

RESEARCH ARTICLE

# Randomized controlled trial demonstrates the benefit of RGTA<sup>®</sup> based matrix therapy to treat tendinopathies in racing horses

Sandrine Jacquet-Guibon<sup>1,2\*</sup>, Anne-Gaelle Dupays<sup>3</sup>, Virginie Coudry<sup>1,2</sup>, Nathalie Crevier-Denoix<sup>2</sup>, Sandrine Leroy<sup>4</sup>, Fernando Siñeriz<sup>5</sup>, Franck Chiappini<sup>5</sup>, Denis Barritault<sup>5,6</sup>, Jean-Marie Denoix<sup>1,2</sup>

**1** Centre d'Imagerie et de Recherche sur les Affections Locomotrices Equines, Ecole Nationale vétérinaire d'Alfort, Maisons-Alfort, France, **2** Unité Sous Contrat 957, Biomécanique et Pathologie Locomotrice du Cheval, Institut National de la Recherche Agronomique, Ecole Nationale vétérinaire d'Alfort, Maisons-Alfort, France, **3** Clinique du Molinel, Marcq en Baroeul, France, **4** Episcience, London, United Kingdom, **5** Organ, Tissue, Regeneration, Repair and Replacement Société Actions Simplifiés, Paris, France, **6** Laboratoire de recherche sur la Croissance Cellulaire, Réparation, et Régénération Tissulaire, Faculté des Sciences, Université Paris-Est Créteil, Créteil, France

\* [sandrine.jacquet@vet-alfort.fr](mailto:sandrine.jacquet@vet-alfort.fr)



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## Abstract

A randomized controlled trial was performed on racing horses, to evaluate the efficacy of a new class of therapeutic agents in regenerative medicine—ReGeneraTing Agents<sup>®</sup> (RGTA<sup>®</sup>), to treat tendinopathies. Preliminary uncontrolled studies on tendon healing in racing horses with RGTA<sup>®</sup> (OTR4131)—Equitend<sup>®</sup> showed encouraging results, justifying performing a randomized, controlled, multicenter study with a two-year racing performance follow up. The objective of this study was to evaluate the effect of Equitend<sup>®</sup> versus placebo on acute superficial digital flexor tendonitis in racing French Standardbred Trotters (ST). Twenty-two ST were randomly and blindly assigned to receive with a ratio of 2 to 1, a single Equitend<sup>®</sup> (n = 14) or placebo (n = 8) intralesional injection under ultrasonographic guidance. Horses were evaluated over 4 months, by clinical and ultrasonographic evaluations (day 0, months 1, 2, 4), and their racing performances followed up over the 2 years after treatment. During the first month of treatment, a significant decrease in the cross-sectional area (CSA) was found in the Equitend<sup>®</sup> group (p = 0.04). After 4 months, the number of Equitend<sup>®</sup> treated horses with an improved CSA was significantly higher than the placebo-treated horses (p = 0.03571). The Equitend<sup>®</sup> group returned to their pre-injury performance level, racing in, and winning, significantly more races than the placebo group (p = 0.01399 and 0.0421, respectively). Furthermore, recurrence was significantly higher in the placebo group than in the Equitend<sup>®</sup> group (71.4% vs 16.6%, p = 0.02442). In conclusion, we measured a significant, short-term, reduction effect on CSA and demonstrated a long-term beneficial effect of intralesional injection of Equitend<sup>®</sup> for the treatment of superficial digital flexor tendonitis on racing ST, racing 2.3 times more often than placebo, with 3.3 times fewer recurrences maintaining pre-injury performance level. This study may open the way for the development of a human treatment of tendonitis.

author. The funder had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript. OTR3 provided support in the form of salaries for authors (FS and FC) and research material, but did not have any additional role in the study design, data collection and decision to publish. The specific roles of these authors are articulated in the 'Author Contributions' section. OTR3 provided Equitend<sup>®</sup> treatment.

**Competing interests:** D.B. is OTR3 founder and inventor with patents and a significant OTR3 shareholder. F.S. holds some shares of OTR3 company and is an employee of OTR3. FC is an employee of OTR3. Equitend<sup>®</sup> is a product under development and a dossier has been submitted to the EMA authority. Patents' names and numbers in relation with the Equitend<sup>®</sup> product are described in "Financial Disclosure". This does not alter our adherence to PLOS ONE policies on sharing data and materials, S.J.G, A.G.D, V.C, NCD, S.L and J.M. D. have declared that no competing interests exist. Patents' names and numbers in relation with the Equitend<sup>®</sup> product are described below: Title: Method for sulphonation of compounds comprising free hydroxyl (oh) groups or primary or secondary amines. Family: BIP119919 - BIP119919AUPC AUSTRALIA 2002365302 28/11/2002 2002365302 11/06/2009 BIP119919CADC CANADA 2,468,759 28/11/2002 BIP119919CNPC CHINA 02827691.4 28/11/2002 02827691.4 29/11/2006 Cover Letter BIP119919ILPC ISRAËL 162192 28/11/2002 BIP119919INPC INDIA 314/MUMNP/2004 28/11/2002 211390 26/10/2007 BIP119919JPPC JAPAN 2003-547462 28/11/2002 4394950 23/10/2009 BIP119919USPC UNITED STATES 10/853,323 28/11/2002 7,396,923 08/07/2008 BIP119919BEEP BELGIUM 02803847.9 28/11/2002 1456247 29/08/2007 BIP119919CHEP SWITZERLAND 02803847.9 28/11/2002 1456247 29/08/2007 BIP119919DEEP GERMANY 02803847.9 28/11/2002 1456247 29/08/2007 BIP119919ESEP SPAIN 02803847.9 28/11/2002 1456247 29/08/2007 BIP119919FREP FRANCE 02803847.9 28/11/2002 1456247 29/08/2007 BIP119919GBEP UNITED-KINGDOM 02803847.9 28/11/2002 1456247 29/08/2007 BIP119919IEEP IRLAND 02803847.9 28/11/2002 1456247 29/08/2007 BIP119919ITEP ITALY 02803847.9 28/11/2002 1456247 29/08/2007 BIP119919LUEP LUXEMBOURG 02803847.9 28/11/2002 1456247 29/08/2007 BIP119919NLEP NETHERLANDS 02803847.9 28/11/2002 1456247 29/08/2007 BIP119919SEEP SWEDEN 02803847.9 28/11/2002 1456247 29/08/2007 BIP119919FR00 FRANCE 0115444 29/11/2001 2832708 13/02/2004 Title: Use of biocompatible polymers for the preparation of a composition or a medical device

## Introduction

Tendon injury remains a frequent condition in sport and race horses [1, 2], responsible for substantial wastage in the equine sports industry [3, 4], and justifying continuous development of research into new treatments. In the last decade, studies substantially focused on "biological therapies", such as glycosaminoglycan polysulphate [5, 6], hyaluronic acid (HA) [5, 7], platelet rich plasma (PRP) [8–10], insulin-like growth factor-I [11], several sources of stems cells [12–17]. ReGeneraTing Agents (RGTA<sup>®</sup>) used in the present study belong to these "biological" or "nature mimicking" therapies. RGTA<sup>®</sup> are nano-polysaccharides engineered by substituting dextran side chains with sulfate and carboxymethyl groups, to mimic heparan sulfates (HS). HS are a subset of glycosaminoglycans (GAGs), a key element in the extracellular matrix architecture, bridging and protecting matrix proteins, as well as storing, protecting and positioning heparan binding cytokines, chemokines and growth factors. Efficacy of RGTA<sup>®</sup> on tissue repair was previously demonstrated in several experimental animal models and in human medicine in other tissues (bone, muscle, cornea, etc) [18–21]. These results triggered our interest to investigate the use of RGTA<sup>®</sup> to treat horses' tendinitis.

HS are degraded in injured tissue, due to inflammation mediated enzymatic degradation. The injected RGTA<sup>®</sup> are resistant to enzymatic degradation, and replace HS at heparan-binding sites in the matrix, allowing the extracellular matrix scaffold and growth factors to reposition in the cellular microenvironment [18]. Equitend<sup>®</sup> is an injectable formulation of RGTA<sup>®</sup> OTR4131, which has been adapted specifically for the treatment of tendon injuries, by the addition of supplementary acetate groups which alters hydrophobicity [21]. Results of a previous preliminary clinical trial on 51 horses with superficial digital flexor tendonitis, treated with a single injection of Equitend<sup>®</sup> [22] indicated an improvement of ultrasonographic appearance of tendinous lesion over the first 4 months and returned to racing 2.4 months earlier with higher level of earning (75% versus 15% in the matched controlled group). These encouraging results prompted us to design a double blind randomized, controlled multicenter clinical trial.

The objective of this clinical study was to evaluate the efficacy of Equitend<sup>®</sup> (in terms of ultrasound and performance data) on superficial digital flexor (SDF) tendonitis in French Standardbred Trotters (ST) after a single intralesional injection of Equitend<sup>®</sup> or placebo. The specific objectives were: i/ to compare the short term ultrasonographic healing of SDF tendonitis between groups receiving Equitend<sup>®</sup> or placebo; ii/ to compare the racing performances of both groups with a follow-up period of two years after lesion.

## Materials and methods

### Trial design

A multicentric prospective controlled clinical trial was conducted at the Center of Imaging and Research on Equine Locomotor Injuries (CIRALE, Normandy, France) and in 2 equine practices in Normandy (Clinique Equine du Livet, 14 140 St Michel de Livet;- Clinique Equine de Méheudin, 61150 Echouché).

Institutional Animal Care and Use Committee of the National Veterinary School of Alfort (Ecole Nationale Vétérinaire d'Alfort, Maisons-Alfort, France) approved the study protocol.

Inclusion criteria for client-owned racing French Standardbred Trotters (ST) were clinical and ultrasonographic signs of unilateral, acute or subacute (less than one-mon duration) SDF tendonitis. Horses were only included if the client agreed to the study design and tendons had not received intralesional injections before. Concurrent lesion which could be considered as a predisposing factor of re-injury of the SDF tendon (*i.e.* lameness of the opposite limb), and bilateral lesions were exclusion criteria (S1 Fig and S1 Table).

Family: BIP121524 BIP121524AUPC AUSTRALIA 2004285318 28/10/2004 2004285318 04/03/2010 BIP121524CAPC CANADA 2,543,530 28/10/2004 BIP121524CNPC CHINA 200480036221.9 28/10/2004 BIP121524EAPC EURASIA 200600862 28/10/2004 010945 30/12/2008 BIP121524EPPC01 EUROPE 10006666.1 28/10/2004 BIP121524EPPC02 EUROPE 10184844.8 28/10/2004 Published 15/12/2010 under N. 2260853 BIP121524ILPC ISRAËL 175235 28/10/2004 BIP121524ILPC01 ISRAËL 210249 28/10/2004 BIP121524INPC INDIA 1473/CHENP/2006 28/10/2004 BIP121524JPPC JAPAN 2006-537359 28/10/2004 BIP121524USPC UNITED STATES 10/577,637 28/10/2004 BIP121524ATEP AUSTRIA 04805335.9 28/10/2004 1677807 08/09/2010 BIP121524BEEP BELGIUM 04805335.10 28/10/2004 1677807 08/09/2010 BIP121524CHEP SWITZERLAND 04805335.11 28/10/2004 1677808 08/09/2010 BIP121524CZEP CZECH REPUBLIC 04805335.12 28/10/2004 1677809 08/09/2010 BIP121524DEEP GERMANY 04805335.13 28/10/2004 1677810 08/09/2010 BIP121524ESEP SPAIN 04805335.14 28/10/2004 1677811 08/09/2010 BIP121524FREP FRANCE 04805335.15 28/10/2004 1677812 08/09/2010 BIP121524GBEP UNITED KINGDOM 04805335.16 28/10/2004 1677813 08/09/2010 BIP121524HUHP HUNGARIA 04805335.17 28/10/2004 1677814 08/09/2010 BIP121524IEEP IRLAND 04805335.18 28/10/2004 1677815 08/09/2010 BIP121524ITEP ITALY 04805335.19 28/10/2004 1677816 08/09/2010 BIP121524LIEP LIECHTENSTEIN 04805335.20 28/10/2004 1677817 08/09/2010 BIP121524LUEP LUXEMBOURG 04805335.21 28/10/2004 1677818 08/09/2010 BIP121524NLEP NETHERLANDS 04805335.22 28/10/2004 1677819 08/09/2010 BIP121524PLEP POLAND 04805335.23 28/10/2004 1677820 08/09/2010 BIP121524PTEP PORTUGAL 04805335.24 28/10/2004 1677821 08/09/2010 BIP121524SEEP SWEDEN 04805335.25 28/10/2004 1677822 08/09/2010 BIP121524TREP TURKEY 04805335.26 28/10/2004 1677823 08/09/2010 BIP121524FR00 FRANCE 03/12605 28/10/2003 Published 29/04/2005 under N. 2861308 Title: Biocompatible polymers, their process of manufacture and compositions containing them Family: BIP121532 BIP121532CAPC CANADA 2,337,328 20/07/1999 BIP121532EPPC EUROPE 99932921.2 20/07/1999 Published 03/02/2000 under N. 1117695 BIP121532EPPC01 EUROPE 10182823.4 20/07/1999 Published 02/03/2011 under N. 2289970 BIP121532JPPC JAPAN 2000-561226 20/07/1999 4485058 02/04/2010 BIP121532USPC UNITED STATES 10/695,574 20/07/1999 BIP121532USPC01 UNITED STATES 12/212093

Equitend<sup>®</sup> treatment and placebo solutions were randomly assigned with a ratio of 2 treatments for one placebo (this ratio was chosen to improve client compliance and facilitate recruitment). Randomization procedure and blinding are described in [S1 Fig](#). Briefly, Excel function (RANDBETWEEN(Bottom, Top)) was used to randomize the treatments between the two groups. Blinding was in place throughout the trial and revealed only once all data, including racing performances, were collected. None of the clinicians, experimenters, owners and trainers were aware of the content of the vials at any time.

### Clinical examination

Clinical examination included physical and dynamic evaluation. Thickening and heat of the metacarpal tendon area were evaluated as well as sensitivity to pressure of the SDF tendon on flexed limb, using a four-grade scale: 0 = no reaction at pressure, 1 = reaction to moderate pressure, 2 = reaction to mild pressure, 3 = violent reaction to mild pressure.

Dynamic evaluation was performed on straight line at trot and the grade of lameness was established according to the American Association of Equine Practitioners scoring system [23].

### Ultrasonographic examination

All horses underwent a complete ultrasonographic examination of both metacarpal tendon areas, using a high-resolution machine equipped with 7.5 MHz linear probe coupled with stand-off pad. Severity of the SDFT injury and treatment-associated changes were assessed by measuring the cross-sectional area (CSA) of the tendon at the maximal injured site (MIS), echogenicity, transverse lesion extent, transverse lesion architecture using the angle contrast ultrasound technique (ACUST) [24], longitudinal lesion extent and architecture according to the grading system presented in [S2 Table](#). All the ultrasound images of each horse were scored blindly by four experienced veterinarians (JMD, AGD, SJ, VC) at once, at the end of the follow-up period. Time was not randomized, and images were reviewed and scored chronologically. The scores for all horses were made at the same time by each reviewer.

### Equitend<sup>®</sup>/Placebo treatment

Equitend<sup>®</sup> product contained the GAG mimetic [OTR4131], obtained from OTR3 Inc. (Paris, France). OTR4131 is an alpha 1–6 polyglucose polymer substituted with carboxyl, sulphate and acetate groups as shown in [Fig 1](#) [25], and prepared in 0.9% sodium chloride injectable form (0.15M NaCl), at the concentration of 10µg/mL Placebo vials were 0.9% sodium chloride only and absolutely identical to the Equitend<sup>®</sup> vials.

After aseptic preparation of the tendon area, injection of 1ml of Equitend<sup>®</sup> or placebo was performed on flexed limb, under ultrasound guidance at the level of the MIS, using a 0.5x16 mm needle ([S1 Table](#)) as previously described [22]. Horses were sedated by intravenous administration of detomidine (8 µg/kg) and butorphanol tartrate (16 µg/kg). Following injection, a light bandage was applied.

### Short-term follow-up and rehabilitation program

Clinical and ultrasonographic follow-up were performed at one (M<sub>1</sub>), two (M<sub>2</sub>) and four (M<sub>4</sub>) months after treatment or placebo injection using the same criteria as on inclusion day ([S3 Table](#)).

20/07/1999 BIP121532USPC02 UNITED STATES 09/765,788 20/07/1999 6,689,741 10/02/2004 BIP121532FR00 FRANCE 9809309 21/07/1998 98/09309 08/08/2003.

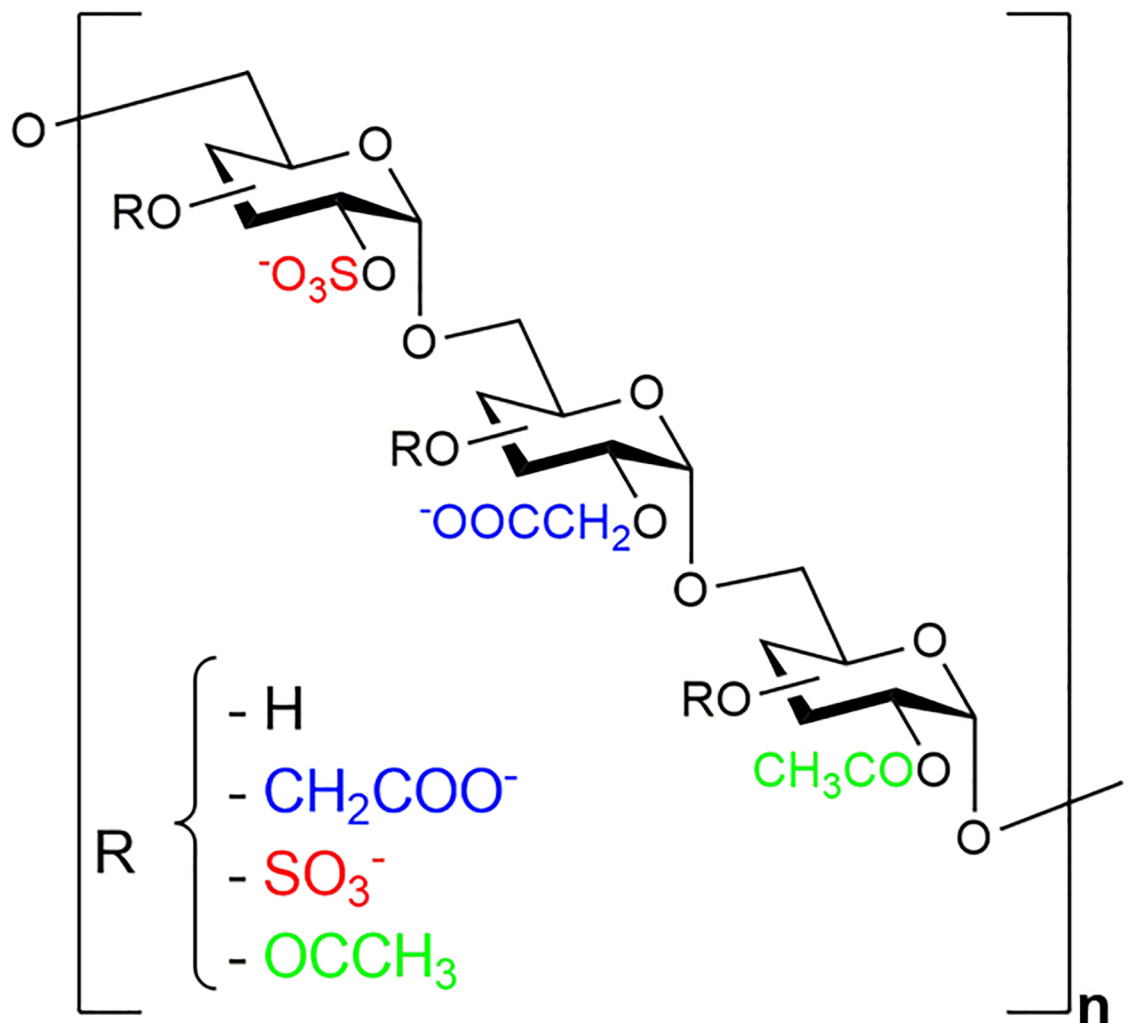
**Abbreviations:** ACUST, angle contrast ultrasound technique; AL-SDFT, accessory ligament of the superficial digital flexor tendon; CSA, cross-sectional area; D, day; GAG, glycosaminoglycan; GEE, generalized estimating equations; HA, hyaluronic acid; HS, heparan sulfate; M, month; PRP, platelet-rich plasma; RGTA<sup>®</sup>, ReGeneraTing Agents<sup>®</sup>; SDF, superficial digital flexor; SDFT, superficial digital flexor tendon; ST, standardbred trotter; TB, thoroughbred.

A standardized exercise program with interval training was recommended for all horses (S4 Table). This program was adjusted in accordance with the clinical and ultrasonographic findings and the expectations of the owner and trainer.

The use of a light shoe with wider toe and beveled branches for the front feet were recommended in all horses.

### Performance follow-up

All horses were followed up for two years after injury. The criteria used were: time to race post injury (established as the number of months from the treatment date to the first post-injury race), the number of races, the number of places, victories, the earnings per start post-injury. The results of performances after treatment were compared with results the year before injury. Racing data was obtained through websites (<http://www.letrot.com/fr/fiche-horse> and <http://www.turf-fr.com/pack-pro>). Re-injuries or end of careers were also recorded based on the information that the owners or the treating veterinarians gave us directly during the consultation or by phone call.



**Fig 1. Chemical structure of Equitend<sup>®</sup> active substance OTR4131.** OTR4131 is an alpha 1–6 carboxymethyl sulfate acetate glucose polymer with a molecular weight ranged between 100 to 150 kDa.

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## Statistical analysis

All calculations were performed using R v.3.3.2 software [26].

**S4 Table** summarized the collected data at the corresponding time, and their format such as continuous, binary or ordinal variables. Because the variables did not follow a Gaussian distribution, we used non-parametrical tests to analyze the data. Using a non-parametric test avoids making any assumptions about the distribution of the variables (*i.e.* Gaussian distribution), the independency of the variables and the minimum number of observations required to perform the test such as in parametrical test. Therefore, Kruskal -Wallis test was used to analyze non-Gaussian variables and as a non-parametric method for testing whether groups originate from the same distribution, it was used for comparing two or more independent groups of equal or different sample sizes.

For the following tests, type I error-set is 5% ( $\alpha = 5\%$ ).

**Analysis of initial data.** Baseline data at inclusion ( $D_0$ , day of treatment) were described and compared between the treatment and placebo groups, using non-parametric Kruskal-Wallis test for continuous variables, chi-squared ( $\chi^2$ ) test for binary variables, and Cochran-Armitage test for ordinal variables. Chi-squared ( $\chi^2$ ) test is the appropriate test to compare the distribution (*i.e.* frequency or proportion) between two or more groups such as recurrence. Finally, Cochran-Armitage test is an extension of the chi-squared ( $\chi^2$ ) test for the particular case when the categorical variable is ordinate (*i.e.* ordinal variable such as a grade from 0 to 4 as in ultra-sonographic variables) [27]. The test can be found in "coin" package in R-software [28].

**Analysis of clinical and ultrasound data.** In order to compare the clinical and ultrasonographic outcomes over time, the difference between data at the time-point considered ( $M_1$ ,  $M_2$  or  $M_4$ ) and baseline ( $M_0$  at  $D_0$ ) was calculated. Linear regression models were built in which the difference between  $M_i$  ( $i = 1, 2$  or  $4$ ) and  $M_0$  (value at inclusion) of clinical and ultrasonographic variables were the dependent variables, and independent variables were treatment/placebo group, age at the individual level and the center at group-level. It provided an estimate of the difference between the two groups because of treatment (or treatment effect) for each time-period ( $M_0$  to  $M_1$ ,  $M_0$  to  $M_2$ , and  $M_0$  to  $M_4$ ), adjusted on the horse age and inclusion centers (considered as fixed effects).

A generalized estimating equations (GEE) model was used for comparison of the cross-sectional area (CSA) between groups. To be closest to the normality, CSA was transformed in  $1/(\text{CSA})^2$ . Horse age and center effect were introduced into the model as fixed-effect covariates [29].

Briefly, GEE is part of marginal models that do not require distributional assumptions for observations, only a regression model for the mean response. Marginal models provide a unified method for analyzing diverse types of longitudinal responses (*i.e.* continuous and ordinal variables), which avoids making assumptions about the distribution of the vector of responses; the method relies solely on assumptions about the mean response and the comparison of the coefficients and the variances [30].

**Analysis of the performances variables.** The performances such as earnings per race, number of races and number of victories before tendinitis (one year) and after treatment (*i.e.* 24-months after) were analyzed by using Kruskal-Wallis non-parametric chi-squared test. The recovery of the performances before tendinitis and after treatment was analyzed using the Wilcoxon signed rank pairwise non-parametric test with continuity correction.

The recurrence of tendinopathy over 2-years follow-up in both placebo and Equitend<sup>®</sup>-treated groups was assessed by Kaplan-Meier "survival" curve ("survival" R-package) and analyzed using Fleming-Harrington test [31, 32] because the effect of the therapy is more

pronounced towards the middle and the end of the follow-up period (“survMisc” and “FHtest” R-packages). The recurrence over the 2-years after treatment was estimated by the odds ratio and the risk ratio using the small-sample adjusted method and compared by chi-squared test (“epitools” R-package) [33].

## Results

### Study population

Between May 2008 and February 2012, forty-two (n = 42) horses met the eligibility criteria, but only twenty-four (n = 24) ST presenting with acute (or subacute) SDF tendonitis in one front limb were included in the study because of lack of owners’ compliance for the eighteen (n = 18) horses not included (S1 Fig). Two horses were excluded during the follow-up period because they presented major colic signs (S1 Fig) that required administration of non-steroidal anti-inflammatory drugs. In the final 22 horses studied, there were 3 females, 8 males and 11 geldings. The mean age was 5.4 +/- 1.4 years old (range, 3 to 8 years). Horses were randomly assigned into Equitend<sup>®</sup> (n = 14) or saline (n = 8) treatment groups and no significant differences in age, genders, duration of SDF tendinopathies, affected forelimb, centers of investigation, lameness grades and sensitivity grades were observed between treatment groups (Table 1 and S1 Table). Hence all conditions were met for further investigation of the Equitend<sup>®</sup> treatment.

### Safety

As expected from preclinical safety and previous studies [22], intralesional injection was well tolerated for all horses and no adverse local or systemic effects were reported after treatment.

### Clinical and ultrasound follow-up

Analysis of clinical parameters on the day of inclusion (day 0) into the study revealed no significant difference between Equitend<sup>®</sup> and saline treated groups. Ultrasonographic examination

**Table 1. Characteristics of the study population at the date of inclusion.**

	Placebo n = 8	Equitend <sup>®</sup> Treatment (1 mL, OTR4131, 10 µg/mL) n = 14	p-value
Age (years, mean ± SEM)	5.3 ± 0.6	5.3 ± 0.4	0.09 <sup>‡</sup>
Gender (F/G/M)	1/5/2	2/6/6	0.7 <sup>†</sup>
Duration of SDF tendinopathy before examination (days, mean ± SEM)	11.9 ± 2.9	25.8 ± 13.1	0.5 <sup>‡</sup>
Affected forelimb R or L (n)	2/6	5/9	0.9 <sup>†</sup>
Centers of investigation (C/CL/CM)	5/3/0	7/6/1	0.7 <sup>†</sup>
Lameness grades n (%)	Grade 0: 2 (25)	Grade 0: 3 (21)	0.8 <sup>+</sup>
	Grade 0.5: 0 (0)	Grade 0.5: 2 (14)	
	Grade 1: 4 (50)	Grade 1: 5 (36)	
	Grade 2: 2 (25)	Grade 2: 4 (29)	
Sensitivity grades n (%)	Grade 1: 3 (38)	Grade 1: 1 (7)	0.1 <sup>+</sup>
	Grade 2: 2 (25)	Grade 2: 5 (36)	
	Grade 3: 3 (38)	Grade 3: 8 (57)	

Horses were randomly assigned into either Equitend<sup>®</sup> treatment or placebo group. All horses included in this study are Standardbred trotter (ST). Comparison between placebo and Equitend<sup>®</sup> by using ‡ unpaired t-test, † Chi-squared test with Yates’ correction, and + Cochran-Armitage test for ordinal variables.

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**Table 2. Ultrasonographic and performance data at D0, and comparisons according to treatment groups.**

Variables	Grade	Overall population (n = 22)	Control group (n = 8)	Treatment group (n = 14)	p-value
<i>Ultrasonographic variables</i>					
Echogenicity	2	3 (14%)	1 (12.5%)	2 (14%)	0.7
	3	12 (55%)	4 (50%)	8 (57%)	
	4	7 (32%)	3 (37.5%)	4 (29%)	
Transversal architecture	2	1 (5%)	0 (0%)	1 (7%)	0.08
	3	8 (36%)	3 (37.5%)	5 (36%)	
	4	13 (59%)	5 (62.5%)	8 (57%)	
Longitudinal architecture	2	2 (9%)	1 (12.5%)	1 (7%)	0.7
	3	14 (64%)	5 (62.5%)	9 (64%)	
	4	6 (27%)	2 (25%)	4 (29%)	
Trans. Lesion extent	1	2 (9%)	2 (25%)	0	0.04
	2	5 (23%)	3 (37.5%)	2 (14%)	
	2.5	2 (9%)	0 (0%)	2 (14%)	
	3	8 (36%)	2 (25%)	6 (43%)	
	4	5 (23%)	1 (12.5%)	4 (29%)	
Long. Lesion extent	3	2 (9%)	2 (25%)	0	0.3
	3.5	1 (5%)	0 (0%)	1 (7%)	
	4	19 (86%)	6 (75%)	13 (93%)	
Cross Section Area—tendonitis side		1.55 (1.27–1.77) 1.57 (0.37)	1.26 (1.14–1.45) 1.33 (0.32)	1.66 (1.13–1.88) 1.70 (0.34)	0.01
Cross Section Area—contralateral limb		0.85 (0.78–0.94) 0.88 (0.16)	0.79 (0.76–0.86) 0.80 (0.07)	0.87 (0.81–1.10) 0.93 (0.18)	0.08

Values are expressed as median (inter-quartile interval) and mean (standard deviation) for continuous variable, and as number (percent) for categorical variables.

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revealed an unexpected increase in the transversal lesion extent ( $p = 0.04$ ) and a higher CSA at MIS ( $p = 0.01$ ) in the Equitend<sup>®</sup> treated group at day 0 (Table 2).

While the CSA did not significantly change in the placebo treated group ( $p = 0.2$ ), there was a significant decrease in CSA from M0 to M1 (from 1.70 to 1.61 cm<sup>2</sup>) in Equitend<sup>®</sup> treated horses ( $p = 0.04$ , Tables 3 and 4) whereas in the placebo group CSA remained constant (from 1.33 to 1.34 cm<sup>2</sup>). These differences could be explained because in Equitend<sup>®</sup>-treated horses CSA was significantly higher compared to placebo group at the time of inclusion (M0) ( $p = 0.01$ ).

Interestingly, the number of Equitend<sup>®</sup>-treated horses ( $n = 7$ ) for which the CSA decreased more than 10%, after 4-months of treatment, was significantly higher ( $p = 0.03571$ ) compared to the number of placebo-treated horses ( $n = 1$ , Fig 2). The average CSA improvement in Equitend<sup>®</sup>-treated horses ( $n = 7$ ) was about 17% during this 4-months of treatment, with a CSA improvement in these 7 horses (Fig 2) ranged from an average of 1.80 cm<sup>2</sup> (M0) to 1.50 cm<sup>2</sup> (M4). In both groups, CSA did not change for 5 horses and deteriorated for 2 horses.

**Table 3. Treatment effect on cross section area (CSA) at the maximal injured site (MIS) on the affected limb in the period M<sub>0</sub> to M<sub>1</sub>—General Estimating Equations (GEE) model.**

Variables	Coefficient (95% CI)	p-value
Change of CSA in the control group between M <sub>0</sub> and M <sub>1</sub>	-0.03 (-0.09 to 0.02)	0.2
Difference between the two groups at baseline -M <sub>0</sub>	-0.18 (-0.28 to -0.76)	0.01
Treatment effect on CSA between M <sub>0</sub> and M <sub>1</sub>	0.07 (0.002 to 0.14)	0.04

CI: confidence interval.

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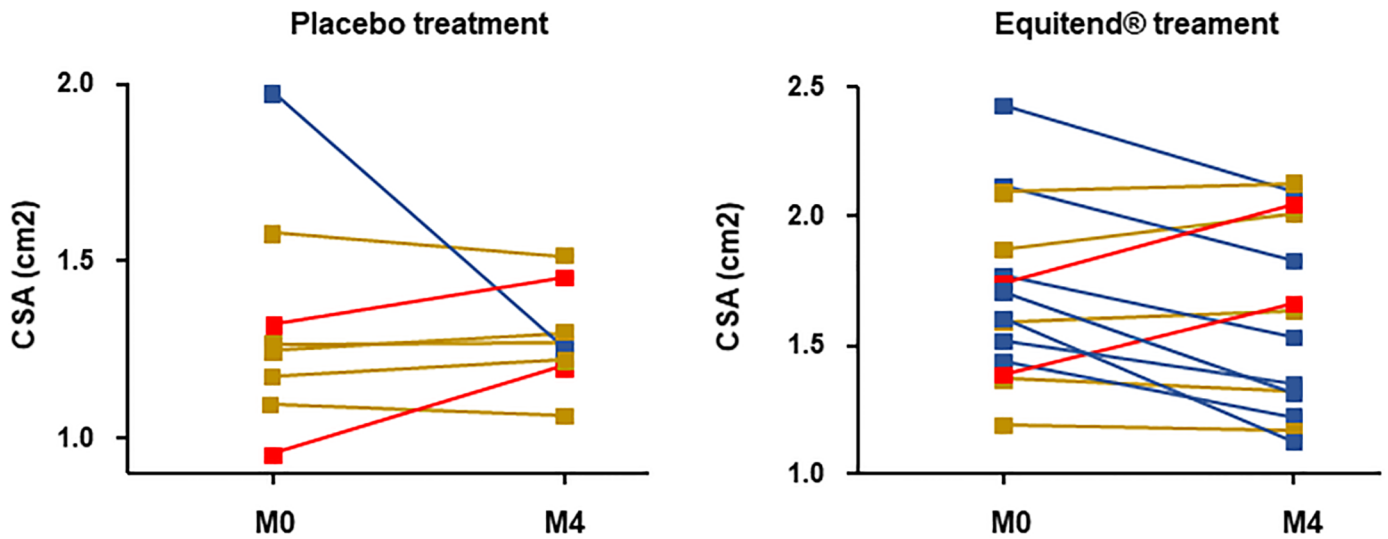
Table 4. Mean CSA measures before and during the first four months of treatment.

	CSA mean $\pm$ SD cm <sup>2</sup>			
	M0	M1	M2	M4
Placebo (n = 8)	1.33 $\pm$ 0.32	1.34 $\pm$ 0.16	1.29 $\pm$ 0.14	1.33 $\pm$ 0.17
Equitend (n = 14)	1.70 $\pm$ 0.34	1.61 $\pm$ 0.34	1.60 $\pm$ 0.36	1.63 $\pm$ 0.35

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### Racing data analysis

Among the 22 included horses, 19 horses returned racing: 7 out of 8 horses (87.5%) in the placebo group and 12 out of 14 horses (85.7%) in the Equitend<sup>®</sup>-treated group. The three horses (one in placebo group and 2 in treated group) which did not resume racing, were retired for reasons unrelated to their tendinopathy. The proportion between groups was not dissimilar (p = 0.974, chi-squared test). Analysis of performance in the year prior to the tendinopathies,



Evolution of CSA from M0 to M4	Placebo n=8	Equitend <sup>®</sup> n=14
Decrease of CSA more than 10% (■)	1	7
Decrease or increase of CSA less than 10% (■)	5	5
Increase of CSA more than 10% (■)	2	2
<b>p-value (Cochran-Armitage test)</b>	<b>0.03571</b>	

Fig 2. Improvement of CSA after 4 months of Equitend<sup>®</sup> treatment. Graphics represent CSA (cm<sup>2</sup>) at the time of inclusion (M0) and after 4-months (M4) treatment for each horse in placebo group (left panel) and Equitend<sup>®</sup>-treated group (right panel). The table represent the number of horses in each treated group that improved more than 10% the CSA (■), not improved (■) Decrease or increase of CSA less than 10%), or worsened more than 10% (■). Data were compared by asymptotic Cochran-Armitage test.

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**Table 5. Equitend<sup>®</sup>-treated horses have better performances and recovery compared to placebo a year before tendinitis and over the two years after inclusion.**

Performances of the horses	12 months before treatment			24 months after treatment			Recovery (Before vs After)	
	Placebo group n = 7	Treatment group n = 12	*p-value	Placebo group n = 7	Treatment group n = 12	*p-value	Placebo #p-value	Treatment #p-value
Earnings/race median [interquartile]	2845 [1484–6259]	2636 [1486–3090]	0.5617	439 [91–3131]	1296 [649–3302]	0.3525	0.1094	0.2593
Number of races median [interquartile]	14 [10–16]	15 [7.5–24.25]	0.5243	6.0 [4.5–9.5]	15.5 [10–22.5]	<b>0.01399</b>	<b>0.0446</b>	0.6382
Number of victories median [interquartile]	1 [1–2.5]	2 [0.75–3.5]	0.8293	0 [0–0]	1.5 [0–2]	<b>0.0421</b>	<b>0.0170</b>	0.2647

Values are expressed as median [inter-quartile range].

\*p-values were calculated using the Kruskal-Wallis non-parametric chi-squared test.

#p-values were calculated using the Wilcoxon signed rank pairwise non-parametric test with continuity correction.

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such as number of races, number of victories and earnings per race did not show any differences between placebo and Equitend<sup>®</sup>-treated groups. Both groups are therefore considered as even.

Equitend<sup>®</sup>-treated horses raced significantly more than the placebo group ( $p = 0.01399$ ) and the number of victories was significant higher ( $p = 0.0421$ , Table 5). Moreover, Equitend<sup>®</sup>-treated horses returned to their pre-injury performance level whereas placebo-treated horses did not (Table 5). In the placebo group the median number of races before treatment was 14 (interquartile [10–16]) whereas 24 months after injection it was 6 (interquartile [4.5–9.5],  $p = 0.0446$ ). In the Equitend<sup>®</sup>-treated group, values before treatment were 15 [interquartile: 7.5–25] and became 15.5 after 24 months of treatment (interquartile [10–22.5],  $p = 0.6382$ ).

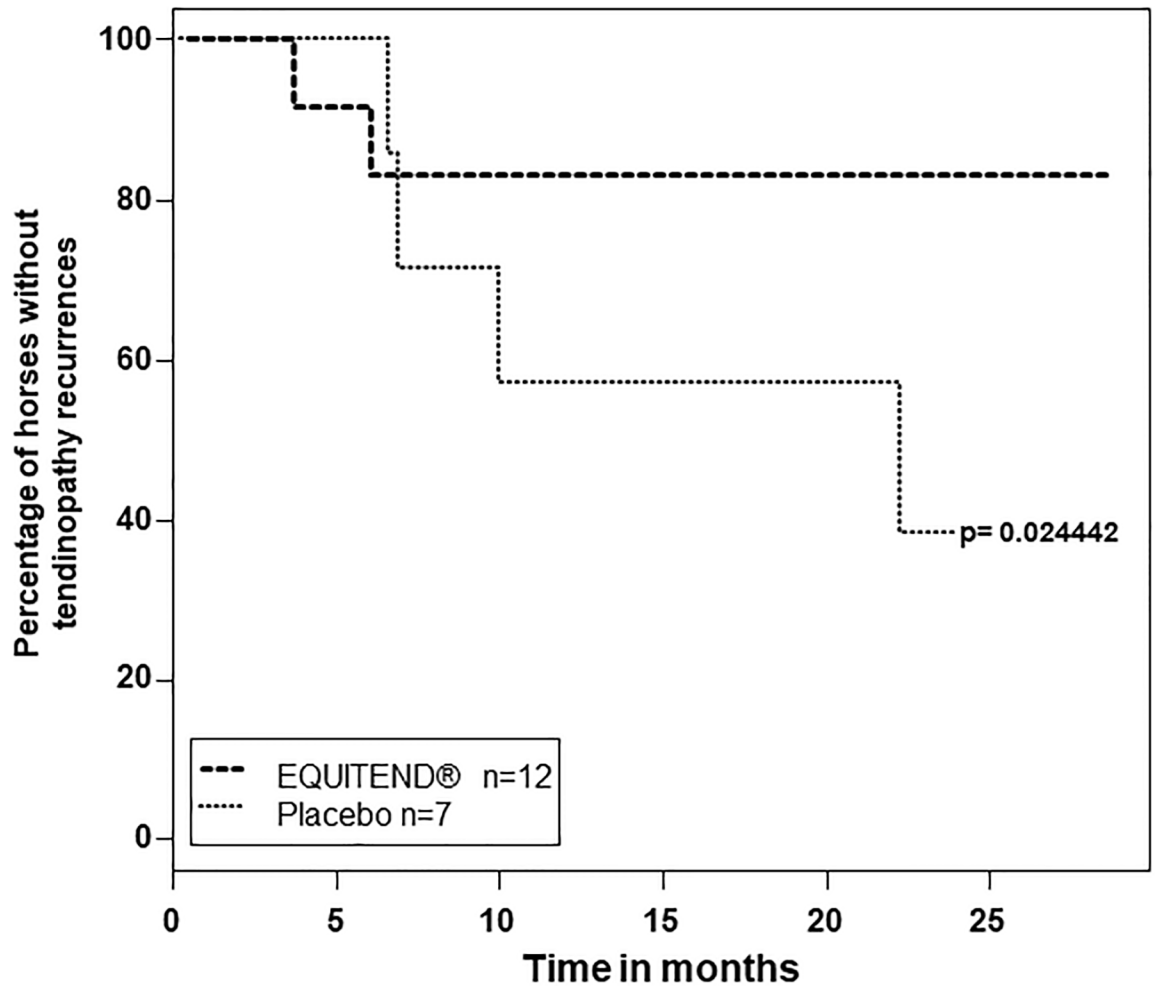
Similar results were observed for the number of victories. In the placebo group, no victories were reported after treatment, while the Equitend<sup>®</sup>-treated horses performed as before injury (Table 5).

The recurrence of tendinopathy over 24-months follow-up was assessed by Kaplan-Meier curve and showed that the placebo-treated group had significantly more recurrence of tendinopathy compared to Equitend<sup>®</sup>-treated group (Fig 3,  $p = 0.024442$ ). Moreover, odds ratio (= 3.333) and risk ratio (= 2.476),  $p = 0.0671$  are largely in favor of the Equitend<sup>®</sup> group compared to the placebo group (Table 6).

## Discussion

This study confirms efficacy of RGTA<sup>®</sup> based matrix therapy in the treatment of tendinopathies. Sport and race horses are considered as a relevant model for humans over use tendonitis such as those developed in repeated motions and intense sport practice [34]. Therefore, the results presented here are expected to be supportive for a human efficacy study, although such a study needs to be designed and adapted. The reduced recurrence rate measured in this study is also a promising indicator to follow for human tendonitis. Another parameter associated with RGTA<sup>®</sup> not reported in this study is pain relief observed in cornea [19, 35] and skin lesion [36]. For a human study, the pain reduction parameter would be of great interest.

Double blind, randomized, controlled, prospective clinical study is unanimously recognized as the gold standard for a clinical trial [37], however it is hard to recruit racing horses to reach a sufficient population to avoid any bias. This limit is due to the lack of owner compliance and represents a weakness of this study since the statistical analysis was limited by the small population. To our knowledge, we identified two field randomized clinical trials showing some



**Fig 3. Equitend<sup>®</sup>-treated horses had significantly fewer recurrences of tendinopathy over 24-months follow-up.** Kaplan-Meier curve shows recurrences of tendinopathy over 24-months follow-up of placebo (n = 7) and Equitend<sup>®</sup>-treated (n = 12) groups. p-value was estimated using Fleming-Harrington weights, emphasizing differences later in time. Fleming-Harrington is a Kaplan-Meier (product-limit) estimator.

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**Table 6. Placebo group has a higher risk of recurrence than Equitend<sup>®</sup>-treated group over the two years after inclusion.**

Recurrence	Placebo group n = 7	Treatment group n = 12
Yes	4	2
No	3	10
Odds ratio	3.333	1
Risk ratio	2.476	1
#p-value	0.0671	

The odds ratio and the risk ratio are estimated by the small-sample adjusted method and # compared by Chi-square test

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efficacy of autologous MSC cells [13, 15]. Dyson (2004) was not able to perform a randomized placebo controlled trial because of this inherent client limitation [5]. To counteract these small groups a very homogeneous population with a detailed and long-term follow-up was studied. Another limit of the trial was the difference of lesion severity between the 2 groups at the time of inclusion, the CSA being significantly higher in the Equitend<sup>®</sup>-treated group compared to the placebo group. This difference could not have been expected nor prevented as the randomization was blinded.

Indeed, CSA is the most objective measure of tendon pathology and can be assessed accurately using ultrasound for the tendinopathy follow-up, as extensively described before [38–40] but not necessarily directly related to the extent of the pathology.

Marr et al. (1993) identified that the severity of the lesions was related to the outcome on a population of 73 National Hunt racehorses [41]. It is reasonable to assume that difference between the 2 groups could have been more demonstrative in favor of the Equitend<sup>®</sup> group if initial lesions were similar.

Most previous published data referred to retrospective [11], uncontrolled [13, 15, 16, 42] or matched [5, 9, 10, 22] trials. A study published by Smith et al. (2013), described a randomized and controlled clinical trial, to estimate the efficacy of autologous bone marrow stem cells on naturally occurring tendinopathy, but no performance follow-up was presented [43]. As the population studied was exclusively composed of retired National Hunt horses, the conclusion was based only on ultrasonographic healing, and results cannot be extrapolated to racehorses in activity. A recent prospective controlled clinical trial evaluated the effect of intralesional PRP treatment on naturally occurring SDF tendonitis [44]. The authors demonstrated that a higher proportion of treated horses were able to reach their previous or higher level of performance compared with placebo treated horses (80 versus 50% at 12 months, 60 versus 50% at 24 months). Nevertheless, the population studied was not homogeneous and only composed of sport and pleasure horses of various ages and levels of activity. To our knowledge the present study is the first prospective controlled clinical trial on racehorses with 2 years follow-up. According to the first Havemeyer workshop on equine tendon disease [37], evaluation of the efficacy of a treatment requires analysis of several objective outcome parameters (*i.e.* re-injury rate, proportion of horses racing more than 3 or 5 times).

Most clinical trials previously published were performed on flat and National Hunt racehorses or sport horses [2, 5, 10, 11, 16, 41, 43]. Few were made on Standardbreds [9, 11, 42, 45], and only three considered superficial digital flexor tendonitis [15, 42, 45]. The choice of ST in this study was based on the large population available in France, the easy accessibility to performance data, and the simple management of racing career (ST resume racing as soon as possible and can race all year long).

As described by Cheetham et al. (2010), several factors as age, sex, breed, discipline, gait, track surface (for Thoroughbred), could interfere with outcome [46]. In the present study, these parameters were very homogeneous between both studied groups. No difference of breed, sex and age, was identified, and trot is the only gait used in France. Furthermore, the placebo and Equitend<sup>®</sup>-treated groups also matched duration of SDF tendinopathies, affected forelimb, centers of investigation, lameness grades and sensitivity grades.

Interestingly, echography analysis before treatment indicated a significant difference between the two groups. The Equitend<sup>®</sup> group showed higher deterioration of the transectional lesion extent and cross-sectional area. After one month of treatment, no improvement was measured in the placebo group while the Equitend<sup>®</sup>-treated group showed significant improvement for both parameters.

CSA was significantly improved by 17% at M4 in 7 Equitend<sup>®</sup> treated horses while only 1 horse in the placebo group spontaneously improved ( $p = 0.03571$ ), suggesting that this

improvement might have been even higher at 6 months as reported in other studies [16, 43, 47].

To ensure homogeneous conditions, the same rehabilitation program and shoeing were recommended for all horses. Activity was adapted according to the findings at ultrasound and clinical examinations as described above in “Short-term follow-up and Rehabilitation program” and S3 Table. A synthesis of the previously reported rehabilitation protocols was built on the 2007 Havemeyer symposium [37], but was dedicated to Thoroughbred (TB) racehorses or sport horses. Interestingly, although recovery under our treatment is faster, performance data registered were similar than those reported on TB [5, 10, 11, 13]. It would suggest that recovery period for SDF tendonitis on ST could be reduced compared to TB racehorses.

Emphasis was put on long-term analysis, with numerous performance data studied and up to 2 years follow-up after injury. Racing performances of the two populations were different: out of 8 Placebo treated horse 7 went back to racing but ran fewer times than previously whereas the Equitend<sup>®</sup> group (12 out of 14 horses) resumed similar activity and performances as before injury, running significantly (2.3 times) more than the placebo group. An average of four times higher recurrence was noted in the placebo group compared to Equitend<sup>®</sup> treated group.

Dyson (2004) reported re-injury rates in various intralesional treatment groups with 42.5% for HA, 44.4% for PSGAG treatment or 45.5% (when including contralateral limb) for  $\beta$ -aminopropionitrile fumarate. Published data on the use of bone marrow mesenchymal stem cells showed lower re-injury rate with respectively 32.7% (when including contralateral limb) in Godwin et al. (2013) study [37, 43], and only 13% in Smith et al. (2013) study [37, 43]. In this last study, the re-injury rate was the unique outcome parameter studied, and analysis of several performance criteria is recommended to evaluate a treatment on tendonitis [37, 43]. In a study comparing performance of TB affected with SDF tendonitis matched with sound horses, O’Meara et al. showed that the assessment of the outcome of horses with SDF tendonitis using the number of races post injury required a minimum of 5 races post injury to be a useful indicator [4].

In the present study, a higher number of races post injury in the Equitend<sup>®</sup> group was noted, with a higher rate of horses who competed more than 5 times. With respectively 78,6% and 64,3% of horses who competed more than 5 and 10 times on the Equitend<sup>®</sup> group, these results compare favorably with our previous analysis for the same category of horses (Standardbreds) [42]. Hogan et al. (1995) published data on horses with desmotomy of the accessory ligament of the superficial digital flexor tendon (AL-SDFT), with a rate of 69% of horses racing more than 5 times [42]. Sawdon (1996) showed that 52% of horses competed more than 5 times with only conservative management [45]. In a more recent study, with use of autologous bone marrow aspirate alone or combined with desmotomy of the AL-SDFT, Russel et al. (2016) observed that 62% of treated horses raced more than 5 times and 53% more than 10 times [15].

Contrary to other treatments such as PRP, bone marrow or stem cells, Equitend<sup>®</sup> is a stable, synthetic molecule. Moreover, only a single intralesional injection with a small volume of product was performed in this study. Therefore, Equitend<sup>®</sup> could be considered by practitioners, as a more practical therapeutic option than PRP or stem cells.

More extensive studies should address the use of Equitend<sup>®</sup> at earlier stage after tendinopathies injury as suggested by the mode of action of this molecule adapted to acute injury and to evaluate the benefit of a second injection in case of inconclusive ultrasonographic follow up data.

## Conclusion

Results of this study combined with the previous preliminary trial performed on Equitend<sup>®</sup> demonstrate that a single intralesional injection of Equitend<sup>®</sup> is safe and has beneficial effect for management of tendonitis in horses. Equitend<sup>®</sup>-treated horses recovered better than placebo treated horses, returning to a performance level similar to their pre-injury level.

Equitend is the first RGTA<sup>®</sup> product developed to be injected for clinical uses and for a veterinary drug application, although many experimental tissue lesion animal models have demonstrated efficacy by injection. The results presented in this study will support development for a human use.

## Supporting information

**S1 Fig. Flow diagram of Equitend<sup>®</sup> clinical trial.** The figure summarized the steps followed to include the 24 horses on the clinical trial based on the consort recommendations (<http://www.consort-statement.org/consort-statement/flow-diagram>). (PDF)

**S1 Table. Description and clinical history and treatment of each horse.** Horses were randomly assigned into either Equitend<sup>®</sup> treatment or placebo groups. Horses were sedated with detomidine (8 µg/kg) and butorphanol (16 µg/kg) and injection sites were prepared by shaving and sterilization. Equitend<sup>®</sup> (1 mL, OTR4131, 10 µg/mL) or control saline solution was administered via intralesional injection under ultra-sonographic guidance into each injured tendon at the maximal injury site (MIS), using 25" gauge disposable sterile syringes. During the entire trial period, the composition of the solutions was known only by OTR3 to ensure blinding throughout the study. Following injection, a light bandage was applied. The horses were kept in stables for 24 h prior to their return to paddocks. Bandages were removed 24 h after injection and the injection site was monitored. (PDF)

**S2 Table. Semi-qualitative and quantitative ultrasonographic criteria for characterizing the SDFT lesions.** MIS: Maximal injury site; CSA: cross-sectional area; SDFT: superficial digital flexor tendon. (PDF)

**S3 Table. Summary of the clinical, ultrasonography and performance variables, their measurement times, and format.** \* All data were collected for the horse, except the CSA that was collected for both side (tendonitis one and healthy counter-side). Meaning of the time-points: D<sub>0</sub>: inclusion date, and day of injection into the forelimb of the experimental treatment or placebo. M<sub>1</sub>, M<sub>2</sub> and M<sub>4</sub>: repeated measurements of the variables. M<sub>12</sub> and M<sub>24</sub>: for the calculation of number of race and victory(ies) 12 months before inclusion and 24 months after inclusion respectively. (PDF)

**S4 Table. Guidelines for the rehabilitation program adjusted according to clinical and ultrasonographic findings at each control (M<sub>1</sub>, M<sub>2</sub>, M<sub>4</sub>).** (PDF)

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## Author Contributions

**Conceptualization:** Sandrine Jacquet-Guibon, Jean-Marie Denoix.

**Data curation:** Sandrine Leroy.

**Formal analysis:** Virginie Coudry, Nathalie Crevier-Denoix, Fernando Siñeriz, Franck Chiappini.

**Funding acquisition:** Nathalie Crevier-Denoix, Denis Barritault, Jean-Marie Denoix.

**Investigation:** Sandrine Jacquet-Guibon, Anne-Gaelle Dupays, Virginie Coudry, Nathalie Crevier-Denoix, Jean-Marie Denoix.

**Methodology:** Sandrine Jacquet-Guibon, Nathalie Crevier-Denoix, Sandrine Leroy, Fernando Siñeriz, Franck Chiappini, Jean-Marie Denoix.

**Project administration:** Denis Barritault.

**Resources:** Fernando Siñeriz, Denis Barritault, Jean-Marie Denoix.

**Software:** Franck Chiappini.

**Supervision:** Anne-Gaelle Dupays, Denis Barritault, Jean-Marie Denoix.

**Validation:** Franck Chiappini, Jean-Marie Denoix.

**Writing – original draft:** Sandrine Jacquet-Guibon, Franck Chiappini, Denis Barritault, Jean-Marie Denoix.

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