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A Phase I trial of prolonged administration of lovastatin in patients with recurrent or metastatic squamous cell carcinoma of the head and neck or of the cervix☆

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Abstract

Squamous cell carcinomas of the head and neck (HNSCC) and of the cervix (CC) are particularly sensitive to the apoptotic effects of lovastatin *in vitro*. In this Phase I study, the safety and maximum related dose (MTD) of lovastatin was evaluated in these specific clinical settings. This was a Phase I open-label study to determine the recommended Phase II dose (RPTD) of lovastatin in advanced HNSCC or CC. This study involved a dose and duration escalation of lovastatin starting at 5/mg/kg/day × 2 weeks, every 21 days, until the MTD was reached. Plasma samples were collected for pharmacokinetic analysis. All 26 patients enrolled were evaluable. Dose-limiting toxicity (DLT) consisting of reversible muscle toxicity was seen at 10 mg/kg/day × 14 days. Toxicity may be related to relative renal insufficiency. The MTD was determined to be 7.5 mg/kg/day × 21 days, every 28 days. The low lipid levels experienced on study did not translate into adverse events. Biologically relevant plasma lovastatin levels were obtained. No objective responses were seen but the median survival of patients on study was 7.5 months (mean 9.2 ± 1.5 months). Stable disease (SD) for more than 3 months was seen in 23% of patients. One patient achieved SD and clinical benefit for 14 months on study and a further 23 months off treatment. The disease stabilisation rate of 23% seen in these end-stage patients is encouraging. We conclude that the administration of lovastatin at 7.5 mg/kg/day for 21 consecutive days on a 28-day schedule is well tolerated in patients with good renal function and warrants further clinical evaluation.

Keywords: Statins; HMG-CoA reductase; Squamous cell; Therapeutics; Phase I

1. Introduction

1.1. Statins

The statin family of drugs block hepatic synthesis of cholesterol, predominantly lowering the low-density serum lipoproteins (LDLs) and hence improve the cholesterol profile [1–4]. Large clinical trials established their ability to safely reduce cardiovascular events, as

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well as all-cause mortality [3–6]. Statins inhibit 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, the rate-limiting enzyme of the mevalonate pathway, a complex pathway yielding vital products for a variety of key cellular functions, including membrane integrity, cell signalling, protein synthesis and cell cycle progression (Fig. 1) [7]. Furthermore, retinoids, which induce cellular differentiation and growth inhibitory responses, are also end-products of the mevalonate pathway in plants. The activity of HMG-CoA reductase is closely regulated by feedback mechanisms [7].

There is growing experimental evidence indicating that statins have anticancer effects ranging from antiproliferative, pro-apoptotic, differentiating, anti-invasive and radiosensitising properties, depending on the particular cell type and circumstances under which they are studied [8–11]. (For recent reviews see Refs. [6,12]). In addition, a retrospective analysis of the large, safety and efficacy trials of statins in coronary artery disease suggested an oncoprotective effect of these agents as the incidence of common cancers appeared to be reduced [4]. Subsequent large and well-designed observational studies conducted on Canadian, Dutch and Israeli populations more strongly support a decrease in the risk of incident cancer following chronic use of statins [13–15]. All of these findings together suggest that inhibition of the mevalonate pathway may offer a novel approach to the treatment of cancer. Direct evidence for statins as cancer agents in patients has not yet been established.

1.2. Lovastatin

Lovastatin, a fungal antibiotic, is a specific and non-reversible competitive inhibitor of HMG-CoA reductase. It is one of the first-generation statins and therefore has a large body of safety and pharmacokinetic data from clinical trials and general use for hyperlipidaemia [1,2]. It is metabolised in the liver by the cytochrome P450 isoenzyme, CYP3A4, with less than 10% being excreted renally [6]. Its role as a cytostatic agent in malignant cells has been appreciated for some time [11]. In addition, *via* its inhibitory action in the mevalonate pathway, lovastatin appears to be a promising pro-apoptotic and differentiating agent, and there is strong evidence for a cytotoxic effect in a number of transformed cell lines including solid tumour lines from squamous cell cancer of the head and neck (HNSCC) and cervical cancer (CC) [8,10]. However, other dividing cells such as normal bone marrow progenitors are not affected by lovastatin [9]. The apoptotic response observed in susceptible cell lines is in part due to the depletion of the downstream product geranylgeranyl pyrophosphate, but not farnesyl pyrophosphate or other products of the mevalonate pathway, including cholesterol or ubiquinone [16]. One pro-apoptotic consequence of lovastatin exposure is downregulation of bcl-2 mRNA and protein, although the mechanism of this downregulation is not known [8,17].

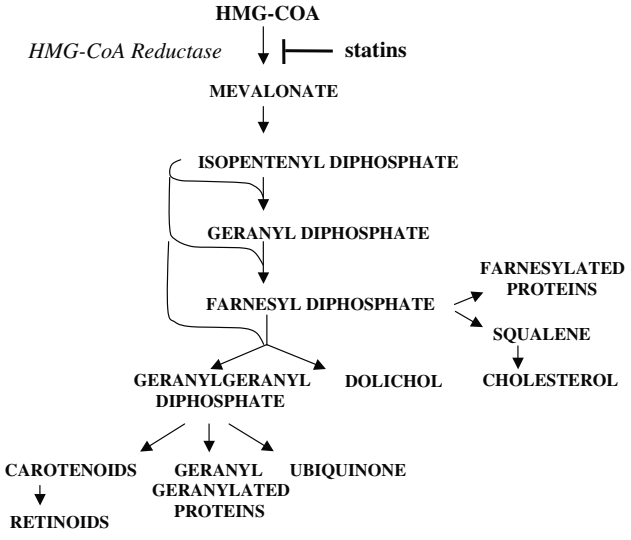

Fig. 1. The mevalonate pathway. 3-Hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase is the rate-limiting enzyme of the mevalonate pathway. Lovastatin is a potent inhibitor of this enzyme.

In a Phase I clinical trial of lovastatin given as a single oral agent [18], 88 patients with solid tumours were treated with repeated courses consisting of 7 days of consecutive dosing, followed by 3 weeks of rest, every 4 weeks. Doses ranging from 2 to 45 mg/kg/day were tested, and the maximum tolerated dose (MTD) was determined to be 25 mg/kg/day. This dose is approximately 25 times the usual upper limit used to lower LDLs. This study showed peak plasma lovastatin concentration of 0.10–3.92 μM were achieved, with trough levels at the MTD averaging 0.28 μM, which corresponded with *in vitro* levels that can trigger apoptosis in sensitive cell lines [10]. Dose-limiting toxicity (DLT) was myopathy that appeared to be associated with low levels of ubiquinone in patients, another end-product of the mevalonate pathway. Only one minor response was documented in a patient with recurrent high-grade glioma. Two small Phase II trials have been reported using a similar dose and schedule which did not show promising activity [19,20]. Interestingly, a randomised Phase II trial in 83 unresectable hepatocellular carcinoma (HCC) suggested a survival advantage when patients were randomised to receive continuous daily pravastatin (another lipid-lowering agent from the statin family) versus no pravastatin following treatment with arterial emobilisation and oral 5-fluorouracil (5-FU) chemotherapy (median survival 18 months *vs.* 9 months, $P = 0.006$) [21]. While survival differences in this small study could be due to an imbalance of prognostic features in the HCC patients between the two treatment arms, the role of statins in HCC warrants further study.

Given the striking cytotoxicity of lovastatin against HNSCC and CC cell lines *in vitro*, we planned to assess

its antitumour activity in these specific clinical settings. Both these tumour types in advanced stages are not cured by any conventional chemotherapy or radiation and median survival estimates are 6–9 months [22]. Novel anticancer agents, if selective against relevant molecular targets, may offer a better therapeutic index over conventional chemotherapy. Since the previous Phase I–II studies utilising the 1-week on, 3-weeks off schedule produced virtually no clinical efficacy, a prolonged oral administration of lovastatin, to provide a sustained exposure, is investigated in this study. *In vitro*, IC_{50}s in the range of 0.5–5.0 μM in HNSCC and CC cell lines are sensitive to lovastatin's apoptotic effects [10]. In fact, lower concentrations (0.1–1.0 μM) were quite sufficient to inhibit proliferation and induce apoptosis if these cells were exposed for a prolonged 5-day period (data not shown). We designed this study to involve a dose and duration escalation of lovastatin, until the recommended Phase II dose (RPTD) was reached aiming to achieve serum concentrations that could produce these anti-cancer effects *in vitro*. Pre- and post-treatment tumour biopsies were planned in consenting patients to evaluate any biological effects of lovastatin.

2. Patients and methods

This was a single-centre, Phase I open-label study to determine the RPTD of lovastatin in the treatment of patients with recurrent or metastatic HNSCC or cervical cancer. Cohorts to evaluate the escalating dose and duration of exposure of lovastatin were planned from 5 to 25 mg/kg/day given over 2–4 week periods. The characteristics of the enrolled patients and the actual dose escalation are summarised in Tables 1 and 2, respectively. Eligible patients had histologically proven HNSCC or CC, that was either recurrent after primary therapy or metastatic at diagnosis. Patients may have had up to two previous lines of chemotherapy for their recurrent or metastatic disease. In addition, prior cisplatin-based therapy given concurrently with radiation for locally advanced disease, and prior adjuvant/neoadjuvant chemotherapy were acceptable. Patients had adequate haematogical, hepatic and renal functions. Adequate renal function was defined as a creatinine clearance (CrCl) >1 ml/s as measured by 24-h urine collection. Biochemical parameters for hepatic function included; total bilirubin ⩽1.5× the upper limit of normal (ULN), alkaline phosphatase ⩽5× ULN, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) ⩽2.5× ULN. Patient had to discontinue any other anticholesterol agents at least 2 weeks prior to starting on the trial. Other exclusion criteria included known brain or leptomeningeal metastases and concomitant treatment with any other anticancer therapy within 4 weeks of study entry, as well as known hypersensitivity or allergy to lovastatin or other HMG-CoA reductase inhibitors.

Table 1
Patient and disease characteristics at baseline ($N = 26$)

Characteristic	Patients	
	N	(%)
Male/female	11/15	(42)/(58)
Median age (range) (in years)	56 (36–76)	
ECOG PS		
0/1	17	(65)
2	9	(35)
HNSCC	14	(54)
CC	12	(46)
Prior therapy		
Radiation	26	(100)
Chemotherapy	19	(73)
1 line	14	(54)
2 lines	5	(19)

HNSCC, head and neck squamous cell carcinoma; ECOG PS, Eastern cooperative oncology group performance status.

2.1. Treatment

Lovastatin was supplied as 40 mg tablets (Apotex, Toronto, Canada). Total daily dose was divided into 4 doses and patients were instructed to take the study drug at 0700, 1200, 1800 and 2200 h. No prophylactic medications were supplied. Standard supportive care

Table 2
Lovastatin Phase I results

	Dose/day mg/kg	Schedule (weeks)	No. of patients[b]	HNSCC/cervix	Total cycles delivered	Number of DLTs	Best response seen
Cohort 1	5	2 on, 1 off	6	3/3	13	0	SD 1 in 6
Cohort 2	10	2 on, 1 off	6 (8)	4/4	31	2 (1 HNSCC, 1 CC)	SD 2 in 6
De-escalate							
Cohort 3	7.5	2 on, 1 off	3	2/1	12	0	SD 2 of 3
Cohort 4[a]	7.5	3 on, 1 off	7 (9)	5/4	17	2 (2 CC)	SD 1 of 8

SD, stable disease; DLT, dose-limiting toxicity.
[a] Recommended Phase II dose and schedule.
[b] Fully evaluable for toxicity: received at least one full cycle at dose (received <1 cycle).

medications were allowed. Oral supplementation of ubiquinone at 60 mg orally (po) q8h was initiated if patient developed grade 3 or 4 muscle toxicity and continued until recovery to baseline.

2.2. Dose escalation per cohort

The study design was a classic Phase I design: Three patients were planned at each cohort. If no DLT was encountered in a cohort of three patients during the first cycle, then dose escalation was planned in the next cohort of three patients. When one patient experienced DLT, then the treatment level was expanded to at least six patients. If no more than one of six patients experienced DLT, then the next cohort of patients was to be treated at the next higher dose level. If $\geqslant$2/6 patients at any dose level experienced DLT, then that level was considered to have exceeded the MTD, and the level immediately preceding that level was designated as the MTD or RPTD.

2.3. Pharmacokinetics

Blood samples were collected on day 1 before drug administration (baseline) and every 3–4 days during cycle 1, immediately before the 1200 dose. Plasma was separated and stored at −20 °C until analysis. Lovastatin was measured by a previously reported method in Ref. [23].

2.4. Toxicity

All toxicity grading was according to the National Cancer Institute – Common Toxicity Criteria (NCI CTC-2). DLT was defined as any first-course, $\geqslant$grade 3 non-haematological toxicity, except alopecia or inadequately controlled nausea/vomiting, grade 4 neutropenia or with fever, grade 4 thrombocytopenia, or dose delay of >2 weeks due to drug-related toxicity. Patients who recovered from DLT were able to continue on the study with a dose reduction to the next lower dose level at the investigator's discretion.

2.5. On study evaluation

Patients had weekly laboratory evaluations including creatine phospo-kinase (CK), liver enzymes and fasting total cholesterol and LDL during cycle 1 and at the start of each subsequent cycle. Physical exams occurred at least at each cycle start and radiological tumour evaluations were completed after every 2 cycles. Tumour response was measured using the World Health Organisation (WHO) bi-dimensional criteria. If patients consented, tumour biopsies were taken at baseline and on treatment at the end of cycle 1 or 2.

2.6. Treatment duration and follow-up

All patients received lovastatin until clinical and/or radiological progression, unacceptable toxicity, or patient refusal. In these cases, the patient went off the protocol treatment. Patients with disease stabilisation were allowed to continue on the treatment at the investigator's discretion. All patients were observed after the last treatment to document ongoing side-effects and any late side-effects. Institutional Research Ethics Board approval was obtained prior to the start of this study, and all study subjects provided written informed consent.

3. Results

Twenty-six eligible patients were enrolled on the study. Four patients were not evaluable for response (one withdrew consent in the first week of treatment, three progressed clinically before completing 1 cycle of treatment). All 26 patients were evaluable for toxicity, but emphasis was placed on the 22 who completed at least one full cycle. Patients' baseline characteristics are summarised in Table 1. All patients had been previously treated with either radiation and/or chemotherapy. The median age at entry was 56 years and most patients had good performance status (65% ECOG PS of 0/1). The median number of cycles received per patient was 2 (range 1–17). Twenty-one patients came off study for disease progression, while 2/26 discontinued because of treatment-related toxicity and 2/26 with stable disease (SD) requested a break from treatment after 6 and 17 cycles. The median survival of patients on the study was 7.5 months (mean 9.2 ± 1.5 months). One patient is still alive and was censored at the time of reporting.

3.1. Pharmacokinetics

There were marked interpatient variability of plasma trough lovastatin levels. Steady-state levels were demonstrated by days 4–8 on treatment. The mean $(Css)_{min}$ (trough level) was 0.07, 0.1 and 0.06 μM for the 5, 10 and 7.5 mg/kg doses, respectively. In addition, ubiquinone levels were measured in seven patients in this study, but found to be highly variable and were not informative.

3.2. Toxicity

DLT, consisting of transient grade 4 CK rises, were observed in the second cohort in 2 of 6 fully evaluable patients treated at the dose level of 10 mg/kg a day × 14 days, every 21 days (Tables 2 and 3). The CK rises developed on days 15–21, which was during the rest week in the 3-week cycle. This biochemical toxicity was associated with only mild and transient symptoms of grade 1

Table 3
Toxicity by cohort

Cohort	Dose and duration	No. of patients/ no. of courses	Increased creatine phospho-kinase (CK)			Muscle weakness			Increased AST/ALT			Anorexia		Nausea	
			1/2	3	4	1/2	3	4	1/2	3	4	1/2	3/4	1/2	3/4
1	5 mg × 2 wks q 3 wks	6/13	0	0	0	1	0	0	1	0	0	2	0	0	0
2[a]	10 mg × 2 wks q 3 wks	8/31	0	0	3[b]	2	0	1	1[b]	2[b]	0	2	1	1	0
3	7.5 mg × 2 wks q 3 wks	3/12	0	0	0	0	0	0	0	0	0	1	0	0	0
4	7.5 mg × 3 wks q 4 wks	9/17	0	0	2[b]	0	1	0	1[b]	1[b]	0	1	0	1	0

wks, weeks; q, every; AST, aspartate aminotransferase; ALT, alanine aminotransferase.

[a] Includes patient #9 who died of acute renal failure as a result of tumour progression and subsequent lovastatin-induced muscle toxicity (not fully evaluable).

[b] Four of five patients with biochemical DLT recovered to baseline after holding lovastatin ×1–2 weeks, and 2 of 5 of these patients continued on the drug with a dose reduction and no further biochemical toxicity.

muscle weakness. These patients were admitted to hospital to receive intravenous (i.v.) fluids, close monitoring and oral ubiquinone, and discharged on improvement of biochemical parameters (within one week for both patients). The cardiac-specific isoenzyme of CK (CK-MB) component was not affected nor was there evidence of myoglobinuria. Both patients developed a concurrent transaminitis, of grade 2 and 3 intensity, respectively, each resolving in 1–2 weeks. Both patients continued on study at the lower dose level of 5 mg/kg/day × 14 days, every 21 days, without further toxicity.

The third cohort continued with a dose de-escalation to 7.5 mg/kg/day × 14 days, every 21 days, without further toxicity. In the subsequent cohorts, it was planned to escalate the duration of therapy, with the daily dose of lovastatin fixed at 7.5 mg/kg. DLT occurred in cohort 4 in 2 of 7 (<2 of 6) fully evaluable patients treated with lovastatin at 7.5 mg/kg/day × 21 days, every 28 days. Grade 4 CK rises developed in both patients on days 22–28, also during the rest week, but 1 week later than the patients who experienced DLT in cohort 2. One of these patients developed associated grade 3 muscle weakness, and grade 3 transaminitis, all of which resolved with supportive care in 1–2 weeks.

The use of concurrent medications known to affect lovastatin clearance *via* CYP3A4 was not found in any of the patients experiencing DLT. Plasma lovastatin levels did not correlate with toxicity as two patients experiencing muscle toxicity had levels slightly above the mean and the other two patients had levels below the mean. The only other toxicity appreciated on the study drug was mild anorexia or nausea.

Four of the five patients enrolled on study who experienced any muscle toxicity had CC, a patient population that commonly has renal dysfunction due to hydronephrosis. We explored relative renal dysfunction as a risk for this toxicity. All patients met the baseline eligibility criteria defining adequate renal function as a CrCl >1 ml/s, as measured by 24-h urine collection. All four women with CC who experienced muscle toxicity had borderline CrCl levels of 1–1.08 ml/s, whereas the remaining patients on the trial had CrCl levels >1.17 ml/s.

The MTD or RPTD of this study was declared at lovastatin 7.5 mg/kg/day × 21 days, every 28 days, as 2 of 9 patients (<2 of 6 evaluable patients) at this dose level experienced DLT. However, since both of these patients had CC, it is reasonable to start these patients at one dose level lower with lovastatin 7.5 mg/kg/day × 14 days, every 21 days, and escalate to a longer duration if the first cycle is tolerated. Restricting CrCl to >1.17 ml/s in patients would also be prudent.

One study patient was not fully evaluable as she developed renal failure soon after enrolling on study, but has a sequence of events that are informative. Patient # 09, a 56-year-old woman with advanced CC involving right kidney and aortocaval nodes (baseline CrCl of 1.02 ml/s) was treated in cohort 2. In cycle 1, week 2, she developed acute tubular necrosis of the functioning left kidney second to renal hypoxia from tumour-thrombosis which led to complete renal failure. Lovastatin was held at the first sign of the renal impairment (day 7), but the patient subsequently developed a grade 4 CK rise (day 12) with progressive, diffuse grade 4 muscle weakness, despite prior initiation of haemodialysis and oral ubiquinone supplementation. This patient died soon after and her death was attributed to disease progression plus lovastatin-induced rhabdomyolysis in the setting of renal failure which pre-dated the rhabdomyolysis. This case also suggests an adequate renal function threshold is still required for safe metabolism of lovastatin, despite its recognised dominant hepatic clearance.

3.3. Response

No objective responses were seen. A best response of SD was noted for six patients, with SD greater than 3 months seen in 5 patients (23% of total, 2 HNSCC, 3 CC). One patient treated in cohort 2 at 10/mg/kg/day × 2 weeks, q 3 weeks, achieved SD and clinical

benefit over 17 cycles and had not progressed when he chose to discontinue treatment. This 51-year-old man had a large SCC of the parapharyngeal space and was treated initially with radical radiotherapy, but recurred within 4 months. He was treated on a trial evaluating single agent epidermal growth factor tyrosine kinase inhibitor; Erlotinib (Tarceva, OSI-774) [24], initially achieved a partial response (PR), but subsequently had clear radiological and clinical progression. Four weeks after discontinuation of the Erlotinib trial, this patient was enrolled in the lovastatin study. At baseline, he had a 4.5 cm growing tumour invading the parapharangeal and pterygoid spaces and was symptomatic with pain and trismus. By 4 cycles of lovastatin, there was improvement in pain, trismus and nutritional status all of which were sustained throughout the treatment (14 months). A correlative biopsy was not feasible due to the inaccessible location of the tumour. After 14 months on the study, he chose to take a break from treatment. At 23 months off treatment, he remains stable, both clinically and radiologically, with the invasive mass measuring 4.3 cm.

3.4. Cholesterol

Fasting cholesterol levels were followed closely on the study. Baseline cholesterol levels of patients entering the study were as follows: mean total cholesterol (Tchol) of 4.8 mMol/l (median 4.6, standard deviation (SD) 1.1) and mean calculated LDL levels of 2.8 mMol/l (median 3.0, SD 0.9). These values fall within the low-normal risk category, based on the heart disease risk assessment-cholesterol level consensus guidelines (where low-risk cholesterol profiles for patients >30 years old include Tchol < 5.2 and LDL < 3.4 mMol/l). LDL cholesterol levels on the study demonstrated a rapid nadir to an average of 50% of baseline by day 14 in all cohorts (mean day-14 LDL of 1.4 mMol/l, median 1.3, SD 0.6). All patients recovered LDL cholesterol levels to within 70% of their baseline by the start of cycle 2, following the 1-week drug break (mean day 1 LDL: 2.0 mMol/l, median 2.0, SD 0.8). This pattern persisted over all cycles treated, without apparent cumulative treatment effects. Lovastatin dosing effects were seen with respect to both the magnitude of the drop of cholesterol and the time to nadir (Fig. 2).

Two patients on the study were noted to have baseline cholesterol levels considerably below the study mean, as well as being low for the general population (baseline Tchol 2.7 and 2.6 and LDL 1.4 and 1.0 mMol/l). Treated in cohorts 3 and 4, respectively, each experienced a LDL nadir to 70% of baseline (1.0 and 0.7 mMol/l, respectively) with full recovery after each drug break. Neither experienced any treatment-related toxicity. Twelve patients over 19 treatment cycles experienced LDL level drops to $\leqslant$1.0 mMol/l, which would

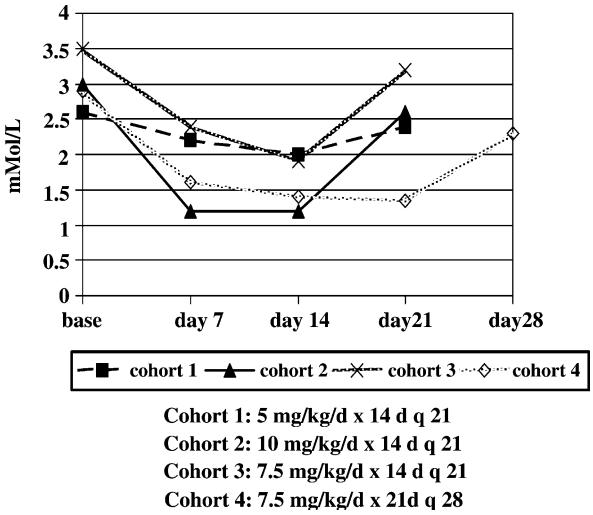

Fig. 2. Mean serum low-density serum lipoprotein (LDL) cholesterol levels of patients within each lovastatin treatment cohort.

be considered very low levels by general population standards. Two of these patients were amongst the 4 who experienced DLT, while the other 10 had no toxicity. One of the 12 patients with low nadirs was the long-standing stable patient who experienced no toxicity over 17 cycles. The other 2 patients with DLT on this study had nadir LDLs above the mean (1.5 and 2.0 mmol/l).

3.5. On-treatment tumour biopsies

Eight of 26 patients consented to have tumour biopsies. Paired baseline and on treatment samples were obtained for 6 of those 8 patients and analysed for anticancer effect of lovastatin using the standard Tdt-mediated dUTP biotin nick-end labelling (TUNEL) assay and immunohistochemistry for Ki67. All 6 were adequate for analysis, but showed no evidence of on treatment increased apoptosis or decreased cell proliferation (data not shown), which also reflected the clinical observations of those patients' progression disease. Unfortunately, none of the 6 patients achieving disease stabilisation were captured with paired biopsies for analysis.

4. Discussion

Inhibition of the mevalonate pathway has appeared a promising anticancer strategy based on pre-clinical studies showing numerous examples of the impact of statins on malignant cell differentiation, cycling and apoptosis [8–11,17,25]. Potential cancer therapeutics capable of inhibiting the mevalonate pathway already exist and are widely used as safe and effective anti-cholesterol agents [6,12]. In our own experience lovastatin appeared a potent cytostatic and pro-apoptotic agent in SCC cell lines of the head and neck and cervix [10].

Based on dose-intensity estimations, patients were initiated at a 14-day exposure at 5 then 10 mg/kg/day. The expected DLT of myopathy was encountered early on in the escalation strategy, in cohort 2 at 10 mg/kg/day × 14 days, every 21 days. DLT at this dose was somewhat surprising given that Thibault and colleagues [18] had shown the same DLT, but at 35 mg/kg/day × 7 days, q 28 days. The 14-day exposure to lovastatin, even at the 10 mg/kg/day dose, was significant. De-escalation to 7.5 mg was well tolerated over 14 days, but DLT was seen with 21-day exposures. Myopathy appeared to be dependent on both maximum daily dose and the length of exposure, or occurs at fairly equivalent cumulative doses or a threshold as the DLT at 7.5 mg occurred 1 week later in the cycle than at the 10 mg dose.

High doses of lovastatin appear more effective at inhibiting the mevalonate pathway than the recommended lipid-lowering doses. We observed a rapid decline of LDL cholesterol levels to 40–60% of baseline within 1–2 weeks, that was more pronounced at the 10 mg than 5 mg dose. By contrast, a 30–40% maximum reduction usually occurs over 4–6 weeks with standard dosing of lovastatin [1,2]. The block of HMG-CoA reductase activity is temporary as intermittent dosing results in the recovery of levels at the start of each cycle. This would suggest that any biological anti-tumour activity initiated would also be interrupted. Because of toxicity we were not able to extend the exposure safely past 3 weeks. The mean trough plasma lovastatin levels, measured in the 0.06–0.10 μM range in these study patients is just at the biologically active *in vitro* level. While this plasma level is more than adequate to inhibit HMG-COA reductase, we do not have any data on intra-tumour levels.

The low lipid levels experienced by patients in this study did not appear to translate into adverse events. Given the nutritional depletion cancer patients experience secondary to anorexia, cachecia and difficulty with swallowing, further lowering of lipids was of concern. There appeared to be no clear association with the low lipids levels on treatment with the toxicity observed. Anorexia and further weight-loss were not significant issues. An interesting comparison would be individuals who are heterozygous for the beta- or hypobetalipoproteinaemia gene defect. They cannot produce sufficient quantities of apo B-containing lipoproteins (LDL, IDL and VLDL) and have lipid profiles similar to a number of patients on this study (LDL < 1.0 mMol/l). These patients are not prone to myopathy [26].

Thibault and colleagues [18] in the previous Phase I lovastatin trial had hypothesised that the depletion of ubiquinone, an end-product of the mevalonate pathway involved in the mitochondrial electron transfer chain, is the important pathophysiological mechanism responsible for lovastatin-induced muscle damage. Ubiquinone is also readily available through diet (e.g., meat, vegetables, and eggs) and as an oral supplement [27]. Patients in the second half of the Thibault study were supplemented with ubiquinone resulting in detectable increased plasma ubiquinone levels. They concluded that ubiquinone prophylaxis can reduce the severity albeit not the incidence of muscle toxicity [16]. While we have not confirmed this, the prophylactic supplementation of ubiquinone in future trials is a reasonable intervention to help reduce serious muscle toxicity.

The disease stabilisation rate of 23% seen in these end-stage patients with CC or HNSCC treated with a prolonged administration schedule of lovastatin is encouraging, in particular the case of the man with over 2 years of SD and clinical benefit. His case raises a question of whether there was some additive anticancer benefit to his having been exposed to an EGFR inhibitor, despite progression, before lovastatin. We conclude that the administration of lovastatin at 7.5 mg/kg/day for 21 consecutive days on a 28-day schedule is well tolerated, in patients with good renal function (CrCl >1.17 ml/s). Further clinical evaluations of statins should evaluate their potential as part of a combination of targeted therapy, combined modality approach or as Phase II cytostatic agents and employ time-to-progression endpoints to gauge activity.

Conflict of interest statement

None declared.

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References

1. Corsini A, Maggi FM, Catapano AL. Pharmacology of competitive inhibitors of HMG-CoA reductase. *Pharmacological Research* 1995, **31**, 9–27.
2. Hunninghake DB. HMG-CoA reductase inhibitors. *Curr Opin Lipidol* 1992, **3**, 22–28.
3. Hebert PR, Gaziano JM, Chan KS, *et al*. Cholesterol lowering with statin drugs, risk of stroke, and total mortality. An overview of randomized trials. *Jama* 1997, **278**, 313–321.
4. Randomised trial of cholesterol lowering in 4444 patients with coronary heart disease: the Scandinavian Simvastatin Survival Study (4S). *Lancet*, 1994, **344**, 1383–9.
5. Farmer JA. Statins and myotoxicity. *Curr Atheroscler Rep* 2003, **5**, 96–100.

6. Wong WW, Dimitroulakos J, Minden MD, *et al*. HMG-CoA reductase inhibitors and the malignant cell: the statin family of drugs as triggers of tumour-specific apoptosis. *Leukemia* 2002, **16**, 508–519.
7. Goldstein JL, Brown MS. Regulation of the mevalonate pathway. *Nature* 1990, **343**, 425–430.
8. Dimitroulakos J, Thai S, Wasfy GH, *et al*. Lovastatin induces a pronounced differentiation response in acute myeloid leukemias. *Leuk Lymphoma* 2000, **40**, 167–178.
9. Dimitroulakos J, Nohynek D, Backway KL, *et al*. Increased sensitivity of acute myeloid leukemias to lovastatin-induced apoptosis: A potential therapeutic approach. *Blood* 1999, **93**, 1308–1318.
10. Dimitroulakos J, Ye LY, Benzaquen M, *et al*. Differential sensitivity of various pediatric cancers and squamous cell carcinomas to lovastatin-induced apoptosis: therapeutic implications. *Clin Cancer Res* 2001, **7**, 158–167.
11. Keyomarsi K, Sandoval L, Band V, *et al*. Synchronization of tumour and normal cells from G1 to multiple cell cycles by lovastatin. *Cancer Res* 1991, **51**, 3602–3609.
12. Chan KK, Oza AM, Siu LL. The statins as anticancer agents. *Clin Cancer Res* 2003, **9**, 10–19.
13. Blais L, Desgagne A, LeLorier J. 3-Hydroxy-3-methylglutaryl coenzyme A reductase inhibitors and the risk of cancer: a nested case-control study. *Arch Intern Med* 2000, **160**, 2363–2368.
14. Graaf M, Beiderbeck A, Egberts A, *et al*. The risk of cancer in users of statins. *J Clin Oncol* 2004, **22**, 2388–2394.
15. Poynter J. HMG CoA reductase inhibitors and the risk of colorectal cancer. *Proc Am Soc Clin Oncol*., abstract #1.
16. Dimitroulakos J, Marhin WH, Tokunaga J, *et al*. Microarray and biochemical analysis of lovastatin-induced apoptosis of squamous cell carcinomas. *Neoplasia* 2002, **4**, 337–346.
17. Agarwal B, Bhendwal S, Halmos B, *et al*. Lovastatin augments apoptosis induced by chemotherapeutic agents in colon cancer cells. *Clin Cancer Res* 1999, **5**, 2223–2229.
18. Thibault A, Samid D, Tompkins AC, *et al*. Phase 1 study of lovastatin, an inhibitor of the mevalonate pathway, in patients with cancer. *Clin Cancer Res* 1996, **2**, 483–491.
19. Kim WS, Kim MM, Choi HJ, *et al*. Phase II study of high-dose lovastatin in patients with advanced gastric adenocarcinoma. *Invest New Drugs* 2001, **19**, 81–83.
20. Larner J, Jane J, Laws E, *et al*. A phase I–II trial of lovastatin for anaplastic astrocytoma and glioblastoma multiforme. *Am J Clin Oncol* 1998, **21**, 579–583.
21. Kawata S, Yamasaki E, Nagase T, *et al*. Effect of pravastatin on survival in patients with advanced hepatocellular carcinoma. A randomized controlled trial. *Br J Cancer* 2001, **84**, 886–891.
22. Boring CC, Squire TS, Tong T, *et al*. Cancer statistics. *CA Cancer J Clin* 1994, **44**, 7–26.
23. Ye LY, Firby PS, Moore MJ. Determination of lovastatin in human plasma using reverse-phase high-performance liquid chromatography with UV detection. *Ther Drug Monit* 2000, **22**(6), 737–741.
24. Bonomi P. Erlotinib: a new therapeutic approach for non-small cell lung cancer. *Expert Opin Investig Drugs* 2003, **12**, 1395–1401.
25. Dimitroulakos J, Yeger H. HMG-CoA reductase mediates the biological effects of retinoic acid on human neuroblastoma cells: Lovastatin specifically targets P-glycoprotein-expressing cells. *Nat Med* 1996, **2**, 326–333.
26. Schonfeld G. The hypobetalipoproteinemias. *Annu Rev Nutr* 1995, **15**, 23–34.
27. Overvad K, Diamant B, Holm L, *et al*. Coenzyme Q10 in health and disease. *Eur J Clin Nutr* 1999, **53**, 764–770.