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# *In situ* photoimmunotherapy: a tumour-directed treatment for melanoma

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## Summary

We report a new immunological treatment for advanced cutaneous melanoma which combines laser stimulation with topical application of a toll-like receptor agonist. This treatment, *in situ* photoimmunotherapy (ISPI), provides an alternative to traditional therapies for melanoma patients with cutaneous metastases. A 6-week cycle of ISPI is carried out on cutaneous metastases located in a designated 20 × 20 cm treatment area: 2 weeks of pretreatment with twice-daily topical applications of imiquimod (5% cream under plastic occlusion), with a laser treatment session at week 2 and again at week 4. Topical imiquimod is continued for the entire 6-week cycle. Two patients with late-stage melanoma were treated with ISPI. Patient 1 had the primary tumour and local metastases on the left arm, as well as metastatic tumours in the lungs [American Joint Committee on Cancer (AJCC) stage IV]. Patient 2 had a head and neck melanoma with multiple local metastases (AJCC stage IIIC), which had failed repeated attempts at surgical resection and high-dose radiation therapy. Patient 1 is now free of all clinically detectable tumours (including the lung metastases) >20 months after the first treatment cycle. Patient 2 has been free of any clinical evidence of the tumour for over 6 months. These two cases demonstrate that ISPI can clear local tumour and trigger beneficial systemic responses, with a side-effect profile that compares favourably with other treatments for advanced melanoma.

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None declared.

Although melanoma accounts for only 4% of skin cancer cases, it causes 79% of all skin cancer deaths. Patients with metastatic melanoma have a poor prognosis, and long-term survival is only about 5%.[1,2] Deaths in advanced disease occur because current treatment modalities are ineffective. Conventional therapies such as surgery and radiation therapy usually do not cure American Joint Committee on Cancer (AJCC) stage III or stage IV melanoma, while traditional chemotherapy is primarily palliative.

The development of cutaneous and subcutaneous metastases in advanced disease is generally associated with a poor prognosis. Patients with stage III disease and skin metastases have a worse prognosis than those with only lymph node metastases.[3,4] In patients with stage III disease, *in-transit* metastases are now recognized as a distinct form of regional disease with a worse prognosis.[1,5] Stage III melanomas with cutaneous involvement have a significantly worse 5-year survival (63% for IIIA vs. 27% for IIIC).[1] In addition, palliative control of widespread cutaneous metastases is difficult with standard techniques. We report a new immunological treatment which provides an alternative to traditional therapies for melanoma patients with cutaneous metastases, as well as the hope of improved long-term outcomes.

Evidence from experimental animal models has demonstrated that antitumour immunity can be enhanced significantly by a judicious combination of immunological stimulation and laser devitalization of tumour nodules *in situ*.[6–8] This knowledge combined with anecdotal cases in humans of cutaneous melanoma metastases responding to topical imiquimod[9–14] suggested that immunostimulation with a toll-like receptor agonist combined with the laser devitalization technique could produce clinically meaningful responses in patients with melanoma. This combination of techniques will subsequently be referred to as *in situ* photoimmunotherapy (ISPI). This

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approach to treating solid tumours is, in effect, a form of *in situ* autovaccination that is analogous to treatment with an autologous tumour vaccine.

## Materials and methods

Tumour devitalization with amelanotic metastases is achieved with an intralesional injection of an energy-absorbing dye (indocyanine green, ICG), followed by irradiation with a near-infrared 805-nm diode laser. A 6-week cycle of ISPI is carried out for each designated 20 × 20 cm treatment area: 2 weeks of pretreatment with twice daily topical applications of imiquimod (5% cream under plastic occlusion), with a laser treatment session at week 2 (1·0 W $cm^{-2}$, applied for 10 min per treated tumour), followed by a second laser treatment session 2 weeks later, assuming that target lesions are still present in the treatment area.

Following local administration of anaesthetic (lidocaine 1% with adrenaline), hypopigmented and nonpigmented melanoma nodules are injected with ICG (a solution of 0·25%) at a dose of 0·5 mL $cm^{-3}$, a few minutes prior to laser irradiation. Topical imiquimod is continued for the entire 6-week cycle except for two applications that are withheld on the night of the laser treatment sessions and during any rest periods that may be required by patient tolerance.

## Case reports

Two patients with late-stage melanoma were treated with ISPI. Patient 1 had the primary tumour and local metastases on the left arm, as well as metastatic tumours in the lungs (AJCC stage IV). Patient 2 had a head and neck melanoma with multiple local metastases (AJCC stage IIIC), which had failed repeated attempts at surgical resection and high-dose radiation therapy. Both patients treated with the ISPI regimen ultimately achieved complete clearance of all detectable tumours.

### Patient 1

A 64-year-old white woman with a nonpigmented birthmark on her left forearm noted a new lesion arising proximal to it in late February 2004. Biopsies of these lesions established the diagnosis of melanoma, presumably arising in a previously unrecognized amelanotic congenital naevus, and a satellite metastasis. Her initial staging chest X-ray was interpreted as within normal limits in late February 2004. No intracranial or intra-abdominal metastases were noted on the initial staging computed tomographic (CT) scans in early March 2004.

In April 2004, she was referred as a possible candidate for ISPI. On physical examination she had *in-transit* lymphatic metastases in the left arm (see Fig. 1a–c), and a satellite lesion adjacent to her primary site. There was also a 5- or 6-cm 'drinking straw'-like chord of metastatic deposits in her left arm proximal to the antecubital fossa on the medial side that probably represented a series of small nodular and diffuse infiltrates along a lymphatic channel. No ipsilateral axillary or other adenopathy was present.

Her largest metastasis in the left medial antecubital fossa was debulked surgically in May, a few weeks prior to ISPI (largest tumour in Fig. 1a). The smaller (< 1 cm diameter), more superficial *in-transit* metastases were left undisturbed, as they were the preferred targets for ISPI. A repeat chest X-ray in early June 2004 revealed two metastatic nodules, one in each lower lobe (Fig. 2a), confirming her status as a patient with stage IV melanoma.

Therapy consisted of 4 weeks of pretreatment with topical imiquimod (5% cream) applied twice daily under occlusion to a defined area (10 × 20 cm in size) that contained most of the easily observable cutaneous metastases. At the end of the first week of imiquimod treatment, an increase in inflammation was already evident in some of her *in-transit* metastases (see Fig. 1c). Other than intermittent itching, she experienced few symptoms during the initial period of imiquimod treatment.

Her first laser treatment occurred in late June 2004. The photograph in Figure 1(d) was taken just after completing laser treatment of her first three *in-transit* metastases. The two most proximal nodules received intralesional injection of ICG prior to laser treatment, while a third (the most distal lesion in the photograph) received only infrared laser treatment. She experienced some burning pain during the initial treatment of one of her nodules due to inadequate infiltration of lidocaine, an error that was corrected in subsequent treatments. Except on the nights of the laser treatment sessions, she continued to apply imiquimod cream twice daily under plastic wrap occlusion during the entire 6-week treatment cycle.

The two areas selected as targets for the second laser treatment session included an area of extensive tumour infiltration shown in Figure 1(b). This treatment site overlapped with one of the previous treatment sites. This diffuse area of subcutaneous involvement was chosen as it represented the largest mass of tumour still present in the arm. The other target for the second laser treatment session was a previously untreated nodule. Because one target site was treated twice, there were only four treatment-related ulcers present at the end of the ISPI (Fig. 1e), even though five areas were treated in the two laser treatment sessions.

Imiquimod induced all of the laser-treated areas to ulcerate. Almost all of the visible *in-transit* metastases showed marked inflammation and were shrinking, although areas of tumour not in the imiquimod-treated area showed less inflammation. The degree of inflammation seen by the end of the treatment cycle was impressive (see Fig. 1e), although the patient tolerated this remarkably well and complained mainly of occasional intense itching. Transient reactive adenopathy was intermittently present during the treatment cycle. As treatment progressed, it became more and more difficult to identify tumour in the left arm on physical examination. In particular, the large area of induration and infiltration of tumour into the subcutaneous tissues (Fig. 1b) softened and became indistinguishable from the normal subcutaneous tissue around it. The

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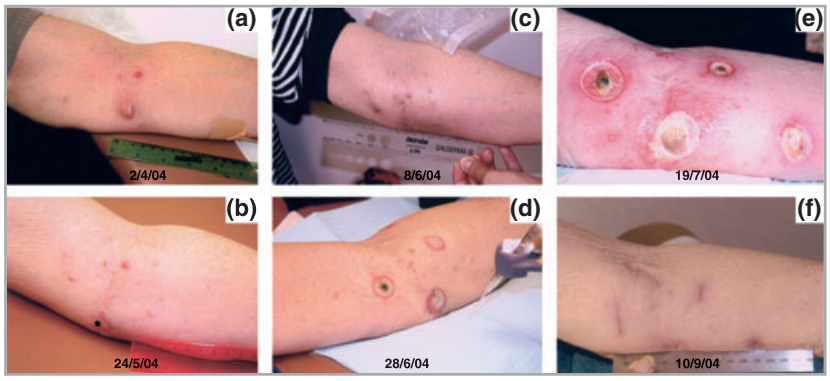


**Fig 1.** Treatment area of patient 1 during *in situ* photoimmunotherapy. (a) The target area on the left arm prior to treatment. (b) The largest *in-transit* metastasis has been removed surgically, and there is an area of diffuse tumour infiltration of the subcutaneous tissues and dermis. (c) The treatment area is shown after application of imiquimod cream under plastic wrap occlusion twice daily for 1 week. (d) The treatment area immediately after the first laser session. (e) The treatment area 1 week after the second laser treatment. (f) The treatment area 6 weeks after completion of the first treatment cycle.

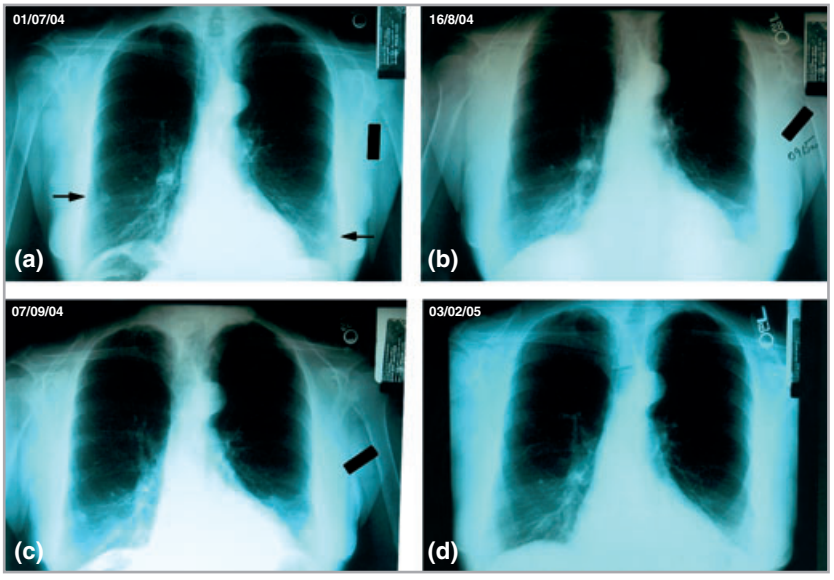


**Fig 2.** Posterior–anterior views of chest X-ray, patient 1. (a) Chest X-ray taken 3 days after the first laser treatment demonstrates a definite (approx. 1·2 cm) metastasis in the right lateral lung base and one (approx. 1·5 cm) near the left costophrenic angle (black arrows indicate locations of the metastases). (b) Three weeks after the second laser treatment, metastases have actually increased in size although their outlines are less distinct; there is a small pleural effusion in the left costophrenic angle. (c) Eight weeks after the second laser treatment, the metastases are clearly shrinking although they are still present both on plain films and in computed tomographic images. (d) Seven months after the second laser treatment, the plain films are back to baseline (no evidence of pulmonary metastases).

palpable, 'drinking straw'-like chord of tumour in the left arm also softened and disappeared during therapy.

The treatment-related ulcers healed rapidly over a few weeks following cessation of her treatment cycle. When she was re-staged in early September 2004, her reactive left axillary lymphadenopathy had resolved, and there was no longer any tumour in the left arm on physical examination (Fig. 1f). Three biopsies taken in areas where there were tumours previously showed no residual tumour, confirming the clinical examination.

Serial chest X-rays taken during and immediately after her treatment cycle showed two lung metastases (Fig. 2a, 3 days after the first laser treatment). Three weeks after the second laser treatment, these nodules had reached maximum size (Fig. 2b). By 7 weeks following completion of the treatment cycle, the nodules had begun to shrink (Fig. 2c). Restaging CT scans of the head, chest, abdomen and pelvis in early September 2004 revealed the presence of the two shrinking pulmonary nodules evident on plain films, but no evidence of disease elsewhere. A positron emission tomographic scan in

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mid-October 2004 showed no tumour in the lungs or elsewhere. In February 2005, 7 months after completing the treatment cycle, the lung nodules had been cleared completely (Fig. 2d) and she was considered free of all clinically detectable disease.

## Patient 2

A 66-year-old white man was diagnosed as having a melanoma on the left postauricular scalp in May 2004. During examination, he was also found to have a previously undiagnosed chronic lymphocytic leukaemia (CLL). The primary melanoma was removed surgically. By August 2004, a preauricular metastasis developed and was removed with a wide local excision. In September, a second metastasis was noted on the preauricular skin, also treated surgically with wide local excision and grafting. In October 2004, diffuse, smaller metastases developed in the graft site, and in other previously treated areas. His condition was then considered beyond surgery and he was treated with local radiotherapy. By December 2004 it was clear that radiotherapy was not controlling the disease, which continued to spread to contiguous areas. At that point he had a surgically unresectable stage IIIC melanoma with extensive radiation damage to the skin around the ear. In late December 2004 he was referred for ISPI.

Treatment began in early January 2005, and he responded to topical imiquimod with greater than expected irritation, probably due to the pre-existing radiation damage. While the irritation was uncomfortable, it did not interfere significantly with treatment, and he was able to complete the first cycle of therapy.

As it initially appeared that he had achieved a complete local response, post-treatment biopsies were carried out 4 weeks after completing the first treatment cycle. One of four post-treatment biopsies showed an area that histologically resembled an atypical actinic lentigo, which may have coincidentally been biopsied. However, as no lesion was noted clinically at the time of biopsy, and because of concern that this histologically bland pigmented lesion might in fact be persistent disease, a second treatment cycle was initiated which was limited to the immediate area of the positive biopsy.

Because the tumours in patient 2 were pigmented, ISPI was performed without injection of ICG. The treatment areas before and after ISPI are shown in Figure 3. Figure 3(a) shows dozens of small (1–2 mm) black metastatic nodules infiltrating the skin and subcutaneous tissues surrounding the left ear. Figure 3(b) shows the acute effects of the infrared laser treatment (note the treatment-related bulla which can be seen immediately following treatment). Figure 3(c) shows scars at the site of treatment-related ulcers that healed more slowly than normal after two treatment cycles. Four weeks following the second treatment cycle, he was restaged. No changes were noted from previous CT scans, indicating that he still had no detectable systemic disease.

## Histological studies

Histology from these two patients is shown in Figures 4 and 5. Figure 4(a,b) shows the appearance of the tumour in patient 1 prior to treatment. Diffuse masses of darkly staining malignant cells were seen in nodules (Fig. 4a), while strands of malignant cells were seen infiltrating other areas (Fig. 4b). Three months after therapy, previously involved areas showed only postinflammatory changes with residual scattered and perivascular mononuclear cell infiltrates (Fig. 4c,d). S100 staining of these sections was negative for tumour cells.

Figure 5 shows a similar histological pattern in patient 2. Pretreatment biopsies showed numerous large, pale-staining malignant cells associated with lymphocytic infiltrates (Fig. 5a). Two months after completion of the second treatment cycle, only diffuse mononuclear infiltrates were seen in previously involved areas (Fig. 5b). As in patient 1, special staining (Melan-A) of these sections was negative for tumour cells.

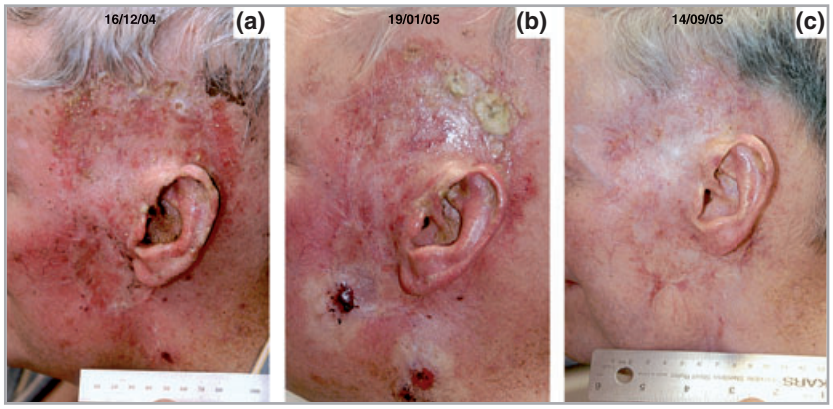


**Fig 3.** Photographs of *in situ* photoimmunotherapy (ISPI) treatment area of patient 2 with stage III melanoma around the left ear. (a) Before ISPI, showing extensive acute radiation damage and numerous small black metastases. (b) One week after the first laser treatment, a treatment-related bulla is present anterior to the earlobe. (c) Five months after completion of two cycles of ISPI, all treatment-related ulcers have healed and the subject is free of clinically and radiologically detectable tumours.

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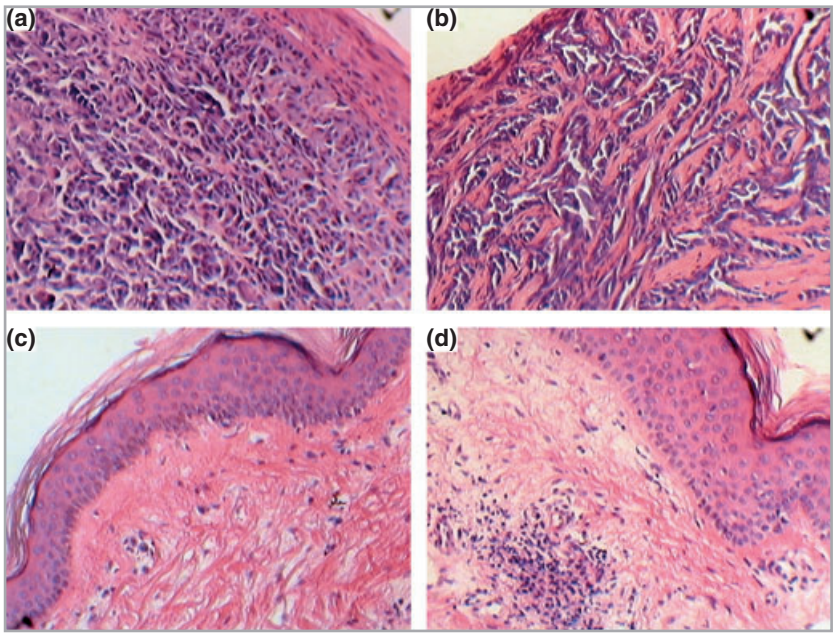


**Fig 4.** Histology from patient 1 (stage IV melanoma). (a, b) Before *in situ* photoimmunotherapy. (a) Appearance of the primary lesion, a 2·8-mm nodular melanoma which arose in a pre-existing congenital naevus. This lesion demonstrates packed masses of darkly staining atypical epithelioid cells. (b) A satellite metastasis, an ulcerated malignant neoplasm containing atypical epithelioid cells arranged in thick strands and linear aggregates. (c, d) Post-treatment sections. Areas previously involved with melanoma show chronic inflammation and fibrosis. S100 staining for melanoma (not shown) was negative.

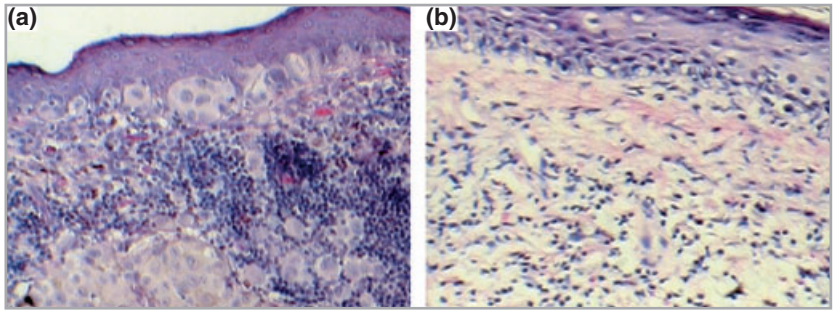


**Fig 5.** Histology from patient 2 (surgically unresectable stage III melanoma). (a) Appearance of the primary lesion of a 1·1-mm nodular melanoma before *in situ* photoimmunotherapy, with large atypical melanocytes with frequent mitotic figures extending singly and in clumps along the dermoepidermal junction and downwards into the dermis. (b) Post-treatment appearance of areas previously involved with melanoma, with chronic inflammation and fibrosis. Melan-A staining for melanoma (not shown) was also negative in these specimens.

## Discussion

These two cases demonstrate useful palliative responses to ISPI. The side-effects of ISPI compare favourably with those of other treatment modalities for advanced melanoma, such as intravenous cytotoxic chemotherapy, isolated limb perfusion, high-dose interferon or high-dose interleukin-2 therapy. The main side-effect appears to be intermittent itching in the treatment site, which can be intense. Pain has not been a treatment-limiting side-effect thus far. Nausea, which was not a problem in these treatments, can be a dose-limiting side-effect of intense topical imiquimod therapy.

Several things are remarkable about patient 2. Firstly, this patient responded to ISPI despite previous failures of standard treatments, including radiotherapy. Secondly, radiation therapy just prior to the start of ISPI did not prevent a useful response to ISPI. Thirdly, the mild immunosuppression from his CLL did not prevent a useful response. Fourthly, prior to ISPI, the progressive temporal pattern of recurrences with relapses or local extension of disease at least every 4–6 weeks suggested rapid progression with a very poor prognosis. During ISPI his tumour responded completely and rapidly, interrupting the pattern of rapid relapse seen with previous conventional therapies.

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The results seen in these subjects suggest that ISPI has a positive impact on the biology of the tumour and is not simply an ablative technique such as $CO_2$ laser. Based on our previous work in animals, and especially based on these human subjects, the treatment positively influences the course of the disease.

While it is arguable that these results may be due to chance, this approach warrants further investigation for the following reasons. Firstly, the infrared laser treatment of tumours as an approach to autologous vaccination therapy has shown consistent and repeatable success in animal models.[6–8] Secondly, this approach ensures that the tumour antigens involved in the 'vaccination' protocol are relevant, as they are in fact the patient's own tumour. Thirdly, there is abundant and growing evidence that toll-like receptor agonists are potent vaccine adjuvants.[15–21] It is therefore likely that the ISPI approach to autologous vaccine therapy significantly and beneficially impacts the immunobiology of the host–tumour relationship. This implies that the results obtained in these first cases are not simply accidental, and that a larger series would also show a high response rate in similar patients.

In summary, toll-like receptor agonists have established themselves as practical tools for treating human skin cancer. Topical imiquimod has demonstrated some degree of effectiveness in individual cases of advanced cutaneous melanoma, even when used as monotherapy. These two cases demonstrate: (i) that ISPI has a remarkable ability to clear local cutaneous and subcutaneous tumour deposits, (ii) that beneficial systemic responses can occur, and finally, (iii) that ISPI is reasonably well tolerated compared with other treatments for advanced melanoma.

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