BJD
British Journal of Dermatology

A phase I clinical trial demonstrates that nfP2X$_7$-targeted antibodies provide a novel, safe and tolerable topical therapy for basal cell carcinoma

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Accepted for publication
25 January 2017

Funding sources
This study was supported in full by the Biosceptre group of companies. Employees of the Biosceptre group of companies were involved in the design and conduct of the study; collection, management, analysis and interpretation of the data; preparation, review and approval of the manuscript, and decision to submit the manuscript for publication.

Conflicts of interest
A.G.B., J.A.B., L.C.T., J.K. and S.M.G are employees of Biosceptre (Australia) Pty Ltd and Biosceptre (U.K.) Limited. S.M.G. received payment from the Biosceptre group of companies for his involvement in this trial.

DOI 10.1111/bjd.15364

Summary

Background Expression of P2X$_7$, an ATP-gated calcium channel, increases cancer cell proliferation and invasiveness. A variant of P2X$_7$ (termed nfP2X$_7$), in which a normally hidden epitope (E200) is exposed for antibody binding, is observed in a variety of different cancers.
Objectives To investigate the safety, tolerability and pharmacokinetics and assess indicative efficacy of a novel antibody ointment as a therapeutic for basal cell carcinoma (BCC).
Methods An open-label, phase I clinical trial was undertaken at three dermatology clinics to evaluate the safety and tolerability of topical administration of an ointment containing 10% sheep polyclonal anti-nfP2X$_7$ antibodies (BIL010t) to primary BCC lesions twice daily for 28 days. Twenty-one patients with primary BCC lesions at least 0·5 cm^2 in area and less than 2·0 cm in diameter were enrolled. The primary end points were safety, tolerability and pharmacokinetics. Change in lesion size after treatment was determined and histology was performed on pretreatment and end-of-treatment (EOT) biopsies.
Results Compliance was very high, with treatment being well tolerated. The most common adverse events were treatment site erythema, pruritus, dryness and pain. There was no evidence of systemic penetration of the sheep antibody. Lesions were measured prior to and after 28 days of treatment, with 65% of patients showing a reduction in lesion area, 20% showing no change and 15% showing an increase. Histopathology of post-treatment excision of lesion sites showed eight patients with stable disease, nine with partial response and three with complete response.
Conclusions Antibodies against nfP2X$_7$ (BIL010t) provide a novel, safe and well-tolerated treatment for BCC.

What's already known about this topic?

- nfP2X$_7$ is a form of the ATP-gated P2X$_7$ receptor in which the E200 epitope is exposed for antibody binding.
- nfP2X$_7$ is highly expressed in several cancers, including melanoma.

What does this study add?

- A mouse model of melanoma is used to demonstrate that topical application of anti-nfP2X$_7$ antibodies is effective in reducing lesion size.
- Expression of nfP2X$_7$ in basal cell carcinoma is demonstrated by immunohistochemistry.

- A phase I clinical trial demonstrates that topical application of an ointment containing anti-nfP2X$_7$ antibodies to basal cell carcinomas is well tolerated and provides early indications of efficacy.

Nonmelanoma skin cancer (NMSC) is extremely common. The lifetime risk of developing NMSC is estimated to be one in five, with the majority of cases being basal cell carcinoma (BCC).[1] Incidence of BCC increases with age, with the main risk factor being ultraviolet exposure.[2] Patients with a diagnosed BCC are at significantly higher risk of developing subsequent lesions.[3,4] Most BCC lesions are successfully treated with surgery. Therapeutic options for BCC lesions with a low risk of recurrence include topical treatment with 5-fluorouracil or imiquimod and localized destructive methods such as cryotherapy, electrodesiccation and curettage.[2] Mohs surgery may be used for more difficult-to-treat recurrent BCC, BCC with high-risk histological features or BCC in high-risk locations, including the mask area of the face, hands, feet and genitalia. However, in some lesions all surgical options are contraindicated or highly invasive and these require new, improved therapeutic approaches.[5]

P2X$_7$ is a trimeric receptor that responds to high concentrations of ATP (EC_{50} 800 μmol L^{-1}).[6,7] In response to brief activation with ATP, P2X$_7$ forms a nonselective cation channel allowing Ca^{2+} and Na^{+} influx into the cell and K^{+} efflux from the cell.[7] Sustained activation causes the opening of a large pore permeable to molecules of up to 900 Da in size, which can result in apoptosis.[8] In normal tissue, the extracellular concentration of ATP is in the nmol L^{-1} range, well below that needed to activate P2X$_7$.[9] However, in the tumour microenvironment, ATP concentrations in the hundreds of μmol L^{-1} range are observed.[9] This has the potential to induce large pore formation in cells expressing P2X$_7$, which can lead to cell death.

The potential to activate P2X$_7$ large pore formation, caused by the high level of ATP found in tumours, suggests that fully functional P2X$_7$ receptors would induce cell death. However, expression of the P2X$_7$ receptor has been shown to increase the growth of tumour cells both *in vitro* and *in vivo*.[10–12] This apparent anomaly may be due to the expression of altered forms of the P2X$_7$ receptor, in which pore function is attenuated while other signalling functionality is conserved or enhanced. A number of studies have demonstrated that various forms of the P2X$_7$ receptor possess an attenuated pore function. This can occur as a consequence of modified receptor glycosylation status,[13] expression of variant SNPs,[14–16] production of different splice variants[17–19] or by binding of proteins associated with the P2X$_7$ signalling complex.[20]

An epitope termed E200 that is associated with nonfunctional forms of the P2X$_7$ receptors has been identified.[21–23] The E200 epitope is exposed on P2X$_7$ on the surface of cancer cells but not on normal, noncancerous cells. This form of the receptor, termed nfP2X$_7$, can be specifically targeted with antibodies raised against the exposed E200 sequence. The nfP2X$_7$ receptor has been shown to be upregulated in multiple cancers including breast, prostate and melanoma.[24–27] Here, sheep polyclonal antibodies raised against the E200 epitope of nfP2X$_7$ have been investigated as a topical treatment for skin cancers with a focus on BCC.

Materials and methods

Preclinical mouse model

An efficacy study, to evaluate the active pharmaceutical ingredient (API) in a mouse melanoma model, was approved by the University of Sydney Animal Ethics Committee. C57BL/6 mice were injected with 1×10^6 B16F10 mouse melanoma cells on day 0 and randomized into four groups (10 mice per group). Group 1 mice received no treatment while the mice in the other groups received treatment with one laser pulse (Epiture Easytouch, Norwood Abbey, Frankston, Australia) set to 480 V (1·1 J cm^{-2}) on days 5, 9, 13 and 16 to remove stratum corneum. Mice in group 2 received laser treatment only. Mice in groups 3 and 4 were treated with laser and topical administration of a polyethylene glycol (PEG)-based ointment containing protein G purified preimmune sheep IgG or protein G purified anti-E200 sheep IgG at 50 mg IgG per gram of ointment. Tumours were measured with a calliper gauge 5 days after injection and subsequently every 2 days until day 19.

Production of sheep polyclonal anti-E200 antibody and BIL010t

Anti-nfP2X$_7$ antibody was manufactured by repeatedly immunizing sheep with the P2X$_7$ E200 sequence (GHNYTTRNILPGLNITC)[21,22] conjugated to keyhole limpet haemocyanin followed by the collection of sera and purification of IgG as the API. The API used in this clinical trial was purified from sera using the mercaptoethylpyridine HyperCel hydrophobic charge induction chromatography fractionation process with acid elution, followed by diafiltration. Virus inactivation was achieved through two orthogonal approaches, a low-pH hold and ultrafiltration. The API was lyophilized, and the freeze-dried powder was formulated into an ointment, BIL010t, containing 59·4% PEG 400, 29·7% PEG 3350 and 0·9% phenoxyethanol. BIL010t drug product contained 100 mg API per gram of ointment (10%).

Immunohistochemistry

Five-micrometre-thick sections were cut from formalin-fixed paraffin-embedded tissue from BCC lesions and mounted on glass slides. Heat-induced epitope retrieval was carried out by incubation in Biocare Universal Decloaker (Biocare, Pacheco, CA, U.S.A.) solution for 50 min at 98 °C. Sections were stained with mouse monoclonal anti-E200 antibody (BPM09) primary for 60 min followed by Mach 4 mouse probe (Biocare) for 15 min and Mach 4 universal polymer horseradish peroxidase for 25 min. Each step was separated by tissue rinsing for 5 min in Tris-buffered saline. Dako liquid diaminobenzidine (DAB; Dako, Carpinteria, CA, U.S.A.) was used as chromagen (5 min) and haematoxylin (5 s) was used as the counterstain. Slides were examined on an Olympus BX41 microscope with 20× objective.

Haematoxylin and eosin staining

Shave biopsies from screening and lesions excised at end of treatment (EOT) were excised for histological examination. Five-micrometre-thick sections were cut from formalin-fixed paraffin-embedded tissue and mounted on glass slides. Each of the tissue sample sections was stained on a Leica XL autostainer by dewaxing in xylene, hydrating through a series of graded alcohols and rinsed in tap water. Tissue sections were then stained with Mayer's haematoxylin and aqueous eosin and rinsed in tap water. The tissue sections were then dehydrated through a series of graded alcohols, cleared in xylene and mounted with Pertex® (Histolab, Askim, Sweden). Three or four sections from each pretreatment shave biopsy and five or six sections from each excision biopsy were examined by a pathologist.

Clinical trial

Study design

The study design was an open-label, multicentre phase I clinical trial to evaluate the safety and tolerability of topical administration of BIL010t to primary BCC lesions. The primary objectives were to evaluate the safety and tolerability of topical administration of BIL010t, to determine the steady-state pharmacokinetics of the API and to determine levels of human antidrug antibody (ADA) in serum. The study was designed by the Clinical Research Organisation, Biosceptre's clinical consultant, the clinical investigators and Biosceptre.

All patients were at least 18 years of age and had one primary, histologically confirmed BCC that was suitable for surgical excision (and not indicated for Mohs surgery). Maximum lesion diameter was 2 cm with a minimum area of 0·5 cm^2. Histological diagnosis was made no more than 4 weeks prior to the screening visit, and the shave biopsy removed no more than 25% of the lesion (histological subtypes are listed in Table 1). Lesions on the hands and feet were excluded. The study protocol was approved by Quorum Review Independent Review Board, Seattle, and all patients provided written informed consent prior to commencement of the study.

Study protocol

Twenty-one patients met the study entry criteria and all received treatment with the investigational product (IP) (BIL010t). Patients applied approximately 50–100 mg of BIL010t to an area of approximately 25 cm^2 containing a single BCC lesion twice daily for 28 days. A large treatment area was utilized to examine the safety characteristics of the treatment on both the lesion and the surrounding normal skin. Prior to the morning dose each day, patients desquamated the entire 25-cm^2 treatment area using a 3M Buf-Puf® Gentle Facial Sponge (3M; St Paul, MN, U.S.A.). After 28 days of treatment the lesion was excised.

The size of each lesion was measured at baseline (day 1) and before excision on day 29 to provide an indication of efficacy. Patients underwent clinical assessment at baseline before the first dose of IP was applied (day 1), then on days 3, 8, 15 and 29 during treatment, and on days 41 and 57 post-treatment.

Blood sampling for pharmacokinetic studies was carried out predose at baseline (day 1) and at day 15. At the EOT visit, blood was drawn predose before the final application of IP and postdose at 1 h, 2 h and 4 h. API concentration in blood samples was measured using an anti-sheep IgG enzyme-linked immunosorbent assay. To determine API immunogenicity, blood samples from screening, baseline, day 29 and day 57 were analysed in a bridging format electrochemilluminescence assay for human antisheep IgG antibody.[28]

Results

Preclinical data

Polyclonal antibodies purified from sheep immunized with the E200 peptide were shown to bind specifically to nfP2X$_7$ expressed on cancer cell lines, but not to functional P2X$_7$ (Fig. S1; see Supporting Information). These antibodies were formulated into a PEG-based topical ointment.

This was first tested as a topical treatment in an orthotopic mouse model of melanoma. Treatment with ointment containing E200 targeted sheep IgG caused a significant reduction in B16F10 tumour growth compared with all control groups (Fig. 1) [two-way ANOVA with Tukey's multiple comparison test (day 17, $P = 0{\cdot}02$; day 19, $P < 0{\cdot}01$)].

As P2X$_7$ has been shown to be expressed in NMSCs, including BCC,[29] this was investigated as an indication for treatment with BIL010t. Shave biopsies from BCCs tested for the presence of nfP2X$_7$ with an E200-targeted monoclonal antibody showed extensive staining for nfP2X$_7$ by immunohistochemistry (Fig. 2).

To investigate the possibility that targeting nfP2X$_7$ may provide a therapeutic option for BCC, the nfP2X$_7$-targeted sheep polyclonal ointment was developed as a treatment for BCC.

Table 1 Patient characteristics and treatment site safety characteristics from a phase I clinical trial of topical treatment of basal cell carcinoma with nfP2X7-targeted sheep polyclonal antibody for 28 days

Baseline characteristics				Treatment site adverse events					
Patient number	Sex and age (years)	Site of lesion	Type of lesion	Dryness	Erythema	Pruritus	Pain	Exfoliation	Completed study
01-001	F, 49	Forehead	Nodular						Yes
01-002	M, 84	Left back	Nodular	Mild (8–15); mild (27–36)					Yes
01-003	F, 72	Left cheek	Nodular						Yes
01-005	M, 51	Left forehead	Nodular						Yes
01-006	M, 75	Left upper back	Nodular						Withdrawn owing to myocardial infarction (49–51)
01-007	M, 51	Left forearm	Superficial						Yes
01-008	F, 53	Chest	Superficial						Yes
01-009	F, 81	Forehead	Nodular						Lost to follow-up
01-010	M, 69	Forehead	Nodular						Yes
02-001	M, 67	Right neck	Superficial						Yes
02-002	M, 79	Right chest	Nodular	Mild (59–63)	Moderate (47–63)	Mild (47–59)			Yes
02-004	F, 70	Right lateral cheek	Nodular	Mild (28–35);	Severe (35–85)				Yes
02-005	M, 47	Chest	Nodular	Mild (43–57)	Moderate (43–71)			Mild (43–57)	Yes
02-006	M, 48	Clavicle			Moderate (12–15); mild (41–62)	Mild (12–15)	Mild (12–15)		Yes
02-008	F, 86	Upper mid chest	Nodular	Mild (3–45)	Mild (10–15); mild (45–58)	Mild (10–45)		Mild (10–45)	Yes
02-009	M, 67	Right back	Superficial	Mild (29–43)	Moderate (3–29); severe (29–57)	Mild (15–57)	Mild (43–57)	Mild (3–8); moderate (29–43)	Yes
02-010	F, 45	Left lower back	Superficial	Mild (3–7); mild (55–ongoing)	Severe (16–32)	Mild (32–55)		Mild (55–ongoing)	Yes
03-002	M, 52	Right upper arm	Nodular						Yes
03-003	F, 67	Left upper arm	Nodular		Mild (3-8)				Yes
03-005	M, 91	Upper left shoulder	Superficial	Mild (1–3)	Mild (1–15); mild (29–57)		Mild (8–15)	Mild (1–3)	Yes
03-006	M, 60	Left upper chest	Nodular		Mild (29–57)				Yes

The severity of each treatment site adverse event is listed together with day of onset and day at which event was resolved (day 1 is start of treatment). F, female; M, male.

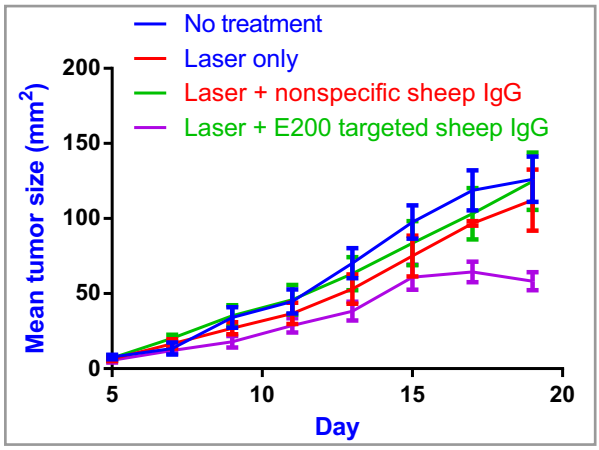

Fig 1. Topical treatment of a mouse melanoma model with nfP2X$_7$-targeted sheep polyclonal antibody causes a reduction in tumour growth. B16F10 mouse melanoma cells were inoculated into 42 C57Bl/6 mice at day 0. Mice were randomized into four groups: group 1 (blue) received no treatment, groups 2, 3 and 4 were treated on days 5, 9, 13 and 16 with a laser pulse to remove stratum corneum. Group 2 (red) received laser pulse treatment only, while group 3 (green) were additionally treated with 50 mg per gram of preimmune sheep IgG ointment and group 4 (magenta) were additionally treated with 50 mg per gram of sheep IgG anti-nfP2X$_7$ ointment. Tumours treated with anti-nfP2X$_7$ ointment (magenta) showed significantly reduced growth compared with tumours treated with preimmune ointment at 15, 17 and 19 days (two-way ANOVA with Tukey's multiple comparison test).

Extensive independent preclinical toxicology studies were performed. These included immunohistochemistry tissue cross-reactivity studies using the API on a full panel of normal human tissues. Binding to the membranes of cells in normal tissues was not seen, but binding to the membrane of control frozen tissue sections from cancer cell lines was observed (data not shown).

Independent preclinical toxicology studies were performed in minipigs. These studies, conducted for 28-day (32 minipigs) and 90-day (40 minipigs) periods, demonstrated that the ointment was well tolerated (data not shown).

Phase I clinical trial

The 10% topical ointment (BIL010t) was then used in an open-label phase I clinical trial (clinicaltrials.gov NCT02587819). Participant flow through the trial is shown in Figure 3. Twenty-six patients with skin lesions initially diagnosed as BCC were assessed for study eligibility. The screening process for each subject included a shave biopsy to confirm the diagnosis of BCC, followed by 25–31 days to enable the site of the shave biopsy to heal. The baseline visit was conducted after the screening period and was designated as 'day 1'. Four patients were excluded as histopathology indicated that their lesion was not a BCC and another patient was excluded based on electrocardiogram results.

Safety and tolerability

The baseline characteristics of the remaining study population of 21 patients are outlined in Table 1. There was a high level of compliance with the treatment regimen. Twenty of 21 patients completed dosing with one patient lost to follow-up prior to the EOT visit. Eleven patients reported treatment site adverse events (AEs) (recorded in Table 1). These included erythema reported in 10 patients, dryness (seven patients), exfoliation and pruritus (both reported in five patients) and application site pain (three patients). Treatment site AEs were

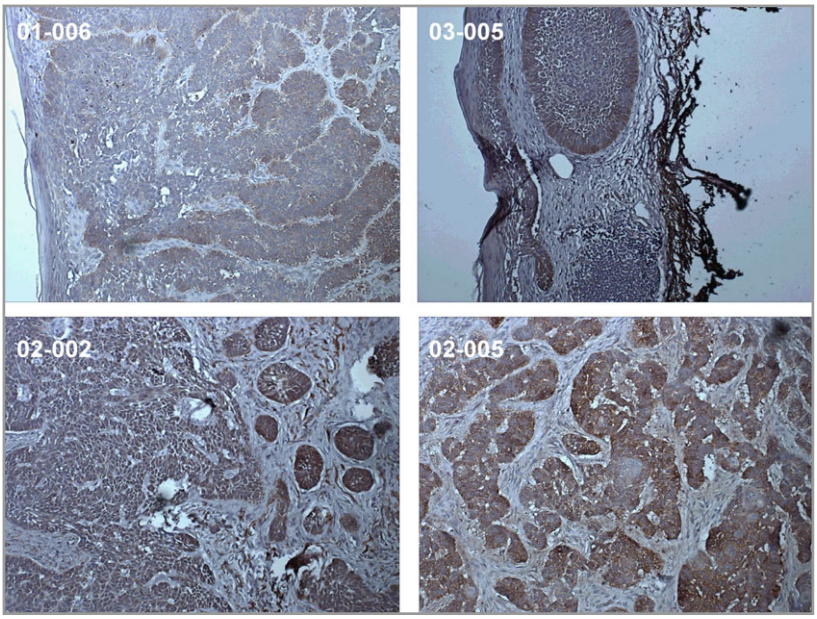

Fig 2. Immunohistochemistry (IHC) staining of shave biopsies demonstrates that basal cell carcinomas (BCCs) express nfP2X$_7$. Shave biopsies were taken from BCC lesions before treatment and IHC staining, using an nfP2X$_7$-targeted monoclonal antibody, showed the nfP2X$_7$ target to be present.

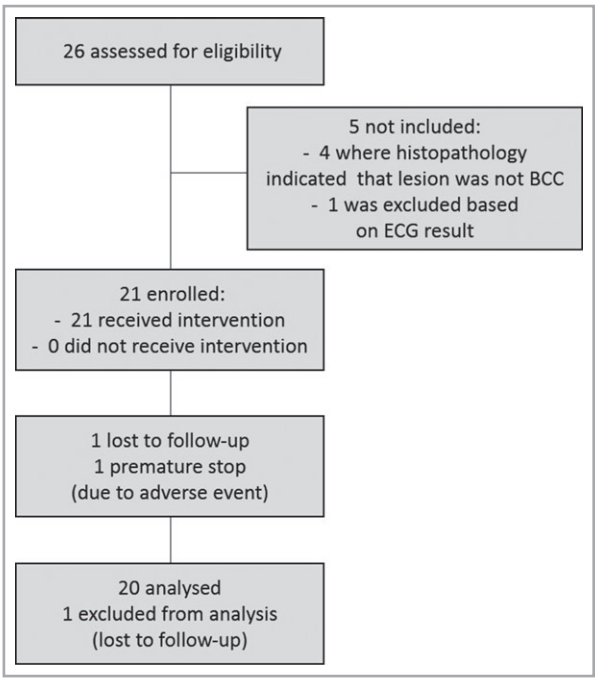

Fig 3. Participant flow through phase I clinical trial of topical treatment of basal cell carcinoma (BCC) with nfP2X$_7$-targeted sheep polyclonal antibody for 28 days. ECG, echocardiogram.

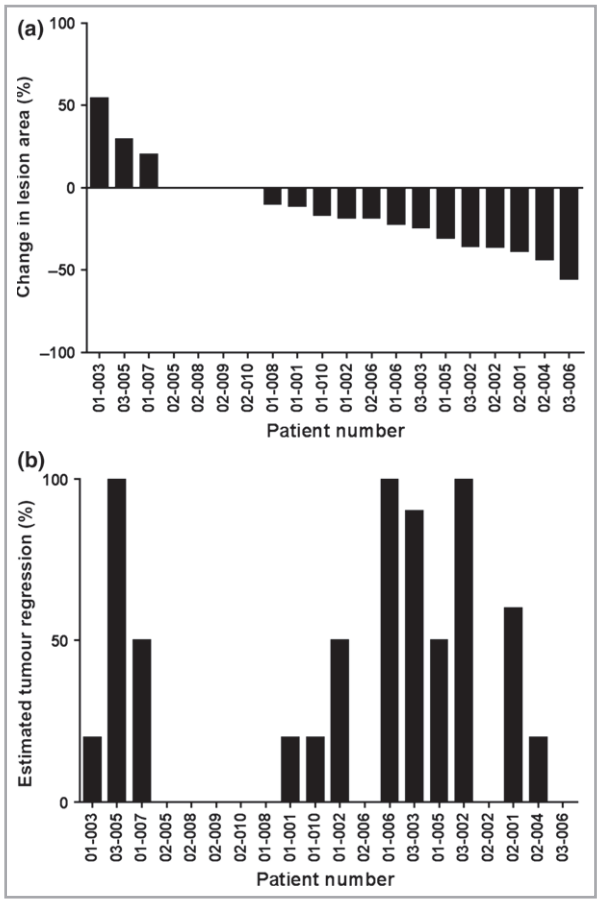

Fig 4. Indications of efficacy in patients with basal cell carcinoma (BCC) treated topically with ointment containing nfP2X$_7$-targeted antibodies. Twenty patients with BCC were treated by topical application of 10% anti-nfP2X$_7$ ointment (BIL010t) twice daily for 28 days. (a) Lesion area was measured before treatment and after 28 days. (b) End-of-treatment sections were stained using haematoxylin and eosin and assessed for evidence of tumour regression.

mild to moderate, except for severe erythema, which was reported in three patients.

In addition to treatment site AEs listed in Table 1, there were three other AEs that were not considered to be related to treatment. Patient 01-006 experienced a serious AE (a cardiac arrest) at day 49 during follow-up. Patient 03-003 experienced mild diarrhoea from day 20 to 24 of treatment and moderate sinusitis from day 41 to 56 of follow-up.

Pharmacokinetics and immunogenicity

Immunogenicity and pharmacokinetics were also examined during the study. Antisheep IgG antibody levels were measured in blood samples taken from patients at screening, baseline, EOT and follow-up on day 57. Results are shown in Figure S2 (see Supporting Information). Eight patients had detectable titres at screening and baseline (prior to first dose) visits, demonstrating that antisheep IgG antibodies exist in the untreated population. Of these eight patients, three showed a twofold increase in titres and one showed a fourfold increase in titres after treatment. Twelve patients were negative at baseline and of these, three patients developed titres (two patients at EOT and one further patient at follow-up). Overall, treatment with BIL010t was not immunogenic in most patients, and in patients who developed ADAs, these were at low titres and did not correlate with AEs.

Pharmacokinetics were examined at the EOT visit. Patients had blood drawn predose and at 1 h, 2 h and 4 h after application of BIL010t. At all time points, the serum concentration of sheep IgG was too low to be quantified in most patients (data not shown). One patient had measurable sheep IgG at 1 h postdose at visit 6 (EOT) and three other patients had measurable sheep IgG at predose time points. These data suggest that there is minimal systemic penetration of BIL010t.

Efficacy

Lesion size in each patient was measured at baseline (day 1) prior to the start of treatment and at day 29 (EOT). A waterfall plot of percentage change in lesion area is shown in Figure 4(a). Overall, 65% of patients showed a reduction in lesion area after 28 days, 20% showed no change and 15% showed increased lesion size. This outcome provides an initial indication of efficacy that will be investigated further in a subsequent clinical trial.

Haematoxylin and eosin-stained sections from shave biopsies obtained at the screening visit and tumour tissue excised

at the EOT visit were examined by an independent pathologist. There were extensive BCC tumour cells present in all screening-visit shave biopsies. Mild inflammation was seen in five of 20 patients. The regression of BCC results in accumulation of fibrous stroma around the tumour.[30,31] Therefore, the ratio of fibrous stromal reaction to remaining tumour in EOT sections was used to approximate tumour regression (Fig. 4b, Fig. S3; see Supporting Information). In EOT sections from three patients (01-006, 03-002, 03-005) there was no evidence of tumour, suggesting that histological clearance had occurred. In a further nine patients there was evidence of increased fibrous stromal reaction within the tumour margins suggesting partial tumour regression. EOT sections from all patients showed inflammatory reaction with accumulation of lymphocytes around the tumour.

Discussion

This clinical trial is the first to assess safety for an antibody-based treatment of BCC and the first to assess antibodies targeting nfP2X$_7$ in humans. Preclinical data demonstrate that BIL010t binds specifically to nfP2X$_7$, a form of P2X$_7$ with attenuated pore functionality, and does not bind to fully functional P2X$_7$. Formal preclinical studies confirmed that the API of BIL010t does not bind to cell membranes of normal tissue (data not shown). Furthermore, formal toxicology studies, undertaken in minipigs, confirmed the necessary safety to support a phase I clinical trial. Together, these observations demonstrated that BIL010t is highly likely to be safe when used as a topical therapeutic owing to its target specificity and minimal potential for off-target effects.

Preclinical studies showed that BIL010t was efficacious in an orthotopic mouse melanoma model, where sheep polyclonal antibodies raised against E200 were able to inhibit the growth of B16F10 cell-line-derived tumours significantly.

P2X$_7$ expression has been previously described in BCC,[29] and immunohistochemistry confirmed the presence of nfP2X$_7$ in BCC lesions. Therefore, BIL010t was examined as a topical treatment for BCC in a phase I clinical trial in which patients demonstrated a high level of compliance with the treatment regimen, with mainly localized treatment site AEs reported, and no patients discontinuing treatment prematurely as a result of AEs. This demonstrates that BIL010t is both safe and well tolerated in patients with BCC and therefore could be used in a phase II study to establish efficacy.

The surface lesion size was measured at baseline and after 28 days of treatment to give an indication of efficacy for BIL010t. Lesion depth was not measured and, therefore, results may not reflect the full extent of efficacy. However, 65% of patients showed a reduction in lesion surface area. In addition, haematoxylin and eosin-stained EOT sections were analysed for signs of regression. Three patients showed no evidence of tumour in EOT sections and a further nine patients showed evidence of partial regression. These data are supportive of the efficacy of BIL010t as a treatment for BCC. However, as the trial was carried out over a short 28-day time frame with limited efficacy measures, these results are not conclusive. Efficacy will be thoroughly evaluated in a carefully designed phase II trial over a longer treatment period. A shave biopsy was taken from all lesions prior to treatment and it is unclear whether this may have affected the penetration of BIL010t into lesions. This will be addressed in further efficacy trials.

There is no apparent correlation between measured lesion size and histological assessment of tumour regression. For example, three patients presenting with increased lesion size all showed evidence of histological regression, including complete histological clearance in patient 03-005. This illustrates the challenges in assessing efficacy using traditional RECIST (Response Evaluation Criteria In Solid Tumours) measurements when macroscopic observations are utilized.

In conclusion, it has been demonstrated that BIL010t, a first-in-class topical antibody therapy targeting nfP2X$_7$, is safe for treating BCC. In addition, BIL010t has shown an initial indication of efficacy based on reduced lesion size and histological evidence of regression. These positive outcomes justify additional clinical trials to assess the potential for BIL010t to treat skin cancer. Because of the widespread expression of nfP2X$_7$ on a broad variety of cancers and the intrinsic safety of the target,[24–27] additional therapeutic studies targeting nfP2X$_7$ on multiple solid-tumour types are being considered.

Acknowledgments

We are indebted to Jeffrey S. Altman MD and Steven A. Davis MD who were lead investigators at two of the study sites involved in this trial. We would also like to thank Dr Jay Birnbaum for his valuable advice and consultation with regard to the preclinical and clinical programmes.

References

1 Lewin JM, Carucci JA. Advances in the management of basal cell carcinoma. *F1000Prime Rep* 2015; **7**:53.
2 Marzuka AG, Book SE. Basal cell carcinoma: pathogenesis, epidemiology, clinical features, diagnosis, histopathology, and management. *Yale J Biol Med* 2015; **88**:167–79.
3 Chuang TY, Popescu A, Su WP, Chute CG. Basal cell carcinoma. A population-based incidence study in Rochester, Minnesota. *J Am Acad Dermatol* 1990; **22**:413–7.
4 Marcil I, Stern RS. Risk of developing a subsequent nonmelanoma skin cancer in patients with a history of nonmelanoma skin cancer: a critical review of the literature and meta-analysis. *Arch Dermatol* 2000; **136**:1524–30.
5 Mohan SV, Chang AL. Advanced basal cell carcinoma: epidemiology and therapeutic innovations. *Curr Dermatol Rep* 2014; **3**:40–5.
6 Surprenant A, Rassendren F, Kawashima E *et al.* The cytolytic P2Z receptor for extracellular ATP identified as a P2X receptor (P2X$_7$). *Science* 1996; **272**:735–8.
7 Michel AD, Chessell IP, Hibell AD *et al.* Identification and characterization of an endogenous P2X$_7$ (P2Z) receptor in CHO-K1 cells. *Br J Pharmacol* 1998; **125**:1194–201.
8 Di Virgilio F, Chiozzi P, Falzoni S *et al.* Cytolytic P2X purinoceptors. *Cell Death Differ* 1998; **5**:191–9.

9 Pellegatti P, Raffaghello L, Bianchi G *et al.* Increased level of extracellular ATP at tumour sites: *in vivo* imaging with plasma membrane luciferase. *PLOS ONE* 2008; **3**:e2599.
10 Adinolfi E, Callegari MG, Ferrari D *et al.* Basal activation of the P2X7 ATP receptor elevates mitochondrial calcium and potential, increases cellular ATP levels, and promotes serum-independent growth. *Mol Biol Cell* 2005; **16**:3260–72.
11 Jelassi B, Chantôme A, Alcaraz-Pérez F *et al.* P2X($_7$) receptor activation enhances SK3 channels- and cystein cathepsin-dependent cancer cells invasiveness. *Oncogene* 2011; **30**:2108–22.
12 Adinolfi E, Raffaghello L, Giuliani AL *et al.* Expression of P2X$_7$ receptor increases *in vivo* tumour growth. *Cancer Res* 2012; **72**:2957–69.
13 Lenertz LY, Wang Z, Guadarrama A *et al.* Mutation of putative N-linked glycosylation sites on the human nucleotide receptor P2X$_7$ reveals a key residue important for receptor function. *Biochemistry* 2010; **49**:4611–9.
14 Gu BJ, Zhang W, Worthington RA *et al.* A Glu-496 to Ala polymorphism leads to loss of function of the human P2X$_7$ receptor. *J Biol Chem* 2001; **276**:11135–42.
15 Wiley JS, Dao-Ung LP, Li C *et al.* An Ile-568 to Asn polymorphism prevents normal trafficking and function of the human P2X$_7$ receptor. *J Biol Chem* 2003; **278**:17108–13.
16 Roger S, Mei ZZ, Baldwin JM *et al.* Single nucleotide polymorphisms that were identified in affective mood disorders affect ATP-activated P2X$_7$ receptor functions. *J Psychiatr Res* 2010; **44**:347–55.
17 Cheewatrakoolpong B, Gilchrest H, Anthes JC, Greenfeder S. Identification and characterization of splice variants of the human P2X$_7$ ATP channel. *Biochem Biophys Res Commun* 2005; **332**:17–27.
18 Giuliani AL, Colognesi D, Ricco T *et al.* Trophic activity of human P2X$_7$ receptor isoforms A and B in osteosarcoma. *PLOS ONE* 2014; **9**:e107224.
19 Feng YH, Li X, Wang L, Gorodeski G *et al.* A truncated P2X$_7$ receptor variant (P2X$_7$-j) endogenously expressed in cervical cancer cells antagonizes the full-length P2X$_7$ receptor through hetero-oligomerization. *J Biol Chem* 2006; **281**:17228–37.
20 Gu BJ, Rathsam C, Stokes L *et al.* Extracellular ATP dissociates nonmuscle myosin from P2X(7) complex: this dissociation regulates P2X($_7$) pore formation. *Am J Physiol Cell Physiol* 2009; **297**:C430–9.
21 Gidley-Baird A, Barden JA. *Antibodies to Non-Functional P2X$_7$ Receptor Diagnosis and Treatment of Cancers and Other Conditions*. Patent WO2002057306 A1, 2002.
22 Gidley-Baird A, Barden JA. *Antibodies to Non-Functional P2X$_7$ Receptor, Diagnosis and Treatment of Cancers and Other Conditions*. Patent WO2003020762 A1, 2003.
23 Barden JA, Sluyter R, Gu BJ, Wiley JS. Specific detection of non-functional human P2X($_7$) receptors in HEK293 cells and B-lymphocytes. *FEBS Lett* 2003; **538**:159–62.
24 Slater M, Danieletto S, Gidley-Baird A *et al.* Early prostate cancer detected using expression of non-functional cytolytic P2X$_7$ receptors. *Histopathology* 2004; **44**:206–15.
25 Slater M, Danieletto S, Pooley M *et al.* Differentiation between cancerous and normal hyperplastic lobules in breast lesions. *Breast Cancer Res Treat* 2004; **83**:1–10.
26 Slater M, Scolyer RA, Gidley-Baird A *et al.* Increased expression of apoptotic markers in melanoma. *Melanoma Res* 2003; **13**:137–45.
27 Barden JA, Yuksel A, Pedersen J *et al.* Non-functional P2X7: a novel and ubiquitous target in human cancer. *J Clin Cell Immunol* 2014; **5**:237.
28 Mire-Sluis AR, Barrett YC, Devanarayan V *et al.* Recommendation for the design and optimization of immunoassays used in the detection of host antibodies against biotechnology products. *J Immunol Methods* 2004; **289**:1–16.
29 Greig AV, Linge C, Healy V *et al.* Expression of purinergic receptors in non-melanoma skin cancers and their functional roles in A431 cells. *J Invest Dermatol* 2003; **121**:315–27.
30 Hunt MJ, Halliday GM, Weedon D *et al.* Regression in basal cell carcinoma: an immunohistochemical analysis. *Br J Dermatol* 1994; **130**:1–8.
31 Kaur P, Mulvaney M, Carlson JA. Basal cell carcinoma progression correlates with host immune response and stromal alterations: a histologic analysis. *Am J Dermatopathol* 2006; **28**:293–31.

Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Fig S1. Sheep polyclonal anti-E200 antibodies bind specifically to nfP2X7 on cancer cell lines but do not bind overexpressed wild-type P2X7.

Fig S2. BIL010t is not strongly immunogenic.

Fig S3. Stromal reaction accumulates around tumour post-treatment indicating partial regression.