Annals of Oncology 23: 1314–1319, 2012
doi:10.1093/annonc/mdr363
Published online 19 September 2011

An intra-patient placebo-controlled phase I trial to evaluate the safety and tolerability of intradermal IMM-101 in melanoma

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Received 7 April 2011; revised 23 June 2011; accepted 4 July 2011

Background: IMM-101 is a heat-killed innate and adaptive immune-activating mycobacterial product; a phase I study aimed to determine its safety and tolerability in individuals with melanoma.

Patients and methods: An intra-patient placebo-controlled study evaluated the safety and tolerability of three doses, namely, 0.1 (1 mg/ml), 0.5 (5 mg/ml) and 1.0 mg (10 mg/ml) of IMM-101 in stage III or IV melanoma. Each dose was administered in ascending order to one of the three cohorts.

Results: Based on observations from patients administered the 0.1-mg dose, it was considered appropriate to proceed with dosing the patients in the 0.5-mg dose cohort and then the 1.0-mg cohort ($n = 6$ per cohort). Treatment-emergent adverse events that would be considered typical of a post-vaccination state (including joint pains/aches, headaches and influenza-like symptoms) occurred at all dose levels, along with injection site reactions. These were mainly mild in intensity, resolved in a matter of days and responded well to supportive care. During post-study follow-up, two clinical responses (15%) were observed in patients with stage IV disease.

Conclusion: IMM-101 is safe and well tolerated and there is a rationale for studying IMM-101 at a nominal 1.0-mg dose to complement conventional cytotoxic therapy for patients with advanced cancer.

Key words: immunotherapy, IMM-101, melanoma, mycobacteria, phase I, trial,

introduction

There are a number of ways, in principle, whereby the power of the immune system can be harnessed to treat cancer [1, 2]. The finding that cancer regression can be achieved by immune rejection of tumour antigens theoretically allows the long-term control of neoplastic cells without toxicity to normal tissues [3–5].

The incidence of metastatic melanoma has increased over the past three decades, and the death rate continues to rise faster than the rate with most cancers [6]. The World Health Organization estimates that, worldwide, there are 66 000 deaths annually from skin cancer, with ~80% due to melanoma. The mortality rate from malignant melanoma has risen ~2% annually since 1960. The median survival of patients with melanoma who have distant metastases (American Joint Committee on Cancer stage IV) is <1 year. Enrolment in a clinical trial has become the standard of care for both early- and late-stage disease [7, 8]. Until recently, the only agents approved by the US Food and Drug Administration for the treatment of metastatic melanoma have been dacarbazine and interleukin (IL)-2, even though clear survival benefits have not been confirmed in randomised studies.

Recently, use of an antibody (ipilimumab) against the cytotoxic T lymphocyte-associated antigen 4 molecule, an inhibitory membrane protein that is expressed after T-cell activation, has shown improvements in overall survival and progression-free survival, with, to date, the best overall response rate benefit in melanoma [9–12], leading to its approval for the treatment of unresectable melanoma. Symptoms related to autoimmunity develop in some patients since host defences subjected to checkpoint blockade lose the ability to discriminate between self and nonself [13, 14]. Since tumours derive from self tissues, the appearance of immune-related adverse effects, such as colitis, hepatitis and hypophysitis, has been correlated with clinical response from ipilimumab therapy. These limitations indicate the need to develop alternative forms of immune-mediated therapy for melanoma and other strategies are currently being investigated [15–19].

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One strategy utilises IMM-101, a suspension of heat-killed whole cell *Mycobacterium obuense*, 1 of >100 named species within the genus *Mycobacterium*. IMM-101 is similar, in concept, to SRL172, a product containing heat-killed whole cell *Mycobacterium vaccae* that has been investigated widely in humans across many different disease areas and has been shown to modulate the immune response, with minimal toxicity, in a way that could be beneficial to cancer patients [20–23]. SRL172 has been administered i.d. to >2500 patients in clinical trials, including several oncology studies [24–26], and has been reported to be well tolerated. Anecdotally, some individuals with stage IV melanoma who volunteered for the original SRL172 studies are alive many years later. Although IMM-101 had not been administered to humans previously, the results of non-clinical *in vivo* studies and *ex vivo* studies using human blood supported the hypothesis that IMM-101 would elicit very similar responses to *M. vaccae*-derived products. Therefore, a first-in-human, placebo-controlled, dose escalation trial was undertaken to evaluate the safety and tolerability of three doses of IMM-101 (0.1, 0.5 and 1.0 mg) administered i.d. as a 0.1-ml volume of 1.0, 5.0 or 10.0 mg/ml suspensions, respectively, to individuals with melanoma.

patients and methods

Eligible patients had stage III or IV melanoma without other ongoing therapy for at least 30 days before screening. The primary objective of the study was to evaluate the safety and tolerability of IMM-101, when administered as a course of three i.d. injections over a 4-week period. The secondary objectives were to (i) investigate local injection site reactions to IMM-101 when administered i.d. to melanoma patients and (ii) compare the local tolerability of i.d. injection of IMM-101 with that of i.d. injection of a placebo control; appropriate ethics committee approval was obtained in accordance with the Declaration of Helsinki.

Each dose was administered in ascending order to one of the three cohorts. Once the consent form had been signed and eligibility had been confirmed, each patient received a placebo injection of borate buffered saline solution (day −3) to provide an intra-patient placebo control and to allow the patient to practise completion of the diary. Patients who were willing and able to proceed with the study were injected with a single dose level of IMM-101 on three subsequent occasions. Doses of IMM-101 were administered over a 4-week period on days 0, 14 and 28 (with up to 2 days variation in the dosing interval). A standard volume of 0.1 ml of suspensions containing IMM-101 at the concentrations 1, 5 and 10 mg/ml was injected. At days 0, 3, 14, 28 and 42, local tolerability was assessed by standardised techniques (measurements at injection site) by a study physician or research nurse (supplemental Figure S1, available at *Annals of Oncology* online). The intensity of each injection site reaction was scored with reference to a Vaccine Toxicology Rating Scale (supplemental Tables, available at *Annals of Oncology* online). Routine safety assessments by means of haematological and biochemical blood tests, urinalysis, electrocardiograms and physical examinations were carried out on each patient at screening and at days 3, 14, 28 and 42.

An initial placebo injection was included in the study to assess whether patients were capable of measuring their own injection site reactions accurately. If any patient had recorded a large site reaction after the placebo injection, this would have indicated a difficulty with patient-recorded measurements. The use of a placebo was also intended to demonstrate that patients were not hypersensitive to the borate buffered saline used in the formulation of IMM-101.

The planned sample size of 18 assessable patients (6 patients at each dose level) was considered appropriate for a study of this type. No formal power calculation was deemed necessary as no formal hypothesis testing was planned.

results

patient characteristics

From March to July 2010, a total of 24 individuals were screened, 19 were randomly allocated to the study and 18 completed treatment (Table 1). Their baseline characteristics were similar for the three cohorts. Thirteen (68%) individuals had stage IV melanoma, three (16%) stage IIIc and three (16%) stage IIIb. The median time since initial diagnosis of melanoma was 4.12 years (range 0.4–37.5 years). Fourteen (74%) patients had had a regional lymph node resection with the mean time since resection of 4.13 years (range 0.2–11.4 years). The mean thickness of the primary lesion was 2.81 mm with a range of 1.5–5.1 mm. Twelve (63%) patients had lymph node metastases, two (11%) had none and five (26%) had no recorded details. All individuals were White/Caucasian.

There was nothing remarkable in the vital signs at screening for any of the patients. Examination of demographic and other baseline data did not reveal any clinically relevant differences between the treatment groups and the three cohorts were considered to be well matched. Eighteen of the 19 patients in the safety population received all three doses of the study medication IMM-101. One patient who was withdrawn from the study due to a protocol violation (pre-existing brain metastases) received two doses at the initial 0.5-mg dose.

local toxic effects

As predicted by the extensive previous clinical experience with *M. vaccae*, the most frequently reported treatment-related adverse events (AEs) were in the 'General disorders and administration site conditions' category. For five of the six patients in the IMM-101 0.1-mg dose group, administration site reactions were reported as AEs. Administration site reactions were reported as AEs for all patients in the IMM-101 0.5-mg and 1.0-mg dose groups. As expected, there was a dose-related increase in the total number of mild and moderate administration site reactions.

It should be noted that some patients reported more than one AE per injection site, e.g. tenderness, pain, itching and discharge were all reported as separate events. Only one patient (in the highest dose group) had a reaction that required treatment, namely, application of povidone-iodine ointment. No injection site reaction was reported as a serious adverse event (SAE) by the investigator and no patient withdrew due to an intolerable injection site reaction.

Based on patients' diary cards, 7 of the 19 patients (36.8%) reported a reaction at the administration site within 24 h of receiving the placebo injection (borate buffered saline; area 4.36 ± 1.1 mm^2). Four reactions to placebo were recorded by patients in the IMM-101 0.5-mg dose group and three in the IMM-101 0.1-mg dose group. None were seen in the 1.0-mg dose group. These reactions are considered most likely to have been due to injection technique and therefore not observed at

the higher dose level that was conducted later, as staff gained injection proficiency.

Every patient reported a reaction in the left upper deltoid region following administration of the first dose of IMM-101. Based on the data reported in the patients' diary cards, 12 of these reactions occurred on the same day as the injection, the other 7 occurred on the day after the injection.

The size of the reaction developed by each patient was followed over the course of the study (visit 4/day 3, visit 5/day 14, visit 7/day 28 and visit 9/day 42). Based on the available data recorded at site visits, regardless of the dose administered, the size of the skin reaction elicited by the first administration of IMM-101 appeared to resolve over time in all patients (Figure 1). Based on the measurements obtained at the time of the visits and, with few exceptions (in particular one patient in the group receiving 0.1 mg), there appeared to be a positive correlation between size of the reaction and the dose received (Figure 2). Patients receiving larger doses tended to develop the larger reactions. Moreover, regardless of dose administered and regardless of subsequent administrations of IMM-101 (second and third dose, visit 5/day 14 and visit 7/day 28, respectively), based on the data collected at the site visit, all reactions elicited by the first IMM-101 dose appeared to resolve over the course of the study and not to undergo exacerbation upon subsequent injection. The last day of observation (visit 9/day 42) permitted an overview of each skin site reaction that had developed after each administration. Overall, there appeared to be a trend for resolution over time, with the earlier and older skin reactions appearing reduced in size compared with the more recent ones (Figure 3). Furthermore, from the available data collected at the time of the site visits, it appears that previous administrations of IMM-101 did not seem to cause larger skin reactions upon

Table 1. Patient characteristics

Variable	Total patients	IMM-101, 0.1 mg	IMM-101, 0.5 mg	IMM-101, 1.0 mg
Total patients	19	6	7	6
Age, median (range)	59 (26–79)	47 (26–69)	68 (32–79)	63 (31–78)
Sex				
Male	11	4	4	3
Female	8	2	3	3
Stage of disease				
Stage IIIb	3 (15.8%)	1 (16.7%)	1 (14.3%)	1 (16.7%)
Stage IIIc	3 (15.8%)	1 (16.7%)	0 (0.0%)	2 (33.3%)
Stage IV	13 (68.4%)	4 (66.7%)	6 (85.7%)	3 (50.0%)

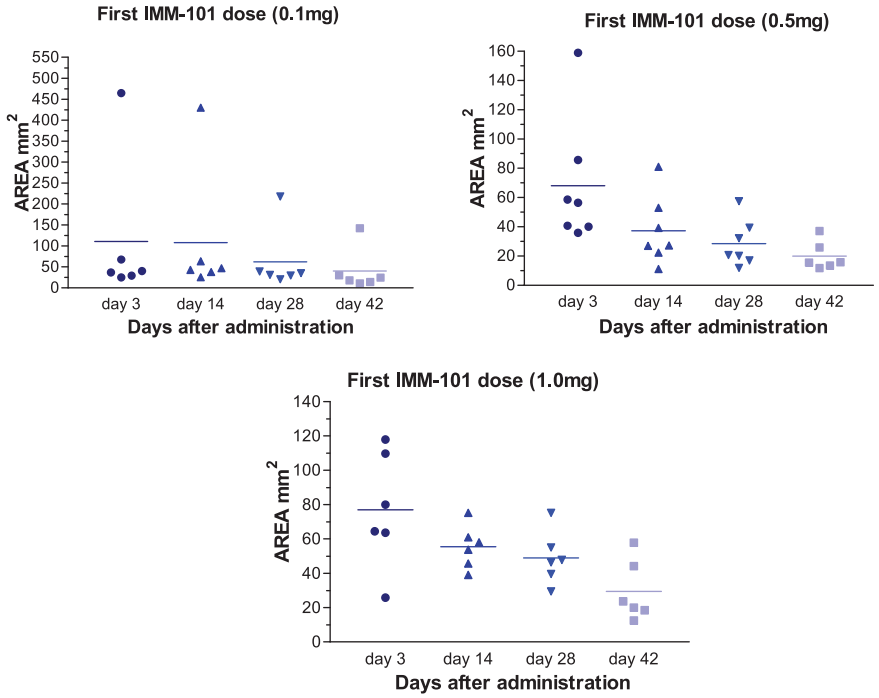

Figure 1. Injection site reactions following first administration of IMM-101. Individual area measurements for each patient and median for the group for all injection site reactions developed following the first administration of IMM-101 (day 0) by each patient recruited in the study. The size of the reactions was followed over time over the course of the study, and data relative to lesions that were 3, 14, 28 and 42 days old are shown. In all cases, the size of the reactions decreased as the reaction resolved over time.

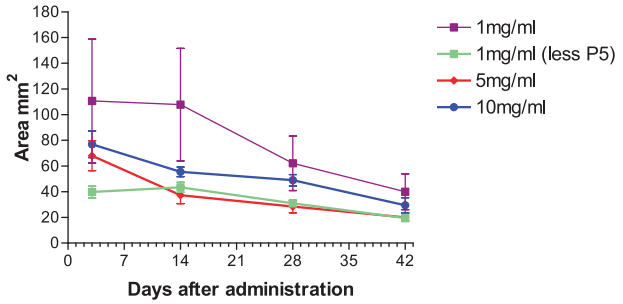

Figure 2. Development of injection site reactions following the first administration of IMM-101. Area measurements (mean ± standard error of the mean) for all injection site reactions developed following the first administration of IMM-101 (day 0) in each group of patients receiving the three strengths of IMM-101 (1, 5 and 10 mg/ml). The size of the reactions was followed over time over the course of the study, and data relative to lesions that were 3, 14, 28 and 42 days old are shown. In all cases, the size of the reactions decreased as the reaction resolved over time. Data for the group receiving IMM-101 1.0 mg/ml are presented in its entirety or without one patient (P5) who developed a large reaction following IMM-101 administration.

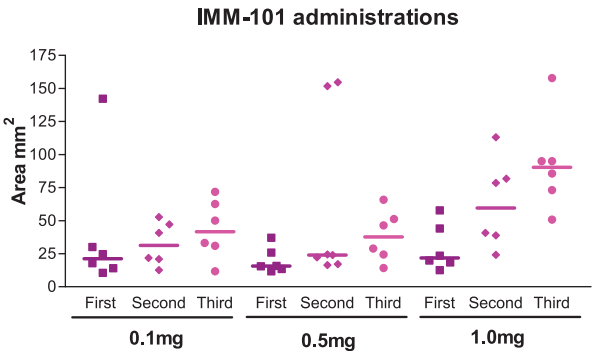

Figure 3. IMM-101 injection site reactions at the end of the study (day 42). Individual area measurements for each patient recruited in the study and median for the group for all injection site reactions developed following administrations of IMM-101 as observed on the day of the last site visit (day 42/visit 9). On day 42, reactions to the first IMM-101 dose were 42 days old, reactions to the second dose were 28 days old and reactions to the third dose were 14 days old. The size of the reactions appeared to decrease over time with older lesions smaller than more recent ones.

subsequent administrations. For example, the size of the reaction developed following the third administration of IMM-101 did not appear to be remarkably different in size from the one developed following the first or second administration, suggesting that over time repeated administration does not predispose the patient towards larger or more intense reactions after subsequent administrations (Figure 3).

systemic toxic effects

There were no dose-limiting toxic effects observed and no evidence to suggest a clinically significant impact upon haematological indices, biochemical parameters or cardiac function. All documented changes in vital signs from screening to the end of the study were unremarkable. There were no treatment-emergent AEs reported in the period following the placebo injection and before the first dose of IMM-101, thus allowing a clear definition of the tolerability of the test product.

Treatment-emergent AEs that would be accepted as typical of a post-vaccination state occurred at all dose levels. These were mainly mild in intensity and mostly resolved in a matter of days, responding well to simple supportive medication such as paracetamol (Table 2). This is entirely consistent with the pooled information available on *M. vaccae* and was generally less intense than symptoms typically observed following Bacille Calmette–Guérin (BCG) vaccination of tuberculin-negative individuals. Only one SAE was reported: the patient was hospitalised and found to have progressive disease, confirmed by the investigator and medical monitor to be unrelated to IMM-101.

As expected, the majority (94.6%) of the treatment-related AEs related to the 'General disorders and administration site conditions' category. There was a dose–response relationship with respect to this category of event (9, 15 and 29 events in the low-, mid- and high-dose groups, respectively) reflecting a normal and expected local response to the injection of a mycobacterial preparation (see supplemental Tables, available at *Annals of Oncology* online).

There was one report of nausea in the 0.1-mg dose group that was considered possibly related to IMM-101 and two reports of headache (one mild, one moderate) that were considered possibly related to IMM-101: one in the 0.1-mg dose group and one in the 1.0-mg dose group. There were two reports of mild pyrexia that were considered probably related to IMM-101: one in each of the 0.5-mg and 1.0-mg dose groups. There was one report of mild fever (pyrexia) in the 0.5-mg dose group that was also considered probably related to IMM-101. One patient in the 1.0-mg dose group reported flushing, a further patient reported redness of the face, three patients in the 0.5-mg group reported joint pain and one patient reported 'severe itching at the left lower deltoid injection site'. All resolved without treatment or with paracetamol.

Although not a formal study of clinical responses, there was no indication that IMM-101 led to a worsening of the tumour status. As a group, the 13 patients with stage IV disease, especially those previously extensively treated, have survived longer than would otherwise have been expected. Two stage IV patients had progressive disease (one died shortly after the study was completed and one only received two doses in the 0.5-mg cohort), nine had stable disease over the course of 6 months and two had objective partial responses (one in the lungs, one in cutaneous disease). Of the six individuals with stage III disease, one progressed and the remainder were stable. In aggregate, IMM-101 does not appear to exacerbate or 'enhance' tumour activity.

discussion

The purpose of this study was to evaluate the safety and tolerability of three escalating doses of heat-killed whole cell *M. obuense* when administered i.d. to melanoma patients. Additionally, it aimed to characterise local responses to IMM-101 using standardised nomenclature and toxicology criteria. The methods employed in this study help to delineate inappropriate/unacceptable local reactions from those indicative of a beneficial immunological response in future

Table 2. Summary of treatment-related adverse events by body system

Description	IMM-101, 0.1 mg ($N = 6$)		IMM-101, 0.5 mg ($N = 7$)		IMM-101, 1.0 mg ($N = 6$)		Overall ($N = 19$)	
	No. of patients reporting	No. of reports	No. of patients reporting	No. of reports	No. of patients reporting	No. of reports	No. of patients reporting	No. of reports
Any	4	11	6	15	6	30	16	56
	66.7%	11	85.7%	15	100%	30	84.2%	56
Gastrointestinal disorders	1	1	0	0	0	0	1	1
	16.7%	9.1%	0.0%	0.0%	0.0%	0.0%	5.3%	1.8%
General disorders and administration site conditions	2	9	6	15	6	29	14	53
	33.3%	81.8%	85.7%	100%	100%	96.7%	73.7%	94.6%
Nervous system disorders	1	1	0	0	1	1	2	2
	16.7%	9.1%	0.0%	0.0%	16.7%	3.3%	10.5%	3.6%

studies of IMM-101 in cancer patients. IMM-101 was demonstrated to be safe and well tolerated at the doses given, with no evidence to suggest a clinically significant impact upon haematological and biochemical indices or cardiac function. Treatment-emergent AEs that would be accepted as typical of a post-vaccination state occurred at all dose levels. These were mainly mild in intensity and mostly resolved in a matter of days, responding well to simple supportive medication. This is entirely consistent with the pooled information available on *M. vaccae* and was generally less intense than symptoms typically observed following BCG vaccination of tuberculin-negative individuals. Only one SAE was reported; the patient was hospitalised and found to have progressive disease.

The injection site reactions were consistent with this class of product and well tolerated by the patients. Local skin reactions should be viewed as a normal and predicted reaction to exposure to a preparation of mycobacterial antigens and a representation of desirable immunological activity. However, there is likely to be a point at which an injection site reaction may become unacceptable to the patient and may require discontinuation of treatment or dose reduction. The injection site reactions resolved over time, with the earlier and older skin reactions appearing reduced in size compared with the more recent ones at the end of the study. Furthermore, it appears that repeated administration does not predispose the patient towards larger or more intense reactions following subsequent administrations.

The survival time of those patients with stage IV melanoma, even allowing for the two individuals who died after the study completion, is longer than expected, especially for patients with extensively pretreated disease, and 2 of 13 patients (15%) showed evidence of partial responses, although we would caution against over-interpretation of such data.

The rationale to use IMM-101 at a nominal 1.0-mg dose (e.g. 0.1 ml of a 10.0 mg/ml suspension) to complement conventional cytotoxic therapy for patients with advanced cancer is sound and warrants further exploration. This is supported by the evidence from this phase I study, which shows safety and tolerability in a multiple-dose schedule.

This immunotherapy is thought to activate both the innate and adaptive immune systems (Figure 4), in response to the effect of the cancer itself leading to dysregulated responses. *Mycobacterium vaccae*, which is closely related to the

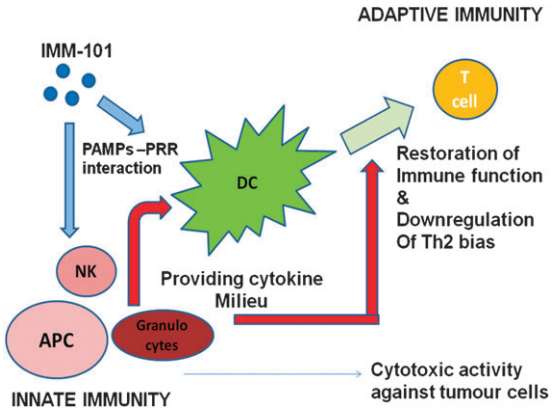

Figure 4. IMM-101 mechanism of action. The proposed mode of action of IMM-101 is based on published experimental models and clinical data with a related heat-killed whole cell mycobacterial preparation, *Mycobacterium vaccae*. It is now widely agreed that cancer is associated with dysregulation of the immune system that may be reflected in depressed innate immune responses, impaired activation of antigen-presenting cells (APCs) such as macrophages and DCs and subdued adaptive immunity. Hence, immunotherapeutic interventions aimed at restoring these immune functions may benefit cancer patients. Because of its very nature, heat-killed whole cell *M. vaccae* acts on both cells of the innate immune system such as natural killer cells and granulocytes, and on APCs, through interaction with a number of receptors. Activation of these cells that are known to have cytotoxic activity against tumour cells may indeed be beneficial to patients. Furthermore, the effects on DC may influence the development of adaptive immunity and effective immune responses. In murine models, treatment with *M. vaccae* was shown to affect the cytokine production potential of putative CD11+ DCs *in vivo*.

mycobacterial preparation used here, is recognised by toll-like receptor 2 (TLR2), but in contrast to other TLRs ligands, it induces specific maturation of monocyte-derived dendritic cells (DCs), which when placed in culture with naive CD4+ T cells is associated with inhibition of IL-4 and induction of CD25+FoxP3+ cells [27]. This finding is of particular significance in advanced cancer that has been associated with a T_h2 bias. *Mycobacterium vaccae* priming of DC promoting Tregs induction may result in the down-regulation of T_h2 responses (as seen when this preparation is used to treat allergic conditions) and a restoration of T_h1 responses when these have

been suppressed (as observed when this preparation is used to treat or prevent overt tuberculosis [28]). Both are likely to be of benefit in the cancer setting.

The burgeoning field of immunotherapy for melanoma has important implications for clinicians and for the novel paradigms of treatment and response assessment that immunotherapies will promote [29–32]. The unique side-effect profile for immune-mediated drugs will be a challenge but new skills for dealing with them in practice will be learnt. It appears, however, that IMM-101 will be unlikely to generate any grade III or IV systemic toxic effects. Overall, the safety of IMM-101 justifies its evaluation in clinical trials but will require different assessment criteria than those applied to conventional chemotherapy studies as responses to immunotherapy may take longer to manifest and may not occur until after a period of progression of the disease. In subsequent studies, the concept that physicians might see late regression or progression followed by regression is likely to cause a sea change in the way patients are treated using immunotherapy. Furthermore, a desirable aim of immunotherapy is not necessarily to totally eradicate the tumour but to revert from a state of escape to one of equilibrium [33–35] so that the patient and the stable tumour coexist.

acknowledgements

We are grateful to the patients who were kind enough to enter into the study and the research nurses and junior doctors who assisted in this trial. This trial is registered at clinicaltrials.gov identifier: NCT01308762.

funding

Immodulon Therapeutics Ltd.

disclosures

CG, GW and LRB are paid consultants to Immodulon Therapeutics Ltd, developers of IMM-101. JG and SM are directors and hold stock in Immodulon Therapeutics Ltd. JS, AD, AGM and AM have no conflict of interest to declare.

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