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Clinical Investigation

Pravastatin Reverses Established Radiation-Induced Cutaneous and Subcutaneous Fibrosis in Patients With Head and Neck Cancer: Results of the Biology-Driven Phase 2 Clinical Trial Pravacur

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Summary

Previous preclinical investigations based on the inhibition of the Rho/Rho-associated protein kinase/connective tissue growth

Purpose: The "PRAVACUR" phase 2 trial (NCT01268202) assessed the efficacy of pravastatin as an antifibrotic agent in patients with established cutaneous and subcutaneous radiation-induced fibrosis (RIF) after head and neck squamous cell carcinoma (HNSCC) radiation therapy and/or radiochemotherapy.

Methods and Materials: The main inclusion criteria were: NSCC in remission, grade ≥2 cutaneous and subcutaneous neck RIF (National Cancer Institute Common

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factor pathway brought the biological rationale for this bicentric phase 2 clinical trial on the use of statins for the treatment of established radioinduced fibrosis. This trial showed that pravastatin (40 mg/d for 12 months) is a safe and efficient antifibrotic agent in patients with established grade ≥2 cutaneous and subcutaneous fibrosis after head and neck cancer radiation therapy. These results support the concept of fibrosis reversibility.

Terminology Criteria for Adverse Events, version 4.0), and no current treatment with statins or fibrates. Patients received pravastatin 40 mg/d for 12 months. The primary endpoint was reduction of RIF thickness by more than 30% at 12 months, as measured by cutaneous high-frequency ultrasonography. Secondary endpoints included RIF severity reduction, pravastatin tolerance, and quality of life.

Results: Sixty patients with grade 2 (n = 37), grade 3 (n = 22), or grade 4 (n = 1) RIF were enrolled from February 2011 to April 2016. The mean interval between RIF diagnosis and pravastatin initiation was 17.1 months. Pravastatin was stopped before 11 months of treatment in 18 patients (because of grade ≥2 adverse events related to pravastatin in 8 patients [13%]). In the 40 patients in whom pravastatin efficacy was assessed by high-frequency ultrasonography at baseline and at 12 months of treatment, a reduction of RIF thickness ≥30% was observed in 15 of 42 patients (35.7%; 95% confidence interval, 21.6%-52.0%). At the 12-month clinical evaluation, RIF severity was decreased in 50% of patients (n = 21; 95% confidence interval, 34.2%-65.8%), and the patients' self-perception, mood state, and social functioning were significantly improved. Pravastatin was well tolerated, with a very low occurrence of grade 3 toxicities (myalgia, n = 1) and grade 2 toxicities (myalgia/arthralgia or esophagitis, n = 3).

Conclusions: This phase 2 prospective study supports the notion of radioinduced fibrosis reversibility. It showed that pravastatin (40 mg/d for 12 months) is an efficient antifibrotic agent in patients with grade ≥2 cutaneous and subcutaneous fibrosis after HNSCC radiation therapy.

Introduction

Head and neck squamous cell carcinoma (HNSCC) can develop in 4 main locations: the oral cavity, oropharynx, larynx, and hypopharynx. In 2015, 932,614 new cases were reported worldwide, and HNSCC was the seventh most common cancer and cause of death.[1] The standard of care is a multimodal treatment approach with surgery followed by adjuvant radiation therapy (RT), or RT as definitive treatment. The control of locally advanced HNSCC, which is common in patients with a history of smoking and alcohol consumption, has been significantly improved by the combination of RT and chemotherapy and by RT altered fractionation. However, this population still has a poor outcome.[2] Conversely, patients with human papillomavirus−positive and non−smoking-related HNSCC have a better prognosis.[3]

Treatment intensification in HNSCC is associated with increased frequency and severity of radiation-induced toxicities, particularly radiation-induced fibrosis (RIF). Neck RIF is a substantial late toxicity. Indeed, at 3 years post-treatment, the risk of grade ≥2 RIF is 56% and 28%, respectively, in patients who received RT after or not after neck surgery and 34% after combined RT and chemotherapy alone.[4] The use of modern RT modalities, such as intensity modulated RT (IMRT), has significantly reduced the incidence of acute and late toxicities.[5] However, grade ≥2 RIF occurrence is still high even with IMRT. In a recent study, Nevens et al observed grade ≥2 RIF in 29.2% of patients at 6 months with an increased occurrence in the case of upfront neck dissection (70.6% vs 18.1%).[6] RIF functional consequences can lead to decreased quality of life (QoL) and to dysphagia, trismus, lymphedema, and limited cervical range of motion.[7]

RIF usually occurs at least 4 months after RT completion and progresses over the years. The main manifestations of cutaneous and subcutaneous RIF are skin induration and thickening. RIF severity is graded using the Common Terminology Criteria for Adverse Events (CTCAE) scale. Because this rating scale may be subjective, other tools have been developed, such as ultrasonography (US) quantification using a high-frequency transducer. Changes in tissue echogenicity (mild, moderate, and severe) are observed in function of RIF severity.[8] Older studies showed that measuring tissue deformation in response to an applied force as assessed using an ultrasound probe is a quantitative method to monitor RIF and correlates with symptoms and neck rotation restriction.[9]

RIF is the result of a dysregulation of the wound-healing process and is characterized by transdifferentiation of fibroblasts into myofibroblasts and by excessive accumulation of extracellular matrix. Tissue injury, inflammation, and repair play a role in RIF development and progression.

The first strategies to reduce or treat cutaneous and subcutaneous RIF were based on top-down approaches, such as the use of superoxide dismutase[10] or of pentoxifylline combined with α-tocopherol (vitamin E).[11,12] A better understanding of RIF molecular mechanisms allowed definition of potential therapeutic targets, such as transforming growth factor β (TGF-β), which is activated by RT and is a fibrosis driver.[13] Galunisertib, a TGF-β receptor type I kinase inhibitor, combined with a platelet-derived

growth factor receptor inhibitor, significantly decreased lung RIF in mice models.[14] The safety of an anti−TGF-β antibody (GC1008) is currently assessed in early clinical trials in patients with idiopathic pulmonary fibrosis (NCT00125385). Aside from TGFβ targeting, we and others reported that the Rho/Rho-associated protein kinase/connective tissue growth factor signaling pathway also is involved in RIF development and maintenance.[15,16] Pharmacologic modulation of this pathway using statins (ie, HMG-CoA reductase inhibitors) limits and reduces RIF in vitro, ex vivo, and in vivo in various preclinical models of normal tissue radiation-induced toxicity.[17-20]

On the basis of these promising preclinical results, we designed a biology-driven phase 2 clinical trial to assess pravastatin efficacy in patients with delayed cutaneous and subcutaneous grade ≥2 RIF after HNSCC RT (NCT01268202).

Methods and Materials

The protocol was approved by all local institutional review boards and was accepted by the ethics committee of Bicêtre Hospital, Paris, France (file number 10-001).

Patients

This bicentric phase 2 clinical trial enrolled 61 patients with cutaneous and subcutaneous RIF (grade ≥2, CTCAE, v4.0) diagnosed at least 6 months but less than 24 months after HNSCC treatment (ie, adjuvant or exclusive RT, combined or not combined with concomitant chemotherapy). Patients were in complete remission at inclusion. Additional inclusion criteria were adequate kidney function (serum creatinine ≤130 μM/L) and hepatic function (aspartate aminotransferase and alanine aminotransferase levels at least 2 times lower than the laboratory upper normal limit; bilirubin level at least 1.5 times lower than the laboratory upper normal limit).

Exclusion criteria were long-term steroid therapy or current treatment with statins, fibrates, or cyclosporine; a history of severe heart failure; a history of muscle toxicity during previous treatments with fibrates or statins; a history of hereditary muscle diseases; and baseline muscle creatine kinase levels 3 times higher than the laboratory upper normal limit.

At baseline, all patients underwent head and neck computed tomography to confirm HNSCC remission and high-frequency US (HF-US; at least 16-MHz linear transducer) to assess RIF thickness (in millimeters) compared with neighboring normal skin (upper part of the chest wall). The radiologists who performed the HF-US assessments (image collection and interpretation) were HF-US experts. Because RIF is a dynamic process as a result of permanent extracellular matrix remodeling, RIF thickness was measured at its thickest point at baseline and at all the study time points.

Before inclusion, written informed consent was obtained from all patients. Investigators from the 2 centers then sent by fax the data required for the patient's registration to the Gustave Roussy Biostatistics Unit, Villejuif, France. After eligibility check and registration, a unique identification number was assigned to each patient and provided by fax to the investigator and the pharmacist. Thus, patient registration was done independently from the study investigators. Pravastatin was given to the patients by the hospital pharmacist only after registration.

Treatment

Treatment (pravastatin 40 mg/d per os for 12 months) began at inclusion. Dose adjustment was not permitted. Premature drug discontinuation was planned in the case of pravastatin-related toxicity (renal, hepatic, or muscle problems), cancer progression, consent withdrawal, or major protocol violation. Patients who discontinued pravastatin were followed in the same manner as all the other patients enrolled in the trial. Neck motion exercises or scar manipulation were not performed during the study because at that time they were not part of the standard of care for HNSCC in France.

Endpoints

The primary endpoint was RIF thickness reduction of at least 30% (compared with baseline) as measured by cutaneous HF-US at 12 months.

The secondary efficacy endpoints were reduction of RIF severity (according to the CTCAE v.4.0) evaluated by clinical examination and QoL changes determined by using the self-administered VQ-Dermato questionnaire.[21] The VQ-Dermato questionnaire is a valid, reliable, dermatology-specific QoL instrument for chronic skin diseases, particularly for assessing the effect on QoL of therapeutic strategies.[21] It includes 7 dimensions (self-perception, daily living activity, mood state, social functioning, leisure activity, treatment-induced limitations, and physical discomfort) explored by 28 items.

Pravastatin tolerance and compliance were evaluated. Pravastatin-related toxicities were assessed by clinical examination and by blood testing (cholesterol and muscle creatine kinase level variations).

Follow-up

Follow-up visits with the investigator were planned each month during the first 3 months and then at 6, 12, 18, and 24 months after inclusion. RIF was evaluated clinically and by HF-US every 6 months after treatment start. Patients were asked to fill in the VQ-Dermato questionnaire at baseline and at 6, 12, 18, and 24 months after treatment start. Pravastatin-related toxicities were assessed monthly during the first 3 months and then at 6 and 12 months.

Ancillary study

Before enrollment, an ancillary study to assess pravastatin biological efficacy was proposed to each patient. After patients signed the written informed consent for the ancillary study, a skin punch biopsy in the RIF area was done before pravastatin treatment initiation and at 12 months. Biopsy results were processed using classical histopathological procedure. Hematoxylin–eosin staining was used for histologic analysis and Sirius Red staining for quantification of collagen infiltration, as previously described.[22] Each patient was his or her own control.

Statistical Analysis

This single-stage, phase 2 trial tested the null hypothesis that the success rate would be lower than 10% versus the alternative hypothesis that it would be higher than 30%. This required the inclusion of 40 evaluable patients (ie, patients who received pravastatin for at least 11 months). If there were more than 8 successes among the 40 evaluable patients, pravastatin would be considered interesting in this setting. With this design, the 1-sided α error rate was 4.2% and the power was 94.5%. According to the expected rate of early discontinuation (30%), it was estimated that 55 patients were needed for the trial.

The success rate (according to the HF-US assessment and CTCAE grading) was described with the 95% confidence interval (CI). The paired t test was used to compare the VQ-Dermato scores at baseline and at 12 months and to compare RIF thickness and normal skin thickness at baseline. Because the sample size was small, the Wilcoxon signed rank test was used when appropriate and gave results similar to those of the paired t test.

This study was registered at ClinicalTrials.gov, number NCT00208273.

Role of the Funding Source

The study funder (Institut National du Cancer/INCa) had no role in the study design; data collection, analysis and interpretation; or report writing. The corresponding author had full access to all data and the final responsibility for the decision to submit for publication.

Results

Pravastatin is well tolerated and shows antifibrotic effect in a subset of patients

From February 2011 to April 2016, 61 patients with grade $\geq$2 cutaneous and subcutaneous RIF after HNSCC were registered (Fig. 1). One patient was not eligible because he was already treated with statins and was excluded from the analyses. The patients' demographic and clinical characteristics at baseline are listed in Table E1 (available online

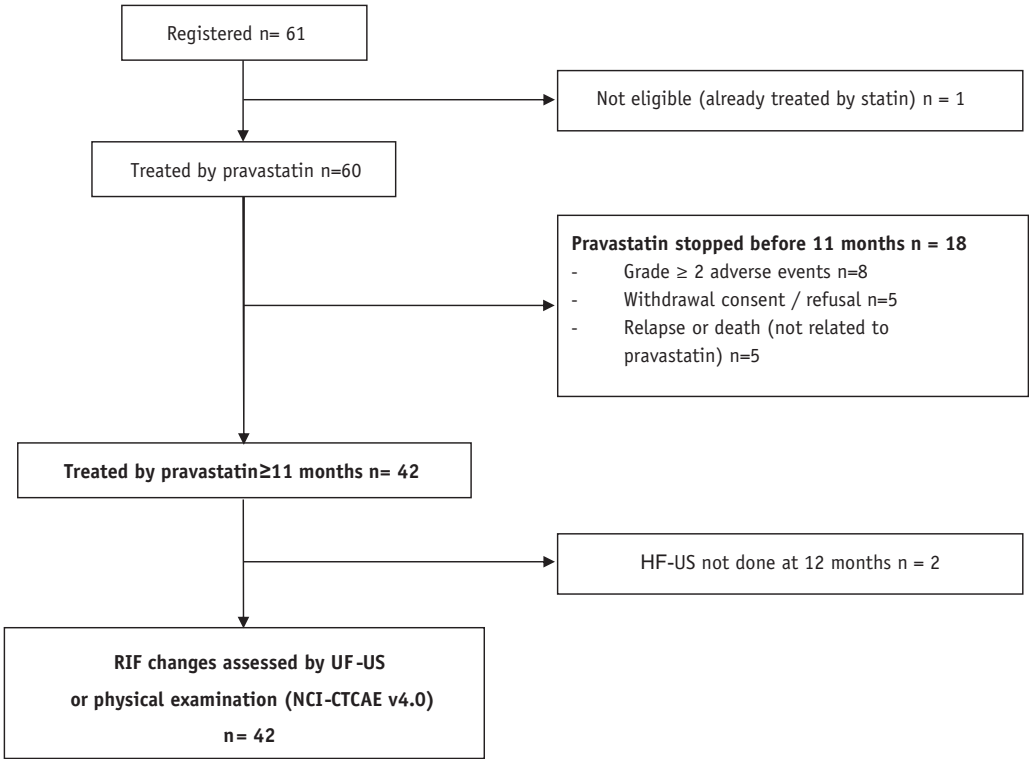

Fig. 1. Study flowchart. *Abbreviations:* HF-US = high-frequency ultrasonography; NCI-CTCAE v4.0 = National Cancer Institute Common Terminology Criteria for Adverse Events, version 4.0; RIF = radiation-induced fibrosis.

Table 1 Evaluation of fibrosis at baseline

Variable	Pravastatin for <11 mo (N = 18)	Pravastatin for ≥11 mo (N = 42)	Total N = 60
Timing of RIF appearance after RT completion, mo			
Mean (SD)	10.7 (8.5)	9.6 (9.8)	9.9 (9.4)
Timing of pravastatin initiation after RIF onset, mo			
Mean (SD)	17.7 (14.1)	16.9 (10.5)	17.1 (11.6)
Baseline RIF severity grade (clinical assessment according to CTCAE, v.4.0), n (%)			
2	11 (61)	26 (62)	37 (62)
3	7 (39)	15 (36)	22 (37)
4	0	1 (2)	1 (2)
Baseline RIF and healthy skin thickness (mm, by HF-US)			
RIF thickness, mean (SD) median [range]	2.30 (0.96) 2.45 [0.70-4.40]	3.92 (4.42) 2.40 [0.70-20]	3.44 (3.80) 2.45 [0.70-20]
Healthy skin thickness, mean (SD) median [range]	1.47 (0.52)* 1.60 [0.40-2.40]	2.17 (3.53)* 1.60 [0.70-24]	1.97 (2.99)† 1.60 [0.40-24]
Thickness difference between RIF and healthy skin, mean (SD)median [range]	0.71 (0.86)* 0.70 [−1.4 to 2.0]	1.38 (2.78)* 0.70 [−4.0 to 15.4]	1.18 (2.39)† 0.70 [−4.0 to 15.4]
Primary endpoint: RIF thickness decrease (between baseline and the 12-mo assessment)			
Success (ie, thickness decrease of 30% or more compared with baseline) [95% confidence interval]	0 (0%) [0%-18.5%]	15 (35.7%) [21.6%-52.0%]	-
Failure (ie, thickness decrease lower than 30% compared with baseline)	4 (22.2%)	25 (59.5%)	-
Unknown (HF-US not done)	14 (77.8%)	2 (4.8%)	-
Secondary endpoint: RIF CTCAE grading modification (12-mo assessment compared with baseline)			
Decrease of severity (−2 points)	0 (0%)	2 (5%)	-
Decrease of severity (−1 point)	3 (16.7%)	19 (45%)	-
Stable (same score)	5 (27.8%)	17 (40%)	-
Increase of severity (+1 point)	0 (0%)	3 (7%)	-
No clinical evaluation at 12 mo	10 (55.6%)	1 (2%)	-
Success (grade decrease) [95% confidence interval]	3‡ (16.7%) [3.6%-41.4%]	21 (50.0%) [34.2%-65.8%]	-
RIF and healthy skin thickness at 12 mo (mm, by HF-US)			
RIF thickness, mean (SD) median [range]		2.24 (1.07) 2.05 [1.00-7.60]	
Healthy skin thickness, mean (SD) median [range]		1.33 (0.42)§ 1.40 [0.50-2.00]	

Abbreviations: CTCAE = National Cancer Institute Common Terminology Criteria for Adverse Events, version 4.0; HF-US = high-frequency ultrasonography; RIF = radiation-induced fibrosis; RT = radiation therapy.

* Data missing for 1 patient.

† Data missing for 2 patients.

‡ Two patients received pravastatin for 1.7 mo, and 1 patient received it for 8.7 mo.

§ No significant difference in healthy skin thickness between baseline and 12 mo.

at https://doi.org/10.1016/j.ijrobp.2019.02.024). The total RT dose ranged from 50 to 70 Gy. Patients were mainly treated with 3-dimensional conformal RT (61%) with others treated using IMRT (37%). At baseline, 62% of patients had grade 2 RIF and 37% had grade 3 RIF (Table 1). RIF was detected after a mean of 9.9 months post-RT. At baseline, skin thickness (measured by HF-US) was significantly increased in the RIF area compared with normal tissue (3.44 mm vs 1.97 mm; $P = .0004$).

Eighteen patients stopped pravastatin before 11 months of treatment because of grade ≥2 pravastatin-related toxicities (n = 8), consent withdrawal or patient refusal to continue treatment (n = 5), or tumor relapse or death (n = 5). In the patients with pravastatin-related toxicities, 6 stopped treatment during the first 3 months after initiation because of grade 2 to 3 myalgia (n = 3; pravastatin treatment duration, 0.3-3 months), grade 3 arthralgia (n = 1; pravastatin treatment duration, 3 months), grade 3 abdominal pain (n = 1; pravastatin treatment duration, 2.8 months), and grade 3 esophagitis (n = 1; pravastatin treatment duration, 1.7 months). These toxicities led to the definitive discontinuation of pravastatin. The other 2 patients reported a hypersensitivity reaction (n = 1 after 6 months of pravastatin treatment) and grade 1 diarrhea and grade 1 erectile dysfunction (n = 1 after 8.7 months of pravastatin treatment).

In total, 42 patients took pravastatin for at least 11.6 months. Among them, 40 patients underwent HF-US assessment both at baseline and at 12 months. In these 40 patients, the mean RIF thickness was 4.04 mm at baseline and 2.24 mm at 12 months (reduction by 16.9% ± 38.8%) (Fig. 2). A RIF thickness decrease of 30% or more was observed in 15 patients (Table 1), corresponding to a success rate of 35.7% (95% CI, 21.6%-52.0%). Therefore, according to the hypotheses tested in this trial, the use of pravastatin as an antifibrotic agent was successful.

In addition to RIF thickness decrease, pravastatin also reduced RIF severity (per CTCAE) in 50% of patients (95% CI, 34.2%-65.8%). In 8 patients, RIF thickness and severity were decreased at the 12-month follow-up visit. After pravastatin completion, no "rebound effect" was observed. RIF thickness, assessed by HF-US, was not significantly different at 12 months (mean, 2.24; standard deviation [SD], 1.07; median, 2.05; range, 1.00-7.60); 18 months (mean, 2.47; SD, 2.28; median, 1.9; range, 1.10-15.0); and 24 months (mean, 2.92; SD, 5.96; median, 1.7; range, 1.20-36.0).

Although the VQ-Dermato questionnaire was not always fully completed by the patients, analysis of the score variations between baseline and the 12-month follow-up indicated that pravastatin treatment significantly improved self-perception ($P = .027$), mood state ($P = .010$), social functioning ($P = .040$), and global scores ($P = .002$; Table E2, available online at https://doi.org/10.1016/j.ijrobp.2019.02.024).

Among the patients who received pravastatin for more than 11 months, compliance was excellent, with a mean treatment duration of 365 days (range, 335-365), and the drug was well tolerated (Table 2).

Skin structure is improved and collagen infiltration decreased after 12-month treatment with pravastatin (ancillary study)

Hematoxylin-eosin staining of skin punch biopsies collected before and after pravastatin treatment (n = 19 patients) showed that after 12 months of treatment, the skin histopathological structure was improved in 14 patients, as indicated by the decreased infiltration of immune cells and normalization of the epidermis thickness (Fig. 3A). In the other 5 patients, no modification was observed. Moreover, collagen infiltration (Sirius Red staining quantification) was decreased in 8 of 14 patients after pravastatin treatment (Figs. 3B, 3C).

Discussion

This phase 2 clinical trial confirmed previous preclinical data on pravastatin antifibrotic potential. To the best of our

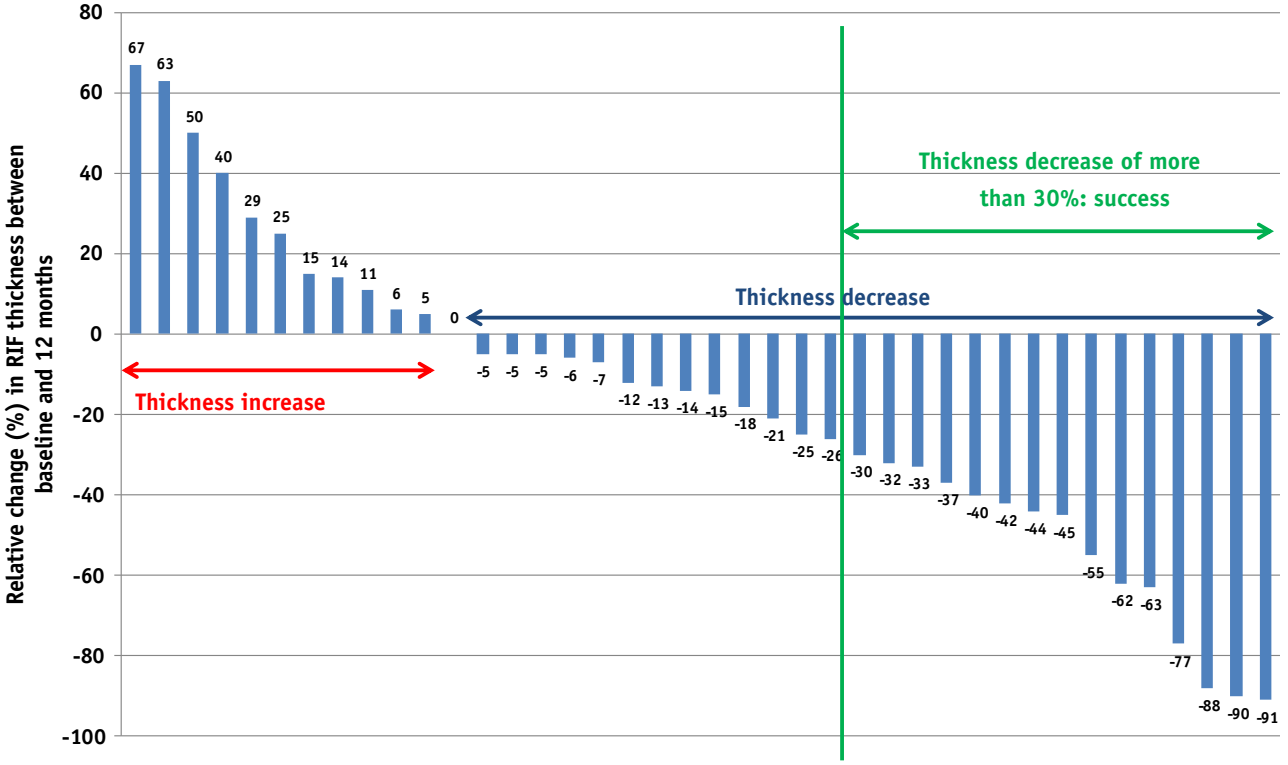

Fig. 2. Relative change in RIF thickness between baseline and 12 months of pravastatin treatment. Negative values indicate a reduction in RIF thickness between baseline and the 12-month follow-up visit; positive values indicate an increase in RIF thickness between these time points. *Abbreviation:* RIF = radiation-induced fibrosis.

Table 2 Pravastatin tolerance in the 42 patients who received pravastatin for more than 11 months

pravastatin side effects	n (%)
Arthralgia	
None	39 (93)
Grade 1	1 (2)
Grade 2	**2 (5)**
Myalgia	
None	32 (76)
Grade 1	8 (19)
Grade 2	**1 (2)**
Grade 3	**1 (2)**
Cramps	
None	34 (81)
Grade 1	8 (19)
Fatigue	
None	39 (93)
Grade 1	3 (7)
Dysphagia-esophagitis	
None	39 (93)
Grade 1	1 (2)
Grade 2	**2 (5)**

knowledge, this is the first study combining objective and subjective criteria (RIF thickness and severity, respectively) to provide evidence of pravastatin antifibrotic efficacy in patients. Although IMRT has significantly reduced the incidence of acute and late toxicities, grade $\geq$2 RIF still occurs, and pravastatin could be proposed to such patients. Pravastatin was well tolerated, and only 6 patients (10%) experienced discomfort (arthralgia, myalgia) that led to treatment withdrawal during the first 3 months of the trial. This outcome suggests that pravastatin could be better tolerated than the current antifibrotic treatment based on pentoxifylline/vitamin E, which caused discomfort (hot flushes, nausea, epigastralgia, severe asthenia, headache, or vertigo) in 45% of patients[11] and definitive treatment discontinuation in 11% of patients (n = 3, of 27 patients) because of myalgia, diarrhea, or nausea during the first 4 months of treatment.[23]

Tissue injury induced by RT can be managed by administering prophylactic agents (or radioprotectors) before RT or mitigators after RT completion and by curative interventions after the appearance of radiation-induced toxicities. Here, pravastatin was assessed as a curative approach (ie, several months after RT completion and in the presence of established $\geq$grade 2 fibrosis). This strategy presents several advantages, including the absence of interference with the anticancer treatments and its administration only to the patients who really need it. Curative treatment after RIF appearance is based on the idea that fibrotic tissue can be mobilized and the fibrogenic process reversed by normalizing tissue homeostasis. The biological basis of fibrosis reversion has been reviewed elsewhere,[24-26] and several pharmacologic strategies have been proposed to achieve this (reviewed by Montay-Gruel et al[26]).

Because the objective evaluation of fibrosis regression is a critical issue in clinical trials, we chose HF-US to assess pravastatin efficacy and to obtain quantitative measurement of RIF thickness. Delanian et al measured the variation in length and width of the cutaneous fibrotic surface to monitor the efficacy of the pentoxifylline−vitamin E combination.[12] However, this measure is operator dependent and consists of the palpation of the fibrotic block edges. Recently, a systematic review by Shaw et al on objective tools, including computed tomography (densitometry and perfusion), Cutometer, and US, found that US is a better objective measurement than palpation.[7] Moreover, 84.9% agreement of interrater reliability regarding RIF grade was reported when physical examination was associated with US[8]; however, the value of US-based assessment of RIF modulation after antifibrotic treatment has never been evaluated. In our study, the decrease of both RIF thickness and severity was observed in only 8 patients. Our findings in normal tissue and RIF areas are in agreement with those of a pilot study using HF-US for RIF assessment presented at the European Society for Radiotherapy and Oncology 35 meeting.[27] Nevertheless, additional investigations are needed to confirm HF-US value for RIF assessment during antifibrotic treatment.

One of the major secondary outcomes of the present phase 2 clinical trial is that pravastatin efficiently reduced RIF severity and improved QoL. Specifically, 50% of patients displayed a RIF severity decrease of at least 1 point in the CTCAE grading system, resulting in a QoL improvement, particularly in self-perception, mood, and social functioning. No other clinical trial assessing antifibrotic agents has focused on these secondary endpoints. Here, we found that pravastatin significantly improved 3 dimensions of the VQ-Dermato questionnaire (self-perception, mood state, and social functioning) and consequently the global score. Conversely, Gothard et al did not observe any QoL improvement after 6 months of pentoxifylline−vitamin E treatment for breast RIF.[28] Our results are consistent with those reported by Delanian et al (pentoxifylline−vitamin E for at least 6 months), with a mean decrease of the Subjective Objective Medical management and Analytic score by 35% ($\pm$20%) and by 48% ($\pm$21%) at 6 months and 1 year, respectively.[29]

Many preclinical studies assessed different antifibrotic agents that target TGF-β 1, inhibit collagen production, or deplete macrophages. Some clinical trials and pilot studies showed RIF reduction after low-dose interferon gamma therapy (n = 4)[30] and higher range of motion after treatment with pirfenidone (n = 6).[31] Ongoing clinical trials are assessing different curative strategies: the Tocovid SupraBio−pentoxifylline combination in bowel radiation-induced disease after pelvic RT (NCT02230800) or topical superoxide dismutase in skin RIF in patients after HNCC (NCT01771991). A phase 1 trial is evaluating the efficacy of umbilical cord mesenchymal stem cells in established lung fibrosis (NCT02277145). Other clinical trials are assessing mitigation strategies, particularly for the

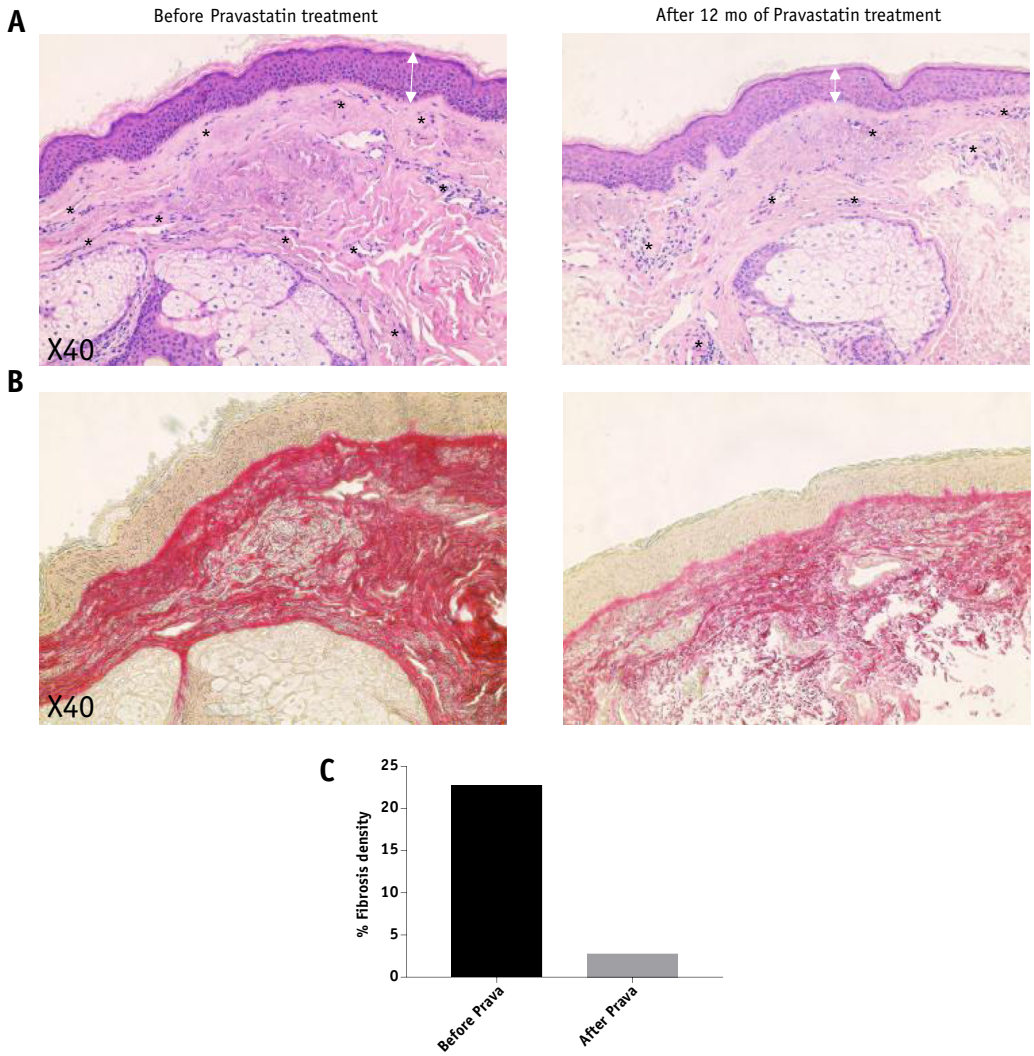

Fig. 3. Histologic analysis of a skin punch biopsy from a patient with radiation-induced fibrosis who responded to pravastatin treatment. Skin punch biopsies in the radiation-induced fibrosis area were performed before pravastatin initiation and at 12 months. (A) Representative images of hematoxylin–eosin stained skin sections before (left) and at 12 months of pravastatin treatment (right) showing decreased immune cell infiltration (asterisks) and normalization of the epidermis thickness (arrows); magnification ×40. (B) Representative images of Sirius Red stained sections before and at 12 months of pravastatin treatment showing the reduction of collagen deposition; magnification ×40. (C) Densitometric analysis of collagen deposition before treatment and at 12 months of pravastatin treatment. *Abbreviation:* Prava = pravastatin. (A color version of this figure is available at https://doi.org/10.1016/j.ijrobp.2019.02.024.)

prevention of lung fibrosis, by administration of enalapril (NCT01754909), captopril (NCT00077064), or nicorandil (NCT02809456). To date, none of them has reported results comparable with those obtained here using a compound selected on the basis of its biological efficacy.[18,20,32]

Finally, the results of our ancillary study are consistent with the clinical data, although the study was performed in a limited number of patients. Results showed that pravastatin treatment induced structural improvement of the skin in 14 of the 19 patients, suggesting that increasing the treatment duration could lead to further improvement. Moreover, collagen infiltration was reduced in 8 of 14 patients. In these 8 patients, RIF thickness also was reduced by more than 30% (by HF-US), or RIF severity grade was decreased. A specific evaluation of the Rho/Rho-associated protein kinase/connective tissue growth factor pathway would have been interesting but was difficult to perform in skin punch biopsy specimens because of their small size. These molecular analyses are more accurate in preclinical and experimental models in which more mechanistic studies can be conducted using both pharmacologic and genetic approaches.

Conclusions

This biology-driven, phase 2, clinical trial shows a curative efficacy and good tolerance of pravastatin in patients with

established cutaneous and subcutaneous grade $\geq$2 RIF in the neck after RT for HNSCC. These results need to be confirmed in a phase 3 randomized trial. However, because very few antifibrotic strategies can be proposed to patients in the clinical practice, statins could be already used as secondary treatment in patients with severe cutaneous RIF. More studies are required to investigate the biological differences between responders and nonresponders to better target the patients who will benefit from this strategy.

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