2. SYNOPSIS

Protocol code:	Protocol DIBA/41
Title:	A monocentre, randomised, double-blind, between-patient study to compare the effect of Vit D 300,000 IU orally vs placebo on bone metabolism, muscular function, vascular system and infection in patients with diabetic foot at high cardiovascular and infection risks.
Clinical Design:	This study is a phase IV, single site, parallel-group, double-blind, randomized, placebo-controlled study to assess the clinical effects of a single dose of Vit D_3 300,000 IU in patients with diabetic foot at high cardiovascular and infections risk.
Clinical Phase	IV
Trial period	February 2009 - September 2010
Centres involved	1 Italian site
Objectives and outcome variables	The objective of this trial is to assess the effects of Vit.D_3 300,000 IU supply on bone metabolism, muscular function, vascular system and infection in patients with diabetic foot, at high cardiovascular (CV) and infections risk. The study secondary objectives are to evaluate the effects of one single administration of Vit. D_3 on: - muscular improvement - risk reduction for progression of vascular calcifications - infections - thrombosis events. As well as Vit D_3 safety.
Subjects Enrolment	188 patients
Diagnosis and main criteria for inclusion	Patients with diabetic foot at high risk of cardiovascular and infection events **Inclusion criteria** • Males and females out patients with type 2 diabetes mellitus; • presence of diabetic foot complication; • age-range: ≥ 60 years; • patients agree to participate and give the written informed consent. **Exclusion criteria** • patients with previous or current tumoral disease, with less than 1 year quoad vitam prognosis; • patients with severe chronic or auto-immune inflammatory diseases; • contra-indications of Vit D (chronic renal failure, renal lithiasis, hypercalcemia, hypercalciuria, allergy to calciferol or excipients); • impossibility to perform follow up envisioned in the protocol of the study; • patients treated with strontium ranelate; • treatments effective on bone turnover , including: -bisphosphonates by I.V. or by I.M. and rhPTH by S.C. within the previous 12 months -bisphosphonates, os, for more than 14 days in the previous 12 months -calcitonin, estrogens, ipriflavon, SERMs during the last 6 months -thyroid hormone, except for in case of stable dose for the last 6 months, together with a state of euthyroidism as documented by TSH test -systemic corticosteroids for more than 3 months, at doses greater than the equivalent of 5 mg of prednisone per day, in the previous 12 months; • patients treated with antithrombotic therapies; • gastrointestinal disorders which impair drug absorption;

	• participation to other clinical trials in the previous 12 months; • poor compliance.
Study populations	Thirty patients (30), out of the 188 requested by protocol, outpatient subjects signed the informed consent and were enrolled into the study. Out of 44 screened patients, 30 were randomised and received treatment: 14 patients were allocated to Vit D_3 group and 16 patients to the Placebo group. Twenty-four (24) out of 30 treated patients completed the study as planned.
Efficacy results	No effect of Vit D_3 on osteocalcin levels emerged from study results, as it could be reasonably expected being the sample size reduced from 188 to 24 evaluable patients. The means of β-CTx at 13 and 26 weeks are reduced but not significantly in both treatment groups. The results of ANCOVA performed on the differences between baseline and final values highlight that the treatment factor is not significant. However, a strong relationship does emerge with the baseline β-CTx. With regard to bone metabolism parameters (vit D, Bone ALP, OPG, PTH), the two groups proved to be homogeneous at baseline for all these parameters except for bone ALP: Vit D3 group present higher values (p=0.0057). The difference between Vit D3 and Placebo is significant for OPG at week 26 (p=0.0361). The analyses (ANCOVA) on mean changes from baseline show significant differences between the two treatment groups only for OPG (p=0.0085): OPG values are increased from baseline to week 26 in Vit D_3 group while are decreased in Placebo group. Between muscular/adipose metabolism parameters (leptine, adiponectin, CPK), all homogeneous at baseline, only leptine presents a significant increase after treatment, that tends to persist at the subsequent time points only in the Vit D3 group (p=0.0316 at weeks 13, and 0.0595 at week 26). The change from baseline in the two groups is significant (p=0.0442) at week 26. Immune parameters (α-TNF) and blood coagulation parameters (hemochromocitometric examination, fibrinogen, PTT) are all homogeneous at baseline. Comparison between Vit D_3 and Placebo in the change from baseline on α-TNF did not reveal any significance. No differences in changes from baseline between Vit D_3 and Placebo are noted in densitometric measurements and aortic calcifications. As regards infections, no significant difference emerges between the two treatment groups and only one acute thrombotic event is recorded during the study in the Placebo group.
Safety results	Twenty-two (22) out of 30 treated patients experiencing an adverse event during the study, 13 (93%) and 9 (56%) in patients receiving Vit D_3 or Placebo respectively. Only 1 patient receiving Vit D_3 reported an adverse drug reaction. Two serious adverse events were reported, none of them related to the study treatment.

Conclusions	From study results is evident the high prevalence of hypovitaminosis D in this cohort of diabetic patients, but unfortunately the premature interruption has not allowed to assess the efficacy of vitamin D3 supplementation in the bone metabolism measured with the serum levels of osteocalcin, because the sample size requested was not reached. What seems to be evident, however, is that the single oral administration of 300000 IU of Vit D3 might not be the best way to supplement these patients, because the blood levels of β-CTX did not reach normal values at 24 weeks, nor at 13 weeks, in spite of a statistically significant increase in the treated group. Based on this clinical observation, among diabetic patients with severe vitamin D deficiency a single oral dose of 300000 IU vitamin D3 is not sufficient to increase 25-hydroxyvitamin D serum concentration in most of them, in spite of a statistically significant increase, in particular in the first three months. Several hypotheses could be put forward to explain these differences, but no conclusion could be drawn from study results. In particular, it could be speculated that the low levels of Vit D3 observed at week 13 and 26 is the result of excess vitamin D trapped in the fat tissue being the patient's average BMI > 28. Nevertheless, from study results it could be possible to detect some significant increases in the level of OPG and leptin in the group under treatment. Study data deserve further attention, because they suggest an action of vitamin D3 supplementation mediated by OPG and leptin in diabetic patients, where the quality of bone is compromised, particularly in the cortical compartment.