Measures of biosimilarity in monoclonal antibodies in oncology: the case of bevacizumab

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Biosimilars have been available on the European market since 2006 and experience with their use is increasing. The next wave of biopharmaceuticals that are about to lose patent protection consists of more-complicated products, including many monoclonal antibodies. Guidance has been released on the particulars of a biosimilarity exercise involving these products. Considerable challenges exist to establish biosimilarity for anticancer products. An especially challenging product is bevacizumab (Avastin®). On the basis of data available for the innovator product (bevacizumab) we will discuss strengths and weaknesses of preclinical and clinical models and explore the application of novel endpoints to the biosimilar comparability exercise.

Introduction

Legislation allowing the approval of competitor versions of biopharmaceuticals, so-called biosimilars, has been in place in the European Union (EU) since 2004. To receive marketing authorization as a biosimilar in the EU, a manufacturer has to provide a full quality dossier, demonstrating that a product is manufactured according to regulatory standards. In addition, comparative, analytical, preclinical and clinical studies should demonstrate comparable quality, safety and efficacy to a product authorized in the EU [1]. Thus far 14 products, including epoetins, filgrastims and recombinant human growth hormones, have received marketing authorization as biosimilars. The patents for some of the best-selling monoclonal antibodies (MAbs) are about to expire and there is a lot of interest in developing biosimilar versions of these products [2,3]. Recently, a guideline outlining the approval criteria for biosimilar MAbs was released in the EU, which states that a case-by-case approach will be adopted to determine the requirements of the comparability exercise [4].

The primary sequence of the biosimilar antibodies should be identical to the reference product and the product attributes should be comparable for biosimilars [4]. However, differences in post-translational modifications such as glycosylation or oxidation can affect the immunogenic potential of a product. In addition, subtle differences in effector functions and pharmacokinetics (PK) and pharmacodynamics (PD) of two products can occur as a result of differences in the production of cell lines and/or production processes. For example, levels of fucosylation can influence the ability of the antibody to induce Antibody dependent cell-mediated cytotoxicity (ADCC), and high mannose concentrations have been linked to altered serum half-life [4]. Furthermore, it can be particularly challenging to establish comparable efficacy and safety for anticancer products based on preferred endpoints such as overall survival (OS) or progression-free survival (PFS). Therefore, novel endpoints, other than patient benefit per se, could be acceptable to establish biosimilarity [4]. The questions that arise are: which strategy is required to establish biosimilarity in MAbs used in an oncology setting? Which instruments are optimal to detect relevant differences between a biosimilar MAb and its reference product? Currently, there are six MAbs authorized as anticancer agents. Here, we have chosen one product, bevacizumab (Avastin®), to assess challenges in establishing biosimilarity of MAbs licensed as anticancer agents (Box 1). On the basis of the available preclinical and clinical experience of bevacizumab we will review possibilities and challenges of a comparability exercise

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BOX 1

About bevacizumab

Bevacizumab (Avastin®) is a 149 kDa humanized IgG1 monoclonal antibody that selectively binds and inhibits vascular endothelial growth factor (VEGF), a 45 kDa homodimeric glycoprotein. VEGF binds and activates a receptor tyrosine kinase stimulating the growth of blood vessels (angiogenesis), which has a central role in the growth, invasion and metastasis of tumors. Overexpression of VEGF has been found in several tumor types and has been linked to a worsened prognosis in patients [50]. Bevacizumab was first authorized in the USA in 2004, for the treatment of metastatic colon and rectal cancer, in combination with 5-fluorouracil-based chemotherapy – and Europe followed in 2005. In Europe, bevacizumab is currently authorized as an add-on therapy to various chemotherapy agents in the treatment of non-small-cell lung carcinoma, colorectal neoplasms, renal cell carcinoma, ovarian neoplasms and breast neoplasms. In the USA bevacizumab is no longer indicated for the treatment of metastatic breast cancer, but is indicated as a second-line treatment as monotherapy for the treatment of glioblastoma. The patent of bevacizumab will expire in the USA in 2017 and in the EU in 2019 [2].

for MAbs in oncology. We assume that the product is sufficiently similar in terms of quality and we focus our review on *in vitro*, *in vivo* and clinical studies.

Preclinical

In vitro *studies*

Nonclinical similarity studies start with *in vitro* studies to demonstrate comparable functional binding. For the development of bevacizumab, specific binding of bevacizumab (and its parent murine antibody, A4.6.1) to vascular endothelial growth factor (VEGF) was demonstrated by various *in vitro* affinity binding assays, such as radioligand binding, immunoprecipitation assays and surface plasmon resonance (SPR) assays [5–7]. These assays enable a quantitative assessment of the affinity of the antibody to its target. Because VEGF is a soluble target, evaluation of ADCC and complement-dependent cytotoxicity (CDC) is not required. Potent inactivation of VEGF by bevacizumab and A4.6.1 was demonstrated in several assays that model hallmarks of angiogenesis [8]. Cell sorting and counting assays demonstrate that endothelial cells (ECs) could be dose-dependently inhibited after incubation with bevacizumab. In addition, an *ex ovo* model of angiogenesis, the chick embryo chorioallantoic membrane (CAM) assay, offered visible semi-quantitative confirmation of inhibition of vessel formation after treatment with bevacizumab [5]. Bevacizumab demonstrated similar efficacy and potency to A4.6.1 *in vitro*. For example, A4.6.1 and bevacizumab showed 90% inhibition of proliferation of capillary endothelial cells at 500 ng/ml. The ED_{50}s of A4.6.1 and bevacizumab were comparable at 48 ± 8 and 50 ± 5 ng/ml, respectively (European Medicines Agency: http://www.ema.europa.eu/ema/index.jsp?curl=pages/medicines/human/medicines/000582/human_med_000663.jsp&mid=WC0b01ac058001d124). Likewise, these assays could be used to demonstrate similarity. Additional assays could include inhibition of migration of ECs in a scratch wound assay, where closure of an inflicted scratch in an EC culture after stimulation with VEGF is measured. A sensitive *in vitro* assay is the 3D spheroid sprouting assay, which simulates the complex behavior of ECs in angiogenesis [9]. Functional *in vitro* studies are highly accurate, sensitive and reproducible and enable a detailed comparison of the affinity, potency and downstream signaling modulation of anti-VEGF antibodies.

In vivo *studies*

The need for *in vivo* studies is determined based on the outcome of the *in vitro* program and they can include PD, PK and safety studies. If novel excipients are used in formulating the biosimilar or information of their use in the clinic is limited, local tolerance studies might also be required. For bevacizumab PD studies were performed in severe combined immune deficient (SCID), athymic and nude mouse or athymic rat xenograft models of various tumors. In these models, bevacizumab reduced tumor growth and increased survival [10–16]. However, for a biosimilarity exercise, xenograft models can have limited value because many variables can influence the outcome, including the choice of mouse strain, tumor type and size at implantation and the implantation site itself, all of which add to the complexity of interpreting the study results and can be difficult to reproduce [14,15]. Directed *in vivo* angiogenesis assays, which can quantitatively compare inhibition of angiogenesis in tumors, could be used as an alternative PD model that would reduce the number of test animals required (European Medicines Agency: http://www.ema.europa.eu/ema/index.jsp?curl=pages/medicines/human/medicines/000582/human_med_000663.jsp). *In vivo* PK studies should provide a quantified comparison between the reference and biosimilar. Single-dose studies, including internal comparability studies between different bevacizumab formulations and batches, were conducted in mouse and rat models and met bioequivalence criteria. Because bevacizumab only binds to cynomolgus monkeys and rabbit VEGF, these were the two model species of choice for most repeated dose PK and toxicity studies. In rabbits, bevacizumab induced the formation of antibodies after eight days. Therefore, non-human primates might be the only valid alternative for longer-term PK studies. However, ethical (and economical) considerations lead to the use of fewer non-human primates in a study, which might affect the sensitivity of the *in vivo* study. Although many MAbs are immunogenic in non-human primates, bevacizumab generally did not induce formation of antidrug antibodies (European Medicines Agency: http://www.ema.europa.eu/ema/index.jsp?curl=pages/medicines/human/medicines/000582/human_med_000663.jsp).

Three repeated dose toxicity studies were conducted in non-human primates to evaluate the safety of bevacizumab (European Medicines Agency: http://www.ema.europa.eu/ema/index.jsp?curl=pages/medicines/human/medicines/000582/human_med_000663.jsp). In these studies, bevacizumab was generally well tolerated and main side effects were related to the pharmacology of the drug including physial displasia of the distal femur, degeneration of the cartilage matrix and disruption of the menstrual cycle in female monkeys. Two common side effects of bevacizumab in clinical trials were hypertension and proteinuria. Interestingly, no adverse effects of bevacizumab on blood pressure or urinalysis were observed in preclinical safety studies with non-human primates (European Medicines Agency: http://www. ema.europa.eu/ema/index.jsp?curl=pages/medicines/human/medicines/000582/human_med_000663.jsp). Repeated dose safety studies in non-human primates are not likely to add substantially to demonstrate biosimilarity and are also not recommended [4].

TABLE 1

Sample size calculations of for noninferiority trials in various authorized indications and possible novel endpoints

	1-Year survival rate	Placebo efficacy (median)	Innovator efficacy (median)	Hazard ratio (95% CI)	NI margin	Sample size required	Refs
Time to event endpoint							
Colorectal cancer							
PFS	30.4%	8.0 months	9.4 months	0.83 (0.72–0.95)[c]	δ = 1.10	3454	[24]
OS[b]	78.9%	19.9 months	21.3 months	0.84 (076–1.03)[c]	δ = 1.10[a]	8546[a]	
Renal cell carcinoma							
PFS	42.9%	5.4 months	10.2 months	0.63 (0.52–0.75)	δ = 1.29	544	[51]
OS	74.2%	21.3 months	23.3 months	0.91 (0.76–1.10)	δ = 1.13[a]	3838[a]	
Non-small-cell lung cancer							
PFS	17.3%	4.5 months	6.2 months	0.66 (0.57–0.77)	δ = 1.25	622	[52]
OS	51.6%	10.3 months	12.3 months	0.79 (0.67–0.92)	δ = 1.13	2502	
Epithelial ovarian cancer							
PFS	55.7%	10.3 months	14.1 months	0.72 (0.63–0.82)	δ = 1.20	1258	[53]
OS	91.0%	39.4 months	39.8 months	0.90 (0.72–1.13)	δ = 1.06[a]	52378[a]	
Breast cancer[c]							
PFS	34.2%	5.7 months	8.6 months	0.69 (0.56–0.84)	δ = 1.22	828	[54]
OS	81.6%	21.2 months	29 months	0.85 (0.63–1.14)	δ = 1.09[a]	11104[a]	
Other endpoints[d]							
Hypertension		6.4%	18.9%	–	δ = 1.97	300	[24]
AMD		62.2%	94.5%	–	δ = 1.26	66	[43]

Sample sizes were calculated using R statistics (Version 2.15, The R-foundation). Time-to-event analyses were calculated using the plansurvct.func function created by Filleron *et al.* [55]. See Supplementary information for an example of a sample size calculation. All calculations were based on a noninferiority trial assuming one control subject per experimental subject, an accrual interval of 12 months and additional follow-up after the accrual interval of 24 months, $\alpha = 0.025$ and $\beta = 0.80$, no dropout is assumed and overall 1-year survival was measured from the primary publications. NI margins (δ) were computed based on the ratio innovator:placebo using the 50% retention method of the point estimate of innovator efficacy vs placebo as described by Tanaka *et al.* [20].

[a] Overall survival was not significantly different in various clinical studies, so care must be taken to interpret a 'noninferiority' margin.

[b] 97.5% confidence interval was provided.

[c] Breast cancer based only on arm receiving bevacizumab in combination with capecitabine (EU authorized indication).

[d] Sample sizes for hypertension and AMD were calculated as binary endpoints using the 'R' gsDesign package for a noninferiority trial with equal efficacy, 1-sided alpha level of 2.5% and power of 80%.

TABLE 2

Incidence of commonly reported adverse events in bevacizumab clinical Phase III trials

Event (%)	Colorectal cancer [24]		Renal cell carcinoma [51]		Non-small-cell lung cancer [52]		Ovarian cancer [53]		Breast cancer[a] [54]	
	Placebo	Bevacizumab	Placebo	Bevacizumab	Placebo	Bevacizumab	Placebo	Bevacizumab	Placebo	Bevacizumab
Hypertension										
(Any)	6.4	18.9	9	26	NA	NA	10	26	NA	NA
(Grade 3)	1.2	3.7	<1	3	0.5	6.8	<1	6	1.0	10.1
Proteinuria										
(Any)	NA	NA	3	18	NA	NA	2	4	NA	NA
(Grade 3)	0	<1	0	7	0	2.6	<1	1	0	2.2
Bleeding										
(Any)	25.9	30.5	9	33	NA	NA	11	38	NA	NA
(Grade 3 or 4)	1	2	<1	3	0.7	4.4	<1	1	0.5	0.2
Neutropenia										
(Any)	NA	NA	7	7	NA	NA	29	28	NA	NA
(Grade 3 or 4)	NA	NA	2	4	16.8	25.5	15	17	1.0	1.2
Thrombotic event										
Venous thrombotic event	4.9	7.8	<1	3	NA	NA	4	7	3.5	5.0
Arterial thrombotic event	1.0	1.7	<1	1	NA	NA	1	4	1.5	1.5
Gastrointestinal perforation	<1	<1	0	1	NA	NA	<1	1	0	0
Wound healing complications	<1	<1	1	1	NA	NA	2	5	0	0.7

Grade 2/3 hypertension = requiring medication.

[a] Breast cancer based only on the arm receiving bevacizumab in combination with capecitabine (EU authorized indication).

Clinical PK and PD

The clearance of MAbs is determined by a nonspecific clearance pathway, mediated by the Fc region, and a specific clearance pathway, which is dependent on target binding via Fab regions. The nonspecific pathway generally shows linear clearance, whereas the specific pathway can be saturated, dependent on dose of the MAb and the expression of the target [16]. To date, there are no PD biomarkers for clinical efficacy of bevacizumab. Given the long elimination half-life and the possibility of immunogenicity of MAbs, the EMA accepts parallel group PK studies, rather than crossover studies [4]. Bevacizumab is not cytotoxic and it should be feasible to study PK in volunteers. However, all PK studies for bevacizumab presented in the European Public Assessment Report (EPAR) involved patients rather than healthy volunteers, which could indicate that regulatory bodies consider the risks associated with bevacizumab unacceptable for healthy volunteers. Single-dose studies are preferred, although ethical considerations might prevent performing a single-dose study in patients, because the current standard of care requires multiple doses of bevacizumab to be administered.

Expression levels of VEGF are low and PK studies investigating bevacizumab doses ranging from 0.1 mg/kg to 10 mg/kg found linear PK for all doses studied, with a terminal half-life of ±20 days (range 11–50) regardless of concomitant chemotherapy (European Medicines Agency: http://www.ema.europa.eu/ema/index.jsp?curl=pages/medicines/human/medicines/000582/human_med_000663.jsp) [17,18]. On the basis of limited available data, there is no interaction between various chemotherapy agents (including carboplatin and paclitaxel) and bevacizumab. PK studies for currently authorized biosimilars studied the authorized dose of the comparator product and guidelines recommended that PK should be studied using the lowest possible dose [4]. Therefore, the recommended starting dose for colorectal cancer of 5 mg/kg will be a probable candidate for PK studies. Eight PK studies were included in the authorization dossier for bevacizumab, two of which examined the authorized dose for colorectal cancer of 5 mg/kg every two weeks. The clearance rate was calculated at 2.79 ± 0.849 ml/kg/day (mean ± SD), the mean area under the curve was calculated at 2009 ± 653 μg/day/ml (mean ± SD), whereas the median elimination half-life was estimated to be 19.9 days [19]. Although there is considerable inter-individual variability in availability of bevacizumab, even at 2.5 mg/kg dosing, it is expected that >98% of VEGF is bound to bevacizumab [19]. Therefore, even significant variability in clearance is not likely to influence efficacy. None of the studies identified antibodies to bevacizumab.

Clinical efficacy and safety

Given the absence of validated PD markers, confirmatory clinical efficacy studies will be needed. Commonly applied endpoints are preferred in the biosimilar MAb guideline, including OS or PFS, but it is recognized that these might not be sensitive enough for establishing comparability. To illustrate the challenges in sensitivity we calculated sample sizes required for trials in indications that are licensed in the EU to demonstrate noninferiority, here defined as retention of 50% of the efficacy of the innovator over placebo [20]. The noninferiority margin used in our example is illustrative and does not necessarily reflect clinical relevance, which is required by regulatory authorities [21]. More-stringent equivalence trials are preferred, but we chose to determine sample sizes for time-to-event analyses assuming an optimistic scenario (Table 1). Source data were obtained from pivotal trials described in the EPARs and publications of these studies that have appeared in peer-reviewed scientific medical journals. Bevacizumab was approved in combination with irinotecan and 5-fluorouracil (IFL) for the treatment of metastatic colorectal cancer. However, the standard of care has evolved and so has the relative improvement in PFS and OS. European guidance on the evaluation of anticancer medicinal products in humans stipulates that the choice of reference regimen should be selected from the best available, evidence-based therapeutic options [22]. This means that a widely used, but not necessarily licensed, regimen with a favorable benefit–risk profile should be chosen. At this moment, European practice guidelines recommend using oxaliplatin plus 5-FU combination or capecitabine plus 5-FU combination plus bevacizumab as the first-line treatment for colorectal cancer [23]. Therefore, for the calculation of the required sample size in colorectal cancer patients we included study NO16966 [24]. For all other authorized indications we included those trials that included treatment regimens that were included in respective treatment guidelines, which were mostly the pivotal trials (Table 2).

Trials designed to establish noninferiority for PFS require sample sizes that range from 544 patients in the metastatic renal cell carcinoma indication to 3454 in advanced metastatic colorectal cancer. Sample sizes required to demonstrate noninferiority using OS as the endpoint were 2502 patients for non-small-cell lung cancer and many more patients for the other authorized indications. Bevacizumab did not show significant improvement in OS versus the respective control groups, and it is not possible to establish a valid noninferiority margin for these endpoints. In principle, to establish noninferiority it is recommendable to perform comparative studies in patients with poor prognosis that benefit most from bevacizumab (e.g. patients with renal cell carcinoma). In various trials, the improvement in PFS was not supported by similar improvements in OS (at least not for the duration of the trial). Other widely used efficacy endpoints in oncology, such as response evaluation criteria in solid tumors (RECIST), might not be good predictors of efficacy, because treatment with bevacizumab does not result in tumor shrinkage [25,26]. Accepted clinical endpoints might thus be unsuitable to establish biosimilarity.

Safety events as the possible endpoint

The monoclonal antibody guideline states that safety outcomes can also be considered a measure of biosimilarity [4]. To be suitable for a biosimilarity exercise, safety issues need to be relatively frequent and occur relatively early. In Table 2 commonly reported adverse events for bevacizumab are presented. Hypertension is one of the most common side effects of bevacizumab occurring in up to 22.4% of the patients in Phase III clinical studies. There are several hypotheses about the biological mechanisms underlying this effect, including a reduction of VEGF-induced nitrogen oxide (NO) production and systemic changes to the vascular network [27]. Several studies have investigated hypertension as a predictor of clinical response. A single-arm observational study reported that

the median PFS was 14.5 months in patients with grade 2/3 hypertension versus 3.1 months in patients without grade 2/3 hypertension [28]. Two other studies found a PFS survival of 15.1 or 10.5 months in patients with hypertension versus 8.3 or 5.3 months in patients without [29,30]. Interestingly, the median time to onset of hypertension was 1 month, and 95% of the cases developed within 6 months. Another study failed to show an improved radiological and clinical response rate in patients with poor-risk colorectal liver-only metastases that developed hypertension [31]. A prospective analysis of 218 patients, including 184 colorectal cancer patients, found that patients who developed grade 2/3 hypertension had improved overall survival versus patients without hypertension (median PFS 29.9 months vs 17.2 months) [32]. Other smaller studies also reported an improved outcome in patients who developed hypertension [33]. A retrospective analysis of the pivotal studies of bevacizumab found that hypertension was predictive in study AV2107g but not in study NO16966 [34]. Most studies investigating hypertension were retrospective and did not use standardized methods. Although hypertension can be a suitable biomarker, its etiology and relation to clinical outcome remain uncertain [35]. Nevertheless, hypertension seems to be a pharmacological, dose-dependent ADR that can have a sufficiently high incidence to be a suitable surrogate endpoint of a clinical comparability study. A trial choosing hypertension as a primary endpoint would require significantly fewer patients; 150 patients per arm can have enough power to demonstrate noninferiority (Table 1).

Other safety endpoints

A meta-analysis reported that proteinuria occurred in as many as 41–63% of patients receiving bevacizumab, is dose dependent and associated with the occurrence of hypertension [36]. Proteinuria has been associated with improved OS and PFS in a limited number of case series [37]. A meta-analysis including bevacizumab for various solid cancers did not identify an association between high-grade proteinuria and OS or PFS [38]. Although proteinuria can have a high incidence, its relationship to the pharmacology of bevacizumab is less well established. Also, bleeding events have been reported frequently in patients receiving bevacizumab, mainly mucocutaneous hemorrhage (20–40%) and epistaxis (22–34%) (European Medicines Agency: http://www.ema.europa.eu/ema/index.jsp?curl=pages/medicines/human/medicines/000582/human_med_000663.jsp). Bleeding events have not been linked to bevacizumab efficacy and increases in bleeding events were mainly observed in non-small-cell lung cancer patients and, to a lesser extent, in colorectal cancer patients [39]. Other adverse events occur too infrequently to be suitable markers for a clinical comparability study.

Other biomarkers

Various candidates, including baseline VEGF levels, circulating VEGF levels, capillary density and intercellular adhesion molecule 1 (ICAM-1) levels have been investigated as biomarkers predictive for VEGF activity, but thus far none has been identified that reliably predicts clinical efficacy [25,40,41]. Other studies have focused on identifying prognostic biomarkers that could help to identify patients that benefit more from bevacizumab, including genotypes, baseline values of various proteins, baseline proteinuria, cell counts and miRNAs, but so far this search has not yielded viable candidates [25]. Although such a biomarker could have clinical importance, its use in a comparability exercise is of questionable value, because a trial including patients that respond well to treatment will increase rather than decrease the number of patients required to establish noninferiority.

Unauthorized indications

It has been demonstrated that bevacizumab is efficacious in the treatment of age-related macular degeneration (AMD). The efficacy of bevacizumab was compared with ranibizumab (Lucentis®), a PEGylated anti-VEGF Fab fragment authorized for the treatment of AMD in two large trials involving >1800 patients [42,43]. Both trials concluded that monthly administered bevacizumab has equivalent efficacy to ranibizumab. The effectiveness of bevacizumab in AMD is considerable and beneficial effects are seen within 12–24 weeks of treatment. A noninferiority trial to establish retention of 50% of the effect of the originator versus placebo would require 66 patients, assuming efficacy measured as percentage of patients losing visual acuity according to Rosenfeld *et al.* (Table 1) [44]. However, in AMD bevacizumab is administered intravitreally at doses that are only a fraction of those used in oncology. This results in a different distribution profile, which makes it challenging to extrapolate the safety profile to the cancer indication.

Discussion

Here, we have reviewed various challenges and opportunities for a biosimilarity exercise of a biosimilar anti-VEGF antibody. A comparability exercise requires a stepwise approach and each step has strengths and weaknesses. *In vitro* assays are the most sensitive ways to detect differences in pharmacological activity. They are easily quantifiable and can be more specific and sensitive than studies in animals [45]. Several assays exist that have a strong discriminative power. However, it is not always clear how much difference can be accepted. For example, ranibizumab shows considerable differences to bevacizumab in various *in vitro* assays but comparable clinical efficacy in the treatment of AMD [43,46–48]. Furthermore, *in vitro* assays cannot evaluate changes that occur by modifications *in vivo*. Internal PK comparability studies for bevacizumab have been successfully conducted in rat and rabbit, but the sensitivity and reproducibility of *in vivo* studies are limited and results should be interpreted with caution. Most common *in vivo* PD models for cancer are rodent xenografts, which do not optimally represent the situation of advanced metastatic cancer. *In vivo* safety studies in non-human primates did not reveal limiting toxicities and are not likely to generate additional information. Therefore, clinical studies will be required regardless of the outcome of *in vivo* studies.

PK studies are likely to be performed in patients at a dose of 5 mg/kg. However, validated PD markers that are indicative of efficacy do not currently exist for anti-VEGF therapy. PK shows linear kinetics and is an important measure to demonstrate comparability. Demonstrating similar PK behavior is even more important for MAbs in oncology given the challenges in establishing similarity based on clinical efficacy outcomes. Longer-term clinical efficacy and safety studies will probably be required. As a principle, a clinical endpoint should be selected that fulfills the following requirements: (i) sensitive enough to detect small

differences; (ii) measurable with sufficient precision; and (iii) clinically relevant for the target population. Given the long time it requires to measure efficacy and the relatively modest benefit of bevacizumab in addition to existing chemotherapy regimens, large trials are required that follow patients for long times to establish noninferiority. Performing clinical studies in patients at high risk of disease progression such as non-small-cell lung cancer or renal cell carcinoma, which are likely to benefit from bevacizumab, could reduce the sample size required but will still require substantial numbers of patients that all need to be followed up for a considerable amount of time. Furthermore, it might be challenging to recruit a sufficient number of patients for trials in some less prevalent cancers. When designing a noninferiority trial, identifying probable responders will not reduce the required sample size. Hypertension could be a suitable surrogate measure because it is widely recognized as a pharmacological effect that has also been linked to improved clinical outcome and becomes apparent within a matter of months. Other adverse events like proteinuria and/or bleeding events might also be considered, but their discriminative power is lower and they lack clinical rationale. Although choosing hypertension as a surrogate endpoint will significantly reduce the amount of patients required in a comparability study, there are also considerable challenges. There is a limited amount of prospective data available linking hypertension to clinical outcomes, and the assessment of hypertension in the various studies has been far from standardized [35].

Another route could be to assess efficacy of bevacizumab in AMD. Establishing comparability in AMD has obvious appeal, because it is highly efficacious and it can be established relatively quickly with fewer patients, when compared with accepted outcomes in cancer trials. Although AMD is not an authorized indication of bevacizumab, the EMA guidelines leave room for establishing comparability in nonauthorized indications. However, 'the applicant should justify that the model is relevant as regards efficacy and safety, and sensitive to demonstrate comparability in the indication(s) applied for' [4]. If it is considered that the mechanism of action is the same in AMD, it is conceivable that a three-armed trial comparing a biosimilar to bevacizumab and a third arm including ranibizumab is conducted to ensure comparability to an authorized indication could be an acceptable surrogate for anti-VEGF activity in humans. Alternatively a third arm including placebo could be chosen to validate the chosen noninferiority margin as recommended by current guidelines [25]. Although this might demonstrate comparable efficacy, questions remain regarding the comparability of the safety of the product when administered systematically in an oncology setting. All residual concerns about the safety and efficacy of the product at the level of the prescribers and patients could prevent the uptake of biosimilars in clinical practice [49].

None of the studies involving bevacizumab identified antibodies against the product. However, it is possible that a biosimilar version of bevacizumab induces antibodies. In this case it is probable that the product's PK and thus its efficacy and possibly safety profile are affected. In this case it will be hard to conclude that a product is biosimilar. Therefore it is of the utmost importance that immunogenicity is studied using validated assays and taken into account in the overall analysis of efficacy and safety in a preplanned manner.

Our case study demonstrates that anticancer products such as bevacizumab might also force regulatory authorities to reconsider the objective of the comparability exercise. Currently, European legislation requires a product to 'demonstrate biosimilarity' (EUROPA: http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CELEX:32003L0063:en:NOT), but this could be altered to 'exclude excessive dissimilarity'. A similar position has been adopted for erythropoietins. The key concern regarding erythropoietins is that a biosimilar product is more immunogenic than the innovator product. Pre-licensing data are expected only to identify 'excessive immunogenicity' and it is recognized that the real frequency of pure red cell aplasia, a severe consequence of immunogenicity for erythropoietins, can only be established post authorization [overview of comments received on the guideline on nonclinical and clinical development of similar biological medicinal products containing recombinant erythropoietins (EMEA/CHMP/BMWP/301636/2008): http://www.ema.europa.eu/docs/en_GB/document_library/Other/2011/01/WC500101123.pdf]. Likewise, for anticancer MAbs it could be conceivable that a biosimilar is granted authorization on an extensive *in vitro* and *in vivo* package, comparable PK, comparable immunogenicity and clinical efficacy using a nonvalidated PD marker, provided that sufficient long-term efficacy data will be obtained post authorization, for example through long-term follow-up of patients included in the clinical efficacy trials or through post authorization observational studies.

Concluding remarks

It can be conceptually challenging to 'demonstrate' that two products are similar. Clearly there is no 'one size fits all' answer to the challenges posed by complicated products such as bevacizumab. Comparability of such products needs to be investigated at all levels. To allow the authorization of biosimilars in oncology at realistic costs requires novel approaches to the clinical development program that might conflict with the existing biosimilar framework. Care must be taken to ensure that an alternative approach generates relevant data to establish comparable efficacy and safety. Although the biosimilarity exercise needs to convince regulatory bodies to grant marketing authorization, ultimately, prescribers and patients need to feel confident that a biosimilar product is as safe and efficacious as the original product.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.drudis.2013.05.004.

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