Safety and tolerability of an intratumorally injected DNAzyme, Dz13, in patients with nodular basal-cell carcinoma: a phase 1 first-in-human trial (DISCOVER)

The MIT Faculty has made this article openly available. Please share how this access benefits you. Your story matters.
Safety and tolerability of an intratumorally injected DNAzyme, Dz13, in patients with nodular basal-cell carcinoma: a phase 1 first-in-human trial (DISCOVER)

Eun-Ae Cho, MBBS MS*,1, Fergal J. Moloney, MD*,†,1, Hong Cai, MD PhD2, Annie Au-Yeung, BSc2, Carlos China, MD3, Richard A. Scolyer, MD4, Benafsha Yosufi, BSc5, Mark J. Raftery, PhD6, Jason Z. Deng, PhD7, Stephen W. Morton, PhD7, Paula T. Hammond, PhD7, Hendrik-Tobias Arkenau, MD PhD8, Diona L. Damian, MBBS PhD1, Douglas J. Francis, BVSc MVSc PhD9, Colin N. Chesterman, MBBS DPhil2, Ross St.C Barnewt, MBBS MD1, Gary M. Halliday, PhD DSc*,1, and Levon M. Khachigian, PhD DSc†,2

1Dermatology, Sydney Medical School, Bosch Institute, Royal Prince Alfred Hospital at University of Sydney, Sydney NSW, Australia
2Centre for Vascular Research, University of New South Wales, Sydney NSW, Australia
3Woolcock Institute of Medical Research, Sydney NSW, Australia
4Tissue Pathology and Diagnostic Oncology, Royal Prince Alfred Hospital, Sydney NSW, Australia
5Melanoma Institute Australia, Sydney NSW, Australia
6Mark Wainwright Analytical Centre, University of New South Wales, Sydney NSW, Australia
7Koch Institute for Integrative Cancer Research, Massachusetts Institute of Technology, Cambridge MA, USA
8Sarah Cannon Research UK, London, United Kingdom
9DF Pre-clinical Services Pty Ltd, Canberra ACT, Australia

Abstract

Background—c-Jun, a nuclear transcription factor, is preferentially expressed in cancer. The study drug Dz13, a deoxyribozyme targeting c-Jun mRNA inhibits the growth of a range of tumors, including basal cell carcinoma (BCC) in murine models.

Aims—The primary objectives of this first-in-class, first-in-human trial (DISCOVER) Phase I trial (ACTRN12610000162011) were to determine the safety and tolerability of Dz13 in patients with BCC. A secondary objective was to assess Dz13 pharmacokinetics, changes in protein expression and cell markers in the tissue following single intratumoral injection.
**Methods**—Nine patients with nodular BCC were recruited from Royal Prince Alfred Hospital, Sydney, Australia between 2010 and 2011 and enrolled in three escalating dose groups of three patients. Each patient received a single intratumoral injected dose of Dz13 (10, 30 or 100µg) and was monitored over four weeks. The highest dose used in the trial (100µg), is at the upper limit of solubility of the formulation for a 50µl injection and was the highest concentration used in the preclinical mouse tumor studies. At each follow up visit, complete physical examination, blood and urine tests, electrocardiogram and pharmacokinetic/pharmacodynamic analyses were performed. BCCs were surgically removed 14 days post-Dz13 injection and compared with the pre-injection biopsy.

**Findings**—All nine patients completed the study with no drug-related serious adverse events. No systemic Dz13 exposure was detected. c-Jun expression was reduced in the BCC of all nine participants treated with Dz13. The DNAzyme increased Caspase-3, -8, -9, and p53, reduced Bcl-2 and MMP-9, and stimulated inflammatory and immune cell infiltration in the tumors. Moreover, five of the nine patients had a reduction in histological tumor depth.

**Interpretation**—Dz13 is safe, well tolerated, inhibits its target, and shows no detectable systemic exposure following single intratumoral injection.

**BACKGROUND**

Basal cell carcinoma (BCC) is the most common cancer in Caucasians and represents about 70% of non-melanoma skin cancers (NMSC) 2–5. In the US, approximately 1 to 3.5 million cases of NMSC are diagnosed annually 6, 7. Australia has the highest incidence of NMSC in the world 8, where skin cancer is about four times as prevalent as all other cancers combined. Approximately two-thirds of Australians will develop at least one NMSC before the age of 70, making it the most expensive cancer in the nation 9, 10. Most BCCs are amenable to primary excision. Radiotherapy is also effective while photodynamic therapy and topical imiquimod are options for treating superficial BCC. Additional treatment options may be required for high risk lesions such as centrofacial, recurrent, large sized or histologically aggressive BCCs. Patients with locally advanced or metastatic BCCs that have recurred following surgery or deemed unsuitable for surgery or radiation can be considered for systemic treatment with Vismodegib GDC-0449 11, a synthetic small-molecule antagonist of hedgehog signaling, following FDA approval in 2012. Targeted molecular therapy may also offer a further novel effective and less invasive therapeutic option for BCC.

A myriad of molecules have been implicated in tumorigenesis, and activating protein-1 (AP-1) is a well-recognized participant in the process. AP-1 transcription factors participate in oncogenic transformation, angiogenesis, dysregulated proliferation and apoptosis, invasive growth and metastasis 12–14. c-Jun is a basic leucine-zipper (bZIP) protein and prototypic member of AP-1. Dominant negative c-jun-expressing transgenic mice are protected against skin tumor promotion 15. GLI1 and GLI2, key transcriptional activators of the hedgehog pathway, directly regulate c-Jun 16. In addition, skin tumorigenesis is suppressed in c-Jun NH2-terminal kinase-2-deficient mice 17. c-Jun expression is minimal in normal adult tissues, whilst increased in a range of pathologic settings and abnormal tissue, including BCC, making it an ideal therapeutic target 18–25.

Dz13 is a deoxyribozyme (DNAzyme) that specifically targets c-jun mRNA 26. DNAzymes are catalytically-active single-stranded synthetic oligonucleotides that bind and cleave their target mRNA via Watson-Crick base-pairing in the flanking recognition arms and a de-esterification reaction 27. DNAzymes differ from ribozymes and siRNA in that the former is composed entirely of DNA, rather than RNA, and differ from antisense oligonucleotides in that DNAzymes contain a catalytic domain and constituent nucleotides are linked by
phosphodiester rather than phosphorothioate bonds. Dz13 cleaves at the G\(^{1311}\)U junction in human c-jun mRNA and exerts its anti-tumor activity via induction of apoptosis, inhibition of angiogenesis and the induction of adaptive immunity. Recent studies have demonstrated the pre-clinical safety of Dz13 in mice, rats, minipigs and monkeys, and \textit{in vivo} efficacy of Dz13 in a range of murine models of cancer including BCC, SCC, melanoma, osteosarcoma, liposarcoma, prostate and breast cancer. However, to the best of our knowledge there are no published reports of the clinical evaluation of this or any other DNAzyme. We therefore conducted a first-in-human, first-in-class (DISCOVER) Phase I trial of a DNAzyme to explore the safety and tolerability of the study drug Dz13 in patients with BCC.

**METHODS**

**Study participants and target lesion selection**

Outpatients attending dermatology clinics at Royal Prince Alfred Hospital (RPAH), Sydney, between 2010 and 2011 with clinically suspected nodular BCC were screened, and nine otherwise healthy consenting patients with histologically confirmed nodular BCC were consecutively recruited into three dose groups (10, 30 and 100µg Dz13) with three patients per group (Supplementary Figure 1A). All recruited patients satisfied the selection criteria outlined in Supplementary Table 1.

Each patient only had a single skin cancer when entered into the trial. Target lesions were selected as follows. Suitable lesions were examined clinically and dermoscopically by a dermatologist (FJM) for features of nodular BCC. Patients with lesions satisfying inclusion and exclusion criteria (Supplementary Table 1) were further assessed for eligibility. The largest tumor measured 5–16 mm diameter and the tumors exhibited clear clinical margins to facilitate study drug administration and dermoscopic measurement. Details of skin cancer history are included in Table 1. There were no interventions during the course of the trial other than the administration of Dz13.

**Study design**

This was a non-controlled, non-randomized, non-blinded, dose-escalating Phase I clinical trial. Following informed consent and screening, the patients were injected intratumorally with Dz13 and monitored at 7, 14 and 28 days post-dose (Supplementary Figure 1B). Study endpoints were analyzed using Wilcoxon signed rank test where applicable and considered significant at \(p=0.05\).

The study was prospectively registered with the Australian New Zealand Clinical Trials Registry (ACTRN12610000162011) and approved by the Sydney South West Area Health Service Human Research Ethics Committee (HREC).

**Study drug**

Dz13 is a synthetic oligodeoxynucleotide of 34 nt length and synthesized using good manufacturing practice (GMP) (Oligos Etc, Wilsonville, OR, USA) with an inverted thymidine at the 3’ position and entirely phosphodiester-linked bases. The nucleotide sequence of Dz13 is 5’-CGG GAG GAA GGC TAG CTA CAA CGA GAG GCG TTG (3’-3’T)-3’. Dz13 was dissolved in sterile water (Baxter, Old Toongabbie, NSW, Australia) to a concentration of 8mg/ml, sterilized through a 0.22µm filter unit (Millipore, Kilsyth, VIC, Australia), then stored in 100µl aliquots at \(-20^\circ\text{C}\) until use. Dz13 was combined with GMP grade synthetic cationic lipid carriers 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE) and 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP) in DMEM (Dulbecco’s...
modified essential medium; Invitrogen, Mulgrave, VIC, Australia) into 50µl doses of 10, 30 or 100µg Dz13 as per Supplementary Table 2.

DOPE and DOTAP were obtained in powder form (Avanti Polar Lipids Inc., Alabaster, Alabama, USA) and dissolved in 100% ethanol (Covidien, Hazelwood, MO, USA) to a concentration of 20mg/ml. A stock solution of mixed lipids was prepared by mixing an equal volume of each lipid to a final concentration of 10mg/ml. The solution was sterilized through a 0·22µm filter unit (Millipore, Kilsyth, VIC, Australia) and stored in 1ml aliquots at −20°C until use.

Details relating to preparation and administration of study drug, stability testing, clinical assessments, safety and tolerability, pharmacokinetic assessments, histological and immunohistochemical analyses, and statistical analyses can be found in the Appendix.

RESULTS

Study design and drug

The Phase I study was a traditional 3 + 3 design, incorporating “sentinel dosing” wherein the first subject at a dose was administered Dz13 and assessed for 14 days before administration to the second subject. The doses used (10, 30, 100µg) were based on recent preclinical analyses in mice bearing dermal BCC and SCC in which doses of Dz13 between 10–100µg (in DOTAP/DOPE) were delivered intratumorally in 50µl with no apparent adverse effects. The full volume of study drug (50µl) was delivered to each of the treated tumors in DMEM for consistency with the formulation used in preclinical toxicological studies and to simulate physiologic conditions. The Dz13 lipoplex, injected into the tumors 20 min after mixing (Supplementary Table 2), formed within 5 min and was morphologically stable for at least 120 min (Supplementary Figure 2). Scanning electron micrograph (SEM) and atomic force microscopy (AFM) of the DNAzyme-lipoplex revealed highly polydispersity and primary particle size of approximately 100–200 nm (Supplementary Figure 3).

Subject demographics

Baseline characteristics of the nine participants in this clinical trial are outlined in Table 1. This involved six males and three females with a mean age of 55 ± 4 years. All nine patients had a prior history of BCC and the mean BCC diameter prior to treatment was 9·7 ± 1·1 mm.

Safety profile

Clinical findings—There were no clinically significant changes on physical examination, hematology (hemoglobin, red cell count, white cells with differential counts and platelet count), biochemistry (electrolytes, renal and liver function tests, blood glucose and lipid profile), coagulation studies, immunology (immunoglobulins G, A, M, and E, and antinuclear antibody), urinalysis and ECG parameters (rhythm, rate and QT/QTc intervals) at any time point in any of the patients.

Adverse events—Over the course of the study a total of seven adverse events occurred (Supplementary Table 3). Of these, only transient (<24 h) injection site swelling, discomfort and nausea were possibly related to the study drug. Notably, all three related adverse events occurred or were reported by the same subject (10µg dose). The adverse events in the other three patients were unrelated to Dz13. All events were mild and self-limiting without treatment, except wound infection post excision and phlebitis, which were moderate and required a course of antibiotics and anti-inflammatory agent, respectively. None of the patients had a severe adverse event or withdrew from the study due to an adverse event. There was no obvious dose-association.
Pharmacokinetics profile

There were no significant levels of Dz13 detected in the plasma samples collected for PK evaluation (0, 0.5, 1, 2, 4, 8, 12 and 24 hours, and 7, 14 and 28 days after intratumoral injection of the DNAzyme formulation) even at the very early times (Supplementary Table 4). The above PK sampling schedule was employed based on preclinical studies where Dz13 was administered intravenously

Dz13 effects on the target lesions

We performed a controlled comparison of pharmacodynamic biomarkers at the completion of the study (14 days post-Dz13 treatment) with baseline levels prior to excision. As this was a Phase I trial, only one test formulation was used. This clinical trial was not powered, nor intended to establish proof-of-principle. A number of important pharmacodynamic changes were nonetheless observed in the treated lesion. Several of the non-established markers (eg. VEGF-A, FGF-2, MMP-2, MMP-9) were selected on the basis of the dependency of these genes on c-Jun/AP-1

**c-Jun**—Expression of c-Jun protein in the target BCC was reduced in all nine of nine participants. After administration of the single dose of Dz13, c-Jun levels were inhibited by 21.2%, 19.7% and 48.4% in the 10, 30 and 100µg dose groups, respectively (Figure 1A). The data suggested a dose-related effect at 100µg (Figure 1B). c-Jun expression in Dz13-treated BCC was significantly reduced compared to pre-treatment samples (Table 2 & Figure 1C).

**Protein markers**—Outcomes of immunohistochemical analyses for c-Jun and a range of protein markers in all nine patients treated with Dz13 (10–100µg) are summarized in Table 2. Caspase-3 (p=0.0039), Caspase-8 (p=0.0039), Caspase-9 (p=0.0039) and p53 (p=0.0078) levels were significantly increased following the single administration of Dz13. The anti-apoptosis mediator Bcl-2 and the matrix metalloproteinase MMP-9 were both significantly decreased by 22.9% (11 to 26.2% over all the doses) and 22.8% (15.7 to 29.4%), respectively. No significant changes were observed in levels of Ki-67, MMP-2, FGF-2 or VEGF-A at the 14 day post dose endpoint (Table 2).

**Cell types**—Dz13 caused a striking change in the population of immune and inflammatory cells in the target BCC (Table 3 & Figures 1D-I). CD3+ (p=0.0039), CD4+ (p=0.0039), CD8+ (p=0.0156) and CD1a+ dendritic cells (p=0.0039) were significantly increased in post-dose excision samples compared to the pre-dose biopsy samples (Table 3).

**Histological tumor depth**—The depth of the target BCC did not increase in any of the nine patients and five of the patients showed a reduction in tumor depth (Figure 2A). Moreover, all three patients in the 100µg dose group demonstrated reduction in tumor depth (Figure 2B). Overall, there was 11.1% (0 to 21.5%) reduction in tumor depth 14 days post-dose compared to baseline, with an average of 6.7%, 9.3% and 17.0% reduction in the 10, 30 and 100µg dose groups respectively (Figure 2). The inhibitory effect of Dz13 did not reach statistical significance at the 14 day post dose endpoint (p=0.063).

**Clinical tumor area**—Dermoscopic measurements showed a mean reduction in tumor area at Day 14 post-dose compared to Day 0 pre-dose by 3.3%, 17.5% and 9.5% in the 10, 30 and 100µg dose groups, respectively. However the reduction as a whole was not statistically significant (p=0.13).
DISCUSSION

It is imperative that an effective yet minimally invasive approach be developed to address the increasing incidence of cancer and the cost burden on society. This Phase I study evaluated, for the first time, a novel class of drug in the setting of a solid tumor and demonstrates that Dz13 is safe and well tolerated when injected intratumorally. In contrast to the hedgehog inhibitor GDC-0449, there was no significant systemic exposure detected following administration of Dz13 and all adverse events were of CTC grade 1 or 2.

Although efficacy was not a primary endpoint of this trial, intratumoral injection of Dz13 reduced c-Jun expression, its primary target, in treated BCCs in nine of the nine patients. The study drug also reduced tumor depth, increased expression of Caspase-3, -8, -9 and p53, and reduced Bcl-2 expression in the tumor nests. Moreover, Dz13 inhibited levels of MMP-9, a protease known to facilitate tumor invasion.

Dz13 also increased immune and inflammatory cell populations in the BCCs, suggesting the involvement of local immunity. It is unlikely that this effect of Dz13 within the tumor is merely a response to a foreign body. We recently showed that Dz13scr (a size-matched counterpart of Dz13 with scrambled binding arms, the same net charge, same 10–23 catalytic domain as that of Dz13, and in DOTAP/DOPE formulation) caused no such response within the tumor. That study revealed that apoptosis induced by Dz13 in the tumors is associated with an inflammatory and adaptive immune response that was not associated with a Toll-like receptor-9 response.

We recently demonstrated that Dz13, in the same DOTAP/DOPE formulation used here, is safe and well-tolerated in a series of GLP-compliant pre-clinical toxicological studies. Repeated intratumoral delivery of Dz13 inhibited BCC and squamous cell carcinoma (SCC) growth in mice in a dose- and time-dependent manner after the tumors became established. Dz13 caused tumor regression, increased apoptotic biomarker expression and stimulated CD4+CD8+ lymphocyte levels in the tumors. Immunodepletion studies in tumor-bearing syngeneic mice confirmed the involvement of adaptive T cell-mediated immunity in Dz13 inhibition of tumor growth. The present study builds on these pharmacodynamic studies and shows similar effects of Dz13 in human BCC as seen in our murine models. It represents the first reported published data of a clinical trial of a DNAzyme of any kind. It also provides window of opportunity data that c-Jun may potentially serve as a target in skin cancer.

BCC was selected as the first human tumor to evaluate Dz13 safety and efficacy for a number of reasons. This includes the unmet clinical need for non-surgical therapy, the visibility and accessibility of BCC, a relative lack of systemic complications associated with a tumor that rarely metastasizes, and the capacity to administer a drug locally. c-Jun is expressed in a range of other skin cancer types, such as SCC and melanoma, and these may potentially be amenable to Dz13 therapy. A clinical trial of Dz13 (in DOTAP/DOPE) is expected to commence in patients with cutaneous in-transit melanoma in 2013. The 28 day post-dose period of monitoring was dictated by what was regarded as an ethically acceptable duration for patients with a biopsy proven BCC to wait to have their target skin cancer or any additionally identified skin cancers excised. This was further justified by the compelling preclinical safety data, the inability to detect Dz13 systemically, and the absence of any study drug-related adverse reactions within the monitoring period.
A broader interpretation of the study results is limited by several factors. First, being a Phase I clinical trial of typical three + three design, the primary objective was to determine the safety and tolerability of Dz13 in humans, which have been validated by the current study. Although encouraging results have been obtained in relation to efficacy, further confirmatory studies are needed whereby pre-dose parameters are not the only comparator. Second, the relatively small number of patients did not allow significance testing of the drug effects. It is nonetheless reasonable for us to conclude that Dz13 had an effect across the nine patients, even though we did not demonstrate a reduction in tumor depth in all nine patients or effects at the level of c-jun mRNA. That c-jun mRNA is inherently unstable with half life of <1h that is halved by Dz13 suggests this may not be possible. Third, we did not assess levels of Dz13 in the tumor even though no plasma levels of study drug were detected. The limited amount of tissue recovered was prioritized for immunohistochemical analyses. This notwithstanding, we recently showed that Dz13 (in DOTAP/DOPE formulation) rapidly escapes from murine tumors within hours of injection. The dermal tumors in the mice were produced by injection of a cell suspension directly into the skin, and are histologically homogenous as compared with human nodular BCC that typically comprise distinct tumor nests and stroma. Nonetheless, the murine intradermal BCC tumor model in Cai et al. demonstrating growth suppression by Dz13 used a poorly differential human facial BCC (BCC-1/KMC) cell line, rather than a murine BCC. There is thus almost no possibility that Dz13 would be detected in the tumor 14 days post injection. Assessment of quantities of Dz13 present in the tumor post injection could be included as part of a Phase II trial. Fourth, tissue analysis was performed at two time points. It was only feasible to collect pre-dose biopsy and post-dose excision samples at a single time point because of ethical and practical considerations. Therefore it is difficult to interpolate the histological and immunohistochemical features of the target lesion following injection as a tumor is a dynamic environment, i.e., Dz13 may have effected changes no longer present at the 14 day post dose time point. In Cai et al. we reported that Dz13 suppressed levels of Ki-67, MMP-2, FGF-2 and VEGF-A in the murine skin tumors. This involved several administrations of Dz13 twice a week. In the present clinical study which involved only a single administration of Dz13, only two time points were examined histologically - the pre-dose and the 14 day post-dose. That no significant changes were observed in levels of Ki-67, MMP-2, FGF-2 or VEGF-A at the 14 day post dose endpoint could simply reflect the one dose of Dz13 administered by necessity in this phase I trial. Alternatively it may reflect catch up in expression levels after a transient suppression by the DNAzyme. This may be overcome in a subsequent trial by increasing the number of patients or target lesions within the same subject to allow collection of tissue samples over various times after Dz13 administration. In addition, the outcomes shown in Figure 1B and 2B suggest a dose-related effect of Dz13 on c-Jun expression and histological tumor depth at the highest dose (100µg). This would argue against a biopsy effect on drug-tumor pharmacokinetics and pharmacodynamics, since the size of the diagnostic biopsy in all patients was identical (2 mm). Additionally, to test the possibility that the biopsy procedure may affect tumour pharmacokinetics we retrieved matched archival biopsy and excision specimens from 9 patients from Tissue Pathology and Diagnostic Oncology Department, Royal Prince Alfred Hospital, processed in an identical manner as those of the 9 patients treated with Dz13. The sections were stained for c-Jun (the target of Dz13), CD3 (a pan T-cell marker) or caspase-9 (a pro-apoptotic marker). We observed no difference in expression in these molecular or cellular markers between biopsy and excision samples (Supplementary Table 5). Additional measurements using image analysis to determine the area within the BCC nests positive for each marker were concordant with the number of positive cells by manual count. Only caspase-8 and Ki-67 showed disagreement between the two analysis methods, however Ki-67 was not significantly altered by Dz13 treatment with either form of analysis. The two analysis were statistically concordant for c-Jun, caspase-9, p53, MMP-9, Bcl-2, caspase-3,
CD3+, CD4+, CD8+ and CD1a+ which showed positively stained cells to be significantly altered by Dz13 (Supplementary Tables 6 and 7). Finally, despite a favorable preclinical toxicology profile 1 we cannot rule out with absolute certainty the possibility that the immune response observed is caused by an off-target effect of Dz13.

In summary, this first-in-human, first-in-class clinical trial demonstrates that Dz13 is safe and well tolerated in BCC patients. Furthermore, our results indicate that the DNAzyme decreases expression of its target, c-Jun in treated BCC. Interventions that avoid or minimize scarring due to surgery, by Dz13 possibly debulking the tumor prior to excision could represent a future treatment option for BCC. With further development, Dz13 may be potentially useful for this and other disease settings where c-Jun regulates disease pathogenesis.

**Supplementary Material**

Refer to Web version on PubMed Central for supplementary material.

**Acknowledgments**

**Funding**

The authors thank Dr Marina Messina PhD (Datapharm Australia Pty Ltd) for helpful regulatory advice, and Dr Anne-Sophie Veillard PhD (NHMRC Clinical Trials Centre, University of Sydney), Prof Miles Davenport MBBS DPhil (UNSW Centre for Vascular Research, Sydney), Dr Jim Sockler PhD (Datapharm Pty Ltd) for statistical analysis, Varsha Tembe (Westmead Institute for Cancer Research and Melanoma Australia) for performing c-Jun mRNA RT-PCR, Trina Lum (Royal Prince Alfred Hospital Sydney) for performing some of the immunohistochemical staining and Oana Crainic (Royal Prince Alfred Hospital Sydney) for assisting in identification of case controls. The DSMB comprised Dr Nikolas K. Haass MD PhD (Dermatology, Royal Prince Alfred Hospital and Centenary Institute of Cancer Medicine and Cell Biology at University of Sydney), Prof Ric Day MBBS MD (St Vincent's Clinical School, University of New South Wales, Sydney) and Dr Hendrik-Tobias Arkenau. This work was supported by a Translational Program Grant from Cancer Institute NSW, and grants from Cancer Council Australia and NHMRC. EAC was supported by an NHMRC Medical and Dental Postgraduate Research Scholarship and LMK with an NHMRC Australia Fellowship.

**REFERENCES**


C

Pre-Dz13 c-Jun

Post-Dz13 c-Jun

Lancet. Author manuscript; available in PMC 2014 March 13.
Figure 1. Dz13 treatment decreases expression of its target protein c-Jun and induces T lymphocyte infiltration into BCC

(A) Each colored line represents an individual patient linking pre- and post-dose c-Jun immunostaining. Green circles represent 10µg Dz13, blue squares 30µg, and red triangles 100µg.

(B, D, E, F) Each point represents post-dose positive cell count for c-Jun (B), CD3 (D), CD4 (E) or CD8 (F) of an individual patient expressed as the percentage of the respective pre-dose cell count, represented as a dotted line at 100%. Straight line generated by linear regression. There were 2 points plotted for the 100µg group for CD8 due to insufficient specimen for patient 7.

(C, G, H, I) Representative photomicrographs at two separate magnifications of pre-dose and post-dose (100µg) immunohistochemical staining for c-Jun (C), CD3 (G), CD4 (H) and CD8 (I) (red/pink positive staining with blue hematoxylin counterstain).
Figure 2. Dz13 decreases histological tumor depth

(A) Each colored line links an individual patient’s pre- and post-dose tumor depth. Green circles represent 10µg Dz13, blue squares 30µg, and red triangles 100µg.

(B) Each point represents post-dose tumor depth of an individual patient expressed as the percentage of the respective pre-dose tumor depth, represented as a dotted line at 100%. Straight line generated by linear regression demonstrates apparent dose-dependency.
Table 1

Subject demographics

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>10µg(^1) (n=3)</th>
<th>30µg(^1) (n=3)</th>
<th>100µg(^1) (n=3)</th>
<th>Overall (^2) (n=9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>52 ± 8(^*)</td>
<td>54 ± 2(^*)</td>
<td>60 ± 10(^*)</td>
<td>55 ± 4(^*)</td>
</tr>
<tr>
<td>BMI(^3) (kg/m(^2))</td>
<td>23·6±1.6(^*)</td>
<td>28·5±3.4(^*)</td>
<td>28·2±2·2(^*)</td>
<td>26·8±1·5(^*)</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>Female</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Race</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caucasian</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>9</td>
</tr>
<tr>
<td>Skin Type</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Burns easily</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Tans after initial burn</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>History of SCC(^4)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>History of BCC(^5)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>9</td>
</tr>
<tr>
<td>History of Melanoma</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>BCC site(^6)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arm</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Chest</td>
<td>0</td>
<td>2</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Back</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>BCC diameter (mm)(^7)</td>
<td>9·3±0·9(^*)</td>
<td>10·7±2·9(^*)</td>
<td>9·0±2·0(^*)</td>
<td>9·7±1·1(^*)</td>
</tr>
</tbody>
</table>

Baseline characteristics of the trial participants within each \(^1\)Dz13 dose group and \(^2\)across all nine patients.

\(^3\) BMI = body mass index;

\(^4\) SCC = squamous cell carcinoma;

\(^5\) BCC = basal cell carcinoma;

\(^6\) Site of target lesion;

\(^7\) Diameter of target lesion measured dermoscopically.

\(^*\) data shown as mean ± SEM.
Table 2
Dz13 alters expression of c-Jun and proteins involved in tumorigenesis in BCC nests

<table>
<thead>
<tr>
<th>Protein Markers</th>
<th>% positive cells in BCC nests</th>
<th>% Difference</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre-Dz13(^2)</td>
<td>Post-Dz13(^3)</td>
<td>% Difference</td>
</tr>
<tr>
<td>c-Jun</td>
<td>75.0 (60.9, 78.4)</td>
<td>49.0 (43.3, 55.5)</td>
<td>−26.0 (−44.1, −15.2)</td>
</tr>
<tr>
<td>Caspase-9</td>
<td>7.0 (4.2, 10.7)</td>
<td>18.0 (17.3, 29.0)</td>
<td>150.4 (92.6, 505.5)</td>
</tr>
<tr>
<td>p53</td>
<td>83.0 (55.0, 91.9)</td>
<td>91.0 (64.0, 98.0)</td>
<td>10.4 (4.1, 20.7)</td>
</tr>
<tr>
<td>MMP-9</td>
<td>65.3 (61.3, 76.2)</td>
<td>51.7 (44.5, 64.3)</td>
<td>−22.8 (−29.4, −15.7)</td>
</tr>
<tr>
<td>Bel-2</td>
<td>92.3 (72.2, 93.5)</td>
<td>70.3 (59.7, 74.0)</td>
<td>−22.9 (−26.2, −11.0)</td>
</tr>
<tr>
<td>Caspase-3</td>
<td>3.7 (1.5, 6.7)</td>
<td>18.3 (9.5, 23.5)</td>
<td>271.7 (196.6, 1470.5)</td>
</tr>
<tr>
<td>Caspase-8</td>
<td>11.3 (4.2, 27.7)</td>
<td>19.0 (17.3, 47.8)</td>
<td>255.3 (34.2, 314.9)</td>
</tr>
<tr>
<td>Ki-67</td>
<td>39.3 (27.7, 51.2)</td>
<td>38.3 (20.0, 43.5)</td>
<td>−12.9 (−24.4, 4.4)</td>
</tr>
<tr>
<td>MMP-2</td>
<td>34.7 (26.2, 53.9)</td>
<td>24.0 (17.4, 43.8)</td>
<td>−8.8 (−45.7, 2.0)</td>
</tr>
<tr>
<td>FGF-2</td>
<td>76.3 (52.2, 79.5)</td>
<td>65.7 (58.5, 68.7)</td>
<td>−4.8 (−14.3, 10.8)</td>
</tr>
<tr>
<td>VEGF-A</td>
<td>86.3 (76.7, 96.2)</td>
<td>78.3 (68.4, 90.5)</td>
<td>−15.3 (−21.9, 4.2)</td>
</tr>
</tbody>
</table>

* indicates statistical significance.
<table>
<thead>
<tr>
<th>Protein Markers</th>
<th>% positive cells in BCC nests&lt;sup&gt;1&lt;/sup&gt;</th>
<th>% Difference&lt;sup&gt;4&lt;/sup&gt;</th>
<th>p-value&lt;sup&gt;5&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-Dz13&lt;sup&gt;2&lt;/sup&gt;</td>
<td>[78.8, 94.3]</td>
<td>[−21.9, 7.1]</td>
<td></td>
</tr>
<tr>
<td>Post-Dz13&lt;sup&gt;3&lt;/sup&gt;</td>
<td>[70.0, 88.2]</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>1</sup>Percentage cells positive in tumor nests for the specific immunomarkers indicated;

<sup>2</sup>pre-dose biopsy sample expressed as median with (interquartile range, IQR) and [95% confidence intervals];

<sup>3</sup>14 day post-dose excision sample expressed as median with (interquartile range, IQR) and [95% confidence intervals];

<sup>4</sup>percentage difference between pre- and post-dose expressed as median with (interquartile range, IQR) and [95% confidence intervals];

<sup>5</sup>statistical comparison between pre and post Dz13 treatment samples performed using Wilcoxon signed rank test;

* denotes statistically significant p-values.
Table 3
Dz13 increases lymphocyte and inflammatory cell infiltration in BCC

<table>
<thead>
<tr>
<th>Cell Types</th>
<th>% positive cells in BCC nests(^1)</th>
<th>% Difference(^4)</th>
<th>p-value(^5)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre-Dz13(^2)</td>
<td>Post-Dz13(^3)</td>
<td></td>
</tr>
<tr>
<td><strong>T Lymphocytes</strong> (CD3(^+))</td>
<td>2·0 (0·3, 2·5)</td>
<td>6·0 (3·4, 7·4)</td>
<td>166·7 (100·0, 1067·5)</td>
</tr>
<tr>
<td></td>
<td>[0·5, 3·3]</td>
<td>[1·4, 13·1]</td>
<td>[57·3, 1064·2]</td>
</tr>
<tr>
<td><strong>(CD4(^+))</strong></td>
<td>1·7 (0·7, 2·3)</td>
<td>8·7 (4·9, 8·9)</td>
<td>411·8 (239·1, 857·1)</td>
</tr>
<tr>
<td></td>
<td>[0·9, 2·2]</td>
<td>[4·6, 10·8]</td>
<td>[198·6, 929·7]</td>
</tr>
<tr>
<td><strong>(CD8(^+))</strong></td>
<td>0·7 (0·3, 1·2)</td>
<td>4·4 (2·8, 11·8)</td>
<td>1350·0 (140·6, 2019·2)</td>
</tr>
<tr>
<td></td>
<td>[0·3, 1·3]</td>
<td>[1·1, 13·4]</td>
<td>[152·2, 2670·0]</td>
</tr>
<tr>
<td><strong>Dendritic Cells</strong> (CD1a(^+))</td>
<td>4·0 (2·5, 6·2)</td>
<td>15·0 (10·2, 28·5)</td>
<td>381·5 (101·6, 803·8)</td>
</tr>
<tr>
<td></td>
<td>[2·4, 6·9]</td>
<td>[10·3, 27·9]</td>
<td>[126·9, 799·5]</td>
</tr>
<tr>
<td><strong>B Lymphocytes</strong> (CD20(^+))</td>
<td>1·3 (1·0, 2·9)</td>
<td>2·3 (2·2, 3·7)</td>
<td>53·8 (12·5, 168·1)</td>
</tr>
<tr>
<td></td>
<td>[1·0, 2·7]</td>
<td>[1·9, 3·7]</td>
<td>[−18·5, 226·5]</td>
</tr>
<tr>
<td><strong>Macrophage</strong> (CD68(^+))</td>
<td>2·3 (1·0, 3·4)</td>
<td>4·3 (1·3, 6·0)</td>
<td>160·9 (−60·9, 230·4)</td>
</tr>
<tr>
<td></td>
<td>[1·2, 3·4]</td>
<td>[2·0, 5·8]</td>
<td>[−11·7, 267·2]</td>
</tr>
</tbody>
</table>

\(^1\) Percentage cells positive in tumor nests for the specific immunomarkers indicated;  
\(^2\) pre-dose biopsy sample expressed as median with (interquartile range, IQR) and [95% confidence intervals];  
\(^3\) 14 day post-dose excision sample expressed as median with (interquartile range, IQR) and [95% confidence intervals];  
\(^4\) percentage difference between pre- and post-dose expressed as median with (interquartile range, IQR) and [95% confidence intervals];  
\(^5\) statistical comparison between pre and post Dz13 treatment samples performed using Wilcoxon signed rank test;  
\(^*\) denotes statistically significant p-values.