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A Phase 1 study for safety and pharmacokinetics of BIO101 (20-hydroxyecdysone) in healthy young and older adults

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Abstract

Background Sarcopenia is an age-related skeletal muscle disorder characterized by loss of muscle mass and strength leading to mobility disability. 20-Hydroxyecdysone (20E) is a polyhydroxylated plant steroid that demonstrates pharmacological effects in many disease animal models including ageing/sarcopenia. BIO101 is a 20E purified investigational drug (≥97%) that previously demonstrated good toxicology profiles in rat and dog. BIO101 is evaluated in healthy young and older adults in a Phase 1 study.

Methods This study is a Single Ascending Dose (SAD) followed by a 14-day Multiple Ascending Dose (MAD). In SAD, BIO101 was administered orally to 16 young adults at doses from 100 to 1400 mg and to 8 older adults (age ≥65 years) at 1400 mg. In MAD, doses of 350 mg once daily (qd), 350 mg twice daily (bid) and 450 mg bid were administered to 10 older adults. The primary objective was to evaluate safety and pharmacokinetics (PK), including dosing of circulating metabolites. Pharmacodynamic effects were investigated with regard to myostatin, procollagen-III-amino-terminal propeptide (PIIINP), myoglobin, creatine-kinase Muscle Brain (CKMB), renin and aldosterone plasma/serum levels.

Results BIO101 showed a good safety profile with only mild to moderate adverse events and a satisfactory pharmacokinetic profile. In SAD, at 100 mg to 1400 mg, mean Cmax and areas under the curve increased less than dose-proportionally. Mean half-life was short (2.4–4.9 h), and mean renal clearance was comparable in all doses (4.05–5.05 L/h). Mean plasma exposure was slightly lower in older adults (22% lower for Cmax and 13%–15% lower for AUCs) compared with young subjects. In MAD, 350 and 450 mg bid led to a slight accumulation over 14 days (mean ratio of accumulation [Rac] of 1.31 in both cohorts). Reduction of biomarkers (myoglobin, CK-MB) mean serum levels (vs. baseline) was observed at 450 mg bid. Two major metabolites of 20E (14-deoxy-20-hydroxyecdysone and 14-deoxypoststerone) were identified and quantified.

Conclusions BIO101 shows a good safety and pharmacokinetic profile that led to the selection of doses for the subsequent interventional clinical trials of Phase 2 in age-related sarcopenia (SARA-INT) and Phase 3 in Covid-19 (COVA).

Keywords 20-hydroxyecdysone; BIO101; sarcopenia; safety; pharmacokinetics; older

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Introduction

Sarcopenia is a geriatric condition characterized by a loss of muscle mass and strength, beginning to develop by the fifth decade and contributing to an increased risk of falls and fractures.[1] Sarcopenia prevalence is approximately 10% in men and women.[2]

Age-related sarcopenia management requires acting not only on muscle mass but also on muscle function to prevent subsequent disabilities,[3–6] based on the Foundation of National Health Institute[7] and the EWGSOP definitions.[8] Many products derived from different classes of molecules were tested over the past decade in clinical studies on sarcopenic patients such as anabolic hormones like testosterone[9] or selective androgen receptor modulators (SARMs[10,11]); myostatin inhibitors—monoclonal antibodies, soluble receptors[12,13]; molecules targeting the renin–angiotensin system (angiotensin-converting enzyme inhibitors [ACEI] and angiotensin II receptor blockers [ARBs like losartan][14]).

Despite these attempts, no drug candidate has proven its efficacy, and sarcopenia still represents an unmet medical need.

20-Hydroxyecdysone (20E) is a polyhydroxylated phytosteroid pharmacologically active in mammals and belonging to a group of structurally related compounds (ecdysteroids), of which more than 500 members have been described.[15] Zooecdysteroids are hormones controlling development and reproduction in arthropods. Phytoecdysteroids protect plants against phytophagous insects (as feeding deterrents and/or endocrine disruptors). In mammals, 20E stimulates protein synthesis, especially in muscles,[16] and accelerates myocytes differentiation.[17]

BIO101 is a drug candidate developed with 20E purified at ≥97% as the active pharmaceutical ingredient (API). It was recently demonstrated that BIO101 activates MasR (one of the receptors of the renin–angiotensin system [RAS]), on its protective arm.[18] MasR activation could be responsible for most of beneficial activities observed in relevant animal models of pathology. 20E has a good safety profile with low oral toxicity ascertained by no LD_{50} (lethal dose 50%) reached at 9 g/kg on mice[18] and no change in organ weight reported after a chronic administration for up to 35 days at 2 g/kg in rabbits.[19] NOAELs in 26 weeks in rats and 39 weeks in dogs were established at 1000 mg/kg, and metabolic studies[20] allowed to develop a set of reference metabolites identified in rodents.

Only a few pharmacokinetic studies have been conducted with 20E in humans. Most analyses were restricted to the urine eliminated following oral intake.[21–26] The authors recovered only a small proportion (approximately 1%–2%) of ingested 20E and tentatively identified two metabolites, 14-deoxy-20E (14d20E)[23] and 14-deoxy-poststerone (14dPost).[26] This deserves more extensive studies, especially of circulating levels of 20E and its metabolites after oral intake.

This article reports the first-in-human randomized double-blind Phase 1 placebo-controlled single and 14-day multiple ascending doses study in young and older (age≥65 years) adults to evaluate BIO101 safety, tolerability, pharmacokinetics and pharmacodynamic effects.

Material and methods

Protocol approvals and amendments, registrations

The study identified as BIO101-C01, dated 23 June 2016, and its amendment dated 19 July 2016 was approved by the Independent Ethics Committee (IEC) of Antwerp under the number EC N°4803. The study was also approved by the National Competent Authority of Belgium, FAHMP under the EudraCT ref. 2016-001434-94 and was conducted under the Good Clinical Practices, the Declaration of Helsinki and Applicable National and Local Regulations of Belgium.

Study design

This study in healthy young and older adults was initiated on 14 July 2016 as the date of the first Inform Consent Forms (ICF) and completed in December 2016 (date of last contact with any subject).

The primary objective was to evaluate the safety and pharmacokinetics (PK) of BIO101 and its main metabolites following single and multiple ascending oral doses administered for 14 days.

The secondary objectives were to assess the safety and PK of BIO101 and its main metabolites in older adults following selected oral doses administration for 14 days and to evaluate the food effect and the age effect on the PK of BIO101 and its main metabolites following a selected single oral dose administration.

Exploratory objectives were to investigate any pharmacodynamic (PD) effects of BIO101with the evaluation of a set of selected plasma biomarkers as tentative PD indexes.

The study consisted in two parts—a single ascending dose (SAD) (Part 1) and a multiple ascending dose (MAD) (Part 2) (*Tables 1* and *2* and *Figure S1A–C*), see also the clinical protocol synopsis in the Data S1.

In the Part1 SAD, subjects were randomized into three cohorts (*Figure S1A,B*; *Table 1*). First two cohorts included 8 young subjects of 18–55 years, extremes of age included, and a third cohort (C) included 8 elderly subjects of 65 years and above. The two first cohorts sequentially received 100- and 700-mg oral doses of the study drug (Cohort A) or 350 and 1400 mg (Cohort B) in an alternating panel manner.

Cohort C (*Table 1*) received a single administration of 1400-mg BIO101 or placebo in fasted condition. At each dose level, six subjects were randomized to BIO101 and two to pla-

Table 1 Demographic data, baseline characteristics and doses of the panels in the single ascending doses for Part 1 (SAD), Cohorts A, B and C.

Parameter		Gender *n* (%)		Age, years median (range)	Race *n* (%) White	Height, cm median (range)	Weight, kg median (range)	BMI, kg/m^2 median (range)	BMI group *N* (%)	
Cohort	Treatment	Female	Male						<28 kg/m^2	>28 kg/m^2
Cohort A	Placebo fast/700-mg BIO fast/ 700-mg BIO Fed; *N* = 2	1 (50.0)	1 (50.0)	47.5 (44–51)	2 (100)	171.95 (169.9–174.0)	77.23 (75.3–79.2)	26.15 (26.1–26.2)	2 (100)	0
	100-mg BIO fast/ placebo fast/ placebo fed; *N* = 2	2 (100)	0	46.5 (46–47)	2 (100)	153.65 (153.5–153.8)	59,03 (49.6–68.5)	25.00 (20.9–29.1)	1 (50.0)	1 (50.0)
	100-mg BIO fast/700-mg BIO fast/700-mg BIO fed; *N* = 4	0	4 (100)	48.5 (37–55)	4 (100)	178.00 (170.7–181.5)	89.90 (86.5–101.9)	29.40 (27.2–31.5)	2 (50.0)	2 (50.0)
Cohort B	350-mg BIO fast/placebo fast; *N* = 2	2 (100)	0	46.0 (39–53)	2 (100)	165.50 (158.0–173.0)	60.25 (59.8–60.7)	22.15 (20.0–24.3)	2 (100)	0
	Placebo fast/1400-mg BIO fast; *N* = 2	0	2 (100)	40.5 (33–48)	2 (100)	177.50 (177.0–178.0)	83.80 (77.8–89.8)	26.65 (24.6–28.7)	1 (50.0)	1 (50.0)
	350-mg BIO fast/1400-mg BIO fast; *N* = 4	3 (75.0)	1 (25.0)	29.5 (20–49)	4 (100)	167.40 (164.0–178.7)	58, 65 (55, 2–81.8)	20.95 (20.5–25.6)	4 (100)	0
All subjects cohorts A and B *N* = 16		8 (50.0)	8 (50.0)	46.5 (20–55)	16 (100)	171.85 (153.5–181.5)	76.53 (49.6–101.9)	25.85 (20.0–31.5)	12 (75.0)	4 (25.0)
Cohort C	Placebo fasted; *N* = 2	1 (50.0)	1 (50.0)	66.0 (66–66)	2 (100)	172.7 (172.1–173.3)	85.4 (79.0–91.8)	28.65 (26.3–31.0)	1 (50.0)	1 (50.0)
	1400-mg BIO fasted; *N* = 6	3 (50.0)	3 (50.0)	69.5 (65–75)	6 (100)	163.3 (151.2–172.7)	69.23 (56.3–77.1)	25.90 (22.6–28.0)	5 (83.3)	1 (16.7)
All subjects Cohort C *N* = 8		4 (50.0)	4 (50.0)	67.0 (65–75)	8 (100)	168.95 (151.2–173.3)	73.78 (56.3–91.8)	26.10 (22.6–31.0)	6 (75.0)	2 (25.0)

Note: BIO, BIO101; fast, fasted; *N*, number of subjects; *n*, number of subjects. Subjects had to fast from 10 h pre-dose until 4 h post-dose. The study drug was administered 30 min after the start of a standard high-fat breakfast that had to be eaten within 20 min.
Abbreviation: BMI, body mass index.

cebo. At each dose, two sentinel subjects (one subject with BIO101 and one subject with placebo) were dosed. At least 2 h after, the last sentinel subject had been dosed and after the investigator determined it to be safe, the remainder of the subjects in the panel was dosed.

In Part 2 MAD, dose levels did not exceed the highest tolerated daily dose administered in Part 1. The study drug was administered orally to a total of 30 subjects in three cohorts of 10 elderly subjects (between 65 and 85 years of age, extremes included). For each cohort, eight subjects were randomized to BIO101 and two to placebo (*Table 2*). Dose, dosing frequency (once or twice daily) and fed condition were decided based on the safety, tolerability and PK results of Part 1. Dosing of the subjects enrolled in Part 2 is shown in *Table 2* and *Figure S1C*.

Subject eligibility

Eligibility criteria, study design, randomization and blinding are described in the clinical protocol synopsis in the Data S1.

Study drug (Sarconeos)

BIO101 was obtained by purifying the active substance 20E at ≥97% (Patheon, Germany) from a *Cyanotis* spp. extract (Nutragreen, Shanghai, China). Identified organic impurities were other phytoecdysteroids, the main ones being 20,26-dihydroxyecdysone (20,26E 0.65% –25*R* and 25*S* isomers) inokosterone (0.48%—2 isomers) ajugasterone C (0.34%), 20E-3-acetate (0.13%) and 20E-2-acetate (0.32%). BIO101 was formulated as 100-mg (batch number 16010816151) and 175-mg (batch number 16010816152) size 1 opaque capsules containing 100 or 175 mg of 20E, respectively, with colloidal silica and microcrystalline cellulose as excipients (Amatsi, Saint-Gely France). The matching placebo capsules (batch number 16010816153) contained colloidal silica and microcrystalline cellulose.

Endpoints and assessments

Safety and tolerability

The safety assessments were based on adverse events (AEs) reporting, clinical laboratory safety tests, 12-lead electrocardiogram (ECG) evaluations, vital sign evaluations and physical examinations. All AEs reported spontaneously during the course of the study were recorded, and all serious AEs (SAEs) independent of the circumstances or suspected cause, had to be reported by the investigator. Details of AE and safety assessments (blood and clinical parameters) are provided in the Data S1 (Endpoints and Assessments: Safety and Tolerability).

Table 2 Demographic data, baseline characteristics and doses for Part 2 (MAD) Cohorts D, E and F.

Parameter	Pooled placebo Fed $N = 6$	350-mg BIO qd Fed $N = 8$	350-mg BIO bid Fed $N = 8$	450-mg BIO bid Fed $N = 8$	All subjects $N = 30$
Gender, *n* (%)					
Female	3 (50.0)	2 (25.0)	6 (75.0)	4 (50.0)	15 (50.0)
Male	3 (50.0)	6 (75.0)	2 (25.0)	4 (50.0)	15 (50.0)
Age. years	67.0	74.0	70.5	70.5	72.0
Median (range)	(65–75)	(66–76)	(65–77)	(65–78)	(65–78)
Race *n* (%)					
White	6 (100)	8 (100)	7 (87.5)	8 (100)	29 (96.7)
Asian	0	0	1 (12.5)	0	1 (3.3)
Height, cm	165.75	166.85	162.00	167.00	165.20
Median (range)	(160.1–175.4)	(157.7–177.3)	(150.0–186.3)	(154.4–176.8)	(150.0–186.3)
Weight, kg	78.65	73.35	65.45	74.95	75.50
Median (range)	(77.4–97.7)	(64.9–98.9)	(60.6–95.0)	(54.5–80.8)	(54.5–98.9)
BMI, kg/m^2	29.80	27.65	25.15	25.75	27.00
Median (range)	(27.8–31.8)	(23.4–31.5)	(23.3–28.8)	(21.2–29.)	(21.2–31.8)
BMI group, *n* (%)					
$<28\ kg/m^2$	1 (16.7)	4 (50.0)	6 (75.0)	7 (87.5)	18 (60.0)
$>28\ kg/m^2$	5 (83.3)	4 (50.0)	2 (25.0)	1 (12.5)	12 (40.0)

Note: BIO, BIO101; fast, fasted; *N*, number of subjects; *n*, number of subjects with that observation. Subjects had to fast from 10 h pre-dose until 4 h post-dose. The study drug was administered 30 min after the start of a standard high-fat breakfast that had to be eaten within 20 min.
Abbreviation: BMI, body mass index.

Pharmacokinetics

The exposure to 20E[27] and its main metabolites (14dPost, 14d20E, poststerone [Post] and 6α-hydroxy-20-hydroxyecdysone [6αOH20E]) was determined in plasma and only 20E in urine using a validated analytical method (liquid chromatography with mass spectrometric detection [LC–MS/MS]) by SGS Life Sciences Services (CEPHAC, Saint Benoit, France) as described in the Data S1 and Table S5. Details of PK parameters (time points, dosing and analyses) for SAD and MAD parts are described in the Data S1 (Pharmakokinetics).

Pharmacodynamics

Plasma PD parameters—renin, aldosterone, myostatin, interleukin (IL-6) and N-terminal procollagen III propeptide (PIIINP) (Parts 1 and 2), myoglobin, MB (muscle-brain) isoenzyme of creatine kinase (CK), C-reactive protein (CRP), high-sensitive C-reactive protein (hsCRP) (Part 2 only)—were measured using validated methods. Blood samples in Part 1 were collected pre-dose on Day 1 and on Day 2. Blood samples in Part 2 were collected pre-dose on Days 1 and 14, except for renin and aldosterone. On Days 1 and 14, collection of blood samples to assess renin and aldosterone had to be done in the morning 2 h after subjects woke up and after 15 to 30 min in a supine or sitting position. Myostatin (Mature GDF-8) and PIIINP were assayed using an ELISA method (R&D Systems, Abingdon, UK) and a radio-immunoassay (RIA; Orion Diagnostica, Espoo, Finland), respectively.

Statistical methods

All statistical methods for pharmacokinetics, correlation between biomarkers, correlation with circulating doses of 20E and regression analyses are described in the Data S1 (Statistics).

Results

Volunteer disposal and demographics

Volunteer demographics and characteristics are summarized in Tables 1 and 2. There were no relevant differences between the cohorts that were thought to influence the outcome of the study.

Safety and tolerability

No deaths, SAEs or treatment emergent AEs (TEAEs) leading to treatment discontinuation were reported during the study. All TEAEs were mild or moderate in severity (*Tables 3* and *S1*). All TEAEs were resolved by the end of the study. Most of them resolved spontaneously. In a few cases, additional treatment was required. All treatment-related TEAEs were considered 'possibly' related to the study drug by the investigator. None of them was considered 'probably' or 'certainly' related to the study drug by the investigator. The most common system organ class involved in AEs was 'gastrointestinal disorders'. AEs related to musculoskeletal disorders were reported as back pain at 350 mg bid, muscle spasms at 350 and 450 mg bid, stiffness at 350 mg bid and myalgia at 350 mg bid. One case of neck pain was reported in the placebo group (*Table S2*).

Table 3 Treatment-emergent adverse events Cohorts D, E and F (MAD study).

	BIO101 350 mg qd Fed (*N* = 8)		BIO101 350 mg bid Fed (*N* = 8)		BIO101 450 mg bid Fed (*N* = 8)		Placebo Fed (*N* = 6)	
System organ class and preferred term	*n*	%	*n*	%	*n*	%	*n*	%
Any AE	2	25.0	7	87.5	8	100	3	50.0
Gastrointestinal disorders	0		7	87.5	4	50.0	2	33.3
Abdominal discomfort	0		0		1	12.5	1	16.7
Abdominal distension	0		1	12.5	0		0	
Aphthous ulcer	0		0		1	12.5	0	
Constipation	0		4	50.0	2		1	
Diarrhoea	0		3	37.5	0	25.0	0	16.7
Dry mouth	0		1	12.5	0		0	
Epigastric discomfort	0		0		1	12.5	0	
Eructation	0		1	12.5	0		0	
Flatulence	0		1	12.5	0		0	
Nausea	0		0		1	12.5	0	
Vomiting	0		0		1	12.5	0	
General disorders and administration site conditions	0		1	12.5	2	25.0	0	
Asthenia	0		0		1	12.5	0	
Fatigue	0		1	12.5	1	12.5	0	
Feeling hot	0		0		1	12.5	0	
Infections and infestations	0		2	25.0	3	37.5	0	
Nasopharyngitis	0		0		1	12.5	0	
Pharyngitis	0		0		1	12.5	0	
Rhinitis	0		1		0		0	
Viral upper respiratory tract infection	0		1	12.5	0		0	
Vulvovaginal mycotic infection	0		0		1	12.5	0	
Injury, poisoning and procedural complications	1	12.5	0		0		0	
Wound	1	12.5	0		0		0	
Musculoskeletal and connective tissue disorders	1	12.5	4	50.0	4	50.0	1	16.7
Back pain	0		0		3	37.5	0	
Muscle spasms	0		1	12.5	1	12.5	0	
Musculoskeletal stiffness	0		1	12.5	0		0	
Myalgia	0		2	25.0	0		1	
Neck pain	0		0		0		0	16.7
Pain in extremity	1	12.5	0		0			
Nervous system disorders	0		1	12.5	5	62.5	1	16.7
Dizziness	0		0		1	12.5	0	
Dizziness postural	0		1	12.5	1	12.5	0	
Headache	0		0		5	62.5	1	16.7
Syncope	0		0		1	12.5	0	
Reproductive system and breast disorder	0		0		1	12.5	0	
Vulvovaginal discomfort	0		0		1	12.5	0	
Respiratory, thoracic and mediastinal tissue disorders	0		1	12.5	2	25.0	0	
Dry throat	0		1		0		0	
Epitaxis	0		0	12.5	1	12.5	0	
Oropharyngeal pain	0		0		1	12.5	0	
Skin and subcutaneous tissue disorders	0		0		1	12.5	1	16.7
Erythema	0		0		1	12.5	0	
Pruritis	0		0		1	12.5	1	16.7
Skin irritation	0		0		1	12.5	0	

Note: *N*, number of subjects; *n*, number of subjects with event. The denominator for the percentage calculations is the total number of subjects per treatment group and per analysis period in the safety population.

Part 1—SAD

Young subjects (Cohorts A and B)

No TEAEs were reported for subjects who received 100 and 700 mg (fasted/fed) BIO101 (*Table S1*). For 2 (33.3%) young subjects who each received 350- and 1400-mg BIO101 in fasted condition, at least 1 TEAE was reported, and at least 1 TEAE was reported for 1 (12.5%) young subject in the pooled fasted placebo group. All TEAEs reported in Cohorts A and B were mild in severity. The reported TEAEs in Cohort B were nausea and postural dizziness (in subjects who received 350-mg BIO101) and food poisoning and headache (in subjects who received 1400-mg BIO101; *Table S1*).

Subjects aged ≥65 years (Cohort C)

For 4 (66.7%) elderly subjects who received 1400-mg BIO101, at least 1 TEAE (headache and back pain (in 3 [50.0%] and 1 [16.7%] subjects, respectively) was reported. No TEAE was reported in the subjects who received placebo in this cohort. Only one TEAE (headache) was considered related to the drug

by the Investigator. All TEAEs reported in this cohort were mild in severity (*Table S1*).

Part 2—MAD

The number of subjects for whom at least one TEAE was reported increased with the dose, that is, 2 (25.0%), 7 (87.5%) and 8 (100%) subjects who received 350-mg BIO101 qd, 350-mg BIO101 bid and 450-mg BIO101 bid, respectively (*Table 3*). For 3 (50.0%) subjects in the pooled placebo group, at least one TEAE was reported. For 2 (25.0%) subjects receiving 450-mg BIO101 bid, reported TEAEs were moderate in severity (nausea and syncope in one subject and vulvovaginal mycotic infection in the other subject). All other reported TEAEs in Part 2 MAD were mild in severity.

The highest number of subjects with treatment-related TEAEs was reported in the group receiving 450-mg BIO101 bid, that is, 5 (62.5%) subjects (for all of whom at least a treatment-related headache was reported). One TEAE (dry mouth) reported in this group was considered treatment-related by the investigator.

Treatment-related TEAEs were reported in at most 1 (12.5%) subject each in the 350-mg BIO101qd and 350-mg BIO101 bid treatment groups. Two TEAEs (wound and pain in extremities) were reported in 1 (12.5%) subject each in the 350-mg BIO101 qd treatment group. The pain in extremities was considered treatment-related by the Investigator. The most frequently reported TEAEs in the 350-mg BIO101 bid treatment group were constipation or diarrhoea (4 [50.0%] and 3 [37.5%] subjects, respectively).

Clinical laboratory and other safety parameters

No clinically significant trends over time in median laboratory values nor in median ECG values or vital signs were observed. None of the observed treatment-emergent abnormalities was considered clinically significant by the investigator, and none was reported as TEAE. For physical examination, apart from hyper-peristalsis, reported at follow-up for 1 (16.7%) subject in Cohort B who received a single dose of 1400-mg BIO101 and associated with the TEAE food poisoning, no other treatment-emergent physical examination abnormalities were reported in the study.

Pharmacokinetics

Part 1—SAD

Dose proportionality plasma and urine Pharmacokinetic parameters of 20E are presented in *Table 4*. 20E was rapidly absorbed and distributed with a median t_{max} of 2–3.5 h for all doses. Median t_{max} slightly increased with increasing doses. Mean 20E C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ increased less than dose-proportionally between 100 and 1400 mg (*Figure 1*).

Mean 20E half-life was quite short (between 2.4 h and 4.9 h) in all panels and slightly shorter at the lowest doses. Mean renal clearance was comparable in all dose levels (4.05–5.05 L/h). The unconverted 20E excreted in urine over the 0- to 24-h period in fasted conditions decreased (3.41%–1.51% of the dose administered) with increasing doses. Despite difficulty to collect and handle such matrix, we were able to assess 20E and metabolites exposure in some samples collected. Only two out of four participants (*Table S3*) showed an exposure to 20E, and only one participant with sufficient material showed an exposure to 20E and metabolites, 14d20E being by far the major one detected (90% of total drug exposure while 20E represented 9%). In total, for Participant 108, 387 mg of the total drug were retrieved in faeces (55% of the dose).

Food effect 20E plasma C_{max} and AUCs were slightly higher in fed than in fasted conditions at 700 mg (+24% for C_{max} and +20%–22% for AUCs) (*Table S4*), but t_{max} was similar in both treatments (3.0 and 3.5 h, respectively). Mean relative amount of 20E excreted in urine over the 0- to 24-h period was higher in fed conditions (2.31% vs. 1.61% in fasted conditions). Renal clearance was slightly higher (+17.6%) in fed conditions.

Age effect At 1400-mg BIO101, mean 20E plasma exposure was slightly lower (by 22% for C_{max} and by 13%–15% for AUCs, *Table S4*) in subjects aged ≥65 years (Cohort C) compared with young subjects (Panel B2). Median t_{max} was 2.5 and 3.5 h, for elderly and young subjects, respectively. 20E terminal half-life was not affected by age (mean values of 3.84 h in young vs. 3.85 h in elderly). Mean relative amount of 20E excreted in urine over the 0- to 24-h period was slightly lower in elderly (0.91%) compared with young subjects (1.51%). Renal clearance was about 29% lower in elderly vs young subjects.

Part 2—MAD

Plasma 20E and metabolites After a single oral administration of BIO101 at 350- and 450-mg, 20E was quantifiable in plasma at the first or second sampling time point (i.e., 0.5 or 1 h). After the first (Day 1) and the last morning dose (Day 14), mean maximal BIO101 plasma concentrations were reached 3 h post-dose (*Table 4*). 20E plasma concentrations were quantifiable 12 h post-morning dose in all subjects of Cohorts E and F (350- and 450-mg bid cohorts, respectively) but not in 350 mg qd. In Cohort D (350 mg qd), BIO101 was still quantifiable 24 h post-morning dose on Day 1 and on Day 14 in all subjects but fell BLQ 48 h post-last dose (Day 14) in all subjects. No accumulation of BIO101 was observed after qd dosing (at 350 mg qd [Cohort D]) for 14 days with a mean Rac of 1.14 compared with slight accumulation after

Table 4 BIO101 dose effect on plasma exposure to 20E in Panels A and B of the single ascending doses part and in the multiple ascending doses part.

B1O101 PK	SAD			
Parameter (unit)	100 mg fasted (Panel A1) $N = 6$	350 mg fasted (Panel B1) $N = 6$	700 mg fasted (Panel A2) $N = 6$	1400 mg fasted (Panel B2) $N = 6$
C_{max} (ng/mL)	141 (16.6)	317 (37.9)	399 (24.7)	710 (20.2)
t_{max} (h)	2.03 (1.00–3.02)	3.00 (1.05–4.00)	3.00 (2.00–4.02)	3.50 (2.00–4.02)
AUC_{0-t} (ng h/mL)	767 (31.1)	1924 (40.1)	2578 (22.9)	4148 (15.9)
$AUC_{0-\tau}$ (ng h/mL)	819 (33.6)	1927 (39.8)	2589 (23.1)	4097 (17.0)
$AUC_{0-\infty}$ (ng h/mL)	797 (32.8)	1946 (39.4)	2658 (20.4)	4283 (17.4) $^{(n = 5)}$
$t_{1/2}$ (h)	2.40 (29.0)	3.26 (43.5)	4.85 (66.9)	3.84 (24.1) $^{(n = 5)}$
CLr (L/h)	4.23 (22.0)	4.05 (17.3)	4.43 (36.1)	5.05 (18.6)
R_{ac}	NA	NA	NA	NA
$Ae_{0-\tau}$ (% dose)	3.41 (35.2)	2.22 (41.4	1.61 (41.3)	1.51 (35.9)
C_{max}/dose (ng/mL/mg)	1.41 (16.6)	0.905 (37.9)	0.570 (24.7)	0.507 (20.2)
AUC_{0-t}/dose (ng h/mL/mg)	7.67 (31.1)	5.50 (40.1)	3.68 (22.9)	2.96 (15.9)

Note: C_{max}, maximum observed plasma concentration; t_{max}, time of occurrence of C_{max}; AUC_{0-t}, area under the plasma concentration–time curve from time zero till the time corresponding with the last observed quantifiable concentration; $AUC_{0-\tau}$, area under the plasma concentration–time curve over the dosing interval; $t_{1/2}$, apparent terminal half-life; CLr, renal clearance; ae, cumulative amount excreted in urine. Values are arithmetic mean (CV%) except for t_{max} (median [min–max]). *N*, number of subjects with data; n, number of subjects with this observation; NA, not applicable.

Table 4 (continued)

B1O101 PK	MAD					
Parameter (unit)	350 mg qd fed (Cohort D) $N = 8$		350 mg bid fed (Cohort E) $N = 8$		450 mg bid fed (Cohort F) $N = 8$	
	Day 1	Day 14	Day 1	Day 14 ($N = 7$)	Day 1	Day 14
C_{max} (ng/mL)	346 (16.6)	388 (23.5)	453 (29.9)	506 (15.7)	524 (32.9)	560 (34.5)
t_{max} (h)	3.00 (2.00–4.00)	3.00 (3.00–4.00)	3.00 (2.02–4.37)	3.00 (2.00–4.00)	3.00 (3.00–3.00)	3.00 (2.00–4.00)
AUC_{0-t} (ng h/mL)	2 1 40 (18.4)	2389 (18.0)	2230 (17.6)	2766 (14.5)	2424 (28.5)	3203 (37.9)
$AUC_{0-\tau}$ (ng h/mL)	2141 (18.4)	2389 (18.0)	2234 (17.6)	2768 (14.5)	2429 (28.5)	3203 (37.8)
$AUC_{0-\infty}$ (ng h/mL)	2193 (18.0)	2414 (18.0)	2451 (16.0)	3028 (15.0)	2566 (28.1) $^{n = 7}$	3486 (38.6)
$t_{1/2}$ (h)	4.39 (20.7)	3.39 (10.9)	3.00 (12.1)	3.06 (21.3)	3.72 (28.1)	2.81 (8.62)
CLr (L/h)	4.08 (23.1)	3.84 (14.8)	3.46 (14.3)	4.00 (12.7)	3.57 (13.8)	4.41 (21.1)
R_{ac}	NA	1.14 (23.6)	NA	1.31 (16.7)	NA	1.31 (20.2)
$Ae_{0-\tau}$ (% dose)	2.17 (34.0)	2.38 (25.6)	2.21 (24.8)	3.14 (13.2)	1.88 (20.3)	3.01 (31.1)
C_{max}/dose (ng/mL/mg)	0.988 (16.6)	1.11 (23.5)	1.29 (29.9)	1.45 (15.7)	1.16 (32.9)	1.24 (34.5)
AUC_{0-t}/dose (ng h/mL/mg)	6.12 (18.4)	6.83 (18.0)	6.38 (17.6)	7.91 (14.5)	5.40 (28.5)	7.12 (37.8)

Note: C_{max}, maximum observed plasma concentration; t_{max}, time of occurrence of C_{max}; AUC_{0-t}, area under the plasma concentration–time curve from time zero till the time corresponding with the last observed quantifiable concentration; $AUC_{0-\tau}$, area under the plasma concentration–time curve over the dosing interval; $t_{1/2}$, apparent terminal half-life; CLr, renal clearance; ae, cumulative amount excreted in urine. Values are arithmetic mean (CV%) except for t_{max} (median [min–max]). *N*, number of subjects with data; n, number of subjects with this observation; NA, not applicable.

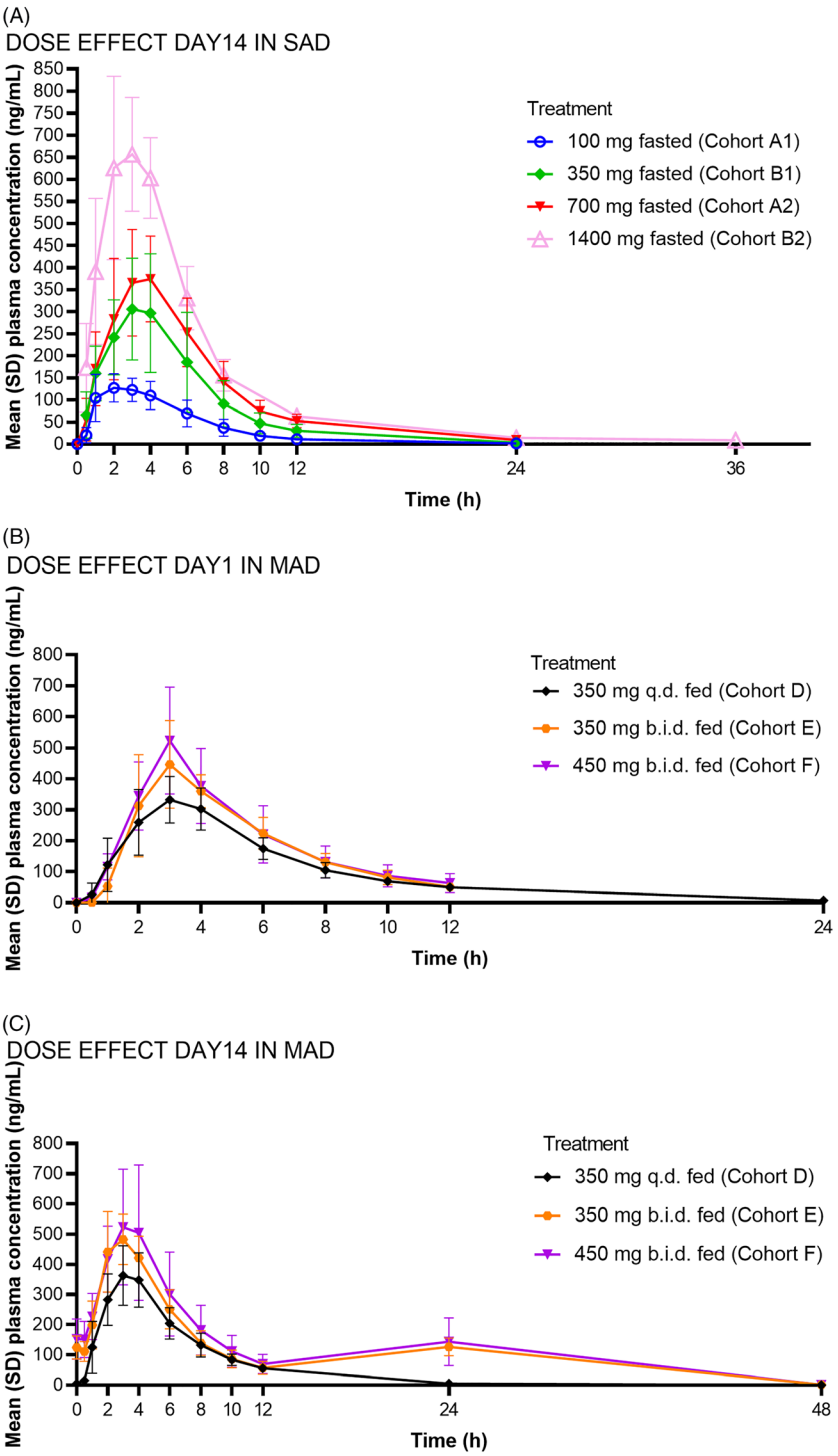

FIGURE 1 Time-course of 20E plasma concentrations following a single oral administration of BIO101 at doses of 100–1400 mg (*A*) and following multiple oral administrations of BIO101 at doses of 350 and 450 mg at Day 1 (*B*) and at Day 14 (*C*).

bid administration of BIO101 at 350 and 450 mg for 14 days (mean Rac of 1.31 in both Cohorts E and F).

After repeated daily administrations of 350-mg BIO101 for 14 days, 20E C_{max} and $AUC_{0-\tau}$ were slightly higher (increase of about 30% for C_{max} and 16% for $AUC_{0-\tau}$), on average, in subjects administered bid (Cohort E) than those administered qd (Cohort D). In Cohorts E and F, after the last morning administration (Day 14), mean C_{max} and $AUC_{0-\tau}$ increased by about 1.11-fold and 1.16-fold for a 1.29-fold dose increase (350–450 mg), so less than dose-proportionally. Mean 20E half-life

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was short (2.8 and 4.4 h in all cohorts). Mean renal clearance was comparable in all treatments after 14 days with values ranging between 3.84 and 4.41 L/h. 20E concentrations were below the limit of quantification (BLQ) in all subjects 48 h post-last administration of BIO101 350 mg qd and BLQ or close to the lower limit of quantification (LLOQ, 1 ng/mL) in almost all subjects 36 h post-last evening administration of BIO101 at 350 or 450 mg bid. Based on the short half-life of 20E (about 3–4 h) and on the trough concentrations profiles, steady state was already reached on Day 2 in all cohorts. Average trough (pre-morning dose) concentrations (Days 2–14) ranged between 4.76 and 7.73 ng/mL in the 350-mg qd cohort, between 105 and 126 ng/mL in the 350-mg bid cohort and between 109 and 152 ng/mL in the 450-mg bid cohort.

The nine metabolites previously characterized in rodents were sought in Cohorts B1, D and E. Three of them (7,8-dihydro-20-hydroxyecdysone [7,8diH20E], 6αOH20E, and 6α-hydroxy-20-R,S-dihydropoststerone [6αOHRSPost]) were not detected. The other six 20E metabolites were detected and quantified in Cohort B1 350 mg (*Figure S2*), Cohort D 350 mg qd (*Figure S3*) and cohort E MAD 350 mg bid (*Figure 2*). The six metabolites are 20,26-dihydroxyecdysone (20,26E), 14-deoxy-20-hydroxyecdysone (14d20E), 6α-hydroxy-14-deoxy-20-hydroxyecdysone (6αOH14d20E, only in cohort B1), poststerone (Post), 14-deoxy-poststerone (14dPost), 20-R,S-dihydropoststerone (20RShPost). *Figure S2* shows the delayed appearance and extended presence of metabolites in plasma, resulting in a significant exposure to 14d20E and (when formed) 14dPost (*Table 5*). While 14-dehydroxylated metabolites were observed in all volunteers, side-chain cleavage metabolites were found in only 5 out of 7 volunteers and this does not appear to be sex related (*Table 5*).

Urinary excretion Our analyses were restricted to the quantification of the excreted 20E. Like for SAD, these amounts were low and the values did not increase linearly with the administered doses, as observed for plasma concentrations. The 20E eliminated over a 12-h period represented between 1.88% and 3.14% of the ingested 20E, and the Day14 values were all higher than the D1 ones. Approximately 50% of these amounts were eliminated during the first 4 h (data not shown).

Pharmacodynamics

BIO101 effects were evaluated on aldosterone and renin, two components of the renin–angiotensin system. BIO101 acts on muscle through the activation of the MAS receptor, one component of this system.[18] Creatine kinase (MM and MB), myoglobin, myostatin and PIIINP levels were also investigated as biomarkers of muscle anabolism/catabolism.

The effects on the immune system

The cytokine IL-6 and hsCRP were investigated as inflammatory biomarkers.

Potential pharmacological effects were expressed as changes from baseline. No clear trend was observed on plasma biomarkers in the SAD part of the study (data not shown). In the MAD part, changes after 14 days of repeated administration of placebo or BIO101 (350 mg qd, 350 mg bid or 450 mg bid) were observed and are reported in *Table 6*.

A trend to a reduction of aldosterone and renin was only observed in subjects administered with 450 mg bid. Of note, the regression analysis of Day 14 change from baseline using the mean values of aldosterone and BIO101 administered doses shows a dose-related decrease of with a coefficient of determination $R^2 = 0.24$.

Mean myostatin levels tended to decrease compared with baseline in subjects who received placebo, while a small increase was observed after 14 days of repeated administration of BIO101, in a dose-dependent manner (Kruskal–Wallis test $P = 0.032$).

A trend to a slight increase in mean PIIINP plasma levels (vs. baseline) was observed after 14 days compared with placebo and mainly at the highest dose. However, the differences were not significant.

Myoglobin and CK-MB data suggest a slight decrease in groups treated with the highest doses of BIO101 (350 mg and 450 mg bid) compared with placebo-treated group. CK-MB levels from all healthy volunteers show a dose effect on Day 14 ($P = 0.043$; Kruskal–Wallis test).

A small increase in the mean plasma levels of hsCRP was observed in placebo subjects and a small decrease in 350-mg bid subjects, but no change at 450 mg bid (*Table 6*). No effect was observed on plasma levels of IL-6.

Discussion

Safety and tolerability of BIO101

The results confirmed BIO101 good safety profiles observed in rats and dogs (data not shown) as well as low toxicity previously observed with 20E in male mice.[19] No SAE occurred during the treatment of healthy human volunteers with BIO101 in the SAD or in the MAD.

Treatment of healthy human volunteers with BIO101 in the SAD or in the MAD showed no SAE. Only three moderate AEs were observed in the highest administered dose at 450 mg bid. Overall, there were more AEs related to musculoskeletal and connective tissue disorders in 350 and 450 mg bid as compared with placebo or 350 mg qd.

Treatment with BIO101 did not show any clinically significant safety changes in vital signs, ECG or laboratory values.

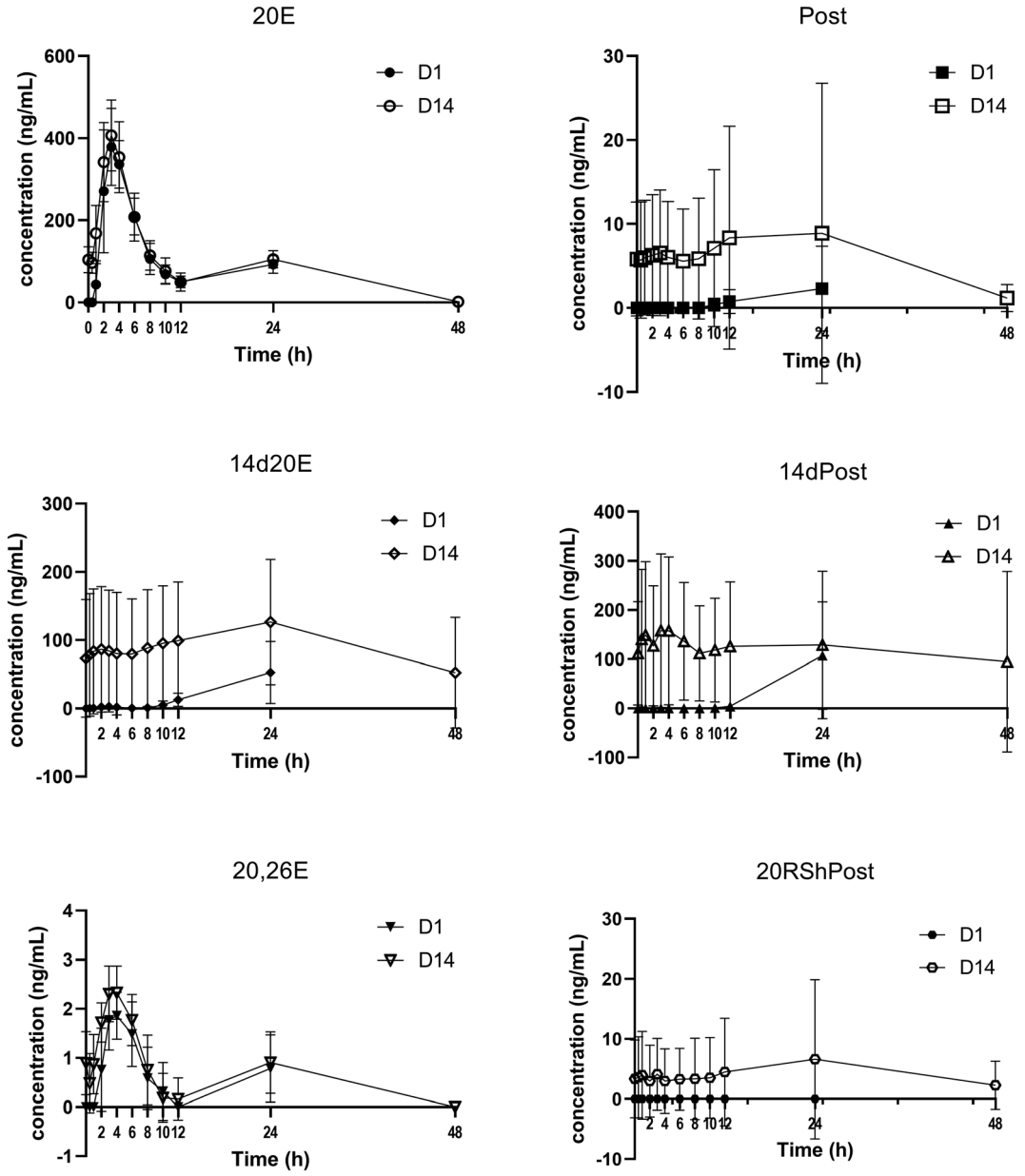

FIGURE 2 Pharmacokinetic profiles for 20E and its detected metabolites (Post; 14d20E; 14dPost; 20,26E; 20R ShPost) at D1 (24 h) and at D14 (48 h) of subjects (mean, *SD*) receiving multiple administration (MAD) of BIO101 at 350 mg bid.

It was concluded that oral repeated dose treatment with BIO101 is safe and well tolerated up to 450 mg bid in the MAD part.

Pharmacokinetics

Low oral bioavailability

C_{max} values indicate a rather low oral bioavailability, consistent with the low quantities of 20E recovered in urine over a period of 0–24 h, with lower relative values at higher doses illustrating a saturation of the absorption process. In the absence of a proper bioavailability study conducted in humans, we hypothesized an equivalence between the BIO101 values in urine and in plasma. Values from urine excreted after BIO101 administration over the 0–12 h after 14 days administration with 2.38%, 3.14% and 3.01% of 350 mg qd; 350 mg bid and 450 mg bid, respectively, can be considered comparable with what was reported in rodents (1%). Given the low oral bioavailability and the small amounts recovered from urine, it is expected that excretion proceeds mainly in the faeces, as in rodents.[20] Actually, exploratory PK analysis in the cohort A2 (700 mg) in the SAD part suggests a heterogeneity of the total drug excretion (BIO101 and metabolites).

Table 5 Estimated individual plasma exposure area under the curve (AUC) to 20E and to its identified metabolites in plasma samples from MAD 350 mg bid subjects at Day 14.

	Raw AUC0–24 h (ng h/mL)										
	Patient ID (gender)										
Compound	711 (F)	713 (F)	715 (F)	717 (M)	718 (M)	719 (F)	720 (F)	Mean AUC (ng h/mL)	% of total AUC (SD)	MW	Mean AUC (nmol h/mL)	% of total AUC
20E (estimated)	5610	5044	4966	4930	3516	3466	5066	4657	45.9 (11.5)	480	9.702	39.21
14dPost	5139	0	1682	2377	0	5229	7439	3124	27.0 (21.4)	346	9.029	36.49
14d20E	1850	5074	1889	357.5	5084	2236	270.8	2394	24.5 (21.9)	464	5.159	20.85
Post	357.7	0	21.02	105	0	60.6	716.5	180	1.4 (1.9)	362	0.497	2.01
20RSPost	188.6	0	0	14.94	0	0	555.6	108	0.8 (1.4)	364	0.297	0.12
20,26E (estimated)	43.12	29.22	35.44	31.46	15.98	19.92	24.36	29	0.3 (0.1)	496	0.058	0.23

Note: Total areas under the curve (AUC) represent exposure of BIO101 and all its detected metabolites. It is estimated from the AUC_{0-12h} for 20E and 20,26E. AUC_{0-12h} is therefore multiplied by 2 to obtain AUC_{0-24h}. The mean AUC and percentage of total AUC is also calculated in molarity (nmol h/mL) as the AUC using mass (ng H/mL) underestimates the relative part of compounds resulting from the side chain cleavage (post, 14dPost, 20RSPost).

One participant showed an excretion of 55% consistent with the data from of the administered dose. The small and sparse amount of samples does not allow us to draw general conclusion at this stage. The time frame of sample collection might also be a limitation in the detection of the compound. This will need further analysis using the same dose of 350 mg bid that were administered in the sarcopenia Phase 2 study.

BIO101 pharmacokinetic properties led us to select a bid-dosing regimen for future clinical trials to ensure a sufficient exposure over a 24-h period. BIO101 bid administration of 350 mg results in a plasma exposure (AUC_{0-12h}) of 2768 ng h/mL, which corresponds to a mean exposure to 2768/12 = 230 ng/mL (0.48 μM), a concentration similar to EC_{50} for the acceleration of myocyte differentiation (0.4 to 0.8 μM) (data not shown) and IC_{50} determined for myostatin expression inhibition in C2C12 cells (between 0.1 and 1 μM).[17] Therefore, 350 mg bid was considered a good candidate active dose to be tested in Phase 2.

Moreover, 350 mg bid was selected as the highest dose in the Phase 2 (SARA-INT) study as it only generated few AEs, all of mild severity. In the Phase 1 study, this dose generated a C_{max} of 506 ng/mL (1.05 μM) and mean circulating ranges (105–126 ng/mL: 0.22–0.26 μM) of BIO101 at pre-dose corresponding to estimated pharmacological active doses obtained in rodents and human myocyte assays.

Food and age effect

20E plasma parameters being slightly higher when BIO101 was administered in fed conditions, led us to administer BIO101 in part 2 MAD in fed conditions, as this was most suitable for older adults, our target population. After a single oral administration of 1400-mg BIO101 in fasted conditions, the non-significant differences in pharmacokinetic parameters (mean plasma exposure [22% vs. 13%–15%] for C_{max} and [2.5 h vs. 3.5 h] for median t_{max}) versus younger healthy volunteers allowed us to conduct the MAD part on elderly healthy volunteers, as well as the further Phase 2 clinical studies.

20E metabolites

In rodents, 20E metabolism occurs mainly through 14-dehydroxylation, side chain cleavage and reduction of the 6-keto group. The major circulating and excreted metabolites in rodents are14d20E, Post, 14dPost and 6αOH20E.[20,27] The current study allowed the identification and quantification of six compounds in human plasma: 20,26E, 14d20E, 6α14d20E, Post, 14dPost and 20RShPost after BIO101 administration. 20,26E showed a parallel kinetics as 20E representing 0.62% of the parent molecule. This similar exposure can be explained by its presence in the drug substance (0.65%).

Concerning the other metabolites, their microbial origin is the most probable, based on (1) the high inter-individual variability of their plasma concentrations; (2) the absence of any observable in vitro metabolism by HepG2 cells or liver micro-

Table 6 Pharmacodynamic parameters evaluated in the MAD part.

Pharmacodynamic markers	Change from baseline at D14						
	Pooled placebo ($N = 6$)	BIO101 350-mg qd fed cohort D ($N = 8$)	BIO101 350-mg bid fed cohort E ($N = 8$)	BIO101 450-mg bid fed cohort F ($N = 8$)	Effect of the dose (Kruskal–Wallis test)	Regression analysis AUC infinity	
						P value	R^2
Aldosterone (pg/mL)	1.767 (−24.00 + 21.60)	3.375 (−31.30 + 36.40)	5.486 ($n = 7$) (−11.70 + 43.10)	−11.200 (−42.80 + 24.10)	0.219 (ns)	All: 0.736 (ns) 350 bid: 0.871(ns)	0.01 0.01
Renin (pg/mL)	0.405 (−1.11 + 2.14)	−0.554 (−5.82 + 1.39)	1.064 ($n = 7$) (−0.33 + 4.25)	−1.131 (−6.02 + 4.20)	0.103 (ns)	All: 0.669 (ns) 350 mg bid: 0.584	0.01 0.06
Myostatin (pg/mL)	−453.5 (−797–29)	100.3 (−410 + 831)	146.4 ($n = 7$) (−574 + 1048)	253.4 (−590 + 876)	0.032 (s)	All: 0.954 (ns) 350 mg bid: 0.906 (ns)	0.0 0.0
Myoglobin (ng/mL)	−3.70 (−18.3 + 12.7)	−2.71 (−21.5 + 9.1)	−8.46 ($n = 7$) (−25.1 + 4.6)	−7.43 (−15.3–3.3)	0.244 (ns)	All: 0.272 (ns) 350 mg bid: 0.012 (s)	0.06 0.75*
CK MB (g/L)	−0.192 (−0.36 + 0.10)	−0.130 (−0.66 + 0.35)	−0.310 ($n = 7$) (−0.92 + 0.15)	−0.428 (−0.95 + 0.14)	0.368 (ns)	All: 0.027 (s) 350 mg bid: 0.008 (s)	0.21 0.79*
CRP (mg/L)	0.00 (0.0 0.0)	0.16 (0.0 + 1.3)	0.00 ($n = 7$) (0.0 0.0)	−3.11 (−23.6 0.0)	nd	nd	nd
hsCRP (mg/L)	0.807 (−0.64 + 3.45)	0.024 (−1.32 + 1.53)	−0.466 ($n = 7$) (−3.30 + 0.59)	−0.018 ($n = 6$) (−0.33 + 0.32)	nd	nd	nd
HSP72 (ng/mL)	−0.1600 (−3.70 0.0000)	0.18375 (−0.0500 + 1.5200)	0.06571 ($n = 7$) (0.0000 + 0.4600)	−0.0940 (−1.2000 + 0.6489)	nd	nd	nd
IL-6 (pg/mL)	0.152 (−1.44 + 3.05)	−1.477 ($n = 6$) (−8.28 + 3.71)	0.160 ($n = 7$) (0.00 + 1.12)	0.4 6 (−2.52 + 3.20)	nd	nd	nd
PIIINP (μg/mL)	−0.13 (−1.95 + 0.65)	0.230 (−1.52 + 1.11)	0.247 ($n = 7$) (−0.91 + 0.93)	0.614 (+0.10 + 1.34)	0.359 (ns)	All: 0.944 (ns) 350 bid: 0.484 (ns)	0.0 0.1

Note: Changes from baseline after 14 days were measured for the different biomarkers. Values are arithmetic mean (min–max). *N*, number of subjects with data. The dose effect on the biomarkers is tested with the Kruskal–Wallis test (ns, non-significant; s, significant [$p < 0.05$]; nd, not done). All, all doses together; 350 mg bid, dose 350 bid only.
*Regression of PD markers depending on area under the curve AUC_{0_∞} is significant at 350 mg bid.

somes (human, rats, dogs, mouse, rat, monkey; data not shown); (3) the observation that IV-injected 20E was recovered unchanged in rat bile[20]. Indeed, 14-dehydroxylation, the major in vivo observed reaction, is a typical prokaryote reaction.[28]

The exposure to the two major metabolites 14dPost and 14d20E was compared with the exposure in rodents (rats[20]). Post, 14dPost, 6OH14dPost and 14d20E were found as 20E metabolites in rat plasma after oral administration of BIO101 at 1000 mg/kg/day (NOAEL in rats), but their relative proportion differed compared with humans. The exposure of Post, 14d20E and 14dPost are respectively 2600%, 37% and 1878% compared with human exposure after 14 days administration of BIO101 at 350 mg bid. Moreover, these findings are consistent with other studies showing an excretion of 14dPost and 14d20E in human urine after 20E administration.[26,29] This clearly shows that 14d20E is the major metabolite that needs special focus and further characterization. Preliminary results showed no off-target activity of these metabolites on steroid receptors and no or moderate activity on protein synthesis for 14dPost and 14d-20E, respectively, in C2C12 cells compared with 20E (data not shown).

Up to now, structure activity relationship studies have been limited to a comparison of various natural phytoecdysteroids,[30] and the biological activity of metabolites is not much documented, with the exception of Post, which showed an anabolic activity similar to that of 20E.[31]

Pharmacodynamic effects

Myostatin is a negative regulator of muscle growth.[32] In the 14-day MAD, we observed a decrease of its plasma levels in the placebo group and an increase in the BIO101-treated group. These possibly counterintuitive results may be explained if we consider myostatin as a negative feedback regulator of muscle protein synthesis by the physical inactivity of elderly healthy volunteers that have triggered a rapid skeletal muscle loss. Indeed, Kortebein et al.[36] observed a 0.95-kg loss of lean leg mass (0.66 kg week^{-1}) after 10 days of bed rest in healthy older adults, as inactivity-induced loss of muscle mass predominantly affects the lower body musculature and is most rapid during the first days/weeks.[37] In this context of reduced proteosynthesis, myostatin production would be reduced.[33] By contrast, BIO101 treatment is expected to stimulate muscle anabolism, as evidenced by in vitro experiments in C2C12.[17] In such a context, BIO101 treatment is counteracting the effects of inactivity in the elderly (muscle loss) and hence would lead to homeostatic increased myostatin levels. Positive correlations between myostatin and lean mass[34] as well as changes in myostatin and lean mass during physical activity were reported.[35]

PIIINP is a product of the proteolytic cleavage of the larger procollagen III molecule during collagen synthesis in connective tissue like skin and muscle.[38] In a study comparing 6-week resistance training with a control group, Fragala et al.[39] observed a significant correlation of the increase circulating PIIINP and the change of lean body mass. In our study, the slight decrease observed in the placebo group after 14 days (−0.130 μg/L) may be explained by the sedentary status of the MAD elderly cohorts in the Phase 1 unit. Interestingly, BIO101 administration led to a trend of an increase of PIIINP plasma level by 0.230, 0.247 and 0.614 μg/L, respectively, at 350 mg qd and 350 mg bid and 450 mg bid. Increase in PIIINP levels is known to be associated with increases in lean body mass and appendicular skeletal mass in the elderly.[40]

Creatine-kinase (*CK*) serum level is routinely measured as an index of muscle damage. *Myoglobin*, the oxygen-binding protein in muscle cells, is also released into the bloodstream in increasing amounts upon muscle damage.[41] In the MAD part, healthy volunteers did experience a slight decrease in myoglobin and creatine kinase muscle brain (CKMB; *Table 6*). BIO101 at the highest doses tended to induce a reduction in CKMB mean serum levels versus baseline compared with placebo. BIO101 lowering effect of circulating CK levels could be explained through MAS receptor activation, as orally administered angiotensin 1-7 (the endogenous ligand of MAS) can reduce plasma CK levels compared to placebo following eccentric exercises: significant decreases are observed at 48 and 72 h after exercises considered as the peak of CK release.[42]

Correlation between pharmacokinetic and pharmacodynamic parameters

Regression analysis performed using the individual values from healthy volunteers (circulating doses AUC) at Day 14 and change from baseline to Day 14 show that aldosterone and renin plasma levels at Day 14 are correlated for the 350 mg bid (R = 0.79). Creatine kinase MB and myoglobin at Day 14 are correlated for all doses (R = 0.62) and especially for the doses 350 and 450 mg bid (R = 0.74). Their changes from baseline to Day 14 values are also correlated for the dose 350 mg bid (R = 0.86). The PIIINP and myostatin change from baseline values are correlated for the 450 mg bid (R = 0.71).

Conclusions

This first in-human study indicates that BIO101 is well tolerated at doses of 350 mg qd, 350 mg bid and 450 mg bid and has good safety profile in young and older adults, with no serious AEs, few mild AEs and only three moderate AEs. BIO101 showed a PK profile suitable for bid oral dosing. The

study also allowed the identification of two major metabolites in human. Finally, BIO101 induced changes in plasma biomarkers involved in the RAS (aldosterone and renin) and in muscle anabolism (PIIINP) or catabolism (CK and myoglobin) that are qualitatively consistent with its mechanism of action.

Overall, this study allowed us to select the doses subsequently used in the ongoing BIO101 Phase 2 (SARA-INT) and Phase 2/3 (COVA) clinical trials (ClinicalTrials.gov Identifier—NCT03452488; ClinicalTrials.gov Identifier—NCT04472728).

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Conflict of interest

Waly Dioh, Cendrine Tourette, Pierre Dilda, René Lafont and Stanislas Veillet are Biophytis company employees. Susanna Del Signore, Louiza Daudigny, Philippe Dupont, are former Biophytis company employees.

Online supplementary material

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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