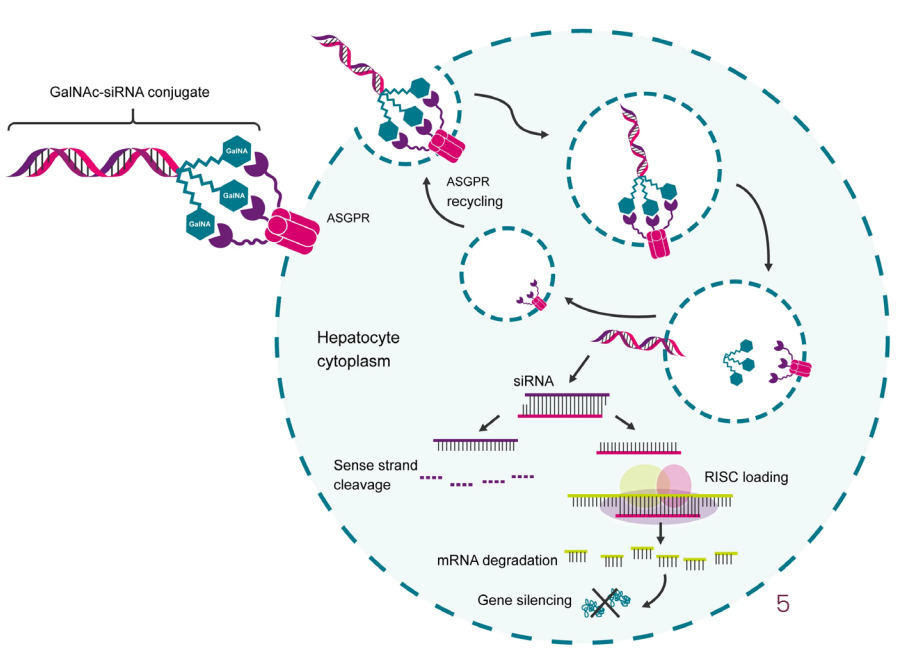
- Enhance stability
- Enhance binding affinity
- Enhance safety
- Enhance (specific) delivery

Types of modifications:

- Conjugates
- Ribose sugar modification
- Backbone modifications

Conjugate modifications

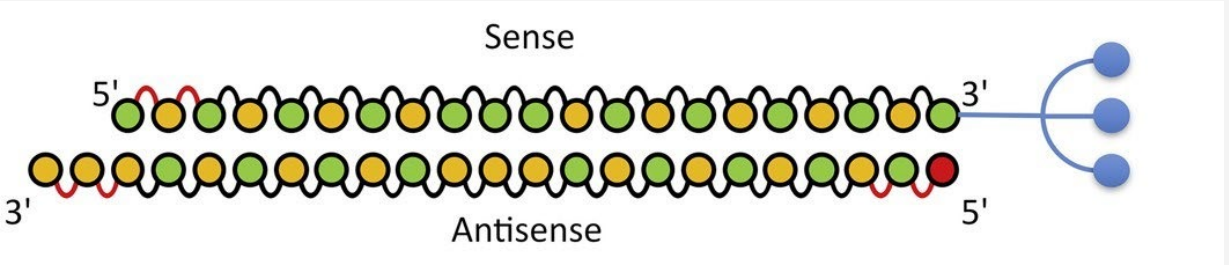
- Enhance delivery potential by targeting, promoting intracellular uptake, reduction of clearance
- Lipids, peptides, aptamers and sugars
- Best known: GalNac-ligands: carbohydrate moiety
- Binds to ASGR1, highly expressed in hepatocytes



Conjugate modifications

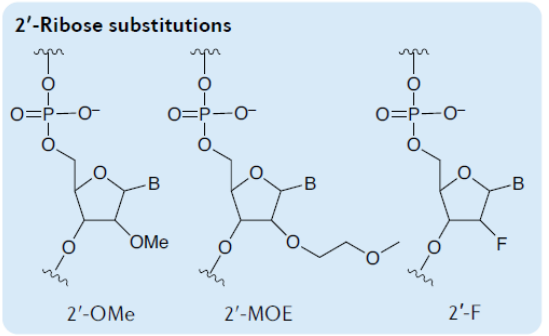
GalNac conjugate:

Feature	ASOs 	siRNAs 
Attachment site	Usually 5′ end or backbone	Usually 3′ end of sense strand
Considerations	Must not block target binding or RNase H activity	Must not block RISC loading
Approved examples	Eplontersen, Olezarsen	Inclisiran, Givosiran, Lumasiran



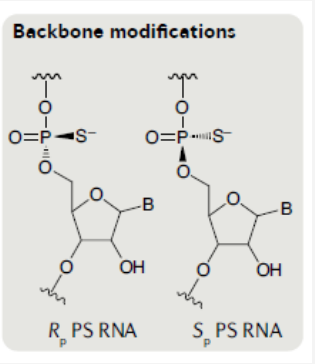
Ribose sugar modifications

- Modifications at the 2' position of the ribose sugar
- Increase nuclease resistance → longer plasma half-life
- Can increase affinity
- Can minimize off-target binding and immune stimulations



Modification	ASOs/siRNAs	Location	Examples
2′-MOE	Common in ASOs Rarely used in siRNAs	At the wings of ASOs	Nusinersen Inotersen
2′-F	Rarely used in ASOs Widely used in siRNA	Guide strand of siRNA	Inclisiran Givosiran
2′-OMe	Common in ASO wings, Widely used in siRNA	At the wings of ASOs Guide strand of siRNA	Inclisiran Givosiran

Backbone modifications



- Phosphorothioate linkages (PS) most common
- Introduces a chiral centre at each modified phosphorous atom (Rp and Sp)

Feature	ASOs 	siRNAs 
PS density	High Often every linkage 20-mer ASO: 2^{19} stereoisomers	Low Mostly terminal linkages
Purpose	Stability, uptake, plasma protein binding	Stability, minimal disruption of RISC loading
Examples	Nusinersen, Inotersen	Vutrisiran, Givosiran, Inclisiran

Oligonucleotides - immunogenicity

- Concern for oligonucleotides as it is a “larger” foreign RNA/DNA molecule
- Can trigger innate immune responses:
  - TLRs and RIG-1 activation → cytokine release and dendritic cell activation
- Can trigger adaptive immune responses:
  - ADAs have been found in patients treated with oligonucleotides

nusinersen

Immunogenicity

The immunogenic response to nusinersen was evaluated in 342 patients with post-baseline plasma samples for ADAs. Overall, 36 Spinraza-treated patients (11%) developed treatment-emergent ADAs, of which 14 (4%) were transient and 22 (6%) were persistent. No discernible effects of ADAs on efficacy or safety have been observed as measured by incidence of AEs including hypersensitivity, anaphylactic reaction, and angio-oedema.

inclisiran

Immunogenicity

In the pivotal studies 1,830 patients were tested for anti-drug antibodies. Confirmed positivity was detected in 1.8% (33/1,830) of patients prior to dosing and in 4.9% (90/1,830) of patients during the 18 months of treatment with inclisiran. No clinically significant differences in the clinical efficacy, safety or pharmacodynamic profiles of inclisiran were observed in the patients who tested positive for anti-inclisiran antibodies.

givosiran

Immunogenicity

Across the 4 clinical studies, there was only 1 case of treatment-induced ADA in 131 subjects (AHP patients + CHE subjects) who received givosiran.

- Modifications in ASOs and siRNAs reduce the potential to elicit an immune response

Generic oligonucleotide requirements

Where do oligonucleotides fit?

	Small molecule generics	Biosimilars
Substance type	Simple, chemically synthesized	Complex, biologically produced
Replication	Identical copy possible	Similar but not identical
Approval requirements	Bioequivalence only	Analytical, functional, PK/PD, clinical studies
Regulatory pathway	Generic approval EU: 10(1) US: ANDA	Biosimilar pathway EU: 10(4) US: BPCIA

Usually parenteral solutions
→ Waiver of BE study possible

Non-biological complex drug

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- Not a distinct category for FDA and EMA
- Chemically synthesized, non-biological active substance
- Complex formulation, e.g. liposomal formulations
- Active substance is not a single, simple, chemical entity, e.g. iron-carbohydrate complexes and glatiramer acetate
- Currently no approved generic oligonucleotides

Glatiramer acetate

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- Indicated for multiple sclerosis
- Strengths of 20 mg/mL and 40 mg/mL of solution
- Mode of action: not fully elucidated, presumed to involve modulation of immune processes
- Heterogenous mixture of synthetic polypeptides
- Contain 4 amino acids: L-glutamic acid, L-alanine, L tyrosine and L-lysine in molar fraction ranges of 0.129-0.153, 0.392-0.462, 0.086-0.100 and 0.300-0.374, respectively
- Average MW ranges from 5 to 9 kDa
- Not completely random: tightly controlled manufacturing process

Glatiramer acetate
Regulatory requirements

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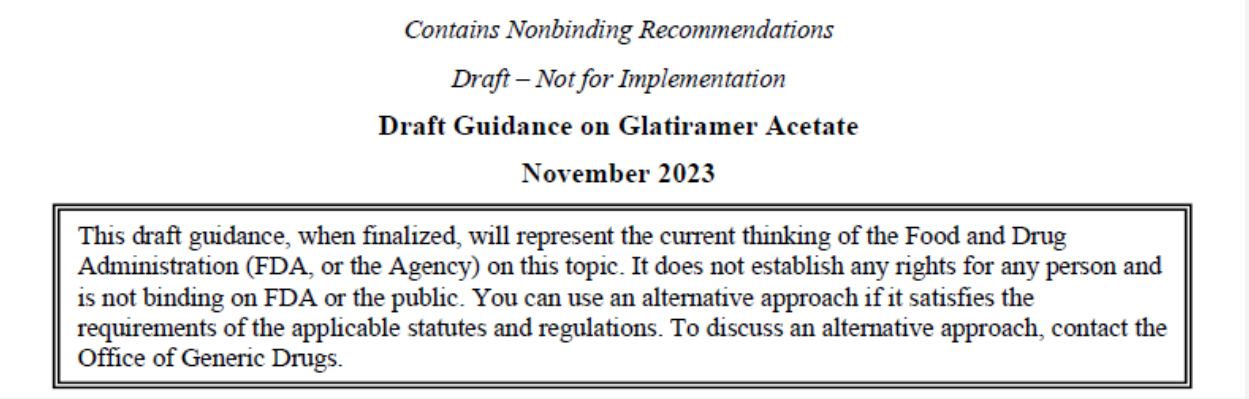
EMA:

- No specific guidelines → Scientific advice
- Type of submission?

FDA:

- Draft guidance on glatiramer acetate (Apr 2016, revised: Jul 2018, Nov 2023)
  - API sameness between Test and RLD
  - Should be done on at least 3 test batches and 3 RLD batches
- ANDA submission



Contains Nonbinding Recommendations

Draft – Not for Implementation

Draft Guidance on Glatiramer Acetate

November 2023

This draft guidance, when finalized, will represent the current thinking of the Food and Drug Administration (FDA, or the Agency) on this topic. It does not establish any rights for any person and is not binding on FDA or the public. You can use an alternative approach if it satisfies the requirements of the applicable statutes and regulations. To discuss an alternative approach, contact the Office of Generic Drugs.

Glatiramer acetate - FDA

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API sameness can be established based on 4 criteria:

a) Equivalence of fundamental reaction scheme;
b) Equivalence of physicochemical properties including compositions;
c) Equivalence of structural signatures for polymerization and depolymerization;
d) Equivalence of biological assay results

API sameness

- (1) polymerization of activated amino acids → intermediate copolymer, and (2) partial depolymerization of intermediate copolymer to yield glatiramer
- Same or equivalent activated amino acids, initiator and chemical reagents for acid-catalyzed cleavage

Glatiramer acetate - FDA

Synthon

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API sameness

- AA content, optical purity, MW distribution (incl. Molar mass moments and polydispersity), and spectroscopic fingerprints
- Using orthogonal and specific analytical tests

Glatiramer acetate - FDA

Synthon

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API sameness

- Needed as manufacturing process of Teva is not publicly available

Glatiramer acetate - FDA

Synthon

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d) Equivalence of biological assay results

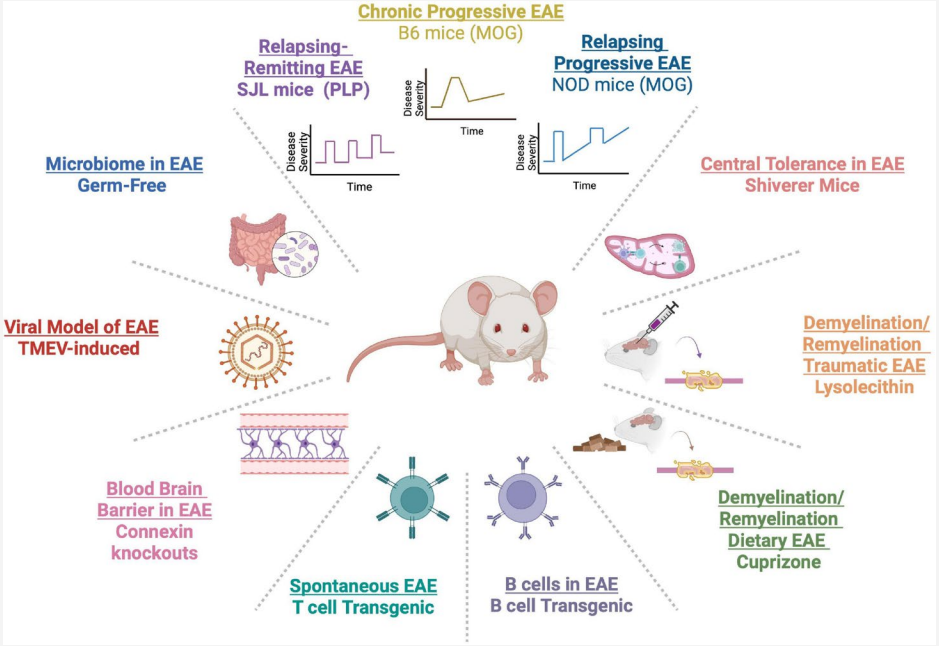
API sameness

- Confirmation of above results
- EAE model

Glatiramer acetate - Europe

Synthon

- 10(3) application (hybrid application)
- Comparative characterization: amino acid composition, chain length, molecular weight distribution etc.
- Non-clinical data: EAE mouse model
- Clinical data: comparative clinical trial GATE



Glatiramer acetate - GATE trial

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- Randomized, multicenter, double-blind, active and placebo-controlled pharmacodynamic phase 3 trial
- Patients with relapsing-remitting multiple sclerosis (RRMS)
- Patients were 18-55 yrs old
- At least 1 relapse in prior year and 1 - 15 gadolinium-enhancing brain MRI lesions
- Treatments: GTR (n=353) : Copaxone (n=357) : placebo (n=84)
- Placebo was requested to demonstrate assay sensitivity
- Primary endpoint: total number of gadolinium-enhancing lesions during months 7, 8, and 9
- Additional endpoints: MRI parameters, annualized relapse rate, expanded disability status score, safety and tolerability

Glatiramer acetate – GATE trial

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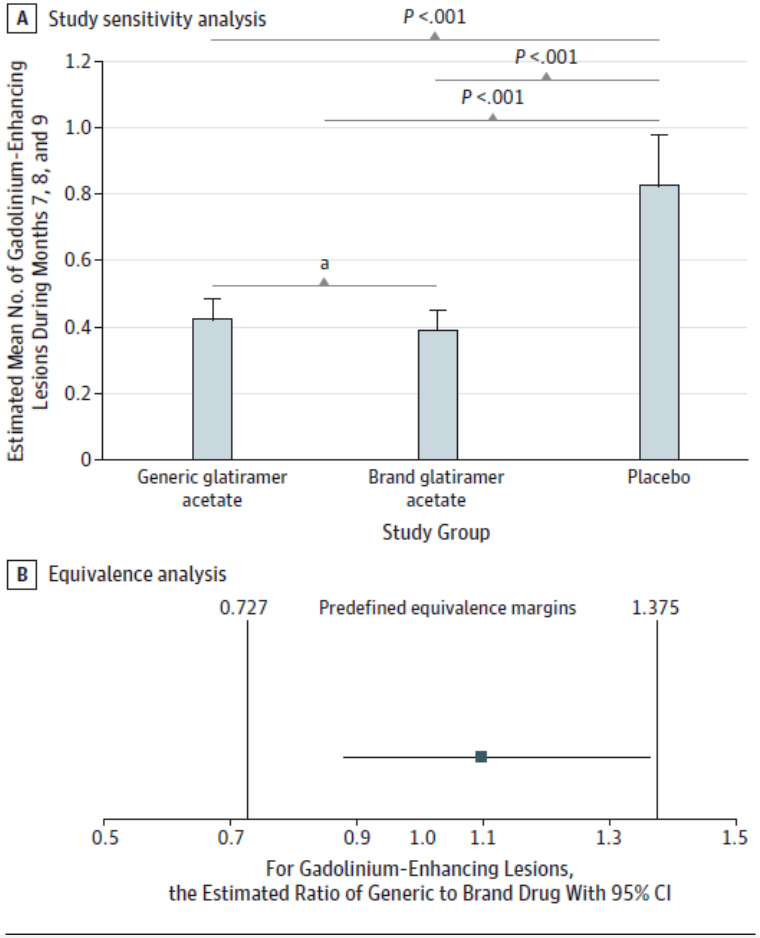

End Point	Generic Glatiramer Acetate (n = 353)	Brand Glatiramer Acetate (n = 357)	Placebo (n = 84)
MRI Outcomes			
Primary end point of total No. of gadolinium-enhancing lesions during months 7 through 9 on T1-weighted images[b]			
Study sensitivity, mean (95% CI)[c]	0.42 (0.31 to 0.57)	0.38 (0.28 to 0.52)	0.82 (0.57 to 1.20)
Ratio of combined active treatments to placebo (95% CI)	0.488 (0.365 to 0.651)		NA
Equivalence, mean (95% CI)[d]	0.45 (0.34 to 0.59)	0.41 (0.31 to 0.54)	NA
Ratio of generic drug to brand drug (95% CI)	1.095 (0.883 to 1.360)		NA
Patients with gadolinium-enhancing lesions during months 7 through 9 on T1-weighted images, No. (%)[e]			
Evaluable patients	333	335	82
0 Lesions	143 (42.9)	142 (42.4)	23 (28.0)
1 Lesion	43 (12.9)	55 (16.4)	8 (9.8)
≥2 Lesions	147 (44.1)	138 (41.2)	51 (62.2)

- Superiority was shown compared to placebo
- T/R ratio was within the predefined equivalence margin of 0.727 - 1.375
- AEs, including injection site reactions, were similar in Test and Reference groups
- Equivalent efficacy, safety and tolerability could be claimed

Glatiramer acetate - discussion

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- The comparative characterization analysis requested by the FDA was much more extensive compared to requirements in Europe
- For Europe clinical trial was needed to assess differences in efficacy and safety
- How ethical is it too include a placebo group for assay sensitivity?
- This was a requirement from the SA, but also not always accepted by EC
- How sensitive is a clinical PD trial to pick-up small differences between Test and Reference API?
- Different requirements resulted in different development approaches

Oligonucleotides - FDA

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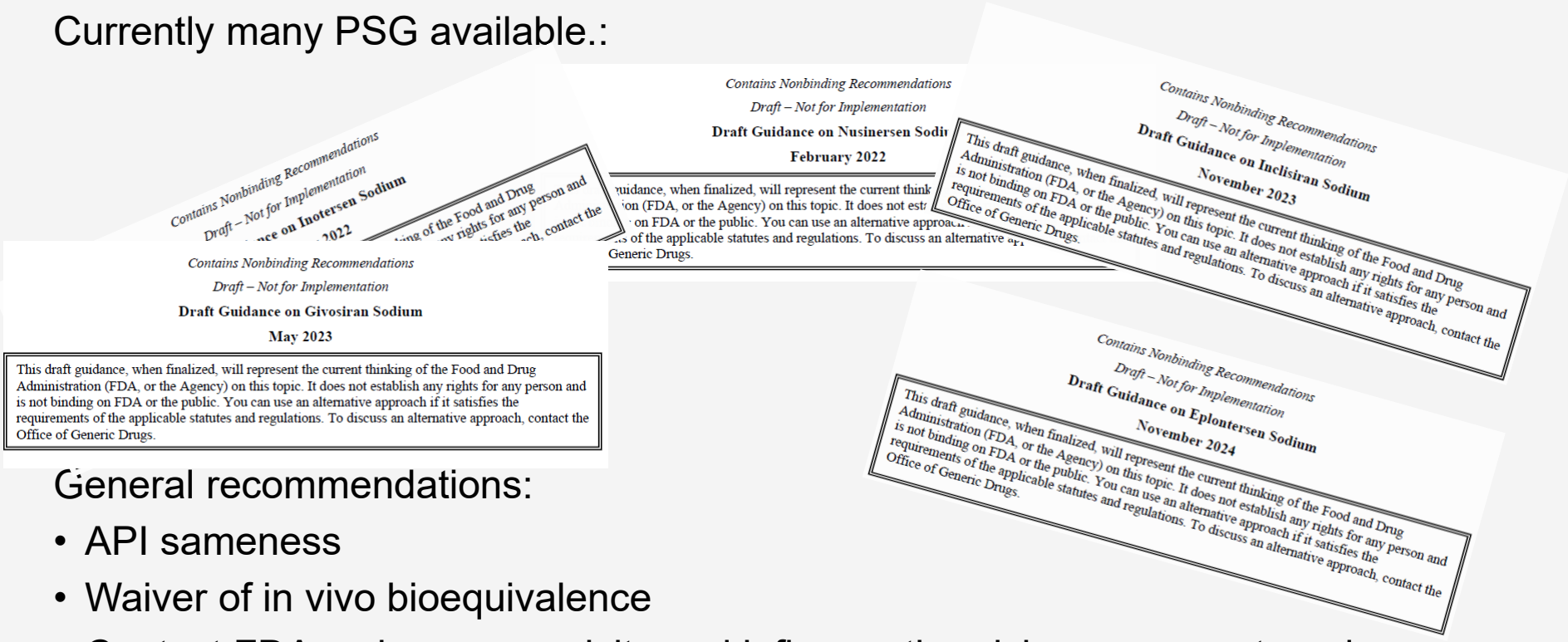
Currently many PSG available.:



General recommendations:

- API sameness
- Waiver of in vivo bioequivalence
- Contact FDA on immunogenicity and inflammation risk assessment, and comparability of impurities in test product

Recommendations are similar to glatiramer acetate

FDA – API sameness

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Primary sequence, chemical structure and diastereomeric composition:

- Diastereomeric composition
  - Reagents and reactions conditions appropriately selected and adequately controlled
  - R/S configuration ratio at each phosphorothioate nucleotide linkage following each elongation cycle should be measured
  - For siRNAs this should also be done for single strands
- Comparison of Test vs. RLD using a broad range of orthogonal analytical methods with sufficient sensitivity, discriminating and resolving power

- Mass spectrometry (MS), including tandem mass spectrometry (MS/MS)
- Nuclear magnetic resonance (NMR) spectroscopy
- Liquid chromatography (LC)
- Duplex melting temperature (Tm) to a complementary strand
- Flame atomic absorption spectroscopy (FAAS)

FDA – API sameness

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Comparative physiochemical properties (including aggregation and higher order structure):

- Circular dichroism (CD) spectroscopy
- Fourier transform infrared spectroscopy (FTIR)
- Differential scanning calorimetry (DSC)
- Size exclusion chromatography (SEC)
- Sedimentation velocity analytical ultracentrifugation (SV-AUC)
- Fourier transform infrared spectroscopy (FTIR)

FDA – immunogenicity

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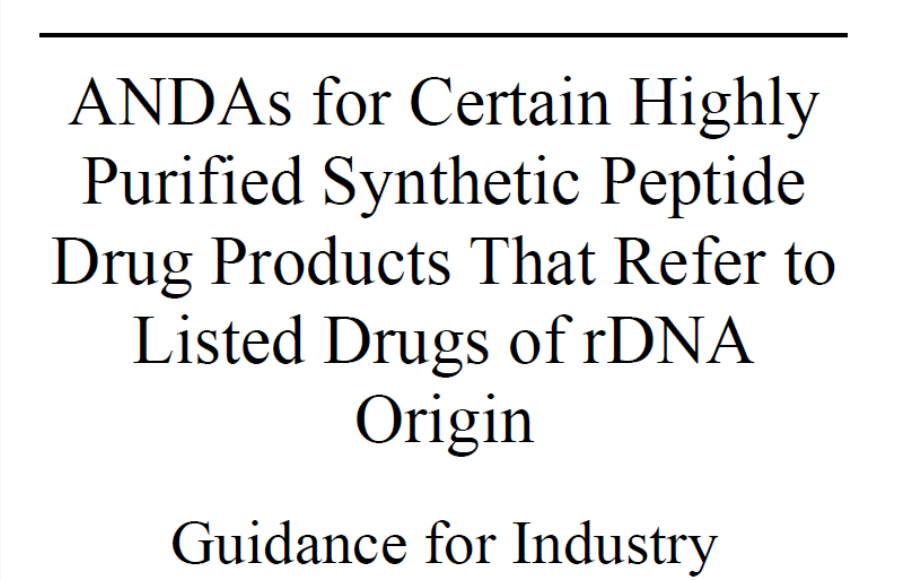
- In PSG unclear what should be done in relation to immunogenicity



- Most likely related to impurity profile
- If impurity profile under control: immunogenicity testing needed?
- Can it be a theoretical assessment?
- Are innate immune in vitro assays needed, to measure e.g. cytokine release and dendritic activation?

→ Case by case assessment that needs to be verified with the FDA

Oligonucleotides requirements?

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	EMA	FDA
Regulatory requirements known	No	Yes*
API sameness assessment	?	Yes
BE waiver	?	Yes, if it is an aqueous parenteral solution
Immunogenicity testing	?	Yes*
Clinical study needed to show similar efficacy and safety	?	No

* FDA immunogenicity testing requirements still unclear

Oligonucleotides conclusion

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- PSGs for numerous oligonucleotides available at FDA
- FDA immunogenicity requirements still unclear
- No regulatory framework for EMA → SA needed
- BE study waiver possible for EMA?
- Clinical PD or efficacy/safety trial, like needed for glatiramer acetate, not the best platform to pick up difference between Test and Reference API
- Possibly a sensitive biological activity test would be better to pick up differences between Test and Reference?

THANKS!