Recent phase I and II clinical trials have shown promise for the use of inhibition of the hedgehog pathway for the treatment of two rare but highly morbid forms of basal cell carcinoma (BCC): locally advanced (laBCC) as well as metastatic (mBCC). Basal cell carcinoma is the most common type of cancer in Caucasians, and worldwide incidence increases annually by approximately three to 10 percent. Local surgical procedures, topical chemosurgery, photodynamic therapy, or radiation are used to cure most cases of BCC. Nevertheless, in some instances, BCC may metastasize or locally progress to advanced stages, which are life-threatening, unresectable, or only resectable by mutilating and/or deforming procedures. The estimated rate of BCC metastasis is 0.03 percent per year with the majority of cancers localizing to regional lymph nodes. In addition, BCC may metastasize to lung and bone. No currently available treatment successfully treats BCC that is locally advanced (laBCC) or metastatic (mBCC). Since the median survival time of mBCC is eight months, the need for a successful treatment is imperative.

The Hh Pathway and Vismodegib (GDC-0449)

The hedgehog (Hh) pathway is essential for normal embryonic development but remains mainly dormant in most tissue of healthy adults. Adult hair follicles and tongue taste buds are among the tissues that remain dependent on Hh. However, in the case of BCC and medulloblastomas, Hh signaling becomes active. Data suggest that activation of the SHH pathway occurs in about 90 percent of acquired BCC. Additionally, activation occurs in conjunction with mutation of SHH pathway proteins Ptch1, Ptch2 and/or SUFU in patients with Basal Cell Nevus Syndrome (BCNS, or Gorlin’s syndrome).

The Hh pathway begins with the binding of Sonic (SHH), Desert (DHH), or Indian (IHH) ligand to the Patched (PTCH1) receptor. This interaction alleviates the repression of SMO by PTCH1, in turn promoting calcium dependence of the SHH pathway and theoretical implications in oral treatment of locally advanced or metastatic basal cell carcinomas.
activation of GLI transcription factors. These factors facilitate transcription of Hh target genes. In a phase I clinical trial of GDC-0449, Von Hoff, et al. (2009) reported high levels of GLI1 mRNA expression in tumors from patients with advanced BCC. These findings are consistent with activation of the Hh pathway.

Vismodegib (GDC-0449) is a small molecule inhibitor of smoothered (SMO), which functions by inhibiting Hh signaling. The drug has shown promising antitumor activity in patients with advanced BCC. Overall tumor response rates for patients with mBCC and lBCC were approximately 50 and 60 percent, respectively. However, accompanying side effects to treatment with vismodegib dishearten patients who continue treatment with GDC-0449. Side effects included fatigue, hyponatremia, muscle spasm, dysgeusia, atrial fibrillation, and hair loss. Efforts to mitigate these adverse effects must begin with an understanding of their underlying cause. We would like to suggest and discuss the important role calcium plays in the function of Hh signaling along with its possible role in the manifestation of adverse effects and resistance to vismodegib.

**SHH Pathway and Calcium**

The SHH pathway may well be dependent upon calcium. Heo, et al. (2007) have shown that SHH induced an increase in intracellular calcium in mouse embryonic stem cells. This calcium influx promoted subsequent cell proliferation by collaboration of calcium and protein kinase C (PKC) as well as GLI1 activation. SHH additionally activated matrix metalloproteinases (MMP) to stimulate the epidermal growth factor receptor (EGFR), contributing to downstream cell cycling activity. Upon binding of epidermal growth factor (EGF) to its receptor, calcium is mobilized from both an extracellular medium through kinase-activated calcium channels and through inositol trisphosphate (IP3)-mediated release of intracellular calcium store.

Another report identified an intracellular calcium spike in developing spinal neurons after SHH activation of coreceptor SMO. Of note, the degree of extracellular calcium may modulate the SHH pathway. In rat gastric mucosal cells, SHH failed to activate extracellular signal-regulated kinases under calcium-free culture conditions. Contrastingly, cells in a calcium-rich media had prolonged intracellular calcium levels compared to calcium free media. These accounts might indicate that extracellular calcium is necessary to initiate the SHH pathway. By implication, decreases in extracellular calcium may lead to disruption of the pathway.

As might be expected, data support that the ratio of extracellular to intracellular calcium may lead to SHH antagonist drug resistance. Normal physiologic extracellular calcium concentration is about 1.3 mM. Under these conditions, physiologic SHH regulatory antagonists such as hedgehog interacting-protein (Hhip) will fully occupy the binding sites on SHH, rendering therapeutic drug non-efficacious. In one trial, novel SHH-antagonist 5E1 required a 1,000-fold higher serum concentration than the SHH receptor affinity for one week before therapeutic effect was noted. This appears to be consistent with vismodegib observations. With once per day dosing at 150mg for 11 days, the fraction of unbound vismodegib was elevated in a 3:1 ratio compared to a single dose of study drug. Once and thrice per week dosing regimens were associated with declines in unbound steady-state vismodegib concentrations.

Calcium-Dependent Adverse Effects

Fluctuation in calcium levels may well contribute to the adverse effects seen in patients treated with vismodegib. Such adverse effects include dysgeusia, hair loss, muscle spasms, and hyponatremia. What appears of interest is that these side effects may all be dependent on calcium flux.

Given the known importance of calcium in generation of muscle contraction and neurotransmitter signaling, it seems intuitive that flux in calcium levels would manifest in muscular involvement. The role of calcium in muscle spasms is demonstrated by the use of quinine and gabapentin treatments. Anticonvulsants such as gabapentin and pregabalin exert their effect by binding to the δ-subunit protein of the calcium channels located in the brain and spinal cord. As a result, the drugs inhibit the release of excitatory neurotransmitters involved in the production of pain. Additionally, in skeletal muscle, gabapentin binds the dihydropyridine receptor (DHPR), an L-type calcium channel. DHPR is a voltage gated channel involved in the transport of extracellular calcium such that its activation brings extracellular calcium into the skeletal muscle, activating a series of events leading to release.
of the cell’s intracellular calcium stores from the sarcoplasmic reticulum. Drugs that inhibit the transport of extracellular calcium into the skeletal muscle may be able to prevent muscle spasm. Such ability suggests that the muscle spasms experienced with vismodegib treatment may be the result of rises in extracellular calcium levels.

Additionally, hair loss experienced with vismodegib treatment can be accounted for by changes in calcium level. Hair growth on the scalp occurs in a calcium dependent manner involving adenosine A1 receptor. As such, treatment with minoxidil induces proliferation of dermal papilla cells which contribute to growth at hair follicles. Minoxidil is an ATP-sensitive potassium channel opener which induces cell proliferation with an increase in intracellular calcium and vascular endothelial growth factor. Li, et al. (2001) demonstrated that the ability of minoxidil to increase intracellular calcium in dermal papilla cells was inhibited by application of an agonist for the adenosine A1 receptor. This finding highlights the dependence of calcium signaling on the G-protein coupled receptor adenosine A1 receptor.

Interestingly, the adenosine A1 receptor is allosterically regulated by extracellular calcium such that elevated calcium increases the affinity of agonist-receptor binding. Increased binding of agonist to the adenosine A1 receptor may invariably lead to desensitization, possibly via uncoupling by β-arrestin.

In addition, hair remains dependent on the SHH pathway. Vismodegib may well block the pathway, creating an imbalance in the calcium hemostasis, resulting in the hair loss observed.

Concordantly, the adenosine A1 receptor also plays an important local role in perception of taste. Therefore the dysgeusia experienced by patients treated with vismodegib may also be explained by calcium effects on the adenosine A1 receptors. One report suggests that dysgeusia is related to increased intracellular calcium. Since the tongue remains dependent on SHH pathway; vismodegib may well inhibit this pathway leading to increased intracellular calcium. The resultant extracellular calcium may well ‘wash away’ in the saliva, thus possibly reducing the feedback inhibition of the inhibitor.

Vismodegib, as well as other Hh inhibitors are associated with hyponatremia, an effect that is reversible with temporary discontinuation of the drug. These Hh inhibitors induce cell death by shifting these cells from apoptosis induced by tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) Type II to TRAIL Type I apoptosis. This type of apoptosis involves a sodium calcium exchange pathway. In response to the cell injury, calcium increase intracellularly. Sodium then enters the cell as calcium is exchanged to the extracellular spaces. The influx of sodium is associated with the ultimate apoptotic cell death. Thus, one may conclude that this process will lead to excess calcium associated with the side effects above as well as a loss of sodium. If there is enough tumor induced apoptosis then the shifts in sodium from extra-cellular to intracellular may account for the hyponatremia.

Correcting Calcium Imbalance
The calcium-induced outcomes brought about by vismodegib therapy may be corrected on a case-by-case basis regarding a particular side effect. For instance, as described above, a transport inhibitor of extracellular calcium into skeletal muscle, such as gabapentin, may be able to prevent muscle spasm. Dependence of hair growth upon appropriate intracellular/extracellular mineral levels suggests that patients experiencing hair loss with vismodegib therapy may benefit from treatment with zinc pyrithione (an active ingredient based in specific shampoos) and minoxidil (topical), which shift mineral ion balance.

The issue of combating dysgeusia may be more complex. The literature points out that the calcium-dependent adenosine A1 receptor plays a role in sensory taste. It is also presently known that taste disorders have been linked to zinc deficiencies. One study found that exposure of salivary gland cells to ionic zinc propagated the release of intracellular calcium from cell stores with the subsequent stimulation of zinc receptors but only in a co-dependent manner where both intracellular calcium and zinc were required for activation. This implies that zinc administration may well counter dysgeusia, such as that induced by the calcium-dependent effects of vismodegib, as this use for zinc is well documented in current literature. Epidemiologic studies point to drug-induced mechanisms as the number one cause of dysgeusia, so it is not surprising that decrease of taste-sensation is related to vismodegib in those being treated.

Further Study
Pathways involving Ca+ and maintenance of its intracellular and extracellular levels may be implicated to play an integral role in the mechanism of vismodegib resistance and adverse effect with vismodegib treatment. These findings beg the questions of how vismodegib disrupts calcium homeostasis and how adverse effects can be corrected. This article lays the ground-
work for the exploration of further compounding factors.

Among these are the roles of PAKinase and Gli transcription. In non-cancerous cells PAKinase, along with Slimb, phosphorylate and cleave Ci which represses transcription of Gli genes. Gli transcription involves zinc finger protein transcription factors, such that disruption in mineral balance may influence zinc finger proteins.

Extending view to malignancies other than BCC and BCNS, prostate cancer has been shown to be SHH dependent only in a hypoandrogenic state. In this hypoandrogenic state, as exists in castration-resistant prostate cancers, cell migration and invasiveness were shown to be dependent on cation channel mediation of basal intracellular calcium. Clinical trials are ongoing to determine whether or not the addition of SHH inhibitor will positively affect the therapeutic benefit of anti-androgens in advanced prostate cancer (www.clinicaltrials.gov).

When considering the systematic effects of vismodegib, it becomes important to look at the big picture implication of calcium-dependent pathways throughout the body. The mechanism or mechanisms targeted by vismodegib which lead to adverse effects seem to be active in a range of body systems. Avenues of further study thus include: absorption of Ca+ and involvment of Vitamin D, interaction of calcium with other metalloproteins or cations, use of Ca+ channel blockers, activity of the Na+/Ca+ transporter on the plasma membrane and function of the Ca+/ATPase sequestering intracellular calcium ions.

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A CLOSER LOOK: Inside Incidence Data

Lead time bias occurs when two or more different detection methods are compared, producing some changes in apparent progression or survival rates over time. In recent decades dermatologists, perhaps aided by clinical tools such as the dermoscope as well as improved patient self-surveillance, may be identifying melanoma at its earliest stages. Lead time bias does not suggest that physicians are simply finding cases that would have been otherwise overlooked (i.e., more cases overall); they are identifying cases earlier. Lesions that eventually would be discovered anyway are being discovered earlier, producing a statistical anomaly defined as “lead time bias.” Lead time bias may produce a slight surge in new diagnoses for a certain period following the introduction of a new diagnostic tool/approach. Clinicians would be identifying all the cases they otherwise would have diagnosed plus making earlier diagnoses (otherwise delayed for months or years) with the new tool. As such, with a consistent increase in incidence seen over several decades and a failure to see a significant drop in the frequency of thick melanomas, lead time bias doesn’t seem to explain the current “melanoma epidemic.”

Length time bias refers to a selection bias that can become evident when data are followed for an arbitrarily defined period of time. Increased surveillance of a given population results in detection and excision of increasingly less aggressive lesions. This leads to a shift, where increasingly earlier lesions of questionable biologic significance are detected. Length time may be particularly problematic in the study of cancers, as variable rates of progression may lead to misrepresentation of certain cancers or types of cancers in prevalence studies. We would expect to see increased diagnosis of primarily thin melanomas, which, in fact, has been suggested worldwide.