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Recombinant Oligoclonal Antibody Botulinum Antitoxins Are a Low-Cost Alternative to Equine Antitoxin

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Abstract

We have developed highly potent botulinum neurotoxin (BoNT) antitoxins consisting of three mAbs that bind non-overlapping epitopes and act synergistically to neutralize and rapidly clear circulating toxin. The resulting potency permits a low total antibody dose for treatment (34 mg for a seven-serotype product under the assumptions used here). Based on this high potency, the cost of producing the drug substance per dose is relatively low relative to chronic, high-dose antibody therapeutics. Recombinant human IgG also provides a longer serum half-life than equine $F(ab')_2$ antitoxin and therefore has potential for both treatment and prevention of botulism lasting for months. Here, we analyze the manufacturing costs of the recombinant antitoxin and the development costs of multivalent products and compare them to those of the currently approved equine-derived antitoxin. Approaches to reducing manufacturing complexity, including co-expression and co-purification, formulation, stockpiling, clinical use, analytical control, and multivalent antibody formats, are discussed. Our analysis indicates that recombinant oligoclonal BoNT antitoxins are not intrinsically cost-prohibitive and that development and overall manufacturing costs of Drug Product do not increase linearly with the number of serotypes covered.

Keywords: botulinum neurotoxin; botulism; monoclonal antibody; oligoclonal antibody; antitoxin; cost of goods; biomanufacturing; biodefense; polyclonal

Key Contribution: This paper addresses factors that impact the cost of manufacturing antitoxin for botulinum neurotoxin. Findings show that the primary factor determining the cost per dose is the antitoxin's potency. The extremely high potency of recombinant antitoxin renders a low cost per dose of a heptavalent antitoxin amenable to stockpiling.

1. Introduction

1.1. Recombinant Antibody Drugs

The increasing number of approved antibody-based drugs can be credited to the integration of mice transgenic for the human immunoglobulin locus, antibody humanization through the cloning and engineering of antibody V-genes, and human V-gene display [1]. These advancements enabled the generation of human antibodies against nearly any protein target. Moreover, genetic engineering techniques facilitate the creation of human and humanized antibodies, stable antibody domains, and tuned pharmacokinetic (PK) and affinity properties. Advances in expression systems and bioprocessing have substantially increased antibody production yields and reduced the cost of producing drug substance. Improvements in protein engineering, cell-line development, intensified fed-batch and

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perfusion culture [2], downstream purification, and single-use manufacturing have made recombinant IgG production an increasingly standardized industrial platform.

1.2. Cost of Recombinant Antibody Drugs

Existing studies report manufacturing costs for typical therapeutic monoclonal antibodies, but do not examine the cost implications of low-dose, multi-antibody products intended for stockpile use, nor do they compare those costs with the acquisition price of the current equine product. The cost of goods (COGS) for antibody therapeutics is often perceived as high, particularly compared with small-molecule drugs. However, true manufacturing cost is largely decoupled from the price charged for a commercial antibody drug. Drug price incorporates research and development, clinical trials, regulatory costs, market size, reimbursement, exclusivity, and the cost of failed programs, whereas manufacturing COGS reflects the cost of producing and releasing the drug substance and drug product [3]. For highly potent antibodies given once or infrequently, COGS can therefore represent only a small fraction of the commercial price.

For antibody therapeutics, COGS is strongly influenced by the required dose. Most marketed antibody drugs are intended for chronic administration in oncology or autoimmune disease and are dosed repeatedly at high milligram or milligram-per-kilogram quantities. BoNT antitoxins present a different economic case. They are intended for single-dose or prophylactic administration, and the extraordinary potency of oligoclonal neutralizing antibodies permits total doses in the tens of milligrams. Table 1 compares the potency of the individual botulinum equine polyclonal antitoxin (BAT) serotype components with representative recombinant antitoxin combinations and illustrates the dose advantage that drives the COGS analysis.

Table 1. Comparison of the required dose of equine antitoxin with recombinant antibodies.

Serotype	Equine BAT Potency (IU) [a]	Recombinant Antitoxin Potency (IU/mg)	Recombinant Dose (mg) Equivalent to the Potency of BAT
BoNT/A	4500	>4000	1.12 [4]
BoNT/B	3300	>4000	0.825 [5]
BoNT/C	3000	1600	1.875 [6]
BoNT/D [b]	600	40	15 [6]
BoNT/E	5100	16,000	0.32 [7]
BoNT/F	3000	660	4.5 [8]
BoNT/G [b]	600	213	2.8 [9]

[a] 1 IU = 10,000 mouse intraperitoneal (i.p.) LD_{50}'s except for BoNT E, which is 1 IU for 1000 mouse i.p. LD_{50}'s. [b] The lower potency of antitoxins reflects lower specific activity of BoNT/D and BoNT/G.

In addition to these pharmacologic considerations, biologics manufacturing technology has improved substantially over the past decade. Advances in cell-line engineering, intensified fed- and perfusion culture, high-capacity purification, single-use bioreactors, and expanded CMO capacity have increased productivity while reducing facility and changeover requirements [10–12]. These advances are especially relevant to biodefense products, for which manufacturing campaigns may be intermittent and flexible capacity is advantageous.

Recombinant BoNT antitoxins, therefore, represent a particularly favorable application of antibody technology. Their low dose requirement, single-use administration, standardized IgG format, and high potency decouple manufacturing COGS from the pricing experience of many commercial antibody therapeutics. The relevant question is not whether antibody drugs are expensive in the marketplace, but whether the quantity of

antibody required for an effective BoNT antitoxin can be manufactured economically. The calculations below indicate that it can.

1.3. Cost and Efficacy of Polyclonal Antisera for Prevention and Treatment of Botulism

In the United States, equine-derived botulinum antitoxin is the sole specific therapy available for non-infant botulism and is released by CDC for essentially all suspected cases in which the diagnosis remains plausible [13,14]. Infants with botulism receive human botulinum immune globulin [15], which is not used to treat adults due to limited supply.

Equine-derived heptavalent (targeting serotypes A–G) botulinum antitoxin (BAT®) remains the licensed countermeasure for adult botulism in the United States and is maintained within the Strategic National Stockpile [13,16]. BAT is an $F(ab')_2$ preparation with reported serum half-lives ranging from approximately 7.5 to 34.2 h [16]. This short persistence prevents its use for prophylaxis and reduces the duration of post-treatment protection. BoNT complexes enter the host via the intestinal tract [17], and relapse following antitoxin treatment has been reported in clinical cases [18]. Because BAT contains heterologous equine immunoglobulin fragments, it is inherently immunogenic and requires slow intravenous infusion with monitoring for hypersensitivity reactions, including serum sickness and anaphylaxis [16,19,20]. These characteristics constrain rapid deployment and administration. In contrast, human IgG mAbs have an elimination half-life between two and four weeks [21–24], and NCT05348993 [25], thereby presenting a viable option for both prevention and treatment of botulism.

Besides the seven known serotypes of BoNT (or eight if BoNT H is included [26,27]), significant sequence variability exists within BoNT serotypes [28]. Differences among subtypes are greatest for BoNT/F and BoNT/A (approximately 33% and 15%, respectively), followed by BoNT/B and BoNT/E [29]. These differences can alter toxin epitopes and substantially affect antitoxin potency. If intoxication is caused by a subtype that differs substantially from the toxin used for horse immunization, a larger dose of polyclonal antitoxin may be required. A key advantage of a recombinant mAb-based antitoxin is the ability to select individual antibodies for cross-neutralization of clinically relevant subtypes and to replace components if new sequence variants compromise activity.

The production of equine antitoxin, which relies on repeated immunization and plasmapheresis, also poses challenges for large-scale and renewable manufacturing. Historical U.S. procurement targeted approximately 200,000 adult doses of heptavalent antitoxin and required a substantial horse-production program [30]. Human botulinum immune globulin (BabyBIG®) [15] is likewise constrained by the availability of human immune plasma and is reserved for infant botulism. These animal- and donor-dependent manufacturing approaches are difficult to scale rapidly in response to a major exposure. In contrast, recombinant antibodies are renewable from sequence-defined cell banks, reproducible between lots, and transferable to qualified manufacturing facilities.

The National Institute of Allergy and Infectious Diseases (NIAID) prioritized the development of recombinant, human-compatible antitoxins, and subsequent federal investment generated monoclonal antibody combinations against the major BoNT serotypes. Several serotype-specific products have progressed to human studies, with no unexpected adverse events [21–24], and NCT05348993 [25]. This body of work provides the scientific and clinical foundation for a multivalent recombinant antitoxin and substantially reduces the development risk compared with starting de novo.

1.4. Monoclonal Antibodies for the Prevention and Treatment of Botulism

The assumption that COGS for a recombinant BoNT antitoxin would be prohibitively high should be reevaluated. The relevant determinants are the amount of antibody required

per dose, the productivity of the manufacturing process, and the number of independent manufacturing and release campaigns—not the commercial price of unrelated antibody drugs. The following sections use conservative assumptions to estimate these costs and identify where multicomponent products do and do not add expense.

mAbs for treatment or prevention of botulism must be sufficiently potent to achieve the required mechanism of action at a practical dose. For example, the adult BAT dose contains thousands of International Units (IUs) of activity against BoNT/A. Because oligoclonal recombinant antibodies can achieve very high specific neutralizing potency, comparable activity can be delivered with milligram quantities of IgG. Table 1 summarizes the potency assumptions used to estimate recombinant doses equivalent to the individual BAT components. These values are intended to support the economic model; the final clinical dose of a heptavalent recombinant product would be established during product development.

2. Results

2.1. Relationship of Development Costs to and Cost of Goods to Valency

Cell line generation, process development, and analytical characterization costs increase proportionally with the number of component antibodies. A bivalent product (AB antitoxin, 6 IgGs total) required approximately $12 million for cell line development, $6 million for process development, and $12 million for bioanalytical work ($12 + $6 + $12 = $30 M). Extending to a pentavalent product (CDEFG, 12 IgGs) adds $5 million more for cell line work, $2 million for process development, and similar analytical costs (total $37 M). For a heptavalent antitoxin (ABCDEFG, 18 IgGs), development costs are estimated at $67 million ($30 M + $37 M).

We estimate that the cost of the drug substance for a heptavalent antitoxin treatment, with potency equivalent to equine BAT, would be USD 22.50. For a bivalent treatment covering the two most common serotypes, BoNT A and B, the cost of drug substance for an antitoxin treatment with potency equivalent to equine BAT would be USD 3.75 (see Methods Section and Table 2).

A key consideration for the total heptavalent cost is that regulatory and manufacturing validation costs are independent of the number of constituent antibodies. Non-clinical safety testing ($2 million), Phase I/II clinical trials and animal efficacy studies ($15 million), process validation for drug product formulation ($2 million), and fill-finish operations ($2 million) are independent of serotype coverage. Consequently, the incremental cost to expand from a serotype-specific product to multivalent coverage is modest.

As shown in Table 2, expansion from serotype-specific to multivalent coverage results in modest incremental cost relative to the fixed expenditures required for any biologic countermeasure program. Advances in CHO cell line engineering, intensified fed-batch and perfusion processes, single-use bioreactor platforms, and expanded global contract manufacturing capacity have increased productivity and reduced production costs [10–12]. Contemporary IgG manufacturing is now standardized and scalable.

Table 2. The estimated cost of drug substance per dose for cGMP recombinant botulinum antibody products.

Product (Total mAbs)	Indication, Route of Delivery	Total Dose of Antibodies [a]	Manufacturing Batches Required	Campaign DS Cost [c]	Complete Doses from that Campaign	COGS/Dose
AB (6)	Treatment, IV (BAT-equivalent dose)	5 mg	6	$9.0 M	2,400,000	$3.75
AB (6)	Treatment, IV (conservative)	50 mg [d]	6	$9.0 M	240,000	$37.50
AB (6)	Prophylaxis, 1 month IM	50 mg	6	$9.0 M	240,000	$37.50
AB (6)	Prophylaxis, 3 month IM	100 mg	6	$9.0 M	120,000	$75.00
AB (6)	Prophylaxis, 6 month IM	200 mg	6	$9.0 M	60,000	$150.00
CDEFG (12) [b]	Treatment, IV (BAT-equivalent)	24 mg	12	$18.0 M	1,000,000	$18.00
CDEFG (12) [b]	Treatment, IV (conservative)	40 mg	12	$18.0 M	600,000	$30.00
ABEF (12)	Treatment, IV (BAT-equivalent)	15 mg	12	$18.0 M	1,600,000	$11.25
ABEF (12)	Treatment, IV (conservative)	55 mg	12	$18.0 M	436,364	$41.25
ABCDEFG (18) [b]	Treatment, IV (BAT-equivalent)	30 mg	18	$27.0 M	1,200,000	$22.50
ABCDEFG (18) [b]	Treatment, IV (conservative)	74 mg	18	$27.0 M	486,486	$55.50

[a] The prophylactic clinical doses for antitoxin to serotypes A and B are informed by G03-52-01 IM Phase 2 trial NAC duration (NCT05348993 [25]). 50 mg IM is modeled as approximately one month of dual A, B coverage; 100 mg IM as approximately 3 months. The conservative dose for IV treatment is an assumption for rapid Cmax. [b] Four antibodies cover both serotypes C and D [6], and two antibodies will cover serotype G due to the lower potency of this toxin [9]. [c] Costs are estimated based on a 500 L scale expression with a minimum titer of 5 g/L and 80% overall recovery. A sensitivity analysis is provided in Supplementary Table S1. [d] The maximum dose supported by Phase 1 safety studies [21,22] is 50 mg.

2.2. CMC and Analytical Testing

Because multiple antibodies bind the same toxin target, component-specific assays are required to monitor identity, relative concentration, potency, pharmacokinetics, and stability of the individual antibodies. We have developed protein-domain-based assays that selectively quantify individual antibodies in mixtures directed against a common antigen [31,32]. Antibody-specific domains have been generated for components spanning the seven BoNT serotypes. These tools provide a practical analytical strategy for monitoring individual mAbs during development, manufacturing, release, and stability testing and address an important CMC challenge for highly multicomponent products.

3. Discussion

3.1. Cost of BoNT Existing Antitoxins

The current stockpile of BAT from the horse serum has an unknown amount of remaining stability. Animals have variable responses to immunization, and the antibodies derived from immunizing animals are non-renewable. The production of additional doses as BAT expires requires re-immunization of horses with toxoid, plasmapheresis, and processing, purification, and characterization of the equine antibodies.

3.2. Implications for Development of Antitoxin to Novel Botulinum Toxins

New BoNT serotypes and mosaic toxins continue to be reported [33]. For example, a novel BoNT, serotype H was reported, produced by the bivalent *C. botulinum* strain IBCA10-7060 [26,34]. The novel BoNT is chimeric with an H_C most homologous to BoNT/A1 (~84%) and an H_N and LC most homologous to BoNT/F5 (~64% and ~81% respectively) [26].

Should a new BoNT serotype of concern be identified, the novel toxin would need to be produced and formalin-treated to form a toxoid. The amount of toxoid required per horse has been reported as 0.36–0.39 mg [35]. This is a large amount of toxin given that the LD_{50} for humans of BoNT/A is 2 ng per kilogram [36], producing large quantities of toxin poses significant safety and logistical challenges. Horses would then need to be immunized, serum tested for antibody production against the new toxin, and efficacy studies conducted. In contrast, developing recombinant antibodies against a novel BoNT requires only sufficient toxin to test antibody potency in mouse models of protection, since catalytically inactive toxin or toxin domains can be used for antibody discovery.

Furthermore, the horse immunization strategy is not necessarily faster than recombinant techniques. Horse plasmapheresis is performed 130 days after immunization or later [35], and individual horses have unique and variable responses to immunization.

3.3. Costs Associated with Complying with Regulatory Requirements

Recombinant antibody products are renewable and can be manufactured to reproducible specifications for identity, purity, potency, and composition. Polyclonal products, in contrast, reflect the immune response of the individual animals or donors used for production and therefore require control of biological lot-to-lot variability. Recombinant antibody lots can be released on validated potency assays (e.g., a competitive ELISA bridged to a reference standard) without repeating animal efficacy studies. This is not the case for animal-derived hyperimmune sera. Equine responses are polyclonal and variable; they change with the boosting regimen and when individual horses are replaced. Consequently, each commercial lot of equine botulinum antitoxin must be released on a mouse neutralization assay rather than binding titer alone.

A multicomponent recombinant product still presents substantial CMC and regulatory work, particularly for component-specific assays, comparability, and stability, but those challenges occur within an established biologics regulatory framework. For BoNT countermeasures, efficacy may be established under the FDA Animal Rule when human efficacy trials are not ethical or feasible, with human studies used to establish safety and pharmacokinetics [37].

3.4. Reducing Manufacturing Costs with Co-Expression and Co-Purification

The low total dose of ultrapotent mAbs means that even when manufactured separately, they can remain economical; nevertheless, reducing the number of manufacturing campaigns provides additional opportunities to simplify production. However, significant cost reductions could be achieved through co-expression and co-purification strategies [38–41] instead of individual IgG expression. If compatible antibodies are expressed and purified

together rather than separately, process validation costs for the drug substance would decrease, with fewer process validations of manufacturing runs. We and others have shown that recombinant antibody mixtures can be manufactured using co-culture or co-expression strategies that consolidate multiple antibodies into a single upstream and downstream process [38,42,43]. In the recent six-mAb BoNT/A+B manufacturing study, three antibodies per serotype were co-expressed in CHO-K1 cells at 10 L scale and purified through a shared Protein A, anion-exchange, and cation-exchange process. Individual chromatographic steps recovered >95% of antibody, with overall process yields of approximately 63–75% [38]. The principal advantage is not necessarily increased volumetric productivity; it is the reduction in separate manufacturing campaigns, purification operations, batch records, testing, and validation activities. Measurement of component antibody ratios remains an important requirement in CMC.

3.5. Reducing Manufacturing Costs with Multivalent Antibody Formats

Neutralization of BoNT is achieved by benefits from simultaneous binding of multiple, non-overlapping epitopes, thereby promoting rapid toxin clearance and reducing the likelihood that a single epitope variant will eliminate activity. This mechanistic requirement has motivated the development of multivalent molecular formats that consolidate two or three binding specificities into fewer protein species [44]. Such formats could reduce the number of cell lines, manufacturing campaigns, and analytical components required for a broad-spectrum product, reducing the cost of goods compared to IgG. However, the manufacturing advantage must be balanced against the biophysical complexity of the engineered molecule.

We have designed and produced tri-epitopic and bi-epitopic BoNT antibodies using dual-variable-domain (DVD) architectures [45–47]. The bispecific antibodies have the same potency in vivo as two IgG antibodies and a combination of three DVDs can recapitulate the potency of 6 IgGs (unpublished). Bispecific IgG-like antibodies represent a mature and commercially validated strategy for consolidating epitope coverage while preserving many favorable properties of conventional IgG. Numerous bispecific architectures have now entered clinical development or reached the market. For BoNT antitoxin, bispecifics could potentially reduce the number of independently manufactured molecules, therefore reducing the analytical burden while retaining high-affinity binding to non-overlapping epitopes. Their value will depend on whether they can match the potency, stability, and manufacturability of the corresponding oligoclonal mixtures. IgG mixtures provide a well-characterized manufacturing benchmark, while co-expression and bispecific formats provide a credible strategy to further reduce manufacturing. We are investigating bispecific antibodies as a potential alternative to IgGs (unpublished).

4. Conclusions

Economic modeling using conservative cGMP manufacturing assumptions demonstrates that recombinant oligoclonal antitoxins are not intrinsically cost-prohibitive. Published benchmarks indicate that process development and manufacturing account for 13 to 17 percent of total research and development costs and that commercial drug substance costs range from the tens to the low hundreds of dollars per gram [3,48,49]. Using a 500 L single-use bioreactor, a minimum yield of 5 g/L, and approximately 80% recovery, the modeled drug-substance COGS is approximately USD 0.75/mg. At total doses in the tens of milligrams, antibody drug-substance COGS therefore remains in the tens of dollars per dose. These estimates are not acquisition prices and will vary with manufacturing scale, release testing, fill-finish, yield, and product configuration. Their significance is that the number of

antibodies in a multivalent antitoxin does not, by itself, make recombinant manufacturing economically unrealistic.

Recombinant mAb-based antitoxins offer structural advantages that directly address limitations of equine-derived products: renewable and scalable production independent of animal immunization, defined composition and reproducible potency, the ability to select antibodies for subtype breadth, longer serum persistence, and the possibility of formulations designed for facile stockpiling and rapid administration. Co-expression and co-purification can further reduce the number of manufacturing campaigns, while multi-epitopic or bispecific formats may provide additional consolidation. The remaining challenges are primarily development and CMC questions, final dose, component control, scale-up, formulation, stability, regulatory strategy, and procurement, not a fundamental inability to manufacture the required quantity of antibody economically. Recombinant oligoclonal antitoxins therefore represent a technically credible and economically feasible alternative to animal-derived BoNT antitoxin. Finally, while this analysis is based on the BoNT antitoxin, these findings will apply to other potent recombinant polyclonal products derived from horses, such as snake antivenoms [50,51].

5. Materials and Methods

5.1. Cost of Goods for Recombinant Botulinum Antitoxin

The cost of a therapeutic or prophylactic recombinant antibody product for botulism depends on the number of BoNT serotypes targeted, the total antibody dose, and the manufacturing configuration. We estimated COGS per dose for five product combinations, as shown in Table 2. The base manufacturing model assumes a 500 L single-use bioreactor, a minimum expression titer of 5 g/L, approximately 80% overall recovery, and a total batch cost assumption of USD 1.5 million, including cGMP manufacturing and release activities. This estimate is based on our experience with 500 L single-use cGMP antibody campaigns and is consistent with published benchmarks [3,48,52]. A sensitivity analysis is provided in Supplementary Table S1. Published process-economic models report fed-batch operating costs of goods of approximately USD 99/g, with continuous processes as low as USD 51/g, and show sensitivity to titer, scale, and yield [49,53]. Under these assumptions, approximately 2 kg of released antibody drug substance is produced per batch, at a cost of approximately USD 0.75/mg. We have two dose estimates in Table 2. The values in Table 2 for prophylaxis are based on the proposed clinical dose determined by the duration of protective neutralizing antibody concentration (NAC) in G03-52-01 (NCT05348993, [25]). The values in Table 2 for treatment are estimated using the BAT potency-equivalent estimates (shown in Table 1) and a more conservative estimate (i.e., higher dose) based on the highest dose used in Phase 1 safety studies [21,22].

5.2. Generation of Individual Cell Lines and Cell Banks

Each antibody component can be produced from an independent clonal CHO-K1 cell line. For each monoclonal antibody, a master cell bank (MCB) and working cell bank (WCB) are generated to support long-term manufacturing. This conventional approach provides maximum control over individual antibody yield and quality but creates a separate upstream campaign for each component. Cell-line generation and banking are therefore among the development costs that scale most directly with the number of antibodies.

5.3. Cell Culture and Antibody Production

The mechanism of action of the three-antibody mixture requires simultaneous binding of three antibodies to non-overlapping epitopes, which promotes rapid clearance of circulating toxin [4–9]. To preserve this mechanism while minimizing the total amount of

antibodies, the antibodies are generally combined at approximately equimolar ratios unless potency data support another composition. The low total dose means that even when mAbs are manufactured separately, they can remain economical; nevertheless, reducing the number of manufacturing campaigns provides additional opportunities to simplify production.

Furthermore, we and others have shown that recombinant antibody mixtures can be manufactured using co-culture or co-expression strategies that consolidate multiple antibodies into a single upstream and downstream process [39,42]. In the recent six-mAb BoNT/A+B manufacturing study, three antibodies per serotype were co-expressed in CHO-K1 cells at 10 L scale and purified through a shared Protein A, anion-exchange, and cation-exchange process. Individual chromatographic steps recovered >95% of antibody, with overall process yields of approximately 63–75% [38]. The principal advantage is not necessarily increased volumetric productivity; it is the reduction in separate manufacturing campaigns, purification operations, batch records, testing, and validation activities. Measurement of component antibody ratios remains an important requirement in CMC.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/toxins18110484/s1, Table S1: Sensitivity analysis of modeled drug-substance cost.

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Conflicts of Interest: J.D.M. is an inventor of patents covering the botulinum neurotoxin antibodies described in this article and received royalties from these patents. Those patents are assigned to The Regents of the University of California. M.T.T. was previously employed by commercial entities that manufactured these antibodies; those entities have since ceased operations or been acquired. M.T.T. is affiliated with Albrem BioPharma Inc. S.F.-J. declares no conflict of interest.

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