Protocol synopsis

Protocol title		Treatment of complex perianal fistulas of Crohn's disease with tissue transplantation by local injection of microfragmented autologous adipose tissue. Prospective, multicenter, randomized controlled trial
Short title		ATTIC
Version no. (date)		2 (11 January 2023)
Study design		Interventional, non-pharmacologic, prospective, randomized, controlled, double blind, parallel groups, profit, multicentric
EudraCT		--
Principal investigator		Prof. Silvio Laureti
Sponsor		Lipogems International S.p.A.
Experimental center		IRCCS Azienda Ospedaliero-Universitaria di Bologna, Policlinico San Orsola-Malpighi, Bologna, Italy
1.	**Rationale**	Crohn's disease is a chronic inflammatory process that can affect the entire digestive tract and, in about one-third of cases, leads to the development of perianal fistulas or abscesses. Perianal Crohn's disease causes pain, release of purulent or fecal material, and anal sphincter destruction, which all have deleterious effects on patients' quality of life. Perianal fistulas rarely heal spontaneously or after medical or surgical treatment. The role of surgery is to sanitize sepsis, improve quality of life, and defer the need for ileostomy or colostomy. The availability of anti-TNF antibodies (e.g. infliximab), however, has made fistula healing a realistic therapeutic goal. While infliximab is a powerful drug, if not used correctly patients may have septic complications. Thus, surgical drainage is a fundamental step in the treatment algorithm. The preferred procedure is cone-like fistulectomy, which creates a surgical wound that heals from the inner opening outwards, avoiding abscess development. Combined therapy (surgery and a biological drug) is the gold standard for perianal Crohn's disease. Still, it is ineffective in about one-third of patients, necessitating the need for new therapies. A new approach to promote healing of perianal fistulas is the local injection of mesenchymal stem cells (MSC), which have immunomodulatory activity. Some studies tested the efficacy of allogenic MSCs that had been expanded in vitro under laboratory conditions of good manufacturing practice. An alternative is to use

			autologous preparations that are rich in MSC-like cells but have not been expanded in vitro. These minimally manipulated materials can be prepared from adipose tissue using a Lipogems medical device, which mechanically fragments lipoaspirates into submillimeter particles suitable for transplantation. We previously tested microfragmented autologous adipose tissue for efficacy against perianal Crohn's fistulas in a non-controlled study of 15 patients and obtained healing in 10 patients at 24 weeks. This new randomized controlled trial will test the efficacy and safety of transplantation of microfragmented autologous adipose tissue in patients with perianal Crohn's disease refractory to standard therapy.	
2.	**Study objectives**		**Objective**	**Outcome**
		Primary	To determine the efficacy of circumferential, submucosal infiltration of microfragmented autologous adipose tissue around the internal fistula opening and in the perianal adipose tissue around the fistula: combined remission	Combined remission: both (i) absence of drainage at gentle finger compression of all treated external openings that were draining at baseline and (ii) absence of pelvic MRI evidence of abscesses > 3 mm near the treated fistulas, 24 weeks after the intervention Study hypothesis: combined remission rates of 10 % in the control group and 40 % in the treatment group
		Secondary	To determine the efficacy of circumferential, submucosal infiltration of microfragmented autologous adipose tissue around the internal fistula opening and in the perianal adipose tissue around the fistula: clinical remission, clinical response, failure, time to response, safety, and improvement in quality of life	Clinical remission: absence of drainage at gentle finger compression of all treated external openings that were draining at baseline*[1] Clinical response: closure of ≥ 50 % of all treated external orifices* Time to clinical remission in the subgroups of patients with combined remission: time from intervention to combined remission Quality of life: PDAI, IBDQ and WPAI scores* C-reactive protein* Safety: adverse events, serious

[1]After the study was over, we discovered that the definition of this outcome had been written incorrectly in the original protocol in Italian. The correct definition is reported here and has been communicated to ClinicalTrials.gov.

				adverse events, and suspected unexpected serious adverse reactions (SUSARs)* * Evaluated at 2 weeks (± 2 d), 4 weeks (± 2 d), 8 weeks (± 2 d), 12 weeks (± 3 d) and 24 weeks (± 5 d)
3.	**Study design**		Interventional, non-pharmacologic, prospective, randomized, controlled, double blind, parallel groups, profit, multicentric	
4.	**Patient selection**			
	Target population		Patients with Crohn's disease and complex perianal fistulas refractory to standard treatment (surgical sanitization and local or systemic biological therapy). Complex fistulas will be defined according to the American Gastroenterological Association and will include transsphincteric fistulas involving over 30% of the external anal sphincter, suprasphincteric fistulas, extrasphincteric fistulas, horseshoe fistulas, and fistulas with multiple tracts.	
	Inclusion criteria		– Age > 18 years at surgery – Crohn's disease confirmed by histology and diagnostic imaging – Complex perianal, fistulizing disease refractory to standard treatment (surgical drainage and local or systemic administration of anti-TNF for at least one year) – Written informed consent – Ability to understand the study and participate for the entire duration	
	Exclusion criteria		– More than one internal opening or three external openings – Ileostomy or colostomy – Anovaginal or rectovaginal fistulas – Active infection with HIV, HCV, HBV or tuberculosis – Abdominal Crohn's disease that could require general surgery during the study – Active oncologic or lymphoproliferative disease that could make it difficult to obtain at least 60 cc lipoaspirate – Clinical conditions that could compromise the outcome of surgery or follow-up – Pregnant or breastfeeding women	
	Concomitant treatments		Use of biological drugs, low-availability steroids or mesalamine in the 4 weeks before enrollment and azathioprine or mercaptopurine in	

		the 6 months before enrollment will be allowed in a stable dosage for the entire study. Concomitant treatments will be reported.
	Study groups	Treatment group (A): surgical sanitization with cone-like fistulectomy and infiltration of microfragmented autologous adipose tissue at the internal orifice (40 patients) Control group (B): surgical sanitization with cone-like fistulectomy, infiltration of physiological saline, and placement of a suture at the internal orifice (40 patients)
5.	**Study procedures**	Patients will be enrolled after protocol approval by the ethics committee of each center. Enrollment will be competitive, i.e. each center will enroll at least 8 patients until the total number (80) is reached.
	Randomization	Randomization will be centralized (i.e. not done at any study center). A computer-generated randomization list will be made, and patients will be assigned to the two groups in a 1:1 ratio. At each center, an investigator will identify eligible patients according to the inclusion and exclusion criteria and will present to them the scope of the study, the protocol and the procedures. After a patient provides consent, another investigator (not involved in enrollment, surgery or postoperative assessment) will contact the randomization center to obtain this patient's assignment (i.e. the first place available on the randomization list).
	Data collection	Each patient will be assigned an alphanumeric identification code generated by Lipogems International S.p.A. using the *blockrand* package v1.5 in R Statistical Software v4.2.0. The code will be compliant with rules on the privacy of subjects of clinical trials. All the data from the study will be registered by an investigator uninvolved in study procedures (including randomization and surgery). Radiological data will be saved in digital form on each institute's computer system. The other data will initially be recorded on case report forms (paper). For each patient, the study center will keep a folder with all the completed forms, including the signed informed consent form and privacy agreement. Information such as the identification code, personal data, clinical reports, informed consent, and privacy agreement will be kept in the patient's file and made available only in the case of audit or inspection. During the study, data from the paper forms will be progressively inserted into a database (regularly backed up).
	Enrollment and randomization	Potentially eligible patients will undergo a screening evaluation by an investigator, uninvolved in the surgical procedures, according to the inclusion and exclusion criteria. Eligible patients will be told of the study's goals and procedures and asked to provide informed consent

		and authorize the use of their data. Once a patient has been enrolled, an investigator will collect baseline clinical data, and the patient will undergo basic blood testing, measurement of serum C-reactive protein, and pelvic MRI. After enrollment, participants were randomly assigned in a 1:1 ratio to either the intervention group or control group through an Interactive Web Response System (IWRS). Patients in the treatment and control groups will undergo the same pre- and postoperative procedures and will be operated on by a surgeon uninvolved in their assessments. The study will be double blind, as follows: randomization and statistical analyses will be done by an investigator not involved in assessments or surgery. Clinical assessments will be done by investigators uninvolved in surgery and blinded to group assignment. The radiologist who evaluates the MRI scans will be blind to patients' assignment. The surgeons will not be allowed to share information on the treatments and will not participate in clinical assessments. In case of emergency, the principal investigator can decide to unblind a patient, who will then be excluded from the study. In these cases, the study sponsor will be notified as soon as possible. The date and reason for unblinding will be reported in the patient's clinical file.
	Preoperative assessment (T0)	On the day of surgery, the following data will be collected or measured: – Any use of biological drugs, low-availability steroids or mesalamine in the 4 weeks before enrollment and azathioprine or 6-mercaptopurine in the 6 months before enrollment – Serum C-reactive protein level – Pelvic MRI scan – PDAI score – IBDQ score – WPAI scores
	Preparation of microfragmented adipose tissue – Lipogems system	Lipogems is a class II A sterile medical device for the fragmentation of adipose tissue (with CE mark subsequent to assessment by notified body 0476). The US Food and Drug Administration approved the use of Lipogems devices for liposuction, microfragmentation without additional manipulation, and lipofilling in plastic surgery, reconstructive surgery and other surgical applications (22 December 2014; class II device under 21 CFR 878.5040, Section 510(k) premarket notification). The single-use Lipogems device reduces lipoaspirates to

			submillimeter clusters enriched in pericytes, with intact vascular structure, free of oil or blood. Because microfragmentation is achieved by mechanical means, without centrifugation or additives (e.g. enzymes), the resulting tissue product is considered minimally manipulated. With the Lipogems 60 cc kit, approximately 60 mL lipoaspirate is obtained in less than 20 min.	
	Surgical procedures		Liposuction and tissue transplantation will be done in a single surgical procedure.	
		Treatment group (A)	Phase 1. Liposuction	Liposuction will be done under general or spinal anesthesia. A plastic surgeon will choose a donor site (lateral or lower abdomen or thigh) in which 100 cc modified Klein solution (500 cc physiological saline, 1 cc epinephrine 1/1000 IU) will be injected using a 17G cannula on a 60 cc Luer-lock syringe. Adipose tissue will be aspirated (50-100 cc) using a 13G cannula on a 20 mL VacLok syringes.
			Phase 2. Tissue processing	The lipoaspirate will be immediately processed in a Lipogems device. The microfragmented tissue will be collected in a 60 cc syringe and allowed to settle for the removal of excess physiological saline. The remaining product will be transferred to 10 cc syringes.
			Phase 3. Surgery and tissue transplantation	Each patient will undergo a proctological exam under anesthesia for the identification of fistula tracts and any abscesses. Purulent material will be drained, and the tracts will be curetted. Necrotic or inflamed tissue will be removed by cone-like fistulectomy. Then, 15 cc microfragmented autologous adipose tissue will be injected using a 21G needle into the submucosa around the internal fistula opening and along the remaining fistula tract.
		Control group (B)	Phase 1. Sham liposuction	A 6-0 absorbable suture will be placed on the skin of the thigh to simulate liposuction.
			Phase 2. Surgery	Each patient will undergo a proctological exam under anesthesia for the identification of fistula tracts and any abscesses. Purulent material will be drained, and the tracts will be curetted. Necrotic or inflamed tissue will be removed by cone-like fistulectomy. Then, 10 cc physiological saline will be injected using a 21G needle around the internal fistula opening and along the remaining fistula

				tract. The internal fistula orifice will be closed using a 2-0 absorbable polyglactin suture.
	Postoperative assessments			
		2 weeks (T2)	C-reactive protein, clinical exam (assessment of drainage at gentile finger compression), PDAI, IBDQ and WPAI scoring; registration of any adverse events	
		4 weeks (T4)	C-reactive protein, clinical exam (assessment of drainage at gentile finger compression), PDAI, IBDQ and WPAI scoring; registration of any adverse events	
		8 weeks (T8)	C-reactive protein, clinical exam (assessment of drainage at gentile finger compression), PDAI, IBDQ and WPAI scoring; registration of any adverse events	
		12 weeks (T12)	C-reactive protein, clinical exam (assessment of drainage at gentile finger compression), PDAI, IBDQ and WPAI scoring; registration of any adverse events	
		24 weeks (T24)	C-reactive protein, clinical exam (assessment of drainage at gentile finger compression), PDAI, IBDQ and WPAI scoring; registration of any adverse events. Pelvic MRI	
			Control patients who do not achieve combined remission will be offered treatment with microfragmented autologous adipose tissue.	
	Pelvic MRI		MRI scanning will be done on a 1.5-3T MR scanner (Signa HDxt, GE Medical Systems) with an 8-channel phased array body coil (without endoanal coil). Axial T1- and T2-weighted fast spin-echo sequences with 5 mm slices will be used to assess sphincter anatomy and fistula extent. Short tau inversion recovery sequences, in axial, sagittal and coronal planes, and 3 mm slices will be used to distinguish fistulas from adjacent tissue and to assess soft tissue edema and inflammation in the perianal and perirectal areas. Radiological fistula healing will be defined as a lack of T2 hyperintensity and lack of abscesses > 3 mm.	
	Safety		At every follow-up visit, an investigator will record the occurrence of adverse events, serious adverse events and SUSARs between the date when the patient gave written informed consent and the end of the study.	
		Adverse events	An adverse event is any sign, symptom or disease, unfavorable and unwanted, that has a temporal association with use of the experimental product, even in the absence of a causal relation. The clinical conditions and pathologies that patients had before the	

			beginning of the study are considered adverse events only if they worsen during the study. Every adverse event that occurs during the study will be registered. For every adverse event, the investigators will promptly provide appropriate standard care. If necessary, the patient will be withdrawn from the study.
		Serious adverse events	A serious adverse event is an undesired sign, symptom or clinical condition that: 1. Is fatal, 2. Is life threatening, 3. Requires or prolongs hospitalization, 4. Results in disability or serious or prolonged incapacity, 5. Causes a congenital anomaly or birth defect. Hospitalization for a preexisting condition will be considered a serious adverse event, unless it had been planned prior to the start of the study.
		Classification of causality	The causal relation between an adverse event and the treatments will be classified as: – Unknown – Unrelated – Impossible – Possible – Probable – Certain The investigators will rank the severity of each adverse event to one of the following categories: – Mild: the symptom is evident but tolerable. – Moderate: it negatively affects normal activity. – Severe: a severe effect, incapacity to work, need to withdraw from the study.
		Reporting of adverse events	All adverse events will be documented in the case report forms. The investigators are responsible for correctly registering the events and for notifying healthcare authorities of all undesired events during the study. For each adverse event, the following will be recorded: – Identification code and initials of the patient – Randomization code

			– Description of the symptom or event – Classification of the event and its severity – Dates of onset and outcome – Frequency (once, occasionally, frequently, continually) – Treatment (none, pharmacological, non-pharmacologic, dose reduction, temporary suspension, hospitalization) – Causal relation with the study product – Outcome (resolved, not yet resolved, sequelae of disability or incapacity, death, unknown) Any serious adverse event or any adverse event that can affect the safety of the patient or affect the conduct of the study, whether correlated or not to the treatments, will be notified immediately (within 24 h) to Lipogems International Spa. Lipogems will forward the information to healthcare authorities as required by law.
		Withdrawal	Patients can withdraw from the study for the following reasons: a. personal decision (even without motivation) b. as decided by an investigator c. in case of an adverse event. The reason for withdrawal will be reported in the case report form.
6.	**Sample size and statistical analyses**		Sample size was calculated assuming the use of Fisher's exact test to assess the difference between groups in combined remission at T24. Assuming combined remission rates of 10 % in the control group and 40 % in the intervention group, a two-tailed test at a significance level of 0.05 would require a sample of 72 patients (36 per group) to achieve 80 % power. Assuming a drop-out rate of 10 %, the sample size was set to 80 (40 per group). Qualitative variables will be reported as counts and range. Quantitative variables will be reported as mean, standard deviation and range. Differences between groups in categorical variables, including the primary outcome, will be tested for significance using Fisher's exact test or chi-squared test. The distribution of continuous variables will be assessed using the Shapiro-Wilk test. Normally distributed variables will be assessed for significance using Student's *t* test; otherwise, the Mann-Whitney test will be used. Longitudinal data will be analyzed using two-way ANOVA for repeated measures and multiple regression.
7.	**Study duration**		Total, 24 months: enrollment, 12 months; follow-up, 6 months; data analysis, 3 months; writing, 3 months

8.	**Publication**	The results of the study will be written up in a manuscript, which will be sent to a peer-reviewed medical journal for publication.
9.	**Ethical and regulatory issues**	The study will adhere to the ethical principles of the Declaration of Helsinki (64th WMA General Assembly 2013), to ICH-GCP guidelines and ISO 14155 - Clinical Investigation of medical devices for human subjects, and to Italian laws on clinical trials.
	Ethical approval	The study protocol will be approved by each center's ethics committee before the study begins.
	Protocol changes	Any change to this protocol will be presented by the sponsor to the ethics committees for approval.
	Informed consent	Written informed consent will be obtained from each patient before entering the study. The informed consent form will be approved by the ethics committees.
	Privacy	The privacy of patients in the study will be protected in accordance with ICH/GCP and local legislation. Patients' identities will be known only to the investigators and the Clinical Monitor (in accordance with UE regulation 2017/745).
	Data access	The investigators will allow study monitoring by the sponsor. They will also allow healthcare authorities and ethics committee members direct access to study documentation during audits.
	Data ownership	Study data will be the property of Lipogems International S.p.A.
	Funding	The study is funded by Lipogems International S.p.A. without costs to the Italian healthcare system.
	Ethical guidelines	The study will adhere to the Declaration of Helsinki (Fortaleza 2013) and to the principles of good clinical practice as expressed in Italian law (D.leg. 24 giugno 2003, n. 211 alla G.U. n. 184 del 09/08/2003).
	Insurance	Lipogems International S.p.A will provide insurance for all study subjects.
10	**Premature study termination**	The investigators have the right to stop the study at any moment for medical or administrative considerations. Reasons for the interruption will be documented, and the ethics committee of the Italian Ministry of Health will be informed.