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Challenges posed to the European pharmaceutical regulatory system by highly personalized medicines

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The European pharmaceutical regulatory system has not yet been challenged by issues related to highly personalized medicines such as those to be found with active substances that affect RNA biochemistry. We review the current status of RNA-based pharmacology and present three possible case histories. The implications for the European pharmaceutical regulatory system are discussed.

Introduction to RNA-based pharmacology

Since the 1980s, our understanding of RNA has evolved from it being simply an intermediate between DNA and protein to a class of molecules that regulate the functions of genes and cells in all living organisms. In turn, this has led to a currently emergent pharmacology based on RNA biochemistry. This new pharmacology may be classified according to the site and mechanism of action of the active substance and includes (i) inhibitors of mRNA translation (antisense oligonucleotides), (ii) agents of RNA interference (RNAi), (iii) catalytically-active RNA molecules (ribozymes) and (iv) RNAs that bind proteins and other molecular ligands (aptamers) [1, 2].

At present, about 50 chemically-modified single-stranded antisense oligonucleotides between 20 and 30 bases long are being investigated as potential medicinal agents in clinical trials for a variety of diseases including cancers, genetic disorders and viral infections. This review briefly outlines the pharmacology of antisense oligonucleotides and then describes possible scenarios that will test the ability of the regulatory agencies to oversee this new area of highly personalized medicine.

Mode of action of antisense oligonucleotides

Each antisense oligonucleotide has a primary base sequence that complements its mRNA target molecule so that binding occurs by Watson–Crick base-pairing. The act of binding leads to suppression or alteration of translation of the target RNA by one of the following mechanisms:

- exon exclusion by interfering with splicing of a pre-mRNA
- promotion of degradation of the target RNA
  - by RNase H (the target RNA is degraded whereas the exogenously introduced antisense oligonucleotide remains intact and moves on to inhibit another transcript)
  - by small double-stranded interfering RNAs that guide an mRNA of interest into the RNA-induced silencing complex where it is cleaved
- steric hindrance of ribosome activity
- binding to micro-RNAs and so preventing these molecules from regulating translation

Antisense oligonucleotides therefore present new therapeutic strategies to treat diseases associated with aberrant gene expression [3, 4].

Structure of antisense oligonucleotides

An antisense oligonucleotides is usually between 20 and 30 bases long. Unmodified antisense oligonucleotides are susceptible to degradation by nucleases in serum and cells and so a variety of chemical alterations of the sugar and phosphate moiety of the oligonucleotide backbone have been developed to decrease susceptibility to nuclease degradation whilst increasing stability and potency of the

molecule. Recent advances in the manufacturing processes of synthesizing modified RNA and DNA molecules have increased the efficiency and reliability of the finished molecule at reduced production cost.

Those molecules that seem most likely to go on to clinical use bear the following structures in a variety of combinations: phosphorothioate backbone modification, 2′-O-methyl, 2′-fluoro or 2′-O-methoxyethyl sugar substitutions, 2′-O, 4′-C-methylene linked bicyclic ribonucleotides also known as a locked nucleic acid, L-RNA (enantiomer of natural RNA) oligonucleotides also known as 'spiegelmers', hexitol nucleic acids, and tricyclo-DNA.

Oligonucleotides intended for exon skipping, steric hindrance of ribosome activity or binding to micro-RNAs are more likely to be successful if composed of building blocks that confer stability and high-affinity such as the hexitol nucleic acids or tricyclo-DNA.

Oligo-nucleotides intended to promote degradation of the RNA target molecule have different considerations. Thus, phosphorothioates elicit RNase H activity but their affinity to complementary RNA is moderate. On the other hand, high affinity RNA binders such as locked nucleic acid, hexitol nucleic acids or tricyclo-DNA do not elicit RNase H activity. In order to take advantage of the different properties of the different chemical modifications, chimeric molecules have been developed that have a central sequence composed of structures that will elicit RNase H activity whilst the flanks are composed of structures that combine stability with a high affinity for RNA. These chimeric molecules are also known as 'gapmers'.

Within a structural class, many of the physico-chemical properties of an antisense oligonucleotide such as solubility, hydrophilicity and protein binding properties are common to all members of the class irrespective of the primary sequence of bases. As a result, pharmacokinetic properties and toxicological profiles are similar within any one class of antisense oligonucleotide.

Irrespective of the large number of possible permutations and combinations of chemistries, however, there are not any data to support the general superiority and versatility of any one chemically-modified antisense construct. Indeed, perhaps it is because of the large variety of possible antisense structures that there is not a consensus on single 'best' design [5, 6].

Selection of antisense oligonucleotide

Selecting an antisense sequence that will bind to an RNA target molecule requires more information than just the primary sequence of the RNA target molecule. Many oligonucleotides that are complementary to the primary sequence of an mRNA are found to have little or no antisense activity. The chosen region on the RNA target molecule needs to be physically accessible for hybridization. Those regions of an RNA target molecule that are single stranded are most likely to bind successfully to an antisense molecule whereas stable secondary and tertiary structures arising from intramolecular base-pairing within the RNA target molecule are likely to impede binding. Further, the number of possible conformational states grows exponentially with the chain length. For example, the number of hairpin conformations increases from 138 for a 10 nucleotide chain to 24 666 for a 16 nucleotide chain. Considerations of secondary structure also apply to the antisense oligonucleotide which may take part in dimer formation and intramolecular folding and so impede binding to the proposed target.

Examples of sites on the RNA target molecule that are most likely to be single stranded include the 5′ and 3′ terminal ends, internal loops, hairpins, bulges of 10 or more consecutive nucleotides and the join region between exons and introns of pre-mRNA.

Initial coding regions of the mRNA target molecule usually lack secondary structure but although they seem to present a target that is likely to result in successful binding of an antisense molecule, such initiation sites may display shared homology with other genes thereby increasing the likelihood of 'off-target' effects.

The strength and stability of interactions between the antisense oligonucleotide and the complementary target mRNA also depend on factors such as thermodynamic stability, the kinetics of duplex formation, the proximity of the hybridization site to functional motifs on the designated transcript and cellular factors such as the type of cell being targeted and the intracellular association of binding factors such as AUF1 (an adenine- and uracil-rich binding factor) leading to the formation of condensed, complex RNA structures.

Strategies that are used to screen and choose a base sequence for an antisense oligonucleotide include use of:

(i) mRNA walking – oligonucleotides of a given length and complementary to sequences along the target RNA sequence are synthesized and screened for antisense activity
(ii) computer algorithms to predict RNA structure
(iii) oligonucleotide arrays – oligonucleotides complementary to the target mRNA sites are synthesized and hybridized with mRNA transcripts. Antisense sequences are chosen by selecting the ones with high affinity to the mRNA on the array
(iv) RNase H mapping – RNase H is used to cleave mRNA that hybridizes to a random oligonucleotide library. Appropriate antisense oligonucleotides are selected by identifying RNase H cleavage sites of the target mRNA.

In practice, a variety of approaches are applied to design the most appropriate antisense sequence and, apart from an obvious property such as biostability, selection still depends ultimately upon a process of trial and error [4, 6].

Delivery of antisense oligonucleotide

Liposomes, polymers or peptides are used as vectors to facilitate cellular uptake by endocytosis or by means of membrane channels because only very small amounts of naked oligonucleotides permeate across the plasma membrane when added to cells.

When administered parenterally, most oligonucleotide will distribute to organs of the reticulo-endothelial system such as the liver, spleen, lungs and kidneys. Unmodified oligonucleotides tend to accumulate in the kidneys whilst the use of vectors such as liposomes leads to uptake by the liver. For applications that require a targeted systemic delivery, the accompanying vector may be enhanced by cell-specific ligands that promote receptor-mediated uptake or by the inclusion of PEGylated lipids or by modulation of the surface charge of the vector. As an alternative, target cells may be collected from the patient, modified by exposure to the oligonucleotide and then re-infused back.

Free oligonucleotides are cleared from plasma in a biphasic manner with a first half-life (distribution phase) measured in minutes and a second half-life (elimination phase) measured in hours. Vector systems will prolong the half-lives.

In vivo toxicity reflects the capture and long term deposit of oligonucleotides in the reticulo-endothelial system causing adverse effects such as renal tubule dysfunction and necrosis, splenomegaly, thrombocytopenia and elevation of the serum activity of liver transaminases. These adverse events may be mitigated by adjustment of the chemistry of the oligonucleotide or selecting an appropriate vector delivery system [5].

Examples of clinical scenarios

We now describe three clinical scenarios with consequent conundrums based on the new pharmacology of oligonucleotides.

The players:

- Company A
- Company B
- Company C
- Patient 1 who has a rare genetic disease causing reduced lifespan
- The regulatory agency

Scenario 1

Company A identifies a genetic disease with orphan status that would be amenable to treatment with an antisense oligonucleotide, ASO-999. The primary sequence of ASO-999 is 20 bases long. Company A holds the patent for the chemically modified structure of the sugar phosphate backbone of ASO-999. The clinical development programme and application for a licence to place ASO-999 on the marketplace takes 10 years at a cost of £•• million.

As part of its post-marketing surveillance programme, Company A identifies one patient (Patient 1) who does not respond to ASO-999. A blood sample is obtained from Patient 1 and the primary sequence of the RNA target molecule from Patient 1 is determined within 12 h of receipt. It is found that the RNA target molecule from Patient 1 differs from the RNA primary sequence of bases of all patients so far tested by three bases only. Company A considers that the alteration in primary sequence of the RNA target molecule has prevented ASO-999 from binding adequately to the RNA target molecule of Patient 1 and so ASO-999 lacks clinical efficacy when administered to Patient 1.

Within a further 12 h, Company A manufactures an antisense oligonucleotide, ASO-998, that matches the primary sequence of the RNA target molecule of Patient 1. Company A wishes to make ASO-998 available to Patient 1 for immediate use.

Company A is unwilling to embark on a new clinical development programme for ASO-998. Company A argues that, although ASO-998 is a different molecule from ASO-999, the oligonucleotides have the same mode of action at the RNA target molecule and will have similar pharmacokinetic and toxicological profiles because the ASO-999 and ASO-998 belong to the same class of compound. Company A argues that exposure of Patient 1 to ASO-998 will result in a benefit–risk profile that matches that described for ASO-999 in all patients so far studied. Company A wishes to create an intellectual bridge between ASO-998 and clinical data for ASO-999.

Company A wishes to be reimbursed for supplying ASO-998 to Patient 1.

Company A is unwilling to apply for a separate marketing licence for ASO-998 because there are not any clinical data to support such an application. Clinical data for ASO-998 are unlikely to be obtained because, at present, Patient 1 is the only person known to have a primary base sequence of the RNA target molecule that matches ASO-998. A formal variation procedure for the existing licence for ASO-999 (if this were possible) would take about 1 year to complete. In the meantime, the genetic disease will lead to a deterioration in the health of Patient 1 and cause premature death unless it is treated without delay. Company A wishes the regulatory agency to handle ASO-998 as part of the existing licence for ASO-999 so that Patient 1 may receive treatment without delay and so that Company A may be reimbursed.

Conundrums:

The regulatory agency wonders if molecule ASO-998 is a new active substance or a derivative of ASO-999. And if ASO-998 is a derivative of ASO-999, at what point does ASO-998 become a new active substance? When the difference is by five bases, 12 bases or the entire 20 base length? Can any or all of the new molecules related to ASO-999 be incorporated into the licence for ASO-999 and

so avoid lengthy and expensive development programmes for each new product? The regulatory agency is concerned that ASO-998 will have a different 'off-target' activity compared with ASO-999 and that this has not yet been adequately investigated or described.

Scenario 2

Company B makes antisense oligonucleotides using its own patented chemically modified sugar phosphate backbone. Patient 1 presents to Company B instead of Company A. Company B confirms that Patient 1 has an RNA target molecule that has a primary sequence of bases that differs from the usual sequence by three bases. Using its own patented sugar phosphate backbone, Company B manufactures an antisense oligonucleotide that matches the RNA target molecule of Patient 1.

Company B claims that this antisense oligonucleotide is a new active substance (it has a different sugar phosphate backbone from ASO-999 and a different primary sequence of bases) and calls it ASO-B1. Company B does not wish to agree a private contractual arrangement with Company A because the active substance of Company B is very different chemically from that of ASO-999.

Company B wishes to be reimbursed for supplying ASO-B1 to Patient 1.

Company B is unwilling to embark on a clinical development programme for ASO-B1 for the same reasons as Company A gave for ASO-998. Company B wishes to create an intellectual bridge between ASO-B1 and clinical data for Company A's active substance ASO-999 on the grounds that ASO-B1 has the same mode of action at the RNA target molecule. The pharmacokinetic and toxicology profiles of ASO-B1 will be similar to those of other antisense oligonucleotides in Company B's portfolio that use the same sugar phosphate backbone. Company B argues that the genetic disease will lead to a deterioration in the health of Patient 1 and cause premature death unless it is treated without delay.

Conundrums:

How much protection should the regulatory agency afford to Company A and how much permission should the Regulatory Agency give to Company B? How and when should an intellectual bridge be made to the clinical data of active substance ASO-999?

Scenario 3

Company C makes antisense oligonucleotides using its own patented chemically modified sugar phosphate backbone. Patient 1 presents to Company C and it is confirmed that Patient 1 has an RNA target molecule that has a primary sequence of bases that differs from the usual sequence by three bases. Company C carries out computer modelling of the RNA target molecule and finds that the change in primary sequence will render it unlikely that altering the primary sequence of the antisense oligonucleotide will result in binding to the target because of novel secondary and tertiary structure in the RNA of Patient 1.

By means of computer modelling, Company C identifies a different 30 base stretch on the RNA target molecule that will result in tight binding and that will lead to the same biochemical consequence as Company A's active substance ASO-999 in patients with the 'usual' RNA sequence. It is predicted also that clinical efficacy will be similar.

Using its own patented sugar phosphate backbone, Company C manufactures an antisense oligonucleotide that matches the RNA target molecule of Patient 1 and claims that this is a new active substance that it calls C-A1.

Company C does not intend to enter into a contractual arrangement with Company A because the active substance of Company C has a different primary sequence of bases and a different site of action on the RNA target molecule compared with the active substance of Company A. Nevertheless, Company C wishes to create an intellectual bridge between C-A1 and clinical data for Company A's active substance ASO-999 because exposure of Patient 1 to C-A1 will result in the same biochemical consequence and presumed clinical efficacy that Company A's ASO-999 has for 'usual' patients. Company C states that the pharmacokinetic and toxicology profiles of C-A1 will be similar to those of other antisense oligonucleotides in its portfolio that use the same sugar phosphate backbone.

Company C wishes to be reimbursed for supplying C-A1 to Patient 1. Company C argues that the genetic disease will lead to a deterioration in the health of Patient 1 and cause premature death unless it is treated without delay.

Conundrums:

How much protection should the regulatory agency afford to Company A and how much permission should the Regulatory Agency give to Company C? How and when should an intellectual bridge be made to the clinical data of active substance ASO-999?

Regulatory and legal issues

The current method of assessment of a new medicine (or a new indication for an existing medicine) by the regulatory agencies relies on submission of data arising from a clinical development programme that has produced a statistically adequate evaluation of efficacy of the new medicine arising from properly designed and conducted trials that are randomized, blinded and placebo-controlled. Such trials also provide a description of safety. The decision to recommend that a new medicine be granted a licence to be placed on the marketplace is then based upon the initial formulation of a risk–benefit analysis that is subject to on-going scrutiny by regular updates of safety derived from the post-marketing experience. These activities are

described by (the Medicines) Directive 2001/83/EC of the European Parliament and the European Council, as amended.

Under current EC legislation on orphan medicinal products, Regulations (EC) nos. 141/200 and 847/2000 of the European Parliament and of the Council, a similar active polynucleotide substance is one where 'the addition or deletion of nucleotide(s) not significantly affecting the kinetics of hybridization to the target would normally be considered similar'. In scenarios 1 and 2, although the new substances each differ from the originator only by three bases, the Companies have made changes to the primary base sequence of their antisense oligonucleotides because the kinetics of the original molecule were inadequate when used with Patient 1. Companies A and B therefore have grounds to claim that their active substances are *not similar* to the originator because the kinetics of hybridization of their active substances are significantly different for Patient 1. According to the current legislation, modifications to the ribose or deoxyribose sugar backbone or to the replacement of the backbone by synthetic analogues would not be adequate to make a claim that the new active substance is *not similar*. Company B may therefore not make a claim for the product of scenario 2 being *not similar* solely on the basis of the different sugar phosphate backbone structure. The active substance of Company C in scenario 3 has an entirely different primary sequence of bases from the originator and therefore may claim to be *not similar*.

At the time of writing, only one oligonucleotide has been granted a licence to permit access to the European marketplace: Macugen, EU/1/05/325/002, indicated for the treatment of neo-vascular age-related macular degeneration in adults, authorized in 2006. There is, therefore, limited experience of efficacy and safety of oligonucleotides in the present clinical setting. As more understanding of these compounds is acquired, however, it is anticipated that clinical safety issues of compounds with the same backbone may be shown to be similar and that quality issues of purity and identity will be overcome [4]. In order for a patient to gain access to a highly personalized licensed medicine, we are left, then, with the issue of how to address clinical efficacy. In this regard, should the regulatory agencies request individual $n = 1$ trials prior to granting a licence knowing that $n = 1$ trials have pitfalls and are prone to issues of therapeutic misconception(s) that make it difficult to interpret results [7]? Or should there be a presumption of clinical efficacy based upon chemical structure of the oligonucleotide that would permit a licence to be granted in the absence of clinical data for that particular molecule, as for the active substance produced by Company C in scenario 3? If the latter option is applicable, then should the $n = 1$ trial be carried out in the post-marketing setting and how would data from such a trial be processed? Whether the intellectual bridges to data (as described in scenarios 1, 2 and 3) may be created and to what extent remains to be ascertained. Irrespective, we are concerned that the Medicines Directive 2001/83/EC, as amended, does not appropriately describe methods for the assessment of risk–benefit of highly personalized medicines that have been tailor-made for individual patients. We are also concerned that the current structures are not adequately responsive to the needs of a patient who has a rapidly progressing disease that requires immediate (within days) access to a tailor-made licenced medicine.

From the legal perspective, it is not possible to predict how the legal issues will unfold should cases of drug-induced injury from highly personalized medicines come to Court. Common law negligence, statutory consumer protection and product liability are already known to be difficult to reconcile with regulatory decisions on clinical efficacy and safety and are likely to be made more so by products that rely on conclusions of benefit–risk balance by inference to a related product. Although actions in negligence allow for the question of risk and benefit in finding breach of duty of care, strict liability in the consumer protection regime does not allow for the benefit–risk balance of a product as a defence for defective products. Causation of injury has to be proven and such proof will be extremely difficult in actions for these tailor-made products. There are also concerns on how current law on intellectual property, in particular patent law, will be applied to these 'made to order' products. Clearly, legal considerations will need to be taken into account and are highly likely to influence development of highly personalized medicines.

Discussion

Medicine has traditionally used the model of 'one pill for all'. Forthcoming legislation for personalized medicines that make use of accompanying diagnostic tools will introduce the concept of 'one pill for a defined subset of all'. We now introduce the concept of highly-personalized medicine based on the 'new pharmacology' related to RNA biochemistry that will facilitate the development of made to order medicines for patients. Such RNA-based therapeutics are currently in development for cancers, inherited metabolic diseases and viral infections.

In this review, we have described three clinical scenarios with consequent conundrums that, at present, the current regulatory and legal systems do not seem able to address in a manner that will lead to agreeable conclusions for all parties concerned. Consequent upon the issues described in this review and with the presumption that oligonucleotides are shown to have a place in clinical medicine, we envisage that the ability to formulate tailor-made oligo-nucleotides will render redundant the traditional model of phase 1, 2 and 3 clinical trials and that there will be a need to find solutions to gather meaningful data on clinical efficacy and safety in patient groups that may

only have one participant. Further, the rapid advances in technologies accompanied by the speed of development of tailor-made medicines will challenge the current regulatory and legal frameworks to develop new, adaptive and responsive systems to cope with the stresses imposed.

We believe that the existing regulatory and legal frameworks will require major and on-going reviews in order to maintain their fitness for purpose. Such reviews of procedures will require input from patient advocacy groups, clinicians, researchers, industry and regulators.

Competing Interests

All authors have completed the Unified Competing Interest form at http://www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and declare no support from any organization for the submitted work, no financial relationships with any organizations that might have an interest in the submitted work in the previous 3 years and no other relationships or activities that could appear to have influenced the submitted work.

Contributors

Both JDJ and PF contributed and wrote the review. Although the authors work for regulatory authorities, the views expressed are their own and do not reflect those of any agency or institution.

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