**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. It affects about 11,000 people in the US. Patients 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 and tumors of multiple organ systems have been described in these patients.

Malignations result in the up regulation of hedgehog (HH) signaling are associated with the development of BCCs. Mutations in the PTCH1, SUFU, and GLI1 genes are implicated in the growth of both sporadic and familial BCCs that develop in patients with Gorlin syndrome. PTCH1, known as the primary antagonist of HH signaling, is an integral membrane protein that signals in loss of repression of the HH cascade, and three other genes have gain-of-function mutations in SMO, leading to over-activation of the pathway (Figure 1).

Currently, there is no cure for Gorlin syndrome. Dermatologic treatment centers on the prevention and management of cutaneous BCCs tumors caused by the disease. The current treatment option consists of surgical excision, topical 5-FU and imiquimod, photodynamic therapy or cryotherapy, often resulting in dermalization. In addition, two systemic Pathway Inhibitors (IPI) are approved for management of either metastatic and/or locally advanced and recurrent and/or surgically inoperable cutaneous BCCs. These FDA approved only administered HHs are small molecules that target transmembrane components of the pathway, such as the protein SMO. Although these in vitro treatments affect HH signaling, early tumor histologic studies note the need for treatments that target proteins located downstream in the pathway (Figure 2). In addition, these proteins are frequently associated with tissue-based side effects when used long-term, resulting in discontinuation of treatment in up to 70% of patients by 12 months.

In addition to certain mutations in the SUFU protein, located downstream in the PTCH1 and SMON proteins, non-canonical signaling also can lead to the activation of the GLI-2 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.

In recent studies, the down-regulation of GLI1 in tumor tissue is more effective than the canonical upstream inhibition of SMO (Figure 3). Therefore, it is a logical target of interest in the development of therapies targeting HH-dependent cancers, such as BCC. Several inhibitors targeting GLI1 are currently under development (Field). Feldan has selected this target, via the intracellular delivery of a GLI1-specific antagonist, adenosine/concentrins (Figure 4).

**Objectives**

Our objective was to evaluate an intratumoral treatment for BCC based on the 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-inducible mice treated with the development of BCCs. Mutations in the PTCH1, SUFU, and GLI genes are implicated in the growth of both sporadic and familial BCCs that develop in patients with Gorlin syndrome. PTCH1, known as the primary antagonist of HH signaling, is an integral membrane protein that signals in loss of repression of the HH cascade, and three other genes have gain-of-function mutations in SMON, leading to over-activation of the pathway (Figure 1).

**Results**

The evaluation of tumors during treatment with the intratumoral injection of an ASO targeting GLI1 using the Feldan shuttle technology showed complete regression of the tumors. In the untreated control, a partial regression of the tumors was observed, and the tumor tissue was harvested and analyzed by both routine (H&E) and specialized (IHC, IF) histologic methods.

**Conclusion**

We observed that intratumoral treatment with the ASO alone is not associated with tumor regression and in fact permitted continued tumor growth (Fig 7 and Fig 10). However, intratumoral treatment of BCCs in the inducible mouse model with the GLI1 ASO and Feldan Shuttle peptide combination (FDD103) resulted in rapid and marked tumor regression, and sometimes complete eradication, over a few weeks (Fig 10 A, B, C and D).

Healthy peripheral skin in the mouse model appears unaffected by the treatment, demonstrating that treatment with the intratumoral injection of an ASO targeting the production of the GLI1 gene, 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 (FDD101) and GLI1-ASO (FLD105), the procedure resulting in the introduction of the ASO into the tumor cells. Injections were performed using a syringe fitted with a 27G needle and 25 µl of dilution formulation in sterile phosphate buffered saline (PBS). The evolution of the tumors was monitored several times a week with upper measurements and photography. Following the treatments, mice were sacrificed, and the tumor tissue is harvested and analyzed by both routine (H&E) and specialized (IHC, IF) histologic methods.

**References**

4. Feldan Therapeutics, Quebec, PO, Canada, 2. TransBioTech, Levis, PO, Canada, 3. Dermatology Consultant to Feldan Therapeutics – Department of Medicine, University of Ottawa, Canada.