Management of Cutaneous Neurofibroma: Current Therapy and Future Directions

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Abstract

Neurofibromatosis Type I (NF1) is a life-long neurocutaneous disorder characterized by a predisposition to tumor development, including cutaneous neurofibroma (cNF), the hallmark of the disease. cNF is a histologically benign, multicellular tumor formed in virtually most individuals with NF1. It is considered the most burdensome feature of the disorder due to their physical discomfort, cosmetically disfiguring appearance, and psychosocial burden. Management of cNF remains a challenge in the medical field. Effective medicinal treatment for cNF does not exist at this time. Trials aimed at targeting individual components of the neoplasm such as mast cells with Ketotifen have not shown much success. Physical removal or destruction has been the mainstay of therapy. Surgical removal gives excellent cosmetic results but risk general anesthesia and may require trained specialists. Destructive laser such as CO_2 laser is effective at treating hundreds of tumors at one time but have high risk of scarring hypopigmentation or hyperpigmentation that alter cosmetic outcomes. A robust, low-risk surgical technique has been developed which may be performed in clinic using traditional biopsy tools that may be more accessible to NF1 patients worldwide than contemporary techniques including ER:Yag or Nd:Yag lasers. In this review, specific recommendations for management of cutaneous neurofibromas are made based on symptoms, clinical expertise, and available resources. Additionally, anti-proliferative agents aimed at stimulating cellular quiescence are explored.

Introduction

Neurofibromatosis Type I (NF1) is a neurocutaneous disorder characterized by the loss of NF1 (Neurofibromin) tumor suppressor gene due to a de novo mutation or through autosomal dominant inheritance[1]. The genetic alteration leads to a diverse spectrum of manifestations that can be clinically diagnosed by at least two or more of these features of 1) six or more café-au-lait macules 2) two or more neurofibromas or one plexiform neurofibroma 3) freckling in the axillary or inguinal region 4) Lisch nodules (iris hamartomas), 5) optic gliomas, and 6) osseous lesions[2].

Neurofibromas, both cutaneous (dermal) neurofibroma and plexiform neurofibroma, arise from the biallelic loss of NF1 in Schwann cells lineage[1,3,4]. The cutaneous neurofibroma is a neoplasm of peripheral nerve Schwann cells that presents as a soft nodule in the dermis of the skin at virtually any location in the body[5]. The plexiform neurofibroma occurs in more than 30% of those with the NF1 but confers risk to transformation into malignant peripheral nerve sheath tumor which portends a poor 5-year survival prognosis[6]. On the other hand, the cutaneous neurofibroma (cNF) is present in more than 95% of those with the disease as 2mm-3cm, soft, skin-colored nodules covering the skin to the order of tens to thousands[7]. They are histology benign and are made up of many cell types without risk of malignant transformation[8].

Despite their benign nature, people with NF1 consider cNF to be the most burdensome feature of the disease. Neurological symptoms include irritation, pain, and itching[7]. Improper drying after wetting may lead to other complications including maceration, skin breakdown and superficial infections. Physical disfigurement occurs due to the hundreds to thousands of the cNF

that can be present upon one individual[9]. Evidence links cNF to lower quality of life due to feelings of embarrassment, interference with daily activities including shopping, trouble with affection towards partners, sexual difficulties, and adverse social implications. People with NF1 may suffer from lower socioeconomic status as a result of their lower self-esteem and risk aversion and half of those with NF1 suffer from major depressive disorder likely contributed by their cNF burden[10].

The biology of cutaneous neurofibroma is complex that composed of multiple cellular components in a disorganized interaction with extracellular matrix[5,11,12]. A nerve is a necessary component for proliferation, development, and maintenance of NF1 deficient Schwann cells through the perineural microenvironment that releases factors such as Neurogulin 1 (NRG1)[11]. Immune cells are essential constituents of cNF development. Specifically, mast cells are histological hallmarks of cNF and are recruited into the cNF through kit-receptor activation leading to its migration[13]. Mast cell degranulation (through trauma or other mechanisms) releases histamine, serotonin, TGF-b and other neurotransmitters may be important to cNF development and maintenance[14]. Macrophages, the phagocytic leukocytic immune cells, are also present in cNF but their function in propagation of pathology is currently unknown. Fibroblasts are present in abundance in the cNF and react to TGF-b from mast cells with deposition of excessive, disorganized collagen and continual reorganization[15]. Importantly, these neurofibroma-associated fibroblasts contain separate properties to their fibroblast counterparts in keloids or scar tissues by lacking classical markers such as smooth-muscle actin[16]. Other cell types including keratinocytes, melanocytes, and adipocytes are present around cNF but not found to be necessary for driving their development[5]. Although the mechanism of pathogenesis is not

completely understood, the primary theory is maladaptive response to molecular or physical trauma through hyperactive immune response and excessive fibrosis in the setting of NF1 tumor suppression inactivation in the neoplastic Schwann cells.

Anatomical classification of cNF are ordered by stage according to appearance[17,18]. During their nascent stage, cNF cannot be seen by the visible eye but ultrasound or other forms of imaging can detect the dermal mass[12]. The cNF can be classified as flat when their appearance on the skin shows hyperpigmentation or mild epidermal thinning. The sessile stage of the cNF occurs when a visible papule is located on the skin. Subsequently, it moves to the globular stage, which is a larger nodule with a 20-30mm height and comparable base. The final stage is the pedunculated stage signified by the extrusion of dermal cNF contents into a mass above the skin attached by a stalk.

Currently, no gold-standard treatment exists for cNF. Physical removal remains the most effective method for treating cNF. Physical removal may encompass modalities such as surgical excision with primary closure and modified biopsy removal methodology (Figure 1) or destruction by CO_2 laser, electrodessication, and ablation[18-22]. Challenges facing removal include tumor regrowth from incomplete excision, significant scarring, and cost burden. Cost remains high because cNF is still classified as an elective, cosmetic treatment by most insurance companies. Additionally, physical removal has no preventive effect on cNF development which can be improved upon by medicinal therapies.

Current medicinal therapies are still under investigation and none is fully effective nor reliable. The past and present therapeutic options are targeting key components to cNF or signaling pathways involved tumor formation and maintenance, including mTOR, c-Kit, MAPK/MEK, mast cell biochemistry, and cellular proliferative properties[23]. Medicinal therapies applied topically will limit systemic exposure to medication[24]. Unfortunately, individuals with NF1 may have an extensive tumor burden covering over most of their body surface area making the application of a topical medicine unreasonable. The skin barrier may also prevent dermal penetration of the medicine in the collagenous mass. Systemic therapy would be ideal given the holistic treatment of potentially all cells affected by biallelic loss of NF1 mutation in those with NF1 but risks exposure to agents that may alter normal biology.

Herein, we review the current treatments, both physical and medicinal, for cNF and guide specific recommendations for cNF treatment based upon this outline. We will also comment on future directions of treatment based on cellular quiescence and genomic editing.

Treatments of Cutaneous Neurofibromas: Physical Removal

To date, physical removal is the most assured method for cNF treatment. Surgery through excision and primary closure was the first technique developed for treatment. Since, several more modalities have been developed each with their pros and cons (Table 1).

The first recorded publication for cNF removal was from Bromley et al., who utilized surgical excision to remove cNF on 32 patients.[19] The technique involves an elliptical excision

with removal of the overlaying epidermis and dermal tumor and suturing or healing by primary intention on multiple cNF in each session. Surgical excision has been utilized in a "mega-session" fashion where numbers of cNF are removed in one operation with healing by primary or secondary intention depending on the size [25]. The "mega-session" technique requires general anesthesia, prior IV antibiotics or topical antibiotics, sterile surgical field, and post-operative pain management. Surgical excision yields favorable post-operative results with minimal scarring and consistently high patient satisfaction[19,26]. Additionally, surgical excision can remove giant cNF or cNF in sensitive areas including the eyelids, nipples, genitals, or near neurovascular structures over other physically destructive methods [26]. Excision requires highly-trained medical specialists including dermatologists, general surgeons, and plastic surgeons who are familiar with the anatomy. Clinics and operational sites should be prepared for hemostasis with aluminum chloride, hyfrecator, or deeper arterial suturing [27]. Costs are highly dependent on the method used for excision thus access to this technique may be difficult for all patients with NF1. Excision of a few lesions using local anesthesia (1:1000 epinephrine with lidocaine) would cost significantly less than a multi-hour operation requiring general anesthesia in the operating room[28]. Operations with elliptical excision may take significantly more time due to the longer excision and accounting the time for suturing.

In the 1985, the CO_2 laser was introduced to treat a variety of dermatologic skin manifestations[29]. The laser employs a 9.4-10.6 μm wavelength laser capable of destroying tissue by rapidly heating and vaporizing intra-cellular water[30]. Since its release, the CO_2 has been tested for cNF treatment by a variety of groups[20,21,29,31-35]. In most cases, the CO_2 was aimed at the tumor to destroy both the superficial and dermal components leaving a charred center to which

healing by secondary intention would occur. The CO_2 laser simultaneously seals small nerve endings, rather than leaving frayed endings as occurs with steel scalpel surgery, potentially resulting in less postoperative pain[35]. Small lymphatics are also sealed resulting in less post-operative edema. Because this technique achieves hemostasis without sutures, hundreds to thousands of cNF can be treated in one sitting. In all cases, majority of lesions were replaced with a flat, dyspigmented or depigmented scar that corresponded to the size of the cNF[29,34]. Patient satisfaction, despite the resulting obvious adverse coloration or aberrant scarring, was high[21,31,32,34]. Recurrence or tumor regrowth was rare at 3-10%[29,31]. This method is primarily employed to treat sessile, globular, and pedunculated cNF under 2cm and should not be used for excessively large cNF with significant dermal mass. Problems include high cost of the machine, expertise or training required for equipment handling, and overall patient access may be limited in certain body areas. Skin-pad burns of uninvolved skin locations may occur when using this equipment and a 25-50uM area surrounding the cNF is expected to have thermal necrosis as a byproduct of normal operation[36]. Destructive modalities also make challenges for examination by histopathology.

A recent study by Chamseddin et al. developed a robust surgical technique for surgical removal of cNF that differs by targeting the distinct anatomy of cNF[18]. The cNF first begins as a nascent tumor in the dermis that eventually grows to become visible on the patient's skin surface. A superficial shave or biopsy will miss a sizeable portion of the tumor in the dermis which may lead to more significant scarring, regrowth, and will not relieve the pain or itch. The technique comprises a shave biopsy of the soft mass above the skin with a dermablade or surgical razer then using forceps to grasp the dermal component of the tumor, extruding its contents for more

visibility, and removing its entirety with the same blade. The end product will be an empty hole the same size or smaller than the cNF base excised. It can be closed using sutures, surgical glue, or staples dependent on the location of the excision. This technique has excellent post-operative cosmetic results featuring minimal scar size that is less than one required for a complete elliptical excision. In 84 tumors excised, one (1.2%) lesion in an African American male developed hypertrophic scarring and post-inflammatory hyperpigmentation in 12% of cases which improved significantly after 5-month follow-up[18]. The Dermatology Life Quality Index (DLQI) showed statistically significantly increase in quality of life[18,37]. The procedure also does not require antibiotics or sterile gloves due to the superficial nature of the procedure and low risk for infection. The technique relies on local anesthesia and has benefits of world-wide accessibility due to its low-risk setting and reduced costs. Implementation of suturing prevents the removal of hundreds or thousands of cNF in one sitting. Patients can be advised to return for multiple rounds for removal of tens of cNF until acceptable results are obtained.

Contemporary technology has placed focus on cNF treatment with photocoagulation using erbium-doped yttrium aluminium garnet laser (Er:YAG) or neodymium-doped yttrium aluminum garnet (Nd:YAG) utilized for cNF treatment in many studies to date[21,38,39]. Photocoagulation occurs with Nd:YAG lasers by emitting light at 1064 nm at both pulse and continuous modes for laser-induced thermotherapy to produce tissue destruction by thermal necrosis[40]. Er:YAG lasers use an analogous mechanisms of light emission but due to its absorption by water molecules it may have different outcomes on the heterogenous, extracellular cNF tumors[41]. Kriechbaumer et al. prospectively compared Er:Yag lasers and CO_2 lasers in 21 patients with 15,580 tumors showing Er:YAG lasers had improved the postoperative pain,

shorter time to re-epithelialization, decreased the duration of postoperative erythema, less thermal necrosis area, and a subjectively improved cosmetic outcome[21]. The photocoagulation laser in the large study had no instances of hypertrophic scaring, tumor recurrence at 3.1%, and dyspigmentation in 9% of cases. One subject had a severed subcutaneous bleed requiring deep suturing for homeostasis[21]. Another study examined Nd:YAG in 12 subjects on 253 cNF which revealed the 40% of cNF treated regressed by at least 75% but around 15% of cNF did not decrease significantly in size[38]. A case report where combination of a superficial shave biopsy of the cNF with added laser photocoagulation of the dermal component showed another option for cNF treatment but also risks hypertrophic scarring and dyspigmentation[39]. The photocoagulation lasers may prove to be a superior method for cNF treatment due to its continued high patient satisfaction and cosmetic results[21]. This procedure can be performed in an outpatient setting but the laser is highly expensive and likely only found in well-funded practices and large academic hospital settings, making it inaccessible to most NF1 patients in the world.

Physical destruction of cNF may also be performed by thermal necrosis through other forms including electrodessication and diathermy loop excision. Monopolar diathermy loop uses a heated, metal loop to simultaneously remove and necrotize the cNF tissue and provide cauterization for healing by secondary intention[42]. This technique rapidly treats hundreds of tumors in a "mega-session" technique with reported high patient satisfaction[43]. This technique is not recommended in cosmetically sensitive areas due to inevitable depigmented scarring at each site of removal[43]. Additionally, diathermy loops are not found in every clinical site thus may be inaccessible to most patients with NF1. Electrodessication, a form of radiofrequency ablation, uses needle pointed tip cautery at alternating electrical currents to illicit minimal thermal damage

to tissue with instant hemostasis. This technique has been used to treat >500 cNF at one sitting but will require general anesthesia under these circumstances[44]. Electrodessication should not be employed as the primary mode of cNF removal in patients with limited cNF burden due to epidermal and dermal damage which will result in dyspigmented scarring as well as in patients with larger cNF[22,44]. It is also important to note that patients with cardiac pacemakers should not undergo electrodessication on the trunk, back, or neck.

Treatments of Cutaneous Neurofibromas: Medicinal Topical & Systemic Therapy

To date, there is no topical or systemic medical treatment recommended for cNF. This report will highlight successes and failures of past trails for cNF in addition to highlight current progress for treatment (Table 2). It is important to stress the similarities and differences between plexiform neurofibromas and cutaneous neurofibromas given many therapies intended for pNF may have consequences for cNF.

Upon degranulation by trauma or other triggers, mast cells present in the dermis release a host of cellular signals critical to cNF development. Transforming growth factor beta (TGF-B) for collagen production from cNF fibroblasts, histamine, vascular endothelial growth factor (VEGF), platelet-derived growth factor and fibroblast growth factor all contribute to cNF maintenance[23,45]. Ketotifen, non-competitive H_1 antihistamine antagonist and mast cell stabilizer, has seen off-label utility by blocking degranulation of mast cells[46]. For symptoms from cNF, ketotifen fumarate was shown to have an unequivocal decrease in symptoms of pain and itching of cNF in a study of ten NF1 patients, likely due to its antihistamine properties[47]. Growth rate of

cNF slowed over three years of treatment but results were not consistent. One long-term, prospective case report proactively gave an infant with NF1 ketotifen daily for 30 years and reported a paucity of cutaneous neurofibromas and the distinctive monotonous uniformity of those present, which were small and flat or barely sessile[48]. No double-blinded controlled trials have been performed for ketotifen for cNF size treatment. Based on our current understanding, ketotifen is not useful for mature cNF treatment but could theoretically prohibit the initial growing event. Side effects of the medication are mild, with the most common being mild and transient drowsiness.

Stem cell factor receptor kit (c-KIT) is found on mast cells and has been extensively correlated to NF1-deficiecnt tumor growth in animal studies[13]. Imatinib, the monoclonal antibody inhibitor of c-KIT, effects on pNF size in a phase-2 clinical showed a 6/36 (17%) response of participants but cNF were not measured [49]. A case of an NF1 individual with cutaneous vasculopathy that was treated with Imatinib had no change or reduction in burden of cNF[50]. The trial was stopped due to adverse effects which for Imatinib may include gastrointestinal upset, hematologic cytopenia, cardiovascular effects, and pulmonary complications[50,51]. Despite these trials, there have not been controlled study investigation cNF volume or growth rate in the setting of Imatinib treatment, thus no conclusions can be made regarding c-kit inhibition for cNF treatment.

Imiquimod is an immune-response modifier that acts as a toll-like receptor 7 (TLR-7) agonist to modify of the innate immune responses [52]. A topical application of 5% imiquimod study was performed with a primary objective to assess tumor volume by calipers and secondary

objectives to evaluate the degree of infiltrating inflammatory cells around the region of application[53]. After 4 months, cNF showed a 15% reduction in tumor volume while the control group showed a 10% reduction. Skin inflammation after prolonged treatment was low (5-10%), suggesting targeting immunogenic response with Imiquimod may not be effective[53]. This study likely discredits the use of TLR-7 for cNF therapy.

Cells of inflammation including leukocytes and macrophages are present within cNF yet their function is unknown. Nonsteroidal anti-inflammatory agents (NSAIDS) target the pro-inflammatory enzymes, COX-1 and/or COX-2, to prevent release of PGE and prostaglandins[54]. Decreased inflammation through NSAIDS were hypothesized as possible mechanisms for treatment. A controlled local injection study with diclofenac showed that 48% of tumors had partial or complete response while others had tumor growth on treatment[55]. In one open, controlled, prospective, proof-of-concept study, 25mg/ml Diclofenac is applied topically twice daily on cNF after microporation with a laser device[56]. Results on 7 patients have currently not been published. The primary objective is to identify inflammatory process with the presence of tissue necrosis while observing adverse events associated with the study drug. In a related study, researchers injected doxycycline to achieve an 89% total response.

Neoplastic Schwann cell biology is also a primary target for cNF medicinal therapies. Naturally, the tumor Schwann cell utilizes growth-factor initiated RAS signaling cascade to upregulate a PI3K-mTOR survival pathway and the RAF-MEK-ERK transcription and proliferation pathway[57]. The NF1 protein, which is absent in tumor cells, inhibits excessive RAS activation and thus preventing activation of these two pathways[58]. Drug therapies aimed at

downregulating these two pathways at the level of tumor Schwann cell were developed to prevent and treat cNF.

The mTOR pathway is a master regulator of cell growth and metabolism and is important in Schwann cell survival[59]. Rapamycin, also known as Sirolimus, is a macrolide compound that inhibits mTOR. Sirolimus and everolimus, other mTOR inhibitors, have been examined in the setting of clinical trials for treatment of pNF and MPNST, respectively[60,61]. Although they were not the primary outcome, cNF were not reported to change or alter under the treatments. A single-arm trial examining everolimus for cNF found that it did not reduce size nor change growth under the intervention[62]. Although the study lacked a control arm, tumor growth was likely not observed due to the quiescence of the matured cNF in the study or the inhibition of mTOR by everolimus. The benefit to Rapamycin in a topically regimen applied daily for 6 months did not have significant systemic absorption and side effects such as pancytopenia were not observed[63]. Despite the necessity of mTOR in Schwann cell survival, trials with mTOR antagonists did not have a significant impact on cNF.

The RAF-MEK-ERK pathway is an important regulator of transcription and cell growth and is tightly linked to pathology involved in cNF development through upstream activation by unregulated RAS. Selumitinib is an oral selective inhibitor MAPK kinase (MEK) that has shown activity against several adult cancers[64,65]. The drug is undergoing a phase II clinical trial of cNF specifically[66]. An earlier study investigated 24 NF1 children who had inoperable pNF with administered Selumitinib twice daily at a dose of 20 to 30 mg per square meter of body-surface area every month [60]. Complications included elevated creatinine kinase, urticaria, acneiform

rash, and in one case decreased left ventricular ejection fraction. cNF sizes were not measured but plexiform neurofibromas did have partial responses (>20% decrease volume) in 70% of children, thus signifying growth of pNF relies on MEK [60].

VEGF Inhibitors have also been trialed stemming from data which shows VEGF, the angiogenic signaling molecule, is expressed highly in NF1-deficient tumors[23,67]. Ranibizumab, a VEGF antibody, was injected into cNF[68]. Uninjected tumors served as internal controls and primary outcomes were cNF volume changes and interstitial pressure. Reports of outcomes are still expected to be released. Another VEGF inhibitor, Sorefenib, had significant effects on lowering pNF volume by MRI, although cNF response was not measured[69,70]. A trial targeting both mTor inhibition with everolimus and VEGF with bevacizumab in order to examine the pNF and MPNST growth also revealed minimal changes to cNF development or growth [71]. One possible reason for this unresponsive nature of cNF to anti-proliferative molecules may be the quiescent nature of mature cNF on the skin which do not rely on these signaling pathways after development or whether the trial outcome measure was not sensitive enough to quantify the changes in cNF.

Hormones play a significant role in cNF development. For example, women with NF1 have been reported to have rapid growth in cNF size and numbers during puberty and again, during pregnancy[72]. Both progesterone receptors and estrogen receptors have been found in varying degrees within cNF[73,74]. In fact, neurofibroma-derived Schwann cells respond by increased proliferation to hormonal (progesterone and estrogen) treatment in vitro and studies in vivo also support this observation[75,76]. Interestingly, two patients with NF1 that took high-dose

progesterone had an increased tumor size burden[77]. However, a study of 59 women with NF1 who took hormonal contraceptives (progesterone-estrogen combination or progesterone alone) did not reveal an association with cNF growth[77]. Thus, the link between hormones and cNF is highly evident in some studies but not in others and further research to characterize this relationship will be beneficial[73,78]. This research should encompass single-sample gene-set enrichment analysis of hormonal pathways in cNF that can reveal hormone impact on individual types of cells within a cNF. To date, growth hormone hypersecretion has been noted in some NF1 patients, but other studies have revealed growth hormone under secretion[79-82]. Therefore, the exact role of hormone in NF1 remains undetermined.

Photodynamic therapy (PDT) has been used to treat a variety of dermatologic, hyperproliferative disease including actinic keratosis, basal cell carcinomas, and cutaneous T cell lymphoma[83]. PDT also can kill bacteria and fungi and destroy viruses that cause warts or molluscum contagiosum[84]. A photosensitizer agent, 5-aminiolevulinic acid (ALA), are precursors to human body's endogenous photosensitizer Protoporphyrin IX. When illuminated with broadband red-light source 570-670nm, cells that up take the photosensitizer succumb to death secondary to reaction causing chemical tissue destruction, recruitment of inflammatory cells, and vascular compromise [83]. In-vitro studies with MPNST cells show a cytotoxic affect[85]. However, a case study examining PDT with ALA for pNF specifically did not note any changes to this mass[86]. Two clinical trials NCT01682811 (recruiting) and NCT02728388 (not yet recruiting) are examining the impact of ALA-PDT on cNF[87,88].

Recommendations for cNF Treatment

Specific recommendations for treatment of cNF rely on several factors that include equipment availability, time, tumor burden, tumor size, location, and desired cosmetic outcomes (Figure 2).

Due to its benign nature, cNF ultimately do not contribute to differences in mortality for NF1 patients. Thus, asymptomatic lesions without cosmetic concern should be managed by reassurance alone. Symptomatic lesions, such as itching or pain, can be removed physically. Lesions that trouble the patient due to cosmetic disfigurement should be removed given the strong link between cNF burden and lower quality of life [89]. For larger (over 2 cm) cNF with globular morphology, elliptical excision with primary suture closure should be reserved to reduce infection and support faster skin healing. If cosmetically unappealing or in a sensitive area including the face, neck, and breast, the modified biopsy removal method [18] or primary excision may be employed to reduce scar size given patients are low risk for hypertrophic or keloidal scarring. Photocoagulation could replace the modified biopsy removal method if equipment and trained specialists are available – though reliability for complete removal of the lesion remains unclear. Given an extensively high cNF burden in the abdomen, chest, or back, more rapid, "mega-session" and cosmetically insensitive techniques can be utilized including CO_2 laser for tumors >5mm and electrodessication for tumors <5mm without the need for suturing and healing by secondary intention. Risks and benefits for each available procedure should be discussed with the patients.

Future Directions

Surgical and destructive removal is the mainstay and golden-standard of therapy for cutaneous neurofibromas. Destructive modalities including CO_2 lasers, electrodessications, and photocoagulation are effective at treatment of tackling hundreds of cNF at one sitting. At this time, future research and controlled clinical trials are necessary to target cNF in early stages of development prior to requiring overt treatment. The ideal cNF therapy for patients with NF1 would prevent tumor development from the very beginning. This could come in the form of genetic therapy with genomic editing techniques. The application of CRISPR in theory could be used to correct the initial mutation[90]. However, the technique is still incomplete, not yet fully developed and controversial. The advances provided by understanding the biology of cNF derived from recent animal models may afford new opportunity for specific target therapies[23] . Lessons learned from the molecular interactions between the neoplastic Schwann cells and their tumor microenvironment within the cNF will provide us new approaches to develop novel therapies to delay and to prevent neurofibroma development in NF1 patients. In this arena, cellular quiescence, halting of the cell-cycle, is at the cornerstone of cNF evolution and should be a prime target for prevention of cNF[91]. It has been known that cNF rapidly proliferate in size at the early stage but eventually becomes quiescent in mature stage[17] as it shuts down proliferation of the mass when it reaches a certain size and remains unchanged for years. The mechanisms behind quiescence are unknown. Additional studies should be invested to characterize cNF quiescence. Clear targets for this endeavor are examining the cells of origin in early stage and neoplastic cells as well as the tumor microenvironment in the quiescent stage[23]. Additionally, prevention of growth and reducing tumor size by minimizing the microenvironment collagen will contribute to overall cNF mass[92].

Conclusion

Within the report lies discussion involving current therapy guidelines for cNF management through physical removal and examination of medicinal clinical research which targets cNF biology. Importantly, future directions for research in understanding cellular quiescence in cNF as well as interaction between the neoplastic Schwann cells and its tumor microenvironment in initiating and maintaining cNF will be essential to develop specific and effective therapy for the most common tumor in NF1.

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Figure Legends

Figure 1. Modified Biopsy Removal of Cutaneous Neurofibroma with 5 month-follow up [18].

Before: 2-cm, globular cutaneous neurofibroma before biopsy removal. **1**: Dermablade or razor blade shave biopsy of cutaneous neurofibroma above the skin. **2**: Soft, pale, dermal component of tumor present. **3**: Forceps grasping dermal component to extrude its contents. **4**: Empty hole after removal of dermal neurofibroma. **5**: Suture to close the skin. **After**: cutaneous neurofibroma was removed with minimal scar at five-month follow-up. (* = site of tumor removal)

Figure 2. Recommendations for Physical Removal of Cutaneous Neurofibromas.

Table

Table 1: List of studies pertaining to physical removal of cutaneous neurofibromas

Physical Removal	Use, Features, Efficacy	Limitations and side effects	Sources
Surgery	Large Tumors > 4cm Cosmetically Sensitive Histology is available	General Anesthesia required Highly trained specialists required Suture Removal Required More Expensive	18, 25, 26
CO2 Laser	Small Tumors> 1cm Remove > 100 cNF at once Rapid Surgery	High Risk for Scarring Expensive Equipment Highly Trained specialists required Histology is unavailable	20, 29, 31-35
Modified Biopsy Removal	Small/Medium Tumors up to 2cm Accessible equipment Can performed by MD, PA, NP Increased Quality of Life Cosmetically Sensitive Histology is available	Cannot remove > 10 at once Suture Removal Required	18
Photocoagulation	Minimal Discomfort Local Anesthesia Small/Medium Tumors < 1cm Low Scar Risk Cosmetically Sensitive Healing by secondary intention	Expensive Equipment Highly Trained Specialist required Histology is unavailable	21, 38, 39
Electrodessication	Removal > 100 cNF at once Very small tumors < 5mm Accessible Equipment Performed by MD, PA, NP Healing by secondary intention	High Risk for Scarring Histology is unavailable	22, 44
Radiofrequency Ablation / Diathermy Loop	Rapid Surgery Healing by secondary intention	High Risk for Scarring Histology is unavailable	43

Table 2. *List of studies pertaining to medicinal therapy for cutaneous neurofibromas*

Medicinal Therapy	Target	Benefits/Outcomes	Limitations and side effects	Sources
Ketotifen	Mast Cell H1 histamine receptor	Decreased symptoms of pain, itch Prophylaxis for 30 year case showed decreased in tumor burden	Drowsiness	46-48
Imiquimod	TLR 7/8	Minimal changes in cNF by calipers	Erythema, irritation	53
NSAIDS	COX1/COX2	Local injection of diclofenac leads to 48% of tumors had partial or complete response while others had tumor growth	Erythema, irritation	55
Photodynamic Therapy	Photosensitizer	Results not yet available	Erythema, irritation	86, 87
Imatinib	c-KIT	No change in cNF tumor burden	Pancytopenia, Cardiovascular Risks, Gastrointestinal upset, Pulmonary complications	49, 50
Rapamycin Sirolimus, Everolimus	mTOR	cNF were not reported to change or alter under treatments. A single-arm trial examining everolimus for cNF found no reduction size nor change in growth under the intervention.	Relatively safe	61-63
Selumitinib	MEK (MAPK kinase)	cNF was not measured	Elevated creatinine kinase, urticaria, acneiform rash, and in one case decreased left ventricular ejection fraction	60, 66
Ranibizumab	VEGF	Variable responses, minimal efficacy	Vision changes	68
Sorefenib	VEGF	cNF was not measured	Vision changes	69, 70
Everolimus & Bevacizumab	mTor, VEGF	Minimal changes in cNF by calipers		71
High-Dose Progesterone	Progesterone Receptor	Increased in tumor burden No changes in cNF	High Blood Pressure Mood changes Drowsiness	73, 77, 78

cNF, cutaneous neurofibroma. TLR, toll-like receptor. COX1/COX2, cyclo-oxygenase.

Figure 1

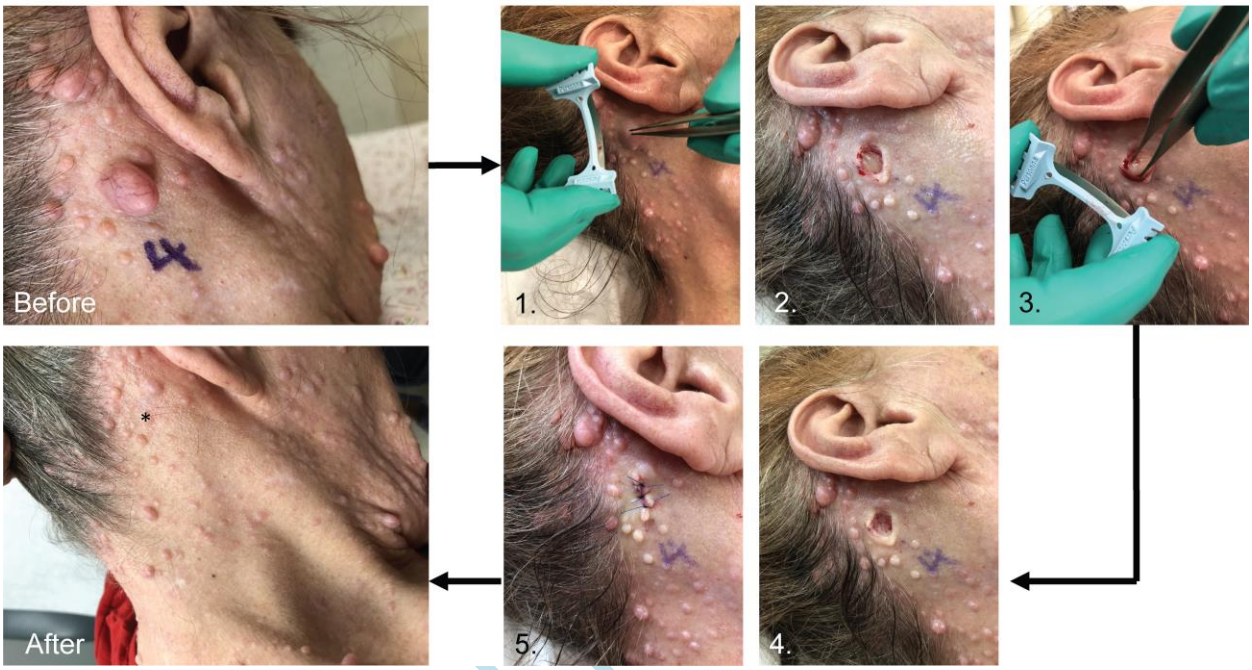

Figure 2