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Strategies for designing clinical trials for oligonucleotide therapeutics

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Recent Phase III clinical trials for oligonucleotide therapeutics have yielded disappointing results. There is growing evidence that trial designs that consider the specific mode of action of these compounds are of crucial importance for their clinical testing. Early trials for oligonucleotide therapeutics should consider additional endpoints for the definition of a biologically active dose rather than focusing on the traditional concept of maximal tolerated dose. In later phases, alternative clinical endpoints and enriching sensitive study populations through innovative trial designs could improve the efficiency of clinical trials for oligonucleotide therapeutics.

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▼ Over the past decade, the unravelling of the human genome has led to a fundamental change in our understanding of tumour biology. Insights into the complex network of molecular-controlled pathways inspired the challenging idea of developing drugs that are targeting the molecular level of disease with the promise for more effective treatments [1]. A steadily increasing number of drug candidates with molecular targets have emerged and, in addition to antibodies and kinase inhibitors, several oligonucleotide therapeutics (OTs) are currently under clinical evaluation. OTs are a group of nucleic-acid-based compounds that comprises not only antisense oligonucleotides (ASO) for the targeted knockdown of gene expression, nucleic acids with catalytic activity (ribozymes) and small interfering RNA (siRNA), but also oligonucleotides that act by specifically binding to proteins (aptamers) and immunostimulatory oligonucleotides (CpG ODN) [2–5]. ASO are the most advanced OTs in terms of clinical development and their progress and applications are reviewed here. The hybridization of an ASO to complementary target mRNA leads to the destruction of that mRNA, thus the use of these single-stranded DNA molecules is an attractive approach for the downregulation of selected proteins.

The clinical development of ASO began in the 1990s when an ASO that targeted p53 was administered to acute myeloid leukaemia patients [6]. Since that time, several ASO have entered clinical trials, with the majority having applications for oncological indications [7]. Most of the clinical trials were designed towards traditional endpoints in drug development and evaluated safety and tolerability in Phase I, activity in Phase II and clinical benefit compared with standard treatment in Phase III [8].

Although this stepwise approach is well-established in drug development, there are several concerns whether trial designs and endpoints used to date for Phase I–III trials meet the requirements for targeted therapeutics such as OTs [9]. For targeted drugs, not only OTs, there is a growing list of drugs that showed promising activity in early development but failed in late phases [10]. Indeed, this failure rate could be the result of the clinical inefficacy of these drugs, but there is an increasing understanding that inappropriate trial design and endpoints might contribute to negative outcomes in clinical trials for OTs. For example, in oncology, the use of trial designs that were established for non-specific cytotoxic drugs does not necessarily guarantee the acquisition of conclusive data for the assessment of targeted therapeutics (i.e. you do not get the right answers if you ask the wrong questions). Notably, even for 'traditional' drugs, the methodology of clinical trial design is currently the source of controversial debate, which has been prompted by the relabelling of ~21% of FDA approved drugs, primarily because of inadequate dosing [11].

There is clearly a need for higher efficiency in drug development. In the past, the number of potential drug candidates was a rate-limiting factor in drug development. Today, there

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are more potential drug targets and OTs that are worthy of testing than the clinical trial infrastructure can simultaneously perform. It will be necessary to accelerate the performance of clinical trials to optimize the use of limited time and financial resources. Therefore, it seems appropriate to reconsider the design of clinical trials for OTs; here, strategies for future trials will be discussed.

Traditional Phase I trial design: assumptions of the maximum tolerated dose concept

The initial step in the clinical development of all novel drugs is a Phase I trial in humans with the primary objective being testing the safety and tolerability of the treatment. Thereby, drug doses are escalated in a limited number of patients to a level where a portion of patients experience dose-limiting toxicity (DLT), which is defined individually for each protocol. The dose level that is below the level associated with an unacceptably high rate of DLT is typically specified as maximum tolerated dose (MTD); in subsequent trials, a dose that is at or near the MTD will be suggested as the recommended Phase II dose (RPTD). For decades, this procedure has proven to be an acceptable strategy in oncology trials. The underlying concept was created for anticancer drugs exerting their antitumour activity predominantly by non-specific DNA damage. This unspecific DNA damage not only resulted in the antitumour activity of the drug but was also responsible for the observed side effects. Therefore, the dose-related toxicity observed could be regarded as a surrogate for therapeutic efficacy. This assumed dose–response relationship led to the supposition that higher dose sizes would have a superior therapeutic outcome.

Alternative endpoints: the concept of minimal effective dose and optimal biological dose

For targeted therapeutics like OTs, the paradigm of 'more is better' does not have inherent validity. As a result of their specific mode of action, OTs might have therapeutic activity that is far below toxic doses. Moreover, any potential side effects can be mediated by a mechanism that is completely independent from the therapeutic mode of action and are therefore not necessarily surrogates for clinical activity. In contrast to the parallel increase of therapeutic and toxic effects for traditional, non-specific anticancer drugs, an alternative dose–response relationship could exist. Dose escalation of an OT might result in a saturable maximum target activity measured by protein modulation at substantially lower doses than the MTD (Figure 1a). In that setting, defining a minimal effective dose (MED) instead of MTD as RPTD would not only minimize potential side effects for patients but also facilitate reducing OT treatment costs.

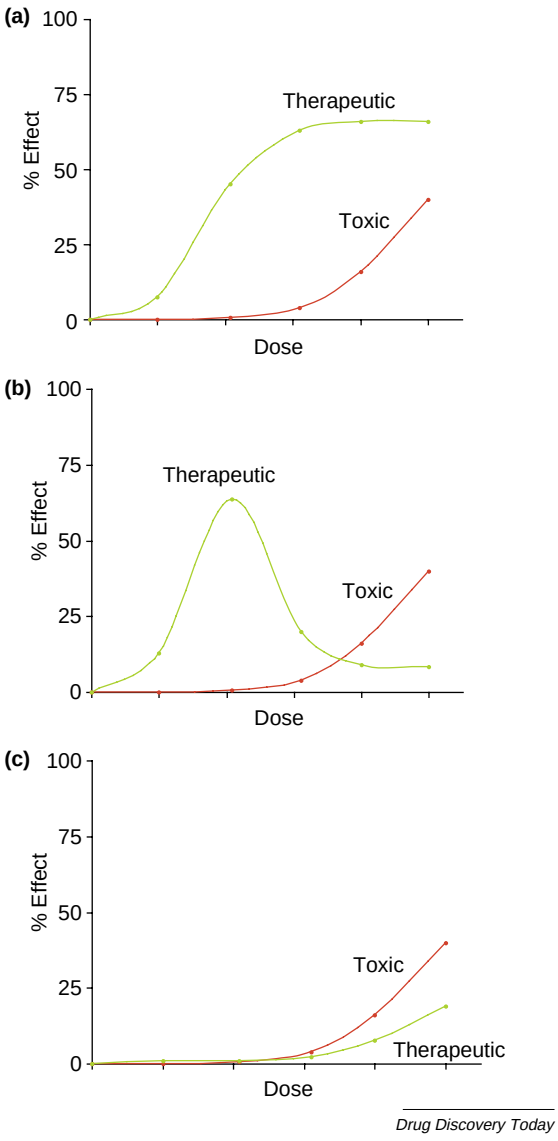

Figure 1. Hypothetical scenarios of dose–response relationships for therapeutic targeting activity (green) and toxic side effects (red) of oligonucleotide therapeutics.

Indeed, research performed in the Wacheck group (Medical University of Vienna; http://www.meduniwien.ac.at) in preclinical animal models indicates that target regulation by ASO could reach a plateau phase before any toxic effects are observed (unpublished results). For other molecularly targeted therapeutics, such as aprepitant, a neurokinin-1 receptor antagonist, the concept of a MED has already been demonstrated in clinical trials [12–14].

Although some therapies might have the effective dose determined by the traditional MTD concept in a conventional Phase I to Phase II sequence, there might be therapies for which the intended maximum biological activity

of a drug is missed by overdosing to MTD. In this scenario, biological activity will increase initially by dose escalation and peak at an optimal biological dose (OBD) with maximum target modulating activity (Figure 1b). Further dose escalation results in a decrease of specific target regulation and, at the MTD, biological activity might be considerably lower than at lower, non-toxic doses. In this instance, a conventional Phase I design that relies solely on the MTD concept would clearly fail to define the dose with the best therapeutic index. An example of this phenomenon for molecularly targeting therapeutics is the epidermal growth factor receptor blocker Iressa® (AstraZeneca; http://www.astrazeneca.com), which showed a tendency towards lower treatment efficacy (and more side effects) at higher doses compared with lower doses [15].

Finally, the endpoint of DLT in a dose-escalating Phase I study for OTs might be reached at a dose level that demonstrates no target regulation activity at all (Figure 1c). In this case, adhering to the traditional MTD concept, a non-toxic, but also inefficient dose, would be recommended for Phase II testing, resulting in a waste of time and financial resources.

There is still limited data available about the dose–target-effect relationship of OTs in clinical trials and the existing data does not enable definite conclusions to be reached. It is not yet known whether the OBD of targeted therapeutics directly translates into the optimal therapeutic dose, which is the ultimate goal for patients. The hypothetical scenarios outlined here might help to illustrate a rationale for designing Phase I trials to define the MED and/or OBD as (additional) alternative endpoints for OTs, as opposed to the traditional Phase I MTD concept. From a pharmacological point of view, it would still be of interest to determine the MTD. However, in the case of target-regulating OTs, knowing the MTD is not a mandatory requirement for further clinical trial design. For some OTs, it might be difficult to define a DLT at all because of their low toxicity profiles at target-regulating doses (e.g. second generation ASO). Furthermore, compound costs of OTs for dose escalation to DLT might exceed the fundable range ('maximal fundable dose').

Surrogates: biomarker, clinical endpoints and surrogate endpoints

To determine the optimal dose for OTs, a surrogate of activity must be identified to enable the establishment of dose–effect relationships in Phase I trials. For example, the intended mechanism of action of ASOs is the specific downregulation of their target gene product. Therefore, measuring a reduction of target expression in response to ASO treatment could be considered as a surrogate for activity of these compounds. In an ideal setting, the concept of a surrogate implies that the surrogate could be regarded as a substitute for the ultimate clinical outcome. Unfortunately, the term surrogate has been often misused in the past, resulting in inaccurate classification of trial endpoints and an unfavourable perception of the use of the overall concept [16]. In an attempt to improve communication about this topic, in 2001, the Biomarker Definitions working group, convened by the National Institute of Health (http://www.nih.gov), proposed clarified definitions for the terms biomarker, clinical endpoint and surrogate endpoint to describe a conceptual model for use in drug development (Box 1) [17]. Biomarkers are of greatest value in the early phases of clinical development for the establishment of 'proof of concept', for guidance in dosing and for selection of lead compounds [14]. For example, Jansen *et al.* [18] measured Bcl-2 expression in serial melanoma biopsies in a combined Phase I–II study of a Bcl-2 ASO as a biomarker to establish 'proof of concept' for this OT in patients. Furthermore, pharmacodynamic data demonstrated that ASO plasma levels in patients above a threshold of biological activity, as determined in animal models, could be reached via different routes of administration. For the use of biomarkers as surrogate endpoints in later stages of clinical trials, a robust linkage with a clinical endpoint is essential and only a subset of biomarkers could ever attain the status of a surrogate endpoint. Thus, biomarkers require intensive validation of the statistical assay used to measure activity, as well as the predictive utility of the biomarker [14,19].

Even if the concept of biomarkers and surrogate endpoints is appealing, it must be stressed that this model bears a considerable risk: an effect on a biomarker is not in itself of any value to the patient [20]. Thus far, none of the genes currently under investigation for targeted ASO approaches has been validated sufficiently as a therapeutic

Box 1. Glossary of terms for conceptual model of drug development

Biomarker: a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes or pharmacologic responses to a therapeutic intervention.
Clinical endpoint: a characteristic or variable that reflects how a patient feels, functions or survives.
Surrogate endpoint: a biomarker that is intended to substitute for a clinical endpoint. A surrogate endpoint is expected to predict clinical benefit based on epidemiologic, therapeutic, pathophysiologic or other scientific evidence.

target to achieve acceptance as a surrogate endpoint by the regulatory authorities. This does not mean that the concept of biomarkers and surrogate endpoints is to be discouraged with regards to the development of OTs in oncology; rather, it emphasizes the necessity for more research activities with high scientific scrutiny and rigour in this area [14,17]. Moreover, because most ASO therapeutic strategies are designed to target a single gene (single gene addiction), the majority of efforts in translational studies were focused only on the target gene as the biomarker. However, there is growing evidence that it might be necessary to study multiple biomarkers (e.g. target gene interacting partners) to obtain a more conclusive dataset to judge the safety and efficacy of targeted therapeutics in oncology [21].

Correlative studies for oligonucleotide therapeutics

How can the concepts outlined here be implemented in clinical trials for OTs? Clinical trials for OTs should comprise a focus on the study of biomarker(s) regulation by OTs through intensified pharmacokinetic (PK) and pharmacodynamic (PD) analyses. Correlating the plasma concentration, target regulation and clinical outcome of a particular OT could potentially lead to a more effective and more rapid performance of clinical trials. Preferably, the PD effects of OTs should be evaluated at their intended site of action (i.e. in the tumour tissue). To enable the assessment of changes from baseline to post-treatment, a minimum of two time points must be studied. The neo-adjuvant setting, where biopsy material obtained for diagnosis can be compared with specimens removed during surgery, is particularly well-suited for such target expression studies. Alternatively, if there is no significant fluctuation in the interpatient baseline target expression, it might be justified to reduce the number of tumour biopsies to one biopsy post-treatment. If a chemosensitising strategy with an OT requires optimized scheduling of the combined chemotherapeutic, the inclusion of multiple biopsies for the evaluation of the time course of target regulation should be a particular consideration. However, in many cases, repeated biopsies represent an invasive procedure with an unacceptable risk for the patient. With the exception of haematological diseases and easily accessible solid tumours (e.g. melanoma), scientists are frequently obliged to study time-course target regulation in surrogate tissues (i.e. peripheral blood mononuclear cell or skin).

The design of clinical trials for OTs must start during preclinical development. This stage of development affords a unique opportunity to gain information about dose- and schedule-dependency of the effects of OTs on target and surrogate matrices with unlimited access to tissues. Optimal Phase I trial design is only possible with conclusive preclinical data. From this perspective, preclinical animal studies could be regarded as 'Phase 0', with a strong influence on the design and outcome of all further clinical development. Ideally, preclinical 'proof of concept' studies with PK and/or PD analyses should be performed in a centre that is experienced in translational research rather than in separate units.

Phase I–III trial designs for oligonucleotide therapeutics

Although there is no 'one size fits all' trial design that covers all potential objectives perfectly, there are some basic elements that should be fulfilled by a trial design for OTs. Ideally, a Phase I trial should provide a fast (and adaptive) dose-escalation scheme to biomarker-modulating doses to avoid subtherapeutic treatment, as well as minimizing the total number of patients to be enrolled and the duration of trial completion. Furthermore, a trial design that enriches a sensitive study population would be favourable from Phase II onwards.

Phase I

The classical Phase I '3+3' design with a fixed dose escalation (e.g. modified Fibonacci) is still the most often applied design for ASO trials. In this design, three patients per dose level will be enrolled and, depending on the observed outcome (i.e. toxicity), doses will be escalated, repeated or de-escalated in the next cohort of three patients. Although the safety of the '3+3' design is well-documented, this design has several shortcomings in the evaluation of OTs. In oncology, an average of 8–13 dose escalation levels is required for defining RPTD trials using this design [22]. Therefore, it is a time-consuming trial design with the potential for treating many patients at subtherapeutic doses without any target regulation.

An alternative approach is the use of an accelerated dose escalation (ADE) scheme [23]. In this design, doses will be escalated with only one patient at each dose level provided that no toxicity is observed. As soon as any moderate toxicity occurs (common toxicity criteria grade ≥2), further dose escalation will be switched to the '3+3' design. Whereas the ADE design minimizes the number of patients treated at subtherapeutic doses, it does not expedite the completion of the trial and has the inherent risk of overshooting the MTD [24]. Therefore, ADE should preferentially be applied in trials with OTs that have shown no, or minimal, toxicity in preclinical testing.

An attractive Phase I trial design for OTs is the PK-guided dose escalation (PGDE) [25]. This design is founded on the assumption that dosing based on weight (or body surface

area) might result in different drug plasma concentrations in preclinical studies performed in animals rather than in humans (because of interspecies variability in metabolism, elimination and binding). The PGDE design is intended to achieve rapid dose escalation to a target plasma concentration for the study drug that has been shown to be effective in preclinical animal studies. In the first cohort of patients, drug plasma concentrations are determined as 'area under curve' (AUC) and further dose escalation is dictated by a prespecified target AUC and is typically performed in 3–4 dosing levels. Thus, the number of patients treated at subtherapeutic doses is reduced and the study duration is shortened. A well-described preclinical dose–response relationship with definition of a target AUC and feasibility of a real-time PK-monitoring are requisites for this approach [24].

Another exciting approach that is suited to improving OT development is the design of trials based on the continual reassessment method (CRM) [26]. This method relies on the Bayesian concept of learning from all previously gathered information for further dose escalation. Data from all patients enrolled in the study is used to determine the next dose level [27]. Analogous to PGDE, fewer patients are treated at subtherapeutic doses by CRM and the trial can be completed more quickly. However, the total number of patients enrolled might be higher and the ongoing assessment of toxicity during dose escalation must be performed by an experienced study centre.

Phase II

In general, Phase II is regarded as the most crucial phase in clinical trial development. Whereas the overwhelming majority of OTs demonstrated manageable safety profiles and successfully passed Phase I testing, their activity is challenged in Phase II by either confirming biological activity in a greater patient sample at OBD (Phase IIa) or validating clinical activity (Phase IIb).

Although target regulation at OBD might be shown to be statistically significant by expanding a Phase I study (combined Phase I and Phase II), Phase IIa trials provide the chance to achieve far greater insight into target regulation. The extent and persistence of target regulation at OBD might be tested in this setting through alternative dosing regimes (e.g. continuous versus multiple bolus administration) with the goal of optimizing patient convenience and chemotherapy combination treatment schedules.

The aim of Phase IIb trials is to provide provisional evidence of clinical activity to justify further clinical evaluation. In addition to choosing an appropriate endpoint to demonstrate preliminary clinical activity (e.g. time-to-progression), determination of an appropriate study population is another major challenge in this stage of drug evaluation. The traditional classification of most human tumours solely by organ allocation results in study populations with considerable heterogeneity in tumour biology. Even if target expression at baseline is used as an inclusion criterion, it might not be enough to predict a response to an OT, because the targeted gene might not be the only determinant for tumour response in a particular patient. Therefore, study designs that identify sensitive study populations are considered essential for OT clinical trials.

One trial design that considers this issue is the randomized discontinuation design (RDD) [28]. In a first phase, all patients are initially treated with the novel therapeutic treatment and will be restaged after a defined treatment period. Patients that are progressive under this treatment will be excluded from the study – only those patients exhibiting at least stabilized disease will be randomized to either continue the novel therapy or the standard treatment. Alternatively, placebo could be administered in some cases at the time of treatment discontinuation. The difference in the outcome of these two study arms is used for the assessment of clinical activity. By randomizing responding patients to the novel treatment, this trial design allows an 'enrichment' of a sensitive population in the second phase. Although carry-over effects in the control arm cannot be ruled out, the RDD provides an attractive approach for studying patients sensitive to an OT without the requirement of a predefined biological inclusion criterion (i.e. target expression).

Phase III

Phase III trials are large randomized trials that compare the novel therapeutic versus the established standard therapy, in terms of a meaningful clinical benefit. In addition to the primary-endpoint total-survival, the prospective evaluation of secondary endpoints, such as progression free survival, response rate or quality of life, could add supportive data for the approval process.

In addition to defining a sensitive target population, other aspects of Phase III trial design with OTs warrant consideration. Phase III trials are commonly performed as randomized, dose-controlled clinical trials (RDCT), which means that a fixed dosing regime is administered to every patient. However, the interpatient variability in drug metabolism that is caused by distinct pharmacogenetic profiles or concomitant medications for individual patients might result in substantial differences in plasma concentrations of the study drug. Instead of RDCT, with a fixed dosing regime, randomized, concentration-controlled trials (RCCT) have been proposed, where dosing is individually adapted to obtain prespecified plasma concentrations.

By controlling PK variability, active and non-toxic OT plasma levels can be assured. As a result, a RCCT study design allows for a reduced sample size in Phase III trials and more open eligibility criteria. The principal limitation is that real-time PK monitoring is required. However, given the serious indications and the expected high treatment costs for OTs in oncology, the benefit might outweigh the additional costs. It should be noted that plasma concentration-controlled dosing is standard of care for some antiepileptic or immunosuppressive drugs.

Conclusions

The ultimate goal of drug development is to provide a safe and effective therapy with meaningful clinical benefit. For OTs, innovative trial designs with alternative endpoints apparently have the potential for improving the efficiency of clinical testing. For that reason, it will be essential to guide the dose escalation in the early phases of clinical trials based on the intended target inhibition (OBD), rather than relying exclusively on the traditional concept of MTD. Indeed, intensified PK and PD monitoring will be more intensive in terms of time and labour, thus necessitating the use of research centres experienced in translational research. In later phase trial designs, enriching a sensitive study population, as well as selecting alternative endpoints, could be crucial for demonstrating clinical activity. Finally, more efforts should be invested in the retranslation of information (e.g. from biopsies) from late-stage clinical trials back to the early oligonucleotide drug development process, thus allowing for the selection of more effective OTs or more promising targets.

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