Intralesional Treatment of Basal Cell Carcinoma in a Genetically Inducible Mouse Model

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Introduction

Gorlin syndrome, also known as nevoid basal cell carcinoma syndrome, is a rare genetic disorder with a prevalence of approximately 1 in 31,000 to 60,000 people [1,2,3], therefore affecting about 11,000 people in the US. People with Gorlin syndrome are at increased lifetime risk of developing hundreds to thousands of cutaneous basal cell carcinoma (BCC) skin cancers, from a few dozen to hundreds every year. In addition, cutaneous anomalies, cysts of the jaws, abnormalities and tumors of multiple organ systems have been described in these patients [4,5,6,7].

Mutations that result in the up regulation of hedgehog (HH) signaling are associated with the development of BCCs. Mutations in the tumor suppressor gene Patched (PTCH1) are implicated in the growth of both sporadic BCCs and those that develop in Gorlin syndrome. PTCH1 is a transmembrane receptor protein that suppresses the hedgehog (HH) signaling cascade. The majority of sporadic BCCs have loss-of-function mutations in at least one allele of PTCH1, resulting in loss of repression of the HH cascade, and others have gain-of-function mutations in SMO, leading to over-activation of the pathway [8] (Figure 1).

Figure 1. Hedgehog pathway

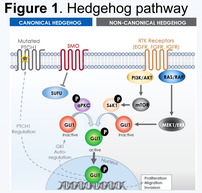
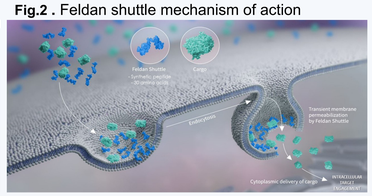


Fig.2 . Feldan shuttle mechanism of action

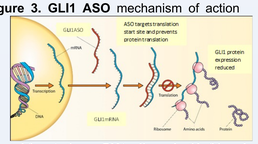


Currently, there is no cure for Gorlin syndrome. Dermatologic treatment centers on the prevention and management of cutaneous BCC tumors caused by the disease. The current treatment options consist of surgical excision, topical 5-FU and imiquimod, photodynamic therapy[9] or cryotherapy, often resulting in disfigurement. In addition, two systemic Hedgehog Pathway Inhibitors (HPI) are approved for management of either metastatic and/or locally advanced and/or recurrent and/or surgically ineligible cutaneous BCC. These FDA approved orally administered HPI are small molecules that target transmembrane components of the pathway, such as the protein SMO. Although these inhibitors effectively suppress HH signaling, early tumor resistance illustrates the need for treatments that target proteins located downstream in the pathway [10,11]. In addition, these products are frequently associated with troublesome side effects when used long term [12,13], resulting in discontinuation of treatment in up to 70% of patients by 12 months [14].

In addition to certain mutations in the SUFU protein, located downstream to the PTCH1 and SMO proteins, non-canonical signaling also can lead to the activation of the GLI1 protein and therefore of the HH pathway. This results in the development of BCC tumors that are unresponsive to traditional hedgehog inhibitors targeting SMO protein activity. GLI transcription factors play a central role in the intracellular signaling cascade as they are the primary mediators of the HH signaling pathway. As several authors have shown in recent studies, the downstream inhibition of GLI1 in tumor therapy is more effective than the canonical upstream inhibition of SMO [15,16]. GLI1 is therefore a target of great interest in the development of therapies against HH-dependent cancers, such as BCC. Several inhibitors targeting GLI1 are currently under development [3]. Feldan has selected this target, via the intracellular delivery of a GLI1-specific antisense oligonucleotide (ASO) (Figure 3).

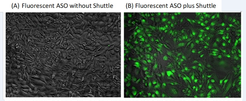
The Feldan Shuttle, a second-generation cell penetrating peptide (CPP), is a 30 amino acid amphiphilic peptide developed by Feldan to deliver different types of cargo of therapeutic interest into the cytoplasm of cells (Figure 2 and 4). Unlike first-generation CPPs, the Feldan shuttle's unique characteristics facilitate release of endosomal contents into the cytoplasm. In conjunction with rapid catabolism of the shuttle peptide itself this results in any significant effect being strictly due to the intracellular presence of the cargo.

Figure 3. GLI1 ASO mechanism of action



Adapted from Antisense_DNA_oligonucleotide (biomedcentral.com)

Figure 4. Intracellular delivery of a labeled ASO



(A) Without Feldan Shuttle the fluorescent ASO does not enter the HeLa cultured cells and is washed away. (B) With the Feldan Shuttle the fluorescent ASO enters and remains in the cells. (Echo Revolve microscope, Digital zoom 1.0, Objective 10X, Overlay of TRANS and FITC channels).

Objective

Our objective was to evaluate an intralesional treatment for BCC based on the intracellular delivery of an ASO targeting GLI1 using the Feldan shuttle technology. To test the efficacy of our treatment, we used our inducible NBCCS-like mouse model, genetically modified at the level of the expression of the PTCH1 and p53 genes in the skin.

Methods

The inducible mouse model uses PTCH1- and P53-knockdown mice treated with tamoxifen and doxycycline, resulting in repression of the PTCH1 and P53 proteins specifically at the level of the skin. Induced mice were then irradiated with UVB three times a week until BCCs appeared, between 16 to 28 weeks (Figure 5 and 6). Some of these BCCs were treated twice a week with injections of the ASO targeting the production of the GLI1 protein, with and without the use of the "Feldan Shuttle" peptide and others only once a week with the ASO and Feldan shuttle peptide combination (FLD103), composed of Feldan Shuttle (0.05%) and GLI1 ASO (0.05%), the process resulting in the introduction of the ASO into the tumor cells. Injections were performed using a syringe fitted with a 28G needle and 25 µl of either formulation in sterile phosphate buffered saline (PBS). The evolution of the tumors was monitored several times a week with caliper measurements and photography. Following the treatments, mice were sacrificed, and the tumor site tissue was harvested and analyzed by both routine (H&E) and specialized (IHC, IF) histologic methods.

Figure 5. Lesions on induced NBCCS mice

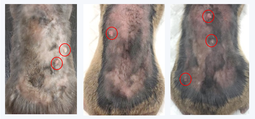
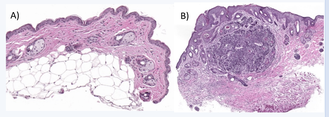
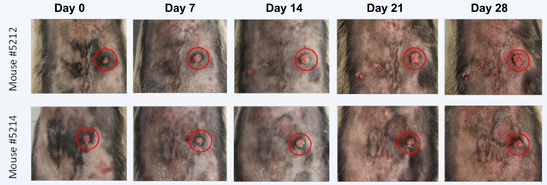


Figure 6. H&E of (A) non-induced NBCCS-like mouse skin (normal) and (B) a BCC in an induced NBCCS-like mouse



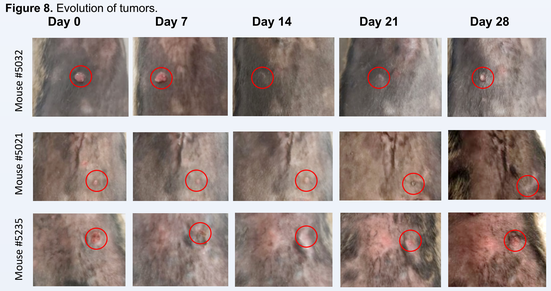
Results

Figure 7. Evolution of tumors.



Evolution of tumors during twice a week intralesional injection with 25 µl of GLI1 ASO (0.05%) alone (red circles identify treated lesions)

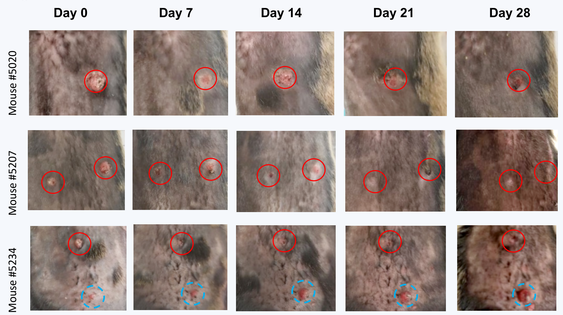
Figure 8. Evolution of tumors.



Evolution of tumors during weekly intralesional injection with 25 µl of FLD103 (red circles identify treated lesions)

Results

Figure 9. Evolution of tumors



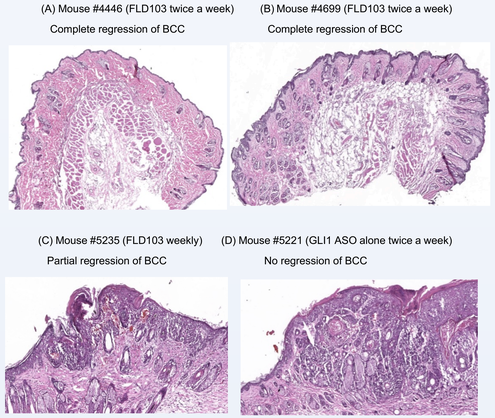
Evolution of tumors during twice a week intralesional injection with 25 µl of FLD103 (red circles identify treated lesions and blue circles identify untreated lesion)

Table 1. Overall responses to intralesional injection after four weeks of treatment, based on analysis of images and caliper measurements

	GLI1 ASO alone twice a week (n=4)	FLD103 weekly (n=10)	FLD103 twice a week (n=12)
Complete	0	1	5
Partial Response	0	4	6
Stable	1	2	0
Progression	3	3	1
Overall Response (%)	0%	50%	92%

n = no. of BCC lesions

Figure 10. H&E of lesions after 28 days of intralesional treatment twice a week



Conclusion

We observed that intralesional treatment with the ASO alone is not associated with tumor regression and in fact permits continued tumor growth (Fig.7 and Fig. 10 (D)).

However, intralesional treatment of BCCs in the inducible mouse model with the GLI1 ASO and Feldan Shuttle peptide combination (FLD103) resulted in rapid and marked tumor regression, and sometimes complete eradication, over a few weeks (Fig 10 (A), (B) and (C)).

Healthy perilesional skin in the mouse model appears unaffected by the treatment, demonstrating targeted treatment specificity. The healed skin appeared grossly normal. After recovery, histologic examination of treated sites showed partial BCC regression in some (Fig.10 (C)), and no residual BCC in most. Areas with no residual tumor showed complete healing of the epidermis, dermis and subcutaneous tissue and no obvious scarring (Fig.10 (A) and (B)).

Twice a week intralesional treatment of mouse tumors (Fig.9) seems more effective than weekly treatment (Fig.8 and table 1) in these pre-clinical studies. It should be noted however that the tumors of these NBCCS-like mice show faster growth compared to those observed in humans. This mouse model has a lesion doubling time of about 10 days compared to approximatively 150 days in humans [17]. Based on the data presented herein and this difference in BCC doubling times, twice a month or monthly intralesional treatment with FLD103 could be effective in treating human nodular BCCs, which are less aggressive than those seen in the NBCCS-like mouse model.

We have demonstrated the utility of a specific intralesional treatment for BCC in a genetically inducible mouse model. This alternative and novel treatment produces rapid results with normal healing of the treated site. This treatment could offer an attractive option for patients with multiple BCCs who wish to avoid systemic treatments or multiple surgeries.

References

(1) Gold NB et al. Sci Rep. 2021 Oct 5;11(1):19791. doi: 10.1038/s41598-021-98752-9

(2) Juan HY et al. Arch Dermatol Res (2022). https://doi.org/10.1007/s00403-022-02447-8

(3) Witmanowski H et al. Adv Dermatol Allergol 2017; XXXIV (4): 381–387

(4) Gorlin, RJ. Medicine (Baltimore). 1987 Mar; 66(2):98-113

(5) Evans DG, Ladusans EJ, Rimmer S et al. J Med Genet 1993; 30:460–4

(6) Kimonis VE et al. Am J Med Genet 1977; 69:299–308

(7) Bree AF et al. Am J Med Genet A 2011; 155A:2091–7

(8) Bakshi A et al. Mol Carcinog 2017 Dec; 56(12): 2543–2557

(9) Basset-Seguin et al. J Eur Acad Dermatol Venereol 2014 May; 28(5):626-32

(10) Ng JM, Curran T. Nat Rev Cancer. 2011;11(7):493-501.

(11) Peer E, Tesanovic S, Aberger F. Cancers (Basel). 2019; 11(4):538

(12) Sekulic A et al. PLoS One. 2022 Jan 14; 17(1)

(13) Sekulic A et al. J Am Acad Dermatol 2015; 72:1021-6

(14) Migden MR et al. Cancer Treatment Reviews 64 (2018) 1–10

(15) Sabol M et al. Int J Mol Sci 2018; 19(9):2562.

(16) Pandolfi, S., & Stecca, B. Expert Reviews in Molecular Medicine, 17, E5. doi:10.1017/erm.2015.3

(17) Khoo ABS et al. Acta Derm Venereol 2019; 99: 1266–1269

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