Effect of topical imiquimod as primary treatment for lentigo maligna: the LIMIT-1 study*

J.R. Marsden,[1] R. Fox,[2] N.M. Boota,[3] M. Cook,[4] K. Wheatley,[2] L.J. Billingham[2] and N.M. Steven,[2] on behalf of the NCRI Skin Cancer Clinical Studies Group, the U.K. Dermatology Clinical Trials Network and the LIMIT-1 Collaborative Group

[1]University Hospitals Birmingham NHS Foundation Trust, Queen Elizabeth Hospital, Edgbaston, Birmingham, B15 2TH U.K.

[2]Cancer Research U.K. Clinical Trials Unit, School of Cancer Sciences, The University of Birmingham, Edgbaston, Birmingham B15 2TT, U.K.

[3]Nottingham Clinical Trials Unit, University of Nottingham, Nottingham, U.K.

[4]Royal Surrey County Hospital, Guildford, U.K.

Summary

Background Topical imiquimod is sometimes used for lentigo maligna (LM) in situ melanoma instead of surgery, but frequency of cure is uncertain. Pathological complete regression (pCR) is a logical surrogate marker for cure after imiquimod, although residual LM and atypical melanocytic hyperplasia may not be reliably distinguished. A trial comparing imiquimod vs. surgery might be justified by a high imiquimod pCR rate.

Objectives Primary: to estimate the pCR rate for LM following imiquimod. Secondary: to assess the accuracy of prediction of pCR, using clinical complete regression (cCR) plus negative post-treatment biopsies, tolerability, resource use, patients' preferences and induced melanoma immunity.

Methods This was a single-arm phase II trial of 60 imiquimod applications over 12 weeks for LM then radical resection. A pCR rate ≥ 25 out of 33 would reliably discriminate between pCR rates < 60% and ≥ 85%. Clinical response was assessed and biopsies taken after imiquimod. Patients recorded adverse events in diaries. Patient preference was measured after surgery using a standard gamble tool. Results The pCR rate was 10 of 27 (37%, 95% confidence interval 19–58%). The rate of cCR plus negative biopsies was 12 of 28, of whom seven of 11 had pCR on subsequent surgery. The median dose intensity was 86·7%. Of the 16 surveyed patients, eight preferred primary imiquimod over surgery if the cure rate for imiquimod was 80%, and four of 16 if it was ≤ 40%.

Conclusions The pCR rate was insufficient to justify phase III investigation of imiquimod vs. surgery. Clinical complete response and negative targeted biopsies left uncertainty regarding pathological clearance. Some patients would trade less aggressive treatment of LM against efficacy.

Lentigo maligna (LM) in situ melanoma characteristically presents as a slowly developing brown or dark brown macule on chronically sun-exposed skin in people aged > 50 years. In the U.K. guidelines, complete surgical resection is recommended with curative intent.[1] Five per cent of patients with typical LM may actually have early invasive melanoma, and the risk of progression to invasive lentigo maligna melanoma (LMM) is poorly quantified.[2] Reported outcomes following surgery vary, including a 30% probability of recurrence at 66–98 months and a 1·5% probability of transformation to LMM for 81 patients,[3] a crude failure rate (recurrence plus incomplete excision) of eight of 102 following resection excision with 2-mm margins,[4] and crude recurrence rates of 16 of 269 (5·9%) following wide local excision and three of 154 (1·9%) following Mohs micrographic surgery.[5] LM occurs most frequently on the head and neck, so surgery can cause significant functional and cosmetic disability and, in some cases, might not be feasible.

In 2001, a U.K. survey showed that the most widely used treatments for LM were surgery, cryotherapy, radiotherapy and observation, in that order, with nonsurgical approaches possibly associated with higher recurrence rates and used more for patients aged > 70 years.[6] Radiotherapy may have a place in LM management, with the aim of trading less invasive intervention and better function and cosmesis against a possibly higher risk of recurrence or progression to melanoma.[7–10] No trials have been undertaken comparing the outcomes of surgery and radiotherapy or other nonsurgical treatments.[11]

Imiquimod is a synthetic imidazoquinoline nucleoside analogue available as a 5% strength topical formulation, with low systemic availability. Skin application induces local inflammation with intensity related to the frequency of application. The use of topical imiquimod as a nonsurgical treatment for LM has increased following an initial case report in 2000 of disease control for 9 months after treatment for 7 months.[12] Treatment duration is not defined, but 12 weeks is widely used;[13] any benefit of longer treatment is unclear. An effect against LM has been confirmed in subsequent case reports and small uncontrolled trials, and in a systematic review,[14] with response rates of 77–90%. These studies lacked long-term follow-up to substantiate disease control and post-treatment histology: the majority of cases involved biopsy only, with the possibility of sampling error.[15–22] One small study used complete surgical excision following imiquimod treatment, and reported complete response in four of six patients recruited.[23] A recent retrospective series reported recurrence of LM following imiquimod alone in six of 22 patients vs. two of 36 having surgery plus imiquimod with a mean follow-up of around 40 months.[13]

The routine use of imiquimod as primary therapy for LM requires proof of efficacy in a large trial compared with the outcomes of surgical excision. We reasoned that, to justify this investment, imiquimod treatment should be shown to have a high probability of achieving pathological complete response (pCR). Patients and clinicians might take into account the probability of cure with imiquimod and avoiding surgery for a premalignant condition, assuming progression is susceptible to surveillance. We surveyed U.K. dermatologists' opinions regarding what threshold of pCR rate would justify routine use of imiquimod instead of surgery. We then designed a single-arm trial to justify progression to a larger randomized trial. We sought opinion from trial participants regarding what threshold of efficacy they would trade against avoiding surgery, using a structured questionnaire.

Patients and methods

Participants

This study was coordinated through the Nottingham Clinical Trials Unit, and approved by Nottingham Research Ethics Committee 2 and eight hospitals recruited between October 2010 and August 2011. The trial was registered with clinicaltrials.gov identifier NCT01161888.

To be eligible, patients had to give informed written consent, have a clinical diagnosis of primary untreated LM (acquired pigmented macule present for > 12 months, no change in skin surface texture or contour, no palpability, diameter > 10 mm, sited on the head or neck) and histologically confirmed LM without invasive melanoma in one or more 4-mm punch biopsies from the darkest area, reported by a pathologist member of a recognized National Health Service skin cancer multidisciplinary team. The LM had to be suitable for complete surgical excision using a 5-mm lateral margin, and to be easily definable visually around its entire circumference. Patients had to be aged > 45 years, fit, and willing for surgery, without coexisting or adjacent melanoma or nonmelanoma skin cancer that might compromise study treatment; neither pregnant nor breastfeeding; without hypersensitivity to imiquimod or excipients; not taking immunosuppressive medication and not participating in another intervention study.

Treatment and follow-up

The trial interventions and investigations are detailed in Figure 1. The pretreatment lesion was photographed, tattooed at 360, 90, 180 and 270 degrees, outlined in ink and traced on a transparency. Training in mapping and tattooing was

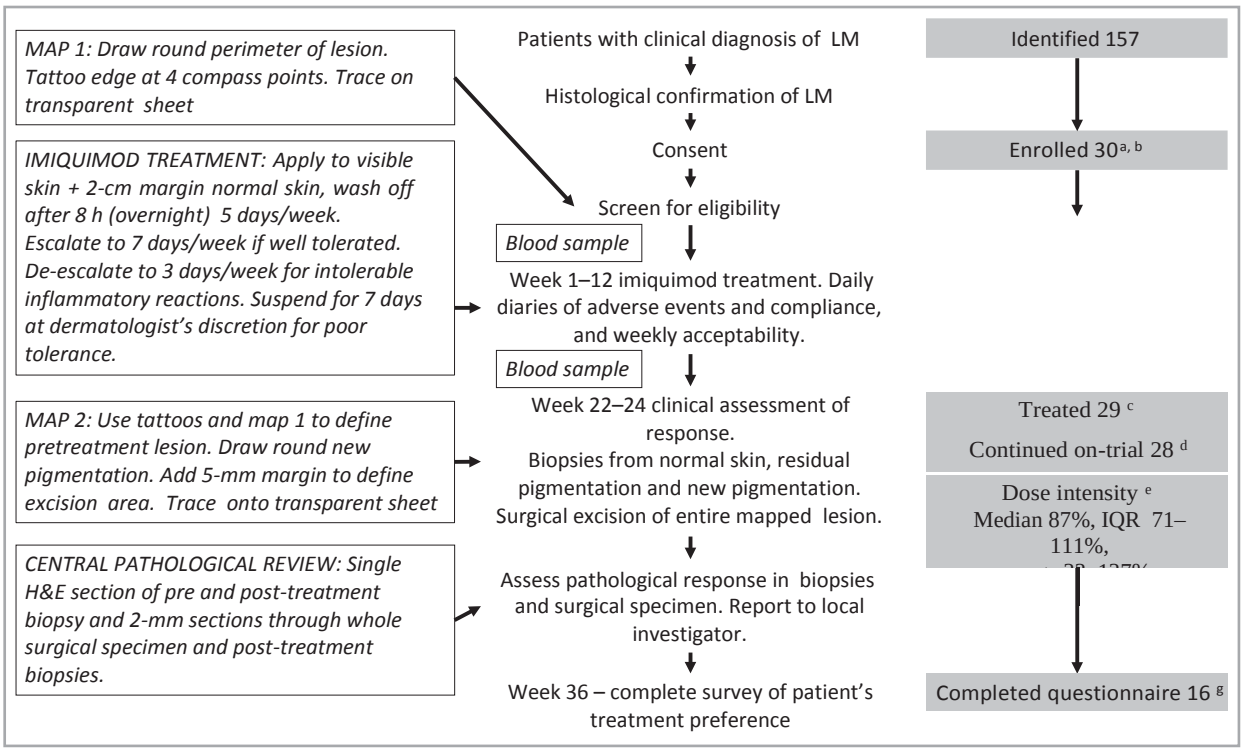

Fig 1. Trial design and recruitment. The sample constituted 28 patients evaluated on an intent-to-treat basis and 27 treated patients with post- treatment surgical specimens. (a) Seventy-five patients were ineligible, most commonly because of a lentigo maligna (LM) size < 10 mm, invasive or recurrent disease, not on head or neck, LM histology not conclusive, steroid treatment, ill-defined lesion, inadequate surgical clearance or LM present < 12 months. (b) Fifty-two patients declined trial entry; the most common reasons were burden of travelling (10) or time involved (15), delay in surgery (13) and concerns over side-effects (three) or lack of efficacy (two) of imiquimod. (c) One patient withdrew consent before starting imiquimod. (d) One patient withdrew consent after 5·3 weeks of treatment. (e) Dose intensity was the number of imiquimod applications as a proportion of the expected 60 applications over 12 weeks. (f) One treated patient declined surgery after imiquimod. (g) The questionnaire was completed by the first 16 patients on the trial. H&E, haematoxylin and eosin; IQR, interquartile range.

provided. Patients applied imiquimod (Aldara®; Meda Pharma- ceuticals Ltd, Bishops Stortford, U.K.) 5 days per week to the visible lesion plus a 2-cm margin of normal surrounding skin for approximately 8 h (overnight). It was then washed off with soap and water as defined in the patient information leaf- let. After 12 weeks of treatment, lesions were remapped, biopsied and excised with central pathological reporting. The User Opinion Questionnaire was undertaken 12 weeks post- surgery by the first 16 patients.

Outcome measures

The primary outcome measure was pCR, defined as the absence of LM in both post-treatment biopsies and resected LM. Imiquimod is an experimental treatment in which post-treatment biopsies would be part of the assessment of clearance. Resection is the standard of care and, in this trial, permits assessment of LM clearance in the whole lesion. Other pathological outcomes were partial regression (pPR, atypical melanocytes present in the epidermis with abnormal distribu- tion and number, but insufficient features to define LM), no change (presence of LM) or progressive disease (invasive melanoma).

Clinical outcomes were complete regression (cCR, complete disappearance of abnormal pigmentation), partial regression (cPR, reduction in size of pigmented area or obvious reduc- tion in its intensity), no change (appearance identical to that pretreatment) and progressive disease (increased size or inten- sity of pigmentation or development of a papule or nodule within LM). Targeted biopsies were scored as for the resection specimen, detailed above. For each individual, the prediction of pCR using clinical examination of the mapped regions (weeks 22–24) plus targeted presurgical biopsies was com- pared with the pathological response in the whole post-treat- ment resected lesion, including any LM outside the clinically mapped margins of disease.

Patients kept weekly diaries through 12 weeks of treatment of adverse reactions, numbers of treatments, treatment accept- ability on a visual analogue scale and reasons for treatment withdrawal. The number of consultations was recorded.

Following surgery, the participants completed a question- naire selecting between 'I want to have imiquimod as first treatment' vs. 'I want to have surgery now' for 15 hypotheti- cal trial results ranging from 'imiquimod cures 100% of peo- ple; surgery cures 95% of people' to 'imiquimod cures 10% of people; surgery cures 95% of people'. The explanation

included that imiquimod treatment included surveillance and deferred surgery in the event of progression.

Whole-blood samples (40 mL) were harvested before and after imiquimod treatment and sent by first-class post to the Human Biomaterials Resource Centre, Birmingham, where peripheral blood mononuclear cells were isolated by differen- tial centrifugation and cryopreserved in the vapour phase of nitrogen. Circulating T-lymphocyte responses against melano- cyte differentiation antigens melan A, gp100 and tyrosinase, and against cancer testis antigens MAGE A1, MAGE A3 and NY-ESO-1 were measured using an ex vivo ELIspot assay,[24] using overlapping peptides covering the whole of each antigen.

Statistical design and analysis

In 2009 all consultant members of the British Association of Dermatologists were asked to mark on a visual analogue scale the pCR rate for imiquimod below which 'I do not think that imiquimod has any potential at all to be used for primary treatment for lentigo maligna' and the pCR rate above which 'I would be persuaded that imiquimod definitely should be used in the primary treatment of lentigo maligna'.

This was a single-arm phase II trial with a sample size of 33, requiring pCR in ≥ 25 participants to justify progression to phase III (A'Hern's method, p0 = 60%, p1 = 85%, a = 5%, 1 — b = 95%).[25] The bounds (p0 and p1) were derived from the upper quartile of pCR thresholds for derma- tologists responding to the two questions above. The recruit- ment target was 40 participants to account for attrition.

The primary intent-to-treat analysis included patients who discontinued imiquimod treatment early but proceeded to sur- gery as per the protocol requirements. The pCR rate for patients undergoing surgery after imiquimod was estimated with 95% confidence intervals (CIs).

The accuracy of clinical assessment was reported as the pro- portion of cases where cCR plus negative biopsies correctly predicted pCR in the subsequent surgical resection specimen.

Imiquimod dose intensity was calculated from patient diary returns, assuming 60 applications (5 days per week for 12 weeks) to be 100%.

Patients' opinions are presented descriptively as the propor- tion of patients preferring imiquimod over surgery in relation to a range of hypothetical cure rates for imiquimod compared with a cure rate for surgery of 95%. Analyses were performed using Stata v12 (StataCorp, College Station, TX, U.S.A.).

Results

Responses from 174 U.K. consultant dermatologists each iden- tified a lower pCR rate below which they considered imiqui- mod to have no potential to treat LM [median 40%, interquartile range (IQR) 30–60%] and an upper pCR rate above which they could definitely be persuaded of the poten- tial of imiquimod to treat LM (median 80%, IQR 60–85%). This was interpreted as indicating that only 25% of clinicians would reject further investigation of imiquimod as a treatment for LM even if the pCR rate was ≥ 60%, and only 25% of clinicians would demand a pCR rate ≥ 85% to justify further investigation of imiquimod. We determined that an observed pCR threshold rate of ≥ 25 out of 33 would reliably exclude a true pCR rate < 60% and be powered not to miss a true pCR rate ≥ 85% (see statistical design).

Twenty-nine patients consented; one withdrew consent and 28 were evaluable. Their median age was 72 years (IQR 65–79), 18 were male, the median size of LM was 14 mm (IQR 12–22; range 10–70) and lesions were located on the cheek (11), ear (four), forehead (four), nape of neck (one), nose (seven) and scalp (two). The median dose intensity over 12 weeks was 86·7%, including three patients stopping treat- ment early after 4, 8 and 11 weeks; 27 underwent surgical excision after imiquimod (Fig. 1).

Twenty-seven patients were evaluable for the primary out- come. Ten achieved pCR (37%, 95% CI 19–58%). None showed LM at the surgical margins. The patients with pCR had achieved imiquimod dose intensity below (seven) and above (three) the median of 86·7%.

Central review of a single pretreatment section did not con- firm the diagnosis of LM in three patients: one with epidermal hyperpigmentation without melanocyte atypia; one with com- pound melanocytic naevus and one with pigmentation and elongated rete ridges without melanocyte atypia. The pCR rate was eight of 24 (33%, 95% CI 16–55%) if these patients are excluded. However, priority was given to the fuller clinico- pathological diagnosis made by the multidisciplinary team at the site.

Post-treatment resection specimens from a further nine patients were scored as pPR (abnormal features falling short of defining persistent LM – see methods), seven had definite residual LM in the resection specimen and one had evidence of LMM.

Clinical evaluation showed that 13 of 28 patients had com- plete disappearance of the LM after imiquimod (i.e. cCR; 46%, 95% CI 28–66%). Of these, on the post-treatment biop- sies, 12 were negative for LM and one showed probable resid- ual LM. Thus clinical and targeted pathological evaluation yielded a response rate of 12 of 28 (43%, 95% CI 24–63%). Of these 12, one declined resection, and seven of the remain- ing 11 (64%, 95% CI 31–89%) had pCR on the resection specimen. Three of 15 who did not achieve cCR were observed nonetheless to have pCR (Fig. 2). Regarding the three with pathological evidence falling short of LM on central review of a single diagnostic slide, the outcomes were cCR + pCR, cCR + pPR and cPR + pCR.

Eleven of 29 patients (38%) had a severe local-site reaction over the study period; 10 (34%) had a moderate reaction and eight (28%) had mild or no reaction. This peaked at week 4, when 24%, 48% and 14% had mild, moderate and severe reactions, respectively. By week 12, 11% and 15% still had moderate or severe reactions, respectively. Nine of 19 patients (47%) having a moderate or severe local-site reaction had a pCR, whereas one of eight patients (13%) with mild or no

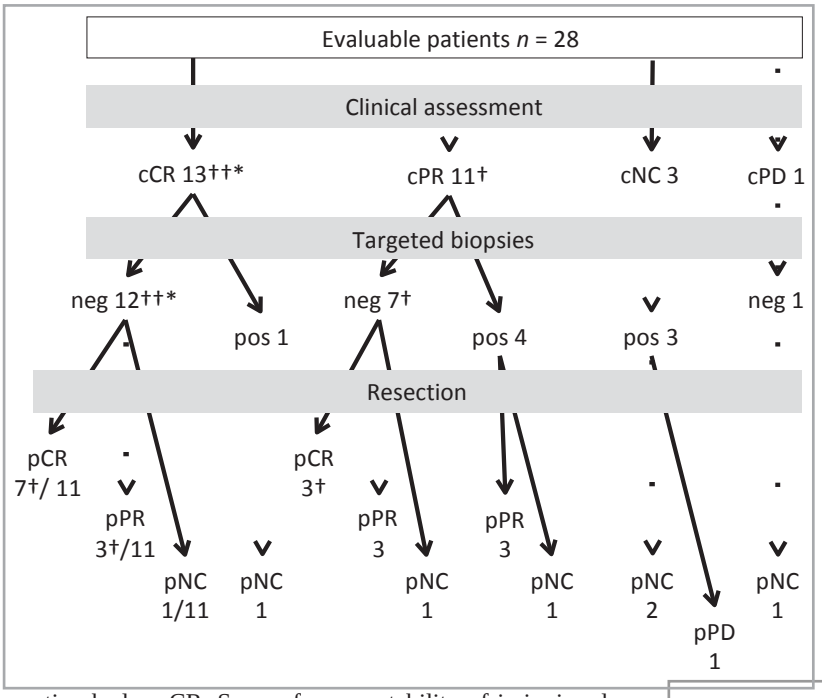

Fig 2. Clinical and pathological responses of lentigo maligna (LM) to treatment. cCR, clinical complete response; cPR, clinical partial response (i.e. clinical evidence of improvement in the LM falling short of complete regression); cNC, clinical no change; cPD, clinical progressive disease; pCR, pathological complete response; pPR, pathological partial response; pNC, pathological no change (i.e. continuing evidence of LM in specimen); pPD, development of LMM; neg, negative; pos, positive. †marks a patient whose local diagnosis of LM was not confirmed on central review of the pretreatment biopsy; *marks a patient who declined resection of LM after imiquimod.

reaction had a pCR. Scores for acceptability of imiquimod through 12 weeks of treatment were reported by 24 of 29 patients. Dose reductions occurred in five of 12 reporting con- sistently good tolerance and eight of 12 reporting variably poor tolerance.

There were 143 adverse events, of which 84 (59%) were definitely related to treatment. Medication was provided for 117 (82%) adverse events, and 140 (98%) resolved. There was no additional health service use for 18 of 29 (62%), one unscheduled visit for eight (28%) and more than one unscheduled visit for three patients (10%).

Sixteen patients completed the treatment preference survey having experienced both treatments (Fig. 3). One expressed a strong preference for immediate surgery even with a hypo- thetical cure rate of 100% for imiquimod, and four strongly preferred imiquimod, tolerating hypothetical cure rates for imiquimod ≤ 40% vs. 95% for surgery. Half of the patients stated they would opt for surgery if the cure rate for imiqui- mod was ≤ 85%.

Imiquimod might work by inducing immune responses against proteins characteristic of melanoma cells. We tested blood samples from 16 patients for such responses, of whom 11 had paired samples analysed about 3 months apart. Target proteins were a number of proteins characteristic of melanoma (melan A, gp100, tyrosinase, NY-ESO-1, MAGE A1 and MAGE A3) and, to confirm the patients' cells were working, proteins were made by common infections (termed CEPT). Positive recognition of target proteins was defined conventionally as a number of reacting immune cells against target proteins that was more than double the background immune reactivity.

Twenty-seven samples from 16 patients were tested, and of these, 18 from 12 patients made a clear positive recognition of

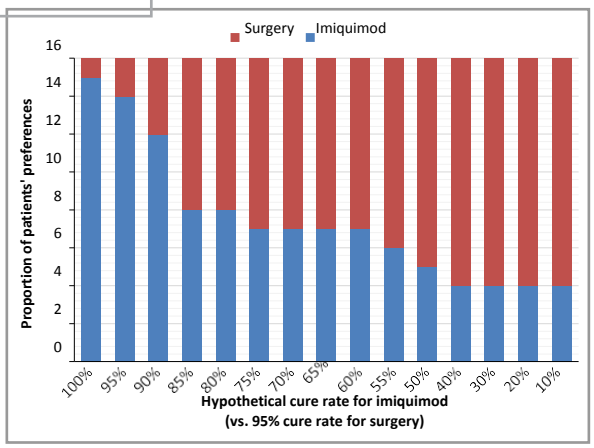

Fig 3. User Opinion Questionnaire. Bar chart depicting the number of patients (y-axis) reporting preference for either surgery (red) or imiquimod (blue) for varying hypothetical imiquimod cures rates (x-axis) vs. a fixed surgical cure rate of 95%. Patients were surveyed having experienced both treatments.

the CEPT control. Of the 18 samples with a positive CEPT response, three samples from two patients exhibited recognition of a melanoma antigen – both MAGE A1. Only six patients had paired samples with positive CEPT recognition on both samples, and of these, one of six (who had pPR and cPR) showed an amplified response over time, defined as the later recognition value being double that of the earlier reading.

Discussion

It is reasonable to try to spare patients surgery for LM; it can impair function and be disfiguring, and may not be feasible.

Nonsurgical approaches need not equal surgery in efficacy, provided treatment failure could be recognized and surgery undertaken before progression to invasive melanoma.

Imiquimod promotes an inflammatory state through the activation of macrophages and antigen-presenting cells via Toll-like receptor 7 signalling, and this localized inflammation can result in regression of neoplastic cells.[26] We undertook a single-arm phase II trial to determine whether investment is justified in a phase III trial comparing imiquimod with sur- gery. Clinical regression following imiquimod treatment can be followed by relapse.[3] Therefore, pCR, measured by detailed histological examination of LM resected after imiquimod ther- apy, was selected as the surrogate outcome measure for possi- ble long-term disease control.

The pCR rate of LM to imiquimod was estimated as 37% (10 of 27), with CIs indicating that a true pCR rate > 60% was unlikely and > 85% very unlikely. Even had accrual con- tinued to target, the highest possible observed pCR rate would have been 16 of 33, falling short of the preplanned efficacy threshold of 25 of 33.

It is improbable that we missed a true effect. Firstly, 27 of 29 patients completed the study and were available for analyses. Secondly, 21 of 29 patients had moderate or severe skin inflam- mation, similar to the imiquimod toxicity described in case reports of apparently successful imiquimod treatment. Thirdly, the median dose intensity was 87%, and reduced dose intensity across 12 weeks did not obviously associate with lower proba- bility of achieving pCR. Our pCR rate by detailed pathological examination is lower than the > 75% regression rate judged by clinical inspection and biopsies in a systemic review of pre- dominantly retrospective series and cases. Note that these case reports and series lacked consistent definition and were suscep- tible to selection and publication bias.[14] Recently, another trial observed a pCR rate of 20 of 38 assessable patients with LM treated with imiquimod for 12 weeks.[19] A further study com- pared topical imiquimod with topical imiquimod plus topical tazarotene, followed by Mohs excision of the treatment site, with pCR rates of 57 vs. 66%, respectively.[18]

Can we rationally offer imiquimod as first-line treatment for LM, reserving surgery for treatment failure? Persistent or progressive clinical abnormality after imiquimod might rea- sonably be taken to indicate proceeding to surgery, because only three of 15 such patients had pCR on the resected speci- men. Conversely, apparent cCR and negative biopsy was an unreliable predictor of pCR, with only seven of 11 cases with cCR plus negative biopsies confirmed as pCR on examination of the excision specimen. We recognize that pPR as defined in this study is indistinguishable from actinic melanocytic hyper- plasia, likely to be present in chronically subdamaged skin.[27] Thus there is uncertainty whether pPR induced by imiquimod might also be a marker for long-term clinical remission, which was not addressed in our study.

The efficacy threshold to justify phase III evaluation had been selected to exclude, at the 5% probability level, proceed- ing to phase III despite a true pCR rate of < 60% and rejecting further investigation despite a true pCR rate > 85%. These thresholds were based on a survey of a large group of U.K. dermatologists. However, reported opinions were diverse. Only half would definitely reject imiquimod treatment even if the true pCR rate was < 40%, and one-quarter would have settled for a true pCR rate < 60% definitely to proceed to phase III. Offering imiquimod treatment for a premalignant condition, with surgery reserved for progression, is a credible strategy for some clinicians despite a low pCR rate. How might patients view this issue? We surveyed the opinion of the first 16 consecutive patients after each had experienced both imiquimod and surgery, and again observed diversity of opinion: four of 16 would still have opted for imiquimod even with the probability of cure ≤ 40%. However, we had not expected the very high early attrition on accrual, in which only one-fifth of identified patients enrolled for the trial. This might cause bias favouring imiquimod because this attrition itself may reflect patients' preference for surgery.

Destroyed cells in a proinflammatory milieu might act as an autologous vaccination against melanoma, resulting in sys- temic immunity. However, we observed that our participants generally had a frequency of circulating activated T cells rec- ognizing melanoma differentiation or cancer germline anti- gens below the limit of detection of the ex vivo ELIspot assay (around 50 per 10^6 peripheral blood mononuclear cells) even after imiquimod treatment. A larger sample would be needed to estimate confidently the immune response rate, and this would also require assays able to detect and measure lower- abundance immune reactivities.

In summary, imiquimod causes local skin adverse reactions, which are variably tolerated by patients and can be managed by adjusting the frequency of applications. We estimated the pCR rate of LM to imiquimod to be 37%. This fell short of our predefined end point that would justify progression to a randomized comparison vs. surgery, assuming that pCR is a prerequisite for long-term disease control. The uncertainty over the interpretation of pPR and the possibility that imiqui- mod given for a longer duration might convert pPR to pCR might still justify such a trial. We observed that clearance of LM clinically and on targeted biopsies missed patients either in whom LM was pathologically persistent or in whom patho- logical persistence was, at best, uncertain. Based on this, with- out a larger long-term trial, the use of imiquimod cannot be recommended as standard first-line treatment for LM outside exceptional circumstances.

Acknowledgments

The Clinical Trials Pharmacy Team at Nottingham University Hospitals. We would like to thank our participants, without whom we would not have been able to complete this study.

References

1 Marsden JR, Newton-Bishop JA, Burrows L et al. Revised U.K. guidelines for the management of cutaneous melanoma 2010. Br J Dermatol 2010; 163:238–56.

2 Weinstock MA, Sober AJ. The risk of progression of lentigo maligna to lentigo maligna melanoma. Br J Dermatol 1987; 116:303–10.
3 Osborne JE, Hutchinson PE. A follow-up study to investigate the efficacy of initial treatment of lentigo maligna with surgical excision. Br J Plast Surg 2002; 55:611–15.
4 Preston PW, Matey P, Sanders DSA, Marsden JR. Surgical treatment of lentigo maligna using 2-mm excision margins. Br J Dermatol 2003; 149 (Suppl. 64):109–10.
5 Hou JL, Reed KB, Knudson RM et al. Five-year outcomes of wide excision and Mohs micrographic surgery for primary lentigo maligna in an academic practice cohort. Dermatol Surg 2015; 41:211–18.
6 Mahendran RM, Newton-Bishop JA. Survey of U.K. current practice in the treatment of lentigo maligna. Br J Dermatol 2001; 144:71–6.
7 Farshad A, Burg G, Panizzon R et al. A retrospective study of 150 patients with lentigo maligna and lentigo maligna melanoma and the efficacy of radiotherapy using Grenz or soft X-rays. Br J Dermatol 2002; 146:1042–6.
8 Orten SS, Waner M, Dinehart SM et al. Q-switched neodymium:yt- trium-aluminum-garnet laser treatment of lentigo maligna. Oto- laryngol Head Neck Surg 1999; 120:296–302.
9 Schmid-Wendtner MH, Brunner B, Konz B et al. Fractionated radiotherapy of lentigo maligna and lentigo maligna melanoma in 64 patients. J Am Acad Dermatol 2000; 43:477–82.
10 Tsang RW, Liu FF, Wells W et al. Lentigo maligna of the head and neck: results of treatment by radiotherapy. Arch Dermatol 1994; 130:1008–12.
11 Tzellos T, Kyrgidis A, Mocellin S et al. Interventions for melanoma in situ, including lentigo maligna. Cochrane Database Syst Rev 2014; 12: CD010308.
12 Ahmed I, Berth-Jones J. Imiquimod: a novel treatment for lentigo maligna. Br J Dermatol 2000; 143:843–5.
13 Swetter SM, Chen FW, Kim DD et al. Imiquimod 5% cream as pri- mary or adjuvant therapy for melanoma in situ, lentigo maligna type. J Am Acad Dermatol 2015; 72:1047–53.
14 Mora AN, Karia PS, Nguyen BM. A quantitative systematic review of the efficacy of imiquimod monotherapy for lentigo maligna and an analysis of factors that affect tumor clearance. J Am Acad Der- matol 2015; 73:205–12.
15 Mahoney MH, Joseph MG, Temple C. Topical imiquimod therapy for lentigo maligna. Ann Plast Surg 2008; 61:419–24.
16 Naylor MF, Crowson N, Kuwahara R et al. Treatment of lentigo maligna with topical imiquimod. Br J Dermatol 2003; 149 (Suppl. 66):66–70.
17 Powell AM, Robson AM, Russell-Jones R et al. Imiquimod and len- tigo maligna: a search for prognostic features in a clinicopathologi- cal study with long-term follow-up. Br J Dermatol 2009; 160:994–8.
18 Powell AM, Russell-Jones R, Barlow RJ. Topical imiquimod immunotherapy in the management of lentigo maligna. Clin Exp Dermatol 2004; 29:15–21.
19 Rajpar SF, Marsden JR. Imiquimod in the treatment of lentigo maligna. Br J Dermatol 2006; 155:653–6.
20 Spenny ML, Walford J, Werchniak AE et al. Lentigo maligna (melanoma in situ) treated with imiquimod cream 5%: 12 case reports. Cutis 2007; 79:149–52.
21 Ormond P, Blasdale C, Leonard N, Lawrence CM. Treatment of lentigo maligna with imiquimod. Br J Dermatol 2002; 147 (Suppl. 62):57.
22 Wolf IH, Cerroni L, Kodama K et al. Treatment of lentigo maligna (melanoma in situ) with the immune response modifier imiquimod. Arch Dermatol 2005; 141:510–14.
23 Fleming CJ, Bryden A, Evans RS et al. A pilot study of treatment of lentigo maligna with 5% imiquimod cream. Br J Dermatol 2004; 151:485–8.
24 Hui EP, Taylor GS, Jia H et al. Phase I trial of recombinant modified vaccinia ankara encoding Epstein-Barr viral tumor antigens in nasopharyngeal carcinoma patients. Cancer Res 2013; 73:1676–88.
25 Tan SH. Sample size software. In: Sample Size Tables for Clinical Studies (Machin D, Campbell MJ, Tan SB, Tan SH, eds), 3rd edn. Chich- ester: Wiley, 2009; 235.
26 De Giorgi V, Salvini C, Chiarugi A et al. In vivo characterization of the inflammatory infiltrate and apoptotic status in imiquimod-trea- ted basal cell carcinoma. Int J Dermatol 2009; 48:312–21.
27 Glass LF, Raziano RM, Clark GS et al. Rapid frozen section immunostaining of melanocytes by microphthalmia-associated transcription factor. Am J Dermatopathol 2010;32:319–25.

Appendix

Contributors. LIMIT-1 Collaborative Group. Nottingham Clini- cal Trials Unit Coordinating Team: N. Boota (trial manage- ment), D. Simpkins (data management), D. Whitham. U.K. Dermatology Clinical Trials Network: J. Chalmers. Chief inves- tigator: J. Marsden. Trial design: N. Steven, L. Billingham. Sta- tistical analysis: R. Fox, K. Wheatley. Trial steering committee:
C. Bunker (chair), S. Wharton (independent member), S. Brothwell (patient representative), L. Hague (patient represen- tative). Data monitoring committee: C. Harwood (chair), N. Ives (independent statistician), H. Ramsay (independent mem- ber). Active LIMIT-1 collaborators. Salisbury District Hospital (7): L. Burrows; Ninewells Hospital (6): C. Proby; University Hospital of Wales (5): R. Motley; Selly Oak Hospital (3): J. Marsden; Norfolk and Norwich University Hospital (3): N. Levell; Royal Cornwall Hospital (2): T. Lucke; Solihull Hospi- tal (2): I. Zaki; Monklands Hospital (1): G. Gupta. Histopathology: Professor M. Cook; L. Collucci and the histopathology team at the Royal Surrey County Hospital, Guildford. Translational substudy, NIHR Clinical Research Facility, Birmingham and Birmingham Experimental Cancer Medicine Centre: N. Steven, J. Williams, J. Steele. Sponsor: University Hospitals Birmingham NHS Foundation Trust.