Clinical Infectious Diseases

BRIEF REPORT

Deployment of Transchromosomal Bovine for Personalized Antimicrobial Therapy

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For decades, intravenous immunoglobulin (IVIg) has provided safe and effective therapy for immunodeficient patients. This proof-of-principle study describes a novel approach to generate personalized IVIg for chronic, antibiotic-resistant infection in real time.

Keywords. polyclonal antibody; personalized medicine; hypogammaglobulinemia; *Mycoplasma hominis*; transchromosomal bovine.

Antibodies, or immunoglobulins (Igs), are composed of 4 polypeptides—2 identical heavy and 2 identical light chains [1]. Igs are critical for host defense against microbial invaders including mycoplasma [1, 2]. In this regard, up to 23% of hypogammaglobulinemic patients in one study developed septic arthritis, of which mycoplasmas were a common isolate (~38%) [3]. Hyperimmune animal serum against mycoplasma has been effective in cases of chronic erosive arthritis in the setting of immunodeficiency, and in some cases resulted in cures [4]. However, animal-derived polyclonal antibody products typically have very high reactogenicity, causing adverse effects such as serum sickness and anaphylaxis.

SAb Biotherapeutics (SAB) has developed a transchromosomic (Tc) bovine platform technology to rapidly produce potent, antigen-specific, fully human Ig against varied disease targets including viruses and bacteria. Tc bovines carry knockouts of the bovine antibody genes, and carry a human artificial chromosome containing human Ig heavy and light chain loci. Tc B lymphocyte development results in the expression of a diverse human Ig repertoire [5, 6]. Immunization of Tc bovine triggers the bovine adaptive immune response, enabling secretion of human polyclonal Ig from Tc bovine B lymphocytes. This technology has the advantages of scale, simplicity, and broad applicability of animal polyclonal production systems without the toxicity associated with animal IgGs.

We describe the application of Tc bovine technology to generate and utilize a biologic therapy personalized for a patient-specific isolate of a drug-resistant organism causing severe, atypical infection. We report on the safety and feasibility of this approach in a single human subject lacking humoral immunity. To assess safety, we monitored patient laboratory values, physical examination, and subjective reports for any adverse reactions to the drug product. To evaluate potential therapeutic value, we collected samples from the primary site of known infection, the right hip, to monitor and quantify *Mycoplasma hominis* burden, and followed serum markers of inflammation during the treatment trial. We obtained longitudinal, patient-reported data using a clinical journal characterizing pain, narcotic use, and hip mobility. In summary, production of SAB-136 and treatment was both feasible and safe. Intermediate- and high-dose therapy correlated with reduced *M. hominis* burden and improved clinical parameters.

CASE REPORT

Our subject is a 68-year-old man diagnosed with *M. hominis* septic polyarthritis in 2009, following recovery of *M. hominis* from a chronic draining right hip incision wound that failed to close after initial hip resurfacing and subsequent replacement in 2009. *Mycoplasma hominis* was also recovered from an aspirate of the right acromioclavicular joint. In January 2010, the patient was found to be hypogammaglobulinemic, likely related to prior therapy for lymphoma in 2004 with rituximab, a monoclonal antibody linked to persistent hypogammaglobulinemia [7–9]. He has been treated with intravenous or subcutaneous Ig replacement therapy (IVIg and SCIg, respectively) since that time. The patient also has chronically undetectable peripheral B cells even though >10 years have passed since his rituximab therapy.

From May 2009, despite multiple courses of antibiotic therapy, the hip wound remained open and *M. hominis* was consistently recovered from wound cultures (Supplementary Table 2). Due to clinical futility, side effects of available antibiotics, case reports, and low minimum inhibitory concentrations in vitro, the veterinary antibiotic, valnemulin, was initiated after US Food and Drug Administration approval for emergency investigational use in 2012. Valnemulin therapy did not elicit wound healing, but while administered, wound cultures sterilized. Valnemulin was used intermittently due to limited availability and associated thrombocytopenia.

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Without valnemulin therapy, *M. hominis* again was recovered from hip wound cultures.

Because hyperimmmune rabbit or goat serum had been reported to ameliorate chronic *M. hominis* infection in hypogammaglobulinemic patients, IVIg lots were tested for activity against the patient's mycoplasma isolate in a bactericidal antibody assay. Available lots with higher relative titers were banked and administered to potentially improve *M. hominis* clearance. Treatment provided modest subjective improvement, but no impact on wound healing. Due to continued symptoms, hip wound drainage, and exhaustion of available therapies, we reasoned that generation of highly specific and potent polyclonal antibodies targeted against the patient-specific *M. hominis* could be a more effective therapeutic strategy.

In partnership with SAB, we immunized a Tc bovine with isolates of the patient's *M. hominis* inactivated with thimerosal. Polyclonal human IgG was purified under current good manufacturing practice from hyperimmune Tc bovine plasma and designated SAB-136. It was shown to have high *M. hominis* binding activity and potent in vitro mycoplasmacidal activity (Figure 1A).

In April 2016, after written informed consent, we began infusing SAB-136 to the patient.

No significant adverse reactions or examination findings were noted with any SAB-136 infusion over the 1-year treatment period. The patient had expected effects from a resection arthroplasty with placement of an antibiotic-impregnated cement spacer and a second operation to remove the spacer during the SAB-136 treatment period. The patient experienced mild thrombocytopenia in treatment month 2, a known side effect of valnemulin, with resolution upon drug discontinuation. Transient mild thrombocytopenia also occurred in treatment months 7–10 and with both hip surgeries. The patient had a microcytic anemia prior to SAB-136 initiation, which was monitored and improved by treatment month 10. Urinalysis and liver function testing was conducted and showed no concerning abnormalities. Transient mild fluctuations in sodium and creatinine occurred in months 7 and 9, respectively. Given that SAB-136 is bovine derived, exposure to bovine factor V and the emergence of anti-bovine factor V antibodies were considered. Because such antibodies can cross-react to human factor V, we monitored coagulation studies (Supplementary Figure 2), which remained within normal limits. Inflammatory markers, erythrocyte sedimentation rate and C-reactive protein, showed marked elevations before SAB-136 treatment, which downtrended during treatment.

To evaluate *M. hominis* burden in the right hip, the primary site of chronic infection, we performed quantitative polymerase chain reaction (qPCR) analyses of *M. hominis* genomic DNA (gDNA) over time, using samples from deep swabs of the hip wound (Figure 1B). Qualitative (i.e. end-point) PCR and culture for *M. hominis* were undertaken independently at a mycoplasma reference laboratory for comparison with the data generated at the Brigham and Women's Hospital (Supplementary Table 1). The data indicate that intermediate and high SAB-136 doses correlated with decreased *M. hominis* gDNA burden. *M. hominis* cultures remained negative while the patient received SAB-136 and following discontinuation of valnemulin, which was the first time cultures were sterile without valnemulin.

We then used a patient-reported clinical diary and health records to evaluate the primary clinical concerns, chronic pain and impaired mobility, during SAB-136 administration. Patient-reported opioid use tapered considerably during SAB-136 treatment (Supplementary Figure 1). Patient hip mobility increased during treatment, improving sit-to-stand maneuvers and standing stability. Early SAB-136 treatment correlated with decreased pain but poor mobility, while later treatments indicated improved mobility with increased exertional pain. The patient experienced resolution of preexisting spasm affecting right shin, hamstring, and gluteal musculature, though pain with hip rotation ensued following removal of a faulty hip prosthesis. The patient noted increased energy and sense of well-being lasting 1–2 weeks after each infusion. Postoperatively, patient hip wounds temporarily closed but then failed to heal. Evaluation of all clinical endpoints was potentially confounded by the removal of the patient hip prosthesis and antibiotics.

DISCUSSION

Ig replacement is a treatment cornerstone for humoral immunodeficiency [10]. However, effectiveness is limited by lot-to-lot variability and may not provide consistent humoral coverage for infections not prevalent in healthy source donors [11, 12]. Tc bovine technology can offer a feasible platform for drug product development personalized for individual patients. This study shows that large quantities of fully human, polyclonal IgG to a select patient isolate may be generated within a time frame permissive for unique, real-time treatment for chronic, antibiotic resistant infections.

Although a single patient study cannot be used to demonstrate overall treatment efficacy and safety, SAB-136 had a high degree of mycoplasmacidal activity (Figure 1A) and was well tolerated. Use of SAB-136, alongside hip surgery and antibiotics, correlated with subjective clinical benefits but did not lead to wound healing, which was not unexpected given prolonged tissue injury. Decreased *M. hominis* qPCR signal was observed at higher SAB-136 doses. However, *M. hominis* cultures, which were negative during the entire study period, converted to positive after the end of the study, despite continued high dose SAB-136. The Tc bovine technology offers the flexibility to inoculate additional animals with newly recovered patient isolates to potentially refine therapeutic antibody specificity in the setting

A

	Concentration	Description	Mycoplasmacidal Titer	Mycoplasmacidal Titer Activity (μg/mL)
1	41.13 mg/mL	SAB-136 α-*M. hominis* hIgG	512	80
2	63.67 mg/mL	α-*M. hominis* hIgG purified (bench scale) from Tc bovine sera	128	497
3	40.81 mg/mL	Negative control hIgG purified from Tc bovine plasma	1	>40 810
4	200 mg/mL	SCIg	32	6250
5	100 mg/mL	IVIg	8	12 500

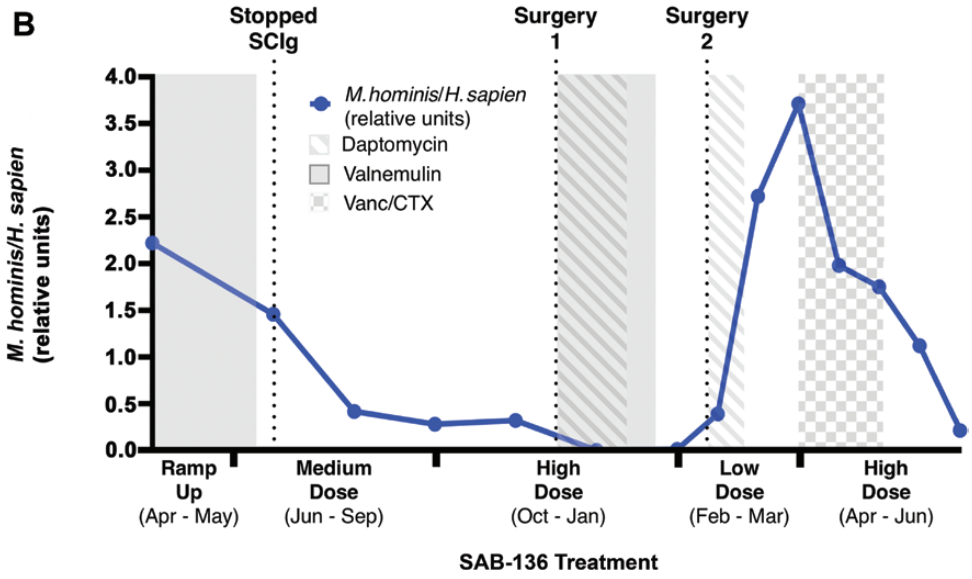

Figure 1. Analysis of in vitro SAB-136 mycoplasmacidal titer/activity and *Mycoplasma hominis* burden over time following SAB-136 administration. *A*, Potency of SAB-136 transchromosomic (Tc) bovine-derived polyclonal immunoglobulin (Ig) for neutralization of mycoplasma in vitro. Shown are the titers and mycoplasmacidal activity of SAB-136 (α-*M. hominis* human IgG [hIgG]) relative to negative control Tc bovine-derived human IgG. A subcutaneous Ig (SCIg) formulation and intravenous Ig (IVIg) formulation were also tested. Mycoplasmacidal activity (μg/mL) is the Ig concentration needed to inhibit mycoplasma growth. Mycoplasmacidal titer provided indicates the Ig dilution corresponding to inhibition of mycoplasma growth (eg, 512 represents an Ig dilution of 1:512). The concentration of each product is provided. SAB-136 α-*M. hominis* hIgG (41.13 mg/mL) was produced at SAb Biotherapeutics in accordance with current good manufacturing practice for clinical use in this study. α-*M. hominis* hIgG purified (63.67 mg/mL) represents research-grade material. See the Supplementary Methods for more detail on this assay. *B*, Quantitative polymerase chain reaction (blue) of *M. hominis* DNA (using *rpoB*, the β subunit of *M. hominis* RNA polymerase) normalized to human DNA (using human oxoglutarate receptor 1 sequence). The x-axis represents the 4 dosing phases over the 1-year treatment period. "Ramp-up" dosing includes a range of SAB-136 from 5 to 50 mg/kg. Medium dose indicates 50–75 mg/kg. High dose indicates 75–100 mg/kg, and the low dose indicates 25 mg/kg. SAB-136 doses were administered typically at 3- to 4-week intervals with 1 exception in January 2017, where the patient received split dosing (25 mg/kg and 75 mg/kg just after surgery). Additional therapeutic interventions coinciding with the SAB-136 treatment period, including hip surgeries (surgery 1 = resection arthroplasty; surgery 2 = removal of cement spacer), SCIg, and *M. hominis*–targeted antibiotic use (valnemulin and daptomycin), are indicated. Also indicated is a course of vancomycin and ceftriaxone (CTX) administered for treatment of polymicrobial hip wound growth that occurred after surgery 2. Last, after SCIg was discontinued, intravenous Ig was infused during all treatment phases following each SAB-136 infusion.

of microbial escape mutants. This would provide a platform for ongoing mycoplasma suppression despite potential microbial escape from polyclonal antibodies.

In summary, we report the generation and administration of personalized polyclonal Ig therapy to potentially expand treatment options for immunodeficient patients suffering from unusual and refractory infections, which may have low coverage in normal IVIg preparations. The use of Tc bovine platform technology may warrant controlled studies in other patients with chronic multidrug-resistant infections or viral infections for which there is no effective treatment or prevention strategy.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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Author contributions. D. R. W. conceptualized and designed the study with the patient. J. N. S. and D. R. W. generated inactivated *M. hominis* for vaccination. SAb Biotherapeutics completed the vaccine development and generated SAB-136. J. N. S. and C. D. A. collected clinical data, and J. N. S., C. D. A., and D. R. W. were involved in clinical care and generated laboratory data. SAb Biotherapeutics generated pharmacokinetic data. The authors had access to all data, and all authors performed data analysis. J. N. S. and D. R. W. wrote the manuscript, which was further edited and approved by all the authors. The authors vouch for the accuracy and completeness of the data and adherence to the protocol. The Partners Institutional Review Board as well as the US Food and Drug Administration reviewed and approved the protocol.

Potential conflicts of interest. J. J. M., H. W., J. A. J., M. A., and E. J. S. have financial relationships with SAB Biotherapeutics. All other authors report no potential conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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Supplementary Material

Supplementary Methods

The Tc Bovine Model
Tc bovines have been previously described[1, 2]. Briefly, Tc bovines used in this study are homozygous for triple knock-outs in the endogenous bovine immunoglobulin genes (*IGHM*$^{-/-}$ *IGHML1*$^{-/-}$ *IGL*$^{-/-}$) and carry a human artificial chromosome (HAC) vector labeled as KcHACD. This HAC vector consists of two human chromosome fragments. A human chromosome 14 fragment contains the entire human immunoglobulin heavy chain locus except that the IGHM constant region remains bovine and the key regulatory sequences were bovinized. A human chromosome 2 fragment contains the entire human immunoglobulin κ light chain locus[1, 3, 4]. Tc bovines produce fully human polyclonal IgG antibodies in plasma up to 15 g/liter.

Generation of SAB-136
M. hominis was isolated from the patient's hip wound and inactivated using thimerosal. A 1% stock thimerosal solution (Sigma) was added to the *M. hominis* in mycoplasma culture medium to a final thimerosal concentration of .02%[5, 6]. The *M. hominis* culture with thimerosal was then incubated for 5 days at 37°Celsius (C) with agitation. The resulting culture was then centrifuged to precipitate a *M. hominis* bacterial pellet. To confirm bacterial inactivation, culture of the inactivated bacteria was performed at BWH for 5 days and then independently at a reference laboratory. The remainder of the inactivated *M. hominis* bacterial pellet was then used for Tc bovine immunization.

The hyperimmune plasma was collected and one qualified Tc bovine was immunized with the inactivated *mycoplasma hominis* formulated with SAB's proprietary adjuvant (SAB-adj-2). A total of five immunizations were administrated via intramuscular injection with 3 to 4-week intervals. Then, hyperimmune plasma was collected from the immunized Tc bovine on Day 10 and Day14 post each immunization from the third immunization to the fifth immunization. The pooled hyper immune plasma was extensively purified, and concentrated to enrich for Tc bovine-derived human IgG (hIgG), to remove bovine plasma proteins (BPP), and to further remove residual IgG molecules that contain a bovine heavy chain (HC) or crystallizable fragment (Fc) of Bovine HC. The enriched polyclonal human IgG, called SAB-136, was manufactured, tested, and released per FDA guidelines.

Mycoplasmacidal Activity Assay
This assay was developed and adapted for this study with the aid of a mycoplasma reference laboratory. Testing for mycoplasmacidal activity is premised on the previously demonstrated concept that mycoplasma is lysed and killed by the combined action of antibodies and complement [7]. The method is described in detail in Razin and Tully's Methods in Mycoplasmology [7]. In brief, samples of SAB-136, IVIg, or SCIg, were used to create a series of dilute suspensions in the presence of guinea pig complement serum and SP4 mycoplasma growth media. These suspensions are then incubated with known quantities of the patient mycoplasma isolate or control mycoplasma at 36.5 Celsius for up to 5 days. A series of positive and negative controls are included to evaluate for the contributions to mycoplasma growth/growth inhibition from media alone and complement alone. The suspensions are plated and colony growth on agar quantified to ensure the proper number of organisms were used to produce the suspensions. Positive control suspensions containing mycoplasma were subjected to serial dilution and monitored for growth by visual inspection of color indicator media. The last dilution to change color (and indicate growth) is then used to determine antibody titer. The highest antibody dilution able to inhibit growth in the serially-diluted mycoplasma suspension is reported as the

mycoplasmacidal titer. The designation of mycoplasmacidality is based upon the previously described method and the observation in this application that inhibition of mycoplasma growth is complement dependent.

Administration of SAB-136

SAB-136 dosing was organized into four phases—ramp up (5-50 mg/kg), intermediate (50-75 mg/kg), high (75-100 mg/kg), and low (25 mg/kg). The first dose of SAB-136 was administered via slow infusion in the Brigham and Women's (BWH) hospital intensive care unit to a total dose of 5 mg/kg[8, 9]. Subsequent infusions at the BWH ambulatory infusion center were conducted at 3-4 week intervals through the four treatment phases in the order listed (see Figure 1). Infusion rates were gradually increased to standard IVIG protocols. The patient was given cetirizine and famotidine prior to each infusion. Solumedrol was initially used as a premedication but later discontinued. Infusion protocols are provided below Supplemental Table 4 and 5.

Extraction of Genomic DNA from Clinical Specimens

Culture swabs were inserted into the patients wound fistula and then immersed in lysis buffer (0.05M KCl, 0.01% Triton X, 0.01M Tris-HCL, pH 9.0) for 1 hour. Cell suspensions were lysed using incubation with Proteinase K (20 mg/ml) to a final concentration of 0.1 mg/ml at 56 C for 1 hour followed by incubation at 80 C for 30 minutes. Samples were briefly vortexed and centrifuged at 14000 rpm for 10 minutes. Genomic DNA (gDNA) isolation then proceeded using isopropanol/ethanol extraction. The supernatants were combined with 500 μl of isopropanol, inverted for 10 seconds, and then centrifuged for 2 minutes at 14000 rpm. The DNA pellet was washed in 500 μl of 70% ethanol, vortexed, and centrifuged for 2 minutes at 14000 rpm. The supernatants were aspirated. The genomic DNA pellet was air dried for 15 minutes, resuspended in Qiagen elution buffer, incubated overnight at room temperature, and then stored at 4C for short-term use and -80C for long-term use.

***M. hominis* Quantitative and Qualitative PCR:**

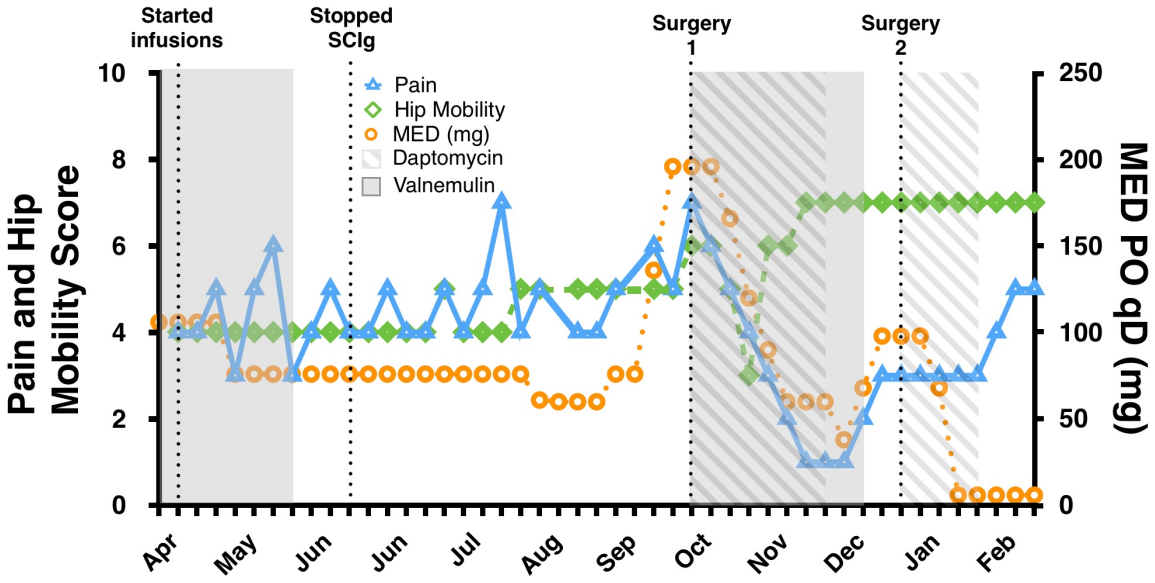
Quantitative PCR (qPCR) was performed on isolated total gDNA from patient culture swab specimens at various treatment time points. Isolated gDNA (5 μl, undiluted or in the dilution range of 1:3 to 1:100) from clinical specimens collected at various time points during the SAB-136 treatment period were subjected to quantitative PCR using FastStart Universal SYBR Green Master Mix and the associated protocol (Roche). Rox was utilized as the reference dye. Primers were designed to amplify *M. hominis* rpoB sequence (encoding the β-subunit of RNA polymerase) [10]. Primers were also targeted to the human oxoglutarate receptor 1 (OXGR1)(Qiagen). Amplification of OXGR1 was used to normalize *M. hominis* amplicon signal to human material. Primer concentrations were typically 10 μM and individual reaction volumes, 10 μl. The assay was performed on a Stratagene Mx3000P device and initially analyzed using MxPro software. The PCR protocol entailed 3 phases: 1) 10 minutes at 95C 2) 40 cycles of 95C for 30 sec with 60C for 1 minute 3) 1 min at 95 C, 30 sec at 55 C and 30 seconds at 95 C. Final analyses were then performed using Microsoft excel. *M. hominis* samples were run in quadruplicate and all control reactions in duplicate for any given qPCR experiment. At least two independent experiments were conducted for analysis of each sample. Positive control gDNA for *M. hominis* amplification was isolated from the cultured patient hip isolate used for bovine inoculation. Human gDNA was isolated from peripheral blood and used as a positive control for OXGR1 amplication. Qualitative PCR for *M. hominis* was also performed at BWH and at a mycoplasma reference laboratory and the results are included (Supplementary Table 1).

Clinical Laboratory Analyses:
Laboratory analyses conducted for this study included complete blood counts with differential, complete metabolic panels, erythrocyte sedimentation rates, C-reactive protein levels, urinalyses, and coagulation studies, including PTT. All clinical testing for all patient encounters during the SAB-136 treatment period was performed in BWH laboratories certified by the Clinical Laboratory Improvement Amendments (CLIA) program and the Joint Commission.

Patient Clinical Logbook/Journal:
A clinical logbook/journal was provided to the patient to monitor pain, pain medication usage, hip and generalized mobility during the treatment period. The patient provided the clinical log data incrementally during the treatment period and data was verified whenever possible in the electronic health record (eg opiate doses). This journal was IRB approved and the following information was requested of the patient: The patient was asked to rate on a scale of 1 to 10, pain intensity. A rating of "1" indicates no pain while "10" indicates the most severe pain the patient has ever experienced. The journal instructs the patient to provide information on the frequency with which pain was experienced, pain medication usage, and noticeable observations of pain other than that occurring at the primary site of infection, the right hip. The patient was further asked to assess hip mobility as defined by the observed range of motion of the affected hip. A 1-10 point scale was provided with "1" indicating no ability to flex, extend, or rotate the affected (or non-affected) hip while a rating of "10" was consistent with normal range of motion without limitation. Overall mobility was assessed with a 1-10 point scale to provide data regarding subjective ability to walk or bear weight with "1" suggesting no ability to walk or bear weight and "10" indicating the ability to walk or bear weight without difficulty. The journal also includes an evaluation of wound discharge, requesting a description of appearance and an indication of volume. Relative volume measurements could be provided as number of dressing changes in a 24 hour period or on a 1-10 point scale, with "1" indicating no discharge and "10" indicating the most prolific discharge the patient has experienced. Lastly, the patient was asked to provide any miscellaneous observations such as rashes, fevers, chills, etc. The data was requested for SAB-136 infusion days along with day 7 and 14 time points following each infusion day during the treatment period. Rigorous adherence to patient provision of data sets on the exact day for which each data set was requested was not monitored. Logbook data reported in Supplementary Figure 1 represents the key, interpretable findings with discernible trends over time

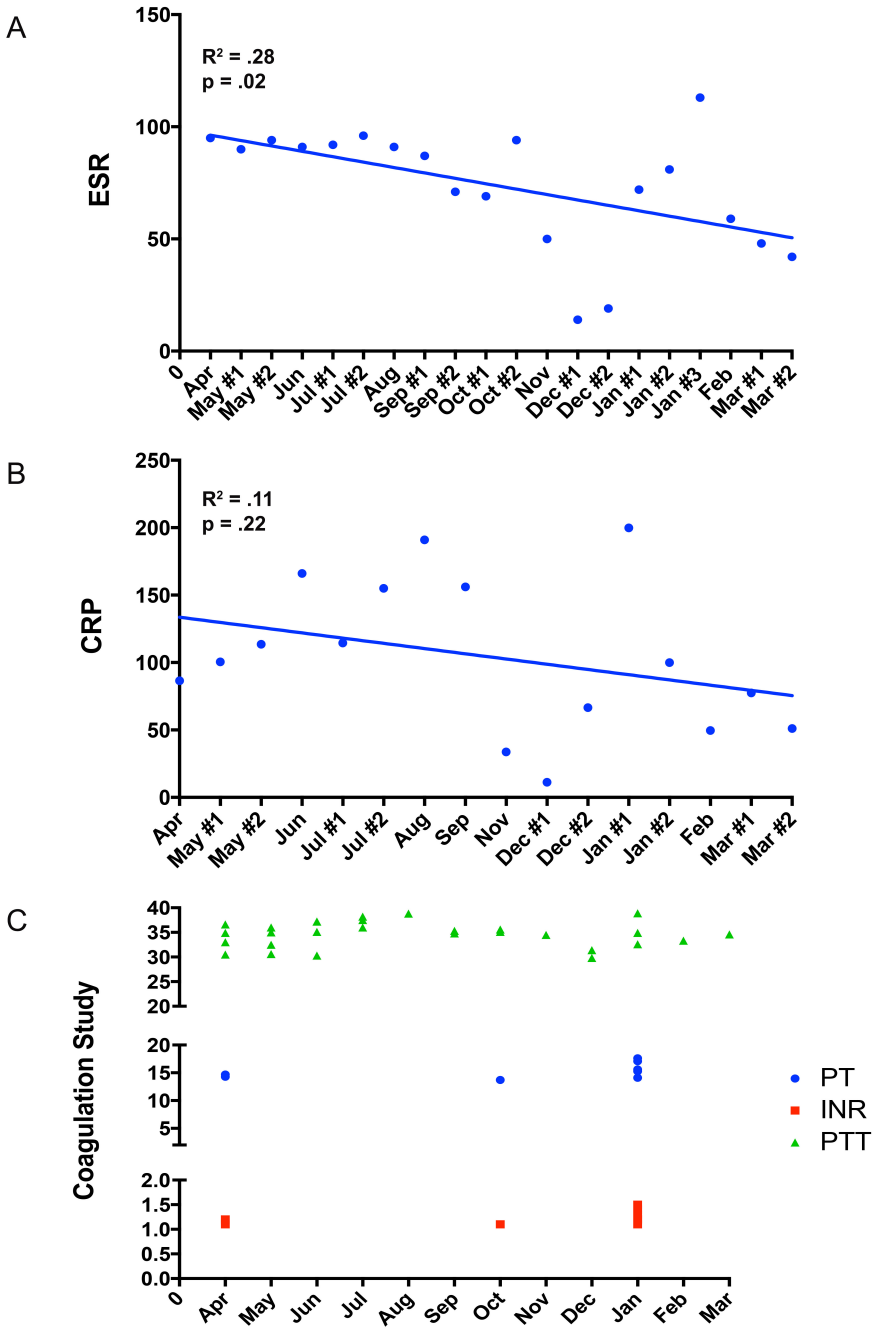
Supplementary Figures

Supplementary Figure 1



Supplementary Figure 1: Pain, hip mobility, and opioid use over time. Patient pain (blue triangles), opioid use (orange circles), and hip mobility (green diamonds) trends are shown over the 1 year SAB-136 treatment period alongside standard treatment modalities including antibiotics and two hip surgeries. Weekly pain and hip mobility scores were self-reported in a journal on a 10-point scale for the duration of the trial. Hip mobility reflects self-reported aggregate subjective range of motion. Opioid use was aggregated from chart review and patient interviews. It consisted of oral hydromorphone and fentanyl patches and is reported as a morphine equivalent dose (MED) in mg taken orally once a day. MED was calculated using the 2016 CDC guideline for prescribing opioids for chronic pain. Equianalgesic dose conversions are only estimates and cannot account for individual variability in genetics and pharmacokinetics. The shaded areas indicate periods of antibiotic usage. Surgery 1 represents right total hip explant with antibiotic spacer placement. Surgery 2 represents removal of cement spacer. See the Online Repository Methods for details.

Supplementary Figure 2



Supplementary Figure 2: Inflammatory markers and coagulation during SAB-136 treatment period. A) Erythrocyte sedimentation rate (ESR in millimeters (mm) /hour, normal current range 0-12 mm/hr) (Y-axis) and, B) C-reactive protein (CRP in milligrams(mg)/Liter(L), normal current range 0-10 mg/L) levels (Y-axis). When multiple values were obtained for a given treatment month (X-axis), these are indicated by the designation of #1 or #2. Linear regression analysis was performed for A & B and is shown with corresponding R values for goodness of fit to the data set. This analysis indicated the trend was significant for ESR but not for CRP. C) Coagulation as designated by PT (prothrombin time in seconds (sec) – blue circles, normal current range 11.5-14.5 sec), PTT (partial thromboplastin time in seconds – green triangles, normal current range 23.8-36.6 sec), and INR (international normalized ratio – red squares, normal current range 0.9-1.1) along the segmented Y-axis plotted against treatment month (X-axis). When multiple values

for coagulation were obtained for a given treatment month, individual values are represented with a single colored shape. The number of values obtained per month for coagulation studies ranged from 1 to 6. All laboratory values and ranges reported here are validated and tested internally at the BWH clinical laboratory.

Supplementary Tables

Supplementary Table 1

Date	V	S	M. hominis Culture	M. hominis PCR
1/4/10[b]			-	ND
1/6/10[c]			+	ND
1/15/10[b]			-	ND
2/18/10[b]			-	ND
4/15/10[b]			-	ND
4/19/10[b]			-	ND
5/12/10[a]			+	+
6/23/10[a]			+	ND
8/17/10[b]			+	ND
9/20/10[b]			+	ND
10/4/10[a]			-	ND
10/4/10[a]			+	ND
10/28/10[b]			+	ND
11/22/10[b]			+	ND
1/28/11[b]			+	ND
1/29/11[a]			+	+
5/5/11[b]			+	ND
7/14/11[b]			+	ND
9/8/11[b]			+	ND
1/5/12[b]			+	ND
2/16/12[a,b]			+[a,b]	+[a]
3/29/12[b]			-	ND
4/26/12[b]			+	ND
5/23/12[a,b]			+[a,b]	ND
9/20/12[a,b]			+[a,b]	+[a]
3/7/13[b]			-	ND
4/18/13[b]			-	ND
6/7/13[a,b]			+[a,b]	+[a]

Date	V	S	M. hominis Culture	M. hominis PCR
2/4/14[b]			-	ND
3/20/14[b]			-	ND
5/13/14[a]			-	+
11/20/14[b]			-	ND
12/8/14[a]			+	+
12/8/14[b]			-	ND
11/30/15[b]			-	ND
4/12/16[b]			-	ND
4/19/16[a]			-	+
5/5/16[a]			ND	+
5/5/16[b]			+	ND
5/26/16[a,b]			-[a,b]	+[a]
6/17/16[b]			-	ND
7/7/16[b]			-	ND
9/8/16[b]			-	ND
10/20/16[b]			-	ND
11/29/16[b]			-	ND
12/2/16[b]			-	ND
12/22/16[a]			-	ND
12/23/16[a]			-	-
1/5/17[b]			-	ND
2/17/17[b]			-	ND
3/2/17[b]			-	ND
3/10/17[a]			-	+
3/23/17[b]			-	ND
4/13/17[b]			-	ND
5/4/17[b]			-	ND

Supplementary Table 1: *M. hominis* burden over time by qualitative PCR and culture. Table illustrating qualitative PCR analyses and culture results for *M. hominis* performed independently at BWH and at a reference laboratory at the given time points before and during the SAB-136 treatment period (column "S" with gray shading). The designation " [a] " represents data analyzed at the mycoplasma reference laboratory, " [b] " represents data produced from the clinical laboratory at BWH, and " [c] " represents data produced at the Massachusetts General Hospital (MGH) clinical laboratory. Dates are provided based upon sample collection. Use of valnemulin (column "V" with black shading) over time is also shown to correlate treatment of known activity against *M. hominis* with culture results, PCR data, and SAB-136 administration. Start and stop dates of valnemulin represent best approximations based on review of the medical record. For PCR and culture results within 1-3 days of valnemulin cessation, valnemulin

was still considered to be present. For PCR and culture data on days when valnemulin was restarted, valnemulin was considered to be absent. When no assay was conducted at a given time point, this is indicated by the designation "ND" for not done.

Supplementary Table 2: Summary of antibiotics used to treat patient prior to use of SAB-136.

Antibiotic	Pre Rx MIC	Post Rx MIC	Clinical response	Reason for stopping
Doxycycline	0.5 mcg/ml	128 mcg/ml	Initial improvement then deterioration	High MIC in *in vitro* testing
Clindamycin	0.5 mcg/ml	64 mcg/ml	Initial improvement then deterioration	High MIC in *in vitro* testing
Azithromycin	N/A	>256 mcg/ml	No clinical response	High MIC in *in vitro* testing
Linezolid	N/A	N/A	No improvement	Pancytopenia
Levofloxacin	N/A	8 mcg/ml	No improvement	High MIC *in vitro* testing
Tigacycline	0.125 mcg/ml	N/A	Clinical improvement	Unable to tolerate
Daptomycin	0.25 mcg/ml	1 mcg/ml	No clinical improvement	No improvement, Difficulty of IV administration
Josamycin	0.5 mcg/ml	4 mcg/ml	No clinical improvement	High MIC in *in vitro* testing
Pristinamycin	N/A	N/A	No clinical improvement	No improvement

Supplementary Table 2: Identification of each antibiotic utilized, the pre and post-prescription (Rx) mean inhibitory concentration (MIC) given in units of microgram (mcg) per milliliter(ml), clinical outcome, and reason for cessation of each treatment are provided.

Supplementary Table 3: Confirmation of deactivation of *M. Hominis* inoculum for Tc Bovine Immunization.

Tube #	Description	Culture Result
1	$5x10^{11}$ CFU *M. Hominis* with Thimerosal 0.02%	Negative
2	$5x10^{11}$ CFU *M. Hominis* without Thimerosal	Negative
3	SP4 control with media alone	Negative
4	$4x10^{12}$ CFU *M. Hominis* with Thimerosal 0.02%	Negative
5	Repeat passage of Tube 2	Negative

Supplementary Table 3: Following thimerosal deactivation of $5x10^{11}$-$4x10^{12}$ colony forming units (CFU) of *M. Hominis* at BWH, test aliquots were cultured in SP4 mycoplasma growth media and agar, then read twice weekly for three weeks for media color indicator change and colony growth, respectively, at a mycoplasma reference laboratory. The results of this analysis are provided for deactivated, non-deactivated, and growth media control samples.

Supplementary Table 4: Method to Administer the First Dose of Intravenous SAB-136.

Name of medication:	**SAB-136**	
Target Dose (mg)	342.0	Pt (ABW) 2/8/16 200 lbs = 90.9 kg
Standard volume per bag (ml)	200	Dose 5 mg/kg, x68.4 (IBW) = 342 mg
Final rate of infusion (ml/hr)	80	Ab concentration 41.13 mg/ml
		Total volume = 8.3 ml
Calculated target concentration (mg/ml)	1.71	**Ideal body wt = 68.4kg**
Standard time of infusion (minutes)	150	

					Total mg per bag	Amount of bag infused (ml)
Solution 1	200	ml of	0.001	mg/ml	0.171	9.38
Solution 2	200	ml of	0.017	mg/ml	3.420	9.38
Solution 3	200	ml of	0.171	mg/ml	34.200	18.75
Solution 4	200	ml of	1.693	mg/ml	338.625	200.00

Step	**Solution**	**Rate (ml/hr)**	**Time (min)**	**Volume infused per step (ml)**	**Dose administered with this step (mg)**	**Cumulative dose (mg)**
1	1	2.5	15	0.625	0.001	0.001
2	1	5	15	1.25	0.001	0.002
3	1	10	15	2.5	0.002	0.004
4	1	20	15	5	0.004	0.008
5	2	2.5	15	0.625	0.011	0.019
6	2	5	15	1.25	0.021	0.040
7	2	10	15	2.5	0.043	0.083
8	2	20	15	5	0.086	0.168
9	3	5	15	1.25	0.214	0.382
10	3	10	15	2.5	0.428	0.810
11	3	20	15	5	0.855	1.665
12	3	40	15	10	1.710	3.375
13	4	10	15	2.5	4.233	7.607
14	4	20	15	5	8.466	16.073
15	4	40	15	10	16.931	33.004
16	4	80	136.875	182.5	308.996	342.000
		Total time (minutes) =	361.875	= 6.03 hrs		

Supplementary Table 4: This intravenous protocol represents a slow infusion and is modeled upon that which is utilized for rapid desensitization to drugs known to elicit type 1 hypersensitivity reactions [8, 9]. Shaded colors of the table include *orange*, highlighting the region of the table which includes target dose, volume per bag of intravenous infusion solution, and final rate of infusion. *Blue* represents the target concentration of the infusion. *Pink* represents the total standard infusion time in the upper component of the table and the target goal infusion rate at the bottom of the table. *Yellow* indicates the volume of infusion per bag when displayed adjacent to each corresponding concentration of antibody per bag of IV solution. ABW refers to actual body weight while IBW refers to ideal body weight.

Supplementary Table 5: Representative Protocol for Maintenance Infusion by Intravenous Administration of SAB-136.

Name of medication:	**ID-SAB-136**	
Target Dose (mg):		3420
Standard volume per bag (ml):		83.14
Calculated target concentration (mg/ml):		41.13

	Volume		Concentration		Total mg per bag	Amount of bag infused (ml)
SAB-136	83.14	ml	41.13	mg/ml	3420	83.14

Step	**Solution**	**Rate (ml/hr)**	**Time (min)**	**Volume infused per step (ml)**	**Dose administered with this step (mg)**	**Cumulative dose (mg)**
1	SAB-136	80	62.4	83.14	3420	3420
	Total time(minutes)		62.4			

Supplementary Table 5:
The upper portion of the table provides the infusion parameters (dose, volume, concentration) necessary for infusion of SAB-136 at 50 mg/kg, a treatment dose utilized during the treatment period. The lower portion of the table provides infusion rate and total infusion time.

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