CLINICAL STUDY PROTOCOL

Protocol Number	IEDAT-02-2015
Study Title	A Multi-center, Randomized, Double-blind, Placebo-controlled Trial to Evaluate the Effects of ***I***ntra-***E***rythrocyte ***D***examethasone Sodium Phosphate on Neurological Symptoms in Patients with ***A***taxia ***T***elangiectasia
Short Study Title - Acronym	**A**taxia **T**elangiectasia **T**reatment with **E**ryDex **S**ys**T**em - **ATTEST**
Phase	Phase III
EudraCT Number	**2015-005241-31**
IND Number	115929
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Trial Protocol Version	Version 11.0; 24 June 2020, Final

GCP Statement

This study will be performed in compliance with Good Clinical Practice (GCP), the Declaration of Helsinki (with amendments) and local legal and regulatory requirements.

1 SYNOPSIS

Title of the Study	A Multi-center, Randomized, Double-blind, Placebo-controlled Trial to Evaluate the Effects of ***I***ntra-***E***rythrocyte ***D***examethasone Sodium Phosphate on Neurological Symptoms in Patients with ***A***taxia ***T***elangiectasia
Protocol Number	IEDAT-02-2015
Phase of Development	III
Center(s)/ Country(ies)	20-25 centers in North America, Europe, Africa, Asia and Australia
Planned Trial Period (first subject enrolled - last subject out)	First Subject In (FSI): 15th of March 2017 Last Subject In (LSI): 30th of March 2020 Last Subject Last Visit (LSLV): 1st Quarter 2021
Study Objectives	***Initial Treatment Period (6 months)*** **Primary Efficacy Objective**: • To evaluate the effect of two dose ranges (~5-10 and ~14-22 mg DSP/infusion) of EryDex System end product [EDS-EP; the EDS is a combination product that is used to load dexamethasone sodium phosphate (DSP) into autologous erythrocytes, creating the EDS end product, which is infused into the patient], compared to placebo, on central nervous system (CNS) symptoms measured by the 'Modified' International Cooperative Ataxia Rating Scale (mICARS) in patients with ataxia telangiectasia (AT). **Key Secondary Efficacy Objective:** • To evaluate the effect of EDS-EP, compared to placebo, in this population on the Clinical Global Impression of Change from baseline (CGI-C). **Safety Objective**: • To evaluate the safety and tolerability of EDS-EP compared to placebo in AT patients, based on the occurrence of Treatment-Emergent Adverse Events (TEAEs), including Serious AEs and discontinuations due to AEs, and changes in vital signs, laboratory parameters, ECGs and physical/neurological examination findings. **Secondary Efficacy Objectives**: • To evaluate the effect of EDS-EP, compared to placebo, in this population on the following efficacy measures: o Clinical Global Impression of Severity (CGI-S) of neurological symptoms of AT; o Adaptive behavior measured by the Vineland Adaptive Behavior Scales (VABS); **Tertiary Objectives:** • To evaluate the effect of EDS-EP on health-related Quality of Life (QoL) using the EQ-5D-5L scale; • To assess the pharmacokinetic and pharmacodynamic relationships between dexamethasone administered through EDS-EP and safety, tolerability, and demographic variables. • To evaluate the pharmacokinetic (PK) profile of dexamethasone administered through EDS-EP at two dose levels based on pooled data from all patients in each treatment group.

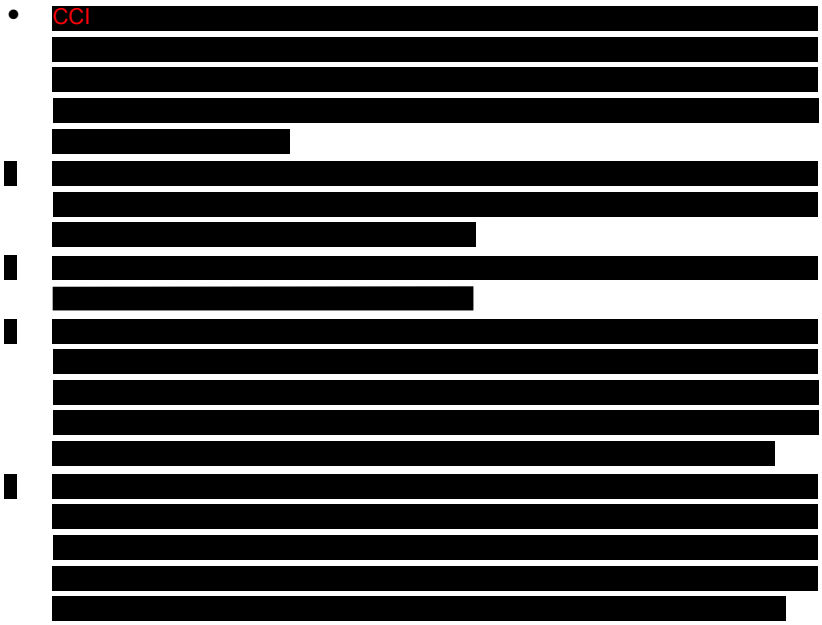
	A determination of individual PK parameters will be performed for patients with an adequate number of PK blood samples after the initial infusion. **Exploratory Objective**: • To collect data on the use of the A-T NEST concurrent with the ICARS, CGI-S and CGI-C, as well as its use in languages other than English, and to compare it with the data from the scales referenced to assess the psychometric properties of the A-T NEST. ***Extension Treatment Period (6 months)*** **Primary Objective**: • To evaluate the efficacy of two dose ranges (~5-10 and ~14-22 mg DSP/infusion) of EDS-EP compared to placebo in treating CNS symptoms in AT patients during long-term treatment (up to 12 months), as measured by the 'Modified' ICARS. **Secondary Objectives:** • To evaluate the long-term (up to 12 months) safety and tolerability of EDS-EP in AT patients; • To compare the effects of the two dose ranges of EDS-EP on the clinician's global impression (CGI-C and CGI-S), adaptive behavior (VABS), and QoL (EQ-5D-5L scale).
Study Design	This is an international (North America, Europe, Africa, Asia and Australia), multi-center, one-year, randomized, prospective, double-blind, placebo-controlled, phase III study, designed to assess the effect of two non-overlapping dose ranges of EDS-EP, administered by IV infusion once per month, on neurological symptoms of patients with AT. All patients who complete the assessments as designed over the initial 6 months of the trial will be eligible to continue in an additional 6-month, double-blind, placebo-controlled extension designed to collect information on the long-term safety and efficacy of the trial treatments. Upon completion of all screening assessments for eligibility patients meeting all selection criteria at baseline will be randomized in a 1:1:1 fashion to one of the two EDS-EP dose levels or placebo. A minimization procedure will be employed to ensure that the proportions of male and female, and younger (6 to <10 years) and older (≥10 years), patients are comparable across the three treatment groups. Every attempt will be made to ensure the same balance is achieved across different regions. A minimum of 180 patients were anticipated to be enrolled, then the last subject was enrolled on the 30th of March 2020, with 175 enrolled patients, due to the Covid-19 outbreak. Each group will consist of 60 patients randomly assigned to receive one of the two dose ranges of EDS-EP or placebo, as follows: • **Group 1:** EDS-EP low dose range of ~5-10 mg DSP/infusion, • **Group 2:** EDS-EP high dose range of ~14-22 mg DSP/infusion, • **Group 3:** Placebo EDS infusion. The initial 6-month treatment period will be considered complete when the endpoint assessment (at Visit 9/Month 6 or at early discontinuation) has been performed for all patients.

	As a results of the COVID-19 pandemic, temporary changes to the protocol have been implemented. These changes are described in Appendix 14.Patients who are not experiencing severe side-effects, or have deteriorated significantly while on the treatment and provide informed consent will be eligible to continue treatment for an additional 6 months in a double-blind, placebo-controlled extension treatment period (or longer, in case a patient is willing to be enrolled in the open-label study IEDAT-03-2018, but the study has not yet received IRB/EC approval at the time the patient reaches Visit 15/Month 12). Patients meeting all entry criteria will be treated as follows: • Patients originally randomized to EDS-EP treatment groups (Group 1 or Group 2) will continue on the same treatment; • Patients originally randomized to the Placebo group (Group 3) will be re-randomized in equal proportions (1:1) to receive either the EDS-EP ~5-10 mg DSP/infusion or ~14-22 mg DSP/infusion, as follows: o Following 6 months of treatment, one third of the originally randomized placebo patients will be re-randomized to treatment with EDS-EP, as described above; o After 9 months of treatment, one third of the originally randomized placebo patients will be re-randomized to treatment with EDS-EP, as described above; o At 12 months, all remaining placebo patients who continue open-label treatment will receive treatment with EDS-EP, as described above. The ICARS will be administered by a site rater and scoring verified by a central remote qualified rater, based on a video recording of the assessment at the site. The scores provided by the central remote raters will be used for the primary analysis of the 'Modified' ICARS (primary efficacy endpoint). The site ICARS rater will not be involved in the rating of the CGI-S and CGI-C, VABS, QoL or A-T NEST scale. The CGI rater will not have access to the ICARS ratings, but may refer to other scales in scoring the CGI. All patients who complete 12 months of treatment in the trial, complete the study assessments, and provide informed consent will be eligible to continue treatment with EDS-EP in an open-label, extension study (IEDAT-03-2018). In case a patient is willing to be enrolled in the open-label study IEDAT-03-2018, but the study has not yet received IRB/EC approval at the time the patient reaches Visit 15/Month 12, the patient may undergo unscheduled monthly assessments/infusions (according to the assigned blinded treatment) starting from the time Visit 15/Month 12 should be performed, and continuing until the site has been initiated for the open-label study IEDAT-03-2018 and he/she has been evaluated for eligibility for the study. All patients, including those previously treated with placebo, who meet all of the selection criteria will receive monthly infusions of EDS-EP (dose range of ~14-22 mg DSP/infusion).
Planned Number of Patients	A minimum of 180 patients meeting all selection criteria were anticipated to be enrolled and randomized to one of the 3 treatment groups (approximately 60 patients per group). Due to the Covid-19 outbreak, the enrollment was closed with 175 randomized patients.
Inclusion and Exclusion Criteria	**Inclusion Criteria** 1. Patient meets clinical criteria for diagnosis of AT. The neurological signs of AT (incoordination of the head and eyes in lateral gaze deflection, gait ataxia

	associated with an inappropriately narrow base) must be documented. Such signs of AT illustrate the body systems in which changes shall be confirmed but the listed changes are examples and other changes in those systems may be observed and documented to confirm the diagnosis of AT. 2. Patient is in autonomous gait or is helped by periodic use of a support (i.e. local ICARS score for *Item 1 – Walking Capacities* between 0 and 4, included). 3. Patient will be investigated for the proven genetic diagnosis of AT (prior documentation or by central laboratory test report). 4. Patient is at least 6 years of age, of either sex 5. Body weight > 15 kg. 6. The patient and his/her parent/caregiver (if below the age of consent), or a legal representative, has provided written informed consent to participate. If consent is provided solely by the caregiver in accordance with local regulations, the patient must provide assent to participate in the study. **Exclusion Criteria** *General* 1. Females that are a. pregnant, or are breast-feeding (for EU countries only); b. of childbearing potential, pregnant, or are breast-feeding (for US and Rest of World countries). *Females of childbearing potential using adequate birth control, as determined by their Health Care Provider, will be eligible.* 2. A disability that may prevent the patient from completing all study requirements. 3. Current participation in another clinical study. *Medical History and Current Status* 4. CD4+ lymphocytes count <400/mm3 (for patients 6 years of age) or <150/mm3 (for patients > 6 years). In presence of oral infections, like oral candidiasis, documented at the screening or recurrent as per medical history documentation, the limit increases to <200/mm3 (for patients > 6 years). 5. Loss/removal of 250 mL or more of blood within the past 4 weeks prior to screening. 6. Current neoplastic disease or previous neoplastic disease not in remission for at least 2 years. 7. History of severe impairment of the immunological system. 8. Severe or unstable pulmonary disease. 9. Uncontrolled diabetes. *Patients with diabetes that has been stabilized (i.e. no hypoglycemic or hyperglycemic episodes in the past 3 months) will be eligible.* 10. Any other severe, unstable, or serious disease or condition that in the Investigator's opinion would put the patient at risk for imminent life-threatening morbidity, need for hospitalization, or mortality. 11. Any clinically significant abnormality on standard laboratory examinations (hematology, biochemistry, urinalysis) at screening that remains abnormal on repeat testing. Eligibility of patients with abnormal laboratory test values will be determined by the Investigator in consultation with the Medical Monitor. 12. Confirmed hemoglobinopathies, e.g. hemoglobin C disease, sickle cell anemia, or thalassemia. 13. Moderate or severe renal and/or hepatic impairment.

	Prior/Concomitant Medication 14. Any previous oral or parenteral steroid use within 4 weeks before Baseline. Treatment with inhaled or intranasal steroids for asthma or allergies, as well as use of topical steroids will be permitted. 15. Chronic condition or prior allergic reaction representing a contraindication to the use of dexamethasone or other steroid drugs. 16. Has participated in any other trial with an investigational drug and received a dose within 30 days or 10 half-lives (whichever is greater) from the start of the 30-day Screening Period. 17. Has participated in a previous trial with EDS. 18. Requires any concomitant medication prohibited by the protocol. 19. Has taken a drug or treatment known to cause major organ system toxicity during the past year. 20. Use of any drug that is a strong inducer/inhibitor of CYP3A4 within 4 weeks before baseline.
Schedule of Visits and Assessments: Screening period (Days -30 to -1)	Every potential patient will provide informed consent or assent prior to the initiation of any screening procedure. During the 30-day screening period, any previous treatments with other corticosteroid compounds will be withdrawn (washout from previous treatment). The following screening evaluations will be conducted: • Medical history and demographics • Physical examination • Vital signs (including height and weight in triplicate) • Neurological examination • ICARS (administered by the qualified site-specific rater. • CGI-S • Electrocardiogram (ECG) • Routine laboratory tests: hematology, biochemistry, urinalysis • Special laboratory tests: HbA1c, CD4+ lymphocytes count, α-fetoprotein, CRP, and RBC antibodies (IgG, IgM and Qualitative Direct Coombs test) • Blood collected to assess RBC osmotic resistance (in selected centers) • Serum pregnancy test (women of childbearing potential) • Plasma cortisol – sample to be collected before 8:00 AM and prior to randomization at baseline. If the basal cortisol level is within the reference normal range, the patient can be enrolled in the study. If the 8:00 AM cortisol level is below 3-5 µg/dL (depending on assay) regardless of symptoms, or the patient exhibits signs or symptoms of adrenal insufficiency (see Appendix 12) and has a cortisol <10 µg/dL, the patient will receive a high dose ACTH stimulation test, regardless of weight, within 24 hours. If the ACTH stimulation test is normal, the patient can be enrolled after the effects of the ACTH have dissipated (e.g., 30 days after ACTH administered). If the patient fails the ACTH stimulation test they will be excluded from the study and referred to a pediatric endocrinologist, with a recommendation to prescribe stress dose steroids. • Sample for AT genetic assay, if not previously reported (test results must be obtained from a certified lab, but do not need to be available before baseline).

	• Mini-ATM detection - 2.5 mL of blood collected • Assessment of prior and concomitant medications • Assessment of AEs If any abnormal laboratory test results, vital sign measurements, or ECG findings of clinical significance are noted at the screening visit, these must be repeated during the 30-day screening period and the results made available prior to making the final decision on a patient's eligibility for the study at Baseline. Adverse events, reported by the patient or observed by the investigator, and the use of concomitant medication will be recorded from the time of signing of informed consent through the end of the study.
Schedule of Visits and Assessments: Baseline (Day 0/1)	The following assessments will be performed on Day 0 and Day 1 before administration of study treatment: • Physical examination • Vital signs (including height and weight in triplicate) • Neurological examinations • Review of all eligibility criteria. • ICARS once on Day 0 (~24 hr before dosing; video recording required) and once on Day 1 (baseline assessment; video recording required). • CGI-S • Vineland Adaptive Behavior Scale (VABS) • Quality of life (QoL): EQ-5D-5L • Columbia-Suicide Severity Rating Scale (C-SSRS) • ECG, only if repeated due to abnormal findings at the screening visit • Routine laboratory tests on the diverted blood sample (see Section 11.6): only if repeated due to abnormal parameters at the screening visit • Special laboratory tests on the diverted blood sample (see Section 11.6): HbA1c, CD4+ lymphocytes count, and CRP • Hemolysis panel [free plasma hemoglobin, haptoglobin, bilirubin (total and conjugated), LDH] • Urine and serum pregnancy test (women of childbearing potential) on the diverted blood sample (see Section 11.6). • Assessment of physical development, sexual maturation, and the effect of treatment with dexamethasone on these aspects (Tanner scale; Marshall and Tanner 1969, 1970). Tanner staging of the breasts in pre-menarchal females and of the scrotum in males who have not completed puberty, will be performed. Post-menarchal females and males who have completed puberty will be excluded from this growth analysis. • Bone mineral density • Assessment of concomitant medications • Assessment of AEs • Blood sample (0 hr) for dexamethasone pharmacokinetics (PK) just before the infusion. Following randomization using the IVRS/IWRS, study treatment will be prepared and administered as follows: • 1 mL blood collected, after blood diversion (see Section 11.6), for aerobic culture. • CCI [redacted]

	• Addition of randomized study medication (DSP or placebo) to the EDS process • CCI [redacted]  After completion of the IV infusion of the study treatment the following assessments will be performed on Day 1: • Blood samples for dexamethasone PK 1, and 4 hr after the end of the infusion. If all samples are taken, the total blood volume collected for PK within the first 24 hours would be 9 mL. • Free plasma hemoglobin (1 hr post infusion) • Vital signs • Physical examination • Assessment of AEs
Schedule of Visits and Assessments: Day 2	At Day 2 the following assessments will be performed: • A-T NEST (in selected centers) • Blood sample for dexamethasone PK 24 hr after the infusion • Hemolysis panel [free plasma hemoglobin, haptoglobin, bilirubin (total and conjugated), CBC, LDH, urinalysis] • Assessment of concomitant medications • Assessment of AEs
Schedule of Visits and Assessments: Day 15	At Day 15 the following assessments will be performed: • Blood sample for dexamethasone PK • Hemolysis panel [free plasma hemoglobin, haptoglobin, bilirubin (total and conjugated), CBC, LDH, urinalysis] • Assessment of concomitant medications • Assessment of AEs
Schedule of Visits and Assessments: Months 1, 2, 4, 5, 7, 8, 10, and 11	At Months 1, 2, 4, 5, 7, 8, 10, and 11 the following assessments will be performed: • Blood sample for dexamethasone PK before infusion on the diverted blood sample (Months 1, 2, 4 and 5 only)

	• Laboratory tests on the diverted blood sample: serum creatinine • Urine pregnancy test will be performed before every infusion (for women of childbearing potential) • A-T NEST (in selected centers) (Months 1, 4, 7, and 10 only) • C-SSRS • Mini-ATM detection on the diverted blood sample, only on Day 60 (Month 2) • Upon completion of the EDS process, fill the satellite sample bag with approximately 6 mL of EDS-EP by gravity (approximately 2mL if no other samples but the ones for sterility culture test will be collected). A 1-mL sterile sample of the EDS-EP will be stored under refrigeration as a "Retention Sample". • Study treatment administration by IV infusion, once all assessments have been completed. • CCI [redacted] After completion of the IV infusion of the study treatment the following assessments will be performed • Physical examination and vital signs 1-2 hr after the end of infusion • Blood sample for dexamethasone PK 1 hr after the end of infusion (Months 2, 4 and 5 only). • Assessment of AEs and concomitant medications.
Schedule of Visits and Assessments: Months 3, 6, 9 and 12	At Months 3, 6, 9 and 12 the following assessments will be performed before treatment administration (unless specified otherwise): • Informed consent/assent at Month 6 for patients continuing treatment • Physical examination • Vital signs • Neurological examinations • ICARS (video recording required) • CGI-S • CGI-C • VABS • EQ-5D-5L • C-SSRS • ECG • Routine laboratory tests on the diverted blood sample

	• Special laboratory tests on the diverted blood sample: HbA1c, CD4+ lymphocytes count, α-fetoprotein, CRP, and RBC antibodies (IgG, IgM, Qualitative Direct Coombs test), only on Months 6 and 12. • Mini-ATM detection on the diverted blood sample, only on Month 6. • Serum pregnancy test (women of childbearing potential) on the diverted blood sample, only Month 6 and Month 12. • Urine pregnancy test will be performed before every infusion for women of childbearing potential. • Assessment of physical development, sexual maturation, and the effect of treatment with dexamethasone on these aspects. Tanner staging of the breasts in pre-menarchal females and of the scrotum in males who have not completed puberty, will be performed at Months 6 and 12. Post-menarchal females and males who have completed puberty will be excluded from this growth analysis. • Hemolysis panel [free plasma hemoglobin, haptoglobin, bilirubin (total and conjugated), LDH] (Months 3, 6 and 9 only) • Bone mineral density [on Months 6 and 12 only] • Assessment of concomitant medications • Assessment of AEs • Blood sample for dexamethasone PK (Months 3 and 6 only) just before the infusion • Upon completion of the EDS process, fill the satellite sample bag with approximately 6 mL of EDS-EP by gravity (approximately 2 mL if no other samples but the ones for sterility culture test will be collected). A 1-mL sterile sample of the EDS-EP will be stored under refrigeration as a "Retention Sample". • Upon completion of the EDS process 5 mL of the EDS from the EryKit_01 bowl at the end of the process will be collected to assess RBC osmotic resistance (in selected centers) • Study treatment administration by IV infusion, once all assessments have been completed. • CCI [redacted] After completion of the IV infusion of the study treatment the following assessments will be performed: • Physical examination and vital signs 1-2 hr after infusion • Blood sample for dexamethasone PK 1 hr after infusion (Month 3 only) • Free plasma hemoglobin (1 hr post infusion)-(Months 3, 6 and 9 only) • Assessment of concomitant medications • Assessment of AEs In case a patient is willing to be enrolled in the open-label study IEDAT-03-2018, but the study has not yet received IRB/EC approval at the time the patient reaches

	Visit 15/Month 12, the patient may undergo unscheduled monthly assessments/infusions (according to the assigned blinded treatment) starting from the time Visit 15/Month 12 should be performed, and continuing until the site has been initiated for the open-label study IEDAT-03-2018 and he/she has been evaluated for eligibility for the study. • Laboratory tests on the diverted blood sample: serum creatinine • Urine pregnancy test will be performed before every infusion (for women of childbearing potential) • C-SSRS • Upon completion of the EDS process, fill the satellite sample bag with approximately 6 mL of EDS-EP by gravity (approximately 2mL if no other samples but the ones for sterility culture test will be collected). A 1-mL sterile sample of the EDS-EP will be stored under refrigeration as a "Retention Sample". • Study treatment administration by IV infusion, once all assessments have been completed. • CCI [redacted] After completion of the IV infusion of the study treatment the following assessments will be performed: • Physical examination and vital signs 1-2 hr after the end of infusion • Assessment of AEs and concomitant medications. The assessments on Month 12, the last or end-of-study visit (Visit 15), is to be performed upon completion of the study or at premature withdrawal from the study. *Safety Follow-up Visit* All patients who discontinue prematurely or who complete the 12-month treatment period, but don't continue in the open-label study will be required to return for a Safety Follow-up Visit (Visit 16) 30 days after their final assessment or at least 60 days after their last infusion, whichever is longer. At this visit, the A-T NEST will be assessed and the occurrence of any AEs or Serious AEs (SAEs) reported by the patient/caregiver or observed by the Investigator since the previous visit will be recorded.
Investigational Medicinal Product(s): dose, mode of administration, and dosing schedule	The study treatment consists of one of two dose ranges of dexamethasone sodium phosphate (DSP) administered via *ex vivo* encapsulation into autologous erythrocytes (EDS), or an infusion of erythrocytes prepared using a placebo solution instead of a DSP solution, that are infused into the patient with AT.

	Autologous whole blood (50 ± 5 mL) to be used for the EDS process will be collected by peripheral venipuncture into heparinized collection tubes. • **Group 1**: EDS-EP low dose range of ~5-10 mg DSP/infusion: CCI [redacted] • **Group 2:** EDS-EP high dose range of ~14-22 mg DSP/infusion: CCI [redacted] • **Group 3:** Placebo: Patients will be treated with autologous erythrocytes prepared with the EDS process using a placebo solution CCI [redacted] Study treatment will be administered by IV infusion once per month for 12 consecutive months (6 months initial treatment + 6 months extension treatment; additional blinded unscheduled treatments will be performed in case a patient is willing to be enrolled in the open-label study IEDAT-03-2018, but the study has not yet received IRB/EC approval at the time the patient reaches Visit 15/Month 12; these unscheduled infusions will be started from the time Visit 15/Month 12 should be performed, and continuing until the site has been initiated for the open-label study IEDAT-03-2018 and he/she has been evaluated for eligibility for the study). CCI [redacted]
Reference Therapy:	For patients randomized to the Placebo group, CCI [redacted]
Planned Treatment Duration per Patient	The study will consist of a 30-day Screening Period followed by two sequential (each one approximately 180-day long) treatment periods, for a total treatment duration of approximately 360 days. Patients completing the Initial Treatment Period that are doing well on their assigned treatment will have the option of continuing treatment in the Extension Treatment Period. One third of patients randomized to the Placebo group (Group 3) in the initial period that continue in the extension period will continue to receive placebo throughout the treatment

	period, while one third of the originally randomized placebo group will be switched to EDS at the start of the extension period, and another third will be switched to EDS after completing 9 months of treatment. All patients who complete 12 (or more) months of treatment in the combined Initial and Extension Treatment Periods will be eligible for continuing treatment with EDS in a 12-month, open-label, extension study (Study IEDAT-03-2018). In case a patient is willing to be enrolled in the open-label-study IEDAT-03-2018, but the study has not yet received IRB/EC approval at the time the patient reaches Visit 15/Month 12, the patient may undergo unscheduled monthly assessments/infusions (according to the assigned blinded treatment) starting from the time Visit 15/Month 12 should be performed, and continuing until the site has been initiated for the open-label study IEDAT-03-2018 and he/she has been evaluated for eligibility for the study.
Statistical Methods (including sample size calculation)	*Sample Size* Based on the very low prevalence of AT, as well as the high disability, morbidity and mortality, the number of patients available for prospective studies is extremely limited. Furthermore, only aggregate Phase II study results are available for the ICARS; no data are available for the 'Modified' ICARS. Sample size calculations are based on the analysis of the primary efficacy variable (i.e., 'Modified' ICARS) under the following assumptions. • For aggregate ICARS, a treatment difference of 3.7 - 4.2 for the ~14-22 mg dose versus placebo with respect to ICARS measurements is expected, with a standard deviation of 5.0 - 7.4 (IEDAT-ERY01-2010 Clinical Study Report). • For 'Modified' ICARS, the treatment difference is expected to be less than the aggregate ICARS, therefore instead of examining a treatment difference between 3.7 and 4.2, the range between 3.0 and 3.7 was examined. The decrease in treatment difference is expected given that most of the questions in the Kinetic Function domain are not included in the 'Modified' ICARS and Chessa et al (2014) reported this domain as demonstrating the greatest overall improvement. • For 'Modified' ICARS, the standard deviation is expected to be more than the aggregate ICARS, therefore instead of examining a standard deviation between 5.0 and 7.4, the range between 5.0 and 8.0 was examined. This increase is expected given that removing questions from health assessment instruments can decrease precision and increase standard deviations (Awad, 2008; McHorney et al, 1992; McHorney, 1997). • The primary efficacy variable will be tested, comparing ~14-22 mg dose versus placebo at the 0.05 two-sided significance level. Testing of the ~5-10 mg dose versus placebo will only proceed if the ~14-22 mg dose is significant. Consequently, no adjustment for multiplicity is needed for testing the two dose levels. Results from a power analysis identified that a sample size of 54 provides sufficient power for a study design with two repeated measures to assess the primary efficacy endpoint of change from baseline in 'Modified' ICARS between two groups with a two-sided 0.05 significance level, when the treatment difference ranges between 3.0 and 3.7, and the standard deviation ranges between

	5.0 and 7.0 (PASS®, Module "Tests for Two Means in a Repeated Measures Design"). The final sample size will be adjusted for 10% loss to follow-up, for a final sample size of 60 per group. It is anticipated that the screen failure rate will be approximately 25%; therefore, at least 240 patients would need to be screened to enroll 180 eligible patients. Due to the Covid-19 outbreak, the enrollment was closed with 175 randomized patients as of the 30th of March 2020. Statistical guidance determined that 175 patients (versus the goal of 180 patients) would not materially change the power of the study. *Efficacy Analysis – Initial Treatment Period* Efficacy analyses will be performed on the Full Analysis Set (defined below). The primary efficacy variable, change from baseline in 'Modified' ICARS total score, will be analyzed using a Mixed Model Repeated Measures (MMRM) approach, with the baseline value as covariate and age, treatment, region, baseline value and treatment interaction, visit and treatment-by-visit interaction as fixed effects. The primary MMRM analysis will assume missing at random (MAR) as a means of handling missing data. Sensitivity analyses will be performed to evaluate the MAR assumption. These will utilize ANCOVA models with missing data imputed using LOCF, OC, and OC + RDO. The analysis of the key secondary efficacy measure, CGI-C, will be performed using ANCOVA, with age, sex, treatment, region, visit and treatment-by-visit interaction as fixed effects. For the primary ANCOVA analysis, missing data will be imputed using LOCF. For sensitivity analyses, missing data will be imputed using OC and OC + RDO. For the other secondary and tertiary efficacy variables, the VABS, CGI-S, and QoL (EQ-5D-5L), the change from Baseline to Month 6 in the total score will be analyzed using either one of the two approaches described for the primary efficacy parameter and key secondary efficacy parameter, as appropriate, for the distribution of the variable being analyzed. For the CGI-C, the proportion of patients with improvement (scores of 1, 2 or 3), vs. those with no change or worsening (scores of 4, 5, 6 or 7), will also be compared between groups at Month 6. Hierarchical Testing Procedure to Control the Type I Error (FWER). The primary endpoint will be tested at the 0.05 two-sided significance level. A sequential testing strategy will then proceed as indicated in the flowchart in the protocol. As long as a significance test is positive, testing will continue to the next indicated test in the sequence. *Efficacy Analysis – Extension Treatment Period* For the Extension Treatment Period, the comparisons will be made for the 'Modified' ICARS, CGI-S, VABS, and QoL [EQ-5D-5L] using either one of the two approaches for the primary efficacy parameter and key secondary efficacy parameter, as appropriate, for the distribution of the variable being analyzed. The CGI-C rating at Months 9 and 12 will be analyzed using ANCOVA, and the categorical ratings for the CGI-C (any improvement vs. no change or worsening) will be analyzed using a Logistic Regression Model with fixed effects for

	treatment and country, comparing the two EDS dose ranges (~5-10 and~14-22 mg DSP/infusion) vs. placebo (as above). The blind will be broken after all patients have completed the 6-month, double-blind, Initial Treatment Period, and the database for this period has been locked, so that the primary efficacy analysis can be performed. Therefore, procedures will be implemented to ensure that the blind is maintained for the 6-month, double-blind, Extension Treatment Period, so that the analyses of the data collected in this extension period are valid (See Section 16.2.8 for details). *Safety Analysis* All AEs will be summarized by body system and preferred term and treatment group. The incidence (%) of SAEs, AEs that are newly occurring or worsened after administration of study medication, and AEs leading to discontinuation (ADOs) will also be summarized. The intensity of AEs and relationship to the study medication, as assessed by the investigators, will also be presented. Results from vital signs, ECGs and laboratory tests, and physical and neurological examination findings will be summarized descriptively, with abnormal and clinically notable values/findings being identified. The change from baseline to endpoint in the C-SSRS will be analyzed using ANCOVA. *Data Safety Monitoring Board (DSMB)* An independent Data Safety Monitoring Board (DSMB) will review safety and tolerability data from the trial. The DSMB will receive unblinded safety data for review at specified intervals and will have the authority to modify or stop the trial if significant safety concerns are detected. The DSMB will review all of the safety data on an ongoing basis, with special emphasis on the incidence and severity of steroid-related events, new infections, and serious AEs and deaths, in addition to the standard safety parameters *Pharmacokinetics (PK)* A population PK analysis will be performed for dexamethasone administered via EDS, based on the pooled data for dexamethasone plasma concentrations from all samples obtained from patients in each of the EDS treatment groups. In addition, for a sub-group of patients for which >80% of planned samples are collected after the first infusion (i.e. at least 5 of 6 post-dose samples are collected through Day 30 [Month 1]), the following PK parameters will be calculated for each patient, based on plasma levels of dexamethasone: C_{max}, t_{max}, AUC, apparent half-life. A mean, standard deviation, median, and range (minimum, maximum) for each of these parameters will be calculated and compared between the two dose groups for EDS. Details of the planned PK analyses will be provided in a separate document.
Analysis Populations	The following analysis sets will be used: 1) *Intention-to-treat population (ITT)*: All randomized patients. 2) *Full Analysis Set (FAS)* (Also referred to as MITT): All randomized patients who received at least one dose of study medication and had at least one post-baseline efficacy assessment of the primary efficacy variable. 3) *Per Protocol Population (PP)*: All patients enrolled into the study who received at least one dose of randomized treatment, fulfilled all Inclusion/Exclusion criteria, did not have any major protocol violations, and

	completed the Initial Treatment Period of the study as planned (i.e. returned for a final evaluation). 4) *Safety Population (SP)*: All patients who received at least one dose of randomized treatment.

Schedule of Visits and Assessments: 6-Month Initial Treatment Period

Visit (V) (z)	**Screening**	**V1**		**V2**	**V3**	**V4**	**V5**	**V6**		**V7**	**V8**	**V9**
Study Day or Month (D/M) #	**D -30 to -1**	**D0/1**		**D2**	**D15**	**M1**	**M2**	**M3**		**M4**	**M5**	**M6 (a,b)**
Procedure (§)		**Pre(c)**	**Post(c)**					**Pre(c)**	**Post(c)**			
Informed Consent Signature	X											
Medical History/Demographics	X											
Inclusion/Exclusion Criteria (v)	X	X										
EDS-EP Infusion (h)		1				2	3	4		5	6	
Culture-based sterility test (aa)			X			X	X		X	X	X	
Neurological Examination	X	X						X				X
Physical Examination	X	X	X			X	X	X	X	X	X	X
Tanner Scale		X										X
Vital Signs (x)	X	X	X			X	X	X	X	X	X	X
ECG	X	X(d)						X				X
Routine Laboratory Tests (e)	X	X(d)						X				X
Serum creatinine						X(w)	X(w)			X(w)	X(w)	
Bone Mineral Density		X										X
Serum($)/Urine(*) Pregnancy Test (o)	X($)	X($,*)				X(*)	X(*)	X(*)		X(*)	X(*)	X($)
ICARS (with video recording)	X(n)	X(n)						X				X
CGI-C								X				X
CGI-S	X	X						X				X
VABS		X						X				X
Quality of Life (EQ-5D-5L)		X						X				X
C-SSRS		X				X	X	X		X	X	X
A-T NEST (in selected centers)				X		X				X		
RBC osmotic resistance (t)	X	X						X				
Special Laboratory Tests (m)	X(f,g)	X(f)										X(f)
Hemolysis Panel (s)		X	X	X	X			X	X			
Genetic AT diagnosis (q)	X											
Mini-ATM detection	X						X					X
Dexamethasone PK sample		X(i)	X(i)	X(j)	X(k)	X(l)	X(l)	X(l)	X(l)	X(l)	X(l)	X(l)
EDS end product sample (u)		X				X	X	X		X	X	
Prior/Concomitant Treatments	Throughout the duration of the study											
Adverse Events	Throughout the duration of the study											

Schedule of Visits and Assessments: 6-Month Extension Treatment Period

Visit (V) (z)	**V9**		**V10**	**V11**	**V12**		**V13**	**V14**	**Unscheduled Visit (£)**	**V15**	**V16**
Study Day or Month (D/M) #	**M6 (b)**		**M7**	**M8**	**M9**		**M10**	**M11**		**M12(y)**	**Safety**
Procedure (§)	**Pre(c)**	**Post(c)**			**Pre(c)**	**Post(c)**					**Follow-up (p)**
Informed Consent Signature	X								X		
Re-Randomization (r)	X				X						
EDS-EP Infusion (h)	7		8	9	10		11	12	#		
Culture-based sterility test (aa)		X	X	X		X	X	X	X		
Neurological Examination					X					X	
Physical Examination		X	X	X	X	X	X	X	X	X	
Tanner Scale										X	
Vital Signs (x)		X	X	X	X	X	X	X	X	X	
ECG					X					X	
Routine Laboratory Tests (e)					X					X	
Serum creatinine			X(w)	X(w)			X(w)	X(w)	X		
Bone Mineral Density										X	
Serum($)/Urine(*) Pregnancy Test (o)	X(*)		X(*)	X(*)	X(*)		X(*)	X(*)	X(*)	X($)	
ICARS (with video recording)					X					X	
CGI-C					X					X	
CGI-S					X					X	
VABS					X					X	
Quality of Life (EQ-5D-5L)					X					X	
C-SSRS			X	X	X		X	X	X	X	
A-T NEST (in selected centers)			X				X				X
RBC osmotic resistance (t)	X				X						
Special Laboratory Tests (m)										X(f)	
Hemolysis Panel (s)	X	X			X	X					
EDS end product sample (u)	X		X	X	X		X	X	X		
Prior/Concomitant Treatments	Throughout the duration of the study										
Adverse Events	Throughout the duration of the study										X

a) Efficacy evaluations on Month 6 (Visit 9) will be used as the Endpoint assessments for all efficacy measures.

b) Month 6 (Visit 9; Endpoint) evaluations should be performed for all patients that complete the Initial Treatment Period, as well as patients that discontinue prematurely; these evaluations will be used as the baseline assessments for the double-blind, Extension Treatment Period.

c) Procedures to be done before (Pre) and after (Post) the EDS-EP infusion.

d) These evaluations will be repeated only if abnormalities requiring follow-up were noted at the Screening evaluation; results from the repeat assessments must be available at baseline to confirm eligibility before the patient can be randomized to treatment.

e) Routine laboratory assessments to include complete hematology, biochemistry, and urinalysis.

f) Special laboratory tests include HbA1c, CD4+ lymphocytes count, α–fetoprotein (not repeated at baseline), CRP, and RBC antibodies (IgG, IgM, Qualitative Direct Coombs test – not repeated at baseline).

g) A sample will be collected before 8:00 AM for measurement of plasma cortisol during the screening period, prior to randomization at baseline. If the basal cortisol level is within the reference normal range, the patient can be enrolled in the study. If the 8:00 AM cortisol level is below 3-5 µg/dL (depending on assay) regardless of symptoms, or the patient exhibits signs or symptoms of adrenal insufficiency (see Appendix 12) and has a cortisol <10 µg/dL, the patient will receive a high dose ACTH stimulation test, regardless of weight, within 24 hours. If the ACTH stimulation test is normal, the patient can be enrolled after the effects of the ACTH have dissipated (e.g., 30 days after ACTH administered). If the patient fails the ACTH stimulation test they will be excluded from the study and referred to a pediatric endocrinologist, with a recommendation to prescribe stress dose steroids.

h) Assigned study treatment: Group 1: EDS-EP dose range ~5-10 mg DSP/infusion; Group 2: EDS-EP dose range ~14-22 mg DSP/infusion; and Group 3: placebo.

i) Samples to be collected before infusion (0 min), and 1,and 4 hr after the end of the infusion.

j) Single sample to be collected 24 hr post-infusion (Day 2).

k) Single blood sample to be taken in the morning.

l) Blood samples to be collected prior to (trough) and 1 hour after (peak) the infusion, except in Months 1 (Visit 4) and Months 6 (Visit 9); only trough levels will be collected.

m) Blood sample to be collected before 8:00 AM for measurement of plasma cortisol at the following times: (1) during the screening period (prior to randomization), (2) when patients are symptomatic, and (3) when patients are at most risk for adrenal insufficiency and adrenal crisis, such as following discontinuation or interruptions of dexamethasone dosing (i.e., loading failures and following discontinuation of the study drug).

n) Video recording will not be required at the screening visit. The ICARS will be performed twice at Baseline, 24 hours apart (Days 0 and 1); the rating on Day 1 (pre-dose) will be used as the baseline value for statistical analyses. ICARS video recording will be required for both the Day 0 and Day 1 (baseline) assessments.

o) For women of childbearing potential only. The test results must be negative at screening (serum) and baseline (urine) for the patient to be eligible for the study. Urine pregnancy test will be performed before every infusion.

p) A Safety Follow-up assessment will be performed 30 days after the final evaluation or at least 60 days after the final infusion, whichever is longer; at this visit, the occurrence of any AEs or Serious AEs since the final evaluation will be reported.

q) If the genetic test for AT has not been performed previously, the test will be done by the central laboratory. For patients who require genetic testing to confirm the diagnosis, test results do not need to be available before baseline.

r) Patients originally randomized to placebo will be re-randomized to receive one of the two doses of EDS-EP, with one third of patients being switched at 6 months and another third at 9 months.

s) Hemolysis panel: free plasma hemoglobin, haptoglobin, bilirubin (total and conjugated), CBC (Day 2 and 15 only), LDH, and urinalysis (Day 2 and 15 only). On Day 1, M3, M6 (b) and M9, only free plasma hemoglobin will be measured, 1 hr post-infusion.

t) RBC osmotic resistance to be measured on a blood sample taken at screening and on a sample from the EryKit_01 bowl at the end of the process in selected centers only.

u) Upon completion of the EDS process, the remaining sample in the satellite sample bag or, if this is not available, a sample collected from another EDS-EP sampling point, will be used for determination of DSP content and CBC.

v) Patients meeting all selection criteria at Baseline will be randomized (1:1:1) to one of the three treatment groups.

w) Blood sample for serum creatinine measurement to be taken before infusion.

x) Vital signs to include height and weight measurements in triplicate at screening, baseline and each monthly visit (pre-dose).

y) Month 12 (Visit 15; Endpoint) evaluations should be performed for all patients that complete the Extension Treatment Period, as well as patients that discontinue prematurely.

z) The monthly infusions should be performed every 21-28 days. A window of ± 7 days will be permitted on the Day 15 visit and of + 10 days on each of the scheduled monthly post-baseline visits (Months 1 - 12). However, no EDS-EP infusion should be performed less than 21 days after the previous infusion. The window between an infusion and the subsequent one should be kept as regular as possible throughout the study, avoiding fluctuations in administration windows. The date of an infusion is not bound to the date of the initial treatment but to the date of the previous IMP administration. .

aa) 1 mL blood collected, after blood diversion, for aerobic culture, before EDS process (see "Revised Study Procedures on Sterility Testing for Study IEDAT-02-2015 (ATTeST)"; Appendix 13). Moreover, a sample of the EDS-EP (approximately 1 ml per inoculum for a total of 2 mL) will be collected from the satellite sample bag to perform a culture-based sterility test. A 1-mL sterile sample of the EDS-EP will be stored under refrigeration as a "Retention Sample".

(£) In case a patient is willing to be enrolled in the open-label study IEDAT-03-2018, but the study has not yet received IRB/EC approval at the time the patient reaches Visit 15/Month 12, the patient may undergo unscheduled monthly assessments/infusions (according to the assigned blinded treatment) starting from the time Visit 15/Month 12 should be performed, and continuing until the site has been initiated for the open-label study IEDAT-03-2018 and he/she has been evaluated for eligibility for the study. An additional Informed Consent Form must be signed prior to the 1st unscheduled visit/infusion.

(§) As a results of the COVID-19 pandemic, temporary additional safety assessments may be requested at some visits. These changes are described in Appendix 14.

2 SIGNATURE PAGE

Study Title:	A Multi-center, Randomized, Double-blind, Placebo-controlled Trial to Evaluate the Effects of ***I***ntra-***E***rythrocyte ***D***examethasone Sodium Phosphate on Neurological Symptoms in Patients with ***A***taxia ***T***elangiectasia
Study Code:	IEDAT-02-2015
Protocol Version/Date:	Version 1.0 – 09 May 2015 Version 2.1 – 16 November 2015 Version 3.0 (including Amendment 1) - 01 February 2016 Version 3.1 (including Amendment 2) – 28 April 2016 Version 3.2 (including Amendment 3) – 25 May 2016 Version 4.0 (including Amendment 4) – 29 November 2016 Version 5.0 (including Amendment 5, Site-specific) – 20 September 2017 Version 6.0 (including Amendment 6) – Final, 29 September 2017 Version 7.1 (including Amendment 7.1) – Final, 13 March 2018 Version 8.0 (including Amendment 8.0) – Final, 23 April 2018 Version 10.0 (including Amendment 10.0) – Final, 16 April 2019 Version 11.0 (including Amendment 11) – Final, 24 June 2020
Number of Centers:	20-25

Protocol Author

PPD

PPD

Sponsor Representative

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Chief Medical Officer
EryDel S.p.A
e-mail: Guenter.Janhofer@erydel.com

I have read this protocol and I approve the design of the trial.

PPD

PPD

Signature Pages (cont'd)

Investigator

Center No. _____

I agree to conduct the clinical trial in accordance with this Protocol and in compliance with Good Clinical Practice and all applicable regulatory requirements.

_____ _____
Investigator's Name (Printed) *Title*

_____ _____
Signature *Date of Signature (dd / mm / yyyy)*

The Sponsor has transferred all responsibilities for the conduct of the trial to the CRO or other designated vendors for specific services.

3 TABLE OF CONTENTS

4 ABBREVIATIONS AND DEFINITIONS OF TERMS

ACTH	Adrenocorticotropic hormone
AEs	Adverse Events
ALT (SGPT)	Alanine-aminotransferase
ANOVA	Analysis of Variance
ANCOVA	Analysis of Covariance
AST (SGOT)	Aspartic-aminotransferase
AT	Ataxia Telangiectasia
ATM	Ataxia Telangiectasia Mutated
A-T NEST	Ataxia-Telangiectasia Neurological Examination Scale Toolkit
ATP	Adenosine triphosphate
AUC	Area under the plasma drug concentration vs. time curve
BARS	Brief Ataxia Rating Scale
BMD	Bone mineral density
BMI	Body Mass Index
BP	Blood Pressure
bpm	Beats per minute
BUN	Blood Urea Nitrogen
CBC	Complete blood count
CD4+ lymphocytes	Cluster differential 4 positive lymphocytes
CGI-S / CGI-C	Clinical Global Impression of Severity / Change
CF	Cystic fibrosis
C_{max}	Maximum plasma drug concentration after dosing
CNS	Central Nervous System
COPD	Chronic obstructive pulmonary disease
CPK	Creatine phosphokinase
^{51}Cr	Chromium-51
CRA	Clinical Research Associate
CRF	Case Report Form
CRO	Contract Research Organization
CRP	C-Reactive Protein
C-SSRS	Columbia-Suicide Severity Rating Scale
CSR	Clinical Study Report
CV	Coefficient of variation
CYP3A4	Cytochrome P450 3A4
D	Day
2,3-DPG	2,3-Diphosphoglyceric acid
DSP	Dexamethasone sodium phosphate
ECG	Electrocardiogram

EDS	EryDex System
EDS-EP	EryDex System end product
EIA	Enzyme immunoassay
EP	Evaluable Population
EQ-5D-3L	EuroQol 5D Three-level version
EQ-5D-5L	EuroQol 5D Five-level version
ESI-MS	Electrospray mass spectrometry
FAS	Full Analysis Set
FSI	First Subject In
FWER	Family-wise error rate
G6PD	Glucose-6-phosphate dehydrogenase
GCP	Good Clinical Practice
GMP	Good Manufacturing Practice
SGOT (AST)	Serum Glutamic-Oxaloacetic Transaminase
SGPT (ALT)	Serum Glutamic-Pyruvic Transaminase
HbA1c	Blood glycosylated haemoglobin
Hct	Hematocrit
HDL	High density lipoprotein
Hb	Hemoglobin
HbA1c	Blood glycosylated hemoglobin
HIV	Human Immunodeficiency Virus
HPLC	High Performance Liquid Chromatography
hr	Hour
IBD	Inflammatory bowel disease
ICARS	International Cooperative Ataxia Rating Scale
ICF	Informed Consent Form
ICH	International Conference on Harmonization
ICSH	International Committee for Standardization in Hematology
IEC	Independent Ethics Committee
IMP	Investigation Medicinal Product
IRB	Institutional Review Board
ISF	Investigator Site File
ITT	Intention-to-Treat
IVRS	Interactive Voice Response System
IWRS	Interactive Web Response System
IU	International Unit
LDH	Lactate dehydrogenase
LDL	Low density lipoprotein
LOCF	Last observation carried forward
LSI	Last Subject In
LSLV	Last Subject Last Visit
M	Month
MAO	Monoamine oxidase
MAR	Missing at random
MCH	Mean corpuscular hemoglobin

MCHC	Mean corpuscular hemoglobin concentration
MCV	Mean corpuscular volume
µCi	Microcuries
mCi	Millicuries
min	Minute
mmHg	Millimeters of mercury (blood pressure)
MMRM	Mixed Model Repeated Measures
mICARS	Modified version of the International Cooperative Ataxia Rating Scale excluding oculomotor items (17-19) and items 8-12
NSAID	Non-steroidal anti-inflammatory drug
OC	Observed cases
OTC	Over-the-counter
pBAR	Probabilistic baseline randomization
PK	Pharmacokinetic(s)
RBCs	Red Blood Cells
RCL	Red Cell Loader
RDO	Retrieved Dropout
RDW	Red blood cell distribution width
RMANOVA	Repeated Measures Analysis of Variance
RT	Room Temperature
SAEs	Serious Adverse Events
SARA	Scale for Assessment of Rating of Ataxia
SD	Standard Deviation
SNRI	Serotonin-norepinephrine reuptake inhibitor
SOPs	Standard Operating Procedures
SP	Safety Population
SSRI	Selective serotonin reuptake inhibitor
$t_{1/2}$	Half-life
T50	Time to disappearance of 50% of the labelled red blood cells from the circulation
^{99m}Tc	Technetium-99m
TCA	Tricyclic antidepressant
TEAEs	Treatment Emergent Adverse Events
t_{max}	Time to maximum plasma concentration after dosing
ULN	Upper limit of normal
WBC	White Blood Cells

5 TITLE OF STUDY

A Multi-center, Randomized, Double-blind, Placebo-controlled Trial to Evaluate the Effects of ***I***ntra-***E***rythrocyte ***D***examethasone Sodium Phosphate on Neurological Symptoms in Patients with ***A***taxia ***T***elangiectasia

6 PROTOCOL NUMBER

This study is being conducted under Protocol No. IEDAT-02-2015.

7 BACKGROUND AND STUDY RATIONALE

7.1 Background Information

7.1.1 Overview of the CCI *System (EDS)*

CCI [redacted]

7.1.2 Non-Clinical Trials with EDS

Pharmacokinetics is the only characteristic of the drug product (dexamethasone sodium phosphate; DSP) pharmacology that differs from that obtained with the ordinary routes of administration (oral, intravenous, intramuscular, etc.) due to the novel method of administration by *ex vivo* encapsulation of the drug into human autologous red blood cells which are then infused. The pharmacokinetics of the drug product in humans was evaluated in clinical trials. It is not possible to study the pharmacokinetics in animal models

because the dephosphorylation rate of the pro-drug, DSP, to the diffusible active drug, dexamethasone, in human RBCs is very different from RBC dephosphorylation rates in other species (Zocchi et al, 1991).

7.1.3 Summary of Human Clinical Trials with EDS

A total of 209 subjects have received at least one dose of EDS-EP. Of these, 115 participated in Investigator-initiated and 89 in Sponsor-initiated studies. A total of 1827 EDS-EP infusions have been administered; 366 of these infusions were performed in the 89 patients enrolled in Sponsor-initiated studies. Twenty-two patients with AT were administered 344 EDS-EP infusions in a phase II study (IEDAT-01-2010) and in subsequent compassionate use.

Table 1 below lists the trials performed to date, the indication, the number of subjects, the doses administered, the treatment duration, and key safety findings.

Table 1. Summary of Clinical Studies Conducted to Date Using EDS-EP

EryDel Study #	Indication	# of unique subjects	Range of doses (mg DSP)	Infusions		Key safety findings	References
				#	Frequency		
Investigator-initiated Studies							
01	Chronic obstructive pulmonary disease (COPD)	10	0.78-8.78	2	Q 15 days	None reported in publication	Rossi et al. 2001
02	Cystic Fibrosis	17	1.2-14.5	3-24	Q 21 days or monthly	None reported in publication	Rossi et al. 2004
02 Extension	Cystic Fibrosis	17	1.2-14.5	24	Q 21 days or monthly	None reported in publication	*Extension study:* Lucidi et al. 2006
03	IBD (Ulcerative Colitis & Crohn's Disease)	10	1.1-10.9	3	Monthly	Fever (n=1 [10%])	Annese et al. 2005
04	Crohn's Disease	1	1.0-21.8	36	Monthly	None reported in publication	Castro et al. 2006
05	Crohn's Disease	18	2.4-14.7	24	Monthly	Dropouts: n=2 (11%; non–compliance with protocol)	Castro et al. 2007
06	Ulcerative Colitis	20	5.0-24.0	2	Every 14 days	None reported in publication	Bossa et al. 2008
07	Cystic Fibrosis	5	1.0-21.8	34	monthly	None reported	Data on file: EryDel SpA
08	COPD	15	1.0-21.8	36	every 28 ± 5 days	None reported	Data on file: EryDel SpA
09	Ulcerative colitis	19	9.9±4.1*	6	monthly	None reported in publication	Bossa et al. 2013

EryDel Study #	Indication	# of unique subjects	Range of doses (mg DSP)	Infusions		Key safety findings	References
				#	Frequency		
Sponsor-initiated Studies							
10	IBD (Ulcerative Colitis & Crohn's Disease)	Total: 33 EryDex: 21 Placebo: 12	1.0-21.8	3 **or** 6	15 days, 30 days	Premature terminations: - Total: 10/33 (30.3%) - EryDex: 6/21 (28.6%) - Placebo: 4/12 (33.3%) *Adverse Dropouts:* None *Lack of efficacy*: 6 (EryDex – 4 [19.0%]; Placebo – 2 [16.7%])' *Lost to Follow-up*: EryDex – 3 (14.3%); Placebo - 0 *Poor compliance*: EryDex – 1 (4.8%); Placebo - 0 SAE: Placebo – 1 (8.3%) – acute pancreatitis (hospitalization) Any AE: EryDex - 11 (53.4%); Placebo - 8 (66.7%) Most frequent AEs: GI Disorders (EryDex – 28.6%; Placebo- 50%); Metabolism & Nutrition Disorders (EryDex –23.8%; Placebo - 0%)	Cro.Co.Dex Clinical Study Report (Data on file: EryDel SpA)
11	Crohn's Disease	EryDex: 28; Placebo: 23	1.0-21.8; 5±2.8*	12	Monthly	Premature terminations: - Total: 44/51 (86.3%) - EryDex: 23/28 (82.1%) - Placebo: 21/23 (91.3%) *Adverse Dropouts* –3 Intestinal occlusion – 2 (EryDex), abdominal hernia complications - 1 (Placebo) *Lack of efficacy* – 22 (EryDex – 13 [56.5%]; Placebo – 16 [76.2%]) *Premature termination of trial by Sponsor* – 7 *Protocol violation* – 1 *Administrative withdrawal* - 2 Death: Placebo - 1(Traffic accident after end of study). SAEs: 10 - EryDex – 6: Crohn's relapse(2), GI haemorrhage, intestinal obstruction, sub-ileus, spinal fracture; - Placebo – 4: sub-ileus(3), septicaemia staph	CRODEX-01 Clinical Study Report (Data on file: EryDel SpA)

EryDel Study #	Indication	# of unique subjects	Range of doses (mg DSP)	Infusions		Key safety findings	References
				#	Frequency		
12 (IEDAT-ERY01-2010)	Ataxia-Telangiec-tasia	EryDex: 22	0.7 – 18.6	6	Monthly	Premature terminations: 4 (18%) - Withdrawal of consent (1); - AE (lab abnormality - 2); - Protocol violation (1) SAEs: 2 (9%); pneumonia and bronchiectasis; both considered not related to study drug; Most frequent AEs (>10%): Otitis (3), Cough (3), Fever (3), Flu syndrome (3)	IEDAT-ERY01-2010 Clinical Study Report (Data on file: EryDel SpA)
13 (IED-PK01-2013)	Healthy volunteers	18 (9/group)	Group 1: 3.28-5.73; 4.2±0.27** Group 2: 14.44-21.28; 16.9±0.9**	1	once	Premature terminations: 2(11.1%) - Withdrawal of consent (1); - Lost to follow-up (1). SAEs: none. AEs requiring treatment: abdominal discomfort (1), GI pain (1), gingival pain (1).	IED-PK01-2013 Clinical Study Report (Data on file: EryDel SpA)
14 (Ery51 Cr-01-2014)	Healthy volunteers	10 (5/group)	Group 1: 12.0-16.2; 14.6±2.0** Group 2: Sham	1	once	Premature terminations: none SAEs: none Most frequent AEs: - Headache (Group 1: 2 of 5; Group 2: 3 of 5) Other: No clinically meaningful changes in routine laboratory tests, hemolysis panel, ECG, vital signs or physical exam	Ery51Cr-01-2014 Clinical Study Report (Data on file: EryDel SpA)

Abbreviations: COPD, chronic obstructive pulmonary disease; IBD, inflammatory bowel disease; *mean ± standard deviation; **mean ± standard error of the mean

A total of 1827 EDS-EP infusions of EDS-EP have been administered to the subjects in these studies, and no toxicity related to RBCs processed for dexamethasone dosing has been observed. These include 51 pediatric patients (< 18 years) suffering from cystic fibrosis (n=11), Crohn's disease (n=19) or AT (n=22, one patient was 19 years of age at inclusion); some of these patients have been exposed to EDS for up 36 months. There was no consistent pattern of dexamethasone-related adverse findings reported in these pediatric populations.

Additional detailed information on pre-clinical and clinical studies evaluating the EDS can be found in the current version of the Investigator's Brochure.

7.1.3.1 Clinical Study IEDAT-ERY01-2010: Pilot Proof of Concept Phase II Trial in Patients with Ataxia Telangiectasia [Chessa et al, 2014]

Title: Evaluation of Effects of **I**ntra-**E**rythrocyte **D**examethasone Sodium Phosphate on Neurological Symptoms in **A**taxia-**T**elangiectasia Patients

Study Design and Patient Population

This was a single-arm, open-label, 6-month, Phase II study to assess the effect of the EryDex System end product (EDS-EP; dexamethasone sodium phosphate [DSP] encapsulated in autologous erythrocytes by the EDS process) on neurological symptoms of patients with Ataxia Telangiectasia (AT) enrolled in two centers in Italy. Males and females, > 3 years of age, with neurological signs of AT (un-coordination of the head

and eyes in lateral gaze deflection, gait ataxia associated with an inappropriately narrow base), an autonomous gait (or helped by support), and a genetic diagnosis of AT (at least one ATM mutation) and/or ATM protein deficiency by Western blot, were included. Patients with low CD4+ lymphocytes counts, current/previous neoplastic disease, history of severe impairment of the immunological system, chronic conditions representing a contraindication to the use of steroid drugs, or any other significant disease that could have put the patient at risk were excluded. Females of childbearing potential that were pregnant, breastfeeding or not using adequate contraception were also excluded. Eligible patients could not have participated in a trial with another investigational agent within 30 days of the start of the Screening Period, and must have discontinued prior steroid medication at least 30 days before the first dose of EDS-EP.

Objectives

The primary objective of the trial was to evaluate the effect of EDS-EP in improving neurological symptoms of AT patients over a 6-month treatment period, assessed by means of the ICARS (Trouillas et al., 1997). The ICARS is a 100-point semi-quantitative scale offering a compartmentalized quantification of the following 4 sub-scores: Posture and gait disturbances, kinetic functions, speech disorders, and oculomotor disorders. The ICARS rating was performed by a trained neurologist at baseline (before the first dose) and at 1, 3 and 6 months after the first EDS-EP administration; analysis of the change from baseline at each visit was performed on the ITT population using Repeated Measures Analysis of Variance (RMANOVA). Secondary objectives of the trial were to evaluate the safety profile of EDS-EP through the assessment of TEAEs, including steroid-dependent adverse reactions, SAEs, standard laboratory parameters, physical examination, vital signs, ECGs and concomitant medications and procedures. Additionally, the effects of EDS-EP were assessed on the patients' global health status (IGA), ocular motility, and adaptive behaviour (VABS). The effect of EDS-EP on special laboratory parameters, i.e. total cholesterol, HDL, LDL, blood glycosylated hemoglobin (HbA1c), $CD4^+$ lymphocytes counts, α-fetoprotein, urinary cortisol and plasma cortisol, was also measured.

Methods

Patients providing informed consent (along with consent of their parents or legal guardian, as required) entered a 30-day screening period, during which any prior corticosteroid treatment was withdrawn. Medical history and demographic information was collected, and physical and neurological examinations, vital signs, ECG and standard laboratory tests were performed. Patients meeting all selection criteria were enrolled in the treatment period and had the physical examination, vital signs and laboratory tests repeated and all efficacy evaluations performed at baseline (Day 0) prior to receiving the first of 6 monthly infusions of EDS-EP. The amount of DSP added to the 50 mL of the subject's blood in the encapsulation procedure was kept constant for all patients, i.e. 500 mg/20 mL DSP solution, and was expected to deliver approximately 10-15 mg of DSP by using the previous EDS version 3.1.2 over the one-month period between doses.

A physical examination, vital signs and routine laboratory tests were performed at each monthly visit. The primary efficacy measure (ICARS) was assessed at one month, and at the 3-month visit, all efficacy assessments were done, along with the special laboratory tests (before drug infusion). A final evaluation was performed at 6 months after the initial infusion (one month after the last dose), during which all efficacy and safety assessments were performed. The occurrence of any AEs and the use of concomitant medication were recorded from the time of signing of the consent form until the end of the study. Blood samples for the PK analysis were collected from selected patients prior to dosing (0 hr), and at 4 hours and 15 and 30 days post-dose for the first and fourth infusions.

Disposition

A total of 26 patients with AT were screened and 22 were enrolled in the study, representing the ITT population. Four subjects discontinued prematurely; one withdrew consent, another patient was dropped due to a protocol violation (CD+4 lymphocytes below cut-off at baseline), and 2 patients experienced laboratory abnormalities (decrease in CD4+ lymphocytes count) that led to their withdrawal.

Demographics and Disease Characteristics

The mean (SD) age of the AT patients was 11.2 (3.5) years, with 11 (50%) patients being males. All patients were Caucasian with a mean weight of 29.1 (9.5) kg and BMI of 15.6 (2.6). The average age of diagnosis of AT was 60.0 (35.2) months, with a mean age of symptom onset of 25.9 (17.5) months. The mean baseline scores for the ICARS and VABS were 50.6 (12.8) and 5.5 (2.0), respectively. Mean baseline scores on the sub-scales of the ICARS were as follows: posture/gait disturbance – 20.9 (7.1); kinetic function – 23.1 (6.2); speech disorders – 3.5 (1.4); and oculomotor disorders – 3.2 (1.0). Two patients were on inhalational steroids at screening, and these medications were discontinued.

Efficacy Results

Sixteen patients (72.7%) received all 6 infusions of EDS-EP, as planned. Results for the primary efficacy measure (change from total baseline ICARS score) for the ITT population (n=22) indicated an overall statistically significant (p=0.02, RMANOVA) improvement with EDS-EP treatment. Significant improvement was observed after 3 and 6 months of treatment with EDS-EP for the kinetic sub-score of the ICARS, the clinician rated the Investigator Global Assessment (IGA), adaptive behavior (as assessed by the VABS) and ocular motility.

Secondary analyses. The mean DSP dose/infusion was 7.5±1.8 mg. There was however, substantial variability in the patient-specific DSP encapsulation in erythrocytes, with mean doses of DSP measured in the EDS-EP for each subject ranging from 0.7±0.1 to 18.6±1.9 mg (patients received 6 doses). Additional analyses were performed to determine if there was a relationship between DSP dose and efficacy response. In these analyses, patients were categorized as "loaders" if their mean DSP dose was 5 mg or higher. Loaders (64%) as compared to non-loaders (27%) showed a clinically significant improvement on the ICARS (defined as > 10% improvement versus baseline) at 6 months. Loaders had a mean percent decrease (improvement relative to baseline) of -14.4% for the ICARS total score, compared to -4.7% for non-loaders.

Safety Results

The safety population included all 22 patients. Overall, 15 (68.2%) of patients experienced at total of 29 TEAEs, most of which were rated as mild (~60% of subjects) and not related to the study medication (> 90% of patients), with the exception of 1 patient with a mild hypercholesterolemia. Two SAEs were reported during the study, including one case of severe pneumonia and a second patient with bronchopneumonia, and bronchiectasis and bleeding; both patients required hospitalisation. Both cases were considered unrelated to study medication and resolved with treatment.

Two patients had a > 20% decrease in CD4+ lymphocytes count during the study period which resulted in premature discontinuation, and were considered related to study medication.

There were no clinically meaningful changes in mean values for routine laboratory parameters with EDS-EP treatment, except for serum iron, which showed a > 20% mean decrease from baseline value, with 8 patients having newly occurring abnormal (low) values at the final visit (one reported as a TEAE). For the special laboratory parameters, no clinically meaningful changes were observed, except for urinary cortisol, which showed an approximately 30% decrease from baseline to the final visit (statistically significant,

p=0.016). Although this could indicate an effect of EDS-EP on the hypothalamo-pituitary-adrenal axis, a comparison of blood and urinary cortisol levels versus dose did not indicate any relationship. No clinically significant changes with EDS-EP treatment were observed for vital signs, ECGs, or physical examination findings.

Conclusions

In this open-label study in AT patients > 3 years of age, EDS-EP treatment for 6 months led to a statistically significant improvement in the primary efficacy measure, the ICARS, which assesses key symptoms of the disease. Additional statistically significant benefits of EDS treatment were noted in the Kinetic Function sub-scale of the ICARS, IGA, adaptive behavior (VABS), and ocular motility (ad hoc scale). EDS-EP was generally well tolerated in this subject population, with only two patients discontinuing prematurely due to adverse events. Two patients experienced serious adverse events related to pulmonary infections; most AEs were mild in intensity and considered unrelated to study medication.

Laboratory findings indicated a low serum iron levels in approximately one third of patients and a mean reduction in urinary cortisol, which did not appear to be related to the dose administered.

The level of DSP loading indicated variability in RBC encapsulation across patients. Almost half of the patients had mean DSP dose of less than 5 mg, whereas a DSP dose between 10-15 mg was targeted. The variability was caused by sub-optimal encapsulation conditions associated with the use of EDS process version 3.1.2 used in this study. Future studies will use the EDS process version 3.2.0 that has demonstrated increased loading and reduced variability in encapsulation.

Pilot observation that higher DSP load appears to be associated with improved response of the ICARS has led the Sponsor to target higher DSP doses in future efficacy studies.

7.1.3.2 Clinical Study IED-PK01-2013

Title: Pharmacokinetic Study to Measure Plasma Concentrations of Dexamethasone following EryDex (Dexamethasone Sodium Phosphate Encapsulated in Autologous Erythrocytes) Infusion in Healthy Volunteers

Study Design, Subject Population and Methodology

This Phase 1, open-label, single-center (US), uncontrolled study was performed to characterize the pharmacokinetic (PK) properties of two planned doses (Group 1: ~ 2.5 to 5 mg; Group 2: ~ 15 to 20 mg) of EDS end product (DSP encapsulated in erythrocytes), when administered as a single infusion to 18 (9 in each group) healthy male and non-fecund female volunteers, using a new version of the EDS process (version 3.2.0) to load the subject's erythrocytes with DSP. The study also evaluated the safety and tolerability of the 2 different dose ranges based on treatment-emergent adverse events (TEAEs), serious AEs (SAEs), laboratory parameters, vital signs, ECGs and physical/ neurological examination findings, with the safety of the lower dose (Group 1) being assessed before proceeding to the higher dose (Group 2).

Subjects were followed for 42 days after EDS end product infusion, with blood samples being taken just prior to dosing (0 min) and at 15 and 30 minutes, at 1, 2, 4, 8, 12 and 24 hours, and at 3, 7, 14, 21, 28, 35 and 42 days post-infusion for measurement of plasma dexamethasone, using a validated HPLC-ESI-MS method, and determination of PK parameters. A follow-up evaluation was performed 84 days post-infusion.

Disposition, Demographics and Doses Administered

Forty-five subjects were screened, and 18 eligible subjects were enrolled (9/group) and received EDS end product. Six subjects in each group were male, and other demographic characteristics were similar between

groups. Of the 18 subjects treated with study drug, 2 (11.1%) subjects discontinued from the study prior to study completion: one withdrew consent (Group 1) and the other was lost to follow-up (Group 2).

The mean (±SEM) actual DSP doses were 4.2±0.27 (range: 3.28–5.73) and 16.9±0.90 (range: 14.44–21.28) mg DSP for Groups 1 and 2, respectively. In Group 1 the actual DSP dose administered was within the target range of 2.5 to 5 mg in 7 of 9 subjects (2 subjects had loaded doses above 5 mg), while in Group 2, the target range of 15 to 20 mg was achieved by 4 of the 9 subjects (3 subjects had loaded doses below 15 mg, and 2 had doses above 20 mg).

Pharmacokinetic Results

One subject in Group 2 missed more than 20% of the PK blood samples; therefore, 9 subjects in Group 1 and 8 subjects in Group 2 were included in the Per Protocol dataset used for the PK analyses. Most of the dexamethasone was rapidly released from the RBCs with a maximum peak occurring approximately 1 hr after the end of the IV infusion, independently of the dose administered. The C_{max} for dexamethasone was approximately 26 ng/mL and 135 ng/mL for Group 1 and Group 2 EDS end product, respectively. Dexamethasone release from the erythrocytes could be detected until 14 and 35 days after the single IV infusion of EDS end product in Groups 1 and 2, respectively.

A dose-relationship was observed for dexamethasone elimination, with a longer terminal elimination half-life for Group 2 ($T_{½el}$ ~ 4.5 day) when compared to Group 1 ($T_{½el}$ ~0.85 day). This difference in the aforementioned terminal half-life estimates could be due to a difference in detectable dexamethasone levels in Group 2 and 1, respectively. The C_{max} and AUC of dexamethasone measured for the actual dosing range of 3.3 mg to 21.3 mg DSP encapsulated in RBCs were in proportion marginally higher than the levels expected based on the dose.

Safety Results

Adverse events. There were no SAEs, or discontinuations due to AEs in either group. Five of 9 (55.6%) subjects in Group 1 reported a total of 5 TEAEs, all of mild intensity. In Group 2, 5 subjects (55.6%) reported a total of 6 TEAEs, 4 of mild and 3 of moderate intensity. Two subjects had TEAEs that required drug treatment, one with abdominal discomfort (treated with an antacid) and the other with gastrointestinal pain (treated with anti-gas agent) and gingival pain (treated with analgesics). All of the TEAEs resolved without any sequelae. No Group 1 subjects received a concomitant medication during the study and only two Group 2 subjects received one or more concomitant medications for their TEAEs (abdominal discomfort, gingival pain, and gastrointestinal pain).

Standard laboratory tests. A number of small but statistically significant transient changes in hematological (absolute eosinophils, hematocrit, hemoglobin, lymphocytes, monocytes, RBC count, MCH, neutrophils, platelet, and WBC count) and biochemical (AST, creatine phosphokinase, CO_2, creatinine, glucose, cholesterol, potassium, sodium, LDH, HDL and BUN) parameters associated with DSP administration were observed. Sporadic abnormal values (high/low) were noted for some of the laboratory hematology and biochemistry parameters at Day 2 and/or the final visit (Day 42); however, none of these were considered to be clinically significant. No meaningful changes from baseline were observed in any urinalysis parameter throughout the study. Both increases and decreases compared to baseline were observed for laboratory analytes in Groups 1 and 2, without any consistent pattern of change.

Other laboratory tests. Serum cortisol levels were significantly reduced up to the 48-hour time point in Group 1, while in Group 2 a significant decrease in serum cortisol levels was observed through the 7-day time point and at Day 42, but not at Day 14. Urinary cortisol showed no significant reduction at any time point.

All 9 patients in each of the treatment groups had normal RBC osmotic resistance values at NaCl concentrations from 0.55% up to 0.85% on Day 1 before the EDS process. The majority of the patients had normal RBC osmotic resistance values at NaCl concentrations between 0.30% and 0.50%; however, 4 patients (2 in Group I and 2 in Group II) showed a pattern of decreased lysis while one subject had a pattern of borderline increase. Results for RBC osmotic resistance for the sample taken from the infusion bag, following the EDS process, confirmed a pattern of decreased lysis at the 0.40%-0.50% concentration range in 3 (1 in Group I and 2 in Group II) out of 4 patients that had this pattern before the procedure. All other patients exhibited RBC osmotic fragility within the normal range.

Free hemoglobin values were above normal (high) on Day 1 (prior to infusion) in 4 of 9 patients in Group 1 and in 6 of 9 patients in Group 2. The mean value for free hemoglobin before infusion in the 9 patients in Group 1 was 6.79 mg/dL (median 5.30 mg/dL) with a maximum subject value of 12.60 mg/dL, and the mean value for the 9 patients in Group 2 was 23.62 mg/dL (median 16.20 mg/dL) with a maximum subject value of 76.00 mg/dL. Free hemoglobin was detected sporadically also after infusion during this trial.

Analysis of plasma samples (taken for PK determination) indicated the presence of free hemoglobin in samples at baseline prior to the EDS end product infusion. The presence of free plasma hemoglobin may have resulted from the blood drawing technique used to collect the samples (e.g., use of IV catheter with a 22-gauge needle). Analysis of urine samples for hemoglobin, bilirubin, urobilinogen, urine clarity and color did not detect any pattern suggestive of hemoglobinuria or associated findings in any subject.

Other safety parameters. Abnormalities in vital signs that were reported as TEAEs included a mild increase in diastolic blood pressure (DBP) on the day of study drug infusion, two reports of mild 'orthostatic tachycardia' on the day of dosing, and mildly rapid heartbeat/palpitations; all resolved without sequelae. Small statistically significant mean changes ($p < 0.05$) were observed for systolic blood pressure, diastolic blood pressure and pulse rate, primarily at early time-points (Days 2 and 3), in both groups, but did not indicate any pattern of adverse change. No statistically significant mean changes from Baseline were observed at any time point for supine respiratory rate, body temperature measures or body weight.

The overall interpretation of the ECG did not indicate any adverse cardiac effects of EDS-EP treatment, as no newly occurring abnormalities were observed post-baseline. In addition, no statistically significant changes in any ECG parameter were noted at Day 42. One physical examination finding was reported (Day 42) as a TEAE (mild severity right eye upper lid inflammation that was not considered to be clinically significant by the Investigator), and one subject had a new abnormality in reflexes reported on the neurological examination (Day 42).

7.1.3.3 Clinical Study Ery51Cr-01-2014

Title: Determination of the *In Vivo* Recovery and Survival of EryDex (Dexamethasone Sodium Phosphate Encapsulated in Autologous Erythrocytes) in Non-patient Volunteer Subjects

Study Design and Methodology

This was a randomized, single-blind, single-center, concurrently controlled, exploratory, Phase I study to determine the *in vivo* kinetics (24-hour post-infusion recovery and T50 survival) of EDS-processed autologous RBCs in non-patient volunteers. RBCs were radiolabeled with Chromium-51 (^{51}Cr) for the *in vivo* kinetic study. In addition, the *in vitro* characteristics of the RBCs processed using the EDS were determined using a standard panel of RBC assays [pH at 37°C, hemolysis, ATP, 2,3-DPG, extracellular potassium, extracellular glucose, extracellular lactate, packed cell volume, RBC morphology, complete blood count (CBC)] and a hemolysis panel was assessed immediately prior to the infusion, and 1 h and 24 h after completion of infusion. Safety assessments included monitoring for adverse events (AEs), routine

laboratory tests (hematology, biochemistry, urinalysis), vital signs, physical examinations, ECG evaluations, serum pregnancy testing for fecund women, and documentation of concomitant medications.

Eligible subjects who provided informed consent underwent a screening period of up to 30 days prior to receiving a single IV infusion of EDS followed by a 49-day follow-up period. A series of blood samples were collected over the first 24 hours from each subject for the measurement of 24-hour recovery of radio-labelled RBCs; additional samples were taken at 48, and 72 hours and 7 days post-infusion, and weekly thereafter through 49 days post-infusion.

A total of 10 non-patient volunteer subjects meeting all selection criteria were enrolled and randomly assigned (1:1) to one of the 2 treatment groups (n=5 each):

- **Group 1 - Active Drug Arm**: 5 mL of dexamethasone sodium phosphate (DSP) solution (25 mg/mL) mixed with 11 mL of water for injection is added to the concentrated RBCs.
- **Group 2 - Sham Arm**: 5 mL of Hypotonic Solution 2 (sham of the 5 mL of Drug) plus 11 mL of Water for injection is added to the concentrated RBCs.

All subjects received the study treatment and completed the 49-day follow-up period.

Results

The study has been completed in the field, and the final results are summarized below.

RBC Recovery and Survival

The results for the mean RBC recovery for Group 1 (77.9%) indicate that DSP-loaded EDS-processed cells met the FDA criteria for 24-hour RBC recovery ≥ 75% (Dumont and AuBuchon, 2008). The mean life span (MLS) of EDS-processed RBCs in Group 1 was 84.3 days with a mean T50 (α/2) of 42.1 days, while these values were 88.9 days and 44.4 days, respectively, in Group 2. These data suggest that there is no adverse impact on the survival of EDS-processed cells based on a comparison with published results for RBCs used for transfusion.

In vitro characteristics of EDS-processed RBCs

Results suggest that RBCs maintain normal ATP-dependent metabolic and biochemical functions after completion of the EDS process. The alterations induced by the EDS process in the characteristics of the RBCs, e.g. MCV, total hemoglobin, hematocrit, MCHC, MCH, platelets and WBC count, do not impact their recovery and survival in the circulation. The quantity of EDS-processed RBCs (roughly 10 mL) that are infused with the EDS-EP constitutes less than 1% of the total RBCs in circulation; therefore, changes in these cells do not have an impact on the vital functions of the blood. The only purpose of this small fraction of EDS-processed RBCs is to transport DSP (and not to carry oxygen, as is the function of RBCs for transfusion) and release it into the circulation over 30 days.

Summary of Safety Data

No consistent pattern of changes of a magnitude that led to values that were abnormal, or were deemed clinically notable, was detected in subjects receiving EDS-processed erythrocytes containing DSP or saline for measures evaluating a pre-specified hemolysis panel of tests, or other routine laboratory hematology, biochemistry, and urinalysis parameters. Similarly, there was no evidence of a systematic effect on vital signs, weight, or ECGs, and no pattern of adverse events was detected with the trial treatments. There were no discontinuations due to AEs, serious adverse events (SAE), or AEs rated severe or unexpected.

7.2 Drug Product and Delivery System

Generic Name

Drug: Dexamethasone sodium phosphate solution (25 mg/ml), referred to as DSP Solution
Process Solutions: Hypertonic/hypotonic sterile solutions
Devices: Blood processing equipment and kit of single use sterile disposable device accessories

Proprietary Name

Drug: Not determined (a proprietary name for dexamethasone sodium phosphate solution, 25 mg/ml has not yet been proposed)
Process Solutions: PIGPA Hypertonic Solution, Hypotonic Solution 1, and Hypotonic Solution 2
Devices: Red Cell Loader and EryKit_01

The CCI System (EDS)

The CCI System (EDS) is used to load dexamethasone sodium phosphate (DSP), a marketed drug, into autologous erythrocytes, creating the EDS end product (EDS-EP), which is infused once per month into the patient.

CCI

CCI [redacted]

7.3 Study Rationale

Study IEDAT-02-2015 is an international, multi-center, one-year, randomized, double-blind, placebo-controlled, Phase III study to assess the effect of two non-overlapping dose ranges of EDS-EP on neurological symptoms of patients with AT. The primary efficacy endpoint will be at the 6-month assessment. Patients completing the initial 6-month treatment period will be eligible to continue treatment in a 6-month, double-blind, placebo-controlled extension performed to evaluate long-term safety and provide preliminary evidence of a long-term effect on symptoms of AT.

Rationale for Using the EDS in Treating AT Patients

AT is considered a *rare disease* by the Office of Rare Diseases of the National Institutes of Health (US), as well as by *Orphanet*, the consortium of European partners. There is no marketed drug approved to treat AT, and there is no treatment available that slows down or stops the progression of AT patients to early debilitating disability and mortality. Symptomatic, preventive, and supportive management of the key disabilities and symptoms of the disease has provided limited benefit. Immune deficiencies are managed by administration of immunoglobulins, and infections are managed by both preventive and symptomatic use of antibiotics. Developmental deficiencies are managed by physical therapies, education, or targeted treatments (Hoche et al, 2012).

Neurological degeneration is the major contributor to the severe outcome of the disease. The progression of the neurologic problems is currently unstoppable. Anti-parkinson and antiepileptic drugs provide limited benefit in the management of extrapyramidal symptoms. The serendipitous discovery of the benefit of short-term betamethasone in a patient with AT (Buoni et al, 2006), and further pilot, uncontrolled, short–term treatment trials in small numbers of patients have suggested that betamethasone at doses of 0.03 mg/kg, but not 0.01 mg/kg, given for a short period has beneficial effects on the neurological impairment of AT patients (Russo et al, 2009; Broccoletti et al, 2008). Treatment discontinuation in these studies was accompanied by the reappearance of symptoms, suggesting that long-term administration may be required to preserve the benefit.

These early findings with administration of betamethasone prompted the search for steroids that could be given at low plasma concentrations for an extended period of time without the associated steroid-like effects. The EDS, consisting of dexamethasone sodium phosphate (DSP) encapsulated in autologous erythrocytes, allows the administration of dexamethasone at low plasma concentrations that are associated with approximately 80-85% occupation of the glucocorticoid receptor (GR), similar to the GR occupancy that occurs with the betamethasone plasma concentrations reported by Broccoletti et al, 2011. The EDS provides the benefit of low and constant plasma delivery of dexamethasone without the associated steroid side effects, despite long-term use in the pediatric population (Rossi et al, 2001; Lucidi et al, 2006; Annese et al, 2005; Bossa et al, 2008; Castro et al, 2007).

The mechanism of action of glucocorticoids in producing the benefits observed in A-T patients was postulated to occur through regulation of expression of corticosteroid-responsive genes, suppression of inflammatory cytokines, or its effect as an antioxidant (Russo L. et al., 2009). Recently, an effect of dexamethasone on ATM (Ataxia Telangiectasia Mutated) gene splicing in an AT lymphoblastoid cell line, leading to production of a new active ATM protein variant, has been demonstrated (Menotta M. et al., 2012). This may explain in part the beneficial effect of treatment with glucocorticoid analogues in AT patients. The results show, for the first time in mammalian cells, a short direct repeat-mediated non-canonical splicing event induced by dexamethasone that leads to the skipping of mutations upstream of nucleotide residue 8450 of *ATM* Coding DNA Sequence (CDS).

A pilot, open-label, 6-month study was performed with EDS-EP in AT patients (Chessa et al, 2014). In this study, statistically significant (p=0.02; RMANOVA) improvement (reduction) in the ICARS score from baseline to endpoint (~4 points; ITT population; n=22) was observed. The decision to initiate the current double-blind, placebo-controlled study in AT patients was based on the results from this pilot study, as well as the other evidence of the benefit of steroids in treating the disease, as described above.

Rationale for the Patient Population

The selection criteria for the proposed confirmatory trial have been based on the cardinal symptoms of ataxia telangiectasia (AT), the limitations posed by the natural history of the disease, the identification of neurological symptoms that may respond to therapy, prior experience gained through a Phase II European study, and feedback from AT experts in Europe (Universities of Rome and Brescia), Israel (Sheba Medical Center, Tel Aviv), and the US (Johns Hopkins University, Baltimore, MD). A brief description of the important features of AT is provided prior to discussing the selection criteria.

Ataxia telangiectasia (AT) is a rare inherited autosomal recessive, multisystem disorder, characterized by the following: progressive neurologic disease, including cerebellar ataxia and oculomotor apraxia; cutaneous and ocular telangiectasia (90%); increased alpha-feto-protein levels (95%); reduced or absent IgA levels (70%) and ATM protein (98%); increased susceptibility to chronic nasal and pulmonary infections (70%); delayed organ maturation; and increased susceptibility to X-rays and malignancies (Hoche et al, 2012). In countries where marriage between cousins is uncommon, such as in the U.S., the incidence of AT is about 1 in 40,000 live births. However, among ethnic groups where cousin-cousin marriages are common, the incidence increases significantly. The carrier frequency is approximately 1 in 100 persons (https://www.ataxia.org/pdf/Ataxia%20Telangiectasia.pdf). The estimated prevalence in Europe is 1 in 100,000 population (Orphanet. 2014). Ataxia telangiectasia is due to mutations in the ATM gene located on human chromosome 11 (11q22.3). These defects in the ATM gene reduce or eliminate the function of the ATM protein that controls cell division and is involved in double-strand DNA repair (Lee and McKinnon, 2000; Boehrs et al, 2007), thus leading to repeated infections and malignancies.

The onset of symptoms becomes evident in early childhood when children demonstrate unsteadiness while walking, or swaying when standing still or sitting. Most children with AT have stable neurologic symptoms for the first 4 to 5 years of life, but begin to show increasing problems in early school years. Oculomotor apraxia, slurred speech, and difficulties in swallowing appear in late pre-school and early school age children. AT patients are unable to control their muscles by the time they are 10 to 12 years of age, and this may lead to problems with fine motor functions (writing, coloring, and using utensils to eat), and slurring of speech (dysarthria) that usually stop progressing after the age of about 12 to 15 years. Most AT patients are restricted to wheelchair use for ambulation around this age. Involuntary movements such as tremors, myoclonic jerks, dystonia, chorea, athetosis, etc., are variable in the age of onset and rate of progression. (Boder, 1985; Perlman et al, 2003; Chun and Gatti, 2004) The immune system becomes progressively weaker, and recurrent respiratory infections and blood cancers are noted in late-stage patients. There is high

variability in life-expectancy; however, most patients die around the age of 25 years, largely due to chronic lung disease or malignancies. (Poupard, 2003; Crawford et al, 2006)

The key selection criteria for the current study in AT patients are based on selecting patients demonstrating the cardinal features of the disease, but devoid of findings that may expose them to high risk of morbidity and/or mortality. The study will include males and females, 6 years of age and older, with neurological signs of AT (in-coordination of the head and eyes in lateral gaze deflection, gait ataxia associated with an inappropriately narrow base) who have a proven molecular diagnosis of AT (at least one ATM mutation) and/or ATM protein deficiency by Western blot. Patients must have an autonomous gait or be helped by a support (i.e. local ICARS score for *Item 1 – Walking Capacities* between 0 and 4, included). Additionally, patients must have a body weight > 15 kg, and CD4+ lymphocytes count of >400/mm3 (for patients 6 years of age) or >150/mm3 (for patients > 6 years). In presence of oral infections, like oral candidiasis, documented at the screening or recurrent as per medical history documentation the limit increases to >200/mm3 (for patients > 6 years). .

The lower age-limit has been increased to 6 years (the completed Phase 2 study used 3 years) to ensure that patients can provide assent. The lower age limit of 6 years was also chosen to ensure the patients have a confirmed diagnosis of AT based on neurological findings and are able to comply with the requirements of the study, e.g. multiple blood samples. The neurological signs listed as the requirement allow a clinical diagnosis of AT. A diagnosis of AT will be confirmed based on genetic tests; however, these test results do not need to be available at baseline prior to randomizing a patient. The principal efficacy measure, the ICARS, assesses neurological signs including gait. Patients who are in wheelchairs cannot be assessed for the effect on gait, a significant component of the treatable symptoms of AT.

Children with AT may have a lower body weight compared to age-matched controls; therefore, the minimum weight requirement was lowered to 15 kg. CD4+ lymphocyte counts have been included to assess the immune status of these patients as the ATM gene compromises their ability to combat infections. CD4+ lymphocyte cut-off limits have been proposed stratified by age as recommended by AT experts. Patients whose counts are consistently below 150/mm3 (for patients > 6 years), or below 200/mm3 (for patients > 6 years and in presence of oral infections, like oral candidiasis, documented at the screening or recurrent as per medical history/adverse events documentation) or below 400/mm3 (for patients 6 years of age) during treatment will have their treatment interrupted.

The exclusion criteria for the study are designed to exclude patients who would be at increased risk for an adverse outcome related to treatment with EDS-EP or their participation in the trial due to their medical history/current status or use of concomitant medication. Dexamethasone has teratogenic potential in rodents; therefore, females of childbearing potential will be included only in case they are practicing adequate contraception as described at section 9.2.2. Pregnant women will be excluded. Dexamethasone is excreted in breast milk; therefore, patients who are breast-feeding are also excluded. Patients with AT are at increased risk of neoplastic and immunological diseases due to the decreased ability of the ATM gene to repair DNA; therefore, patients with these conditions are excluded. Patients who are currently on steroids or have taken them within the last month, are excluded, as their effects may confound the assessment of the efficacy and safety of EDS-EP.

Rationale for the Study Design

Double-blind Design

The double-blind design is being used to minimize the systematic bias in ratings of efficacy that may result from the rater, patient or caregiver knowing the treatment that the patient is receiving. The double-blind design also minimizes bias in assessing the potential relationship of safety findings to the study treatments.

Use of a Placebo Control

The study will evaluate two doses of EDS-EP, compared to placebo, in the treatment of patients with AT. Currently, there is no approved treatment for AT; therefore, no active control has been included in the study. Patients with AT have a progressive disease course with fluctuating symptoms; therefore, the use of a placebo control group is needed to define the course of the illness in 'untreated' patients receiving the usual standard of care. The placebo control will allow testing of the null hypothesis and provide a statistically valid assessment of the effects of EDS-EP treatment.

The initial double-blind, placebo-controlled treatment period will last for 6 months, with patients having a 1 in 3 chance of being randomized to placebo. After the initial 6-month treatment period, one third of the patients in the Placebo group will be randomized (1:1) to receive treatment with one of the two EDS-EP doses (Group 1: ~5-10 mg DSP/infusion; Group 2: ~14-22 mg DSP/infusion). At Month 9, one third of the placebo group will be randomly switched to EDS-EP, as above, while the remaining patients will continue to receive double-blind treatment with placebo for the remainder of the extension period. The randomization of the placebo group in a 1:1:1 fashion to be switched to EDS-EP at 6 or 9 months or remain on placebo throughout the extension treatment period will be done at baseline to avoid bias. This procedure will minimize the number of patients remaining on placebo for one year, while still providing placebo data for up to one year for comparison with EDS-EP group in the evaluation of long-term safety and efficacy. All placebo-treated patients will be eligible to start open-label treatment with EDS-EP at the one-year time-point, provided they have completed the visits at 6, 9 and 12 months (or more), meet entry criteria and provide informed consent/assent for the open-label, extension study (Study IEDAT-03-2018). In case a patient is willing to be enrolled in the open-label study IEDAT-03-2018, but the study has not yet received IRB/EC approval at the time the patient reaches Visit 15/Month 12, the patient may undergo unscheduled monthly assessments/infusions (according to the assigned blinded treatment) starting from the time Visit 15/Month 12 should be performed, and continuing until the site has been initiated for the open-label study IEDAT-03-2018 and he/she has been evaluated for eligibility for the study. This design allows all placebo-treated patients the opportunity to be treated with the active drug, while still maintaining a small number of patients on placebo for up to 12 months to facilitate collection of placebo-controlled data for analysis of long-term efficacy and safety. The optional open-label extension study will allow patients who are doing well to continue treatment with EDS as long as their physician determines they are benefitting from it and not experiencing any adverse effects that would necessitate discontinuation of treatment.

Minimization Procedure to Equalize Randomization by Gender, Age, and Region

Ataxia telangiectasia is a progressive illness, with symptoms worsening over time (Crawford et al, 2000). Therefore, to decrease potential heterogeneity among treatment groups in baseline symptomatology related to differences between groups in the number of younger and older patients, as well as by gender (symptoms of the disease and disease course may differ between males and females) and geographical region, a minimization procedure will be employed to ensure that the proportions of male and female, and younger (6 to <10 years) and older (≥10 years), patients are comparable across the three treatment groups. Every attempt will be made to ensure the same balance is achieved across different regions.

Primary Efficacy Endpoint at 6 Months

The primary efficacy endpoint ('Modified' ICARS total score change from baseline) will be assessed at Visit 9 (Month 6 or at early discontinuation), at the end of the initial double-blind, placebo-controlled treatment period. At this point in the study, the patient would have received 6 monthly infusion of EDS-EP (~5-10 or ~14-22 mg DSP/infusion) or placebo. In a previous open-label pilot study in AT patients, statistically significant improvement from baseline in the ICARS score was noted at the 6-month time-point

following 6 monthly infusions of EDS. It is anticipated that similar results will be observed following 6 months of treatment in this double-blind, placebo-controlled trial. The 6-month endpoint was selected as it represents an adequate amount of time to assess an effect on a neurodegenerative disorder (e.g. Alzheimer's disease; Parkinson's disease).

Open-label Treatment

The option to continue open-label treatment with EDS in a separate study (Study IEDAT-03-2018) will be available to all patients who complete 12 months (or more) of double-blind treatment, and have assessments at 6, 9 and 12 months (Visits 9, 12 and 15), including those patients originally randomized to placebo. All patients will receive the high dose range of EDS-EP (~14-22 mg DSP/infusion). The Investigator will assess the patients at the 12-month time-point (Visit 15), to ensure that there are no safety concerns that would preclude the patient from continuing treatment with EDS-EP. Infusions will be performed on a monthly basis during the open-label extension treatment phase, and assessments of safety and efficacy will be done at regular intervals. In case a patient is willing to be enrolled in the open-label study IEDAT-03-2018, but the study has not yet received IRB/EC approval at the time the patient reaches Visit 15/Month 12, the patient may undergo unscheduled monthly assessments/infusions (according to the assigned blinded treatment) starting from the time Visit 15/Month 12 should be performed, and continuing until the site has been initiated for the open-label study IEDAT-03-2018 and he/she has been evaluated for eligibility for the study.

Rationale for EDS Doses

CCI

[redacted]

CCI [redacted]

[redacted]

Rationale for Efficacy Measures

The efficacy measures for this trial were selected based on feedback from Regulatory Authorities. The efficacy measures to be used in the study are described below.

International Cooperative Ataxia Rating Scale (ICARS)

The ICARS will be used to measure efficacy in the study. The internal consistency, criterion-related validity, and internal construct validity of the ICARS have been established in patients with focal cerebellar lesions (Schoch B et al, 2007). The inter-rater reliability, test-retest reliability and internal consistency have also been validated in patients with spinocerebellar ataxia (Weyer et al, 2007, Schmitz-Hubsch et al , 2006a). The ICARS total score satisfied all psychometric criteria in a validation study in patients with Friedrich's Ataxia (Cano et al, 2005; Metz et al 2013) The ICARS has been used as an outcome measure in interventional studies in patients with ataxia, but there are no clinical data to suggest the magnitude of change on the ICARS that would correspond to a clinically relevant improvement, due to the absence of any drug producing such a benefit. The ICARS has been used in several studies in patients with Friedreich's Ataxia over the age of 8 years, in phase 3 potentially pivotal trials (Lynch et al, 2010), as well as in open-label long-term studies (Meier et al, 2012).

The ICARS was used also in two interventional studies in children with AT. Zanolli et al, 2012 showed a statistically significant decrease in the ICARS total score in a placebo-controlled crossover study in 13 children with AT treated with oral betamethasone (Zannolli et al, 2012). Nissenkorn et al, using ICARS as a secondary endpoint, showed improvement in the static and kinetic subscales in a short-term open-label study in 17 children (from 4 years of age) treated with amantadine (Nissenkorn et al, 2013).

Incoordination of eye movements, nystagmus and loss in saccadic eye movement control is observed early in the AT disease process (Hoche et al, 2012). The ICARS items relating to ocular motility do not provide a comprehensive assessment of this domain. Also, no scale has been validated for the measurement of ocular motility in AT patients. The investigator will be asked to assess the ocular motility item based on the site's clinical paradigm and the instructions for rating the ICARS.

As AT is a severe, relentlessly progressive disorder that leads to loss of independent movement in most patients by the age of 10 to16 years, any statistically significant difference from placebo over a 6-month period should be considered as a treatment success.

Most of the developmental and validation studies of the ICARS were performed in patients who were 10 years of age or older. An international study has been completed (ClinicalTrials.gov: NCT01942850) to extend its validity to patients in the age-range of 5 to10 years. Data collected from this validation study in selected centers [Data on file, EryDel S.p.A] indicated that the distribution of scores for the ICARS for

patients with AT under 10 years of age was similar to the scores for these patients on other validated scales such as the SARA (Schmitz-Hubsch et al, 2006b) and the BARS (Schmahmann et al, 2009). Data collected from the validation study in selected centers confirmed that the severity of AT increased with age in patients under the age of 10 years, as demonstrated by a significant correlation of the total scores on the ICARS, SARA, and the BARS, but not for patients above 10 years. Overall, the distribution of scores for the ICARS for 5 to 10 year-old patients with AT was similar to the scores for other validated scales such as the SARA, BARS, CGI-S and CGI unstructured.

The variability associated with having multiple centers across different countries and languages, and multiple raters for the ICARS within each center, will be minimized by the use of central remote raters who will provide ratings of the ICARS based on video recordings of the patients' ICARS assessments performed by the site raters. These videos will be provided to the remote rater blinded to the treatment condition and in random order, without specifying the visit of the study. These remote ratings will be used in the analysis of the primary efficacy endpoint.

Although the ICARS will be rated in its entirety, the primary analyses will be limited to the 'Modified' ICARS; this version excludes Items 8-12 related to kinetic function and Items 17-19 related to oculomotor functions that are required for visual control, as these items are not directly predictive of change in functioning.

CGI-C and CGI-S

The Clinical Global Impression (CGI) consists of two 7-point, clinician-rated, Likert-type scales assessing change from baseline (CGI-C) and severity of illness (CGI-S) (Guy, 1976). In the current study, an overall assessment of the change in the patient's neurological symptoms of AT, compared to the status at baseline, will be performed at each visit and used as the key secondary efficacy measure. To ensure independence of this rating from the rating of the ICARS (the primary efficacy measure), a different rater will perform the CGI-C.

In addition, the severity of illness at baseline and at each subsequent timepoint will be assessed using the CGI-S. No version of the CGI-S exists which has been specifically adapted for use in AT patients; therefore, a 5-point structured version was developed which takes into account the severity of the following symptoms of AT: ataxia (walking), dysarthria, dysmetria, extrapyramidal symptoms (chorea, myoclonus, dystonia, and tremor), and eye movements. Ratings of none, mild, moderate, severe, and very severe are selected based on the level of symptomatology. A pilot validation study has been performed in AT patients using the CGI-S scale, and the results indicate that the CGI–S adequately discriminated patients with mild, moderate, or severe disease [Data on file, EryDel S.p.A.]. The distribution of patients to different scores on the CGI-S of AT was similar using the ICARS, BARS, and SARA in 6 to 9 year-olds. The CGI-S for patients ≥10 years of age was greater than in 6 to 9 year-old patients; however, the distribution of scores on the ICARS, BARS, and SARA was similar across severity categories.

Vineland Adaptive Behaviors Scale (VABS)

Functional intelligence generally is defined as one's ability to behave adaptively in daily life. Specifically, it includes the person's ability to express and comprehend language, behave appropriately in interpersonal situations, understand and use social behaviors, protect himself/herself, and care for himself/herself, in terms of personal hygiene and domestic independence. Although several scales and observation systems for measuring adaptive behavior have been developed since the 1940s, when the construct of adaptive behavior was formally introduced, one of the most widely accepted scales is the Vineland Adaptive Behavior Rating Scales (VABS; Sparrow and Cicchetti, 1985; Sparrow et al, 2005).

The VABS is used primarily with children and adolescents from birth to age 18 years, but may be used for adults suspected of having a mental handicap based on obtained IQ. The scale measures adaptive behavior in four major domains, Communication, Daily Living Skills, Socialization, and Motor Skills. These domains include several subdomains, as follows:

- **Communication:** Receptive, Expressive, Written
- **Daily Living Skills:** Domestic, Personal, Community
- **Socialization:** Interpersonal Relationships, Play & Leisure, Coping Skills
- **Motor Skills:** Gross, Fine

In the current study, this scale will be used to assess the strengths and weaknesses of the AT patients in each of these specific areas of adaptive behavior which may be impacted by the disease.

*QoL Scale (EQ-5D-*5L)

Improvements in quality of life (QoL) are an important goal for treatment of patients with AT. Therefore, the EQ-5D-5L scale assessing QoL has been included as a secondary efficacy measure. The EQ-5D is a standardized instrument for assessing health-related QoL in a variety of health conditions (Rabin and de Charro, 2001). The EQ-5D-5L is a newer version of the scale, which includes five levels of severity for each of the five EQ-5D dimensions (Herdman et al, 2011), and has been validated in a diverse patient population in 6 coutries, including 8 patient groups with chronic conditions and a student cohort (Janssen et al, 2013). For children and adolescents, the EQ-5D-5L administrators will be trained to help the younger population in the study complete the scale. Assessment of the EQ-5D-5L will be performed by the patient, or the patient's parent/caregiver, at regular intervals throughout the study.

A-T NEST

The A-T NEST represents a refinement of the A-T scale published by Crawford et al. (2000)[17] that was developed to quantitatively describe the diverse range of abnormalities seen in patients with A-T and record their rate of progression. The development of the A-T scale was based on data collected from 52 patients with confirmed A-T attending the Johns Hopkins Hospital, who were between the ages of 1 to 19 years. Based on agreements on the ratings of video-taped examinations between 2 highly experienced raters, and the use of a weighted kappa score, principal component analysis, 9 items were selected; 2 items were dropped due to poor inter-rater agreements, and replaced by 3 other items. The final 10 items capture the essential neurologic features of the disorder.

The A-T NEST index offers advantages over other scales used to assess A-T as it was developed specifically for this disorder and thus, captures the broad range of deficits experienced by these patients. The index may be able to capture progression of the disorder faithfully. The A-T NEST scale has been used internationally over the last decade by A-T clinical experts, as part of the Ataxia Telangiectasia Children's Project (ATCP), who have made further changes and recommendations for its use internationally.

The inter-rater reliability, test-retest reliability and internal consistency have not been validated concurrently in large number of patients with A-T from multiple countries with a wide variety of raters. The ability of the A-T NEST to assess treatment-induced change has also not been determined, and there are no clinical data to suggest the magnitude of change on the A-T NEST that would constitute a clinically relevant change.

The use of the A-T NEST in this Study would allow collection of data for the validation of psychometric properties of the scale, from experienced international raters. It would also allow determination of its intra

and inter-rater reliability, based on the data from the CGI-S of A-T and the ICARS, and concordance of A-T NEST scores with these validated scales. Lastly, the study would help assess if the A-T NEST possesses treatment sensitivity.

8 STUDY OBJECTIVES

Initial Treatment Period (6 months)

Primary Efficacy Objective:

- *Efficacy*: To evaluate the effect of two dose ranges (~5-10 and ~14-22 mg DSP/infusion) of EryDex System end product [EDS-EP; the EDS is a combination product that is used to load dexamethasone sodium phosphate (DSP) into autologous erythrocytes, creating the EDS end product, which is infused into the patient], compared to placebo, on central nervous system (CNS) symptoms measured by the 'Modified' International Cooperative Ataxia Rating Scale (ICARS) in patients with ataxia telangiectasia (AT).

Key Secondary Efficacy Objective:

- To evaluate the effect of EDS-EP, compared to placebo, in this population on the Clinical Global Impression of Change from baseline (CGI-C).

Safety Objective:

- To evaluate the safety and tolerability of EDS-EP, compared to placebo, in AT patients, based on the occurrence of Treatment-Emergent Adverse Events (TEAEs), including Serious AEs and discontinuations due to AEs, and changes in vital signs, laboratory parameters, ECGs and physical/neurological examination findings.

Secondary Efficacy Objectives:

- To evaluate the effect of EDS-EP, compared to placebo, in this population on the following efficacy measures:
    - Clinical Global Impression of Severity (CGI-S) of neurological symptoms of AT;
    - Adaptive behavior measured by the Vineland Adaptive Behavior Scales (VABS).

Tertiary Objectives:

- To evaluate the effect of EDS-EP on health-related QoL using the EQ-5D-5L scale;
- To assess the pharmacokinetic and pharmacodynamic relationships between dexamethasone administered through EDS-EP and safety, tolerability, and demographic variables.
- To evaluate the pharmacokinetic (PK) profile of dexamethasone administered through EDS-EP at two dose levels based on pooled data from all patients in each treatment group. *A determination of individual PK parameters will be performed for patients with an adequate number of PK blood samples after the initial infusion.*

Exploratory Objective:

- To collect data on the use of the A-T NEST concurrent with the ICARS, CGI-S and CGI-C, as well as its use in languages other than English, and compare it with the data from the scales referenced to assess the psychometric properties of the A-T NEST.

Extension Treatment Period (6 months)

Primary Objective:

- To evaluate the efficacy of two dose ranges (~5-10 and ~14-22 mg DSP/infusion) of EDS-EP, compared to placebo, in treating CNS symptoms in AT patients during long-term treatment (up to 12 months), as measured by the 'Modified' ICARS.

Secondary Objectives:

- To evaluate the long-term (up to 12 months) safety and tolerability of EDS-EP in AT patients;
- To compare the effects of the two dose ranges of EDS-EP on the clinician's global impression (CGI-C and CGI-S), adaptive behavior (VABS), and QoL (EQ-5D-5L scale).

9 INVESTIGATIONAL PLAN

9.1 Study Design

This is an international (North America, Europe, Africa, Asia, and Australia), multi-center, one-year, randomized, double-blind, placebo-controlled, Phase III study, designed to assess the effect of two non-overlapping dose ranges of EDS-EP (dexamethasone sodium phosphate in autologous erythrocytes), administered by IV infusion once per month, on neurological symptoms of patients with ataxia telangiectasia (AT). All patients who complete the assessments as designed over the initial 6 months of the trial will be eligible to continue in an additional 6-month, double-blind, placebo-controlled extension, designed to collect information on the long-term safety and efficacy of the trial treatments.

Listings of the study procedures to be performed at each visit are provided in Section 11.0.

9.2 Study Population

9.2.1 Inclusion Criteria

To be eligible, the patient must meet all of the following criteria:

1. Patient meets clinical criteria for diagnosis of AT. The neurological signs of AT (incoordination of the head and eyes in lateral gaze deflection, gait ataxia associated with an inappropriately narrow base) must be documented. Such signs of AT illustrate the body systems in which changes shall be confirmed but the listed changes are examples and other changes in those systems may be observed and documented to confirm the diagnosis of AT.
2. Patient is in autonomous gait or is helped by periodic use of a support (i.e. local ICARS score for *Item 1 – Walking Capacities* between 0 and 4, included).
3. Patient will be investigated for the proven genetic diagnosis of AT (prior documentation or by central laboratory test report).
4. Patient is at least 6 years of age, of either sex.
5. Body weight > 15 kg.
6. The patient and his/her parent/caregiver (if below the age of consent), or a legal representative, has provided written informed consent to participate. If consent is provided solely by the caregiver in accordance with local regulations, the patient must provide assent to participate in the study.

*9.2.2 **Exclusion Criteria***

To be eligible, the patient must not meet any of the following criteria:

General

1. Females that are
    a. pregnant, or are breast-feeding (for EU countries only);
    b. of childbearing potential, pregnant, or are breast-feeding (for US and Rest of World countries).

    Females of childbearing potential using adequate birth control, as determined by their Health Care Provider, will be eligible.
2. A disability that may prevent the patient from completing all study requirements.
3. Current participation in another clinical study.

Medical History and Current Status

4. CD4+ lymphocytes count <400/mm3 (for patients 6 years of age) or <150/mm3 (for patients > 6 years). In presence of oral infections, like oral candidiasis, documented at the screening or recurrent as per medical history documentation, the limit increases to <200/mm3 (for patients > 6 years).
5. Loss/removal of 250 mL or more of blood within the past 4 weeks prior to screening.
6. Current neoplastic disease or previous neoplastic disease not in remission for at least 2 years.
7. History of severe impairment of the immunological system.
8. Severe or unstable pulmonary disease.
9. Uncontrolled diabetes.
    Patients with diabetes that has been stabilized (i.e. no hypoglycemic or hyperglycemic episodes in the past 3 months) will be eligible.
10. Any other severe, unstable, or serious disease or condition that in the Investigator's opinion would put the patient at risk for imminent life-threatening morbidity, need for hospitalization or mortality.
11. Any clinically significant abnormality on standard laboratory examinations (hematology, biochemistry, urinalysis) at screening that remains abnormal on repeat testing. Eligibility of patients with abnormal laboratory test values will be determined by the Investigator, in consultation with the Medical Monitor.
12. Confirmed hemoglobinopathies, e.g. hemoglobin C disease, sickle cell anemia, or thalassemia.
13. Moderate or severe renal and/or hepatic impairment.

Prior/Concomitant Medication

14. Any previous **oral or parenteral** steroid use within 4 weeks before Baseline. *Treatment with inhaled or intranasal steroids for asthma or allergies, as well as use of topical steroids will be permitted.*
15. Chronic condition or prior allergic reaction representing a contraindication to the use of dexamethasone or other steroid drugs.
16. Has participated in any other trial with an investigational drug and received a dose within 30 days or 10 half-lives (whichever is greater) from the start of the 30-day Screening Period.
17. Has participated in a previous trial with EDS.
18. Requires any concomitant medication prohibited by the protocol, as specified in Section 11.8.
19. Has taken a drug or treatment known to cause major organ system toxicity during the past year.
20. Use of any drug that is a strong inducer/inhibitor of CYP3A4 within 4 weeks before baseline.

9.3 Documentation of Randomization

This is a randomized, double-blind, placebo-controlled, one-year study in which a minimum of 180 patients meeting all of the inclusion/exclusion criteria were anticipated to be randomized equally (1:1:1; 60 patients per group; due to the Covid-19 outbreak, the enrollment was closed with 175 randomized patients.) to receive one of the two dose ranges of EDS-EP or placebo, as follows:

- **Group 1 –** EDS-EP low dose range of ~5-10 mg DSP/infusion;
- **Group 2 –** EDS-EP high dose range of ~14-22 mg DSP/infusion;
- **Group 3 –** Placebo EDS infusion.

Randomization will be done through a centralized Interactive Voice/Web Response System (IVRS/IWRS). In the 6-month Initial Treatment Period, each patient will receive 6 infusions of EDS-EP (~5-10 or ~14-22 mg DSP/infusion) or placebo, given at monthly intervals. After 6 months of treatment, patients in the EDS-EP groups (Groups 1 and 2) who enter the Extension Treatment Period will continue on the same randomized treatment and will receive an additional 6 monthly infusions of EDS-EP. However, patients in the placebo group (Group 3) will be randomly assigned at baseline to be switched to one of the two dose ranges of EDS-EP in a 1:1 ratio in an incremental fashion, with one third of patients being switched at 6 months, another third at 9 months, and the remaining patients at 12 months, provided they continue treatment in the open-label study (IEDAT-03-2018).

At the time of screening, the patient will receive a unique 7-digit *subject alphanumeric code* consisting of 3 digits *(two letters and 1 number)* representing the center and 4 digits *numbers* representing the order in which the subject was screened. For example, subject number IT1-0003 would correspond to the third subject screened at Center IT1. This subject *alphanumeric code*, will be used for the subject throughout the remainder of his/her participation in the trial, and will not be re-assigned to any other subject.

Only subjects who meet all of the inclusion criteria and none of the exclusion criteria at Baseline will be eligible for being assigned to treatment. Assignment to treatment within each of the 3 groups will be done on Day 1, just prior to the time of dosing. At the time of randomization, the subject will be assigned a unique randomization number by the IVRS/IWRS, which will be linked to the subject's treatment assignment. This number will be entered in the eCRF at Baseline, but will not be used as a subject indentifier.

9.4 Premature Discontinuation

Patients who discontinue from the study prematurely must have their reason for discontinuation entered in the Case Report Forms (CRFs). Patients who discontinue from the study after having received a dose of study medication will not be replaced.

All patients who discontinue prematurely prior to Month 6 (final visit of Initial Treatment Period) will be asked to return for all final (Month 6; Visit 9) efficacy and safety evaluations. Similarly, patients who discontinue prematurely during the Extension Treatment Period will be requested to return for all final (Month 12; Visit 15) assessments.

Screening for adrenal insufficiency will be performed in all patients, regardless of weight, via early morning (before 8:00 AM) plasma cortisol testing following discontinuation of the study drug, according to the procedures described in Appendix 12. The timing of the sample collection with regard to the prior EDS-EP infusion will be left up to the clinical judgment of the Investigator.

9.5 Record of Study Participants and Screening Failures

The investigator will be required to maintain a confidential record of all study participants, including all patients who were screened for the study, but were not randomized to treatment. The confidential record must include sufficient information so that it would be possible to contact the study patient. Information on patients who have signed ICFs, but have failed screening, should be entered on the Screen Failure CRF.

The primary reason for screen failure will be recorded using the following categories:

- Did not meet entry criteria
- Major protocol deviation
- Pre-treatment Event/Adverse Event
- Lost to follow-up
- Voluntary withdrawal (specify reason)
- Study termination
- Other (specify reason).

Subject numbers assigned to patients who fail screening will not be reused.

10 STUDY MEDICATION

10.1 CCI

CCI

CCI	CCI
CCI	CCI
CCI	CCI

CCI

CCI

CCI	CCI
CCI	CCI
CCI	CCI

CCI

10.1.2 CCI

The C(CCI

10.1.3 CCI

Processing Solutions

CCI

CCI

CCI

Sponsor's name and contact information, center identification, storage conditions (5 ± 3 °C), lot number and expiry date (Appendix 6).

CCI [redacted]

10.2 **Dose and Administration**

CCI [redacted]

- Group 1 (~5-10 mg/infusion): CCI [redacted]
- Group 2 (~14-22 mg/infusion): CCI [redacted]
- Group 3 (Placebo): CCI [redacted]

A description of the EDS and details of the procedure for the encapsulation process are provided in Appendix 5.

CCI [redacted]

CCI [redacted]

10.3 CCI [redacted]

CCI [redacted]

10.4 Blinding and Randomization

This is a randomized, double-blind study; therefore, the Sponsor, Investigator, site staff (other than the unblinded pharmacist/technical operator) and patient will not be aware of the treatment assignments. A minimum of 180 patients were anticipated to be randomly assigned equally to one of the 3 treatment groups

(1:1:1; 60 patients/ group; due to the Covid-19 outbreak, the enrollment was closed with 175 randomized patients) by the Investigator using an IVRS/IWRS. A minimization procedure will be employed to ensure that the proportions of male and female, and younger (6 to < 10 years) and older (≥ 10 years), patients are comparable across the three treatment groups. Every attempt will be made to ensure the same balance is achieved across different regions.

The customized IVRS will have the capability to process up to twenty-four (24) concurrent phone calls, while maintaining acceptable levels of performance and data integrity. The IVRS/IWRS will be able to capture, store, and process data for all subjects in this project. All data inputs by the user will be checked for validity by the IVRS/IWRS whenever such entries are performed. The user will be notified of any invalid entries or transaction errors that could prevent the user from continuing with the transaction. The IVRS/IWRS will store data in an Oracle relational database. The IVRS/IWRS can be customized to provide additional messages at specific visits. If the users encounter system problems or lock the IVRS/IWRS access during a call, they will be presented with the option to transfer to a customer service representative at the IVRS/IWRS Help Desk. The minimization algorithm will be customized for the protocol and fully tested within the IVRS/IWRS.

The Investigator may break the blind on an individual patient by using the IVRS/IWRS. This should be done only in a medical emergency in which the patient's treatment assignment needs to be known in order to properly treat the patient. The reason for breaking the blind must be provided.

10.5 Accountability

During the course of the study, the unblinded study pharmacist or site personnel designated by the Investigator to manage the study medication must record the study drug disposition and keep the accountability forms updated. The study drug accountability forms will be cross-checked with the Encapsulation Procedure Check-List (Appendix 7). A copy of the accountability forms must be kept in the study files at the site, and the other copy will be withdrawn by the CRO staff responsible for monitoring the unblinded study medication. The used and unused dexamethasone and placebo vials, as well as the Encapsulation Procedure Check-List, will be kept at the site for accounting and reconciliation by the CRA vs. the study documentation. Since the drug accountability records will be unblinded, the CRA responsible for monitoring these records will not be involved in monitoring any other aspect of the study. The used infusion bags containing any remaining erythrocytes (with DSP encapsulated) will be disposed of at the site, with appropriate documentation.

10.6 Overdose

Patients in the active group in this study will receive up to 6 infusions of EDS in the initial 6-month treatment period. Each infusion should contain a maximum of approximately 22 mg DSP encapsulated in autologous RBCs. However, based upon results of prior studies, some inter-individual variability in loaded dose is expected; therefore, administered doses of DSP greater than **30 mg** will be considered an overdose. Treatment for an overdose should be the same as the clinical management for an overdose of dexamethasone.

10.7 Occupational Safety

There are no risks anticipated related to the study medication for the staff involved in administering EDS to the patients in this study. Standard procedures and precautions for handling needles and biological samples should be followed.

11 EVALUATIONS AND PROCEDURES

11.1 Written Informed Consent

Written Informed Consent must be obtained from the patient and his/her parent/caregiver (if below the age of consent), or legal representative (if necessary), at screening prior to initiating any study procedures required by the protocol, according to the procedure described in Section 17.1. If consent is provided solely by the caregiver in accordance with local regulations, the patient must provide assent to participate in the study. The details of the study should be discussed with the patient and parent/caregiver or legal representative (if necessary) prior to obtaining informed consent, and the Informed Consent Form (ICF) must be signed and dated by the patient, parent/caregiver/ legal representative (if necessary), and by the Investigator or his/her designee. A copy of the signed ICF will be provided to the patient, and the original will be retained with the source documents. The initial consent will cover one year of treatment; however, the patient will be asked to provide an additional consent/assent after completing the 6-month initial treatment period, before continuing in the 6-month extension treatment period.

Patients who complete the current study and elect to continue open-label treatment with EDS-EP in Study IEDAT-03-2018 will need to sign a separate consent form for that study. In case a patient is willing to be enrolled in the open-label study IEDAT-03-2018, but the study has not yet received IRB/EC approval at the time the patient reaches Visit 15/Month 12, the patient may undergo unscheduled monthly assessments/infusions (according to the assigned blinded treatment) starting from the time Visit 15/Month 12 should be performed, and continuing until the site has been initiated for the open-label study IEDAT-03-2018 and he/she has been evaluated for eligibility for the study. Before performing the additional infusions or any of the assessments associated with unscheduled visits, the patient or the parent/legal guardian, as well as the Investigator who conducted the consenting process, must sign the relevant ICF approved by Ethical Committee to allow these additional procedures to be performed.

11.2 Study Conduct

This study is divided into three periods: Screening (Days -30 to -1), 6-month Initial Treatment Period (Months 1-6; Visits 1-9), and 6-month Extension Treatment Period (Months 7-12; Visits 10-15). A detailed listing of the assessments and procedures to be performed at each visit is provided in Section 11.4 for the Initial Treatment Period and Section 11.5 for the Extension Treatment Period.

As a results of the COVID-19 pandemic, temporary changes to the protocol have been impleted. These changes are described in Appendix 14.

Screening Period/Baseline

After providing consent/assent, as described in Section 11.1, each patient will undergo a 30-day Screening Period, during which any previous treatments with other corticosteroid compounds will be withdrawn (washout from previous treatment). Demographic information will be collected for all patients, and additional information on the medical management and cost of illness will be collected in selected countries where such information can be reliably collected. Each patient will undergo a series of screening evaluations, including medical history, physical and neurological examinations, measurement of vital signs, electrocardiogram (ECG), clinical laboratory tests [hematology, biochemistry, urinalysis, blood glycosylated hemoglobin (HbA1c), CD4+ lymphocytes count, α–fetoprotein, C-Reactive Protein (CRP), RBC antibodies (IgG, IgM, Qualitative Direct Coombs test), and serum and urine pregnancy test (women of childbearing potential)], and assessment of prior and concomitant medications. In addition, a blood

sample for mini-ATM detection will be collected. In selected centers, a 5-ml blood sample will be taken for assessing RBC osmotic resistance. A sample will be taken for the genetic assay for AT, if not previously reported, however, the results do not need to be available at baseline.

The initial ratings of the ICARS and CGI-S will be performed at the screening visit. Throughout the study, the ICARS rating will be performed by qualified raters trained in the use of the scale and not involved in rating other scales. The ICARS rater will not have access to the results of other scales or to the safety data. In addition, the evaluation will be videotaped and rated by a central remote rater who will be blinded to the treatment assignment and the order in which the ratings were performed for each patient. The CGI rater will be blinded to the ICARS ratings and safety data, but will have access to ratings of other secondary efficacy measures when performing the assessment.

If any abnormal laboratory test results, vital sign measurements, or ECG findings of clinical significance are noted at screening, these must be repeated during the 30-day screening period and the results made available prior to making the final decision on a patient's eligibility for the study at Baseline. Adverse events, reported by the patient or observed by the investigator, and the use of concomitant medication will be recorded from the time of signing of informed consent through the end of the study.

A sample will be collected before 8:00 AM for measurement of plasma cortisol during the screening period, prior to randomization at baseline. If the basal cortisol level is within the reference normal range, the patient can be enrolled in the study. If the 8:00 AM cortisol level is below 3-5 µg/dL (depending on assay) regardless of symptoms, or the patient exhibits signs or symptoms of adrenal insufficiency (see Appendix 12) and has a cortisol <10 µg/dL, the patient will receive a high dose ACTH stimulation test, regardless of weight, within 24 hours. If the ACTH stimulation test is normal, the patient can be enrolled after the effects of the ACTH have dissipated (e.g., 30 days after ACTH administered). If the patient fails the ACTH stimulation test they will be excluded from the study and referred to a pediatric endocrinologist, with a recommendation to prescribe stress dose steroids.

At Baseline (Day 0/1), vital signs and physical and neurological examinations will be repeated and the Columbia-Suicide Severity Rating Scale (C-SSRS) will be performed. The inclusion/exclusion criteria will be reviewed and patients meeting all criteria will be randomized to one of the 3 treatment groups. Two ratings will be done on the ICARS, one 24 hours before baseline (Day 0) and the other on Day 1 (pre-dose); video recording will be required for both assessments. In addition, the baseline ratings of the following efficacy measures will be performed prior to dosing: CGI-S (assess baseline severity of the patient for use in evaluating change at post-baseline visits), VABS, and EQ-5D-5L. The diverted blood sample (see Section 11.6, at least 15 mL, will be drawn prior to dosing to measure baseline values for the foreseen laboratory tests. The serum and urine pregnancy test will be repeated for women of childbearing potential. A baseline measurement of bone mineral density (BMD) will be performed.

Initial Treatment Period (6 months)

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To maintain the blind, all properly prepared RBCs (EDS or Placebo) will be administered to the patients, without knowledge of the actual dose or treatment being administered.

CCI

[redacted]

On Day 1, a blood sample will collected for measurement of free plasma hemoglobin, 1 hr post-infusion. On Days 2 and 15, a complete hemolysis panel [free plasma hemoglobin, haptoglobin, bilirubin (total and conjugated), CBC, LDH, urinalysis] will be performed.

At Months 1, 2, 3, 4 and 5, a blood sample will be taken for dexamethasone PK before and 1 hr after the infusion (except for Month 1). Measurement of body weight and height in triplicate, as well as a C-SSRS assessment, will be performed at each of these visits. The diverted blood sample (see Section 11.6) will be collected for measurement of serum creatinine at Months 1, 2, 4 and 5, whereas creatinine will be part of the routine laboratory tests at Months 3 and 6. Upon completion of the EDS process, 2.5 mL of the treated blood will be collected for measurement of DSP concentration and a CBC. At Month 3, in selected centers, a 5-mL sample will be taken from the EryKit_01 bowl at the end of the process to assess RBC osmotic resistance.

After completion of the IV infusion of the study treatment, a physical examination and vital signs will be performed (1-2 hr after infusion). At Month 3 (Visit 6), a complete set of efficacy (ICARS, CGI-C, CGI-S, VABS, and QoL) and safety (vital signs, ECG, routine laboratory tests, physical and neurological examinations) evaluations will be performed, and a hemolysis panel will be done pre- and post-infusion. In addition, the diverted blood samples will be collected for mini-ATM detection at Months 2 and 6.

The final evaluations for the Initial Treatment Period will take place at Visit 9 (Month 6 or at early termination) and will include all assessments of efficacy (ICARS, CGI-C, CGI-S, VABS, and EQ-5D-5L) and safety (vital signs [including weight and height in triplicate], ECG, routine laboratory tests, physical and neurological examinations, C-SSRS, BMD and assessments of AEs and concomitant medication use), as well as special laboratory tests [HbA1c, CD4+ lymphocytes count, α–fetoprotein, CRP, and RBC antibodies (IgG, IgM, Qualitative Direct Coombs test)]. A serum and urine pregnancy test will be performed for women of childbearing potential. The efficacy evaluations at Visit 9 will be used as Endpoint assessments for statistical purposes. The final blood sample (trough level) for the dexamethasone PK analysis will also be collected. The total volume of blood collected throughout the 6-month study for all PK samples would be approximately 42 mL.

Extension Treatment Period (6 months)

Patients completing the 6-month Initial Treatment Period and doing well on the study medication will be eligible for continuing treatment for an additional 6 months in the Extension Treatment Period. Informed

consent/assent must be obtained in writing for the extension, as in the Initial Treatment Period. Patients meeting all entry criteria will be treated as follows:

- Patients originally randomized to one of the two dose ranges of EDS (Groups 1 or 2) will continue on the same treatment;
- Patients originally randomized to the Placebo group (Group 3) will be re-randomized (randomization to be done at baseline) in equal proportions (1:1) to receive either the EDS-EP ~5-10 mg DSP/ infusion or ~14-22 mg DSP/infusion (or the remaining dose of EDS-EP, if one of the doses has been eliminated following the Interim Analysis), as follows:
    - Following 6 months of treatment, one third of the placebo patients will be switched to treatment with EDS-EP, as described above;
    - After 9 months of treatment, one third of the placebo patients will be switched to treatment with EDS-EP, as described above;
- At 12 months, all remaining placebo patients who continue in the open-label phase will receive treatment with EDS-EP, as described above.

Visit 9 (Month 6) evaluations from the Initial Treatment Period will serve as the Baseline assessments for the Extension Treatment Period. Once these are completed, patients will have blood drawn for the EDS encapsulation procedure and will receive the infusion as before. Vital signs and a physical examination will be performed after dosing.

Subsequently in the Extension Treatment Period, EDS-EP infusions will be performed at Months 7, 8, 9, 10 and 11 (Visits 10 – 14), after which vital signs (including body weight and height in triplicate) and a physical examination will be performed. The C-SSRS assessment will also be performed at each of these visits. A blood sample will be collected for measurement of serum creatinine at Months 7, 8, 10 and 11, whereas creatinine will be part of the routine laboratory tests at Months 9 and 12. In addition, on Month 9 (Visit 12), all efficacy assessments (ICARS, CGI-C, CGI-S, VABS, and QoL) will be done prior to the infusion, and the neurological examination, routine laboratory tests and the ECG will be repeated. Upon completion of the EDS process 2.5 mL of the processed blood will be collected for measurement of DSP concentration and a CBC.

At Months 6 and 9, in selected centers, a 5-mL sample will be taken from the EryKit_01 bowl at the end of the process to assess RBC osmotic resistance. At Months 6 and 9, a hemolysis panel will be done pre- and post-infusion.

The final evaluations for the Extension Treatment Period will take place at Visit 15 (Month 12 or at early termination) and will include all assessments of efficacy (ICARS, CGI-C, CGI-S, VABS, and QoL) and safety (vital signs [including weight and height in triplicate], ECG, routine laboratory tests, physical and neurological examinations, C-SSRS, BMD and assessments of AEs and concomitant medication use), as well as special laboratory tests [HbA1c, CD4+ lymphocytes count, α–fetoprotein, CRP, and RBC antibodies (IgG, IgM, Qualitative Direct Coombs test)]. A final serum and urine pregnancy test will be performed for women of childbearing potential.

In case a patient is willing to be enrolled in the open-label study IEDAT-03-2018, but the study has not yet received IRB/EC approval at the time the patient reaches Visit 15/Month 12, the patient may undergo unscheduled monthly assessments/infusions (according to the assigned blinded treatment) starting from the time Visit 15/Month 12 should be performed, and continuing until the site has been initiated for the open-label study IEDAT-03-2018 and he/she has been evaluated for eligibility for the study.

Patients completing 12 (or more) months of treatment in the combined Initial Treatment and Extension Treatment Periods and doing well on the study medication, will be eligible to continue treatment with EDS-EP in a 12-month, open-label, extension study (Study IEDAT-03-2018). Informed consent must be obtained in writing for participation in this open-label extension study.

Due to the length of time required for the encapsulation procedures (>2 hr), it is anticipated that only 1 or 2 patients will be dosed on any one day.

Safety Follow-up Visit

For all patients who complete the one-year study and do not continue in open-label treatment, as well as those who discontinue treatment prematurely, a Safety Follow-up Visit (Visit 16) will be conducted 30 days after their final assessment or at least 60 days after their last infusion, whichever is longer. At this visit, the occurrence of any AEs or Serious AEs (SAEs) reported by the patient/caregiver or observed by the Investigator since the previous visit will be recorded.

Evaluation and Infusion Schedule

The 12 (or more) monthly infusions should be performed every 21-28 days. A window of ± 7 days will be permitted on the Day 15 visit and of + 10 days on each of the scheduled monthly post-baseline visits (Months 1 - 12). However, no EDS-EP infusion should be performed less than 21 days after the previous infusion. The window between an infusion and the subsequent one should be kept as regular as possible throughout the study, avoiding fluctuations in administration windows. The date of an infusion is not bound to the date of the initial treatment but to the date of the previous IMP administration. Infusions should not be skipped; therefore, if necessary, the infusion schedule can be modified to conform to the above guidelines.

11.3 Schedule of Visits and Assessments

An overview of the schedule of evaluations for the study is presented in the Study Flow Chart for the Screening period and 6-month Initial Treatment Period in Table 2A. The assessments that will be performed during the 6-month Extension Treatment Period are detailed in Table 2B.

Table 2A. Study Flow-Chart: 6-Month Initial Treatment Period

Visit (V) (z)	**Screening**	**V1**		**V2**	**V3**	**V4**	**V5**	**V6**		**V7**	**V8**	**V9**
Study Day or Month (D/M) #	**D -30 to -1**	**D0/1**		**D2**	**D15**	**M1**	**M2**	**M3**		**M4**	**M5**	**M6 (a,b)**
Procedure (§)		**Pre(c)**	**Post(c)**					**Pre(c)**	**Post(c)**			
Informed Consent Signature	X											
Medical History/Demographics	X											
Inclusion/Exclusion Criteria (v)	X	X										
EDS-EP Infusion (h)		1				2	3	4		5	6	
Culture-based sterility test (aa)			X			X	X		X	X	X	
Neurological Examination	X	X						X				X
Physical Examination	X	X	X			X	X	X	X	X	X	X
Tanner Scale		X										X
Vital Signs (x)	X	X	X			X	X	X	X	X	X	X
ECG	X	X(d)						X				X
Routine Laboratory Tests (e)	X	X(d)						X				X
Serum creatinine						X(w)	X(w)			X(w)	X(w)	
Bone Mineral Density		X										X
Serum($)/Urine(*) Pregnancy Test (o)	X($)	X($,*)				X(*)	X(*)	X(*)		X(*)	X(*)	X($)
ICARS (with video recording)	X(n)	X(n)						X				X
CGI-C								X				X
CGI-S	X	X						X				X
VABS		X						X				X
Quality of Life (EQ-5D-5L)		X						X				X
C-SSRS		X				X	X	X		X	X	X
A-T NEST (in selected centers)				X		X				X		
RBC osmotic resistance (t)	X	X						X				
Special Laboratory Tests (m)	X(f,g)	X(f)										X(f)
Hemolysis Panel (s)		X	X	X	X			X	X			
Genetic AT diagnosis (q)	X											
Mini-ATM detection	X						X					X
Dexamethasone PK sample		X(i)	X(i)	X(j)	X(k)	X(l)	X(l)	X(l)	X(l)	X(l)	X(l)	X(l)
EDS end product sample (u)		X				X	X	X		X	X	
Prior/Concomitant Treatments	Throughout the duration of the study											
Adverse Events	Throughout the duration of the study											

Table 2B. Study Flow-Chart: 6-Month Extension Treatment Period

Visit (z)	V9		V10	V11	V12		V13	V14	Unscheduled Visit (£)	V15	V16
Study Day or Month (D/M) #	**M6 (b)**		**M7**	**M8**	**M9**		**M10**	**M11**		**M12 (y)**	**Safety**
Procedure (§)	**Pre(c)**	**Post(c)**			**Pre(c)**	**Post(c)**					**Follow-up (p)**
Informed Consent Signature	X								X		
Re-Randomization (r)	X				X						
EDS-EP Infusion (h)	7		8	9	10		11	12	#		
Culture-based sterility test (aa)		X	X	X		X	X	X	X		
Neurological Examination					X					X	
Physical Examination		X	X	X	X	X	X	X	X	X	
Tanner Scale										X	
Vital Signs (x)		X	X	X	X	X	X	X	X	X	
ECG					X					X	
Routine Laboratory Tests (e)					X					X	
Serum creatinine			X(w)	X(w)			X(w)	X(w)	X		
Bone Mineral Density										X	
Serum($)/Urine(*) Pregnancy Test (o)	X(*)		X(*)	X(*)	X(*)		X(*)	X(*)	X(*)	X($)	
ICARS (with video recording)					X					X	
CGI-C					X					X	
CGI-S					X					X	
VABS					X					X	
Quality of Life (EQ-5D-5L)					X					X	
C-SSRS			X	X	X		X	X	X	X	
A-T NEST (in selected centers)			X				X				X
RBC osmotic resistance (t)	X				X						
Special Laboratory Tests (m)										X(f)	
Hemolysis Panel (s)	X	X			X	X					
EDS end product sample (u)	X		X	X	X		X	X	X		
Prior/Concomitant Treatments	Throughout the duration of the study										
Adverse Events	Throughout the duration of the study										X

a) Efficacy evaluations on Month 6 (Visit 9) will be used as the Endpoint assessments for all efficacy measures.

b) Month 6 (Visit 9; Endpoint) evaluations should be performed for all patients that complete the Initial Treatment Period, as well as patients that discontinue prematurely; these evaluations will be used as the baseline assessments for the double-blind, Extension Treatment Period.

c) Procedures to be done before (Pre) and after (Post) the EDS-EP infusion.

d) These evaluations will be repeated only if abnormalities requiring follow-up were noted at the Screening evaluation; results from the repeat assessments must be available at baseline to confirm eligibility before the patient can be randomized to treatment.

e) Routine laboratory assessments to include complete hematology, biochemistry, and urinalysis.

f) Special laboratory tests include HbA1c, CD4+ lymphocytes count, α–fetoprotein (not repeated at baseline), CRP, and RBC antibodies (IgG, IgM, Qualitative Direct Coombs test – not repeated at baseline).

g) A sample will be collected before 8:00 AM for measurement of plasma cortisol during the screening period, prior to randomization at baseline. If the basal cortisol level is within the reference normal range, the patient can be enrolled in the study. If the 8:00 AM cortisol level is below 3-5 µg/dL (depending on assay) regardless of symptoms, or the patient exhibits signs or symptoms of adrenal insufficiency (see Appendix 12) and has a cortisol <10 µg/dL, the patient will receive a high dose ACTH stimulation test, regardless of weight, within 24 hours. If the ACTH stimulation test is normal, the patient can be enrolled after the effects of the ACTH have dissipated (e.g., 30 days after ACTH administered). If the patient fails the ACTH stimulation test they will be excluded from the study and referred to a pediatric endocrinologist, with a recommendation to prescribe stress dose steroids.

h) Assigned study treatment: Group 1: EDS-EP dose range ~5-10 mg DSP/infusion; Group 2: EDS-EP dose range ~14-22 mg DSP/infusion; and Group 3: placebo.

i) Samples to be collected before infusion (0 min), and 1 and 4 hr after the end of the infusion.

j) Single sample to be collected 24 hr post-infusion (Day 2).

k) Single blood sample to be taken in the morning.

l) Blood samples to be collected prior to (trough) and 1 hour after (peak) the infusion, except in Months 1 (Visit 4) and Months 6 (Visit 9); only trough levels will be collected.

m) Blood sample to be collected before 8:00 AM for measurement of plasma cortisol at the following times: (1) during the screening period (prior to randomization), (2) when patients are symptomatic, and (3) when patients are at most risk for adrenal insufficiency and adrenal crisis, such as following discontinuation or interruptions of dexamethasone dosing (i.e., loading failures and following discontinuation of the study drug).

n) Video recording will not be required at the screening visit. The ICARS will be performed twice at Baseline, 24 hours apart (Days 0 and 1); the rating on Day 1 (pre-dose) will be used as the baseline value for statistical analyses. ICARS video recording will be required on both Day 0 and Day 1 (before infusion).

o) For women of childbearing potential only. The test results must be negative at screening (serum) and baseline (urine) for the patient to be eligible for the study. Urine pregnancy test will be performed before every infusion.

p) A Safety Follow-up assessment will be performed 30 days after the final evaluation or at least 60 days after the final infusion, whichever is longer; at this visit, the occurrence of any AEs or Serious AEs since the final evaluation will be reported.

q) If the genetic test for AT has not been performed previously, the test will be done by the central laboratory. For patients who require genetic testing to confirm the diagnosis, test results do not need to be available before baseline.

r) Patients originally randomized to placebo will be re-randomized to receive one of the two doses of EDS-EP, with one third of patients being switched at 6 months and another third at 9 months.

s) Hemolysis panel: free plasma hemoglobin, haptoglobin, bilirubin (total and conjugated), CBC (Day 2 and 15 only), LDH, and urinalysis (Day 2 and 15 only). On Day 1, M3, M6 (b) and M9, only free plasma hemoglobin will be measured, 1 hr post-infusion.

t) RBC osmotic resistance to be measured on a blood sample taken at screening and on a sample from the EryKit_01 bowl at the end of the process in selected centers only.

u) Upon completion of the EDS process, the remaining sample in the satellite sample bag or, if this is not available, a sample collected from another EDS-EP sampling point, will be used for determination of DSP content and CBC.

v) Patients meeting all selection criteria at Baseline will be randomized (1:1:1) to one of the three treatment groups.

w) Blood sample for serum creatinine measurement to be taken before infusion.

x) Vital signs to include height and weight measurements in triplicate at screening, baseline and each monthly visit (pre-dose).

y) Month 12 (Visit 15; Endpoint) evaluations should be performed for all patients that complete the Extension Treatment Period, as well as patients that discontinue prematurely.

z) The monthly infusions should be performed every 21-28 days. A window of ± 7 days will be permitted on the Day 15 visit and of + 10 days on each of the scheduled monthly post-baseline visits (Months 1 - 12). However, no EDS-EP infusion should be performed less than 21 days after the previous infusion. The window between an infusion and the subsequent one should be kept as regular as possible throughout the study, avoiding fluctuations in administration windows. The date of an infusion is not bound to the date of the initial treatment but to the date of the previous IMP administration

aa) 1 mL blood collected, after blood diversion, for aerobic culture, before EDS process (see "Revised Study Procedures on Sterility Testing for Study IEDAT-02-2015 (ATTeST)"; Appendix 13). Moreover, a sample of the EDS-EP (approximately 1 ml per inoculum for a total of 2 mL) will be collected from the satellite sample bag, to perform a culture-based sterility test. A 1-mL sterile sample of the EDS-EP will be stored under refrigeration as a "Retention Sample".

(£) In case a patient is willing to be enrolled in the open-label study IEDAT-03-2018, but the study has not yet received IRB/EC approval at the time the patient reaches Visit 15/Month 12, the patient may undergo unscheduled monthly assessments/infusions (according to the assigned blinded treatment) starting from the time Visit 15/Month 12 should be performed, and continuing until the site has been initiated for the open-label study IEDAT-03-2018 and he/she has been evaluated for eligibility for the study. An additional Informed Consent Form must be signed prior to the 1st unscheduled visit/infusion.

(§) As a results of the COVID-19 pandemic, temporary additional safety assessments may be requested at some visits. These changes are described in Appendix 14.

11.4 Visit Schedule and Assessments – Initial Treatment Period

11.4.1 Screening (Days -30 to -1)

At Screening (Days -30 to -1) each patient will report to the clinic where the following procedures will be performed to establish eligibility for the study:

a) Obtaining written informed consent (before any study procedures)

b) Demography

c) Medical history

d) Physical/neurological examination

e) Vital signs – body weight and height in triplicate, calculation of BMI, temperature (orally or tympanic, according to the specific medical practice of the Center), pulse, systolic and diastolic BP, and respiratory rate

f) Laboratory evaluations comprising the following tests:

   i. hematology - RBC, WBC, hemoglobin, hematocrit, platelets, neutrophils, eosinophils, basophils, lymphocytes, monocytes, MCV, MCH, MCHC, and RDW

   ii. clinical chemistry – total protein, albumin, bilirubin, AST, ALT, BUN, creatinine, sodium, potassium, chloride, bicarbonate, calcium, phosphorus, serum iron, LDH, alkaline phosphatase, glucose, CPK, triglycerides, and cholesterol (total, HDL and LDL)

   iii. urinalysis (automated) – color, pH, specific gravity, glucose, ketones, nitrites, protein, bilirubin, hemoglobin, urobilinogen and reflex microscopic RBC, WBC and casts (if indicated)

   iv. serum pregnancy test (women of childbearing potential)

g) Special laboratory tests: HbA1c, CD4+ lymphocytes count, α–fetoprotein, CRP, and RBC antibodies (IgG, IgM, Qualitative Direct Coombs test).

h) Blood sample (5 mL) for assessing RBC osmotic resistance (in selected centers)

i) Mini-ATM detection – 2.5 mL of blood collected

j) 12-lead standard ECG

k) ICARS (video recording not required)

l) CGI-S

m) Prior (previous 4 weeks) and concomitant medications

n) Adverse events (AEs) occurring after giving informed consent

o) Blood sample for AT genetic testing, if the test has not been performed previously. *For patients who require DNA sequencing and Western Blotting to confirm the genetic diagnosis of AT, test results do not need to be available before baseline.*

p) Plasma cortisol – sample to be collected before 8:00 AM and prior to randomization at baseline. If the basal cortisol level is within the reference normal range, the patient can be enrolled in the study. If the 8:00 AM cortisol level is below 3-5 µg/dL (depending on assay) regardless of symptoms, or the patient exhibits signs or symptoms of adrenal insufficiency (see Appendix 12) and has a cortisol <10 µg/dL, the patient will receive a high dose ACTH stimulation test, regardless of weight, within 24 hours. If the

ACTH stimulation test is normal, the patient can be enrolled after the effects of the ACTH have dissipated (e.g., 30 days after ACTH administered). If the patient fails the ACTH stimulation test they will be excluded from the study and referred to a pediatric endocrinologist, with a recommendation to prescribe stress dose steroids.

q) Patient eligibility and inclusion/exclusion criteria checklist **(all test results must be available before the Day 0 visit).**

11.4.2 Day 0/1 (Visit 1): Pre-Infusion (Baseline)

Baseline assessments to be performed on Day 0 and Day 1 (pre-dose) are as follows:

a) Inclusion and exclusion criteria checklist; review of all selection criteria.

b) Vital signs - body weight and height in triplicate, calculation of BMI, temperature (orally or tympanic, according to the specific medical practice of the Center), pulse, systolic and diastolic BP, and respiratory rate. BP and pulse will be done 3 times (~10 min apart) starting at least 1 hour prior to dosing;

c) Physical/neurological examination

d) Laboratory evaluations, on the diverted blood sample (see Section 11.6), comprising the following tests:

   i. hematology *
   ii. clinical chemistry *
   iii. urinalysis *
   iv. serum and urine pregnancy test (women of childbearing potential)

e) Assessment of physical development, sexual maturation, and the effect of treatment with dexamethasone on these aspects (Tanner scale)

f) Special laboratory tests: HbA1c, CD4+ lymphocytes count, and CRP.

g) Hemolysis panel [free plasma hemoglobin, haptoglobin, bilirubin (total and conjugated), LDH]

h) 12-lead standard ECG *

i) ICARS – To be performed ~24 hours prior to dosing (Day 0; video recording required) and on Day 1 (baseline assessment prior to infusion; video recording required)

j) CGI-S

k) VABS

l) EQ-5D-5L

m) C-SSRS

n) Bone mineral density

o) 1 mL blood collected, after blood diversion (see Section 11.6), for aerobic culture

p) Autologous blood collection for EDS processing

q) Blood sample for plasma dexamethasone (pre-dose blank – 0 min)

r) CCI [redacted]

[redacted]

t) Upon completion of the EDS process 5 mL of the EDS-EP from the EryKit_01 bowl at the end of the process will be collected to assess RBC osmotic resistance in selected centers.

u) Review of AEs – reported by the patient or observed by the Investigator

v) Review of concomitant medication

w) **Infusion of EDS-EP autologous RBCs.**

*****_These routine laboratory and ECG evaluations will be repeated only if abnormalities requiring follow-up were noted on the Screening evaluations; results from these repeat assessments must be available at baseline to confirm eligibility before the patient can be randomized to treatment._

*11.4.3 **Day 1 (Visit 1): Post-Infusion***

a) Physical examination

b) Vital signs - temperature (orally or tympanic, according to the specific medical practice of the Center), pulse, systolic and diastolic BP, and respiratory rate;

c) Blood samples for plasma dexamethasone –1 and 4 hr after the end of the infusion.

d) Hemolysis panel [free plasma hemoglobin only, 1 hr post-infusion]

e) Review of AEs – reported by the patient or observed by the Investigator

f) Review of concomitant medication

*11.4.4 **Day 2 (Visit 2; 24 hours post-infusion)***

a) A-T NEST

b) Blood sample for plasma dexamethasone collected 24 (± 2) hours post-infusion.

b) Hemolysis panel [free plasma hemoglobin, haptoglobin, bilirubin (total and conjugated), CBC, LDH, urinalysis]

c) Review of AEs – reported by the patient or observed by the Investigator

d) Review of concomitant medication

*11.4.5 **Day 15 (Visit 3)***

a) Blood sample for plasma dexamethasone – single sample to be collected in the morning

b) Hemolysis panel [free plasma hemoglobin, haptoglobin, bilirubin (total and conjugated), CBC, LDH, urinalysis]

c) Review of AEs – reported by the patient or observed by the Investigator

d) Review of concomitant medication

11.4.6 ***Day 30 (Month 1; Visit 4)***

a) Physical examination

b) Vital signs – including body weight and height in triplicate,

c) Laboratory evaluation on the diverted blood sample: serum creatinine

d) Urine pregnancy test will be performed before infusion (for women of childbearing potential)

e) C-SSRS

f) A-T NEST

g) 1 mL blood collected, after blood diversion, for aerobic culture

h) Autologous blood collection for EDS processing

i) Blood sample for plasma dexamethasone (trough) infusion.

j) CCI [redacted]

[redacted]

l) Review of AEs – reported by the patient or observed by the Investigator

m) Review of concomitant medication

n) **Infusion of EDS-EP autologous RBCs.**

11.4.7 ***Month 2 (Visit 5)***

a) Physical examination

b) Vital signs – including body weight and height in triplicate,

c) Laboratory evaluation on the diverted blood sample: serum creatinine

d) Urine pregnancy test will be performed before infusion (for women of childbearing potential)

e) C-SSRS

f) Mini-ATM detection on the diverted blood sample

g) 1 mL blood collected, after blood diversion, for aerobic culture

h) Autologous blood collection for EDS processing

i) Blood sample for plasma dexamethasone prior to (trough) and 1 hr after (peak) the end of infusion

j) CCI [redacted]

[redacted]

l) Review of AEs – reported by the patient or observed by the Investigator.

m) Review of concomitant medication

n) **Infusion of EDS-EP autologous RBCs.**

11.4.8 ***Month 3 (Visit 6)***

Pre-infusion

a) Physical/neurological examination

b) Vital signs – including body weight and height in triplicate,

c) 12-lead standard ECG

d) Laboratory evaluations on the diverted blood sample, comprising the following tests:

   i. hematology

   ii. clinical chemistry

   iii. urinalysis

e) Urine pregnancy test will be performed before infusion (for women of childbearing potential)

f) C-SSRS

g) ICARS (with video recording)

h) CGI-C

i) CGI-S

j) VABS

k) EQ-5D-5L

l) Hemolysis panel [free plasma hemoglobin, haptoglobin, bilirubin (total and conjugated), LDH]

m) 1 mL blood collected, after blood diversion, for aerobic culture

n) Autologous blood collection for EDS processing

o) Blood sample for plasma dexamethasone prior to (trough) and 1 hr after (peak) the end of infusion

p) Upon completion of the EDS process, fill the satellite sample bag with approximately 6 mL of EDS-EP by gravity (approximately 2 mL if no other samples but the ones for sterility culture test will be collected).

q) CCI [redacted]

[redacted]

s) Review of AEs – reported by the patient or observed by the Investigator.

t) Review of concomitant medication

u) **Infusion of EDS-EP autologous RBCs.**

Post-infusion

v) Physical examination

w) Vital signs

x) Hemolysis panel [free plasma hemoglobin only, 1 hr post-infusion]

11.4.9 ***Month 4 (Visit 7)***

a) Physical examination

b) Vital signs – including body weight and height in triplicate,

c) Laboratory evaluation on the diverted blood sample: serum creatinine

d) Urine pregnancy test will be performed before infusion (for women of childbearing potential)

e) C-SSRS

f) A-T NEST

g) 1 mL blood collected, after blood diversion, for aerobic culture

h) Autologous blood collection for EDS processing

i) Blood sample for plasma dexamethasone prior to (trough) and 1 hr after (peak) the end of infusion

j) CCI [redacted]

[redacted]

l) Review of AEs – reported by the patient or observed by the Investigator

m) Review of concomitant medication

n) **Infusion of EDS-EP autologous RBCs.**

11.4.10 ***Month 5 (Visit 8)***

a) Physical examination

b) Vital signs – including body weight and height in triplicate,

c) Laboratory evaluation on the diverted blood sample: serum creatinine

d) Urine pregnancy test will be performed before infusion (for women of childbearing potential)

f) C-SSRS

g) 1 mL blood collected, after blood diversion, for aerobic culture

h) Autologous blood collection for EDS processing

i) Blood sample for plasma dexamethasone prior to (trough) and 1 hr after (peak) infusion

j) CCI [redacted]

[redacted]

l) Review of AEs – reported by the patient or observed by the Investigator

m) Review of concomitant medication

n) **Infusion of EDS-EP autologous RBCs.**

11.4.11 ***Month 6 (Visit 9; Endpoint Evaluation for Efficacy)***

The following procedures will be performed at the final visit on Month 6, or if the patient discontinues from the study prematurely:

a) Physical/neurological examination

b) Vital signs – body weight and height in triplicate, temperature (orally or tympanic, according to the specific medical practice of the Center), pulse, systolic and diastolic BP, and respiratory rate

c) Blood sample for plasma dexamethasone – single sample to be taken in the morning (just before the infusion for patients continuing in the 6-month Extension Treatment Period)

d) 12-lead standard ECG

e) Laboratory evaluations on the diverted blood sample, for patients continuing in the 6-month Extension Treatment Period, comprising the following tests:

   i. hematology

   ii. clinical chemistry

   iii. urinalysis

iv. serum pregnancy test (women of childbearing potential)

f) Assessment of physical development, sexual maturation , and the effect of treatment with dexamethasone on these aspects (Tanner scale)

g) Special laboratory tests on the diverted blood sample for patients continuing in the 6-month Extension Treatment Period: HbA1c, CD4+ lymphocytes count, α–fetoprotein, CRP, and RBC antibodies (IgG, IgM, Qualitative Direct Coombs test)

h) Mini-ATM detection on the diverted blood sample for patients continuing in the 6-month Extension Treatment Period

i) ICARS (with video recording)

j) CGI-C

k) CGI-S

l) VABS

m) EQ-5D-5L

n) C-SSRS

o) Bone mineral density

p) Review of AEs – reported by the patient or observed by the Investigator

q) Review of concomitant medication

11.5 Visit Schedule and Assessments – Extension Treatment Period

11.5.1 ***Month 6 (Visit 9)***

Final evaluations for the 6-month Initial Treatment Period will serve as baseline evaluations for patients continuing in the 6-month Extension Treatment Period.

a) Obtain written informed consent for the extension period

b) Randomized switch of one-third of placebo patients to EDS-EP treatment

c) 1 mL blood collected, after blood diversion, for aerobic culture

d) Autologous blood collection for EDS processing

e) Upon completion of the EDS process, fill the satellite sample bag with approximately 6 mL of EDS-EP by gravity (approximately 2 mL if no other samples but the ones for sterility culture test will be collected).

f) CCI [redacted]

[redacted]

h) Hemolysis panel [free plasma hemoglobin, haptoglobin, bilirubin (total and conjugated), LDH]

i) Urine pregnancy test will be performed before infusion (for women of childbearing potential)

j) Review of AEs – reported by the patient or observed by the Investigator

k) Review of concomitant medication

l) **Infusion of EDS-EP autologous RBCs.**

Post-infusion

m) Physical examination

n) Vital signs

o) Hemolysis panel [free plasma hemoglobin only, 1 hr post-infusion]

*11.5.2 **Months 7, 8, 10 and 11 (Visits 10, 11, 13 and 14)***

a) Physical examination

b) Vital signs – including body weight and height in triplicate,

c) Laboratory evaluation on the diverted blood sample: serum creatinine

d) Urine pregnancy test will be performed before every infusion (for women of childbearing potential)

e) C-SSRS

f) A-T NEST (at Months 7 and 10 [Visits 10 and 13] only)

g) 1 mL blood collected, after blood diversion, for aerobic culture

h) Autologous blood collection for EDS processing

i) CCI [redacted]

[redacted]

k) Review of AEs – reported by the patient or observed by the Investigator

l) Review of concomitant medication

m) **Infusion of EDS-EP autologous RBCs.**

*11.5.3 **Month 9 (Visit 12)***

Pre-infusion

a) Randomized switch of another one-third of placebo patients to EDS-EP treatment

b) Physical/neurological examination

c) Vital signs – including body weight and height in triplicate,

d) 12-lead standard ECG

e) Laboratory evaluations on the diverted blood sample, comprising the following tests:

   i. hematology

   ii. clinical chemistry

   iii. urinalysis

f) Hemolysis panel [free plasma hemoglobin, haptoglobin, bilirubin (total and conjugated), LDH]

g) Urine pregnancy test will be performed before infusion (for women of childbearing potential)

h) C-SSRS

i) ICARS (with video recording)

j) CGI-C

k) CGI-S

l) VABS

m) EQ-5D-5L

n) 1 mL blood collected, after blood diversion, for aerobic culture

o) Autologous blood collection for EDS processing

p) CCI [redacted]

[redacted]

[redacted]

s) Review of AEs – reported by the patient or observed by the Investigator.

t) Review of concomitant medication

u) **Infusion of EDS-EP autologous RBCs.**

Post-infusion

v) Physical examination

w) Vital signs

x) Hemolysis panel [free plasma hemoglobin only, 1 hr post-infusion]

*11.5.4 **Unscheduled Visit(s)***

In case a patient is willing to be enrolled in the open-label study IEDAT-03-2018, but the study has not yet received IRB/EC approval at the time the patient reaches Visit 15/Month 12, the patient may undergo unscheduled monthly assessments/infusions (according to the assigned blinded treatment) starting from the time Visit 15/Month 12 should be performed, and continuing until the site has been initiated for the open-label study IEDAT-03-2018 and he/she has been evaluated for eligibility for the study.

a) Obtain written informed consent (prior to the first additional infusion only)

b) Physical examination

c) Vital signs – including body weight and height in triplicate,

d) Laboratory evaluation on the diverted blood sample: serum creatinine

e) Urine pregnancy test will be performed before every infusion (for women of childbearing potential)

f) C-SSRS

g) 1 mL blood collected, after blood diversion, for aerobic culture

h) Autologous blood collection for EDS processing

i) CCI [redacted]

[redacted]

k) Review of AEs – reported by the patient or observed by the Investigator

l) Review of concomitant medication

m) **Infusion of EDS-EP autologous RBCs.**

*11.5.5 **Month 12 (Visit 15; Final Visit)***

a) Physical/neurological examination

b) Vital signs – including body weight and height in triplicate,

c) 12-lead standard ECG

d) Laboratory evaluations on the diverted blood sample, comprising the following tests:

   i. hematology

   ii. clinical chemistry

   iii. urinalysis

   iv. serum pregnancy test (women of childbearing potential)

e) Assessment of physical development, sexual maturation, and the effect of treatment with dexamethasone on these aspects (Tanner scale)

f) Special laboratory tests on the diverted blood sample: HbA1c, CD4+ lymphocytes count, α-fetoprotein, CRP, and RBC antibodies (IgG, IgM, Qualitative Direct Coombs test)

g) ICARS (with video recording)

h) CGI-C

i) CGI-S

j) VABS

k) EQ-5D-5L

l) C-SSRS

m) Bone mineral density

n) Review of AEs – reported by the patient or observed by the Investigator.

o) Review of concomitant medication

11.5.6 ***Safety Follow-up Visit (Visit 16)***

All patients who discontinue prematurely, as well as those who complete the 12-month treatment period but do not continue treatment in the open-label extension study, will be required to return for a Safety Follow-up Visit (Visit 16) 30 days after their final assessment or at least 60 days after their last infusion, whichever is longer. At this visit, A-T NEST will be administered and the occurrence of any AEs or Serious AEs (SAEs) reported by the patient/caregiver or observed by the Investigator since the previous visit will be recorded.

11.5.7 ***Visit Windows***

A window of ± 7 days will be permitted on the Day 15 visit and of + 10 days on each of the scheduled monthly post-baseline visits (Months 1 - 12). However, no EDS-EP infusion should be performed less than 21 days after the previous infusion. The window between an infusion and the subsequent one should be kept as regular as possible throughout the study, avoiding fluctuations in administration windows

11.6 **Laboratory Sample Collection**

Revised study procedures to ensure sterility of the EDS-EP

New aseptic procedures have been implemented to ensure sterility during blood collection for the EDS process, the various steps of the EDS process, sample collection for routine laboratory tests, sample collection from the EDS-EP for sterility testing, and administration of the EDS-EP to the patient. Details of these procedures are described in a separate document entitled "Revised Study Procedures on Sterility Testing for Study IEDAT-02-2015 (ATTeST)", which will be provided to each site (see Appendix 13). These new procedures include the following measures that must be performed to ensure sterility:

- Blood sampling under sterile conditions.
- Careful hand washing and use of fresh (non-sterile) gloves for each patient, disinfected frequently using a bactericidal rub.
- Frequent and repeated use of a bactericidal solution, including the mandatory use of 2% chlorhexidine in 70% isopropyl alcohol for disinfection of the skin at the venipuncture site, or

during handling of the venous catheter (if used), and for disinfecting ports, connectors and working surfaces/environments.

- CCI [redacted]

Blood sampling

Venous blood samples will be drawn for routine safety evaluations (hematology and clinical chemistry). The maximum volume of blood drawn for laboratory analyses on any one day will be approximately 68 ml:

- 1.0 mL for hematology tests [Screening, Day 0 (optional) and Months 3, 6, 9 and 12]
- 5.0 mL for clinical chemistry [Screening, Day 0 (optional) and Months 6 and 12]
- 3.5 mL for clinical chemistry [Day 0 (optional) and Months 3 and 9]
- 1.0 mL for CD4+ lymphocytes tests (Screening, Day 0, Month 6 (pre-dose), and Month 12); samples for alpha fetoprotein, C-reactive protein, and Hb1Ac to be taken at same timepoints from blood collected for clinical chemistry or hematology.
- 1.0 mL for RBC antibody testing (Screening, and Months 6 and 12)
- Sample for serum pregnancy test (Screening, Day 0 and Months 6 and 12) to be taken from blood collected for clinical chemistry.
- Hemolysis panel (pre and post infusion):
    - 1.0 mL for free hemoglobin (Day 0, Day 1 - 1 hr post-infusion, Months 3, 6, 9 pre- and post-dose, and Days 2, and 15);
    - 3.5 mL for haptoglobin (Days 0, 2 and 15, and Months 3, 6 and 9 pre-dose);
    - Bilirubin, LDH will be measured from the clinical chemistry or haptoglobin samples;
    - 1.0 mL for CBC (Days 2 and 15); CBC at Day 0 and Months 3, 6 and 9, will be measured from the hematology samples.
- 2.5 mL for Mini-ATM detection (Screening and Months 2 and 6).
- 1.0 mL for separate serum creatinine measurement (Months 1, 2, 4, 5, 7, 8, 10 and 11)
- 2.0 mL for plasma cortisol measurement (Screening, and when adrenal insufficiency is suspected [Appendix 12]);
- 6.0 mL for the high dose ACTH stimulation test (to be peformed in response to signs or symptoms of adrenal insufficiency [Appendix 12]) – samples to be taken prior to ACTH administration (0 min) and 30 and 60 min post-dose.

Results of plasma cortisol measurements and the ACTH stimulation test will be made available only to the Principal Investigator, and will not be available to raters performing the primary and secondary efficacy asssessments.

- 3.0 mL for PK samples [Day 1 (0 hr), Day 2 (24 hr), Day 15, and Months 2, 3, 4, and 5 (pre- and post-dose), and Month 1 and 6 (pre-dose)].
- 1.0 mL for PK samples [Day 1 (1 and 4 hr post-dose)].

In addition, on the day of the infusion ~50 mL of blood will be drawn for use in the EDS Process and assessments on EDS-processed RBCs.

The total amount of blood to be drawn on any single day or within any 8-week period conforms to the guidelines specified for pediatric patients by the World Health Organization (Howie, 2011).

Urine collection

Urine needed for the urinalysis will be collected at Screening, Day 0 (optional), Days 2 and 15 (as part of hemolysis panel) and Months 3, 6, 9 and 12, according to local laboratory guidelines.

Pregnancy test

All female patients of childbearing potential will have a blood sample drawn at Screening, Baseline (Day 0), Month 6 and Month 12 (final visit) for a serum pregnancy test. "Non-childbearing potential" is defined as follows:

- surgical sterilization (hysterectomy, bilateral oophorectomy, or bilateral tubal ligation), or
- patient is pre-pubertal, with confirmation that menstruation has not started.

Results of the serum pregnancy test must be negative at Screening for the patient to be included in the study.

A urine pregnancy test will be performed montly before every infusion. Results of the urine pregnancy test must be negative at Baseline (Day 0) for the patient to be randomized.

All blood and urine samples for safety (hematology/ clinical chemistry/ urinalysis) and special laboratory evaluations will be analyzed by an external (central) laboratory.

11.7 Measurement of Dexamethasone Sodium Phosphate and Other Analytes in Infusion Bag Sample

Once the EDS encapsulation procedure has been completed, a sample of the contents of the infusion bag will be collected for analysis of the dexamethasone sodium phosphate (DSP) concentration, so that the actual dose administered to each patient can be determined. The procedure to be followed for this sample is as follows:

1. After the EDS encapsulation procedure is completed, the erythrocyte suspension in the collection bag will be gently mixed and aseptically sampled for the following assays;
2. Two different 500-µL aliquot of the erythrocyte suspension will be transferred to two cryo-tube with a screw cap, which will be stored immediately at < -20°C in a monitored freezer until analysis (samples require immediate freezing to avoid modification of DSP content). The tubes will be labeled with the following information:
    a. Study No.
    b. Sample from Infusion Bag

c. Center No.
d. Subject No.
e. Nominal timepoint of sample collection

3. The tubes will be kept frozen at < -20°C pending shipment for analysis. Samples will be shipped to the laboratory [INC/inVentiv Health Clinique, 2500 Einstein Street, Québec, QC, GIP 0A2, Canada] in batches. The site will organize these shipments when all samples have been collected for more than one patient. The laboratory will analyze the DSP concentration in a sample from one tube using a validated **LC/MA/MS** method (see Appendix 8). The second sample will be kept frozen as a control.
4. INC/inVentiv Health Clinique will provide the results for dexamethasone sodium phosphate concentration, which will be transferred to the database.

11.8 Concomitant Medications

All patients to be included in the study must **not** have received **oral or parenteral** steroid therapy within 4 weeks prior to the administration of EDS. However, treatment with inhaled or intranasal steroids for asthma or allergies, as well as the use of topical steroids, will be permitted. In addition, patients with a history of use or currently using any of the following medications will be excluded from participation, according to the guidelines specified:

- Narcotic analgesics – use within 4 weeks prior to baseline;
- Antipsychotics - unless used at a low, stable dose starting at least 4 weeks prior to baseline, in which case they will be permitted;
- Drugs that are strong inducers (e.g. carbamazepine, St. John's wort) or inhibitors (e.g. clarithromycin, grapefruit juice) of CYP3A4 within 4 weeks prior to baseline;

  Drugs that are inducers or inhibitors of Cytochrome P450 3A4 (CYP3A4) may alter the plasma levels of dexamethasone, which is metabolized by this enzyme. Therefore, in the current study, any drug that is a strong inducer or inhibitor of CYP3A4 will be prohibited from use during the trial. Patients who are taking these drugs upon entry into the screening period should have the medication discontinued, if possible, or be switched to another similar medication that does not have this property. A link to a website with a list of drugs that are strong inducers or inhibitors of CYP3A4 will be provided to the Investigator as a reference.

- Amphotericin-B within 4 weeks prior to screening – combination with corticosteroids or corticotropin (ACTH) may induce hypopotassemia;
- Any immunization, vaccination or skin test, especially using a live, attenuated vaccine, within 4 weeks prior to screening. The use of a vaccine that contains killed viruses is left to the clinical judgement of the Principal Investigator, and the standard of care at the site.

Patients on stable doses of other drugs, such as antihypertensives, benzodiazepines, antihistamines (histamine receptor blockers), birth control, proton-pump inhibitors, vitamins/multi-vitamins, anti-diabetics agents and lipid-lowering agents (e.g. statins), will be eligible for the study. The use of other concomitant medications with CNS effects should be discussed with and approved by the Medical Monitor at the CRO before prescribing to the patient during the study. Use of immunoglobulins either administered by IV or IM route is permitted; the Investigator should determine the optimal timing of the dose with respect to the EDS infusion.

During the entire period of the study, starting with the signing of the ICF, any new treatment that is initiated must be reported in the CRF using the pharmacological name (not the trade name, with the exception of medicines in fixed combination), specifying daily dose, route, duration of treatment, reason for use. After screening, patients should be instructed to contact the Investigator before starting any OTC or prescription medication on their own or as prescribed by their physician. The Medical Monitor at the CRO should be informed of any new medication that may be a prohibited medication and is started during the study, and will make a decision whether or not it is acceptable for the patient to continue in the trial.

12 EFFICACY EVALUATIONS

12.1 ICARS

12.1.1 Description of the ICARS

The primary efficacy endpoint in the AT study will be the mean change from baseline to endpoint (Month 6 or early discontinuation) in the total score on the 'Modified' International Cooperative Ataxia Rating Scale (ICARS).

The ICARS (Trouillas et al, 1997), the most frequently used clinician-(neurologist) rated measure in patients with ataxias, was developed by a Committee of the World Federation of Neurology to help standardize common manifestations of syndromes that lead to cerebellar dysfunction. The ICARS is a 100-point, semi-quantitative scale offering a compartmentalized quantification of the following 4 sub-scores: Posture and Gait Disturbances (34 points), Kinetic Functions (52 points), Speech Disorders (8 points), and Oculomotor Disorders (6 points).

The complete ICARS will be rated in the current study; however, for the primary efficacy endpoint, the 'Modified' ICARS will used. The 'Modified' ICARS excludes all of the Oculomotor Disorders items (items 17-19) and items 8-12 in the Kinetic Functions domain, as these items do not directly contribute to functioning.

12.1.2 Validity of ICARS in AT Patient Population

The internal consistency, criterion-related validity, and internal construct validity of the ICARS have been established in patients with focal cerebellar lesions (Schoch B et al, 2007). The inter-rater reliability, test-retest reliability and internal consistency have also been validated in patients with spinocerebellar ataxia (Weyer et al, 2007, Schmitz-Hubsch et al , 2006a). The ICARS total score satisfied all psychometric criteria in a validation study in patients with Friedrich's Ataxia (Cano et al, 2005; Metz et al 2013) The ICARS has been used as an outcome measure in interventional studies in patients with ataxia, but there are no clinical data to suggest the magnitude of change on the ICARS that would correspond to a clinically relevant improvement, due to the absence of any drug producing such a benefit. The ICARS has been used in several studies in patients with Friedreich's Ataxia over the age of 8 years, in phase III potentially pivotal trials (Lynch et al, 2010), as well as in open-label long-term studies (Meier et al, 2012).

The ICARS was used also in two interventional studies in children with AT. Zanolli et al, 2012 showed a statistically significant decrease in the ICARS total score in a placebo-controlled crossover study in 13 children with AT treated with oral betamethasone (Zannolli et al, 2012). Nissenkorn et al, using ICARS as a secondary endpoint, showed improvement in the static and kinetic subscales in a short-term open-label study in 17 children (from 4 years of age) treated with amantadine (Nissenkorn et al, 2013). As AT is a severe, relentlessly progressive disorder that leads to loss of independent movement in most patients by the age of 10 to 16 years, any statistically significant difference from placebo over a 6-month period should be considered as a treatment success.

Most of the developmental and validation studies of the ICARS were performed in patients who were 10 years of age or older; an international study has been completed (ClinicalTrials.gov: NCT01942850) to extend its validity to patients in the age-range of 5 to10 years. Data collected from the validation study in selected centers [Data on file, EryDel S.p.A] indicated that the distribution of scores for the ICARS for patients with AT under 10 years of age was similar to the scores for these patients on other validated scales such as the SARA (Schmitz-Hubsch et al, 2006b) and the BARS (Schmahmann et al, 2009). Data collected from the validation study in selected centers confirmed that the severity of AT increased with age in patients under the age of 10 years, as demonstrated by a significant correlation of the total scores on the ICARS,

SARA, and the BARS, but not for patients above 10 years. Overall, the distribution of scores for the ICARS for 5 to 10 year-old patients with AT was similar to the scores for other validated scales such as the SARA, BARS, CGI-S (structured and unstructured versions).

*12.1.3 **Standardization of Ratings and Use of Central Remote Rater***

To maximize the consistency of the data obtained from the ICARS, the same neurologist, qualified, trained, and certified in the use of the ICARS and with expertise in the field of AT disorders, will evaluate the same patient at approximately the same time throughout the study. ICARS ratings should be completed without consulting scores from the previous visit. In addition, each ICARS administration will be video recorded according to a standardized procedure. The video recordings from each patient will be sent to central remote raters, where they will be blindly evaluated in random order. The ICARS ratings from the central remote raters will be used for the primary efficacy endpoint analysis.

The ICARS will be performed at screening and repeated twice at Baseline, on Day 0 and on Day 1 prior to administration of study medication. Both the Day 0 and Day 1 assessments will be videotaped and scored by the local rater. The Day 1 assessment video will be rated by the central rater and used as a baseline covariate in the analysis. In the event that the Day 1 assessment could not be performed, or is not available, the Day 0 assessment will be rated by the central rater and will be used as the baseline.

12.2 **Clinical Global Impression (CGI-C and CGI-S)**

The CGI (Guy, 1976) is the general name for two scales, the CGI - Change scale (CGI-C) and CGI- Severity scale (CGI-S). The CGI-C scale assesses the change in the patient's clinical status from **baseline** using a 7-point scale, ranging from 1 (very much improved) to 7 (very much worse), with a score of 4 indicating no change. In the current study, the change from the patient's baseline condition will be assessed at all post-baseline visits, and the mean rating of change at Endpoint (Month 6 or early discontinuation) will be the key secondary efficacy measure The rater performing the CGI-C will be different from the rater performing the ICARS rating, and will assess the patient without access to the ICARS ratings or safety data. A guideline for performing the rating of change using the CGI-C is provided below.

For the CGI-C rating, clinicians will be required to conduct a full clinical interview and examination of the patient, if necessary with the caregiver present. The interview and examination should assess various aspects of the patient's appearance (grooming, evidence of falls, etc.), ataxia, cognition (orientation, calculation ability, language, ability to follow commands, memory, etc.), apraxia, dysarthria, extrapyramidal motor symptoms, activities of daily living and mood. The CGI-C rating should be based on a holistic assessment of the patient, and should not be anchored to specific change or cut-off scores of performance-based measures. It is not necessary for the rater to use specific scales or be guided by their scores for severity; however, the rater may review findings on other measures that are assessed in the trial, e.g. VABS, quality of life scale (EQ-5D-5L), to guide their assessments. The raters may also use additional scales or measures to assess specific domains; however, it is important that the raters use the same methods at baseline and at all subsequent visits.

The CGI-S scale measures global severity of illness at a given point in time, and is usually rated on a 7-point, Likert-type scale ranging from 1 (normal, not ill at all) to 7 (among the most extremely ill patients). However, no version of the CGI-S exists which has been specifically adapted for use in AT patients; therefore, a 5-point version was developed that takes into account the severity of the following symptoms of AT: ataxia (walking), dysarthria, dysmetria, extrapyramidal symptoms (chorea, myoclonus, dystonia, and tremor), and eye movements. Ratings of none (0), mild (1), moderate (2), severe (3) and very severe (4) are selected based on the level of symptomatology. Two independent versions of the CGI-S scale were developed, a structured scale, based on the 5 disease-related anchors and an unstructured scale based on the clinical judgment of an experienced physician. Both the structured and unstructured versions were field-

tested. The final structured CGI-S score is based on the severity of the symptoms in each domain, as indicated below:

- A rating of 0 (not ill/asymptomatic) can be made only if there are no symptoms, or a rating of mild on only one symptom domain;
- A rating of 1 (mild) can be made only if there are not more than 2 domains rated greater than mild;
- A rating of 2 (moderate) can be made only if there are not more than 2 domains rated greater than moderate;
- A rating of 3 (severe) can be made only if there are not more than 2 domains rated greater than severe;
- A rating of 4 (very severe) can be made only if there are at least 2 domains rated as severe and at least 2 rated as very severe.

A pilot validation study has been performed in AT patients using the CGI-S scale, and the results indicate that the CGI-S adequately discriminated patients with mild, moderate, or severe disease [Data on file, EryDel S.p.A.]. The distribution of patients to different scores on the CGI-S of AT was similar using the ICARS, BARS, and SARA in 6 to 9 year olds. The CGI-S for patients 10 years of age or older was greater than in 6 to 9 year old patients; however, the distribution of scores on the ICARS, BARS, and SARA was similar across severity categories.

12.3 Vineland Adaptive Behaviors Scale (VABS)

The VABS has been included as a secondary efficacy measure to assess adaptive behavior (Sparrow and Cicchetti, 1985; Sparrow et al, 2005). The expanded version of the VABS consists of 540 items, 261 of which were taken from the short form, which will be used in this study. The VABS is administered using a semi-structured interview by a qualified interviewer with a parent or caregiver who takes care of the patient. Adaptive behavior is measured according to four scales, each subdivided into eleven subscales. Each subscale is further divided into clusters (2 to 8 items) listed in evolutionary order; each cluster is sorted to a target item. A score is assigned to each item based on whether the patient performs that activity "usually", "sometimes" or "never"; answers of "no chance" or "I do not know" are also provided. Therefore, the complete form indicates the strengths and weaknesses of the patient in specific areas of adaptive behavior, allowing the psychologist or educator to select the program best suited to the patient, explain the activities to emphasize in the program, monitor progress during its use and assess the final outcome.

Scales and Subscales. The VABS is divided into four scales and eleven subscales: 1) *Communication*: Receptive, Expressive, Written; 2) *Daily Living Skills*: Personal, Domestic, Community; 3) *Socialization*: Interpersonal Relationships, Play & Leisure Time, Coping Skills; 4) *Motor Skills*: Gross, Fine. An optional fifth subscale, the Maladaptive Behavior Index, will not be assessed in this study.

Generally, to maximize data consistency, the same rater (psychologist or person experienced in use of the scale) should evaluate the same patient on the same assessments at approximately the same time of day, whenever possible. The assessments must be performed without consulting the results of the prior visit.

12.4 Quality of Life Scale

A patient/caregiver-rated assessment of quality of life (QoL) will be performed using the EQ-5D-5L scale. The EQ-5D is a standardized instrument for assessing health-related QoL, which provides a simple descriptive profile and a single index value for health status in a variety of health conditions (Rabin and de Charro, 2001). The scale was originally designed for use in adult populations, 18 years of age and older. The EQ-5D includes single item measures of five health dimensions: mobility, self-care, usual activities,

pain/discomfort, and anxiety/depression. Each item has three possible response options that allow the subject to rate (no problems/some or moderate problems/extreme problems) their current state with respect to each of the 5 domains. In addition, EQ-5D includes a global rating of current health using a visual analogue scale (VAS) ranging from 0 (worst imaginable) to 100 (best imaginable). The EQ-5D is cognitively simple, and provides a score that can be compared across different conditions (http://www.euroqol.org/).

The EQ-5D-5L-that will be used in this study is a newer version of the scale, which includes five levels of severity (i.e., no problems, slight problems, moderate problems, severe problems, and extreme problems) for each of the five EQ-5D dimensions (Herdman et al, 2011); rather than the three levels included in the previous version (EQ-5D-3L). The EQ-5D-5L has been validated in a diverse patient population in 6 coutries, including 8 patient groups with chronic conditions (e.g. cardiovascular disease, respiratory disease, depression, diabetes, liver disease, personality disorders, arthritis, stroke) and a student cohort (Janssen et al, 2013).

For children and adolescents, the EQ-5D-5L administrators will be trained to help the younger population in the study complete the scale. The EQ-5D-5L administrator will explain in detail the scale to the younger patients and will confirm that they understand how the scale should be completed. They will explain what each domain measures in a child friendly manner and will confirm that the respondent understands how to complete the scale. With regards to the usual activities domain, the administrator will explain that this domain measures if a subject has difficulties going to school, or with hobbies, sports, playing and doing things with family or friends. The administrator will be present during the completion and will be available to help the child if he/she does not understand a question or he/she has questions related to scale completion. If the patient is unable to complete the scale, it will be completed by the patient's parent/cargiver.

12.5 Rater Requirements and Training

Properly qualified raters will need to be identified at each site to perform the ratings on the efficacy measures. The ICARS rater must remain blinded to other assessments and will not have access to the safety data. The CGI rater will not have access to the ICARS ratings or safety data, but may refer to other scales in scoring the CGI. Training and certification on the ICARS and CGI will take place at the Investigator's meeting and additional training and an intra-rater reliability (test-retest) assessment will be performed during the study.

12.6 Order of Test Performance

The ICARS should be the first scale administered. The sequence of performance for the other efficacy assessments is not fixed across the study; however, the order in which these tests are performed for an individual patient should be kept constant throughout the trial.

12.7 Exploratory Efficacy Measure

*12.7.1 **A-T NEST***

Measurement of ataxia is difficult, as assessment of the magnitude of movement error – or "lack of order", the etymologic definition of "ataxia" – necessarily includes many complexities and confounders. Numerous ordinal scales have been developed to account for "lack of order" in various human conditions, and validated to widely varying stringency in different disease populations. The International Cooperative Ataxia Rating Scale (ICARS) was targeted to the 1997 understanding of features of cerebellar ataxia in 1997, and later validated in patients with Friedreich Ataxia (Cano et al, 2005), and focal cerebellar lesions (Schoch et al, 2007) before being used as the primary outcome measure for a therapeutic trial of subjects with ataxia telangiectasia (Broccoletti et al, 2008). Another scale for ataxia, the Scale for the Assessment

and Rating of Ataxia (SARA), was developed and validated for assessment of spinocerebellar ataxia (Schmitz-Hubsch et al, 2006) and later refined and compared to other scales for spinocerebellar ataxia subtypes (Monte et al, 2017).

A critical problem for measurement of the neurologic disability experienced by those children and adults with ataxia telangiectasia, however, is its broad range of abnormalities. Each of these are statistically independent contributors to an overall score, with many features more characteristic of classic models of known extrapyramidal dysfunction than cerebellar degeneration (Crawford et al, 2000). The original description of ataxia telangiectasia was titled as a disorder characterized by athetosis (Syllaba et al, 1926), with the "ataxia" label applied only after cerebellar pathology was identified (Boder et al, 1957). A scale encompassing the broadest range of the ataxia telangiectasia phenotype was developed and validated by multiple observers in a single institution in 2000 (Crawford et all, 2000). The A-T NEST scale, consisting of independent and combined elements assessing the range of the disorder, evolved out of that scale by consensus of international A-T clinical experts convened by the Ataxia Telangiectasia Children's Project (ATCP) over the last decade. Study IEDAT-02-2015 offers an opportunity to validate the A-T NEST scale with its well-defined training materials in a multicenter setting, comparing intra- and inter-rater reliability and individual subject stability from one assessment to the next. As such, it offers the ideal platform for development of a more sensitive and more meaningful outcome measure for future studies of the disorder.

The A-T NEST scale and detailed instructions will be provided to the sites selected for this exploratory analysis.

13 SAFETY EVALUATIONS

The assessment of safety and tolerability will be based on the following:

a) Vital signs

b) Standard laboratory tests (clinical chemistry, hematology, and urinalysis)

c) 12-lead standard ECG

d) Physical and neurological examinations

e) Special laboratory parameters

f) C-SSRS

g) BMD

h) Tanner staging

i) Subjective reporting of any AE by the patient

j) Objective observation of any AE by the Investigator

k) The investigator will be asked to comment on any clinically significant abnormal test results.

The frequency of investigations that involve blood draws have been modified to ensure that the volume of blood taken from patients conforms to the guidelines specified for pediatric patients by the World Health Organization (Howie, 2011).

13.1 Physical and Neurological Examinations

Physical and neurological examinations will be performed as specified on the Schedule of Visits and Assessments. If clinically significant abnormal findings are noted at Screening, the examination should be repeated prior to Baseline. The physical examination will include an examination of general appearance, skin, neck (including thyroid), eyes and ears, nose, mouth, throat, lungs, heart, abdomen, back, lymph nodes, extremities and nervous system. Genital, urinary tract and rectal examinations are not required. The findings will be entered on the Physical Examination section of the CRF.

13.2 Vital Signs

Vital signs assessments will be performed as specified on the Schedule of Visits and Assessments and will include body weight, temperature (orally or tympanic, according to the specific medical practice of the Center), pulse, systolic and diastolic blood pressure, and respiratory rate. Height will be measured by a stadiometer in triplicate at Screening and every subsequent visit, and used along with body weight (also measured in triplicate) to calculate Body Mass Index (BMI). Pulse and blood pressure will be measured after the patient has been in the supine position for at least 5 minutes. At Day 0/1, prior to dosing, measurements of blood pressure and pulse (supine) will be repeated 3 times, at least 10 minutes apart, and the values will be averaged to obtain the baseline values.

If a change *of clinical relevance* from pre-dose to post-dose is observed, the vital signs assessment should be repeated as often as needed, at the discretion of the Investigator. Guidelines for clinically notable vital signs values are provided in Appendix 4. Findings should be documented on the Vital Signs section of the Case Report Form.

13.3 Electrocardiogram (ECG)

All patients will have a standard 12-lead ECG performed as specified on the Schedule of Visits and Assessments. At Screening the ECG will be done in triplicate, at least 10 min apart, and the values will be averaged to obtain the baseline values. If clinically significant abnormal findings are noted at Screening and do not normalize on the repeat evaluation at Baseline (in triplicate), the patient will not be enrolled.

The review and interpretation of the 12-lead ECGs will be performed by a centralized ECG service, to ensure consistency across site. These results will be used for determination of a patient's eligibility for enrolment in the trial, as well as post-dose safety monitoring. An initial review of the ECG will be performed by a cardiologist or qualified physician at the investigational site. The report from the centralized ECG service must be reviewed by the Investigator, initialed and dated, and copies inserted in the patient's records and attached to the CRF. If clinically significant abnormalities are found, the patient's ECG should be repeated at regular intervals until it returns to normal.

Each ECG tracing must have the following information entered on it:

- Study number,
- Site number,
- Subject's number and initials,
- Date and time ECG obtained.

If clinically significant abnormalities are found, the patient's ECG should be repeated at regular intervals until it returns to normal. The cardiologist or qualified physician reviewing the ECGs should use the following guidelines in determining the clinical significance of any abnormal findings:

- PR interval: < 100 msec or > 210 msec
- QRS interval: < 50 msec or > 120 msec
- QTc interval: > 450 msec
- Heart rate: < 50 bpm (sinus bradycardia) or > 120 bpm (sinus tachycardia)
- Morphology: presence of T-wave inversion, abnormal R-waves, pathological Q-waves, or significant ST elevation or depression.

The ECG database created by the centralized service will be used in all analyses. Details of the procedures related to the centralized ECG service will be provided in a separate manual.

13.4 Standard Laboratory Evaluations and Screening Tests

Blood and urine samples for measurement of standard laboratory parameters will be as specified on the Schedule of Visits and Assessments. If clinically significant abnormal findings are noted at Screening and do not normalize on the repeat evaluation at Baseline, the patient will not be enrolled.

Evaluations of the hematology, clinical chemistry and urinalysis analytes listed in Table 3 will be performed at each of the visits listed above. In addition, a serum pregnancy test will be performed for women of childbearing potential at Screening, Baseline (Day 0), Month 6 and Month 12 (or at early discontinuation). For women of childbearing potential also a monthly urine pregnancy test will be performed before every infusion throughout the study. For details on aseptic procedure for blood withdrawal and on blood diversion see "Revised Study Procedures on Sterility Testing for Study IEDAT-02-2015 (ATTeST)" (Appendix 13).

Table 3. Summary of laboratory analytes

LABORATORY ANALYTES			
Hematology or CBC	**Clinical Chemistry**		**Urinalysis (automated)**
Hematocrit	Sodium	Alkaline phosphatase	Color
Hemoglobin	Potassium	LDH	pH
RBC count	Chloride	CPK	Specific gravity
WBC count	Calcium	Triglycerides	Protein
Differential WBC count	Phosphorus	Total cholesterol	Glucose
• Neutrophils	Serum iron	HDL cholesterol	Ketones
• Lymphocytes	Bicarbonate	LDL cholesterol	RBC, WBC, casts *
• Monocytes	Glucose		Nitrites
• Eosinophils	BUN		Bilirubin
• Basophils	Creatinine		Hemoglobin
Platelets	Total bilirubin		Urobilinogen
MCV	Albumin		* Reflex microscopic analysis to be performed only if other analytes are abnormal on automated testing
MCH	Total protein		
MCHC	AST (SGOT)		
RDW	ALT (SGPT)		
Special Diagnostic Tests: *Serum pregnancy test* (to be performed at Screening, Baseline (Day 0), Month 6 and Month 12 for women of childbearing potential). For women of childbearing potential also a monthly urine pregnancy test will be performed before every infusion throughout the study.			

The Investigator must review screening laboratory values, as well as any repeat assessments, prior to the first administration of the study agent, to ensure that the patient meets the protocol's inclusion/exclusion criteria. The Investigator must review laboratory values from each subsequent evaluation within 24 hours of receipt of the laboratory report. After the review is completed, the Investigator must sign and date each laboratory report.

A central laboratory will be used for analyzing all samples from routine laboratory tests. The central laboratory will provide normal reference ranges for the laboratory tests on the laboratory results report. A value is considered **normal** when it falls on or within the upper and lower limits of the reference range for the laboratory. A value is considered **abnormal** when it exceeds the upper or lower limit of the reference range. The central laboratory will provide the normal reference ranges for each parameter, and will verify that the result is not due to pre-analytical problems (e.g., sample taken improperly, sample stored incorrectly, sample labeled incorrectly) or to analytical problems (e.g., machine not accurately calibrated, technical problems with equipment or reagents, or deterioration of analyte). If there are any conflicts between reference ranges in Appendix 4 and the reference ranges for the central laboratory, the central laboratory reference ranges will have precedence in the determination of AEs.

The Investigator must evaluate any change *of clinical relevance* from pre-dose to post-dose in a laboratory test as to whether it meets the definition of an adverse event, and repeat, if needed, any clinically significant abnormal laboratory test. Any laboratory abnormalities meeting the definition of an adverse event should be recorded on the Adverse Events CRF.

Refer to Section 14.0, "Reporting Safety Information" for further directions.

13.5 Special Laboratory Evaluations

Measurement of selected "special" laboratory parameters has been included in the study to evaluate potential effects of dexamethasone (EDS-EP) treatment (for details on aseptic procedure for blood withdrawal and on blood diversion see "Revised Study Procedures on Sterility Testing for Study IEDAT-02-2015 (ATTeST)"; Appendix 13). The following special laboratory parameters will be assessed in the study:

- Blood glycosylated hemoglobin (HbA1c, %), CD4+ lymphocytes, α-fetoprotein (not repeated at baseline), C-reactive protein (CRP), and RBC antibodies (IgG, IgM, Qualitative Direct Coombs test) (not repeated at baseline)– to be performed at Screening and Baseline (Day 0) and on Months 6 and 12 (or at early discontinuation).
- Screening for adrenal insufficiency will be performed in all patients, regardless of weight, via early morning (before 8:00 AM) plasma cortisol testing at the following times: (1) during the screening period (prior to randomization), (2) when patients are symptomatic, and (3) when patients are at most risk for adrenal insufficiency and adrenal crisis, such as following discontinuation or interruptions of dexamethasone dosing (i.e., loading failures and following discontinuation of the study drug). If the 8:00 AM cortisol level is below 3-5 µg/dL (depending on assay) regardless of symptoms, or the patient exhibits signs or symptoms of adrenal insufficiency (Appendix 12) and has a cortisol <10 µg/dL, the patient will receive a high dose ACTH stimulation test, regardless of weight, within 24 hours. If the ACTH stimulation test is normal, the patient can be enrolled, or can continue dosing with EDS-EP, after the effects of the ACTH have dissipated (e.g., 30 days after ACTH administered). If the patient fails the ACTH stimulation test they will be excluded or discontinued from the study and referred to a pediatric endocrinologist, with a recommendation to prescribe stress dose steroids.
- High dose ACTH stimulation test – In the event that patients show signs or symptoms of adrenal insufficiency (Appendix 12) during the study, especially following interruption of the study drug (including loading failures), they will be tested for adrenal insufficiency using a high dose ACTH stimulation test (250 µg given i.v. or i.m.), regardless of weight. If ACTH testing confirms adrenal insufficiency, the patient will be referred for evaluation and treatment. Blood samples (2.0 mL) for measurement of plasma cortisol will be collected prior to ACTH administration (0 min), and at 30 and 60 min post-dose. A rise in plasma cortisol level to greater than 18 µg/dL within 60 min demonstrates a normal result. A rise in cortisol to less than 18 µg/dL within 60 min demonstrates an abnormal response.

  Results of plasma cortisol measurements and the ACTH stimulation test will be made available only to the Principal Investigator, and will not be available to raters performing the primary and secondary efficacy asssessments.
- Hemolysis panel [free plasma hemoglobin, haptoglobin, bilirubin (total and conjugate), CBC, LDH and urinalysis] – to be performed on Day 1 (pre-dose), Days 2 and 15 (CBC and urinalysis on Days 2 and 15 only). On Day 1 and Months 3, 6, and 9 (post-infusion) free plasma hemoglobin only is to be performed;
- Mini-ATM detection will be performed at Screening and at Months 2 and 6.

  Background

  Short-term treatment with glucocorticoid analogues improves neurological symptoms characteristic of AT; however, the mechanism of action has not been elucidated. Short direct repeat-mediated non-

canonical splicing events were induced by low-dose dexamethasone in human AT cell lines, which led to the skipping of mutations upstream of nucleotide 8450 of the ATM coding sequence (Menotta et al., 2012). The resulting transcript provided an alternative open reading frame that translated into a new ATM protein variant with the complete functional kinase domain, the mini-ATM variant. This mini-ATM variant was also identified in lymphocytes from AT patients receiving the EDS-EP treatment. Hence, dexamethasone may partly restore ATM activity in AT cells through a new molecular mechanism that overcomes most of the mutations so far described within this gene. Therefore, in the current study, blood samples (3 mL) to determine the mini-ATM will be taken at Screening, Month 2 and Month 6.

Process description for quantification of mini-ATM expression in the blood of AT patients

Tempus™ Blood RNA Tubes will be provided to each center. Three mL of blood will be drawn directly into the Tempus™ Blood RNA Tubes; filling up the tube to the black mark on the tube label indicates the collection of approximately 3 mL. Immediately after the Tempus tube is filled, the blood will be stabilized by shaking the tube gently for 10 seconds to ensure that the Stabilizing Reagent makes uniform contact with the sample. The collected samples will be transferred to the reference laboratory using the same couriers used to ship routine laboratory samples to the central laboratory, in data-logger equipped containers. Validated molecular diagnostic kits and extraction procedures will be used in the selected reference laboratory for the test procedure.

13.6 Sterility Testing of EDS-EP

CCI [redacted]

13.7 Columbia Suicide Severity Rating Scale (C-SSRS)

The C-SSRS (Posner et al, 2007; Posner et al, 2011) is a standardized suicidal rating system that has provided data for the pediatric suicidal risk analysis of antidepressants conducted by the Food and Drug Administration (FDA). The pediatric version of the scale will be used for patients who are less than 12 years of age at the screening visit (and will be used throughout the study, even if a patient turns 12 during the course of the study). At Screening, an assessment of suicidality will be performed using the "Baseline/Screening" version of the C-SSRS. At the baseline visit, upon admission to the clinical unit, the "Baseline" version of the C-SSRS will be performed. Follow-up assessments will be performed using the "Since Last Visit" version of the C-SSRS. Subjects with suicidal ideation will be excluded from participating in the trial.

13.8 Bone Mineral Density (BMD)

Measurements of BMD will be performed for all patients at Baseline and repeated at 6 and 12 months to assess potential steroid-related changes. Bone mass will be measured in the spine and total body (less the head), following the guidelines provided in the 2013 International Society for Clinical Densitometry Official Pediatric Position (Gordon et al, 2014). The suggested method for assessing BMD in the study is dual energy x-ray absorptiometry (DXA). The analysis will be performed with Z-scores following the above guidelines. The same method of assessment will be used for each patient throughout the study.

13.9 Tanner Staging

Assessment of physical development, sexual maturation, and the effect of treatment with dexamethasone on these aspects will be evaluated using the Tanner scale (Marshall and Tanner 1969, 1970) at Baseline, and 6 and 12 months. The scale defines physical measurements of development in children, adolescents and adults based on external primary and secondary sex characteristics, such as the size of the breast, genitals, testicular volume and development of pubic hair. Due to natural variation, individuals pass through the Tanner stages at different rates, depending in particular on the timing of puberty. Tanner staging of the breasts in pre-menarchal females and of the scrotum in males who have not completed puberty, will be performed. Post-menarchal females and males who have completed puberty will be excluded from this growth analysis.

14 REPORTING SAFETY INFORMATION

14.1 Adverse Events

Assessment of Adverse Events (AEs) will be performed throughout the study, from the time of signing of the ICF at the start of the Screening period through the final study visit (Month 12 or early discontinuation). All Adverse Events will be recorded in the CRF. In addition, all subjects will be followed up through 30 days after the final visit (Month 12 or early discontinuation) or at least 60 days after the final infusion, whichever is longer, for the occurrence of any AEs or SAEs.

14.1.1 ***Glossary***

Adverse Drug Reaction (ADR)

In the pre-approval clinical experience with a new medicinal product or its new usages, particularly as the therapeutic dose(s) may not be established, all noxious and unintended responses to a medicinal product related to any dose should be considered adverse drug reactions. The phrase "response to a medicinal product" means that a causal relationship between a medicinal product and an adverse event is at last a reasonable possibility, i.e., the relationship cannot be ruled out.

Adverse Event (AE)

An AE is any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and that does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product.

Serious Adverse Event (SAE) or Serious Adverse Drug Reaction (Serious ADR)

Any untoward medical occurrence that at any dose:

- Results in death,
- Is life-threatening, i.e., an event which, in the view of the Investigator, places the subject at immediate risk of death from the event as it occurred (it does not include an event which hypothetically might have caused death if it were more severe)
- Requires inpatient hospitalization or prolongation of existing hospitalization,
- Results in persistent or significant disability/incapacity,
- Is a congenital anomaly/birth defect (if exposure to product just before conception or during pregnancy resulted in an adverse outcome in the child), or
- Is an important medical event that may jeopardize the subject or may require medical intervention to prevent one of the outcomes listed above.

Symptoms or medically significant laboratory or instrumental (e.g., electrocardiographic) abnormalities of a pre-existing disease, such as cancer or other disease, should not be considered an adverse event. However, the occurrence of new symptoms, or laboratory or instrumental abnormalities, as well as worsening of pre-existing symptoms, are considered adverse events.

Unexpected Adverse Drug Reaction

An Unexpected Adverse Drug Reaction is an adverse event, the nature or severity of which is not consistent with the applicable product information (e.g., Investigator's Brochure for an unapproved investigational product or Package Insert/Summary of Product Characteristics for an approved product).

Non-Serious Adverse Event

A non-serious adverse event is any adverse event that does not meet the criteria listed above for a serious adverse event.

14.1.2 ***Data Collection***

For each event, record the following information on the Adverse Event section of the Case Report Form:

- **Classification of the Event:** Classify the event as either serious or non-serious
- **Description of Signs or Symptoms:** Whenever possible, record a specific diagnosis for the event. If a diagnosis cannot be made, then record each sign or symptom separately, e.g., record nausea and vomiting as two events. If multiple episodes of an event occur, separated by an appropriate time interval to justify considering the subsequent episodes as a repeat occurrence, record each episode separately on the Case Report Form.
- **Onset Date and Time:** Record the date and time the event started. If a change from baseline/previous evaluation in a laboratory test is reported as an adverse event, record the start date as the date of collection of the first lab sample that shows the change.
- **Stop Date and Time:** Record the date and time the event resolved. If a change from baseline/previous evaluation in a laboratory test is reported as an adverse event, record the stop date as the date of collection of the first sample that shows a return to the previous level.
- **Intensity:**
    1. **Mild:** Event not resulting in disability/incapacity, which resolves without treatment.
    2. **Moderate:** Event not resulting in disability/incapacity, which requires treatment.
    3. **Severe:** Event resulting in temporary and mild disability/incapacity, which requires treatment.
- **Relationship to the Study Agent:** Every effort should be made to determine the cause of each adverse event. The correlation between the study agent and the adverse event should be classified as follows:

1. **Probable**
    a) The event follows a reasonable temporal sequence from administration of the study agent;
    b) The event follows a known response pattern to the study agent;
    c) The event <u>cannot be</u> reasonably explained by:
        - the known characteristics of subject's clinical state, or
        - by other therapy administered, or
        - by the diagnostic/interventional procedure;
    d) There is evidence of partial or complete disappearance of the event after withdrawal of the product (positive de-challenge)

2. **Possible**
   a) The event follows a reasonable temporal sequence from administration of the study agent;
   b) Causation of the event by the study agent cannot be excluded;
   c) The event follows a known response pattern to the study agent but the event could have been produced by:
      - the subject's clinical state, or
      - other therapy administered, or
      - a diagnostic/interventional procedure.

3. **Unlikely**
   a) The adverse event follows a reasonable temporal sequence from administration of the study agent;
   b) Other reasons are more likely to be the cause of the adverse event, based on the present knowledge of the
      - disease under treatment, or
      - other therapy administered, or
      - study drug;
   c) A causal relationship between the adverse event and the study drug cannot be ruled out with certainty.

4. **Not Related** The event is either a pre-dose event or is definitely due to causes separate from the administration of the study agent, i.e.,
      - documented pre-existing condition
      - technical and/or manual procedural problems
      - concomitant medication
      - subject's clinical state.

- **Action Taken, in relation to treatment of the AE:**
  0. **None**
  1. **Drug treatment required** (a medication was prescribed or changed; record on the Concomitant Medication section of the Case Report Form)
  2. **Non-drug treatment required** (a non-drug treatment was prescribed or changed, record under "Comments" in the Adverse Event section of the Case Report Form)
  3. **Hospitalization or prolonged hospitalization** (fill out an SAE report)
  4. **Diagnostic or clinical test(s) conducted** (attach a copy of the results to the Case Report Form)
  5. **Subject discontinued from the study**

- **Action taken with study treatment**

  0. **Dose not changed**
  1. **Drug interrupted**
  2. **Drug withdrawn**
  3. **Not applicable**

- **Subject Outcome:**

  1. **Recovered without sequelae**
  2. **Recovered with sequelae** (describe the sequelae under "Comments" in the Adverse Event section of the Case Report Form)
  3. **Not Recovered, event on-going** (follow the subject until a definite outcome can be determined. When follow-up data are collected, report follow-up information under "Comments" in the Adverse Event section of the Case Report Form; if event is serious, fill in a follow-up Serious Adverse Event Report)
  4. **Died** (list primary cause of death under "Event Description" of the Adverse Event section of the Case Report Form; if available, attach a copy of the autopsy report to the Case Report Form and send a copy to the Sponsor)

- **Comments:**

  Provide other pertinent clinical information and observations under "Comments" in the Adverse Event section of the Case Report Form. For example, record predisposing or contributing conditions, such as previous history, concomitant diseases or medications, and/or procedural risks.

14.1.3 Subject Follow-up

Every attempt should be made to follow the subject until the adverse event has resolved or until the Investigator determines the subject has returned to an acceptable state of health.

14.1.4 Reporting Serious Adverse Events (SAEs)

The Investigator must report **all serious adverse events within 24 hours**, irrespective of the relationship to study medication, to the CRO, by fax or by e-mail (as back-up option), completing the appropriate reporting form. The CRO will then forward this information to **EryDel** within one business day of receipt. The names of the CRO contact will be communicated to the investigators by the CRO prior to the start of subject enrollment.

The minimum information required for an initial report of a Serious AE is as follows:

- Sender of report (name, address of Investigator, site number),
- Subject identification (screening number),
- Protocol number,
- Reportable event.

All SAE reports and any case of overdose leading to an AE or SAE must be faxed using the appropriate reporting form to the following number within 24 hours:

PPD

In case of fax failure, all sites can use the following email address as back-up option:

PPD

In case of death, a comprehensive narrative report of the case should be prepared by the Investigator and sent to the CRO by mail together with the Serious AE Form, retaining a copy on-site. If an autopsy is performed, a copy of the autopsy report should be actively sought by the Investigator and sent to the CRO as soon as available. A copy of the autopsy report will be retained on-site.

A follow-up Serious Adverse Event Report will be completed by the Investigator if important follow-up information (i.e., diagnosis, outcome, causality assessment, results of specific investigations) are made available after submission of the initial form. The follow-up form will be sent to the CRO as described above.

If the Investigator becomes aware of any deaths or Serious Adverse Events after the end of the 60-day follow-up window established in the protocol following investigational product administration, they will be reported to the CRO as described above.

14.2 Safety Reporting to Investigators, IRBs/IECs, and Regulatory Authorities

The Sponsor or their designee will be responsible for reporting all SAEs to regulatory authorities, investigators, and Independent Ethics Committees / Institutional Review Boards (IRBs/IECs), as applicable, in accordance with national regulations for the United States. For all active investigators located in the United States, the Sponsor or their designee will prepare an expedited report for all SAEs that are unexpected and potentially related to the study drug, and copies will be distributed according to all applicable laws and regulations. The investigational site also will forward a copy of all expedited reports to their IRB/IEC.

14.3 Reporting Overdose

If the investigational site staff administering the study medication reports that a subject was given more than the specified dose of study medication, this will be considered an overdose and must be reported immediately to the Investigator. Any instance of overdose, whether symptomatic or not, must be communicated to the CRO within 24 hours and be fully documented as a Serious Adverse Event. Details of any signs or symptoms and their management should be recorded including details of any antidote(s) administered.

14.4 Pregnancy

This study will exclude pregnant and breast-feeding patients, and females of childbearing potential, unless they are practicing adequate contraception, as determined by their Health Care Provider. As a further precaution, a serum pregnancy test will be performed at Screening and Baseline (Day 0), and repeated at

Months 6 and 12 (or at early discontinuation) for all young women who have had a menstrual period within the past year. Results of the pregnancy tests at Screening and Baseline must be negative for the patient to be randomized to treatment.

If a patient becomes pregnant during the study, she will be discontinued from the study immediately. Patients and their parents/caregivers should be instructed to notify the Investigator if it is determined that, after completion of the study they have become pregnant, either during the treatment phase of the study or within 30 days of completing the study. Whenever possible a pregnancy should be followed to term, any premature terminations reported, and the status of the mother and child should be reported to the CRO or EryDel after delivery.

Based on the estimated half-life of dexamethasone given with the EDS, i.e. less than 4 days, a period of one month of continued contraception should follow after study conclusion or early termination due to any reason.

14.5 Breaking the Study Blind by the Investigator

In case of emergency, the Investigator may break the blind for an individual patient, if the knowledge of the administered compound is deemed necessary for the safety of the patient. Breaking of the blind will be performed by the Investigator by calling/logging into the IVRS/IWRS system.

If the study blind is broken, the Investigator must immediately notify the CRO and provide the subject's number. The reason for breaking the blind should be noted on the Adverse Event (or Serious Adverse Event) section of the Case Report Form. EryDel will inform the Independent Safety Monitoring Board of the unblinding.

14.6 Independent Data Safety Monitoring Board

An independent Data Safety Monitoring Board (DSMB) will be established by EryDel to review the safety of all patients enrolled in this trial, on an ongoing basis. No EryDel employee or investigator involved in the EDS clinical studies will be a voting member of this board. The DSMB will regularly review the safety data as it accrues.

The Board will be regularly notified of the occurrence of any fatal or life threatening events immediately (within 7 calendar days) and any other serious adverse events within 15 calendar days. The Board will also receive updates on any adverse dropouts on a regular basis (once per month). The Board will have access to the blinded safety data including serious AEs and adverse dropouts, as well as clinically significant abnormal laboratory tests, vital signs and ECGs at periodic intervals. The Board may request an unblinding of the treatment groups if there is a safety concern.

The DSMB will review all of the safety data on an ongoing basis, with special emphasis on the incidence and severity of steroid related events, new infections, and serious AEs and deaths, in addition to the standard safety parameters. After reviewing the emerging safety profile for EDS-EP, the Board will make a recommendation to EryDel to (a) amend the ongoing study (e.g., increase safety monitoring, modify dosing), (b) terminate the EDS program (e.g., the EDS-EP safety profile is unacceptable), (c) continue the clinical program as designed. The current study protocol will not be amended or changed (this includes the study design and entry criteria) unless mandated by the emerging (unblinded) safety profile of EDS-EP.

Details of the DSMB charter (separate document) will be submitted to regulatory authorities, and will be available to IECs/IRBs upon request.

15 SUBJECT COMPLETION AND DISCONTINUATION

15.1 Definitions

A patient will be considered to have 'completed' the Initial Treatment Period of the study when he/she returns for the final evaluations on Month 6. 'Discontinuation' will refer to any subject who does not complete the full 6 months (±30 days) of this initial post-baseline treatment period of the study. Patients who continue in the Extension Treatment Period will be considered to have 'completed' this period of the study when he/she returns for the final evaluations on Month 12. 'Discontinuation' will refer to any subject who does not complete the full 12 months (±30 days) of this extension.

15.2 Procedures for Handling Withdrawals

In the absence of a medical contraindication or significant protocol violation, every effort should be made by the Investigator to keep the patient in the study; however, should the patient be withdrawn prior to Month 6 during the Initial Treatment Period or Month 12 during the Extension Treatment Period. All efforts should be made to complete all final evaluations and report the observations as thoroughly as possible at the time of the patient's withdrawal, with an explanation of why the subject is withdrawing from the study.

The criteria for discharging a patient from the study prior to the final study examination are listed below. The Investigator must indicate the primary reason (only one can be reported) for discontinuation, as well as the date when the decision was made; these will be specified on the 'End of Study' form.

A patient may be withdrawn from study participation if:

- Any hypersensitivity or allergic reaction, clearly linked to the study medication, has occurred;
- the patient experiences an AE sufficiently severe, in the opinion of the investigator, that it contraindicates the patient continuing in the study;
  If the patient experiences a systemic illness considered unrelated to the study medication, it still must be reported as an AE.
- the patient/caregiver withdraws consent (e.g. subject refuses to have any more blood samples taken for the EDS process; in this instance a specific reason must be recorded by the investigator);
- a major protocol deviation that jeopardizes the continued well-being of the patient or poses an risk to the patient's health;
  If the patient is able to abstain in the future from activities/behaviors that constituted a major protocol violation, the patient should be allowed to continue.
- the patient is lost to follow-up, i.e., the subject did not return to the clinic, and attempts to contact the subject were unsuccessful. Attempts to contact the subject must be documented;
- the Sponsor, IEC/IRB), or regulatory agency terminates the study.

Dropouts will not be replaced.

The Investigator may terminate a patient's study participation at any time during the study if a patient meets the study termination criteria described above. In addition, a patient or his/her parent/guardian may discontinue the patient's participation without giving a reason at any time during the study. Should a patient's participation be discontinued, the primary reason for termination must be recorded. For patients who discontinue prior to Month 6/12, an attempt should be made to perform all final (Month 6/12) assessments on the patient, and to follow up on any safety issues until resolution. Patients who discontinue prematurely, but return for scheduled efficacy evaluations will become part of the RDO analysis population.

Patients who complete 12 months of treatment will be eligible for entry into the open-label study (Study IEDAT-03-2018), provided there are no issues that would preclude their continuing in the trial.

Patients who discontinue prematurely, as well as those patients completing the Initial Treatment Period (Month 6) and not continuing in the Extension Treatment Period, and those completing Month 12 of the study and not entering the optional open-label study (Study IEDAT-03-2018), should be followed up for 60 days after their final infusion regarding the occurrence of any Serious Adverse Events.

16 STATISTICAL METHODS

16.1 Analysis of Efficacy

Primary Efficacy Endpoint

The primary efficacy endpoint will be the change from baseline to the follow-up assessments at Days 90 and 180, in the total score on the 'Modified' International Cooperative Ataxia Rating Scale (ICARS).

Key Secondary Efficacy Endpoint

The key secondary efficacy end point is the CGI-C rating of change from baseline at the follow-up assessments at Days 90 and 180.

Other Secondary Efficacy Endpoints

The following secondary efficacy endpoints will be assessed in the study:

- The change of CGI-S score from baseline to follow-up based on repeated measures at Days 90 and 180
- The change of VABS score from baseline to follow-up based on repeated measures at Days 90 and 180

Tertiary Efficacy Endpoints

The following tertiary efficacy endpoint will be assessed in the study:

- The change of QOL scores (EQ-5D-5L scale) from baseline to follow-up based on repeated measures at Days 90 and 180.

This measure will be considered exploratory.

16.2 Statistical Methods

All data collected on the A-T NEST from the selected centers on Day 2 and subsequent visits will be delivered to John Hopkins Statistical group for evaluation of the data, and performance of any analyses needed to determine/ confirm the psychometric properties of the scale.

16.2.1 Sample Size

Based on the very low prevalence of AT, as well as the high disability, morbidity and mortality, the number of subjects available for prospective trials is extremely limited. Furthermore, only aggregate Phase II study results are available (IEDAT-ERY01-2010 Clinical Study Report). Hence, in the absence of a file with the raw item score for each subject, the treatment effect and variability of the 'Modified' ICARS is unknown. Therefore the parameters needed to identify an appropriate sample size for this study were estimated using the aggregate Phase II results.

Sample size calculations are based on the analysis of the primary efficacy variable (i.e., 'Modified' ICARS) under the following assumptions.

- For aggregate ICARS, a treatment difference of 3.7 - 4.2 for the ~14-22 mg dose versus placebo with respect to ICARS measurements is expected, with a standard deviation of 5.0 - 7.4 (IEDAT-ERY01-2010 Clinical Study Report).

- For 'Modified' ICARS, the treatment difference is expected to be less than the aggregate ICARS, therefore, instead of examining a treatment difference between 3.7 and 4.2, the range between 3.0 and 3.7 was examined. The decrease in treatment difference is expected given that most of the questions in the Kinetic Function domain are not included in the 'Modified' ICARS and Chessa et al (2014) reported this domain as demonstrating the greatest overall improvement.
- For 'Modified' ICARS, the standard deviation is expected to be more than the aggregate ICARS, therefore, instead of examining a standard deviation between 5.0 and 7.4, the range between 5.0 and 8.0 was examined. This increase is expected given that removing questions from health assessment instruments can decrease precision and increase standard deviations (Awad, 2008; McHorney et al, 1992; McHorney, 1997).
- The primary efficacy variable will be tested, comparing ~14-22 mg dose versus placebo at the 0.05 two-sided significance level. Testing of the ~5-10 mg dose versus placebo will only proceed if the ~14-22 mg dose is significant. Consequently, no adjustment for multiplicity is needed for testing the two dose levels.

Table 4 below provides power estimates for the range of potential treatment differences and standard deviations. Estimates must be based on ranges, since point value estimates for 'Modified' ICARS are not available.

Table 4. Power given treatment difference and standard deviation estimates for a sample size of 54

Treatment Difference	Standard Deviation	Power
3.0	5.0	99.3%
	6.0	95.7%
	7.0	88.3%
	8.0	78.7%
3.2	5.0	99.7%
	6.0	97.5%
	7.0	91.9%
	8.0	83.6%
3.4	5.0	99.9%
	6.0	98.6%
	7.0	94.6%
	8.0	87.8%
3.6	5.0	>99.9%
	6.0	99.3%
	7.0	96.6%
	8.0	91.1%
3.7	5.0	>99.9%
	6.0	99.5%
	7.0	97.3%
	8.0	92.5%

As seen in Table 4, a sample size of 54 provides sufficient power for a study design with two repeated measures to assess the primary efficacy endpoint of change from baseline in 'Modified' ICARS between two groups with a two-sided 0.05 significance level, when the treatment difference ranges between 3.0 and 3.7, and the standard deviation ranges between 5.0 and 7.0 (PASS®, Module "Tests for Two Means in a Repeated Measures Design").

The final sample size will be adjusted for 10% loss to follow-up, for a final sample size of about 60 per group. It is anticipated that the screen failure rate will be approximately 25%; therefore, at least 240 patients would need to be screened to enroll 180 eligible patients. Due to the Covid-19 outbreak, the enrollment was closed with 175 randomized patients as of the 30th of March 2020. Statistical guidance determined that 175 patients (versus the goal of 180 patients) would not materially change the power of the study.

16.2.2 ***Populations for Analysis***

The following analysis sets will be used:

1) *Intention-to-treat population (ITT)*: All randomized patients.

2) *Full Analysis Set (FAS)* (Also referred to as MITT): All randomized patients who received at least one dose of study medication and had at least one post-baseline efficacy assessment of the primary efficacy variable.

3) *Per Protocol Population (PP)*: all patients enrolled into the study who received at least one dose of randomized treatment, fulfilled all Inclusion/Exclusion criteria, did not have any major protocol violations, and completed the Initial Treatment Period of the study as planned (ie, returned for a final evaluation). Patients who discontinue prematurely for reasons other than protocol violations, but return for their final evaluation, would still be part of the PP population, e.g. patients who discontinue due to AEs should be included.

4) *Safety Population (SP)*: all patients who received at least one dose of randomized treatment.

16.2.3 ***Background and Demographic Characteristics***

The background and demographic characteristics will consist of age, sex, height, body weight, BMI, past and current medical conditions, and history of disease. Continuous variables will be summarized by mean, standard deviation, median, and range (minimum, maximum), and discrete variables will be summarized using frequencies and percentages.

16.2.4 ***Study Medication***

The number of patients receiving each dosing condition (~5-10 or ~14-22 mg DSP/infusion or placebo) will be reported, and the average dose of EDS-EP (RBC-encapsulated DSP) in patients administered the active treatment (Groups 1 and 2, Safety population), based on measurements of samples taken from the infusion bags prior to dosing, will be summarized by mean, standard deviation, median, and range (minimum, maximum).

16.2.5 ***Concomitant Medications and Therapy***

A listing of concomitant medications administered from the time of dosing of the study medication through completion of the final evaluation (Month 6/12 or early discontinuation) will be provided by treatment group. Concomitant medication taken during the Screening period will be listed separately.

16.2.6 ***Safety Evaluations***

All patients in the Safety Population will be included in the safety analyses. All adverse events will be listed and summarized by body system and preferred term. The incidence of AEs (%) and their intensity and relatedness to the study drug, as assessed by the Investigator, will be reported. Serious adverse events (SAEs) and events which are newly occurring or worsening after administration of the study medication will be summarized. In addition, adverse events that result in death or discontinuation (ADO) of the study medication will be listed separately.

Other safety parameters such as vital signs, standard laboratory parameters, ECGs, physical/neurological examination findings, and special laboratory parameters will be listed and summarized accordingly. Abnormal and clinically notable values will be identified and listed for each parameter, as appropriate. Where feasible, mean changes from baseline will be examined using a paired t-test.

16.2.6.1 Interim Safety Analysis

An independent Data Safety Monitoring Board (DSMB) will be established by EryDel to review the safety of all patients enrolled in this trial, on an ongoing basis. No EryDel employee or investigator involved in the EDS-EP clinical studies will be a member of this board. The DSMB will periodically review the safety data accrued.

The DSMB will meet regularly to assess the safety from the emerging data. The first DSMB meeting will occur prior to the start of the trial, and the DSMB will decide when the first data review safety DSMB meeting will occur. They will also propose a tentative schedule for subsequent meetings that they may alter based on emerging safety data. The time between successive meetings should not be more than three months.

After reviewing the emerging safety profile for EDS-EP, the DSMB will make a recommendation to EryDel to (a) amend the ongoing EDS-EP study, (b) terminate the EDS-EP, or (c) continue the clinical program as designed. The current study will not be amended or changed (this includes the study design and entry criteria) unless mandated by the emerging (unblinded) safety profile of EDS-EP.

A separate DSMB Statistical Analysis Plan will be developed for specifying the analyses, tables, listings and figures to be prepared for DSMB meetings.

The DSMB will be regularly notified of the occurrence of any fatal or life threatening events immediately (within 7 calendar days) and any other serious adverse events within 15 calendar days. The DSMB will also receive updates on any adverse dropouts on a regular basis (once per month). The DSMB will have access to the blinded safety data including serious AEs and adverse dropouts, as well as clinically significant abnormal laboratory tests, vital signs and ECGs at periodic intervals. The DSMB may request an unblinding of the treatment groups if there is a safety concern.

Details of the DSMB charter (separate document) will be submitted to regulatory authorities, and will be available to Ethics Committees/IRBs upon request.

*16.2.7 **Efficacy Analyses***

Primary Efficacy Endpoint

The primary efficacy endpoint will be the change from baseline to the follow-up assessment at Days 90 and 180 in the total score on the 'Modified' International Cooperative Ataxia Rating Scale (ICARS).

The null (H_0) and alternative (H_1) hypotheses for the efficacy analysis of change from baseline in the 'Modified' ICARS total score are:

> H_0: There is no difference between Group 2 (EDS-EP ~14-22 mg Dose) and the Placebo group with respect to 'Modified' ICARS;
>
> H_1: There is a difference between Group 2 (EDS-EP ~14-22 mg Dose) and the Placebo group with respect to 'Modified' ICARS.

Statistical significance for efficacy and the study as a whole will be declared if:

The null hypothesis for the primary efficacy analysis (no statistical difference between Group 2 [EDS-EP ~14-22 mg] and the Placebo group) with respect to change from baseline in 'Modified' ICARS is rejected at the 0.05 two-sided significance level.

Efficacy of the ~5-10 mg dose EDS-EP versus Placebo

If the null hypothesis for the primary efficacy analysis (no statistical difference between EDS-EP ~14-22 mg and Placebo) with respect to change from baseline in 'Modified' ICARS is rejected, the efficacy of EDS-EP ~5-10 mg versus Placebo with respect to change from baseline in 'Modified' ICARS will be tested at the 0.05 two-sided significance level.

Key Secondary Efficacy Endpoint

- CGI-C at the follow-up assessments at Days 90 and 180.

Other Secondary Efficacy Endpoints

- The change of CGI-S score from baseline to follow-up based on repeated measures at Days 90 and 180
- The change of VABS score from baseline to follow-up based on repeated measures at Days 90 and 180.

Tertiary Efficacy Endpoint

- The change of QOL scores (AD-50 or SQL scale) from baseline to follow-up based on repeated measures at Days 90 and 180.

*16.2.8 **Randomization***

The milestones for the randomization process are depicted in Figure 1 and described in items 1- 4 below.

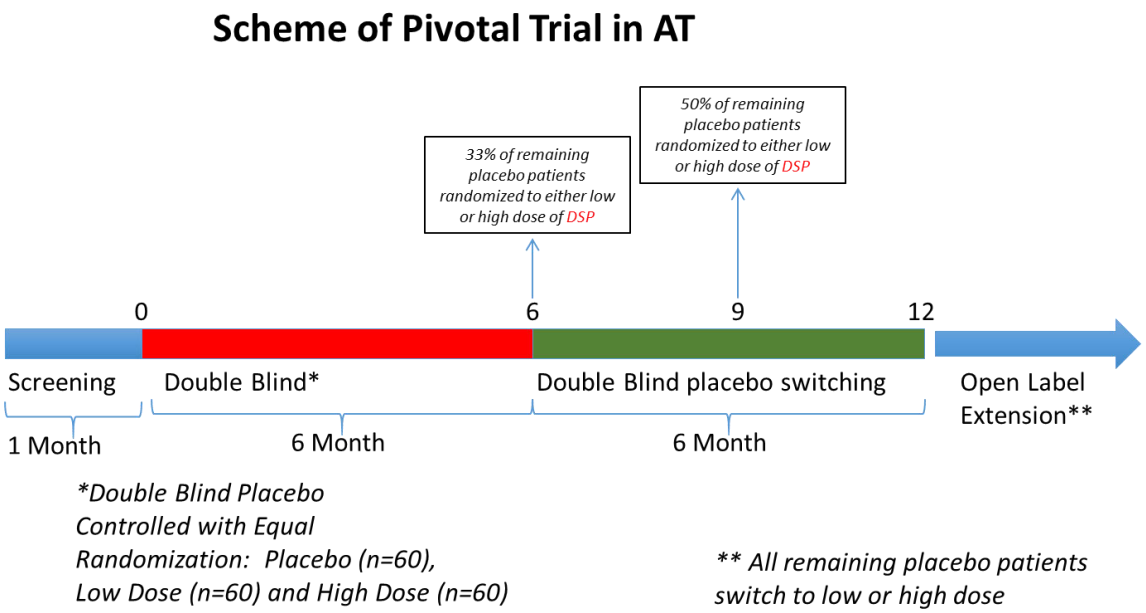

Figure 1. Trial periods and randomization/re-randomization time points

1. Initial Randomization. This will occur at the start of the double-blind period. Because this trial will enroll patients with a rare condition, the sample size will be limited. Important balancing factors include gender and age (age < 10, age ≥ 10), and region. There are three treatment groups. For this situation, probabilistic baseline randomization (pBAR), or equivalently, probabilistic covariate baseline minimization is desirable. The re-randomizations discussed in (2)-(4) will be performed at the time of the initial randomization. (See Figure 2)
2. Re-randomization of remaining placebo patients after 6 months of the Initial Double Blind treatment assignment. Among patients randomized to placebo who complete their initial 6 months of double-blind dosing, every third such placebo patient will be randomized 1:1 to active dose at either the ~14-22 mg or ~5-10 mg dose level.
3. Re-randomization of remaining placebo patients after 9 months of the Initial Double Blind treatment assignment. Among patients randomized to placebo who complete their initial 9 months of double-blind dosing, every other such placebo patient will be randomized 1:1 to active dose at either the ~14-22 mg or ~5-10 mg dose level.
4. Re-randomization of remaining placebo patients after 12 months of the Initial Double Blind treatment assignment. For patients randomized to placebo who complete their initial 12 months of double-blind dosing, all such placebo patients will be randomized 1:1 to active dose at either the ~14-22 mg or ~5-10 mg dose level. These treatment assignments will be made in the open-label extension study (Study IEDAT-03-2018) for those placebo patients who continue treatment in this study.

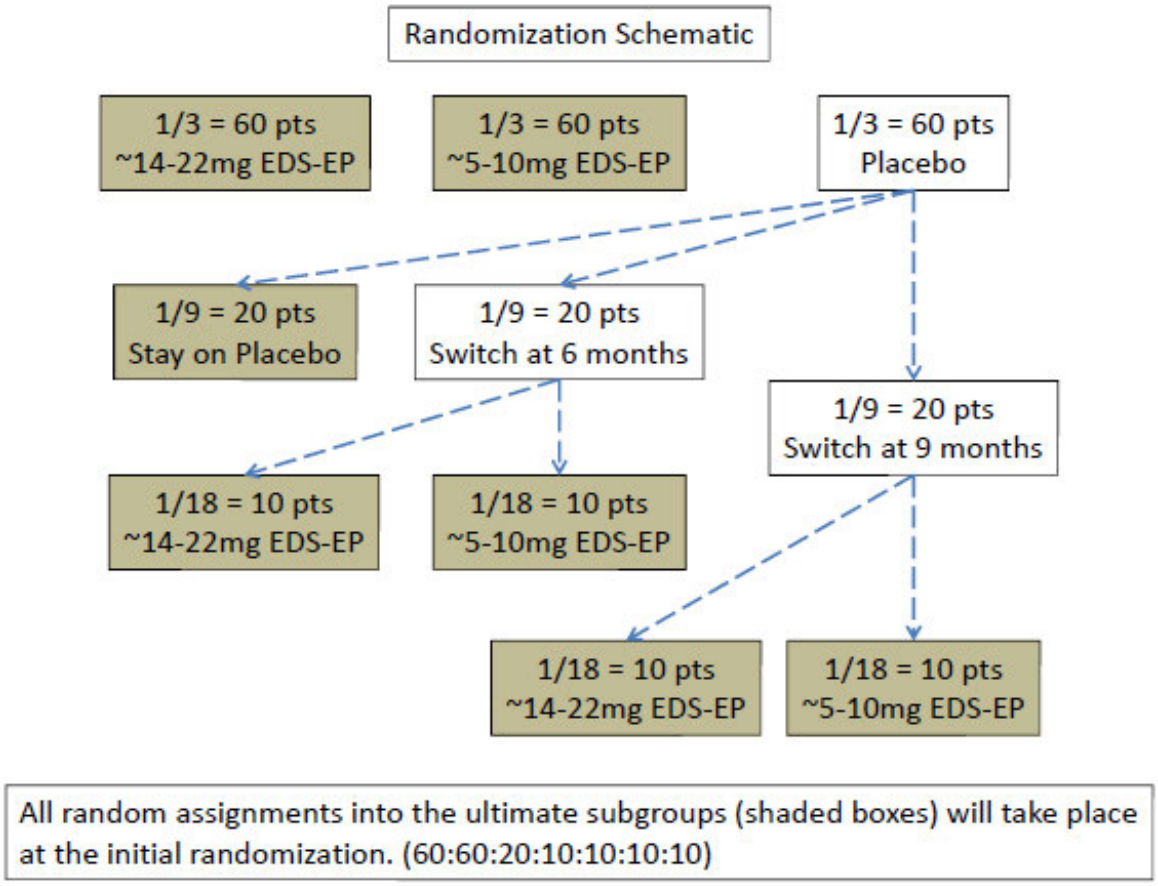

Figure 2. Randomization schematic

The randomization scheme presented in Figure 2 assumes that no patients discontinue from the study. For patients randomized to placebo at baseline, random assignments to switch to either of the active treatment groups (EDS-EP ~14-22 mg or ~5-10 mg) at Month 6 or 9, or to remain on placebo throughout the study, will be done at baseline to avoid any bias in selecting placebo patients for the switch to EDS-EP.

When the design of the pBAR randomization is in place, simulations will be used to test the randomization system. The simulations will be designed

1. to subject the pBAR implementation to conditions beyond the control of the study team, such as the order that patients arrive for randomization, to assess the robustness of the algorithm. The ability of the system to handle re-ordering of the arrival order of patients will be examined to see how well the desired balance is achieved.
2. to examine the extent to which random fluctuation will disturb the balance, both overall, and within the desired strata.
3. to subject the IVRS/IWRS system implementation to various stresses, as a validation.

Handling of Dropouts and Missing Data

Patients who discontinue treatment prematurely are defined as dropouts. Those patients who discontinue treatment prematurely but return for scheduled efficacy assessments at 6, 9 and 12 months are defined as RDOs. Missing data will be handled as described in the analysis of primary efficacy parameter. Retrieved dropouts will be used in sensitivity analyses.

Analysis of the Primary Efficacy Parameter

The primary efficacy variable, change from baseline in 'Modified' ICARS total score, will be analyzed using a Mixed Model Repeated Measures (MMRM) approach, with the baseline value as covariate and age, treatment, region, baseline value and treatment interaction, visit and treatment-by-visit interaction as fixed effects. The estimated treatment difference, together with the associated two-sided 95% CI and p-value, will be calculated. Unstructured covariance matrices will be used. Model effect estimation will be based on restricted maximum likelihood (REML). The region and treatment interaction can be tested in a separate model and if a significant interaction is found, then forest plot will be used to present the region specific treatment difference and associated 95% confidence interval.

The primary MMRM analysis will assume missing at random (MAR) as a means of handling missing data. Sensitivity analyses will be performed to evaluate the MAR assumption. For this, ANCOVA with missing data imputed using Last Observation Carried Forward (LOCF), Observed Case (OC), and OC + RDO will be used.

Analysis of the Key Secondary Efficacy Parameter

The analysis of the key secondary efficacy measure, CGI-C, will be performed using ANCOVA, with age, sex, treatment, region, visit and treatment-by-visit interaction as fixed effects. The estimate of the treatment difference parameter, together with the associated two-sided 95% CI and p-value at will be calculated.

In this analysis, LOCF will be used for missing data. Sensitivity analyses will use OC, OC + RDO for imputing missing values.

Hypothesis Testing and Handling of Multiple Comparisons

The ~14-22 mg dose of the primary efficacy end point will be tested at the two-sided significance level of 0.05. With this requirement, the overall family-wise error rate (FWER) can be no greater than 0.05 two-sided. The study will be judged statistically significant if the ~14-22 mg dose of the primary efficacy endpoint is significant.

The ~5-10 mg dose of the primary efficacy endpoint will be tested only if the ~14-22 mg dose is found to be statistically significant compared to placebo. In this way, the 0.05 two-sided significance level will be maintained and no adjustment will be necessary.

Following the testing for the primary endpoint, an ordered hierarchical testing (step-down) of the key secondary endpoint (CGI-C), and other secondary endpoints, i.e. CGI-S, followed by VABS, will be performed, according to the methodology described by Dmitrienko and D'Agostino (2013), as shown in Figure 3.

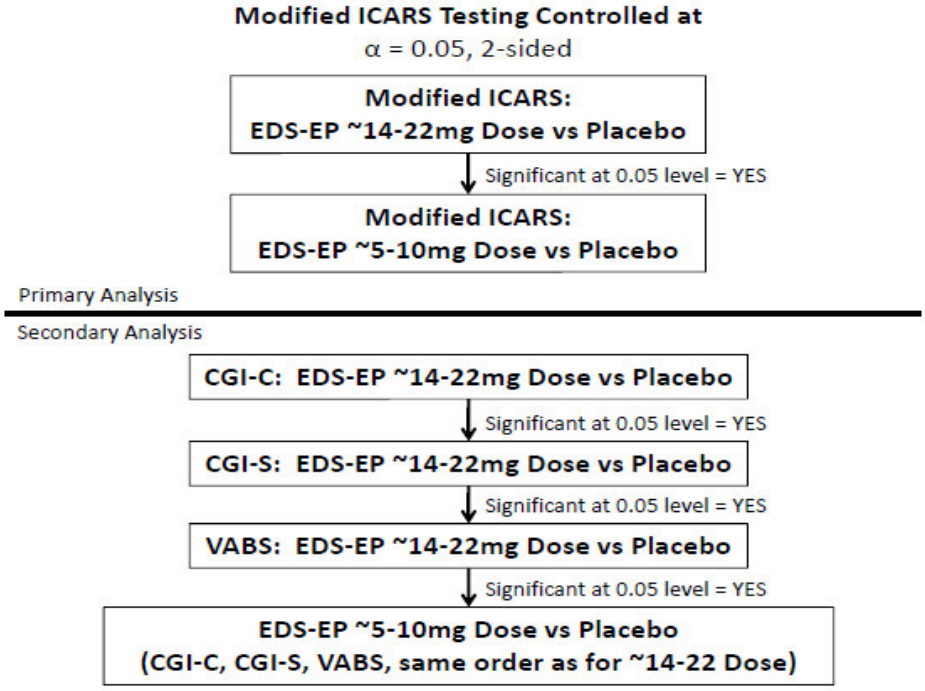

Figure 3. Hierarchical Testing Procedure for Primary and Secondary Endpoints

Secondary endpoints will be tested in the following order (see also Figure 3 flowchart). A sequential testing approach will be used, for which the ~14-22 mg dose versus placebo for the secondary endpoints will be tested in the following order: 1) CGI-C, 2) CGI-S, and 3) VABS. This is to be followed by testing the same secondary variables in the same order for the ~5-10 mg dose versus placebo: 1) CGI-C, 2) CGI-S, and 3) VABS. All tests will be performed at the 0.05 two-sided significance level, and testing will continue to the next variable/dose level only if significance is achieved for the current test.

The analytic approach for these variables will use ANCOVA with missing value imputed using LOCF, OC, and OC + RDO.

The analyses of the EQ-5D-5L will be exploratory.

Efficacy Analysis – Extension Treatment Period

For the Extension Treatment Period, comparisons will be made for the 'Modified' ICARS, CGI-S, VABS, and QoL [EQ-5D-5L] using ANCOVA with missing values imputed using LOCF, OC, and OC + RDO.

Analyses for the various randomization periods

The primary analysis will compare the treatment groups as specified in Figure 3 (in the "Primary Testing" panel) after 6 months of treatment. Only these 6-month evaluations will be controlled at the Type I FWER of 0.05 two-sided.

Further analyses will make the same comparisons after 9 months of treatment, and again, after 12 months of treatment. Because these later analyses will be very underpowered, these analyses will be for descriptive purposes only. However, p-values will be presented strictly as an aid to understanding the results, but will provide no basis for statistical inference.

Maintenance of the blind for the Extension Treatment Period

The blind will be broken after all patients have completed the 6-month, double-blind, Initial Treatment Period, and the database for this period has been locked, so that the primary efficacy analysis can be performed. Therefore, procedures will be implemented to ensure that the blind is maintained for the 6-month, double-blind, Extension Treatment Period, so that the analyses of the data collected in this extension period are valid. Procedures will be put in place to ensure that the investigators, site staff, Sponsor personnel, and CRO personnel, including Data Managers, Project Managers, Clinical Research Associates (CRAs), Medical Monitors, Pharmacovigilance personnel, Medical Writers, etc., who will have access to the data from the Extension Treatment Period, are not made aware of the treatment assignments of any patients continuing treatment in this extension period, prior to the last patient completing the extension period and the database being locked. The personnel mentioned above should have no reason to require access to the individual patient treatment assignments, except in the case of an emergency unblinding necessitated by a significant adverse event.

In contrast, the Biostatistics team [statistician(s), programmer(s), CDISC engineer, etc.] that has been assigned to work on the 6-month Initial Treatment Period, and is responsible for unblinding and performing the primary analysis for the study on the data from this period, will need to have access to the treatment assignments. Therefore, this team will not be involved in the analysis of data for the extension period. Instead, a second Biostatistics team will be trained prior to the start of the unblinded analysis, and will take responsibility from the start of the unblinded analysis through to the completion of the trial.

To further ensure that the blind is maintained, the following restrictions on communications within the CRO, and between the CRO and the Sponsor, will be put in place:

- The Unblinded Biostatistics team will have no direct communication with any blinded personnel within the CRO, other than for purposes of oversight. Any requests for clarifications of data will come through the Sponsor to the Project Manager, Project Management Associate and the Lead Data Manager.
- The Unblinded Statistical team will be removed from all mail circulation lists and from all meeting invites related to the ongoing extension period of the study. Separate oversight calls will be arranged with the CRO Project Manager, Unblinded Biostatistics team and the Sponsor.
- Any communications or documentation generated during the primary efficacy analysis will be filed outside the Trial Master File (TMF) and will be incorporated into the TMF at the end of the study to meet requirements of GCP. The nature of this repository will be agreed with the Sponsor in advance of the primary analysis.

Additional details of the procedures that will be followed to maintain the blind in the Extension Treatment Period, after unblinding of treatment assignments for the Initial Treatment Period, will be included in the Statistical Analysis Plan and Data Management Plan prepared by the CRO.

17 ETHICS

17.1 Ethical Considerations

The study will be carried out in accordance with the Declaration of Helsinki, as amended by the 64th General Assembly of the World Medical Association, Fortaleza, Brazil, October 2013 (Appendix 1).

Subject Information and Informed Consent

The Informed Consent Form, as well as the Subject Information Sheet, must be approved by the IRB/IEC together with the Study Protocol, before the start of the study.

All subjects, or if necessary their parent/caregiver or legal representative (with assent by the subject), must sign and personally date an approved Informed Consent Form after receiving detailed written and verbal information about the reason, the nature, the required procedures, the intended duration and the possible risks and benefits and any discomfort associated with the study. Each subject should be informed that his/her participation in the study is voluntary and that he/she may refuse to participate or withdraw from the trial, at any time, without penalty or loss of benefits to which the subject is otherwise entitled.

The language used in the oral and written information about the trial, including the written Informed Consent Form should be as non-technical as practical and should be understandable to the subject. The subject must be given ample time to read and to understand the Subject Information Sheet and opportunity to inquire and ask any clarification about the trial before signing the Informed Consent Form.

No study procedure can be performed (including the screening visit) before the informed consent form has been signed. The informed consent procedure must be done according to the guidelines provided in the Declaration of Helsinki and the ICH E6 Guideline for Good Clinical Practice.

The subject must be made aware and agree that personal information may be scrutinized during audit / inspection by competent authorities and properly authorized persons. However, personal information will be treated as strictly confidential and will not be publicly available.

By signing the Investigator Statement (Appendix 3), the Investigator assures **EryDel Pharmaceuticals** that Informed Consent will be obtained.

Original signed Informed Consent Forms will be filed with the Investigator's File.

17.2 IEC/IRB Approval

The protocol, Investigator's Brochure, Subject Information Sheet, Informed Consent Form and any advertisement for the recruitment of subjects must be reviewed and approved by an appropriately constituted IEC/IRB, as required in Chapter 3 of the ICH E6 Guideline. A copy of the Committee's dated approval and a list of the members of the IEC/IRB will be given to the Sponsor for the Sponsor's files. A copy will be also included in the Final Report. Written IEC/IRB approval must be obtained by the Sponsor prior to shipment of study agent or subject enrollment. Any amendments to the protocol, Informed Consent Form (ICF) or Subject Information Sheet, other than administrative ones, must be approved by this committee.

18 ADMINISTRATIVE CONSIDERATIONS

18.1 Regulatory Requirements: Sponsor/Investigator Obligations

This study will be conducted in accordance with the Declaration of Helsinki and the ICH E6 Guideline (Good Clinical Practice, see Appendix 2). To ensure compliance the Investigator agrees, by written consent to this protocol, to fully cooperate with compliance checks by allowing access to all documentation, including subjects' hospital files (the source documents), by authorized individuals.

18.2 Curriculum Vitae

The Investigator and any co-Investigator(s) must provide the Sponsor with current copies of their own curriculum vitae.

18.3 Investigator and Study Administrative Structure

The administrative structure of the study (e.g., Investigators, monitoring and evaluation personnel, laboratory facilities, clinical trial supply management) is presented in the Trial Master File.

The listing should include:

a) The Investigator(s);

b) Any other person carrying out observations of primary or other major efficacy or safety variables, such as a nurse, physician's assistant, clinical psychologist, clinical pharmacist or house staff physician.

18.4 Investigator's Statement

This document, signed and dated by the Principal Investigator, describes the Investigator's obligations. The standard text is appended to the protocol (Appendix 3).

18.5 Monitoring Procedures

18.5.1 Study Monitoring

A CRO will be selected by the Sponsor to oversee the conduct of the trial. An appropriate representative of the CRO (Study Monitor) will maintain contact with the Investigator and will visit the study site for the purpose of discussing and/or retrieving data. An initiation (pre-study) visit will be made by the Study Monitor to discuss with the Investigator the protocol and the obligations of both the Sponsor and the Investigator. The Investigator must allow the Study Monitor to perform periodic, interim monitoring visits. The purposes of these visits are:

- To verify that written Informed Consent was obtained prior to each subject's participation in the trial.
- To assess the progress of the study.
- To review the compliance with the study protocol.
- To determine whether all adverse events were appropriately reported.
- To determine whether the Investigator is maintaining the essential documents.
- To discuss any emergent problem.
- To check the Case Report Forms (CRFs) for legibility, accuracy and completeness.

- To validate the contents of the CRFs against source documents.
- To collect completed CRFs.
- To assess the status of drug storage, dispensing and retrieval.

The Investigator will make available the source documents for inspection. This information will be considered as confidential. *Violations of and deviations from the protocol must be notified to the Study Monitor as soon as possible.*

The Study Monitor will perform a closeout visit at the time when all CRFs have been retrieved and all queries have been answered.

*18.5.2 **Case Report Forms (CRFs)***

Electronic Case Report Forms (eCRFs) will be provided for each subject. The study Monitor will review the forms at each site visit.

Case Report Forms must be completed for all subjects who sign Informed Consent, even if the subject fails to complete the study. No section of the CRFs is to be left blank without an appropriate explanation by the Investigator, since the lack of such explanation may necessitate discarding an otherwise usable observation.

If requested, copies of the CRFs are to be made available to the appropriate regulatory agencies.

*18.5.3 **Auditing/Inspecting***

The Investigator will make all pertinent records available, including source documentation, for inspection by regulatory authorities and for auditing by the Sponsor. This information will be considered as confidential. Audits/Inspections may occur any time from start to after conclusion of the study. When an Investigator signs the protocol, he/she agrees to allow regulatory authorities and **EryDel** auditors to inspect his/her study records.

18.6 **Archiving of Records**

Copies of the protocol, subject identification codes, eCRFs, source data, Informed Consent Forms and other documents pertaining to the study conduct must be kept for the maximum period of time as required by the study center. This time period must be at least two years after the last approval of the marketing application of the study agent in an ICH region and until there are no pending or contemplated marketing applications in an ICH region, or at least two years have elapsed since the formal discontinuation of clinical development of the study agent.

No study document should be destroyed without prior written agreement between the Sponsor and the Investigator. Originals of all documentation and copies of outgoing correspondence concerning the study will be stored and retained by the Sponsor in a safe area in the Trial Master File for the lifetime of the product. In particular, the final report sent by the Investigator to the IRB/IEC must be retained by the Sponsor, or the subsequent owner, for five years beyond the lifetime of the study agent.

18.7 **Final Clinical Study Report**

The Final Clinical Study Report (CSR) will be written by the CRO, according to specifications to be defined by **EryDel**.

18.8 Study Documentation and Publication of Results

Study Documentation

All unpublished documentation (including the protocol, CRFs and Investigator's Brochure) given to the Investigator is strictly confidential. All recipients must agree not to disclose the information herein contained to any person not connected with the study without the prior written authorization of **EryDel S.p.A**. The submission of these documents to the IRB/IEC is expressly permitted. The involved parties agree that the results of this study will be used in their original form and/or in a global report for submission to governmental and regulatory authorities of any country.

All information communicated to the investigator(s) by **EryDel** is the exclusive property of **EryDel S.p.A**. The Principal Investigator will ensure this information shall be kept strictly confidential by him/her or any other person connected with the study and shall not be disclosed to any third party without the prior written consent of **EryDel**.

Publication of Results

Any formal presentation or publication of the data from this trial will be considered as a joint publication by the Investigator(s) and EryDel. Authorship will be determined by a Publication Committee consisting of the lead investigator(s) from the trial, representatives from EryDel, and an external consultant with expertise in the field. For multi-center studies, it is mandatory that the first publication is based on data from all centers, analyzed as stipulated in the protocol by a statistician designated by EryDel. Investigators participating in this study agree not to present or publish data gathered from a single center or sub-group of centers before the full initial publication, unless agreed to by all other investigators and EryDel. Authorship of any publication resulting from this trial will include members of each of the contributing centers and key contributors to the design and execution of the study, including EryDel personnel.

EryDel will form a study publication committee to coordinate and develop a publication policy and help in its implementation. The publication committee will be comprised of the Principal Investigators from each of the countries (to be determined by EryDel), based on their contributions to study conduct and their status in the field. The other members of the publication committee will include a representative of EryDel and an external consultant. Members of the publication committee cannot serve as first authors of more than one primary publication.

The authorship of the first multicenter paper will be comprised of members of the publication committee, Principal Investigators of the three sites with the highest numbers of valid patients, and EryDel representatives who have made significant contributions to the design, conduct and analysis of the results. The Principal Investigators of all contributing centers will be acknowledged in the publication. The Publication Committee will decide on the content, journal, and sequence of publications /presentations.

Any publication, abstract, or paper of any information or material relating to or arising out of the present clinical study shall be sent to EryDel for review at least sixty (60) days before presentation at any congress, or publication of the final form(s) by any journal. EryDel will inform the Investigator of any changes or deletions necessary to preserve EryDel's confidential and proprietary technical information. All rights and interests worldwide in any inventions, know-how or other intellectual or industrial property rights, which arise during the course and/or as a result of the present clinical study or which arise from the information or materials supplied under this Agreement, shall be assigned to, vest in and remain the property of EryDel S.p.A.

18.9 Financial Agreement

A financial agreement (separate from the protocol) will be made and signed by **EryDel S.p.A.** or their designee and by a representative of the Institution where the study will be conducted.

18.10 Termination of Study

In the event that the Investigator is unable to continue the study, another suitable sub-Investigator at the site will be designated to serve as the Investigator in the interim, until a new Investigator can be identified. This interim Investigator, if approved by the site IRB/IEC will carry out the responsibilities of the Investigator. Documentation testifying to this will be submitted to the Study Monitor within 10 days of the change. Within 6 months of the appointment of the interim Investigator, the new Principal Investigator must be identified and approved by both **EryDel S.p.A.** and the IRB/IEC for the study to continue at the site.

If the Sponsor and/or the Investigator should discover conditions arising during the study that indicate it should be terminated, an appropriate schedule for termination will be instituted. If the Investigator terminates the study, an explanatory letter will be provided to **EryDel**. Should the study be discontinued due to a decision by **EryDel**, the Investigator will be reimbursed for reasonable expenses incurred and for the subjects actually treated according to the study protocol.

18.10.1 ***Study Discontinuation by the Sponsor***

The Sponsor may terminate the entire study, or the study at an individual site, at any time, for any of the following reasons:

- failure to enroll subjects;
- protocol violations or deviations;
- inaccurate or incomplete data;
- non GCP compliance;
- completion of enrolment;
- administrative reasons.

18.10.2 ***Study Discontinuation by the Investigator***

The Investigator may terminate his/her participation in the study in consultation with the Sponsor due to the occurrence of significant adverse events and/or adverse drug reactions endangering the health of subjects, which make it ethically unacceptable to continue.

18.11 Insurance Policy

EryDel S.p.A., or its designee, will provide insurance coverage for damages emerging from the trial and involving the subjects treated with the test compound, provided that the Investigator(s) have adhered to the terms and provisions of the protocol. The principal Investigator will be supplied with all data concerning the insurance company and policy number for a maximum sum insurable.

18.12 Financial Disclosure

The Principal Investigator and sub-Investigators will provide the Sponsor with adequate and accurate financial information (PD35) to ensure that the Sponsor can make complete and accurate financial certification of disclosure statements to concerned regulatory authorities. It is the duty of the Investigator to promptly update previous information provided to the Sponsor if there are salient changes that occur during the course of the study, and for a period of one year following its completion (last patient last visit).

The study will be performed under a US IND; therefore, all Investigators/sub-Investigators, contractors, etc., are expected to comply with the obligations as specified in the CFR (21 CFR part 54) by the US FDA,

including requirements for full Financial Disclosure (Guidance for Clinical Investigators, Industry and FDA Staff: Financial Disclosure by Clinical Investigators, February 2013).

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