

A Phase IB/IIA Study of ExoFlo, an Ex Vivo Culture-expanded Adult Allogeneic Bone Marrow Mesenchymal Stem Cell Derived Extracellular Vesicle Isolate Product, for the Treatment of Perianal Fistulizing Crohn's Disease

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Study Phase: IB/IIA

Study Type: Multicenter, Single-blind, Placebo-controlled,
Dose-Escalation, Randomized Controlled Trial

Study Investigational Medicinal Product: Bone Marrow Mesenchymal Stem Cell
(bmMSC)-Derived Extracellular Vesicles
(ExoFlo [via Local Injection])

Original Protocol: 09 February 2023

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Sponsor: Direct Biologics
5301 Southwest Parkway, Building 1, Suite 415
Austin, TX 78735

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PROTOCOL AUTHORIZATION

Emil J. Schmidt IV
President/Co-Founder
Direct Biologics, LLC

DocuSigned by:
Emil J. Schmidt IV
 Signer Name: Emil J. Schmidt IV
Signing Reason: I approve this document
Signing Time: 05-Jun-2023 | 19:35 PDT
32EA7640D0064B0C87D2C5C1B821B0D7

Signature

05-Jun-2023 | 19:35 PDT

Date Signed

Amy Lightner, MD
Chief Medical Officer
Direct Biologics, LLC

DocuSigned by:
Amy Lightner
 Signer Name: Amy Lightner
Signing Reason: I approve this document
Signing Time: 06-Jun-2023 | 05:35 PDT
B816CE8CD14D4239899BAB0E732F7CE2

Signature

06-Jun-2023 | 05:35 PDT

Date Signed

Title: A Phase IB/IIA Study of ExoFlo, an Ex Vivo Culture-expanded Adult Allogeneic Bone Marrow Mesenchymal Stem Cell Derived Extracellular Vesicle Isolate Product, for the Treatment of Perianal Fistulizing Crohn's Disease

_____ Signature of Investigator	_____ dd/mm/yy
_____ Printed Name of Investigator and Title	_____ Site Number

With my signature, I agree to supervise and oversee the conduct of this study and to ensure its conduct is in compliance with the protocol, informed consent, IRB/EC procedures, instructions from Direct Biologics representatives, the Declaration of Helsinki, International Conference on Harmonisation (ICH) Good Clinical Practices (GCP) guidelines, and the applicable parts of the United States (US) Code of Federal Regulations (CFR) and local regulations governing the conduct of clinical studies.

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List of Abbreviations

AE	Adverse Event/Adverse Experience
ARDS	Acute Respiratory Distress Syndrome
bmMSC	Bone Marrow Mesenchymal Stem Cell
CBC	Complete Blood Count
cGMP; GMP	Current Good Manufacturing Practices
CD	Crohn's Disease
CRF	Case Report Form
CRP	C-Reactive Protein
DSMB	Data and Safety Monitoring Board
DSS	Dextran Sulfate Sodium
EUA	Exam Under Anesthesia
EV	Extracellular Vesicles
FDA	Food and Drug Administration
GCP	Good Clinical Practice
GMP	Good Manufacturing Practices
HIPAA	Health Insurance Portability and Accountability Act
h-MSC	Human Mesenchymal Stem Cell
IB	Investigator's Brochure
IBD	Inflammatory Bowel Disease
IBDQ	Inflammatory Bowel Disease Questionnaire
IFU	Instructions for Use
IND	Investigational New Drug
IMP	Investigational Medicinal Product
IRB	Institutional Review Board
MRI	Magnetic Resonance Imaging
MSC	Mesenchymal Stem Cell
NS	Normal Saline
PCDAI	Perianal Crohn's Disease Activity Index
PHI	Protected Health Information
PI	Principal Investigator
SAE	Serious Adverse Event
SMA	Superior Mesenteric Artery
SOC	Standard of Care
SOP	Standard Operating Procedure
WOCBP	Women Of Child Bearing Potential

Study Summary

Title	A Phase IB/IIA Study of ExoFlo, an Ex Vivo Culture-expanded Adult Allogeneic Bone Marrow Mesenchymal Stem Cell Derived Extracellular Vesicle Isolate Product, for the Treatment of Perianal Fistulizing Crohn's Disease
Running Title	ExoFlo for Perianal Fistulas
Protocol	DB-EF-PFCD-1a
Phase	IB/IIA
Methodology	Multicenter, Single-blind, Placebo-controlled, Dose-Escalation Design, Randomized Controlled Trial
Overall Study Duration	Up to 12 Months
Single or Multi-Site	Multi-Site
Objectives	Primary Endpoint: - Safety & feasibility of 1 treatment with ExoFlo at a dose of 15 mL or 30 mL for refractory perianal Crohn's disease - Safety & feasibility of 2 treatments with ExoFlo at a dose of 30 mL for refractory perianal Crohn's disease Secondary Endpoints: - Preliminary efficacy assessment of ExoFlo based on clinical and radiographic findings Tertiary Endpoints: - To evaluate ExoFlo in improving patient reported outcomes
Study Population	36 (24 treatment and 12 controls) with perianal fistulizing Crohn's disease
Diagnosis and Main Inclusion Criteria	- Medically refractory perianal Crohn's disease - Male or Female 18-75 years of age
Study Product, Dose, Route, Regimen	ExoFlo 15 mL, 30 mL, or 30 mL at baseline and again after 3 months in the 30 mL arm if not completely healed.
Statistical Methodology	This is a safety study with exploratory assessment of efficacy. The study has insufficient power to confirm efficacy. All assessments of efficacy will be exploratory for the purpose of hypothesis-generation in larger sample sizes.

1 Introduction

This document is a protocol for a human research study. This study will be carried out in accordance with the applicable United States government regulations and individual clinical site policies and procedures.

The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the Institutional Review Board (IRB) for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. In addition, all changes to the consent form will be IRB-approved; a determination will be made regarding whether a new consent needs to be obtained from participants who provided consent, using a previously approved consent form.

1.1 Background

Crohn's disease (CD), a chronic transmural inflammatory disease of the gastrointestinal tract, continues to increase in incidence for unknown reasons¹. According to population based studies, at least 26% of patients with CD will develop perianal fistulas in the first two decades following diagnosis²⁻⁵ particularly those with colonic and rectal involvement². These patients experience significant morbidity due to pain, persistent drainage, recurrent perianal sepsis, and ongoing need to access medical care resulting in increased costs^{6,7} and impaired quality of life⁷.

Unfortunately, perianal fistulizing CD is notoriously difficult to cure with 37% of patients experiencing refractory disease⁸. As a result, patients cycle through numerous immunosuppressive medications that can have significant side effects, and >90% undergo multiple surgical interventions⁵ putting them at risk of incontinence⁹. While up to 56% of patients can achieve healing with infliximab and seton placement¹⁰ and 64% with optimized tissue flaps⁹, 40% of patients are left with active disease, facing a lifetime of debilitating morbidity or, alternatively, a proctectomy^{11,12}. These results have spurred significant interest in identifying better treatment options that have the potential for improved healing efficacy and patient satisfaction, without a risk of incontinence which is an inherent complication of more aggressive surgical options.

The successful use of mesenchymal stem cells (MSCs) for the treatment of a refractory rectovaginal fistula in the setting of CD was first reported in 2003.¹³ These promising results generated a wave of phase I¹⁴⁻¹⁸, phase II^{17,19,20}, and phase III²¹ trials to study the safety and efficacy of using MSCs to treat perianal CD. Despite the heterogeneity in protocols using allogeneic^{15,18,21,22} or autologous MSCs^{13,14,16,17,19,20,23}, derived from both bone marrow^{18,23} or adipose tissue^{13,15-17,21}, administered at various doses, delivered as a singular or repeat injection, delivered with^{13,15,16} or without^{18,21} scaffolding, the results of all completed trials have been encouraging with regard to both safety and efficacy. However, expansion of clinical trials into clinical practice is limited by the required institutional infrastructure for cell-based therapeutics, limited stability of cells on transfer from a GMP grade lab to the treating physician, and prohibitive cost in treating large numbers of patients. Therefore, the expansion of regenerative products with anti-inflammatory and angiogenic properties for the treatment of perianal fistulizing CD offers a promising alternative to drug based therapy.

Given the high safety profile and relative success of MSCs in treating perianal disease, we sought to use a GMP grade MSC product to establish safety and secondarily monitor for healing in patients

with refractory fistulizing perianal CD in a protocol as has been published previously in the studies above. This trial will use allogeneic bone marrow derived mesenchymal stem cells (MSCs) to produce regenerative signals. The specific rationale for MSCs in perianal fistulas is based upon 1) their anti-inflammatory properties; 2) published experience of MSC in this condition and perianal Crohn's fistula demonstrating efficacy and safety; 3) existence of cGMP methods for their isolation and growth.

Given the acceptable safety profile, and relative previous success in treating inflammatory bowel disease with MSCs, we now propose to use a GMP grade extracellular vesicle product derived from allogeneic bone marrow derived MSCs (ExoFlo) to establish safety and secondarily monitor for healing in subjects with medically refractory perianal fistulizing CD.

This trial will use ExoFlo, an extracellular vesicle product derived from allogeneic bone marrow derived mesenchymal stem cells, to produce regenerative signals. The specific rationale for ExoFlo in perianal fistulizing CD is based upon:

1. The investigational product's anti-inflammatory properties,
2. Published experience of ExoFlo in inflammatory conditions demonstrating efficacy and safety,
3. Existence of cGMP methods for their isolation and growth, and
4. Collective safety profile of ExoFlo across a wide range of doses, dosing regimens and therapeutic indications

This study will enroll 36 CD subjects with pharmacologically refractory perianal fistulizing CD (See specific inclusion and exclusion criteria in [Section 4](#)).

1.2 Investigational Medicinal Product

The investigational medicinal product (IMP) is a bone marrow derived extracellular vesicle product notable for its high production standards, purity, and potency. Extensive characterization of the extracellular vesicles contained in the IMP has revealed an absence of immunogenic surface epitopes that make it highly unlikely to cause acute immune reactions, and there are no known product reactions or reported adverse reactions to date. The IMP exerts a powerful anti-inflammatory effect as well as promotes regeneration of tissues damaged by inflammation. Each mL of IMP, from the lots to be used in this study, contains approximately 60-80 billion extracellular vesicles, and each mL is comprised of over 2000 different anti-inflammatory cytokines, anti-oxidative stress, proregeneration, tumor suppressor, and antimicrobial agents; the potential for changing the disease progression of medically refractory perianal fistulizing CD is therefore compelling. To date, the IMP has been used in an independent pilot study for COVID-19 associated ARDS (N=24), which led to the FDA authorization of an IND under which, the Phase II randomized controlled trial for COVID-19 associated ARDS ("EXIT COVID-19", NCT04493242) has been completed with 102 patients randomized. Expanded Access Protocol for Intermediate Population (NCT04657458) is still underway with 103 patients treated. 15 patients have received the IMP for compassionate use through the single patient emergency IND (eIND) pathway. Notably, no severe adverse event has been attributed to the IMP to date.

In addition to the availability of preliminary clinical safety and efficacy data, the IMP (bone marrow-derived extracellular vesicles) manufactured by Direct Biologics, should be considered for use due

the following reasons pertaining to safety, efficacy, reproducibility, and overall quality, summarized in the [table](#) below:

Table 1. Characteristics of IMP and Key Findings

IMP Characteristics	Key Findings
1. Meets Good Manufacturing Practices (cGMP) Standards	The bmMSCs and media both have master files on record at the FDA. The IMP meets donor and manufacturing safety profiles, has lot-specific tissue traceability and a comprehensive labeling including detailed instructions for use.
2. Meets Good Tissue Practices (cGTP) & Single Donor for IMP	The donor has been extensively screened for past medical history, travel history, in addition for specific communicable diseases mandated by the FDA.
3. Standardization	To date, all IMP is human bmMSC derived EVIP and the human MSCs are derived from one single healthy donor, whose cells are registered at an established cell bank registered with the FDA.
4. Safety Profile Clinical Use	No SAE attributable to IMP in clinical investigational use to date. Clinical investigational use includes the following: Pilot study for COVID-19 IND #21669, DB-EF-PHASEII-001 (N=102 randomized), & DB-EF-EXPANDEDACCESS -001 (N=95 treated) as well as 30 single patient Expanded Access
5. No Allogeneic DNA Exposure	IMP does not involve the introduction of foreign DNA.
6. Nanosized Advantage	bmMSC EVIP are 30-170 nanometers in diameter, which is roughly 100x to 1000x smaller than bmMSC, thereby reducing risk of pulmonary vascular occlusion
7. Extensive Characterization in the Literature	bmMSC is more extensively studied in peer-reviewed literature and bmMSC derived EVIP is more extensive studied than another cell source derived EVIP. In addition, the molecular characterization of the IMP is readily available.
8. Lack of immunogenicity	EVIP are not known to express surface epitopes that would be recognized by the immune system as foreign. Additionally, the use of xeno-free medium further reduces the likelihood of an immunologic response. In comparison, allogeneic MSCs have a higher risk of eliciting an immune reaction, GvHD, or other reactions that could be amplified with re-dosing.
9. Scalability	The IMP has been uniformly tested for quality. These signals originate from a single human donor and are abundantly available due to proprietary production technology. In contrast, MSCs and perinatal EVIP must be harvested from different human donors after a meticulous vetting process, posing challenges to uniform quality control, and immediate availability. Furthermore, the use of EVIP precludes the need for a transplant facility to store living cells, which increases the number of facilities that can administer therapy.
10. Product Stability & Storage Ease	The IMP may be stored between -80°C to -60°C for up to 12 months
11. Lack of Carcinogenicity	When harvested from healthy donors, bmMSCs have been extensively characterized without evidence of oncogene expression

1.3 Preclinical Data to Date

An extensive number of animal studies have now been performed demonstrating safety and efficacy of intra-arterial and intravenous delivery of MSCs for various disease processes. Arterial delivery has become the preferred delivery of choice for targeted delivery due to pulmonary trapping of MSCs following intravenous delivery. Dogs, mice, rats, and pigs have now received arterial delivery of MSCs. Most of the studies have involved arterial delivery of MSCs for stroke into the carotid vasculature, while others have studied intracoronary infusion for myocardial infarction and intra-renal delivery for post ischemic kidney injury. Others have looked at intra-arterial delivery of MSCs for a

porcine model of stroke and a mouse model of lower extremity injury without adverse effects; interestingly both studies found improved biodistribution of MSCs when delivered intra-arterially.

A commonly used murine model of colitis is dextran sulfate sodium (DSS) in drinking water for mice. Following the development of colitis, intervention can be performed to see if the colitis reverses. Several animal studies have been done to demonstrate reversal of colitis with mesenchymal stem cells utilizing several modes of delivery including intraperitoneal injection, direct rectal wall injection, intravenous delivery, and intra-arterial delivery. One study showed superiority of intravenous versus intraperitoneal delivery. However, again, due to the intravenous route resulting in pulmonary trapping which can cause adverse effects such as embolus formation and post-transplantation pneumonitis, studies have focused on the safety and efficacy of intra-arterial delivery. Several animal studies have now been performed demonstrating the safety of intra-arterial injection of MSCs specifically for the treatment of DSS induced colitis. A group from Sweden injected 5×10^6 ^{111}In -oxine-labeled human decidual stromal cells (DSCs) into four rabbits via the superior mesenteric artery (SMA) using an angiographical system. The group found that selective injection of human DSCs to the rabbit SMA did not result in acute embolic complications or pulmonary trapping. Rather, selective injections to the SMA resulted in uptake distributed in the intestine supplied by the SMA and in the liver, indicating that this approach could significantly increase the fraction of injected DSCs reaching the target tissue without adverse events. Intravenous delivery corroborated pulmonary trapping. Another study looked at mesenteric injection of adipose derived mesenchymal stem cells relieved colitis in rats without any safety concerns. Another study looked at intra-arterial versus intra-venous delivery of MSCs intravenously versus intra-arterially and found that intra-arterial delivery was safe and had less uptake in the lungs.

While there is not a good animal model for perianal fistulizing CD, there is extensive data of MSCs for the treatment of perianal fistulizing CD. ExoFlo is a product derived from MSCs, thus the clinical data of MSCs is reviewed in detail below.

1.4 Clinical Data to Date

Since the first report of successful healing of a refractory rectovaginal fistula following injection MSCs in 2003¹³, great enthusiasm has spurred several Phase I^{14-18, 24-30} Phase II^{17, 19, 20}, and Phase III²¹ trials designed to study the safety and efficacy of MSCs for perianal CD. Despite the heterogeneity in protocols using allogeneic or autologous MSCs, derived from either adipose tissue or bone marrow tissue, administered at various doses, given as a singular or repeat injection, delivered with or without scaffolding, results of all completed trials have been encouraging with regard to both safety and efficacy. In fact, there have been no reports of serious adverse events (SAEs) related to MSCs during these aforementioned phase I, II, and III clinical trials. Efficacy has been reported at rates higher than conventional medical and surgical options, and healing is sustained to one and four years. (Table 2)

Table 2: Clinical Trial Data to Date with MSCs

Name of Study	Type of Study	Location	# patients with CD	Intervention	Type and source of MSCs	Outcome	Results	Use of MRI	Adverse Events
García-Olmo et al 2003 ¹³	Case report	Spain	1	Local injection of stem cells	Autologous, Adipose tissue	Complete epithelialization of external opening	Fistula healed in 1 week, No recurrence till 3 months post treatment	No	None
García-Olmo et al 2005 ¹⁶	Phase I, open label, single arm	Spain	4	Local injection of 3x 10 ⁶ million MSC	Autologous, Adipose tissue	Complete epithelialization of external opening	3 of 4 rectovaginal or perianal fistula (75%) at 8 weeks	No	None
García-Olmo et al 2009 ¹⁷	Phase IIb, open label, double arm, randomized	Spain	14	Local injection of 2x 10 ⁶ MSC plus fibrin glue as compared to fibrin glue alone; second dose of 4 x 10 ⁶ MSC if fistula healing was not seen at 8 weeks	Autologous, Adipose tissue	Complete epithelialization of external opening	5 of 7 fistulas (71%) in MSC versus 1 of 7 fistulas (14%) healed in fibrin glue alone at 8 weeks	No	15 non serious AE; 4 SAEs, 1 related to MSCs (perianal abscess)
Cho et al 2013 ¹⁴	Phase I, open label, single arm	Korea	10	1x 10 ⁷ , 2x 10 ⁷ , 4x 10 ⁷ , cells/mL based on the size of the fistula (total of 3–40x 10 ⁷ cells)	Autologous, Adipose tissue	Complete epithelialization of external opening	3 of 10 patients (30%) had complete healing at 8 weeks post treatment; sustained at 8 months	No	13 AE were reported in seven patients (70%); 3 SAE in 2 patients (20%, one related with seton placement)
Lee et al 2013 ²⁰	Phase II, open label, single arm	Korea	33	3x 10 ⁷ or 6 x 10 ⁷ cells per 1 cm of fistula length; average number of 15.8x 10 ⁷ cells), followed by a second injection of 1.5 times more cells (average number of 19.1 x 10 ⁷ cells) if fistula closure was not complete at 8 weeks	Autologous, Adipose tissue	Complete epithelialization of external opening	27 of 33 patients (82%) had complete healing at 8 weeks; 88% sustained closure at one year	No	28 AE, all unrelated to MSC; 1 SAE unrelated to MSC
Cho et al 2015 ¹⁹	Phase II extension of Lee phase II	Korea	24	9-42 x 10 ⁷ cells based on length of fistula tract	Autologous, Adipose tissue	Complete epithelialization of external opening	20 of 24 patients (83%) had sustained closure at two years	No	53 AE, all unrelated to MSC
Ciccocioppo et al 2011 ²³	Open label, single arm	Italy	10	1.5 to 3x 10 ⁷ MSC every 4 weeks until an improvement was obtained or when autologous MSCs were no longer available (2–5 injections)	Autologous, Adipose	No drainage on clinical exam as well as healed on MRI	6 of 9 patients (67%) with complete closure at 8 weeks; all sustained closure at one year	Yes	No adverse events
de la Portilla et al 2013 ²²	Phase I/IIa open label, single arm	Spain	24	Local injection of 2x 10 ⁶ MSCs; second injection of 4x 10 ⁶ if unhealed at 14 weeks	Allogeneic, Adipose tissue	absence of drainage and complete epithelization, plus absence of collections measured by MRI	5 out of 18 fistulas (28%) closed at 24 weeks post treatment. 7 out of 18 patients (47%) had closure of external openings at 24 weeks post treatment.	Yes	four SAE (three anal abscesses and one uterine leiomyoma), so the group concluded the treatment had an acceptable safety profile.
Panes J et al 2016 ²¹	Phase III, RCT	Europe/Israel	212	Local injection of stem cells	Allogeneic, Adipose tissue	Absence of drainage and < 2 cm fluid collection on MRI	50% (n=53 of 107) healed in the MSC group compared with 34% (n=36 of 105, p = 0.024) at 24 weeks	Yes	Overall, 68 (66%) in treatment, 66 in placebo (65%); SAE in 18 (17%) and 14

									(14%), majority anal abscess
Molenkijk et al 2015¹⁸	Open label, 4 arms	Netherlands	21	N= 5 in 10 ⁷ MSC dose (G1) N= 5 in 3x 10 ⁷ MSC dose (G2) N= 5 in 9x10 ⁷ MSC dose (G3) N= 6 in placebo (G4)	Allogeneic, Adipose	Absence of drainage and < 2 cm fluid collection	12-week fistula healing: G1:2/5 G2:4/5 G3:1/5 G4:2/6	Yes	50 AE, most common was common cold, 4 abscesses
Dietz et al 2017¹⁵	Phase I, open label, single arm	USA	12	20 million cells on a GORE Bio A Plug	Autologous Adipose tissue on Matrix	Absence of drainage and improvement in Van Assche score on MRI	10 of 12 patients with healing at 6 months (83%)	Yes	No adverse events
Panes J et al 2018²⁴	Phase III, RCT	Europe/Israel	212	Local injection of stem cells	Allogeneic, Adipose tissue	Absence of drainage and < 2 cm fluid collection on MRI	57% (n=49 of 86) healed in the MSC group compared with 39% (n=33 of 84, p = 0.021) at 52 weeks	Yes	Most common anal pain/abscess, study withdraw <10% related to TEAEs
Barnhoon MC, 2019²⁵	Phase I	Europe	15	Local injection of stem cells	Allogeneic, Bone marrow tissue	Absence of drainage and < 2 cm fluid collection on MRI	13/15 (87%) available for 4 year f/u. healing maintained from one year results	Yes	No increased adverse events from one year results

1.5 Study Rationale

MSCs have recently demonstrated safety and improved efficacy for the phenotype of perianal fistulizing CD in phase III randomized control trials. However, cell-based therapy has logistical hurdles to successfully treat thousands of at need patients. Cells have to be delivered fresh to clinical sites, patients scheduled at certain times, and there are significant scalability concerns. Extracellular vesicles derived from MSCs have the potential to overcome many cell-therapy logistical challenges related to shipping, thawing and shelf life.

Given the promising safety and efficacy data with MSCs (as described in the [previous section](#)), and the enthusiasm around their use in refractory patients, further evaluation with a MSC derived product that may have scalability to treat significant numbers of patients is proposed herein.

1.6 Dose Rationale and Risk/Benefits Assessment

1.6.1 Dose Rationale

Dosing of the IMP was calculated based on (1) the 24-patient preliminary COVID-19 clinical case series with the IMP; (2) the START trial, which is a phase I trial of IV administration of bone marrow derived stromal cells as treatment for ARDS, which demonstrated safety of using IV doses of 1 million cell/Kg, 5 million cell/Kg, in addition to a ceiling dose of 10 million cell/Kg; (3) observation of approximately 2,000 extracellular vesicles secreted per stromal cell; and (4) lab analysis of the IMP, which showed that each mL contained approximately 60-80 billion extracellular vesicles. For an adult of 70 Kg, extrapolation from the START trial MSC ceiling dose would yield an IV IMP ceiling dose of 17.5 mL. Given a range of body mass indexes (BMIs), 15 mL of IV IMP was determined as a reasonable starting dose for one treatment arm while 10 mL of IV IMP was

determined as a reasonable lower end dose for a second treatment arm for Phase II randomized controlled trial for COVID-19 associated ARDS “EXIT COVID-19” (NCT04493242).

Independent DSMB on 2/28/21 determined that the two IMP treatment arms were of comparable safety. EXIT COVID-19 has since been completed with database locked. 15 mL IMP treatment arm showed lower 60-day mortality compared to placebo and 10 mL IMP treatment arm though the study was not powered for statistical significance in terms of mortality difference. Repeat dosing has been shown to be safe in both Phase II EXIT COVID-19 (max 2 doses, NCT04493242) and Expanded Access for Intermediate Population for the same indication (max 3 doses, NCT04657458).

We herein describe a protocol of dose escalation between cohorts to better understand the lowest optimal dose. We have chosen 15 mL and 30 mL for local direct injection given the safety data already established with doses at 5 mL, 10 mL and 15 mL given systemically. Given this is a local injection, we need enough volume to fill the perirectal fat tissues surrounding the fistula(s) tract(s). Thus, we picked a starting volume of 15 mL for the first cohort, 30 mL for the second cohort and 30 mL twice (baseline and 3 months) for the last cohort. Each cohort will have a 2:1 randomization of IMP versus placebo (normal saline [NS]).

1.6.2 Known Potential Risks

There is no known potential for malignant transformation based on prior preclinical studies of human bone marrow derived extracellular vesicles. There are no SAEs attributable to the IMP based on independent investigator-initiated trials nor FDA-approved clinical trials (NCT04493242, NCT04657458). Known adverse events include hypotension (one event, NCT04493242) and skin hyperpigmentation at the IV site (one event, NCT04657458).

Theoretical risks include:

- i. Infection: We are injecting IMP into subjects in the perianal region with high bacterial load. The risk of infection is low. At every study visit special care will be taken to thoroughly examine the perianal area for evidence of infection.
- ii. Possible local irritation and increased inflammation secondary to injection of IMP.
- iii. Severe perianal pain.

1.6.3 Known Potential Benefits

Potential benefits include the avoidance of side effects from current immunosuppressive therapies approved for CD and potentially improved efficacy as compared to currently approved therapeutics. In addition, ExoFlo may potentially reduce or eliminate the need for additional surgical intervention, and significantly improve quality of life based on subjects who have reported satisfactory results from other treatments which healed perianal fistulas, including MSCs.

1.6.4 Assessment of Potential Risks and Benefits

The subjects enrolled in this study have perianal fistulas in the setting of CD. We believe that the risks of this study are manageable and minimal. Additional rationale for the safety of ExoFlo in fistulizing CD include:

- This is product derived from a healthy donor
- Large body of literature now demonstrating safety and efficacy of MSCs in perianal Crohn’s fistula

- Large amount of patient data with systemic administration of ExoFlo for inflammatory conditions
- We will space subject accrual to assess safety at each dose utilized. The twelve patients in the 15 mL arm (Cohort 1: 8 treatment and 4 placebo) will all be followed to their two-week post treatment visit to assess for AEs and SAEs related to IMP or delivery of IMP at the 15 mL dose, such that any acute AEs will be identified prior to enrollment of additional subjects at the next dose. Following the last two-week post treatment visit in Cohort 1, the twelve patients in the 30 mL arm (Cohort 2: 8 treatment and 4 placebo) will all be followed to their two-week post treatment visit to assess for AEs and SAEs related to IMP or delivery of IMP at the 30 mL dose. Following the last two-week post treatment visit in Cohort 2, the twelve patients in the 30 mL at baseline and 3 months (Cohort 3: 8 treatment and 4 placebo) will be dosed.

2 Study Objectives

2.1 Primary Objective

To evaluate the safety and feasibility of one 15 mL or 30 mL treatment or two treatments of 30 mL ExoFlo in subjects with perianal fistula(s) in the setting of CD.

2.1.1 Primary Endpoint

To evaluate the number of AEs and SAEs following 1-2 treatments of ExoFlo in subjects with perianal fistula(s) in the setting of CD at doses of 15 mL or 30 mL.

While we do not anticipate any SAEs, we will monitor for the following AEs:

1. Worsening (change in nature, severity, or frequency) of CD present at the time of the study. This will be objectively assessed as a need for escalation of medical therapy, new endoscopic or radiographic findings suggestive of worsening disease.
2. Significant perianal pain increased from prior to IMP delivery that requires intravenous pain medication to control.
3. Significant perianal bleeding that requires a blood transfusion.
4. Need for admission to the hospital due to worsening perianal CD.
5. Abnormal laboratory values (this includes clinically significant shifts from baseline within the range of normal that the investigator considers to be clinically significant).
6. Clinically significant abnormalities in physical examination, vital signs, weight, or drainage from the perianal fistula(s) that changed from prior to the study drug delivery.
7. Formation of injection site abscesses. This will be assessed during scheduled clinical examinations throughout the duration of the study.
8. SAEs within the first six hours after the procedure, including significant changes in vital signs.
9. SAEs throughout the study, including injection-site abscess and/or other significant infection near the injection site, sepsis, severe systemic illness, or need for inpatient hospitalization related to the aforementioned.
10. Need for additional surgical intervention related to the fistula.

2.1.2 Justification for Primary Endpoint

This is the first study of ExoFlo for the treatment of perianal fistulizing CD. Thus, safety is the primary endpoint to determine the rate of AEs or SAEs related to IMP and administration of IMP in this patient population.

2.2 Secondary Objective

To evaluate the efficacy of ExoFlo in inducing complete or partial fistula healing after the delivery of ExoFlo to subjects with perianal fistulizing CD at one dose of 15 mL or 30 mL and two doses of 30 mL.

2.2.1 Secondary Endpoints

The following definitions will be used to describe healing as part of the secondary endpoint:

Complete Healing

- Clinical Healing
 - 100% cessation of drainage on both clinical exam with deep palpation and per subject report and epithelization of the external fistula opening; and
- Radiographic Healing
 - MRI with an absence of a fluid collection >2 cm in 3 of 3 dimensions, lack of edema, inflammation or sign of active inflammatory response. A remnant scar of a fistula, or predominately fibrotic fistula tract may remain

Partial Healing

- Clinical Healing
 - Greater than or equal to 50% cessation of drainage on both clinical exam with deep palpation and per subject report and epithelization of the external fistula opening
 - subject report and epithelization of the external fistula opening; and
- Radiographic Healing
 - MRI with an absence of a fluid collection >2 cm in 3 of 3 dimensions, lack of edema, inflammation or sign of active inflammatory response. A remnant scar of a fistula, or predominately fibrotic fistula tract may remain

Lack of Response

- Clinical or Radiographic healing which does not meet the threshold for Partial Healing

Criteria for treatment failure

- No improvement in clinical symptoms or have worsened symptoms as defined by increased drainage or presence of new fistula tracts.

2.2.2 Justification for Secondary Endpoint

These secondary endpoints were chosen to match the endpoints used in previous clinical trials of MSCs for perianal CD.

2.3 Tertiary/Exploratory Objective

To evaluate the patient reported efficacy of ExoFlo in inducing complete or partial fistula healing after the delivery of ExoFlo to subjects with perianal fistulizing CD at one dose of 15 mL or 30 mL and two doses of 30 mL at baseline and 3 months. .

2.3.1 Tertiary/Exploratory Endpoint

Patient Reported Outcomes:

To evaluate the efficacy of 1-2 doses of ExoFlo in improving disease-specific health related quality of life Patient Reported Outcomes as measured by the following:

- Perianal Crohn's Disease Activity Index (PCDAI)
- Wexner Incontinence Score
- 36 item Short Form Health Survey (SF-36)
- EuroQol 5 Dimensions Survey (EQ-5D)
- IBDQ QoL survey (IBDQ)
- IBD-Patient Reported Treatment Impact Survey (IBD-PRTI)

2.3.2 Justification for Tertiary/Exploratory Endpoint

Patient reported outcomes are a critical part of what is evaluated in clinical trials. These patient reported outcomes and disease activity indexes are validated scoring systems which have been utilized in other IBD clinical trials.

Even though this protocol is a local injection of ExoFlo, any significant worsening in overall Crohn's health status would be important to capture throughout the duration of the subjects' follow up.

3 Study Design

3.1 General Description / Overall Design

This is a multicenter, single-blind, placebo-controlled, dose-comparison randomized controlled trial of 1-2 doses of ExoFlo in 36 subjects (24 treatment; 12 placebo-controlled) with perianal fistulas in the setting of CD.

The current standard of care for virtually all patients with fistulizing perianal disease includes drainage of sepsis with seton placement and maximizing medical therapy. We plan to enroll patients with medically refractory perianal fistula. The next step in the management of these patients is escalation in medical therapy and/or repeat operative intervention.

Subjects will be consented and screened for participation in the trial.

Randomization to treatment with ExoFlo versus placebo with normal saline will be assigned by an Interactive Response Technology (IRT) system.

A total of 15 mL or 30 mL of IMP or placebo will be drawn into three or six separate 5 mL syringes (each containing 5 mL of IMP).

The morning of the ExoFlo injection procedure, urine pregnancy test for women of childbearing potential (WOCBP) only will be performed. Subjects will then undergo an EUA at the study site. The fistula tract will be assessed. A 22-gauge (22G) needle will then be used for IMP injection.

Acute monitoring after ExoFlo delivery: Subjects will be observed following the injection of IMP in the post procedure unit per standard of care.

Long term monitoring: Subjects will return as outlined in the schema below. Tests will include general health assessment and blood work measured on weeks 2 & 6, months 3, 6 and 12. For the last cohort of 30 mL if at 3 months post initial injection of IMP, there is not complete clinical healing (100% cessation of drainage on both clinical exam with deep palpation and per subject report, clinical exam, and epithelization of the external fistula opening), and the subject did not have any IMP related SAEs after the first treatment, a repeat injection IMP, at the same dose, will be administered.

Administration of Patient-Reported Outcome surveys will be done throughout the duration of the study for both the treatment and control cohorts.

3.2 Scientific Rationale for Study Design

The rationale for choosing a placebo controlled, dose escalation RCT is that this is the gold standard for proving safety and efficacy of the IMP + standard of care (SOC) compared to SOC alone. Dose escalation was determined in order to optimize dosing for a larger phase 3 clinical trial design. Randomized block design further minimizes selection bias and the effect of systemic error.

3.3 Duration of Subject Participation

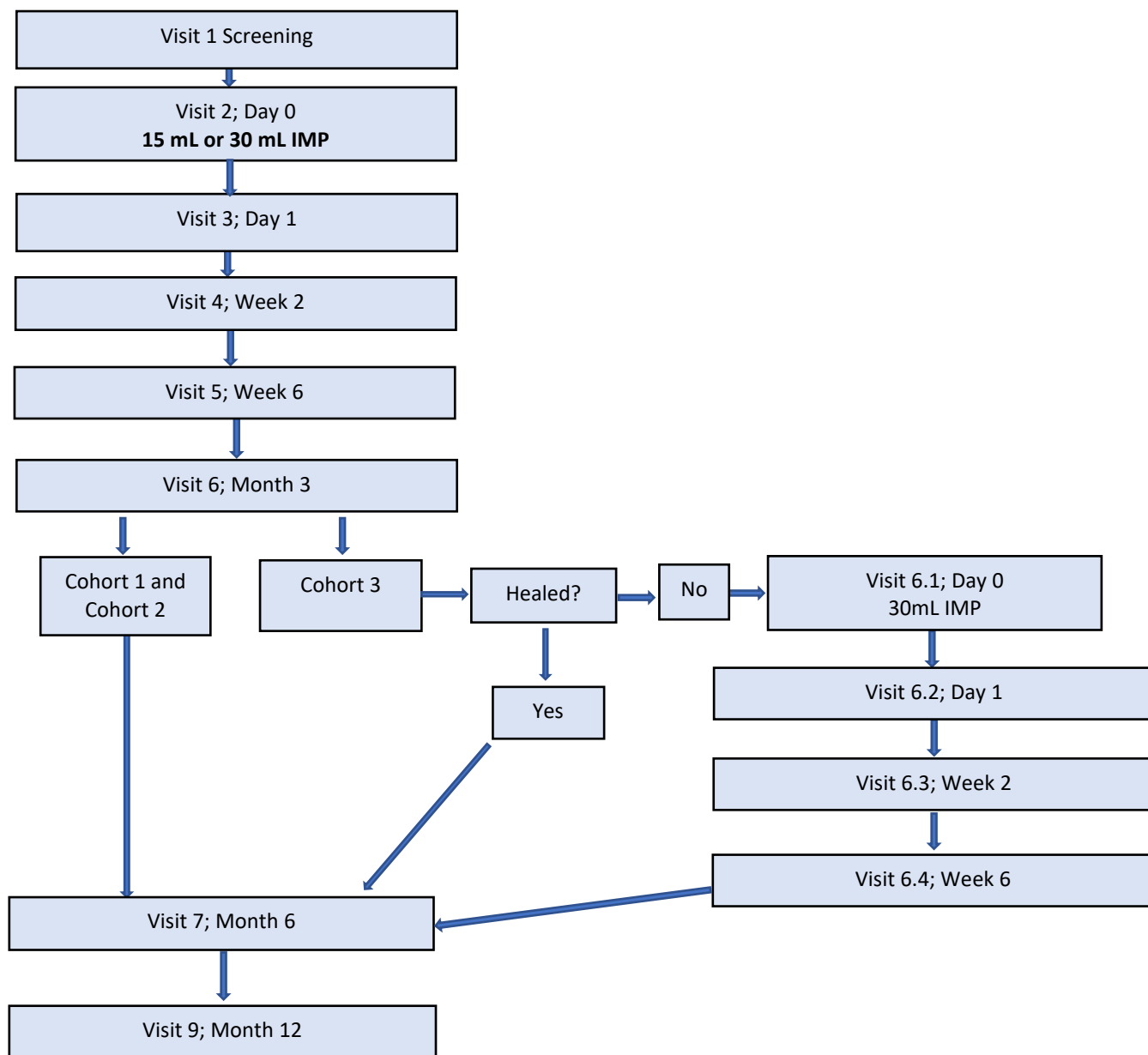
Subjects randomized will be followed for a total of 12 months following their first IMP delivery.

3.4 End of Study Definition

A participant is considered to have completed the study if he or she has completed all study visits visit or the last scheduled procedure shown in the [Schedule of Visits Table](#) in [Section 6](#).

3.5 Study Visit Schema

3.5.1 Visit Schema for Treatment and Control Subjects



3.6 Dose Escalation and Subject Enrollment

Subjects will be randomized to treatment versus control (placebo) in groups of 3 subjects (2 subjects randomized to the treatment arm, and 1 control subject randomized to the placebo arm). This enrollment will continue for 3 Cohorts for a total of 36 patients. The first 12 subjects (Cohort 1) will receive 15 mL IMP or placebo. The next 12 subjects (Cohort 2) will receive 30 mL IMP or placebo. The final 12 subjects (Cohort 3) will receive 30 mL IMP or placebo with the option of a repeat dose at 3 months.

Each cohort will be followed to their two-week post treatment visit to assess for AEs and SAEs related to IMP or delivery of IMP to ensure the absence of severe AEs in the preceding cohort. The DSMB will meet to assess for safety prior to enrollment of additional subjects at the next dosing cohort.

The total duration of follow up for the treatment group will be 12 months, and the total duration of follow up for the treatment and placebo group will be 12 months. In the final 30 mL cohort subjects, if at 3 months post injection of IMP, there is not complete clinical healing (100% cessation of drainage on both clinical exam with deep palpation and per subject report, clinical examination, and epithelization of the external fistula opening), and the subject did not have after the first dose:

- Grade 4 or above AE (CTCAE v5)
- Any Grade 3 or above hypersensitivity
- Pregnancy
- Anesthesia related complications
- Perianal infection or abscess formation
- Any new onset incontinence or reported injury to the sphincter after IMP administration

A repeat injection will be administered. If subjects experience worsening disease that is clinically significant or IMP related AEs, as noted above, subjects will not be retreated but will be followed for the remainder of their trial-related follow up visits.

All safety/adverse event data will be reviewed on an ongoing basis by the medical monitor. If any of the following occur, irrespective of attribution, the study will be paused for additional safety evaluation by the DSMB.

- Any death
- Any CTCAE Grade 4 adverse event (AE)
- Any Grade 3 or equivalent hypersensitivity reaction
- Any two subjects experience a grade 3 AE (CTCAE v.5) in the same system organ class
- Any subject requires surgical intervention within 30 days of product administration
- Any perianal infection or abscess formation within 30 days of the procedure
- Any new onset incontinence or reported injury to the sphincter after IMP administration

4 Subject Selection Enrollment and Withdrawal

4.1 Inclusion Criteria

To be eligible to participate in this study, an individual must meet all of the following criteria:

1. Men and Women 18-75 years of age with a diagnosis of CD for at least six months duration.
2. Single and/or Multi-tract Perianal fistula(s).
3. Failed at least one medical therapy within the last year including, but not limited to, antibiotics, immunomodulators (6-MP, methotrexate, azathioprine), monoclonal antibodies (infliximab, adalimumab, certolizumab, ustekinumab, golimumab, vedolizumab), or small molecule inhibitors.
4. Previous failed surgical intervention, including seton placement at least two weeks prior, or are not candidates for surgical intervention or are not willing to undergo surgical intervention for the management of their fistula.
5. Medical therapy for CD stable for at least 2 months prior to administration of IMP. Changes in dosing or dosing intervals related to serum drug levels are permitted.
6. Ability to comply with protocol.
7. Competent and able to provide written informed consent.

4.2 Exclusion Criteria

An individual who meets any of the following criteria will be excluded from participation in this study:

1. Active perianal abscess or infection at the time of screening, enrollment, or at the time of investigational product administration
2. Clinically significant medical conditions within the six months before administration of IMP that would, in the opinion of the investigators, compromise the safety of the subject.
3. History of cancer including melanoma (with the exception of localized skin cancers) within one year of screening.
4. History of colorectal cancer within 2 years
5. Subjects who have a diagnosis of coagulation disorders and/or are currently on anti-coagulant therapy
6. Investigational drug within one month of treatment
7. Pregnant or breast feeding or trying to become pregnant.
8. Presence of a rectovaginal fistula
9. Presence of an ileal anal pouch and/or history of proctectomy
10. The presence of severe proctitis
11. Any condition which, in the opinion of the investigator, would make it unsafe or unsuitable for patients to undergo MR evaluations (i.e., presence of implantable or external MR unsafe device that cannot be removed, body weight exceeding limitations, claustrophobia etc.).
12. A participant who is unwilling to use medically acceptable contraception methods during participation in study
13. The following out of range laboratory results at screening (result may be repeated)
 - WBC $>11 \times 10^9/L$
 - Hemoglobin $< 8 \text{ g/dl}$

- Platelet count <100,000/mL
- AST/ALT >2 times the upper limit of normal
- Creatinine >2 umol/L
- PT/INR: outside normal limits
- Hemoglobin A1c >6.5 mg/dL

4.3 Subject Recruitment, Enrollment and Screening

Patients who meet eligibility criteria will be offered enrollment into the study.

At the time of the patient enrollment on the trial the following must be verified:

- 1) IRB approval of the trial and open for enrollment
- 2) Patient is willing and able to travel to the study site for all tests, treatments, and follow up required in the protocol
- 3) ExoFlo product availability

At the time of patient enrollment, the following will be confirmed and documented:

- 1) A signed consent form by the patient and the individual obtaining consent
- 2) A signed authorization for the use and disclosure of the patient's protected health information
- 3) Confirmation that the patient has met all inclusion criteria and meets none of the exclusion criteria

Prior to treatment, screening tests must be completed within the guidelines specified on the test schedule.

4.4 Discontinuation of Study Intervention and Early Withdrawal of Subjects

Discontinuation or pausing of the study intervention may occur for many reasons, including physiologic changes during the study intervention suggesting potential infusion reaction, unfavorable physiologic changes within 24 hours following the study intervention, or voluntary patient withdrawal. Unique and unanticipated scenarios will be evaluated by the investigators and the Sponsor on a case-by-case basis.

All safety/adverse event data will be reviewed on an ongoing basis by the medical monitor. If any of the following occur, irrespective of attribution, the study will be paused for additional safety evaluation by as DSMB or equivalent.

- Any death
- Any CTCAE Grade 4 adverse event (AE)
- Any Grade 3 or equivalent hypersensitivity reaction
- Any two subjects experience a grade 3 AE (CTCAE v. 5) in the same system organ class
- Any subject requires surgical intervention within 30 days of product administration
- Any perianal infection or abscess formation within 30 days of the index procedure
- Any newly reported incontinence or reported injury to the sphincter after product administration.

Discontinuation of the study IMP does not mean discontinuation from the study. All participants who received any portion of IMP will be followed according to the Schedule of Events ([Section 6.9](#)).

4.4.1 Screen Failures

Screen failures are defined as participants who consent to participate in the clinical trial but are not subsequently randomized to the study intervention or entered in the study. A minimal set of screen failure information is required including demography, screen failure details, eligibility criteria, and any SAE. Individuals who do not meet the criteria for participation in this trial (screen failure) because of an initially improving or stable clinical course (but is now showing clinical deterioration despite SOC) may be rescreened up to 3 times.

4.5 Subject Discontinuation/Withdrawal From the Study

Participants are free to withdraw from the study at any time upon request. This is the only reason for a subject to be considered withdrawn. If the subject withdraws consent, the Sponsor may retain and continue to use any data collected before such withdrawal of consent, but there will be no further visits or data collected.

Subjects may be discontinued from receiving IMP at the discretion of the Investigator or Sponsor at any time. Discontinuation of the study IMP does not mean discontinuation from the study. All participants who received any portion of IMP will be followed according to the Schedule of Events ([Section 6.9](#)).

Subjects who sign the informed consent form and are enrolled but do not receive the study intervention may be replaced. Subjects who sign the informed consent form and are enrolled and receive any portion of the study intervention, and subsequently withdraw, or are withdrawn or discontinued from the study, will not be replaced.

4.5.1 Lost to Follow-up

A subject will be considered lost to follow-up if the subject stops responding to attempted contacts by the site and subsequently:

- Fails to respond to at least three telephone attempts
- Fails to respond to a certified letter sent to their last known mailing address (or local equivalent methods); and
- Attempts to contact the participant's close contacts per the two bullet points above are unsuccessful.

These contact attempts should be documented in the participant's medical record or study file.

5 Study Drug

5.1 Description

The IMP is a formulation of extracellular vesicles isolated from human donor bone marrow derived MSCs using proprietary technology and processed under cGMP standards. The IMP is notable for its high production standards, purity, and potency ([Table 1, Section 1.2](#)).

5.2 Packaging

The IMP is available in glass vials containing 5 mL or 15 mL frozen liquid product. The vials are shipped in cryoboxes. Refer to the IMP for more details.

5.3 Preparation, Handling, Storage and Accountability

5.3.1 Acquisition, and Accountability

Prior to the study enrollment, IMP will be shipped directly to the hospital pharmacy for proper storage.

5.3.2 Receipt of IMP

Each shipment of IMP will include a packing slip with the specific amount of IMP. Please refer to IMP manual for full details.

5.3.3 Formulation, Appearance, Packaging and Labeling

The sterile biologic product is sealed inside the glass vial. Both the glass vial and product box are labeled with the manufacturer's lot number, product volume, and expiration date (month/day/year). The product box is sealed with Direct Biologics, LLC, logo sticker and labeled with the manufacturer's lot number, the manufacturer's catalog number, product volume, and expiration date (month/day/year); in addition to the contact information for Direct Biologics, LLC. the external packaging exhibits the widely use symbols for single use only, limited to use by a physician, temperature limits (preferred temperature range is -70 ± 10) to which the product can be safely stored prior to immediate use. The IMP manual is to be followed for full storage requirements. At room temperature, the IMP thaws within minutes; once properly thawed, The IMP is a clear liquid that is visually identical to normal saline. Please refer to IMP manual for full details.

5.3.4 Product Storage and Stability

IMP is to be stored between -70 ± 10 Celsius. IMP must be stored while it is still frozen and once thawed, the IP is NOT recommended for sterilization or refreezing for future use. The product boxes will be kept sealed and stored within the pharmacy medication freezer. The IMP manual is to be referred to for further details.

5.3.5 Preparation

For information on IMP preparation and accountability, please refer to the current IMP Manual

5.3.6 Dispensing of Study Drug

The study drug will only be dispensed by an IRB approved treating provider. Regular study drug reconciliation will be performed to document drug assigned, drug dispensed, drug returns, and drug remaining. This reconciliation will be logged on the drug reconciliation form and signed and dated by the study team.

5.3.7 Return or Destruction of Study Drug

Please refer to IMP manual

5.4 Dosing and Administration

Each dose of IMP will be drawn up from 5 mL or 15 mL vials of thawed ExoFlo or Normal Saline (control arm) by a 22G needle into a 5 mL plastic syringe.

If no active sepsis is found, the IMP injection procedure will proceed. At that time, setons will be removed (when present), and IMP will be delivered via direct injection with a 22G needle.

The subject can have multiple branching tracts. If so, the three most dominant tracts will be treated. The dominant tract is the tract with the largest diameter opening which is assessed at the time of EUA with a fistula probe, or if the diameter is equivalent, the longest fistula tract length. The internal opening (on the anal canal/rectal side) will be closed with suture ligation (a stitch of the mucosa and submucosa) using absorbable 2-0 PDS suture.

A total of 15 mL or 30 mL of IMP or placebo (Normal Saline) will be drawn into three or six separate 5 mL syringes (each containing 5 mL of final product) using a 22G needle. A 22G needle will then be used for ExoFlo injection. One third of the total volume will be injected through the 22G needle just deep to the suture ligation of the internal opening, into the submucosal layer of the bowel wall. The external opening of the fistula tract will be identified, and the remaining volume will be injected along the length of the fistula tract (in parallel to the wall of the tract no deeper than 2 mm from the fistula tract wall).

If there are multiple tracts, these volumes will be equally divided between the fistula tracts. If the subject is randomized into the control arm, the subject will still have a suture ligation of the internal opening and injection of NS at 15 mL or 30 mL rather than the ExoFlo product.

Subjects in Cohort 3 may be treated again after 3 months with 30 mL if the fistula is not completely healed clinically.

5.5 Treatment Regimen

Subjects will be randomized 2:1 IMP to NS in 3 Cohorts of 12 subjects as follows:

Cohort 1: Local injection of 15 mL of IMP or NS on Day 0 (8 IMP, 4 NS)

Cohort 2: Local injection of 30 mL of IMP or NS on Day 0 (8 IMP, 4 NS)

Cohort 3: Local injection of 30 mL of IMP or NS on Day 0 and Month 3 (8 IMP, 4 NS)

5.6 Method for Assigning Subjects to Treatment Groups

Subjects will be randomized in groups of three at a time with a ratio of 2:1 IMP to NS within each group. Randomization will be assigned by an Interactive Response Technology (IRT) system.

5.7 Measures Minimize Bias: Randomization, Blinding/Unblinding

5.7.1 Blinding

This is a single blinded study- subjects will be blinded to treatment while the site staff and sponsor will not. When a subject is randomized, the site will follow their internal process for notifying

pharmacy of the treatment assignment (IMP or NS) and administering the treatment while ensuring that the subject remains blinded.

5.7.2 Unblinding

Subjects will remain blinded to their treatment until after their Visit 6.4 assessments.

5.8 Study Intervention Compliance Monitoring

Study visits and patient reported outcome survey requirements will be discussed prior to the subject signing consent. The subject will receive their visit schedule at the time of consent and the dates will be discussed at length to ensure compliance and agreeance. Subject safety will be emphasized during the discussion of study requirements. The subject will be educated on how to reference their study visit schedule at any time and will be provided contact information for any questions. The subject must agree to attend all study visits and complete all study requirements in order to participate in the trial. A courtesy call from the study team to subjects, prior to each study visit, may be made to ensure compliance.

5.9 Prior and Concomitant Therapy

Subject must be on stable medical therapy for CD for 2 months prior to administration of IMP as defined in the inclusion criteria. There are no concomitant medication restrictions, other than Exclusion 5 (no investigational drug within one month of treatment).

All procedures, blood products and concomitant medications such as antibiotics, anti-diarrheals, analgesics, and/or anti-emetics received from screening until the end of the study will be recorded in the subject's source record.

6 Study Procedures

6.1 Visit 1 – Screening (Day -30 to Day 0)

Subjects will sign informed consent and will be evaluated for eligibility (inclusion/exclusion checklist). Subjects will undergo the following tests and procedures at this visit:

- Medical and surgical history review
- General and perianal area exam with vital signs
- ECG
- The Perianal Crohn's Disease Activity Index (PCDAI) score
- Wexner Incontinence Score
- EuroQol 5 Dimensions survey (EQ-5D)
- IBDQ QoL survey (IBDQ)
- The Short Form 36 Health Survey (SF-36)
- IBDQ-PRTI
- MRI of the pelvis will be performed to assess the perianal fistula to verify eligibility if not performed clinically in the previous 90 days.
- Urine pregnancy test will be performed for women of childbearing potential (WOCBP)
- Laboratory studies including:
 - Complete Blood Count

- C-Reactive Protein
 - Comprehensive Metabolic Panel including PT/INR and HbA1c
- Concomitant medications review
- If subject meets eligibility: Randomization to IMP or NS

6.2 Visit 2 (Day 0)

- Confirm Eligibility criteria
- EUA
- Urine pregnancy test (WOCBP only)
- Injection of IMP or NS
- Adverse event (AE) review
- Fecal calprotectin from fistula tract

While the subject is in the operating room for the EUA, designated site personnel will deliver the study treatment. The subject will be dosed if the subject remains eligible. See [Section 5.4](#) for administration.

Subjects will be observed in the post anesthesia care unit (PACU) per EUA standard of care for acute AEs following the IMP injection. Per standard clinical protocol, subjects will be admitted overnight to the hospital for observation if they have a combination of two or more of the following: sustained tachycardia (Heart rate >120 beats per minute above their baseline prior to the procedure), sustained low blood pressure (<30 mmHg below baseline prior to procedure), or sustained fever (>38.3°C on two or more readings spaced at least two hours apart).

Patients may be discharged to home if they do not have a fever >38.0 C, heart rate <50 or >110, systolic blood pressure <80 mmHg or >160 mmHg, and a respiratory rate <8 or >20.

Patients will be admitted to the hospital if the discharge eligibility criteria are not met for more than 4 hours.

Post-operative care instructions will be provided to the subject following the administration of the IMP.

6.3 Visit 3 (Day 1)- Outpatient

- General and perianal exam with vital signs
- AE review

6.4 Visit 4 (Week 2 ± 3 days)- Outpatient

- General and perianal exam with vital signs
 - Clinical healing will be assessed by physical exam of the perianal area and compression of the external opening of the fistula tract to assess for drainage
- PCDAI score
- Wexner Incontinence Score
- EQ-5D
- IBDQ
- SF-36
- IBDQ-PRTI
- Laboratory studies including

- CBC
 - CRP
 - CMP
- Concomitant medications review
- AE review

6.5 Visit 5 (Week 6 ± 5 days)- Outpatient

- General and perianal exam with vital signs
 - Clinical healing will be assessed by physical exam of the perianal area and compression of the external opening of the fistula tract to assess for drainage.
- PCDAI score
- Wexner Incontinence Score
- EQ-5D
- IBDQ
- SF-36
- IBDQ-PRTI
- Laboratory studies including
 - CBC
 - CRP
 - CMP
- Concomitant medications review
- AE review

6.6 Visit 6 (Month 3 ± 7 days)- Outpatient

- General and perianal exam with vital signs
 - Clinical healing will be assessed by physical exam of the perianal area and compression of the external opening of the fistula tract to assess for drainage.
- PCDAI score
- Wexner Incontinence Score
- EQ-5D
- IBDQ
- SF-36
- IBDQ-PRTI
- Laboratory studies including
 - CMP
 - CBC
 - CRP
- Concomitant medications review
- AE review
- Fecal calprotectin from fistula tract (if going for EUA in visit 6.1, will obtain at 6.1. if not EUA will complete at this clinic visit)

Cohort 3 only: If complete clinical healing has not been achieved, and the subject did not have an SAE related to the first treatment, the subject will return to the operating room for an EUA and a repeat injection of IMP.

6.6.1 Visits 6.1 to 6.4

Cohort 3 subjects receiving a repeat injection ONLY

These visits are only for subjects receiving a repeat injection of the IMP or NS because of incomplete clinical healing

	Visit 6.1 (Month 3, Day 0; within 3 days of visit 6.0)- Outpatient • Confirm eligibility criteria • Urine pregnancy test will be performed for WOCBP (if retreated with IMP) • EUA • Fecal calprotectin from fistula tract if not obtained at 6.6 • Repeat injection of IMP or placebo ○ Subjects receiving a repeat injection will complete Visit 6.2 to 6.4. ○ Subjects not receiving additional injection will be seen at Visit 7. • AE review IMP is administered the same way as initial injection per Section 5.4. Post-operative care instructions will be provided to the subject following administration.
	Visit 6.2 (Month 3, Day 1)- Outpatient • General and perianal exam with vital signs • AE review
	Visit 6.3; (Month 3, Week 2 ± 3 days)- Outpatient • General and perianal exam with vital signs • PCDAI score • Wexner Incontinence Score • EQ-5D • IBDQ • SF-36 • IBDQ-PRTI • Laboratory studies including ○ CBC ○ CRP • Concomitant medications review • AE review
	Visit 6.4 (Month 3, Week 6 ± 5 days)- Outpatient • General and perianal exam with vital signs • PCDAI score • Wexner Incontinence Score • EQ-5D • IBDQ • SF-36 • IBDQ-PRTI • Laboratory studies including ○ CBC

- | | |
|--|--|
| | <ul style="list-style-type: none">○ CRP• Concomitant medications review• AE review |
|--|--|

6.7 Visit 7 (Month 6, $\pm$ 14 days)- Outpatient, ALL SUBJECTS

- General and perianal exam with vital signs
 - Clinical healing will be assessed by physical exam of the perianal area and compression of the external opening of the fistula tract to assess for drainage
- PCDAI score
- Wexner Incontinence Score
- EQ-5D
- IBDQ-PRTI
- IBDQ
- SF-36
- Pelvic MRI with fistula protocol
- Laboratory studies including
 - CMP
 - CBC
 - CRP
 - Fecal calprotectin from fistula tract
- Concomitant medications review
- AE review

6.8 Visit 8 (Month 12 $\pm$ 14 days)- Outpatient, ALL SUBJECTS

- General and perianal exam with vital signs
 - Clinical healing will be assessed by physical exam of the perianal area and compression of the external opening of the fistula tract to assess for drainage.
- PCDAI score
- Wexner Incontinence Score
- EQ-5D
- IBD-PRTI
- IBDQ
- SF-36
- IBDQ-PRTI
- Pelvic MRI with fistula protocol
- Laboratory studies including
 - CMP
 - CBC
 - CRP
 - Fecal calprotectin from fistula tract
- Concomitant medications, including blood products
- AE review

All Subjects: Visit 8 completes their participation in this study.

6.9 SCHEDULE OF EVENTS

6.9.1 Subject Schedule of Events

	Visit 1.0 Screening	Visit 2 Day 0	Visit 3 Day 1	Visit 4 Week 2	Visit 5 Week 6	Visit 6.0 Month 3	Visit 6.1 ^b Month 3 Day 0	Visit 6.2 ^b Month 3 Day 1	Visit 6.3 ^b Month 3 Week 2	Visit 6.4 ^b Month 3 Week 6	Visit 7 Month 6	Visit 8 Month 12
Visit window (days)	-30 to 0	0	0	± 3	±5	±7	≤ 14 days of visit 6	0	± 3	± 5	± 7	± 14
Eligibility	X	X					X					
Informed Consent	X											
Medical Surgical History	X											
General & Perianal exam w/vital signs	X		X	X	X	X		X	X	X	X	X
ECG	X											
Patient reported outcome surveys	X			X	X	X			X	X	X	X
MRI	X ^a										X	X
EUA		X					X					
IMP Delivery (ExoFlo or placebo)		X					X ^b					
Pregnancy Test	X ^c	X ^c					X ^c					
Complete Blood Count	X			X	X	X			X	X	X	X
C-Reactive Protein	X			X	X	X			X	X	X	X
Comprehensive Metabolic Panel & PT/INR	X			X	X	X					X	X
Fecal calprotectin from fistula tract		X				X	X ^d				X	X
HbA1c	X											
Concomitant medications	X			X	X	X			X	X	X	X
Adverse Events		X	X	X	X	X	X	X	X	X	X	X

a = If not done clinically in the last 90 days

b = If there is no clinical healing and no allergic reaction to the first treatment Cohort 3 receives repeat dose of 30 mL IMP/Placebo (Complete all visits in grey section, Visits 6.1 to 6.4)

c = Urine pregnancy test for women of childbearing potential only.

d = If not completed at 6.6 due to plan for EUA, then collect at time of EUA

7 Statistical Plan

7.1 Sample Size Determination

This is a phase IB/IIA designed to determine the safety and feasibility of 15 mL and 30 mL of ExoFlo, in the treatment of subjects with perianal fistulizing CD. Safety will be evaluated over the course of 12 months for all subjects.

7.2 Enrollment

This study will enroll 36 subjects. Safety data will be reviewed by the DSMB. The study will be paused for additional safety evaluation by as DSMB or equivalent per [section 8.5](#).

7.3 General statistical considerations

Safety and feasibility will be presented primarily in a descriptive fashion. The small sample size and selective population associated with phase IB/IIA studies restricts the generalizability of complex statistical procedures that are implemented. For ancillary studies, data may be compared to historical controls or baseline data in a manner sufficient to identify drug related events. For example, univariate descriptive statistics and frequency distributions will be calculated, as appropriate for all variables. Baseline values for demographic, clinical, and outcome variables (primary and secondary) will be tabulated for the treatment group. These analyses will help identify potential confounding variables to be used as covariates in sensitivity analyses.

Details regarding each of the variables under analysis are given below.

Interim Analysis

After each Cohort has completed the two-week follow-up visit, the DSMB will review all safety data prior to initiating enrollment in the subsequent cohort.

7.4 Primary outcome measures: Safety & Feasibility

The primary endpoints are safety & feasibility. Safety will be defined as the rate of AEs or SAEs related to IMP and administration of IMP in this patient population. Safety will be monitored through the entire duration of the study by the DSMB.

To evaluate the safety and feasibility of 1-2 treatments of ExoFlo 15 mL or 30 mL and 2 treatment of ExoFlo 30 mL in subjects with perianal fistula(s) in the setting of CD.

7.5 Subject Population(s) for Analysis

All-completed population: Only subjects who completed ALL study related procedures and follow-up will be included.

8 Safety and Adverse Events (AEs)

8.1 Definitions

Unanticipated Problem or AEs

Any unanticipated problem or AE that meets the following three criteria:

- Serious: Serious problems or events that result in significant harm, (which may be physical, psychological, financial, social, economic, or legal) or increased risk for the subject or others (including individuals who are not research subjects). These include: (1) death; (2) life threatening adverse experience; (3) hospitalization - inpatient, new, or prolonged; (4) disability/incapacity - persistent or significant; (5) birth defect/anomaly; (6) breach of confidentiality and (7) other problems, events, or new information (i.e. publications, DSMB reports, interim findings, product labeling change) that in the opinion of the local investigator may adversely affect the rights, safety, or welfare of the subjects or others, or substantially compromise the research data, **AND**
- Unanticipated: (i.e., unexpected) problems or events are those that are not already described as potential risks in the protocol, consent document, not listed in the Investigator's Brochure, or not part of an underlying disease. A problem or event is "unanticipated" when it was unforeseeable at the time of its occurrence. A problem or event is "unanticipated" when it occurs at an increased frequency or at an increased severity than expected, **AND**
- Related: A problem or event is "related" if it is possibly related to the research procedures.

An **AE** is any untoward or unfavorable medical occurrence, including any abnormal sign (for example, abnormal physical exam or laboratory finding), symptoms, or disease. AEs can encompass both physical and psychological harms.

AE means any untoward medical occurrence associated with the use of an intervention in humans, whether or not considered intervention-related (21 CFR 312.32 (a)).

An AE can therefore be:

- Any unfavorable and unintended sign (including laboratory findings), symptom, or disease temporally associated with the use of study agent(s), whether or not apparently related to the study agent(s).
- A concurrent illness.
- An exacerbation, or an **unexpected** increase in frequency or intensity of a preexisting condition, including intermittent or episodic conditions. However, **anticipated** day-to-day fluctuations or **expected** progression of the preexisting condition (based upon the investigator's clinical judgment) are **not** to be considered AEs.
- A significant or **unexpected** worsening or exacerbation of the condition/indication under investigation. However, **anticipated** day-to-day fluctuations or **expected** progression of the disease under investigation (based upon the investigator's clinical judgment) are **not** to be considered AEs.
- A suspected interaction between the study agent(s) **or** between the study agent(s) and concomitant medication(s), including agents used in combination with the study agent(s).
- Any clinically significant laboratory abnormality (including ECG results, radiological interpretations, histopathological findings, sterility testing, etc.).
- Clinically significant abnormalities in physical examination, vital signs, weight, drainage for the perianal fistula

- Sequelae from intentional or unintentional overdose with study agent(s) or other concomitant medication(s).
- An event following (a) study procedure(s).

An AE or suspected adverse reaction is considered "serious" if, in the view of either the investigator or sponsor, it results in any of the following outcomes: death, a life-threatening adverse event, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or a congenital anomaly/birth defect. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

Serious Adverse Event

AEs are classified as serious or non-serious. An SAE is any AE that results in any of the following outcomes:

- death
- a life-threatening experience
- inpatient hospitalization or prolongation of existing hospitalization
- a persistent or significant disability/incapacity
- a congenital anomaly/birth defect
- hospitalization
- a significant infection near the injection site
- severe perianal pain that requires a higher than expected escalation in pain management and/or per protocol may be problems/events that in the opinion of the investigator may have adversely affected the rights, safety, or welfare of the patients or others, or substantially compromised the research data.
- important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

An **Unexpected AE** means any AE not previously known or included in the current Investigator's Brochure, consent form or other risk information.

Related/Possibly Related means there must be reasonable evidence to suggest the event was caused by the investigational medicinal product or investigational intervention.

General Physical Examination Findings

At screening, any clinically significant abnormality should be recorded as a preexisting condition. At the end of the study, any new clinically significant findings/abnormalities that meet the definition of an AE must also be recorded and documented as an AE.

Preexisting Condition

A preexisting condition is one that is present at the start of the study. A preexisting condition should be recorded as an adverse event if the frequency, intensity, or the character of the condition worsens during the study period.

Post-study AE

All unresolved adverse events should be followed by the sponsor-investigator until the events are resolved, the subject is lost to follow-up, or the adverse event is otherwise explained. At the last scheduled visit, the sponsor-investigator should instruct each subject to report, to the sponsor-investigator, any subsequent event(s) that the subject, or the subject's personal physician, believes might reasonably be related to participation in this study.

Abnormal Laboratory Values

A clinical laboratory abnormality should be documented as an adverse event if the abnormality is deemed clinically significant by the clinician investigator, and it is deemed unrelated to the clinical condition being treated. This would also include a positive culture from the sterility testing performed on the final product after it is administered to the subject.

Life-Threatening: An AE or suspected adverse reaction is considered “life-threatening” if, in the view of either the sponsor, its occurrence places the patient or subject at immediate risk of death. It does not include an AE or suspected adverse reaction that, had it occurred in a more severe form, might have caused death.

All AEs that do not meet any of the criteria for serious, will be regarded as **non-serious AEs**.

8.2 AE Reporting Period

For this study, the study treatment follow-up period is defined as 365 days (+/- 14 days) from the first administration of the active IMP. Events occurring after this, last study visit will not be collected, recorded, or reported.

8.3 Recording of AEs

All AEs occurring during the study period must be recorded. The clinical course of each event should be followed until resolution, stabilization, or until it has been ultimately determined that the study treatment or participation is not the probable cause. SAEs that are still ongoing at the end of the study period must be followed up, to determine the final outcome. SAE that occurs during the AE Reporting period and is considered to be at least possibly related to the study treatment or study participation should be recorded and reported immediately.

Hospitalization, Prolonged Hospitalization or Surgery

Any AE that results in hospitalization or prolonged hospitalization should be documented and reported as an SAE.. Any condition responsible for surgery should be documented as an adverse event if the condition meets the criteria for an adverse event.

8.4 Classification of AEs

For AEs not included in the protocol defined grading system, the following guidelines will be used to describe severity.

- **Mild** – Events require minimal or no treatment and do not interfere with the participant's daily activities.
- **Moderate** – Events result in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning.
- **Severe** – Events interrupt a participant's usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually potentially life-threatening or incapacitating. Of note, the term "severe" does not necessarily equate to "serious".

All clinical AEs encountered during the study will be captured in the subjects' source records and entered in EDC. Intensity of AEs will be graded based on the CTCAE, Version 5.0. Grading 1-5 for relationship to the IMP will be the following: Not Related, Unlikely to be Related, Potentially Related, Probably Related, and Definitely Related.

8.5 Reporting of SAEs and Unanticipated Problems

When an AE has been identified, the study team will take appropriate action necessary to protect the study participant and then document the AE.

8.5.1 Principal Investigator reporting

The primary investigator will report any SAEs to the sponsor and local IRB, if applicable. Information collected on the AE will be documented in the subject's source records:

- The date the AE occurred
- Description of the AE
- Relationship of the AE to the research drug and procedure
- If the AE was expected
- The severity of the AE (NCI CTCAE v5 grading scale (above) will be used)
- If any intervention was necessary
- Resolution (was the incident resolved spontaneously, or after discontinuing treatment)
- Date of Resolution

The sponsor will review all AE reports to determine if specific reports need to be made to the FDA. The site should follow their local IRB reporting criteria.

8.5.2 Sponsor reporting: Notifying the FDA

The sponsor will report to the FDA all unexpected, serious suspected adverse reactions according to the required IND Safety Reporting timelines, formats and requirements. The FDA will also be notified of any decision to suspend the study for other safety reasons. The suspended study may only be re-opened after the sponsor-investigator has received FDA approval to do so.

Unexpected fatal or life threatening suspected adverse reactions where there is evidence to suggest a causal relationship between the study drug and the adverse event, will be reported as a serious

suspected adverse reaction. This will be reported to the FDA on FDA Form 3500A, no later than 7 calendar days after the sponsor-investigator's initial receipt of the information about the event.

Other unexpected serious suspected adverse reactions where there is evidence to suggest a causal relationship between the study drug and the adverse event, will be reported as a serious suspected adverse reaction. This will be reported to the FDA on FDA Form 3500A, no later than 15 calendar days after the sponsor-investigator's initial receipt of the information about the event.

Any clinically important increase in the rate of serious suspected adverse reactions over those listed in the protocol will be reported as a serious suspected adverse reaction. This will be reported to the FDA on FDA Form 3500A no later than 15 calendar days after the sponsor-investigator's initial receipt of the information about the event.

Findings from other studies in human or animals that suggest a significant risk in humans exposed to the drug will be reported. This will be reported to the FDA on FDA Form 3500A, no later than 15 calendar days after the sponsor-investigators initial receipt of the information about the event.

8.6 Stopping Rules

All safety/adverse event data will be reviewed on an ongoing basis by the medical monitor. The DSMB will be responsible for safeguarding the interests of trial subjects and assessing the safety of the interventions during the trial. The DSMB will provide recommendations about stopping or continuing enrollment in the trial. If any of the following occur, irrespective of attribution, the study will be paused for additional safety evaluation by the DSMB and the FDA will be informed. Following communication from both the DSMB and FDA, the study can continue enrollment.

- Any death
- Any CTCAE Grade 4 adverse event (AE)
- Any Grade 3 or equivalent hypersensitivity reaction
- Any two subjects experience a Grade 3 AE in the same system organ class determined to be related to IMP
- Subject requires unanticipated surgical intervention related to the fistula within 30 days of receipt of IMP
- Any perianal infection or abscess formation within 30 days of IMP administration
- Any new onset incontinence or reported injury to the sphincter after product administration

8.6.1 Subject/Product Administration Stopping Rules

Product administration will be discontinued and subjects will be discontinued from further injections if any of the following situations are observed during or after administration:

- Grade 4 or above AE (CTCAE v5)
- Any Grade 3 or above hypersensitivity
- Pregnancy
- Anesthesia related complications
- Perianal infection or abscess formation requiring surgical invention within 30 days of IMP administration

- Any new onset incontinence or reported injury to the sphincter after IMP administration

If subjects experience worsening disease that is clinically significant or treatment related AEs, as noted above, subjects will not be retreated but will be followed for the remainder of their trial-related follow up visits.

8.7 Medical Monitoring

It is the responsibility of the Principal Investigator to oversee the safety of the study at his/her site. This safety monitoring will include careful assessment and appropriate reporting of AEs per regulatory requirements. Medical monitoring will include a regular assessment of the number and type of SAEs. All safety/adverse event data will be sent to the Medical Monitor for review and escalation to DSMB review as required. The DSMB will provide recommendations about stopping or continuing enrollment in the trial.

9 Data Handling and Record Keeping

9.1 Confidentiality

Information about study subjects will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). Those regulations require a signed subject authorization informing the subject of the following:

- What protected health information (PHI) will be collected from subjects in this study
- Who will have access to that information and why
- Who will use or disclose that information
- The rights of a research subject to revoke their authorization for use of their PHI.

In the event that a subject revokes authorization to collect or use PHI, the investigator, by regulation, retains the ability to use all information collected prior to the revocation of subject authorization. For subjects that have revoked authorization to collect or use PHI, attempts should be made to obtain permission to collect at least vital status (long term survival status that the subject is alive) at the end of their scheduled study period.

9.2 Source Documents

Source data is all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents. Examples of these original documents, and data records include: hospital records, clinical and office charts, laboratory notes, memoranda, subjects' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, subject files, and records kept at the pharmacy, at the laboratories, and at medico-technical departments involved in the clinical trial.

9.3 Records Retention

The sponsor will maintain records and essential documents related to the conduct of the study. These may include subject case histories and regulatory documents.

The sponsor will retain records and reports for the **longer** of the following:

- Up to 2 years after the marketing application is approved for the drug; or, if a marketing application is not submitted or approved for the drug, until 2 years after shipment and delivery of the drug for investigational use is discontinued and the FDA has been so notified.

10 Study Monitoring, Auditing, and Inspecting

10.1 Study Monitoring Plan

This study will be monitored by the sponsor on a routine basis during the conduct of the trial. Clinical trial monitoring requires review of the study data generated throughout the duration of the study to ensure the validity and integrity of the data along with the protection of research subjects.

The sponsor-investigator will allocate adequate time for such monitoring activities. The investigator will also ensure that the monitor or other compliance or quality assurance reviewer is given access to all the study-related documents and study related facilities and has adequate space to conduct the monitoring visit.

10.2 Auditing and Inspecting

The investigator will permit study-related monitoring, audits, and inspections by the IRB, the sponsor, and government regulatory agencies, of all study related documents (e.g., source documents, regulatory documents, data collection instruments, study data etc.). The investigator will ensure the capability for inspections of applicable study-related facilities (e.g., pharmacy, diagnostic laboratory, etc.).

Participation as an investigator in this study implies acceptance of potential inspection by government regulatory authorities and applicable compliance offices.

11 Ethical Considerations

This study is to be conducted according to United States government regulations and Institutional research policies and procedures.

This protocol and any amendments will be submitted to a properly constituted local Institutional Review Board (IRB), in agreement with local legal prescriptions, for formal approval of the study. The decision of the IRB concerning the conduct of the study will be made in writing to the sponsor-investigator before commencement of this study.

All subjects for this study will be provided a consent form describing this study and providing sufficient information for subjects to make an informed decision about their participation in this study. This consent form will be submitted with the protocol for review and approval by the IRB for the study. The formal consent of a subject, using the Approved IRB consent form, must be obtained before that subject undergoes any study procedure. The consent form must be signed by the subject or the subject's legally authorized representative, and the individual obtaining the informed consent.

12 Study Finances

12.1 Funding Source

Direct Biologics, LLC

12.2 Conflict of Interest

Any study team member who has a conflict of interest with this study (patent ownership, royalties, or financial gain greater than the minimum allowable) must have the conflict reviewed and approved prior to participation in this study.

13 Publication Plan

The sponsor will hold the primary responsibility for publications of the results of this study.

This study will be registered at ClinicalTrials.gov and results will be posted when available.

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15 Protocol Amendment History

Amendment	Personnel	Changes
1, 17 April 2023	Dr. Amy L. Lightner	The original protocol was updated to Amendment 1 based on feedback from the FDA.
2, 02 June 2023	Dr. Amy L. Lightner	- Modified stopping rules (sections 3.6 and 4.4) to include the following: - Any two subjects experience a grade 3 AE (CTCAE v.5) in the same system organ class - Any subject requires surgical intervention within 30 days of product administration - Any perianal infection or abscess formation within 30 days of the procedure - Modified section 3.6 to include: - Perianal infection or abscess formation - Added Protocol Amendment History - Minor corrections.