

## University of Dundee

The effect of topical diclofenac 3% and calcitriol 3 µg/g on superficial basal cell carcinoma (sBCC) and nodular basal cell carcinoma (nBCC)

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1   **Abstract**

2   **Background** Non-steroidal anti-inflammatory drugs and vitamin D derivatives can target signaling  
3   pathways activated in Basal Cell Carcinoma (BCC).

4   **Objective** We investigated the efficacy of topically applied diclofenac sodium 3% gel, calcitriol 3µg/g  
5   ointment and a combination of both in superficial (sBCC) and nodular (nBCC).

6   **Methods** Patients with a primary, histologically proven sBCC (n=64) or nBCC (n=64) were randomized  
7   to topical diclofenac, calcitriol, combination of both or no topical treatment (control group). After  
8   self-application twice daily under occlusion (8 weeks), tumors were excised. Primary outcome: post-  
9   treatment expression levels of proliferation (Ki-67) and anti-apoptosis (Bcl-2) immunohistochemical  
10   markers. Secondary outcomes: histological clearance, adverse events, application-site reactions,  
11   patient compliance.

12   **Results** sBCCs treated with diclofenac showed a significant decrease in Ki-67 ( $p < 0.001$ ) and Bcl-2  
13   ( $p = 0.001$ ), and after combination therapy for Ki-67 ( $p = 0.012$ ). Complete histological tumor regression  
14   was seen in 64.3% ( $P = 0.0003$ ) of sBCCs (diclofenac) and 43.8% ( $P = 0.007$ ) of sBCCs (combination  
15   therapy) compared to 0.0% of controls. No considerable changes were found in nBCCs. Application-  
16   site reactions were mostly mild to moderate.

17   **Limitations** The small sample size.

18   **Conclusion** Our results suggest that topical diclofenac is a promising new treatment for sBCC. Its  
19   mode of action differs from available non-invasive therapies, and thus has an additive value.

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## Introduction

Non-melanoma skin cancer (NMSC) is the most common cancer among Caucasians. Sporadic basal cell carcinoma (BCC) accounts for 80% of all NMSCs and nodular (nBCC) (40%) and superficial BCC (sBCC) (18-31%), are generally considered to be low-risk tumors.<sup>1</sup> Surgical excision, the gold standard treatment, has cure rates of 95-98%.<sup>2, 3</sup> However, because of better cosmetic outcome and lower healthcare costs, non-invasive treatment modalities such as photodynamic therapy (PDT), imiquimod (immune-modulating) and 5-fluorouracil cream (chemotherapeutic) are frequently prescribed, for sBCCs<sup>4</sup> with tumor-free survival rates of respectively 72.8-84.0% , 83.4-87.3% and 80.1%.<sup>4,5</sup>

Current research on BCC focuses on treatments that specifically target key signaling pathways required for tumor growth. The Sonic Hedgehog (SHH) signaling pathway is involved in the pathogenesis of essentially all sporadic BCCs<sup>6</sup> and crosstalk with canonical Wingless (WNT) signaling is described.<sup>7</sup> Both SHH and WNT pathways can either directly or indirectly serve as therapeutic targets for non-steroidal anti-inflammatory drugs (NSAIDs) and the active form of vitamin D (calcitriol ( $1\alpha,25[\text{OH}]_2 \text{D}_3$ )) (fig 1). NSAIDs were found to inhibit canonical WNT signaling in patients with familial adenomatous polyposis and are suggested to be pro-apoptotic in BCC cell lines in a cyclooxygenase-2 (COX-2) dependent and independent manner (fig 1).<sup>8-10</sup> COX-2 is highly expressed in several solid tumors, including BCC.<sup>11</sup> In a phase II clinical trial, systemic NSAIDs reduced both the number and burden of BCCs in patients with basal cell nevus syndrome.<sup>12</sup> Topically applied diclofenac induced a clinical response in the majority of the patients with actinic keratosis, which can be a precursor of squamous cell carcinoma.<sup>13</sup> Calcitriol has anti tumour effects in model systems of several human malignancies derived from prostate, ovary and lungs, which are mainly attributed to stimulation of the vitamin D receptor (VDR).<sup>14</sup> In keratinocytes, the VDR has a regulatory role in SHH and WNT signaling by acting as a tumor suppressor, reducing proliferation and differentiation and inducing apoptosis (fig 1).<sup>14</sup>

We investigated the efficacy of topical application of a NSAID, a vitamin D analogue and the combination on low-risk BCCs, by evaluating the effects on proliferation and apoptosis. Both well-

52 accepted drugs are already available for other indications.<sup>8</sup> We hypothesized that simultaneously  
53 targeting different signaling pathway elements may have a synergistic effect as suggested by several  
54 preclinical and clinical studies.<sup>15</sup>

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## Methods

### Protocol

In this phase II, single-blind, randomized-controlled intervention trial, patients from the dermatology outpatient clinic of the Maastricht University Medical Centre (MUMC), Maastricht, the Netherlands, were included between November 1<sup>st</sup> 2011 and February 15<sup>th</sup> 2013. Histologically proven primary sBCCs or (micro) nodular BCCs  $\geq 4$ mm, not located in the face or on the hairy scalp, were eligible for inclusion and were asked to participate in the trial. Mixed sBCC and nBCC were categorized according to the most aggressive component (nBCC). Patients using oral NSAIDs more than four days a week (chronic users)<sup>16</sup> or vitamin D (containing) supplements in the preceding 30 days were excluded. The local medical ethics and scientific committee approved the protocol and two following amendments. The study was performed in accordance with the Declaration of Helsinki. All participants provided written informed consent.

Enrolled patients with a sBCC or nBCC were randomly assigned to receive either topical diclofenac sodium-3% gel in hyaluronic acid 2.5% (Solaraze®, Almirall, Barcelona, Spain), calcitriol ointment 3 µg/g (Silkis®, Galderma, Rotterdam, the Netherlands) (henceforward called diclofenac and calcitriol respectively), a combination of both (combination therapy), or no medication (control group).

The primary outcome measure was the post-treatment percentage of cells expressing the immunohistochemical stains Ki-67 and Bcl-2. Ki-67, a proliferation marker, can be detected in the nuclei of proliferating cells. The proto-oncogene Bcl-2, regulating apoptosis, is overexpressed in most BCC.<sup>17</sup> Secondary outcomes were histological tumor regression, adverse events, application-site reactions and patient compliance.

### Assignment and masking

Randomization via a computer-generated random allocation scheme was stratified for histological tumor type. Random permuted blocks of eight were used to ensure concealment of allocation.

Although patients and investigators were not blinded for assigned treatment, the pathologists who assessed expression levels of Ki-67 and Bcl-2 and histological tumor regression were.

#### **Participant flow and follow-up**

The investigators provided the study medication directly after randomization. The patients applied the vehicle on the tumor with a radius of 0.5cm and covered it with an occlusive sheet (Tegaderm®, 3M, Leiden, The Netherlands) twice a day for eight weeks. This period was generally the time patients waited for surgical excision. In case of combination therapy, diclofenac gel application was followed by calcitriol ointment with a two-minute interval. Treatment was continued until the day before surgery. In case of a severe local skin reaction, surgery was postponed. Surgical excision was performed with a 3-5mm safety margin. Expression of Ki-67 and Bcl-2 was evaluated in both baseline biopsies and excision specimens. No study related follow-up visits were planned after surgical excision

Treatment reactions were evaluated by a phone interview two weeks after the start of the treatment. Secondary outcome parameters collected from a diary patients completed once a week during the course of the treatment included questions on pain expressed on a visual analogue scale, local skin reactions and compliance. Standardized photographs of all lesions were taken with a ruler and pantone color card (Danes-Picta, Praha, Czech Republic) on day one and 56. Compliance was defined as the number of actual applications as a percentage of the total prescribed number of applications.

#### **Statistical analysis**

We aimed to include 64 nBCC and 64 sBCC patients to enable comparison of the four study arms separately for sBCC and nBCC. Continuous variables were presented as a mean with  $\pm$  standard deviations (if normally distributed) or as a median with an interquartile range (if not normally distributed). Differences in proportions between groups were tested using the Fisher's exact test.

Analysis of covariance (ANCOVA) was used to compare post-treatment expression of Ki-67 and Bcl-2 between treatment groups and control group. Variables indicating treatment group and baseline expression levels of Ki-67 and Bcl-2 were entered as independent variables. The regression coefficients associated with the treatment groups represent the difference in post-treatment expression level between the corresponding treatment group and the control group. In case of skewed baseline distributions of the of Ki-67 and Bcl-2 expression levels data were log transformed to normalize distributions and normality of the distribution of residuals was checked using a normal probability plot. Statistical analyses were carried out using SPSS 20.0 software and [www.openepi.com](http://www.openepi.com). All reported P values are two-sided, and P values  $\leq 0.05$  were considered statistically significant. This study is registered as a controlled trial at [clinicaltrials.gov](http://clinicaltrials.gov), number NCT01358045, since May 17<sup>th</sup> 2011.

## Results

### Patients

All 128 included patients (64 nBCC and 64 sBCC), were randomly assigned to one of the four study arms, with equal distribution of the baseline demographics and tumor characteristics (table 1). One patient withdrew directly after treatment allocation. The primary outcome was not available for eight patients: one patient had a BCC that required treatment by Mohs' micrographic surgery, another patient had a syringoma and for six other patients, the biopsy or excision specimens did not include sufficient tumor tissue to enable immunohistochemistry for Ki-67 and Bcl-2. Subjects with missing primary outcome were evenly distributed among the treatment groups. Totally, 119 patients were included in the statistical analysis of the primary outcome (n=59 sBCC and n=60 nBCC, ). No crossovers occurred.

### Immunohistochemical analysis of proliferation and apoptosis

Figure 2 illustrates the median expression levels of Ki-67 and Bcl-2 in tumor cells before and after treatment for sBCC and nBCC in the four randomized groups. With respect to sBCCs, this figure shows that in the control group median values of endpoint Ki-67 expression levels increased slightly when compared with baseline levels. There was a substantial decrease in Ki-67 expression after treatment with diclofenac and combination therapy and a small increase in the calcitriol group. Median values in Bcl-2 expression show a small increase in the control group, whereas there was a slight decrease in tumors treated with diclofenac, calcitriol and combination treatment.

The distributions of Ki-67 and Bcl-2 expression levels were skewed and a  $\log(x+1)$  transformation was used to normalize the distributions. ANCOVA analyses were used to adjust for imbalances in baseline values of Ki-67 and Bcl-2. The mean differences between the treatment groups and the control group were back transformed from the logarithm scale to raw scale and are presented in Table 2. Ki-67 expression was significantly lower in sBCCs treated with diclofenac and combination therapy, when compared to the post treatment levels in the control group ( $p < 0.001$  and  $p = 0.012$ , respectively, table



2). Also, Bcl-2 expression was significantly lower in sBCCs treated with diclofenac ( $p=0.001$ , table 2). Post treatment expression levels in sBCCs treated with calcitriol and in all nBCCs did not differ significantly from those in the control group, neither for Ki-67 nor for Bcl-2 (Table 2).

### **Clinical response and compliance**

In the sBCC subgroup, histologically complete tumor regression was seen in 64.3% (9 of 14) and in 43.8% (7 of 16) of the tumors treated with diclofenac and combination therapy respectively (fig 3). The difference with the control group (0 of 16) was statistically significant ( $P=0.0003$  and  $P=0.007$ , respectively). None of the participants in the calcitriol group showed complete regression. In the nBCC subgroup 31.2% (5 of 16), 6.2% (1 of 16), 33.3% (5 of 15) showed histological complete regression in patients treated with diclofenac, calcitriol or combination therapy, respectively. No residual tumor was observed in 18.8% (3 of 16) of the nBCC control group (fig 3). Differences between active treatment groups and the control group were not statistically significant.

In the sBCC subgroup with no complete tumor regression, a nodular BCC component was found in four tumors assigned to the combination therapy group. In the calcitriol group two tumors with a nodular, and one with an invasive component were found.

Median compliance rates were generally high (92.7%-98.2%) and comparable between groups ( $n=95$ ).

### **Adverse events**

Adverse events were mostly mild to moderate. Erythema, pruritus and erosions at the target tumor site were most frequently reported (table 3). In eight cases the severity of the application-site reactions led to discontinuation of the therapy and prescription of a topical antimicrobial cream. In 19.4% (6 of 31) of patients treated with diclofenac and 9.4% (3 of 32) of patients treated with combination therapy, surgical excision was postponed two weeks, due to the severity of application-site reactions. Three patients had serious adverse events requiring hospitalization (table 3), but none

173 of these serious adverse events were considered to be related to the study medication. No adverse  
174 events were reported in the control group.

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## Discussion

This phase II trial provides evidence that topical diclofenac has the potential to clear sBCC, with complete histologic tumor regression in 64.3% (9 of 14) and 43.8% (7 of 16) of the patients treated with diclofenac and combination therapy, respectively. These results are in line with significant decreases of expression levels of the proliferative marker Ki-67 and the anti-apoptotic marker Bcl-2 in these treatment groups. Although, application-site reactions were reported frequently, most reactions were of mild to moderate severity, which is in accordance with the literature.<sup>18</sup> Similar reactions are seen in other non-invasive therapies for sBCC, such as imiquimod and 5-fluorouracil cream and probably necessary to achieve tumor regression.<sup>2, 4</sup> Occlusion may have attributed to the severity of the skin reactions.

There was no clinical effectiveness of calcitriol. In calcitriol treated sBCCs, slight increases in expression levels of the proliferative marker Ki-67 and slight decreases in the anti-apoptotic marker Bcl-2 were detected, which were not statistically significant. We found no evidence for the hypothesized synergistic effect of the combination of diclofenac and calcitriol. The observed clinical effect of the combination therapy in sBCC is probably due to the effect of diclofenac, but lower following dilution by the calcitriol. A relatively high ratio of nodular BCC components in tumors diagnosed as sBCC, also found in other studies, is also a possible explanation for lower efficacy.

In the nBCC subgroup, no significant effects of treatment on Ki-67 and Bcl-2 expression levels or in histological tumor clearance were found. However, when comparing post treatment expression levels of Ki-67 between the calcitriol and the control group (fig 2), calcitriol treated nBCCs had even slightly higher Ki-67 and Bcl-2 expression. Previous *in vitro* studies found that high doses of calcitriol can inhibit keratinocyte proliferation, while lower doses may stimulate proliferation.<sup>19, 20</sup> Also, topical application of low dose calcitriol to mouse skin can stimulated epidermal proliferation<sup>21</sup>, which might explain our findings in nBCC.

Excipients such as hyaluronic acid may enhance the penetrance and bioavailability of a substance.<sup>22</sup> Whereas the diclofenac sodium-3% gel contains 2.5% hyaluronic acid, calcitriol does not. Possibly, a higher concentration of calcitriol and/or a different vehicle might be needed.

Complete tumor regression was seen in 18.8% of the controls in the nBCC subgroup. As we included tumors  $\geq 4$ mm and punch biopsies were 3mm, total tumor clearance could be a result of a biopsy-induced local immune response<sup>23</sup>, or a lack of sensitivity of regular histological techniques to detect small amounts of residual tumor. "Therapeutic biopsy", could have occurred in all other treatment groups and underlines the importance of a control group. In other studies the response on non-invasive therapies also differs between nBCC and sBCC and is presumably caused by insufficient penetration of the drug into the deeper dermis.<sup>24</sup>

The clinical effectiveness of diclofenac was not as high as that of currently available non-invasive BCC treatments. However, the ability to attack different molecular pathways activated in BCC is an important finding. Evidence has already suggested that simultaneously targeting SHH and other signaling pathways may have a synergistic effect.<sup>15</sup> Combination of therapies is therefore a logical next step in improving topical treatments. Combining diclofenac with imiquimod cream could be promising, as imiquimod is known to inhibit the SHH-pathway<sup>25</sup> and is currently the most effective non-invasive therapy for BCC. However, with a 1-year efficacy of 83.4%<sup>26</sup>, it is still not as effective as surgery. By adding a drug targeting a different pathway, such as diclofenac cream, the imiquimod resistant cells could be attacked, resulting in higher long-term cure rates.

Limitations of the study are the small sample size and imbalances in baseline levels of Ki-67 and Bcl-2. Despite the small sample size, significant effects of diclofenac treatment in targeting key signaling pathways could be demonstrated and ANCOVA was used to adjust for the differences in baseline levels.

This trial provides evidence that topical application of diclofenac 3% gel in 2.5% hyaluronic acid in sBCC significantly reduces proliferation, induces apoptosis and moreover results in significant histological clearance compared to the control group. We therefore conclude that although surgical

227 excision remains the gold standard for all BCC, topical diclofenac may be a promising new treatment  
228 for low risk sBCC. Efficacy of topical calcitriol was not observed. Other trials using different  
229 concentrations, excipients or combinations of both investigated drugs may be useful to optimize  
230 treatment strategies. Also, given the effectiveness of diclofenac gel in treatment of both BCCs and  
231 actinic keratosis with only limited side effects, the role for topical diclofenac as a prophylactic agent  
232 in NMSC is an interesting subject for future studies.

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## References

1. Raasch BA, Buettner PG, Garbe C. Basal cell carcinoma: histological classification and body-site distribution. *The British journal of dermatology* 2006;155:401-7.
2. Telfer NR, Colver GB, Morton CA, British Association of D. Guidelines for the management of basal cell carcinoma. *The British journal of dermatology* 2008;159:35-48.
3. Bath-Hextall F, Ozolins M, Armstrong SJ, Colver GB, Perkins W, Miller PS et al. Surgical excision versus imiquimod 5% cream for nodular and superficial basal-cell carcinoma (SINS): a multicentre, non-inferiority, randomised controlled trial. *The lancet oncology* 2014;15:96-105.
4. Arits AH, Mosterd K, Essers BA, Spoorenberg E, Sommer A, De Rooij MJ et al. Photodynamic therapy versus topical imiquimod versus topical fluorouracil for treatment of superficial basal-cell carcinoma: a single blind, non-inferiority, randomised controlled trial. *The lancet oncology* 2013;14:647-54.
5. Roozeboom MH, Arits AH, Nelemans PJ, Kelleners-Smeets NW. Overall treatment success after treatment of primary superficial basal cell carcinoma: a systematic review and meta-analysis of randomized and nonrandomized trials. *The British journal of dermatology* 2012;167:733-56.
6. Epstein EH. Basal cell carcinomas: attack of the hedgehog. *Nature reviews Cancer* 2008;8:743-54.
7. Yang SH, Andl T, Grachtchouk V, Wang A, Liu J, Syu LJ et al. Pathological responses to oncogenic Hedgehog signaling in skin are dependent on canonical Wnt/beta3-catenin signaling. *Nature genetics* 2008;40:1130-5.
8. Barker N, Clevers H. Mining the Wnt pathway for cancer therapeutics. *Nature reviews* 2006;5:997-1014.
9. Fecker LF, Stockfleth E, Nindl I, Ulrich C, Forschner T, Eberle J. The role of apoptosis in therapy and prophylaxis of epithelial tumours by nonsteroidal anti-inflammatory drugs (NSAIDs). *The British journal of dermatology* 2007;156 Suppl 3:25-33.
10. Tjiu JW, Liao YH, Lin SJ, Huang YL, Tsai WL, Chu CY et al. Cyclooxygenase-2 overexpression in human basal cell carcinoma cell line increases antiapoptosis, angiogenesis, and tumorigenesis. *The Journal of investigative dermatology* 2006;126:1143-51.
11. Muller-Decker K. Cyclooxygenase-dependent signaling is causally linked to non-melanoma skin carcinogenesis: pharmacological, genetic, and clinical evidence. *Cancer metastasis reviews* 2011;30:343-61.
12. Tang JY, Aszterbaum M, Athar M, Barsanti F, Cappola C, Estevez N et al. Basal cell carcinoma chemoprevention with nonsteroidal anti-inflammatory drugs in genetically predisposed PTCH1+/- humans and mice. *Cancer Prev Res (Phila)* 2010;3:25-34.
13. Criscione VD, Weinstock MA, Naylor MF, Luque C, Eide MJ, Bingham SF et al. Actinic keratoses: Natural history and risk of malignant transformation in the Veterans Affairs Topical Tretinoin Chemoprevention Trial. *Cancer* 2009;115:2523-30.
14. Deeb KK, Trump DL, Johnson CS. Vitamin D signalling pathways in cancer: potential for anticancer therapeutics. *Nature reviews Cancer* 2007;7:684-700.
15. Brechbiel J, Miller-Moslin K, Adjei AA. Crosstalk between hedgehog and other signaling pathways as a basis for combination therapies in cancer. *Cancer treatment reviews* 2014.
16. Schnitzer TJ, Kong SX, Mavros PP, Straus WL, Watson DJ. Use of nonsteroidal anti-inflammatory drugs and gastroprotective agents before the advent of cyclooxygenase-2-selective inhibitors: analysis of a large United States claims database. *Clin Ther* 2001;23:1984-98.
17. Vidal D, Matias-Guiu X, Alomar A. Efficacy of imiquimod for the expression of Bcl-2, Ki67, p53 and basal cell carcinoma apoptosis. *The British journal of dermatology* 2004;151:656-62.
18. Ulrich C, Johannsen A, Rowert-Huber J, Ulrich M, Sterry W, Stockfleth E. Results of a randomized, placebo-controlled safety and efficacy study of topical diclofenac 3% gel in organ transplant patients with multiple actinic keratoses. *European journal of dermatology : EJD* 2010;20:482-8.
19. Bikle DD, Oda Y, Xie Z. Vitamin D and skin cancer: a problem in gene regulation. *The Journal of steroid biochemistry and molecular biology* 2005;97:83-91.

20. Bollag WB, Ducote J , Harmon CS. Biphasic effect of 1,25-dihydroxyvitamin D3 on primary mouse epidermal keratinocyte proliferation. *Journal of cellular physiology* 1995;163:248-56.
21. Lutzow-Holm C, De Angelis P, Grosvik H , Clausen OP. 1,25-Dihydroxyvitamin D3 and the vitamin D analogue KH1060 induce hyperproliferation in normal mouse epidermis. A BrdUrd/DNA flow cytometric study. *Experimental dermatology* 1993;2:113-20.
22. Ulrich M , Stockfleth E. Field treatment of actinic keratoses - focus on COX-2-inhibitors. *Actas dermo-sifiliograficas* 2009;100 Suppl 2:55-8.
23. Swetter SM, Boldrick JC, Pierre P, Wong P , Egbert BM. Effects of biopsy-induced wound healing on residual basal cell and squamous cell carcinomas: rate of tumor regression in excisional specimens. *Journal of cutaneous pathology* 2003;30:139-46.
24. Rippey JJ. Why classify basal cell carcinomas? *Histopathology* 1998;32:393-8.
25. Wolff F, Loipetzberger A, Gruber W, Esterbauer H, Aberger F , Frischauf AM. Imiquimod directly inhibits Hedgehog signalling by stimulating adenosine receptor/protein kinase A-mediated GLI phosphorylation. *Oncogene* 2013;32:5574-81.
26. Arits AHMM, Mosterd K, Essers BAB, Spoorenberg E, Sommer A, De Rooij MJM et al. Photodynamic therapy versus topical imiquimod versus topical fluorouracil for treatment of superficial basal-cell carcinoma: A single blind, non-inferiority, randomised controlled trial. *The lancet oncology* 2013;14:647-54.
27. Takahashi-Yanaga F , Sasaguri T. The Wnt/beta-catenin signaling pathway as a target in drug discovery. *J Pharmacol Sci* 2007;104:293-302.
28. Bijlsma MF, Spek CA, Zivkovic D, van de Water S, Rezaee F , Peppelenbosch MP. Repression of smoothened by patched-dependent (pro-)vitamin D3 secretion. *PLoS biology* 2006;4:e232.
29. Tang JY, Xiao TZ, Oda Y, Chang KS, Shpall E, Wu A et al. Vitamin D3 inhibits hedgehog signaling and proliferation in murine Basal cell carcinomas. *Cancer Prev Res (Phila)* 2011;4:744-51.



## Figure legends

Figure 1. Actions of NSAIDs and Calcitriol in Basal Cell Carcinoma: a schematic overview.

Sonic Hedgehog Pathway (green). The extracellular protein Sonic hedgehog (SHH) binds to and inhibits Patched (PTCH1), a transmembrane receptor, which relieves the inhibition of another transmembrane protein, Smoothened (SMO). SMO activates glioma-associated oncogene homolog 1 (GLI1) and GLI2, transcription factors that travel into the nucleus to activate the expression of tumor-promoting genes.<sup>6</sup> Canonical WNT signaling pathway (pink). Binding of a WNT ligand to its specific receptor complex containing a Frizzled (FZD) family member and LRP5 or LRP6 co-receptors, initiates WNT- $\beta$ -catenin signaling. Axin relocates to the LRP 5/6 tail at the membrane that is bound to WNT through its interaction with dishevelled (DVL), which forms a complex with GSK3 $\beta$  and prevents  $\beta$ -catenin ( $\beta$ -cat) degradation.<sup>8</sup> This allows  $\beta$ -catenin to accumulate and enter the nucleus, where it interacts with members of the TCF/LEF family. In the nucleus,  $\beta$ -catenin converts the TCF proteins into transcriptional activators. Suppressor of fused (SUFU) functions as a tumor suppressor by inhibiting both SHH and WNT signaling.<sup>7</sup>

NSAIDs inhibit WNT signaling by reducing nuclear  $\beta$ -catenin localization.<sup>8, 27</sup> Furthermore, NSAIDs inhibit cyclo-oxygenase-2 (COX-2), which is overexpressed in basal cell carcinoma (BCC) and catalyzes the conversion of arachidonic acid (AA) to prostaglandins (PGE2). A subsequent reduction of PGE2 and a direct down regulation of the anti-apoptotic Bcl-2 family proteins by NSAIDs induces apoptosis.<sup>9</sup> A down regulation of Bcl-2 is also induced by  $1\alpha,25(\text{OH})_2\text{D}_3$  (calcitriol), resulting in caspase cleavage leading to apoptosis. Calcitriol directly inhibits SMO *in vitro*, resulting in repression of SHH signaling. SHH-signaling is also suggested to be directly repressed by the Vitamin D Receptor (VDR) by inhibition of GLI. Finally, activation of the VDR by calcitriol induces the expression of the transmembrane protein E-Cadherin, which recruits  $\beta$ -catenin to the cell membrane and prevents translocation of  $\beta$ -catenin to the nucleus.<sup>14, 28, 29</sup>

Figure 2: Pre- versus post-treatment scatterplot of changes in Ki-67 and Bcl-2 expression.

Data are median %. Abbreviation: BCC=Basal Cell Carcinoma. Diclofenac=diclofenac sodium-3% gel, Calcitriol=calcitriol 3µg/g ointment, Combination therapy=diclofenac and calcitriol. Pre- versus post-treatment changes in Ki-67 and Bcl-2 expression after 8 weeks treatment according to treatment groups.

Figure 3: Complete histologic tumor regression rates after 8 weeks of topical treatment.

Abbreviation: BCC=Basal Cell Carcinoma. Diclofenac=diclofenac sodium-3% gel, Calcitriol=calcitriol 3µg/g ointment, Combination therapy=diclofenac and calcitriol. P-values were calculated with Fisher exact test. Complete histologic tumor regression for superficial BCC and for nodular BCC according to treatment groups, compared to control group.

**Abbreviations:**

BCC, basal cell carcinoma

sBCC, superficial basal cell carcinoma

nBCC, nodular basal cell carcinoma

SHH, Sonic Hedgehog

SMO, Smoothened

NSAIDs, non-steroidal anti-inflammatory drugs

VDR, vitamin D receptor.

