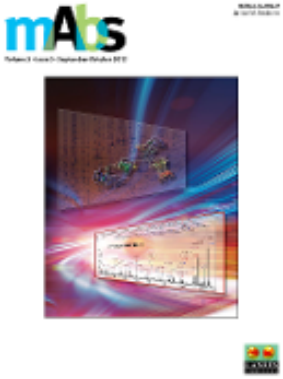

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Immunogenicity of mAbs in non-human primates during nonclinical safety assessment

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Immunogenicity of mAbs in non-human primates during nonclinical safety assessment

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The immunogenicity of biopharmaceuticals used in clinical practice remains an unsolved challenge in drug development. Non-human primates (NHPs) are often the only relevant animal model for the development of monoclonal antibodies (mAbs), but the immune response of NHPs to therapeutic mAbs is not considered to be predictive of the response in humans because of species differences. In this study, we accessed the drug registration files of all mAbs registered in the European Union to establish the relative immunogenicity of mAbs in NHPs and humans. The incidence of formation of antidrug-antibodies in NHPs and patients was comparable in only 59% of the cases. In addition, the type of antidrug-antibody response was different in NHP and humans in 59% of the cases. Humanization did not necessarily reduce immunogenicity in humans. Immunogenicity interfered with the safety assessment during non-clinical drug development when clearing or neutralizing antibodies were formed. While important to interpret the study results, immunogenicity reduced the quality of NHP data in safety assessment. These findings confirm that the ability to compare relative immunogenicity of mAbs in NHPs and humans is low. Furthermore, immunogenicity limits the value of informative NHP studies.

Introduction

The advent of recombinant technology some 30 years ago led to revolutionary novel methods of drug development that allowed the production of any protein-based drug in cell culture. These protein-based biopharmaceuticals offer important advantages over classical small-molecule drugs, such as longer half-lives and very high specificity. In contrast to small molecules, which are metabolized, therapeutic proteins are degraded into their constituent amino acids. As a result, most adverse effects are a result of exaggerated pharmacodynamics.[1] An unsolved challenge in biopharmaceutical development is that these proteins ultimately become immunogenic in some patients, provoking an immune response. The immunogenicity of the agents is dependent on specific properties, such as protein folding, aggregation, post-translational modifications and the presence of B and T cell epitopes. In addition, the presence of impurities in the formulation, the route of administration, mode of action, patient population and treatment regimen may all affect immunogenicity.[2] In clinical practice, a drug-evoked immune response can lead to a host of side effects, such as serum sickness, hypersensitivity and injection site reactions or, in some rare cases, hazardous cross-reactivity with endogenous proteins.[3] More commonly, the immune response leads to a loss of drug efficacy because of the development of neutralizing or clearing antidrug antibodies (ADAs).[4]

The engineering of proteins may yield potentially marked reductions in immunogenicity of protein-based drugs.[5] It is, however, difficult to evaluate the immunogenicity of mAbs because there are few robust and predictive bioinformatics approaches or in vitro screens to measure and characterize the immune response. Bioinformatics approaches have been developed that can identify immunogenic T cell epitopes,[6] and removal of these T cell epitopes is suggested to reduce immunogenicity.[7] Harding et al. have shown that removal of $CD4^+$ T-helper cell epitopes from V-region peptides of the chimeric antibody cetuximab by humanizing these peptides results in a reduction in immunogenic potential.[8] T cell activation assays could also be used to measure the potential of protein drugs to evoke an immune response,[9,10] but, in non-clinical safety assessment, these studies are not required and laboratory animals are routinely used to evaluate immunogenicity. The predictive value of immunogenicity measured in common animal models such as rodents and dogs, however, is low because these models generally overpredict immunogenicity in humans.[11] Non-clinical immunotoxicity studies in animals are also considered inadequate to evaluate safety issues related to immunotoxicity such as hypersensitivity and auto-immunity.[12] The shortcomings of animal studies are reflected in international

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Table 1. Monoclonal antibodies approved in the European Union from 1988 to 2010

INN	Brand name	Approval date
Abciximab	Reopro, CentoRx	1995**
Adalimumab	Humira, Trudexa	2003
Alemtuzumab	MabCampath	2001#
Anti-melanoma antibody fragments*	Tecnemab-K1	1996#
Arcitumomab*	CEA-scan	1996#
Basiliximab	Simulect	1998
Besilesomab*	Scintimun	2010
Bevacizumab	Avastin	2005
Canakinumab	Ilaris	2009
Catumaxomab	Removab	2009
Certolizumab pegol	Cimzia	2007
Cetuximab	Erbitux	2004
Daclizumab	Zenapax	1999#
Denosumab	Prolia	2010
Eculizumab	Soliris	2007
Efalizumab	Raptiva	2004#
Golimumab	Simponi	2009
Ibritumomab tiuxetan	Zevalin	2004
Igovomab*	Indimacis 125	1996#
Infliximab	Remicade	1999
Muromonab	Orthoclone-OKT3	1988**
Natalizumab	Tysabri	2006
Ofatumumab	Arzerra	2010
Omalizumab	Xolair	2005
Palivizumab	Synagis	1999
Panitumumab	Vectibix	2007
Ranibizumab	Lucentis	2007
Rituximab	Mabthera	1998
Sulesomab*	Leukoscan	1997
Tocilizumab	RoActemra	2009
Trastuzumab	Herceptin	2000
Ustekinumab	Stelara	2009
Votumumab*	Humaspect/ Oncospect CR	1998

INN: International nonproprietary name. The infixes that immediately precede -mab indicate the sequence source: u, human; zu, humanized; xi, chimeric; o, mouse; axo, rat/mouse. *Diagnostic/imaging agent. **Country specific approval. #Withdrawn from use in the European Union.

and European immunogenicity guidelines.[13,14] Although the assessment of immunogenicity in non-clinical studies is not recommended as a way to estimate the response in humans, animals may be useful to study some aspects of immunogenicity, such as determining the relative immunogenicity of a biosimilar compared with its reference product[15] and to interpret the findings from animal studies.[16-18]

Besides the low predictive value of immunogenicity in animals, a major handicap is that assays used to assess the immunogenicity of therapeutic proteins are not standardized. A recent industry survey showed that several assays are being used that, although complying with general guidelines, often yield variable results that cannot be compared because of different assay formats. Moreover, the lack of a reference standard, among others, makes these assays semi-quantitative.[19] This makes direct comparisons of immunogenicity between products and species particularly challenging, if not impossible. The relative immunogenicity of mAbs in humans and animals has been assessed in the past.[11,17] Here, we provide an overview and comparison of the immunogenicity in NHPs and humans of all mAbs approved for use in the European Union (EU) through 2010. We also studied the influence of immunogenicity on the ability to interpret non-clinical study findings. For this study, we had access to the marketing authorization applications, which contain all animal studies done to support marketing authorization of mAbs approved in the EU.

Results

Immunogenicity in NHPs. Of 33 mAbs in our analysis (**Table 1**), the safety of 27 mAbs was evaluated in NHPs. MAb29 and MAb31 were not immunogenic in NHP, whereas the remaining 25 MAbs (93%) were. Moreover, the presence of ADAs led to changes in the pharmacokinetic or pharmacodynamic profiles of all but four mAbs: MAb8, MAb13, MAb14 and MAb17. For the remaining 21 products, immunogenicity influenced pharmacokinetics and pharmacodynamics with varying magnitude and severity (**Table 2**). Repeated dose studies for five MAbs (MAb7, MAb9, MAb14, MAb15 and MAb21) were limited in duration by immunogenicity.

Murine antibodies. Three murine mAbs were assessed in NHPs. MAb5 was highly immunogenic because all NHPs developed clearing antibodies by the end of a one-month repeated dose study. MAb7 was moderately immunogenic in NHPs, with repeated dose studies being restricted to two weeks because ADA development was expected to interfere with the safety assessment. MAb8 had immunogenic potential only because ADAs were detected after the last dose in an escalating repeated dose study and it was assessed in only one NHP.

Chimeric antibodies. The safety of five chimeric mAbs was assessed in NHPs. MAb13 had low immunogenic potential and a low-titer ADA response was measured in one control group chimpanzee that was accidentally dosed with MAb13. MAb10 and MAb11 had moderate immunogenic potential. In a two-week repeated dose study with MAb11, the death of one animal with high titers of ADAs was attributed to thrombocytopenia. Two animals with high ADA titers that received a second MAb11 dose rapidly developed thrombocytopenia. MAb9, MAb12 were highly immunogenic. Repeated dose studies with MAb9 were limited to eight weeks because of the development of ADAs, which resulted in the rapid clearance and decreased pharmacodynamics. The considerable ADA response made it difficult to generate conclusive data on the effects of long-term treatment in NHPs.

Humanized antibodies. Eleven of the 12 humanized mAbs included in this study were evaluated in NHP. MAb14, MAb16, MAb17, MAb21 and MAb25 had low immunogenicity in NHPs, whereas MAb20, MAb22, MAb23 and MAb24 had moderate immunogenicity. In the case of MAb21, the duration of meaningful repeated dose studies was limited to one month. Anti-MAb24 antibodies were only measured in repeated dose studies. Interestingly, serum concentrations of MAb24 were increased in NHPs positive for anti-Mab24 antibodies. In addition, perivascular sheathing in some NHPs was associated with high anti-Mab24 antibody titers. MAb15 and MAb18 were highly immunogenic. Antibodies to MAb15 developed in most NHPs within two weeks of single or multiple doses and increased clearance. Immunogenicity was reduced when the dose was increased. In repeated dose studies with MAb18, antibodies to MAb18 were always associated with rapid clearance of the drug. Reliable estimates of pharmacokinetic parameters could only be obtained after the first dose because the development of ADAs interfered with the distribution and pharmacokinetics.

Human antibodies. NHPs did not develop an immune response to MAb29 or MAb31. MAb30 was poorly immunogenic and MAb26, MAb37, MAb32 and MAb33 were moderately immunogenic in NHPs. In the case of MAb30, clearing antibodies developed only in single dose, but not repeated dose, toxicity studies. Antibodies to MAb26 were detected after repeated dosing. In some cases, the presence of ADAs was associated with increased plasma clearance. Two NHPs developed clinical hemolytic anemia that may have been secondary to high antibody titers. It is likely that ADAs developed more often in MAb26-treated animals because a direct Coombs' test, which is used to determine autoimmune hemolytic anemia, suggested that most positive animals were slowly developing anemia. The pharmacokinetic profile of MAb33 was affected by the development of ADAs after repeat dosing, leading to an inverse dose–response relationship. Similarly, clearing antibodies to MAb32 were detected more frequently in NHPs receiving low doses. There, the presence of ADAs was associated with an increased clearance and reduced half-life and anti-MAb32 antibodies developed in up to 97% of the animals after a single low dose. One human mAb, MAb28, was highly immunogenic in NHPs and led to the formation of binding and neutralizing antibodies, and higher doses were needed to maintain exposure. Antibodies to MAb28 were formed in more than 50% of animals tested.

Comparison of immunogenicity in humans and NHPs. Data on the clinical immunogenicity of all mAbs was included in the SPC (29 products) or EPAR (4 products). The induction of ADAs to 20 mAbs affected clinical efficacy, altered pharmacokinetic profiles or caused adverse effects. The presence of ADA to nine mAbs did not have consequences, and 4 mAbs did not give rise to ADA development (**Table 3**). For seven mAbs the incidence of ADAs in NHPs overpredicted the induction of ADAs in humans, and for four mAbs the reverse, underprediction was the case. Sixteen mAbs had comparable ADA incidences in both NHPs and humans. The ADA response was similar for nine mAbs, including four mAbs that did not cause an ADA response in either NHPs or humans. Two mAbs (MAb15 and MAb22) induced clearing and neutralizing antibodies in NHPs, but neutralizing antibodies only in humans. In NHPs, ADAs were more often directed against the Fc-region, resulting in clearing antibodies (17 out of 27 cases) whereas in humans, ADAs were most often formed against the complementarity-determining region (CDR), resulting in neutralizing antibodies (10 out of 33 cases).

Table 2. Incidence and response level, either clearing or neutralizing, of anti-drug antibodies in non-human primates

	Incidence of clearing or neutralizing antibodies in NHP		
ADA incidence in NHP	**Low**	**Intermediate**	**Majority**
Low (0–6%)	MAb12	MAb21	MAb30
	MAb14		
	MAb16		
	MAb17		
	MAb25		
	MAb29		
	MAb31		
Intermediate (6–45%)	MAb9	MAb7	MAb20
	MAb26	MAb10	MAb22
	MAb23	MAb24	MAb32
	MAb27		MAb33
High (> 45%)			MAb5
			MAb8
			MAb11
			MAb13
			MAb15
			MAb18
			MAb28

ADA: Anti-drug antibodies. NHP: Non-human primate.

Discussion

Minimizing immunogenicity remains a considerable challenge in the development of mAbs. While the humanization of mAbs has been successful in reducing the immunogenicity of some products, clinically relevant immunogenicity can still occur despite such modifications.[20-22] Most mAbs in the clinic can be categorized as negligibly or tolerably immunogenic. The onset of ADA formation in the clinic usually occurs after multiple injections that can cover months of treatment. For physicians, treatment management should include frequent monitoring for neutralizing ADA and, when these occur, treatment should be stopped or the patient should switch to a new treatment.[23] Immunogenicity of single-use products such as diagnostics is generally not an issue, but it should be considered that when ADAs to the diagnostic agent develop, they can negatively influence the imaging. Immunogenicity can also result in profound adverse effects after only a few administrations. For example, thrombocytopenia caused by antibodies specific to the murine-derived CDR regions of abciximab is seen in 1% of patients treated with the product.

Table 3. Incidence and effect of anti-drug antibodies in clinical trials in comparison with non-clinical data

Product	Clinical immunogenicity	ADA response clinical	Non-clinical immunogenicity	ADA response non-clinical
		Murine antibodies		
MAb1	Marked	Reduced efficacy due to interference	Not available	Not available
MAb2	Negligible	Diminished efficacy and allergic or hypersensitivity reactions	Not available	Not available
MAb3	Negligible	None	Not available	Not available
MAb4	Negligible	Diminished efficacy	Not available	Not available
MAb5	Tolerable	Diminished efficacy possible	High	Clearing
MAb6	Marked	Neutralizing and hypersensitivity	Not available	Not available
MAb7	Tolerable	Unknown	Intermediate	Clearing
MAb8	Marked	Neutralizing	High	None
		Chimeric antibodies		
MAb9	Tolerable	Allergic or infusion site reactions in few patients	High	Clearing
MAb10	Tolerable	Clearing (in few patients)	Intermediate	Clearing
MAb11	Tolerable	Thrombocytopenia	Intermediate	Thrombocytopenia
MAb12	Tolerable	Unknown	High	Clearing
MAb13	Marked	Neutralizing and hypersensitivity	Low	None
		Humanized antibodies		
MAb14	Negligible	None	Low	None
MAb15	Negligible	Positive Coombs' test, Neutralizing antibodies	High	Clearing and neutralizing
MAb16	Negligible	Allergic reaction in 1 patient	Low	Clearing
MAb17	Negligible	None	Low	None
MAb18	Tolerable	Neutralizing	High	Clearing
MAb19	Tolerable	None	Not available	Not available
MAb20	Tolerable	Clearing	Intermediate	Clearing
MAb21	Tolerable	None	Low	Neutralizing and anaphylaxis
MAb22	Negligible	Neutralizing and hypersensitivity	Intermediate	Clearing and neutralizing
MAb23	Tolerable	Clearing	Intermediate	Clearing
MAb24	Tolerable	Possible role in inflammation	Intermediate	Perivascular sheathing, increased exposure
MAb25	Negligible	None	Low	Clearing
		Human antibodies		
MAb26	Negligible	Unknown	Intermediate	Anemia
MAb27	Tolerable	Neutralizing and binding	Intermediate	Clearing
MAb28	Negligible	None	High	Neutralizing
MAb29	Negligible	None	Low	None
MAb30	Tolerable	Neutralizing	Low	Clearing
MAb31	Negligible	None	Low	None
MAb32	Tolerable	Neutralizing and infusion reactions	Intermediate	Clearing
MAb33	Tolerable	Neutralizing	Intermediate	Clearing

The incidence of this effect could be increased 4-fold after a second administration of abciximab to patients.[24]

Interestingly, the level of humanization did not appear to influence the ADA incidence in humans (**Table 4**). This is surprising because the aim of humanization is to reduce immunogenicity. The merits of humanization have been questioned before.[25] An interesting hypothesis put forward by Clark suggests that the basic idea of humanization to create self-like-proteins is flawed because every B cell clone with a unique specificity also has a unique V-region sequence, and it not likely that tolerance to each clone exists for every new sequence. A complete converse immunological concept is that B cell clones provoke anti-idiotype responses forming an antibody network that regulates immune responses. An equilibrium of these opposites more reflects the

Table 4. Immunogenicity in non-human primates vs. human

		Immunogenicity in NHP			
		Not evaluated in NHP	**Low**	**Intermediate**	**High**
Immunogenicity in clinical trials	Low	Murine Murine Murine	Humanized Humanized Humanized Humanized Human Human	Humanized Human	Humanized Human
	Intermediate	Humanized	Humanized Human	Murine Chimeric Chimeric Humanized Humanized Humanized Human Human Human	Murine Chimeric Chimeric Humanized
	High	Murine Murine	Chimeric		Murine

real situation. Therefore, immunogenicity and humanization of the variable region are not necessarily correlated and decreasing immunogenicity is not a simple matter of increasing the sequence homology.[25,26] There also appeared to be little difference between the relative immunogenicity of chimeric, humanized and human mAbs in NHPs. This is not unexpected because mAbs are probably readily recognized as non-self in NHPs because of species differences in major histocompatibility complex classes and T cell subsets. Most ADA responses in NHPs were directed against the Fc-region (anti-isotype) of the mAbs, resulting in enhanced clearance. In some cases, loss of efficacy and adverse effects were reported after the induction of ADAs. Conversely, in humans, antibodies were more often directed against the CDR (anti-idiotype), resulting in neutralization of the function of the antibody and loss of efficacy. This may occur because the CDR, which is a unique sequence, is the most foreign region of a mAb in humans, whereas both the CDR and Fc-regions are foreign in NHPs.

Because it is difficult to compare directly the immunogenicity of different products in different species, their relative immunogenicity is used to make between-species and -product comparisons. Even with this type of normalization, immunogenicity in laboratory animals is not considered predictive of immunogenicity in humans.[18,27,28] This was confirmed by our analysis, with only 59% of the tested antibodies having comparable incidence of immunogenicity in NHPs and humans. While the incidence of ADAs was comparable in some cases, immunogenicity in NHPs over-predicted the immunogenicity of 30% of the mAbs and underpredicted the immunogenicity of 11% of the mAbs in humans. Bugelski and Treacy established immunogenic classes of recombinant therapeutic proteins based on their source.[11] Prokaryotic and mammalian protein would have very low homology to human protein and these are generally highly immunogenic. Other classes were novel constructs and chimeric, humanized or human antibodies. The homology of these proteins is high, although immunogenicity could be variable. And even NHP studies had limited ability to predict immunogenicity with a trend to over-predict, despite the perceived extensive identity of V-regions (93% at amino acid level for the VH-framework regions and 88–99% for Vκ of cynomolgus monkeys to human antibody sequences). Similarly, comparison of macaque V-regions with that of humans revealed identity between 84% and 97%; however, here differences were considered to possibly result in increased immunogenic response.[29]

The presence of ADAs often interfered with the assay used to detect mAb concentrations in serum. Therefore, pharmacokinetic or safety data should be interpreted with caution. Improvements in assay design could partially overcome some of these difficulties;[30,31] however, proper validation of assays is impeded by the lack of relevant reference standards, and other animal species are often used as controls, which makes the assay less specific. Animals are also the source of antisera needed to develop and validate assays for antibodies to be used during clinical development.

Safety and dose-finding studies make use of laboratory animals, but the potential for immunogenicity complicates the interpretation of kinetic and toxicity data, especially because the development of ADAs can alter or abolish exposure and in some cases result in loss of efficacy. Immunogenicity is not a problem if study groups are of sufficient size and a sufficient number of animals do not develop ADAs, but ethical constraints typically limit NHP studies to small sample sizes. This means that safety studies are particularly difficult to interpret if most or all of the NHPs develop a significant ADA response. For example, in the case of MAb18 the majority of NHPs developed clearing ADAs after the first dose, which affected the interpretability of study results. Therefore, the value of using NHPs during non-clinical drug development will be limited if there is a significant immune

response to the test substance. Continuing the study beyond this point will not yield relevant data and subsequent long-term studies should be reconsidered.[32]

Our study had some limitations. Grouping the NHP data into three operative categories is a necessary over-simplification of immunogenicity. In addition, the various studies differed in their reporting of the rate and effect of ADA development. We used the scale established by Hwang and Foote to classify the immune response, and as the immunogenicity of mAbs is probably higher in animals (the mAbs are foreign) than in humans, we chose to increase the ranges 3-fold.[33] This choice could be considered arbitrary; however, higher or lower ranges would lead to either an under- or over-estimation of immunogenicity in NHPs, respectively. Even though our data set included all mAbs approved in the EU through 2010, there were not enough samples to perform statistical analyses. Therefore, we could only observe and describe trends. Lastly, we only investigated mAbs that received marketing authorization. Inclusion of mAbs that failed during drug development or regulatory review would have provided a larger study cohort, but sufficient immunogenicity data for these mAbs are not publically available.

In conclusion, the results of this study suggest that the immunogenic response in NHPs is poorly predictive of the response in humans, even when using broad categories of immunogenicity. The development of clearing or neutralizing antibodies against the test mAb in NHPs might limit exposure or the duration of repeated dose studies, which in turn can influence the reliability and interpretability of pharmacokinetic, pharmacodynamic and safety data. Lastly, it is difficult to compare the immunogenicity across products and species because of species differences and limits in assay technology. Therefore, NHPs may not be a suitable species for testing mAbs that are immunogenic in NHP, even if these are the only species available.

Materials and Methods

The drug registration files of mAbs and immunoglobulin fragments approved in the EU through 2010 (**Table 1**) were accessed at the Dutch Medicines Evaluation Board. The non-clinical summary and overview, including the tabulated study reports, were used to evaluate the immunogenicity of the mAbs in NHPs. This evaluation was done by assessing the presence of antidrug antibodies in serum from mAb-treated NHPs. Reporting of immunogenicity in animal studies is not standardized in marketing authorization applications, which could be quantitative or qualitative. To normalize the data and to enable comparison between products and species, the incidence of ADAs was classified into three categories, namely, low, intermediate or high. The incidence of ADAs was scored regardless of titer, time of occurrence and persistence of the response. The incidence was scored as "Low" when no or fewer than 6% of animals were positive for ADAs, "Moderate" when between 6% and 45% of animals were positive for ADAs and "High" when more than 45% of the animals were positive for ADAs. These categories were based on those established by Hwang and Foote and were three times higher than similar categories in humans based on higher baseline immunogenicity of NHPs.[33] Three authors individually categorized immunogenicity of the mAbs. When opinions deviated, the mAbs were discussed to come to a final distribution. If changes in pharmacokinetic or pharmacodynamic profiles occurred, the type and incidence of these changes were recorded. Lastly, ADA incidence and the rate of pharmacokinetic or pharmacodynamic changes were aggregated in a 3x3 matrix, with ADA incidence in the rows and rate of pharmacokinetic or pharmacodynamic changes in the columns (**Table 2**).

The Summary of Product Characteristics (SPC) or the European Public Assessment Report (EPAR) was used to determine the immunogenicity of therapeutic mAbs in humans, defined as the proportion of patients in Phase 3 clinical trials that were positive for ADAs. When these could not be retrieved, other online sources such as company websites or web databases were used. The categories established by Hwang and Foote were used: negligible if immunogenicity was seen in 2% of patients or less, tolerable when the incidence was between 2% and 15% and marked if immunogenicity occurred in more than 15% of the patients. Changes in efficacy mentioned in the SPC or EPAR as a result of the development of ADAs were noted. Immunogenicity in NHPs was considered predictive if human and NHP immunogenicity fell into the same operative category. MAbs evaluated in NHP were anonymized.

Disclosure of Potential Conflicts of Interest

PJK van Meer, M Kooijman, V Brinks, C Gispen-de Wied, B Silva-Lima and EHM Moors declare no conflict of interest. H Schellekens participated in meetings and publications sponsored by Amgen, Johnson and Johnson, Roche, Sandoz and Hospira. Part of his research is directly or indirectly sponsored by Roche and Amgen.

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