Protocol Cover Page

Protocol Title: A pilot crossover study to investigate *the safety and efficacy of administering intravenous autologous cord blood versus personalized noninvasive treatments* to children with developmental disorders of the autistic spectrum.

Protocol Number: Bio-Forum Foundation 011 (B-F011)

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Study Phase: I-II

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Confidentiality Agreement

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LIST OF ABBREVIATIONS AND DEFINITIONS

The following abbreviations and specialist terms are used in this study protocol:

Abbreviation	**Explanation**
AE	Adverse Event
ALT	Alanine aminotransferase
ASD	Autistic Spectrum Disorder
AST	Aspartate aminotransferase
b.i.d	*bis in die* = twice daily
CD45+	count of CD45 expressing cells
CNS	Central Nervous System
CRF	Case Report Form
ECG	Electrocardiogram
FC	FlowCytometry
GCP	Good Clinical Practice
GGT	gamma glutamyl transpeptidase
Hb	Haemoglobin
Hct	Haematocrit
HDL-C	High Density Lipoprotein –cholesterol
HLA	Human Leukocyte Antigen
ICH	International Conference of Harmonisation
IEC	Institutional Ethics Committee
IP	Inflammatory Panel
IRB	Institutional Review Board
LCMS	Liquid chromatography linked mass spectrometry
LDH	Lactate dehydrogenase
LDL-C	Low Density Lipoprotein -cholesterol
MB	Microbiology (testing)
MCH	Mean corpuscular haemoglobin
MCHC	Mean corpuscular haemoglobin concentration
MRI	Magnetic Resonance Imaging
ml	milliliter
PQQ	Pironoquinolinquinone
RDW	Red cell distribution width
RBC	Red Blood Cells
SAE	Serious Adverse Event
SEM	Standard error of the mean
T3	Triiodothyronine
T4	Thyroxine
TSH	Thyroid-stimulating hormone
UCB	Umbilical Cord Blood
WBC	White blood cells

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PROTOCOL SYNOPSIS

TITLE	A pilot crossover study to investigate the safety and efficacy of administering intravenous autologous cord blood versus personalized noninvasive treatments to children with developmental disorders of the autistic spectrum		
Investigational site:	Spitalul Angiomedica, Str Cosminului Nr 13, Bucharest, Romania		
Investigators:	Dr Felician Stancioiu, Dr Marie-Elene Niculita		
Sponsor:	Bio-Forum Foundation, Bucharest, Romania		
Representatives:	Dr Felician Stancioiu		
Study number:	B-F001		
Final Protocol:	June 2019	**Clean File:**	June 2019
Ethics Approval:	January 2019	**Statistical analysis:**	
Clinical Phase:		**Study Report:**	
OBJECTIVES:	To compare the safety and efficacy of administering intravenous autologous cord blood versus personalized noninvasive treatments to children with developmental disorders of the autistic spectrum		
STUDY DESIGN:	An open-label, pilot crossover study over a 12-month period. Participants will be screened and randomized at Visit 0 and at visit V1 will be receiving either intravenous umbilical cord blood or noninvasive treatment. At V2, at 2 months after V1, the other treatment is administered and subsequently the child will be evaluated by questionnaires and child psychiatrist. Parents will report adverse events and complete the M-CHAT, Q-CHAT and CAST questionnaires. Modifications on these questionnaires will be analized statistically as a quantitative measure of child's improvement.		
SUBJECTS:	**Inclusion criteria** Males and females between 3 and 7 years of age: • parents informed of the nature of the study and will give written informed consent, • have body weights between 10-30 kg, • have no clinically significant abnormal values on hemoleucogram during screening, • be on no regular medical treatment • have no known allergies to treatments **Exclusion criteria** • Any genetic or metabolic disease or condition which might compromise the haematopoietic, renal, endocrine, pulmonary, central nervous, cardiovascular, immunological, dermatological, gastrointestinal or any other body system. • History of allergic conditions – asthma, urticaria, eczema. • History of autoimmune disorders • Intake of any medication within 14 days before start of the study		

	• Presence of significant abnormal hemoleucogram results during screening.
TREATMENT TO BE EVALUATED	**Active Treatment A:** Autologous umbilical cord blood administered intravenously as single treatment . **Active Treatment B:** Personalized daily treatment following evaluation with a combination of intranasal Oxytocin; Curcumin; Lecithin, Piracetam, Tecfidera; PQQ; Magnesium citrate and vitamin B6
DURATION OF STUDY	12 months
ENDPOINTS:	**EFFICACY** 1. Behavioral improvement with the validated scales for autistic spectrum disorders 2. Examination by child psychologist **SAFETY** 1. Adverse events, especially frequency and intensity of agitation episodes 2. Modification of vital signs: blood pressure, heart rate, respiratory rate, body temperature. 3. Modification of laboratory tests: Haematology: RBC, MCHC, MCH, MCV, RDW, Hct, Hb, WBC Differential count –lymphocytes monocytes, neutrophils, eosinophils, basophils; CD45+; blood cultures Biochemistry: Serum - Bilirubin (total) AST, ALT, LDH, glucose, urea, creatinine, sodium, potassium, chloride, calcium., MB microbiology; IP – Inflammatory panel
SAMPLE SIZE:	at least 25 patients, optimum 40 patients enrolled
STATISTICAL ANALYSIS:	Summary statistics will describe the safety variables. Significance testing will be done using analysis of variance for continuous and chi-square test for categorical variables. ($\alpha = 0,05$)

1. INTRODUCTION

1.1 Abstract

1. The treatment of children with autistic spectrum disease (ASD) represents a serious challenge for both the patients and their families, and also the medical staff involved.

Recently (Dawson G, 2017) prof Geraldine Dawson from Duke University showed that the use of stem cells from autologous umbilical cord blood (UCB) is a safe and efficacious therapy, opening a new and exciting avenue for therapeutic redress in children with ASD. This new therapeutic venue has yielded more dramatic improvements than any previous treatment including intranasal oxytocin.

We know that ASD has multiple determinants - genetic, environmental, neurological, and immunological (Young 2016) – and the pathological processes involved in ASD are various and complex. ASD is after all a spectrum of diseases involving brain development, not just a well-defined condition, and the implication is that the one-molecule-fits-all type of treatment will not be likely to address the multitude of aspects and deficiencies which are present in individuals with ASD. UCB itself contains a plethora of molecules which can modulate the activities of neurons and the supporting microglia, so comparing it with just one therapeutic substance would not be a good strategy.

With this in mind, we propose that ASD treatment should be very much personalized after a careful evaluation of the patient, and a noninvasive treatment option should include two or more substances from the following: oxytocin, curcumin, magnesium citrate with vitamin B6, pironoquinolinquinone (PQQ), lecithin, piracetam, and optionally dimethylfumarate, a medication known to be both neuroprotective and immunomodulatory.

Below we propose a pilot clinical study which aims to compare the safety and efficacy of UCB administration versus a less invasive and costly therapeutic option which consists of a patient-specific combination of medications and supplements. The study will be open-label, crossover type, meaning that all children will get sequentially both treatments after a 2-week washout interval. This study design has the advantage of eliminating the ethically questionable administration of a placebo.

1.2 Background

There is ample information in the medical literature on ASD, due to the increasing incidence of the condition, impact on families and society and the healthcare costs associated with it. As it affects the neuropsychological status of the developing child, it is classified and studied as a psychiatric ailment, and as such DSM-5, characterizes ASD behavior by the following traits:

- difficulties in social reciprocity and communication,
- unusually narrow interests,
- repetitive behaviours and speech,
- insistence on sameness, and
- idiosyncratic sensory responses

We also know that genetic, environmental, neurological, and immunological factors contribute to its etiology and among these we mention:

- increased rate of amygdala growth (Nordahl, 2012)
- atypical pruning of synapses (Geschwind 2007)
- decreased connectivity between ventromedial prefrontal cortex, which is implicated in emotion and social communication and the middle temporal gyrus/superior temporal sulcus region, which is implicated in face expression processing involved in social behaviour; also the precuneus/superior parietal lobule region with reduced functional connectivity, which is implicated in spatial functions including of oneself, and of the spatial environment (Xydakis 2015)
- neuron density in the prefrontal cortex was shown to play a significant role (Courchesne 2011)
- neuroinflammation in the anterior areas of the cortex (Pardo, 2005;

- increased head size in ASD children correlated with a history of allergic/immune disorders, (Sacco, 2007, Nordahl, 2011)
- increased titers of maternal autoimmune antibodies (Croen 2008, Braunschweig 2012)

Considering all the above, the consensus is that he prevailing pathological modification in children with ASD is the presence of a persistent pro-inflammatory status in the central nervous system (CNS) of the patient (Young 2016).

There are not many treatment options which are known to be effective in treating ASD. Secretin, oxytocin (Parker 2017), vaccines were among the treatments studied, but the results of the respective clinical studies were not at all encouraging.

1.2 Study Rationale

Even though it is a recent development, UCB has had the best results so far in treating children with ASD and its results recommend it for treatment to any child with ASD and who had UCB collected at birth. Two important questions arise:
- what other treatment can be recommended to the ASD children without UCB collected at birth, and with what comparative efficacy, and
- being a one-time intervention, can we improve/add to UCB infusion other substances which have a very good safety profile (well tolerated) and at least theoretically have a benefit in improving neuronal function?

To begin to answer these questions we have devised an open-label clinical study with a crossover design (all children receive both treatments, albeit in a different sequence).
One important novelty with our study is that the treatment to which UCB is compared is not the same to all children, but is individually tailored to address specifically the most important problems the child has (inflammation, anxiety/social avoidance, myelination, global delay, etc)
Based on the results of the testing with the M-CHAT-R, Q-CHAT and CAST questionnaires and the evaluation from child psychologist, a recommendation for treatment will be made. The noninvasive treatment consists of a combination of at least two substances from the

following: Oxytocin 10 UI/day intranasally, Magnesium citrate and vitamin B6 ½ cp qd, pironoquinolinquinone (PQQ) 20 mg qd, Lecithin 1000 mg qd, Curcumin 500 mg qd.
The specific treatment components will be recommended and administered according to the criteria below, after performing all the tests (imagistics, labs, questionnaires) which can suggest a deficiency on one or more of the following directions:
- inflammatory problem (IP markers on labs, white matter changes, diffuse or no specific modification on MRI)
- myelination (MRI changes),
- anxiety/social avoidance (questionnaires),
- severe developmental delays in cognition, logic, verbalization

1. ***Curcumin 250 mg bid*** will be included in the child's regimen if the imagistics (MRI/CT scan) and/or laboratory values (IP) suggest the presence of an inflammatory status, either in the CNS (white matter modifications on the brain scan) or systemic (abnormal values in the Inflammatory Panel done from blood);
2. ***Oxytocin 10 UI/day*** via intranasal administration will be administered if a social-communication deficit is clearly shown at evaluation
3. ***Lecithin 500 mg bid*** will be administered if brain imaging reveals any demyelination/myelination deficits, global cerebral atrophy or regional atrophy
4. ***Magnesium citrate with vitamin B6 (150 mg/6 mg qd)*** will be included if restlessness/agitation/anxiety is present significantly in the clinical picture
5. ***Pironoquinolinquinone (PQQ) 20 mg/day*** will be administered in situations of apathic/disinterested behavior, marked introversion, marked developmental delays
6. ***Optional medication for special situations***, with parent's clear understanding, a few other treatment options will be included – ex.
   - Tecfidera (dimethyl fumarate) 60 mg bid with food (presence of hypo/demyelination with an autoimmune component); immune modulation is involved in ASD as documented in Kornberg, 2018
   - piracetam 400 mg bid (presence of severe developmental delays with low energy)
   - Mentat (Himalaya) 1 po qd (presence of demyelination in absence of immune component)

The decision of which of these components should be given is taken after all tests are done and the child psychologist/psychiatrist and the study investigator discuss the case and afterwards discuss the practical issues of administering the recommended treatment with the parents.

2 STUDY OBJECTIVES

The primary objective of this study is to investigate the comparative efficacy of the UCB infusion and the non-invasive treatments for improving the functioning of children with ASD.

A secondary objective is to determine whether there are significant differences in the safety profiles of the two treatments administered

In addition there will be an evaluation of the structural changes in the CNS of the children with ASD, done by MRI

3 STUDY PLAN AND PROCEDURES

3.1 Study design

This is a twelve months long, open-label, crossover study to evaluate the comparative efficacy and safety of UCB versus non-invasive treatments in children with ASD. Each participant will receive both treatments in a random sequence.

The UCB is autologous, previously harvested at the child's birth and cryopreserved in liquid nitrogen for later use.
The UCB infusion will be performed at Angiomedica Hospital, Str Cosminului nr 13, Bucharest by aanesthesiologist, based on the Duke protocol.
Prior to UCB infusion, HLA typing of the child will be performed at the National Institute of Hematology, as well as the HLA typing of the UCB prior to thawing – for positive identification. During this time the UCB collection will be kept in liquid nitrogen at Angiomedica for a maximum of 72 hours, after which thawing and preparing of the UCB for infusion will be performed by Dr Felician Stancioiu; the thawing is done according to the StemSure/Vita 34 protocol, testing for viability will be done with Trypan Blue and flowcytometry

The non-invasive treatment combination is prescribed by the study investigator after the three questionnaires are answered and an evaluation by child psychiatrist/psychologist is peformed independently. This treatment is

3.1.1 Scheduled study visits

Recruitment of patients will occur during initial contacts with Dr Bogdan Ivanescu and the study coordinator Marie-Elene Niculita who will present the parents with the opportunity to enroll in the study, and when general information will be given about the study on timeline, procedures involved, tests, costs.
After agreeing in principle to participate in the study, parents and child will be scheduled for ***Visit 0***, at which the patients' blood is drawn for testing, questionnaires are completed, the patient is evaluated by the child psychiatrist and is randomized for receiving treatment A (UCB infusion) or B (noninvasive treatment), and the necessary arrangements are made for UCB release from the respective banking site.
Visit 1 is scheduled within 7-14 days from V0, and during this study visit the child will receive either treatment A or B. Treatment B (noninvasive) is administered daily for 6 weeks, and is followed by 2 weeks with no treatment (washout period)
Visit 2 follows at 8 weeks distance from V1, which includes the 2 week washout period after the first treatment is administered. New blood tests are performed, also questionnaires and evaluation by child psychiatrist, and the other treatment in sequence is administered.
Visit 3, ***Visit 4***, and ***Visit 5*** are scheduled at 16, 24 and 52 weeks after first treatment was administered (V1) and involve the neuropsychological evaluation of the child by the psychiatrist and with the 3 questionnaires; brain imaging is recommended at 52 weeks

	Visit 0	V1	V2 (+ 8 weeks)	V3 (+16 wks)	V4 (+24 wks)	V5 (+52 wks)
Labs	CBC, LFTs, Els, HLA; IP	HLA MB	CBC, LFTs, Els, HLA; IP; MB			
Imagis-tics	MRI/CT					MRI/CT
Neuro/ Psi Evalua-tion	M-CHAT-R; Q-CHAT; CAST; - Child psychiatrist	x	M-CHAT-R; Q-CHAT; CAST; - Child psychiatrist	M-CHAT-R; Q-CHAT; CAST; - Child psychiatrist	M-CHAT-R; Q-CHAT; CAST; - Child psychiatrist	M-CHAT-R; Q-CHAT; CAST; - Child psychiatrist
Treat-ment started	randomi-zation	A/B	B/A	X	X	X

CBC – complete blood count with differential; LFTs – liver function tests, Els – electrolytes; IP – Inflammatory panel – ex ESR, CRP, serum protein electrophoresis, neopterin, IL-6; HLA – Typing of patient HLA- A, HLA-B, HLA-DR; MB - microbiology

3.2 Study population

This clinical study will include children diagnosed with ASD, 3-7 years, and who meet the inclusion criteria outlined above.
Recruitment will continue until a minimum of 25 patients have received both treatments.

3.2.1 Inclusion criteria

To be enrolled, the following criteria have to be fulfilled at Visit 2:

1. Children of either sex between 3 and 7 years.
2. Body weight between 10 and 30 kg
3. Diagnosed with ASD
4. No significant diseases or clinically significant abnormal laboratory values during screening
5. On no regular medical treatment
6. Parents able to be informed of the nature of the study and willing to give written informed consent (Consent must be obtained before any study-related procedures are conducted).

3.2.2 Exclusion criteria

Any of the following is regarded as a criterion for exclusion from the study:

1. Hypersensitivity or allergic reaction to any drugs or supplements.
2. Any disease or condition which might compromise the haematopoietic, renal, endocrine, pulmonary, central nervous, cardiovascular, immunological, dermatological, gastrointestinal or any other body system.

3. History of allergic conditions – asthma, urticaria, eczema.
4. History or presence of dyspepsia, gastric ulcer or duodenal ulcer.
5. History of autoimmune disorders e.g. systemic lupus erythematosus, haemolytic anemia.
6. Intake of steroid medication within 7 days before start of the study
7. Presence of clinically significant abnormal laboratory results during screening.
8. Participation in a clinical study of any investigational product 1 month prior to visit 1 or during the study.

3.2.3 Justification for inclusion and exclusion criteria

The criteria are set to minimize the risk to the patients, to ensure a study population that will enable the investigation of the set objectives, and to provide equal opportunity for inclusion.

3.2.4 Criteria for discontinuation

Subjects may be discontinued from study treatment and assessments at any time, at the discretion of the investigator. Specific reasons for discontinuing a subject from the study are:

1. Withdrawal of informed consent.
2. Development of exclusion criteria, pregnancy or other safety reasons during the study.
3. Protocol non-compliance.
4. Incorrect enrollment or randomization of the subject.

For subjects withdrawn from the study, the same measurements and assessments should be performed as done at Visit 5. Adverse events should be followed up and questionnaires completed by parents.

3.3 Investigational Products and Treatments

3.3.1 Treatment Schedule

Each treatment arm is administered and considered active for a period of maximum 8 weeks; the non-invasive treatment is administered for 6 weeks followed by a 2-week “wash-out” period, and the UCB infusion will be followed by supplement administration after an 8-week interval.
Patient evaluation, registration informed consent and randomization occur at initial visit 0 (V0); followed after 14-21 days by V1 (longer for the preparation of UCB infusion), during which the first treatment is administered; V2 after another 8 weeks, at which the second treatment is administered, and V3, V4, and V5, at 16, 24 and 52 weeks respectively after V1.
A tabulated summary of the study visits was given above Section 3.1.1

3.3.2 Randomisation

Because it is an open-label study, there is no need for blinding/assigning participant numbers. Each participant will be randomly assigned to begin the study either with the UCB infusion or the non-invasive treatment.
Eligible patients, who for whatever reason, do not report to the clinic to receive the first treatment after having been selected for the study will be referred to as discontinuers in the report. A patient who, for whatever reason, withdraws or is withdrawn from the study after

having been enrolled, will be classified as a dropout, and identified as such in the relevant Case Report Form (CRF).

3.3.3 Identity of study Treatments

After receiving it from the storage bank, UCB will be tested for HLA determinants at the National Institute of Hematology laboratory by gene sequencer.
Afterward positive confirmation UCB will be thawed according to the Duke/Rubinstein protocols. A vial containing 1.5 ml thawed UCB will be sent for TNC/viability by flowcitometry at Victor Babes Laboratory. A label with patients' name, date is affixed to each UCB bag prepared for infusion.

Study code: Bio-Forum Foundation001 Visit no: Investigator: Dr Subject no: Investigational product: UCB Patient name **Keep at room temperature** **Exp Date:** **For clinical study use only**

3.3.4. Storage and Accountability

UCB collections will be kept in liquid nitrogen, vapor phase – 170 degrees Celsius - at all times prior to thawing of collection, and there will be periodic recording of this condition. Transport recipients (dry shippers come with temperature sensors, which will be photographed, and during HLA testing, the collection is kept also in liquid nitrogen and its storage at below – 170 degrees Celsius will also be documented via periodic photography of the measuring device.
The Principal Investigator of the study – Dr Felician Stancioiu – is directly responsible for this stage of UCB preparation.

3.3.5. Allowed medication

Preferably no other medicine should be taken by the subjects. The use of any incidental medication (e.g. mild analgesics, NSAID, etc) must be recorded.

3.3.6 Compliance

The parents will attest to the use of study medication on a daily basis and the adherence to the prescribed treatment will be checked at every clinic visit.

4 STUDY MEASUREMENTS AND ENDPOINTS

In this study the following endpoints will be measured/recorded
1. Efficacy - difference in the scores on the questionnaires M-CHAT, Q-CHAT and CAST before and after each treatment
2. Safety – type, severity, duration, treatment and amelioration of each of the adverse effects occurring during respective treatments.

4.1 Primary Safety Endpoints.

1. Adverse events (type and frequency)
2. Physical examination parameters (body mass, height, etc)
3. Vital signs: blood pressure, heart rate, respiratory rate, body temperature
4. Laboratory tests
   HLA testing by genetic sequencing
   Haematology: CBC - RBC, HB. MCHC, MCH, MCV, RDW, Hct, Hb, WBC Differential count –lymphocytes monocytes, neutrophils, eosinophils, basophils,
   flowcytometry - CD34+, TNC, viability %
   Microbiology – presence and sensitivity of pathogens
   LFTs Serum - Bilirubin (total and conjugated) AST, ALT, GGT, LDH, glucose
   IP – serum protein electrophoresis, ESR, CRP, fibrinogen
   Els - sodium, potassium, chloride, calcium, magnesium.

4.2 Measurements at each visit.

See section 3.1.1 for the visit schedule.

Visit 0: Screening Visit

Patients will be examined within 14 days prior to the first ingestion of the investigational products to assess their eligibility to participate. For each child both parents will consent in writing (Appendix 1, Informed Consent Form) to the screening process before the start of the examination. The consent form will also include the study information leaflet.
The examinations and investigations will include:

- Medical history, including history of past use of medications, demographics (date of birth, sex, race) and ASD diagnosis.
- Physical examination including assessment of general appearance, cardiovascular, lungs, mouth, throat and abdomen and measurement of height and body weight. The examination will be made in accordance with the normal clinical routines at the trial center.
- Vital signs: systolic and diastolic blood pressure in supine (after 5 minutes rest) and standing positions (1minute), pulse, respiratory rate and oral temperature.
- Laboratory tests: HLA, Haematology, (see section 4.4.3.2); baseline plasma levels of IP, Els, LFts (these can be drawn also at V1 if considered by the study PI)

Based on the results of the testing with the M-CHAT-R, Q-CHAT and CAST questionnaires and the evaluation from child psychologist, a recommendation for treatment will be made for either UCB administration or the noninvasive treatment for 6 weeks

Visit 1: The first treatment is administered

- The pre-dose safety assessments and eligibility checks will be recorded on the relevant pages in the individual CRFs.
- The parents will also be given the 3 questionnaires and instructed on how to complete it.
- The parents will be issued with prescriptions for trial medication, explained how to take the medication and given next appointment date; or
- The UCB infusion is administered

Visit 2: Second Treatment is administered

For these visits the examinations and investigations will include:

- Physical examination (as for visit 1)
- Vital signs: systolic and diastolic blood pressure in supine, pulse, respiratory rate and oral temperature.
- Laboratory tests
  HLA testing by genetic sequencing
  - Haematology: CBC - RBC, HB. MCHC, MCH, MCV, RDW, Hct, Hb, WBC
  Differential count –lymphocytes monocytes, neutrophils, eosinophils, basophils,
  - flowcytometry - CD34+, TNC, viability %
  - Microbiology – presence and sensitivity of pathogens
  - LFTs Serum - Bilirubin (total and conjugated) AST, ALT, GGT, LDH, glucose
  - IP – serum protein electrophoresis, ESR, CRP, fibrinogen
  - Els - sodium, potassium, chloride, calcium, magnesium.

Note: For any patient in which any of the above tests indicated levels outside the normal range, a follow-up visit (visit A-C) will be scheduled for an appropriate time (1 or 3 months post visit). All or the above tests will be repeated at this visit.

4.3 Specific detail on measurements

4.4.1 Adverse events

An adverse event is the development of an undesirable medical condition - e.g. symptoms or abnormal results of an investigation - or the deterioration of a pre-existing medical condition (not relevant in this study). AE's will be collected by means of a standard question: "Have you had any health problems since the previous visit?" AE's will be recorded at every visit. Spontaneously reported AE's and/or observed AE's and the subject's response to this question will be recorded on the AE form with information about seriousness, action taken, date of onset and recovery, maximum intensity and outcome. The subjects will be asked to assess the intensity of the reported Adverse Event according to the following scale:

Mild = awareness of sign or symptom, but easily tolerated
Moderate = discomfort sufficient to cause interference with normal activities
Severe = incapacitating, with inability to perform normal activities.

A Serious Adverse Event is an adverse event occurring during any phase of the study and at any dose of the investigational product or placebo, which fulfils one or more of the following criteria:

- Results in death;
- Is immediately life-threatening;
- Requires in-subject hospitalization;
- Results in persistent or significant disability or incapacity.

The causality of Serious Adverse Events (i.e. the relationship to study treatment) will be assessed by the investigators, who in completing the relevant Case Report Form must answer 'yes' or 'no' to the question "Do you consider that there is a reasonable possibility that the event may have

been caused by the study medication?" The following factors should be considered when deciding if there is a "reasonable possibility" that an Adverse Event may have been caused by the investigational product.

- Time course of events and exposure to suspect drug – did the AE occur in a reasonable temporal relationship to the administration of suspect drug?
- Dechallenge experience – did the AE resolve or improve on stopping or reducing the dose of the suspect drug?
- Rechallenge experience - did the AE reoccur if the suspected drug was reintroduced after having stopped?
- Laboratory tests – has a specific laboratory investigation confirm the relationship?
- No alternative cause - the AE cannot be reasonably explained by another aetiology such as an underlying disease (not previously present), other drugs or environmental factors.

There would not be a "reasonable possibility" of causality if none of the above criteria apply or where there is evidence of exposure and a reasonable time course, but any dechallenge is negative or there is another more likely cause of the AE.

In this study the Adverse Events will be noted from the interview at the time of visit and from the daily input in the diary.

4.4.2 12- Lead ECG

Standard 12-lead ECGs will be performed at the indication of the anesthesiology doctor if necessary.

The Bioclinica Laboratory will perform all the clinical laboratory investigations.

The samples will be processed and shipped as per instructions of the pathology laboratory.

For all laboratory investigations the investigator will receive actual printouts of the data from the Laboratory. A signed original printout will be attached to the CRF and a copy thereof will be filed in the subject file. The reference ranges will be provided in the clinical trial manual (investigator's file) together with the descriptions of the laboratory methods used. The laboratory will provide normal ranges on each report to ease the investigator's assessment.
As part of the overall safety monitoring plan, the safety monitor (Dr Marie-Elene Niculita) will also assess the data, particularly those falling outside the normal range.

The blood samples will always be taken before the first trial medication dose for that day is taken. The blood samples will be collected in tubes labeled with the following information: patient name; test type (HLA, biochemistry, hematology, flowcytometry); blood sample number (visit 2, 3,4 or 5) and sampling time.

Blood samples will be handled according to the protocols for testing of the respective laboratories

4.4 Measurements recorded daily in a diary

A diary will be filled in at home during the entire study in the morning after taking the morning dose and at night after the evening before going to bed. The following variables will be recorded:

i) Intake of study medication (if, time and if with food)
ii) Occurrence of any adverse effects.
iii) Intake of any incidental medication

The subjects will record the time (morning and evening) that the study medication has been taken. The recordings are a measurement of compliance.

5 STATISTICAL METHODS

5.1 Determination of sample size

Based on literature we expect that a sample size of minimum 25 participants will be needed in order to observe an alpha value of 0.05 (95% probability) with a p value of 0.05 and a power of 80%. ANOVA testing will be performed on the respective groups of patients

5.2 Statistical analysis

A single statistical analysis will be performed at the end of the study. An intention to treat (ITT) approach will followed, i.e. statistical analysis of safety will be based on data from all patients and from whom meaningful data were collected. Data will be displayed graphically for visual inspection. Descriptive statistics will be presented as means, SEM and ninety percent confidence levels of the means.

Baseline characteristics. The subject disposition will be summarized. Protocol violations will be listed per subject, describing the nature of the violation. Subjects failing to complete the study (as well as the times and reasons for discontinuation) will be displayed. The demographic, background and baseline data will be presented descriptively.

Analysis of Safety. Adverse Events, as reported throughout the course of the trial will be listed individually, per treatment group. Pre-, study and post-study findings of **physical examination, vital sign variables, laboratory variables** (haematology, clinical chemistry and urinalysis), and 12-lead ECG if needed will be listed individually and summarized; values outside the normal range will be listed.
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5.3. Changes to the Clinical Study Protocol

The Angiomedica Hospital (as the clinical site for the study) and the Local Ethics Committees will be notified of any amendments to the Clinical Study Protocol. Such changes will also be registered at the www.clinicaltrials.gov site

6. ETHICS

6.1 Ethics review

The final study protocol, including the final version of the Informed Consent Forms, must be approved in writing by The Ethics Committee before enrolment of any subject into the study. The Principle Investigator (Clinical Trial Manager) is responsible for informing the IEC of any serious adverse events (SAE) and amendment to the protocol as per regulatory requirement.

6.2. Ethical conduct of the study

The study will be performed in accordance with the ethical principles in the Declaration of Helsinki of the World Health Organisation (see Appendix 10.1), and that are consistent with Good Clinical Practice and applicable regulatory requirements.

6.3. Subject information and consent

The Investigator will ensure that parents are given full and adequate oral and written information about the nature, purpose, possible risk and benefit of the study. Subjects must also be notified that they are free to discontinue from the study at any time. The subject should be given the opportunity to ask questions and allowed time to consider the information provided. The subject's signed and dated informed consent must be obtained before conducting any study specific procedure. The investigator must store the original, signed Subject Informed Consent Form and a copy must be given to the subject. Samples of the English version of the Subject Information and Consent Forms are enclosed (Appendix 1).

6.4. Subject data protection

The Subject Information and Consent Form will explain that study data will be stored in a computer database, maintaining confidentiality. Subjects in this database will be identified by initials or enrolment code / subject number only. Authorized representative of a regulatory authority (e.g. ANM) may require direct access to parts of the trial site records relevant to the study, including subjects' medical history for data verification purposes.

The Investigator must keep a Subject Identification List of all subjects that have signed the informed consent, including subject number, full name and last known address.

7. DATA QUALITY ASSURANCE

Data from the study will be collected in CRFs. Data editing will be performed at the trial center, comparing source and CRF entries. Data will be entered in a blind mode.

During the study an independent monitor will visit the investigational site to confirm that the facilities remain acceptable, that the investigational team is adhering to the protocol and that data are being accurately recorded in the CRFs. Source data verification (a comparison of the data in

the CRF with the subjects laboratory test results and other source documents) will also be performed.

Authorized representatives of the regulatory authority (e.g. ANM) may visit the center to perform inspections, including source data verification.

Clean File for the final database will be declared when all data have been entered and a quality check on a sample of the data has been performed. The database will be locked after Clean File has been declared and data extracted for statistical analysis. Treatment code will not be broken until clean file.

The medical, nursing and other staff involved in the study will receive proper education/information on how to conduct the study according to the protocol.

8. STUDY TIME TABLE AND TERMINATION

First participant in	July 2019
Last participant out	July 2020
Clean File	August 2020
Study Report	September 2020

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APPENDIX A

DECLARATION OF HELSINKI

WORLD MEDICAL ASSOCIATION DECLARATION OF HELSINKI
Ethical Principles for Medical Research Involving Human Subjects
Adopted by the 18th WMA General Assembly, Helsinki, Finland, June 1964, and amended by the
29th WMA General Assembly, Tokyo, Japan, October 1975
35th WMA General Assembly, Venice, Italy, October 1983
41st WMA General Assembly, Hong Kong, September 1989
48th WMA General Assembly, Somerset West, Republic of South Africa, October 1996
and the 52nd WMA General Assembly, Edinburgh, Scotland, October 2000
Note of Clarification on Paragraph 29 added by the WMA General Assembly, Washington 2002

INTRODUCTION

1. The World Medical Association has developed the Declaration of Helsinki as a statement of ethical principles to provide guidance to physicians and other participants in medical research involving human subjects. Medical research involving human subjects includes research on identifiable human material or identifiable data.

2. It is the duty of the physician to promote and safeguard the health of the people. The physician's knowledge and conscience are dedicated to the fulfillment of this duty.

3. The Declaration of Geneva of the World Medical Association binds the physician with the words, "The health of my patient will be my first consideration," and the International Code of Medical Ethics declares that, "A physician shall act only in the patient's interest when providing medical care which might have the effect of weakening the physical and mental condition of the patient."

4. Medical progress is based on research, which ultimately must rest in part on experimentation involving human subjects.

5. In medical research on human subjects, considerations related to the well-being of the human subject should take precedence over the interests of science and society.

6. The primary purpose of medical research involving human subjects is to improve prophylactic, diagnostic and therapeutic procedures and the understanding of the aetiology and pathogenesis of disease. Even the best-proven prophylactic, diagnostic, and therapeutic methods must continuously be challenged through research for their effectiveness, efficiency, accessibility and quality.

7. In current medical practice and in medical research, most prophylactic, diagnostic and therapeutic procedures involve risks and burdens.

8. Medical research is subject to ethical standards that promote respect for all human beings and protect their health and rights. Some research populations are vulnerable and need special protection. The particular needs of the economically and medically disadvantaged must be recognized. Special attention is also required for those who cannot give or refuse consent for themselves, for those who may be subject to giving consent under duress, for those who will not benefit personally from the research and for those for whom the research is combined with care.

9. Research Investigators should be aware of the ethical, legal and regulatory requirements for research on human subjects in their own countries as well as applicable international requirements.

No national ethical, legal or regulatory requirement should be allowed to reduce or eliminate any of the protections for human subjects set forth in this Declaration.

B. BASIC PRINCIPLES FOR ALL MEDICAL RESEARCH

10. It is the duty of the physician in medical research to protect the life, health, privacy, and dignity of the human subject.

11. Medical research involving human subjects must conform to generally accepted scientific principles, be based on a thorough knowledge of the scientific literature, other relevant sources of information, and on adequate laboratory and, where appropriate, animal experimentation.

12. Appropriate caution must be exercised in the conduct of research, which may affect the environment, and the welfare of animals used for research must be respected.

13. The design and performance of each experimental procedure involving human subjects should be clearly formulated in an experimental protocol. This protocol should be submitted for consideration, comment, guidance, and where appropriate, approval to a specially appointed ethical review committee, which must be independent of the investigator, the sponsor or any other kind of undue influence. This independent committee should be in conformity with the laws and regulations of the country in which the research experiment is performed. The committee has the right to monitor ongoing trials. The researcher has the obligation to provide monitoring information to the committee, especially any serious adverse events. The researcher should also submit to the committee, for review, information regarding funding, sponsors, institutional affiliations, other potential conflicts of interest and incentives for subjects.

14. The research protocol should always contain a statement of the ethical considerations involved and should indicate that there is compliance with the principles enunciated in this Declaration.

15. Medical research involving human subjects should be conducted only by scientifically qualified persons and under the supervision of a clinically competent medical person. The responsibility for the human subject must always rest with a medically qualified person and never rest on the subject of the research, even though the subject has given consent.

16. Every medical research project involving human subjects should be preceded by careful assessment of predictable risks and burdens in comparison with foreseeable benefits to the subject or to others. This does not preclude the participation of healthy volunteers in medical research. The design of all studies should be publicly available.

17. Physicians should abstain from engaging in research projects involving human subjects unless they are confident that the risks involved have been adequately assessed and can be satisfactorily managed. Physicians should cease any investigation if the risks are found to outweigh the potential benefits or if there is conclusive proof of positive and beneficial results.

18. Medical research involving human subjects should only be conducted if the importance of the objective outweighs the inherent risks and burdens to the subject. This is especially important when the human subjects are healthy volunteers.

19. Medical research is only justified if there is a reasonable likelihood that the populations in which the research is carried out stand to benefit from the results of the research.

20. The subjects must be volunteers and informed participants in the research project.

21. The right of research subjects to safeguard their integrity must always be respected. Every precaution should be taken to respect the privacy of the subject, the confidentiality of the patient's

information and to minimize the impact of the study on the subject's physical and mental integrity and on the personality of the subject.

22. In any research on human beings, each potential subject must be adequately informed of the aims, methods, sources of funding, any possible conflicts of interest, institutional affiliations of the researcher, the anticipated benefits and potential risks of the study and the discomfort it may entail. The subject should be informed of the right to abstain from participation in the study or to withdraw consent to participate at any time without reprisal. After ensuring that the subject has understood the information, the physician should then obtain the subject's freely given informed consent, preferably in writing. If the consent cannot be obtained in writing, the non-written consent must be formally documented and witnessed.

23. When obtaining informed consent for the research project the physician should be particularly cautious if the subject is in a dependent relationship with the physician or may consent under duress. In that case the informed consent should be obtained by a well-informed physician who is not engaged in the investigation and who is completely independent of this relationship.

24. For a research subject who is legally incompetent, physically or mentally incapable of giving consent or is a legally incompetent minor, the investigator must obtain informed consent from the legally authorized representative in accordance with applicable law. These groups should not be included in research unless the research is necessary to promote the health of the population represented and this research cannot instead be performed on legally competent persons.

25. When a subject deemed legally incompetent, such as a minor child, is able to give assent to decisions about participation in research, the investigator must obtain that assent in addition to the consent of the legally authorized representative.

26. Research on individuals from whom it is not possible to obtain consent, including proxy or advance consent, should be done only if the physical/mental condition that prevents obtaining informed consent is a necessary characteristic of the research population. The specific reasons for involving research subjects with a condition that renders them unable to give informed consent should be stated in the experimental protocol for consideration and approval of the review committee. The protocol should state that consent to remain in the research should be obtained as soon as possible from the individual or a legally authorized surrogate.

27. Both authors and publishers have ethical obligations. In publication of the results of research, the investigators are obliged to preserve the accuracy of the results. Negative as well as positive results should be published or otherwise publicly available. Sources of funding, institutional affiliations and any possible conflicts of interest should be declared in the publication. Reports of experimentation not in accordance with the principles laid down in this Declaration should not be accepted for publication.

C. **ADDITIONAL PRINCIPLES FOR MEDICAL RESEARCH COMBINED WITH MEDICAL CARE**

28. The physician may combine medical research with medical care, only to the extent that the research is justified by its potential prophylactic, diagnostic or therapeutic value. When medical research is combined with medical care, additional standards apply to protect the patients who are research subjects.
29. The benefits, risks, burdens and effectiveness of a new method should be tested against those of the best current prophylactic, diagnostic, and therapeutic methods. This does not exclude the use of placebo, or no treatment, in studies where no proven prophylactic, diagnostic or therapeutic method exists. See footnote

30. At the conclusion of the study, every patient entered into the study should be assured of access to the best-proven prophylactic, diagnostic and therapeutic methods identified by the study.

31. The physician should fully inform the patient which aspects of the care are related to the research. The refusal of a patient to participate in a study must never interfere with the patient-physician relationship.

32. In the treatment of a patient, where proven prophylactic, diagnostic and therapeutic methods do not exist or have been ineffective, the physician, with informed consent from the patient, must be free to use unproven or new prophylactic, diagnostic and therapeutic measures, if in the physician's judgement it offers hope of saving life, re-establishing health or alleviating suffering. Where possible, these measures should be made the object of research, designed to evaluate their safety and efficacy. In all cases, new information should be recorded and, where appropriate, published. The other relevant guidelines of this Declaration should be followed.

FOOTNOTE:
NOTE OF CLARIFICATION ON PARAGRAPH 29 of the WMA DECLARATION OF HELSINKI
The WMA hereby reaffirms its position that extreme care must be taken in making use of a placebo-controlled trial and that in general this methodology should only be used in the absence of existing proven therapy. However, a placebo-controlled trial may be ethically acceptable, even if proven therapy is available, under the following circumstances:
- Where for compelling and scientifically sound methodological reasons its use is necessary to determine the efficacy or safety of a prophylactic, diagnostic or therapeutic method; or
- Where a prophylactic, diagnostic or therapeutic method is being investigated for a minor condition and the patients who receive placebo will not be subject to any additional risk of serious or irreversible harm.
All other provisions of the Declaration of Helsinki must be adhered to, especially the need for appropriate ethical and scientific review.

The Declaration of Helsinki (Document 17.C) is an official policy document of the World Medical Association, the global representative body for physicians. It was first adopted in 1964 (Helsinki, Finland) and revised in 1975 (Tokyo, Japan), 1983 (Venice, Italy), 1989 (Hong Kong), 1996 (Somerset-West, South Africa) and 2000 (Edinburgh, Scotland). Note of clarification on Paragraph 29 added by the WMA General Assembly, Washington 2002.

APPENDIX 1

PATIENT INFORMATION LEAFLET AND INFORMED CONSENT

PATIENT INFORMATION SHEET AND INFORMED CONSENT FORM

Patient identification number: . . . . / . . . . . **Patient initials:**

Title of the study: **A pilot crossover study to investigate *the safety and efficacy of administering intravenous autologous cord blood versus personalized noninvasive treatments* to children with developmental disorders of the autistic spectrum.**

Protocol number: B-F011

Sponsor: The Bio-Forum Foundation

Name of Principal Investigator: Dr Felician Stancioiu
Name of Study Coordinator: **Dr Marie-Elene Niculita**

Address of site/institution where study will be conducted:
Spitalul Angiomedica, Str. Cosminului Nr, 13, Bucuresti

INTRODUCTION
You are being invited to take part in a clinical research study sponsored by The **Bio-Forum Foundation.** Bio-Forum Foundation is engaged in joint research with Angiomedica Hospital on stem cell treatments. Before deciding to take part in the study, it is important for you to understand why the research is done and what will happen to participants in the study. Please take the time to read this information carefully and ask the investigator when anything is not clear, or if you would like more information.

What is the purpose of this study?
The purpose of this study is to determine the comparative safety and efficacy of the ***umbilical cord blood versus noninvasive treatments*.**
All participants will be administered both treatments (umbilical cord blood and the noninvasive treatment).

Do I have to participate in the study?
Participation in this study is entirely voluntary. It is up to you to decide whether to take part or not.
If you do not want to take part in this study or if you wish to withdraw from the study at any time you may do so without giving a reason and you will not lose any benefits to which you would otherwise be entitled. If you withdraw from the study the data collected up to the point of withdrawal will be analyzed for the purpose of the study. If you decide to participate, you will be given this information sheet to keep and be asked to provide your signature indicating your consent.

Who is eligible to participate in the study?
About 25-40 children age 3-7 years will take part in this study. In order to participate in this trial, the child needs to be diagnosed with ASD, be in the appropriate weight range, not on any regular medicine, and meet the inclusion criteria of the study, which will be told by the principal investigator.

Safety of medications is discussed for each of the recommended treatments at time of enrollment in the study.

What will happen to me if I participate?
If you decide to take part you must sign the consent form and will then first be examined to see if you meet all the requirements for participation in the study. These examinations and investigations (at visit 1) will include the taking of a medical history history, including history of past use of medications; a physical examination; the withdrawal of 40 ml (approximately 8 teaspoons) of blood for laboratory tests; and other tests as deemed appropriate for each patient.

If you qualify for inclusion you will thereafter, within 21 days of the first examination, at Visit 1 will either be prescribed study medication that you must take for 6 weeks or prepare for UCB infusion which will take place at the Angiomedica Hospital.
You will have to take the study medication for a total of 6 weeks and return for visits to the trial center for a further 3 visits, at 16, 24 and 52 weeks.
If UCB infusion is given first, then at 8 weeks after UCB infusion, the child will start taking the non-invasive treatment recommended after the evaluations.
This means that the study will last around 12 months.

What do I have to do?

As a subject in this study, you are responsible for:
- keeping all clinic appointments;
- taking the study medication as explained by the study doctor;
- completing and returning the questionnaires that you will receive at each study visit;
- telling your study doctor before taking any other medication;
- telling your study doctor as soon as possible if there is a change in your health;

If you experience any injuries and need to see a doctor, you must also tell that doctor that you are participating in this research study.

What risks or discomforts might occur if I participate?
As with any medication and treatment, there may be side-effects associated with the administered therapies, the most common of which is cited in literature – episodes of agitation seen after UCB administration

Some procedures used in this study may cause some discomforts (e.g. the withdrawal of blood may cause slight bruising or possible fainting, there may be discomfort during infusion or after administration of sedation, etc), but these will be no more than that experienced in a typical medical examination.

What are the possible benefits of participating?
You may or may not receive any direct benefit from the two treatments, the only guarantee is that the information we get from this study will extend our existing knowledge and will help to decide if this medication is safe to use in other people such as you.

Will there be any cost to me if I participate?

For participating in this research study, you will need to cover the costs for the materials, medications, consumables, hospital stay, UCB preparation and infusion. testing, clinic visits; the total cost is estimated at between 4.000 and 4500 euros, which will be paid to Fundatia Bio-Forum in two equal installments, half (2000-2250) at enrollment in the clinical study and the remainder half at UCB treatment completion (8 weeks after first visit).

What if something goes wrong or if I have problems while I am in the study?

In case of study related questions please contact:

Doctor:	Dr Felician Stancioiu	or	Dr. Marie-Elene Niculita
Contact Numbers:	0727500402	at	0213266030

If you are harmed by your participation in the study, you will be compensated according to the guidelines of the pharmaceutical industry relating to clinical trials (Association for British Pharmaceutical Industry ABPI guidelines). If you are harmed due to someone's negligence then you may have grounds for legal action. You are not waiving your legal rights by signing this form.

Can I withdraw or be withdrawn from the study?
Taking part in the study is voluntary. If you decide to take part in the study, you are free to withdraw from the study at any time. If you decide to withdraw from the study, you should inform your study doctor immediately. Your study doctor will not be upset and you will not be penalized in any way, and your future care will not be affected. Should you withdraw from the study, the study data collected before your withdrawal may still be processed along with other data collected as part of the study.

In addition, circumstances may arise that will lead to the ending of your participation in this study. Such circumstances could include:

- not taking your medication or not regularly keeping to the study appointments, or if you do not follow the doctor's instructions;
- taking a medication that interferes with the treatments in this study (eg steroids, immune supressant, benzodiazepine, other psychotropic medication);
- having blood tests which are outside of normal
- if the Ethical Committee of Fundatia Bio-Forum stops or suspends the study.

Will my participation in this study be kept confidential?

The records that identify participants will be kept confidential and, to the extent permitted by the applicable laws and regulations, will not be made publicly available. If you consent to take part in this study, any of the medical records and data recorded in this study may be directly inspected by representatives of the study Sponsor (Bio-Forum Foundation), StemSure and Angiomedica Hospital. By signing this written informed consent form you are giving permission for this to be done.

The information collected during the study will be stored in a computer but your name will not be stored. Only your study doctor will know that the information is related to you.

The results of the study may be published in the medical literature and/or presented at a scientific conference or symposium, but your identity will not be revealed. The information disclosed will be collective summarized data.

You may ask to see your medical information as prescribed by law.

Who is organising and funding the study?
This research study is being organized and funded by Bio-Forum Foundation in close collaboration with Angiomedica Hospital and StemSure. Dr Felician Stancioiu is the clinical investigator responsible for the conduct of the trial and the contact person for Bio-Forum Foundation and Dr Marie-Elene Niculital, the representative of StemSure.

Who has reviewed the study?

The study is in accordance with the guidelines of the International Conference on Harmonization (ICH) for Good Clinical Practice (GCP) and with the Declaration of Helsinki (version 2000).This study has been approved by the Research Ethics Committee. The study will also be reviewed by the Institutional Review Board of the Angiomedica Hospital (partner in the study with Bio-Forum Foundation). These are groups of scientific and non-scientific people. They review and approve or disapprove research involving human subjects.

You should inform your general practitioner (your house doctor) that you are taking part in this study.

Contacts for further information.
If during the course of this study, you have questions about the nature of the research or your rights, or you believe that you have sustained a research-related injury, you should contact one of the following:

Dr Felician Stancioiu (study doctor) at 0727500402

INFORMED CONSENT FORM

Clinical study title: A pilot crossover study to investigate *the safety and efficacy of administering intravenous autologous cord blood versus personalized noninvasive treatments* to children with developmental disorders of the autistic spectrum

Patient identification number: . . . . / . . . . . Patient initials:

Protocol number: B-F001

By signing and dating this document,

- I confirm that I have had time to carefully read and understand the patient information sheet provided for this study.
- I confirm that I have had the opportunity to discuss the study and ask questions and I am satisfied with the answers and explanations that I have been provided.
- I give permission for my medical records to be reviewed by the Sponsor or designee, and/or representatives of the Medicines Control Council or Committee for Pharmaceutical Trials.
- I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason and without my medical care or legal rights being affected.
- I confirm that I have received a signed and dated copy of the Patient Information Sheet and Informed Consent Forms.

Child's Name, Mother: _____ _____ _____

Name (capital letters) Signature Date

Father: _____ _____ _____

Name (capital letters) Signature Date

PERSON OBTAINING CONSENT:

_____ _____ _____

Name (capital letters) Signature Date

CLINICAL INVESTIGATOR:

_____ _____ _____

Name (capital letters) Signature Date

ANNEX 1
COSTS

Infusion of the UCB collection 4000 euro
includes
- transport and storage of UCB in liquid nitrogen (StemSure, Bio-Forum, Angiomedica)
- HLA testing of the child (National Institute of Hematology Lab)
- laboratory testing - CBC, LFTs, Els, IP (Bioclinica Lab)
- HLA testing of the UCB (National Institute of Hematology Lab)
- thawing, conditioning of UCB (Bio-Forum, Angiomedica)
- Trypan Blue and microbiological testing (Angiomedica and Bioclinica Lab)
- flowcytometry (TNC and viability) – Victor Babes Research Lab
- preparation of UCB collection for infusion according to StemSure/Vita34 protocols (Bio-Forum, Angiomedica)
- hospital admission (24 hours), pre-medication, administration of UCB by anesthesiologist (Angiomedica)
- other testing (EKG, blood, etc) and medical care, as needed (Angiomedica)

Also included:
- interview/recruitment by study coordinator (StemSure)
- evaluation by child psychiatrist/psychologist (4 visits)
- M-CHAT/Q-CHAT/CAST questionnaires
- evaluation by study investigator and prescription of supplements for 6 weeks
- a second set of blood tests (CBC, LFT, El, IP)

Additional possible, optional costs
- MRI at 9-12 months after UCB infusion (Monza Hospital), 400 euro
- evaluation by another child psychiatrist (per visit) 50 euro

Annex 2
CAST Questionnaire

The Childhood Autism Spectrum Test (CAST)
Child's Name: ..... Age: ..... Sex: Male / Female
Birth Order: ..... Twin or Single Birth: .....
Parent/Guardian: .....
Parent(s) occupation: .....
Age parent(s) left full-time education: .....
Address: .....
.....
.....
Tel.No: ..... School: .....

Please read the following questions carefully, and circle the appropriate answer. All responses are confidential.

1. Does s/he join in playing games with other children easily? Yes No

2. Does s/he come up to you spontaneously for a chat? Yes No

3. Was s/he speaking by 2 years old? Yes No

4. Does s/he enjoy sports? Yes No

5. Is it important to him/her to fit in with the peer group? Yes No

6. Does s/he appear to notice unusual details that others miss? Yes No

7. Does s/he tend to take things literally? Yes No

8. When s/he was 3 years old, did s/he spend a lot of time pretending (e.g., play-acting being a superhero, or holding teddy's tea parties)? Yes No

9. Does s/he like to do things over and over again, in the same way all the time? Yes No

10. Does s/he find it easy to interact with other children? Yes No

11. Can s/he keep a two-way conversation going? Yes No

12. Can s/he read appropriately for his/her age? Yes No

13. Does s/he mostly have the same interests as his/her peers? Yes No

14. Does s/he have an interest which takes up so much time that s/he does little else? Yes No

15. Does s/he have friends, rather than just acquaintances? Yes No

16. Does s/he often bring you things s/he is interested in to show you? Yes No

17. Does s/he enjoy joking around? Yes No

18. Does s/he have difficulty understanding the rules for polite behaviour? Yes No

19. Does s/he appear to have an unusual memory for details? Yes No

20. Is his/her voice unusual (e.g., overly adult, flat, or very monotonous)? Yes No

21. Are people important to him/her? Yes No

22. Can s/he dress him/herself? Yes No

23. Is s/he good at turn-taking in conversation? Yes No

24. Does s/he play imaginatively with other children, and engage in role-play? Yes No

25. Does s/he often do or say things that are tactless or socially inappropriate? Yes No

26. Can s/he count to 50 without leaving out any numbers? Yes No

27. Does s/he make normal eye-contact? Yes No

28. Does s/he have any unusual and repetitive movements? Yes No

29. Is his/her social behaviour very one-sided and always on his/her own terms? Yes No

30. Does s/he sometimes say "you" or "s/he" when s/he means "I"? Yes No

31. Does s/he prefer imaginative activities such as play-acting or story-telling, rather than numbers or lists of facts? Yes No

32. Does s/he sometimes lose the listener because of not explaining what s/he is talking about? Yes No

33. Can s/he ride a bicycle (even if with stabilisers)? Yes No

34. Does s/he try to impose routines on him/herself, or on others, in such a way that it causes problems? Yes No

35. Does s/he care how s/he is perceived by the rest of the group? Yes No

36. Does s/he often turn conversations to his/her favourite subject rather than following what the other person wants to talk about? Yes No

37. Does s/he have odd or unusual phrases? Yes No

SPECIAL NEEDS SECTION

Please complete as appropriate

38. Have teachers/health visitors ever expressed any concerns about his/her development? Yes No

If Yes, please specify.....

39. Has s/he ever been diagnosed with any of the following?:

Language delay Yes No
Hyperactivity/Attention Deficit Disorder (ADHD) Yes No
Hearing or visual difficulties Yes No
Autism Spectrum Condition, incl. Asperger's Syndrome Yes No
A physical disability Yes No
Other (please specify) Yes No

Annex 3
M-CHAT-R Questionnaire

www.m-chat.org

Child's name Date
Age Relationship to child

M-CHAT-RTM (Modified Checklist for Autism in Toddlers Revised)

Please answer these questions about your child. Keep in mind how your child usually behaves. If you have seen your child do the behavior a few times, but he or she does not usually do it, then please answer no. Please circle yes or no for every question. Thank you very much.

1. If you point at something across the room, does your child look at it? Yes No
(FOR EXAMPLE, if you point at a toy or an animal, does your child look at the toy or animal?)

2. Have you ever wondered if your child might be deaf? Yes No

3. Does your child play pretend or make-believe? Yes No
(FOR EXAMPLE, pretend to drink from an empty cup, pretend to talk on a phone, or pretend to feed a doll or stuffed animal?)

4. Does your child like climbing on things? Yes No (FOR EXAMPLE, furniture, playground equipment, or stairs)

5. Does your child make unusual finger movements near his or her eyes? Yes No
(FOR EXAMPLE, does your child wiggle his or her fingers close to his or her eyes?)

6. Does your child point with one finger to ask for something or to get help? Yes No
(FOR EXAMPLE, pointing to a snack or toy that is out of reach)

7. Does your child point with one finger to show you something interesting? Yes No
(FOR EXAMPLE, pointing to an airplane in the sky or a big truck in the road)

8. Is your child interested in other children? (FOR EXAMPLE, does your child watch Yes No
other children, smile at them, or go to them?)

9. Does your child show you things by bringing them to you or holding them up for you to Yes No
see – not to get help, but just to share? (FOR EXAMPLE, showing you a flower, a stuffed animal, or a toy truck)

10. Does your child respond when you call his or her name? (FOR EXAMPLE, does he or she look up, talk or babble, or stop what he or she is doing when you call his or her name?)
Yes No

11. When you smile at your child, does he or she smile back at you? Yes No

12. Does your child get upset by everyday noises? (FOR EXAMPLE, does your child scream or cry to noise such as a vacuum cleaner or loud music?)
Yes No

13. Does your child walk? Yes No

14. Does your child look you in the eye when you are talking to him or her, playing with him Yes No
or her, or dressing him or her?

15. Does your child try to copy what you do? (FOR EXAMPLE, wave bye-bye, clap, or make a funny noise when you do) Yes No

16. If you turn your head to look at something, does your child look around to see what you are looking at? Yes No

17. Does your child try to get you to watch him or her? (FOR EXAMPLE, does your child look at you for praise, or say “look” or “watch me”?) Yes No

18. Does your child understand when you tell him or her to do something? (FOR EXAMPLE, if you don’t point, can your child understand “put the book on the chair” or “bring me the blanket”?) Yes No

19. If something new happens, does your child look at your face to see how you feel about it? (FOR EXAMPLE, if he or she hears a strange or funny noise, or sees a new toy, will he or she look at your face?) Yes No

20. Does your child like movement activities? (FOR EXAMPLE, being swung or bounced on your kne Yes No

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Annex 4
Q-CHAT Questionnaire

Please answer the following questions about your child by ticking the appropriate circle.
Try to answer EVERY question if you can.

1. Does your child look at you when you call his/her name?
○ always
○ usually
○ sometimes
○ rarely
○ never
2. How easy is it for you to get eye contact with your child?
○ very easy
○ quite easy
○ quite difficult
○ very difficult
○ impossible
3. When your child is playing alone, does s/he line objects up?
○ always
○ usually
○ sometimes
○ rarely
○ never
4. Can other people easily understand your child's speech?
○ always
○ usually
○ sometimes
○ rarely
○ never
○ my child does not speak
5. Does your child point to indicate that s/he wants something (e.g. a toy that is out of reach)?
○ many times a day
○ a few times a day
○ a few times a week
○ less than once a week
○ never

Please answer the following questions about your child by ticking the appropriate circle.
Try to answer EVERY question if you can.

6. Does your child point to share interest with you (e.g. pointing at an interesting sight)?
○ many times a day
○ a few times a day
○ a few times a week
○ less than once a week
○ never
7. How long can your child's interest be maintained by a spinning object (e.g. washing machine, electric fan, toy car wheels)?
○ several hours
○ half an hour
○ ten minutes
○ a couple of minutes
○ less than a minute

8. How many words can your child say?
○ none—s/he has not started speaking yet
○ less than 10 words
○ 10–50 words
○ 51–100 words
○ over 100 words
9. Does your child pretend (e.g. care for dolls, talk on a toy phone)?
○ many times a day
○ a few times a day
○ a few times a week
○ less than once a week
○ never
10. Does your child follow where you're looking?
○ many times a day
○ a few times a day
○ a few times a week
○ less than once a week
○ never
11. How often does your child sniff or lick unusual objects?
○ many times a day
○ a few times a day
○ a few times a week
○ less than once a week
○ never
12. Does your child place your hand on an object when s/he wants you to use it (e.g. on a door handle when s/he wants you to open the door, on a toy when s/he wants you to activate it)?
○ many times a day
○ a few times a day
○ a few times a week
○ less than once a week
○ never
13. Does your child walk on tiptoe?
○ always
○ usually
○ sometimes
○ rarely
○ never
14. How easy is it for your child to adapt when his/her routine changes or when things are out of their usual place?
○ very easy
○ quite easy
○ quite difficult
○ very difficult
○ impossible
15. If you or someone else in the family is visibly upset, does your child show signs of wanting to comfort them (e.g. stroking their hair, hugging them)?
○ always
○ usually
○ sometimes
○ rarely
○ never
16. Does your child do the same thing over and

over again (e.g. running the tap, turning the light switch on and off, opening and closing doors)?
○ many times a day
○ a few times a day
○ a few times a week
○ less than once a week
○ never

17. Would you describe your child's first words as:
○ very typical
○ quite typical
○ slightly unusual
○ very unusual
○ my child doesn't speak

18. Does your child echo things s/he hears (e.g. things that you say, lines from songs or movies, sounds)?
○ many times a day
○ a few times a day
○ a few times a week
○ less than once a week
○ never

19. Does your child use simple gestures (e.g. wave goodbye)?
○ many times a day
○ a few times a day
○ a few times a week
○ less than once a week
○ never

20. Does your child make unusual finger movements near his/her eyes?
○ many times a day
○ a few times a day
○ a few times a week
○ less than once a week
○ never

21. Does your child spontaneously look at your face to check your reaction when faced with something unfamiliar?
○ always
○ usually
○ sometimes
○ rarely
○ never

22. How long can your child's interest be maintained by just one or two objects?
○ most of the day
○ several hours
○ half an hour
○ ten minutes
○ a couple of minutes

23. Does your child twiddle objects repetitively (e.g. pieces of string)?
○ many times a day
○ a few times a day
○ a few times a week
○ less than once a week
○ never

24. Does your child seem oversensitive to noise?
○ always

○ usually
○ sometimes
○ rarely
○ never

25. Does your child stare at nothing with no apparent purpose?

○ many times a day
○ a few times a day
○ a few times a week
○ less than once a week
○ never

Annex 5
UCB thawing protocol (Duke/Rubinstein protocols by Vita 34/StemSure)

Form
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CBU Thawing Procedures

Principle
Cryopreserved CBU is protected against freezing damage by subsequent addition of a cryoprotector. Before administering the CBU to the patient, its content of cryoprotector should be removed as quickly as possible after thawing. The quick removal of cryoprotector restores the osmolarity of the blood cell suspension. Furthermore a cryoprotector like DMSO
causes adverse effects in vivo after reinfusion.
Specimen
Cryopreserved umbilical cord blood that has been designated for administration to a particular recipient.
Equipment
Laminar Flow Hood
Centrifuge
Plasma Expressor
Digital Balance
Tube Sealer compatible for PVC tubing
Automated Cell Counter or Microscope and Chamber for counting of cells
Waterbath (4 litres or more at 38°C)
Reagents
Albumin (5% human USP)
10% Dextran 40 (Gentran 40) and 0.9% NaCl, USP
Methyl violet acetic acid, Türk's solution, Leukoplate (or equivalent) for staining of cells
Supplies
Cell Wash/Infusion Bag

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Note: If there is ANY error or ambiguity, advise StemCare and the Transplant team. Do not proceed until the problem is resolved. If your LN2 storage tanks have no space to store the unit in its canister, add LN2 to the Dry Shipper container to maintain the unit frozen until a complete satisfactory determination is made.
5. Leave the CBU in the vapor phase for 5-10 minutes before proceeding.
6. Prepare the thawing solutions as described in point 16 and 21.
7. Open the cardboard box. Work carefully to avoid damaging the frozen plastic bag. Remember that plastic at this temperature is very brittle and breaks easily.
8. Remove the CBU unit from the canister.
Note: Do not allow the unit to bend or it will crack!
9. Place the unit inside a zipper-locked plastic bag, let the air out and close the bag. Put the

unit in a water bath at 38°C. Take care not to break the plastic tube segments, if still attached.
10. To accelerate thawing, carefully move the unit in the water and knead its contents gently but continuously.
Note: Inspect for leaks. Plastic bags for frozen blood may have pin-holes, other defects or develop leaks. It is most important to prevent serious blood losses or bacterial contamination caused by cracks or leaks. If blood leaks out into the zipper-locked bag, find the site of the break and position the unit so as to prevent further escape of blood. While maintaining the position, thaw the unit.
11. As soon as the blood has thawed, transfer the bag from the water bath into a laminar flow hood.
12. Remove the unit from the zipper-locked bag. Using an alcohol or iodine swab disinfect the covers of both ports of the freezing bag.
13. With scissors, cut off both the hermetically sealed covers to the spike ports on the freezing bag. Disinfect the scissors before using.
14. Using an alcohol or iodine swab, disinfect the cut surfaces of the spike port area of the freezing bag.
15. Close all clamps on the Transplant/washing set. Insert the spikes of the transplant set in the ports of the freezing bag.
Note: Label the transplant bag with the unique CBU unit number and the name of the recipient, or according to local standard practice!
16. Fit an 18 gauge needle to a 50 ml syringe. Draw 12.5 ml of 10% Dextran 40 and 12.5 ml of 5% human albumin into the syringe. This is the thawing solution and can be prepared before thawing the CBU.

17. Attach the syringe with thawing solution to the female luer lock on the Transplant set. Open pinch clamp 1, 2 and 3 (PC-1, PC-2 and PC-3), and then slowly introduce the 25 ml of dextran/albumin solution to the 25 ml CBU in the freezing bag. Mix the fluids during transfer. Close PC-3.
18. Allow 5 minutes for equilibration.
19. Open PC-4. Transfer the diluted CBU from the freezing bag to the transplant bag.
20. Pass the diluted CBU back and forth between the transplant bag and the freezing bag in order to more completely wash all cells out of the freezing bag into the transplant bag. Close PC-1 and PC-2.
21. Fit a 18 gauge needle to a 60 ml syringe. Draw 30 ml of 10% Dextran 40 and 30 ml of 5% human albumin into the syringe. Prepare 4 x 60 ml syringes in total as described. The syringes can be prepared before thawing the CBU.
22. Attach a 60 ml syringe with dextran/albumin solution to the luer lock. Open PC-3. Transfer the 60 ml solution to the diluted CBU in the transplant bag. Mix during transfer. Repeat with a second 60 ml syringe. The final volume is now approximately 170 ml (50 ml diluted CBU and 120 ml dextran/albumin solution).
23. Close PC-3 and open PC-1 and PC-2. Pass the diluted CBU back and forth between the transplant bag and the freezing bag in order to more completely wash all cells out of the freezing bag and into the transplant bag. Close PC-4.
24. Seal tubing between PC-4 and Injection Port 1 (IP-1) and disconnect. Discard the spikes, luer lock and connecting tubing.
25. Place the transplant bag and the waste bag in a centrifuge cup. Support the transplant

bag to prevent formation of creases during centrifugation. Make sure PC-5 and Screw Clamp 1 (SC-1) are closed.
26. Centrifuge at 400 x g for 20 minutes at 10°C.
Note: You may now call the Transplant Unit and advise them that the transplant will be ready in about 30 minutes.
27. Place the centrifuged transplant bag in the plasma expressor. Open PC-5. Use SC-1 to adjust the flow and very slowly transfer most of the supernatant to the waste bag. Leave approximately 10-15 ml supernatant with the cells. Empty the tubing between the bags by transferring air from the waste bag to the transplant bag. Close PC-5.
Note: If you detect passage of cells to the waste bag, return contents to the transplant bag, re-suspend the cells and repeat the centrifugation.
28. Inspect the supernatant for escaped cells.
a. Centrifuge 10 ml of supernatant at 600 x g for 10 min.
b. Inspect the sediment for leukocytes in a microscope. If cells are found, count them and calculate the total number of cells that would be lost.
c. Decide whether the escaped cells should be recovered in a second centrifugation for adding to the transplant cell dose. In that case, centrifuge the waste bag at 400 x g for 20 min. and transfer the supernatant to another waste bag (300 ml). Proceed following the guidelines d + e.
d. If <75% of total viable nucleated cells are recovered, re-spin the supernatant in the waste bag to recover additional cells.
e. If ≥1x106 cells/kg of patient body weight are recovered, transport residual cells to the patient's room for reinfusion. If >1x105 but <1x106 cells/kg are recovered, cells should be cryopreserved in 10% DMSO for future testing, storage or other uses. These cells can be stored in a small vial or cryo bag at the discretion of the transplant center lab.
29. Seal the tubing between the bags close to the transplant bag. Disconnect the waste bag with the supernatant.
30. Resuspend the cell pellet by slowly adding 25-50 ml of dextran-albumin solution in a 60 ml syringe through the IP-1. Mix cells and solution thoroughly during transfer.
31. Calculate the weight of the cells by subtracting the weight of an empty transplant bag.
32. Remove a small volume for cell count and viability determination. Samples for bacteriology can be taken from the supernatant.
33. If cells are recovered from the first post-thaw supernatant, add 10 ml of dextran-albumin solution to the bag through IP-2. Resuspend the cells carefully.
Note: The volumes injected in step 30 may be modified if the Transplant Physician prefers injecting the patient with a smaller total volumen. In this case, resuspend the cell pellet to the desired volumen by injecting the desired volume of dextran-albumin solution.
34. Bring the transplant bag to the Transplant Unit even if the second centrifugation is being prepared; the second can be infused separately afterwards.
35. Prepare a report on the procedure. Note the condition of the bag and whether leaks or cracks were detected. Include in the report:
- CBU ID
- Date of receipt of the CBU
- Storage conditions in your facility
- Date of thawing
- Cell count
- Viability of the cells recovered

Submit the report to the Transplant Unit and to StemCare. Alternatively or in addition fill out the Form QA/007E "Transplant Center Feedback Sheet" and return it to StemCare.
36. Return the dry shipper to StemCare according to Form QA/007J "Return Shipping Information".

Annex 6 – Infusion protocol

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CBU Infusion Procedures

Principle

At the transplant center, the cryoprotected cord blood unit (CBU) is stored in the vapor phase of liquid nitrogen until the day of transplant, when it is thawed and washed in dextran / albumin, a process which increases cell viability and removes approximately 90% of the DMSO/dextran cryoprotectant. The washed cells are resuspended in dextran/albumin and transported to the patient's bedside in a labelled transplant bag for infusion.
The unit is infused into the patient's blood via the central venous catheter over 2-30 minutes. Every effort should be made to be sure that all the cells in the transplant bag and IV tubing are delivered to the patient, maintaining a closed system during the infusion.
Specimen
Thawed and washed cells from a cryopreserved cord blood unit (CBU) in a labelled transplant bag.
Reagents
500 ml bag of Normal Saline
Supplies
Y-infusion set
IV extension tubing
Blood filter, 170-260 microns
Procedure
1. Verify that the patient is stable and has received premeds for transplant.
2. Verify that the transplant bag label matches patient identifiers via institutional procedures.
3. Examine transplant bag to be sure that cells are in solution and that large clumps are not present in the bag. If clumps are present, prepare to use blood filter in infusion set-up.
4. Close all clamps on the tubing on the Y-infusion set and blood filter.
5. Spike one arm of the Y-infusion set into the bag of normal saline.
6. Connect extension tubing to the distal arm of the Y-infusion set.
7. Prime all tubing with normal saline.
8. Spike the other arm of the Y-infusion set into the transplant bag.
9. Connect the distal end of the extension tubing to the patient's central venous catheter.
10. Open the clamps between the transplant bag, the extension tubing, and the patient's central line and allow cells to infuse into the patient's blood.
11. Rinse residual cells in the transplant bag and tubing. After all cells have dripped out of the transplant bag, close the clamps on the extension tubing and Y-set connected to the transplant bag. Open the clamps between the normal saline bag and transplant bag and allow approximately 25 cc of saline to run into the transplant bag. Close the clamps between
the saline bag and transplant bag and open the clamps between the transplant bag and the

patient and infuse this saline to the patient. Repeat rinse x 1.
12. Monitor the patient's vital signs before, during and after infusion per institution