Review Article

Targeted genetic and molecular therapies in neurofibromatosis – A review of present therapeutic options and a glimpse into the future

Kaberi Feroze, Feroze Kaliyadan[1,2]

Department of Ophthalmology, Al Azhar Medical College, Thodupuzha, Kerala, India, [1]Department of Dermatology, College of Medicine, King Faisal University, Hofuf, Saudi Arabia, [2]Department of Dermatology, Sree Narayana Institute of Medical Sciences, Chalakka, Kerala, India

Abstract

Neurofibromatosis type 1, the most common phakomatoses, can present with a host of signs and symptoms, usually involving the skin and the peripheral nervous system. It is characterized by a mutation in the neurofibromatosis type 1 gene on chromosome 17q11.2 that codes for the protein neurofibromin. Neurofibromin acts as a tumor suppressor gene by inhibiting rat sarcoma (Ras) activity and its deficiency leads to increased Ras activity, cellular proliferation and tumor formation. This review was conducted to analyze the various targeted therapies at the genetic and molecular level employed to manage the tumors and other clinical presentations associated with neurofibromatosis type 1. Twenty-eight studies of treatment modalities for the conditions associated with neurofibromatosis and which involved either targeted gene therapy or molecular level therapies, including the latest advances, were included in this review. Mitogen-activated protein kinase kinase inhibition, mammalian target of Rapamycin inhibition and Tyrosine kinase inhibition, represent some of the newer treatment options in this category. Although there are a number of trials for providing therapeutic options at the genetic and molecular level for the various physical and psychological morbidities associated with neurofibromatosis type 1, most of them are in the preclinical stage. Increased clinical trials of the molecules and gene therapies could significantly help in managing the various chronic and sometimes, life-threatening conditions associated with neurofibromatosis 1 and these will probably represent the preferred treatment direction of the future.

Key words: Neurofibromatosis type 1, neurofibromin, targeted therapy

Introduction

Neurofibromatosis type 1 or von Recklinghausen's disease, one of the most common autosomal dominant disorders, is characterized by a constellation of signs and symptoms, usually attributable to the skin and the peripheral nervous system.[1,2] First described by von Recklinghausen in the year 1882, it affects about 1 in 3000–1 in 4000 people the world over.[3] Major clinical features include café au lait macules, cutaneous neurofibromas, plexiform neurofibromas, axillary freckling, optic nerve gliomas, iris Lisch nodules and skeletal abnormalities.[3] From a genetic point of view, there is a mutation in the neurofibromatosis type 1 gene on chromosome 17q11.2 that codes for the ubiquitous cytoplasmic protein, neurofibromin, which is highly expressed on Schwann cells, oligodendrocytes, neurons, astrocytes and leukocytes.[4] Neurofibromin acts as a tumor suppressor gene by functioning as a Ras-GTPase activating protein, which is an inhibitor of Ras activity.[3] Deficiency of neurofibromin leads to increased Ras activity, promoting cellular proliferation and tumorigenesis. Unregulated Ras activity leads to activation of growth promoting pathways such as the Raf/MEK/ERK (rapidly accelerated fibrosarcoma/mitogen-activated protein kinase/extracellular signal-regulated kinases) and phosphatidylinositol 3-kinase/protein kinase B/mammalian target of rapamycin pathways and there is enhanced production of signaling molecules like the mammalian target of rapamycin protein.[5,6] Abnormalities of regulation of the

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Corresponding author: Dr. Feroze Kaliyadan, Department of Dermatology, College of medicine, King Faisal University, Hofuf, Saudi Arabia. ferozkal@hotmail.com

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Raf/MEK/ERK signaling are footprints of many cancers and abnormalities in the phosphatidylinositol 3-kinase/protein kinase B/mammalian target of rapamycin pathways are correlated with the occurrence and development of tumors and many other human diseases, such as leukemia, diabetes and schizophrenia.[7-10] As a consequence, there is unchecked growth and cellular proliferation, promoting the formation and growth of various tumors. All these cellular pathways and the growth enhancing molecules provide avenues for genetic modification and molecular therapy to help stem unrestricted growth and tumorigenesis, as well as manage miscellaneous clinical abnormalities associated with neurofibromatosis type 1 without simultaneously affecting other organ systems of the individual. There are various therapeutic options targeting these molecular pathways such as inhibition of Ras signaling, inhibition of downstream pathways of the Ras system like the Raf-MEK-ERK pathway or the phosphatidylinositol 3-kinase/protein kinase B/mammalian target of rapamycin pathways.[3] Receptor tyrosine kinase pathways are another growth promotion pathway which acts by molecules like receptor tyrosine kinase and platelet-derived growth factor receptor and that also presents a locus for action at the genetic level.[3]

This study was conducted to review the various targeted therapies at the genetic and molecular level employed to manage the tumors and other pathologies associated with neurofibromatosis type 1.

Materials and Methods

Search strategy

The search strategy included the following key words "NF1," "Malignant peripheral nerve sheath tumor," "Plexiform neurofibroma," "neurofibroma" "Ras," "Optic nerve glioma," "clinical trials," "experimental studies" and either singly or combined. Databases searched included PubMed, Embase, Cochrane library and Science direct. The search strategy was to identify therapeutic options already in use, completed clinical trials, ongoing clinical trials used for managing the various complications associated with neurofibromatosis type 1, as well as preclinical studies of newer agents/agents already in use for other indications.

Inclusion and exclusion criteria

Research studies were included if they fulfilled all these inclusion criteria:

a. Studies involving therapeutic options for neurofibromatosis type 1 and its complications
b. Clinical and preclinical trials, including animal and cell studies
c. Therapeutic options working at the genetic/molecular level.
d. Studies in English for which the full text could be obtained
e. Published on or before December 15, 2020.

Data selection

All the obtained data was screened according to the inclusion/exclusion criteria. Eighty-three articles were retrieved about the therapeutic options in neurofibromatosis type 1 and associated morbidities of which those not involving treatment options at the genetic and molecular level were excluded from the study. Thirty-seven articles were obtained which fulfilled the inclusion criteria, and, of which 28 were included in this review. Of these, 21 were preclinical trials, 1 Phase I, 5 Phase II clinical trials and 1 randomized cross over trial [Figure 1].

Results

Twenty-eight studies were included in this review. Of these, 11 were related to malignant peripheral nerve sheath tumors, 14 to Plexiform neurofibromas and five were related to the other complications of neurofibromatosis type 1.

Malignant peripheral nerve sheath tumors

Malignant peripheral nerve sheath tumors are highly aggressive tumors arising from the connective tissues surrounding the peripheral nerves, which are resistant to conventional treatments and have a poor prognosis.[11] Up to 10% of neurofibromatosis type 1 patients are prone to develop malignant peripheral nerve sheath tumors.[12] Eleven studies related to malignant peripheral nerve sheath tumors in neurofibromatosis type 1 were retrieved, all of which were preclinical. Ras inhibition,[13] MEK inhibition,[14,15] and mammalian target of rapamycin inhibition were the targets of the trials, all of which were successful in reducing cellular proliferation. There were novel study designs such as bromodomain and extra-terminal bromodomain inhibition, pro-apoptotic molecule compound 21 and oncolytic measles virus, all of which had good results in the trials [Table 1].[16-22]

Plexiform neurofibromas

Plexiform neurofibromas are locally invasive tumors, which may be cosmetically disfiguring, may cause pain and even be life threatening, if located near vital structures.[16,17] Fourteen studies on plexiform neurofibromas and other neurofibromas were included in this review. They comprised of one randomized controlled trial (RCT), 5 Phase II clinical trials, one phase I trial and seven preclinical trials.[18-29] Receptor tyrosine kinase inhibitors and MEK inhibitors were the common agents studied.[11,14,18-20,25] The RCT involved Tipifarnib.[28] Phase II trials involving Imatinib, peg Interferon alpha-2b, Sirolimus and Pirfenidone.[19,21-23,27] The Phase I trial involved Selumetinib [Table 2].[24]

Other conditions associated with neurofibromatosis type 1

There are certain associations of neurofibromatosis type 1 which cause significant morbidity. They include defective fracture healing, optic nerve gliomas, neurofibromatosis type 1 associated pain and cognitive impairment. Five studies were included in this Group II concerning orthopedic complications and one each about optic nerve gliomas, hyperalgesia and cognitive impairment. All were preclinical studies [Table 3].

The different pathways involved in the pathogenesis of neurofibromatosis and the available drugs acting on these pathways are summarized in Figure 2.

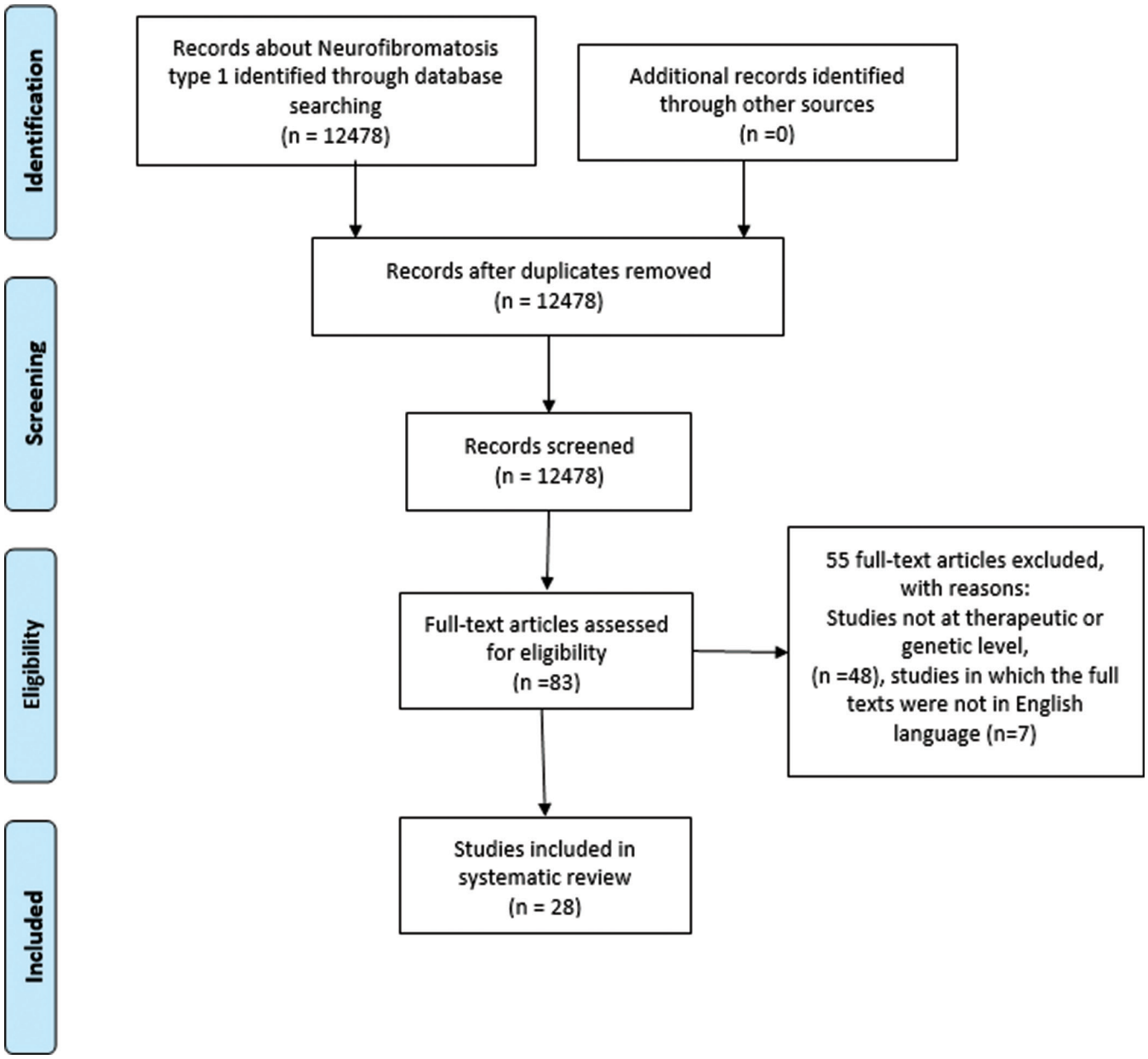

Figure 1: Study inclusion decision tree

Discussion

A number of preclinical trials are available for malignant peripheral nerve sheath tumor which include Ras inhibition, MEK inhibition and receptor tyrosine kinase inhibition.[13-15] Nilotinib, a receptor tyrosine kinase inhibitor, which targets receptor tyrosine kinase, platelet-derived growth factor receptor-α, platelet-derived growth factor receptor-β and discoidin domain receptors, inhibited proliferation and viability of Schwann cell lines and malignant peripheral nerve sheath tumor cell lines.[30] It was also pro apoptotic on malignant peripheral nerve sheath tumor cells lines, had anticollagenase activity and was highly selective against tumor cells. Nilotinib was found to be superior to Imatinib, another drug of the same group, with lower incidence of edema and hematological side effects. Efficacy of Nilotinib is being examined *in vivo* in immunodeficient mice. Another drug studied, the mammalian target of rapamycin inhibitor, Everolimus, which is already in use for certain cancers, was found to have notable antiproliferative actions *in vitro*.[31] Malignant peripheral nerve sheath tumors in deeper locations are found to express more p-protein kinase B and are difficult to excise; these represent ideal therapeutic targets for Everolimus. Further *in vitro* and *in vivo* studies are needed to evaluate its efficacy either singly or in combination. Bromodomain and extra-terminal bromodomain inhibitor JQ1 and pro-apoptotic agent compound 21 represent unique treatment options for malignant peripheral nerve sheath tumor. Bromodomain and extra terminal bromodomains are thought to play a role in malignant peripheral nerve sheath tumor pathogenesis. JQ1, a bromodomain and extra-terminal bromodomain inhibitor, represents a novel therapeutic option

Table 1: Studies related to MPNST

Sl. No.	Article	Type of research	Agent used	Indication	Results	Inference and future	Comments
1.	Barkan *et al*.[13]	Preclinical (mice)	Farnesyl thiosalicylic acid (Ras inhibitor)	MPNST	Tumor growth in neurofibromatosis type 1 associated MPNST cell line was inhibited disappearance of strong actin stress fibers associated with neurofibromatosis type 1 cell lines was noted	Farnesyl thiosalicylic acid may be considered as a potential therapeutic option for neurofibromatosis type 1	Although encouraging, further animal studies are needed to supplement the findings
2.	Jessen *et al*.[14]	Preclinical (neurofibromatosis mouse model and n neurofibromatosis type 1 patient MPNST cell xenografts)	PD0325901 (MEK inhibitor)	MPNST and neurofibromas	Treatment with PD0325901 reduced abnormal cellular proliferation and prolonged survival of mice implanted with human MPNST cells	Further studies are needed to extrapolate it use clinically	More animal studies are needed to monitor for effectiveness and adverse effect profile
3.	Fischer-Huchzermeyer *et al*.[15]	Preclinical (Michigan cancer foundation 7) cell line and neurofibromatosis type 1 associated MPNST human cells, normal human and rat Schwann cells as control)	MEK inhibitors U0126 and PD0325901	MPNST	Addition of MEK inhibitors to all trans retinoic acid was found to have an additive effect and reduced MPNST proliferation in 2 of the 3 cell lines	Combination of MEK inhibitors and All trans retinoic inhibitors shows promise in treatment of MPNST cell lines	Further experimental studies are need to validate effectiveness
4.	Patel *et al*.[32]	Preclinical (MPNST mouse model)	A small molecule BET bromodomain inhibitor JQ1	MPNST	Mice having induced sMPNST (skin precursor derived) tumors had reduced size of tumors following treatment with JQ1	JQ1 may potentially turn into an important therapeutic agent in combination with surgical excision in management of MPNSTs	More studies are required for refining the dosage and adverse effect profile for its clinical use
5	Chau *et al*.[33]	Preclinical (genetically engineered MPNST mouse model)	Compound 21, a proapoptotic agent	MPNST	Treatment with compound 21 reduced tumor burden important in cell cycle) inhibitors	compound 21 reduced tumor burden and appeared to act preferentially on tumor cells	compound 21 represents a unique treatment option for MPNST, but has to be supplemented by more studies
6	Patwardhan *et al*.[11]	Preclinical (MPNST cell lines)	PLX3397(a selective c-Fms and c-Kit inhibitor) and rapamycin (TORC1 inhibitor)	MPNST	PLX3397, in combination with rapamycin causes further inhibition of macrophages and growth suppression which is seen to continue even after stopping the treatment	The authors suggest that the combination with rapamycin should be considered for drug development in MPNST.	Since PLX3397 is at present in clinical trials for certain other tumors, early clinical trials could accelerate drug development
7	Varin *et al*.[34]	Preclinical (human neurofibromatosis type 1 derived MPNST cell lines and plexiform neurofibroma derived Schwann cells)	AZD8055 (inhibitor of both mTORC1 and mTORC2)	MPNST	AZD8055 was noted to inhibit cellular proliferation and migration in both MPNST and Schwann cell lines	Absence of paradoxical AKT activation and presence of mTORC2 inhibition makes this agent superior to rapamycin	As this a cell line study, animal trials could assess efficacy and safety criteria
8	Jiang *et al*.[30]	Preclinical (plexiform neurofibroma derived Schwann cells and MPNST cell lines)	Nilotinib (tyrosine kinase inhibitors, prototype of Imotinib, acting against the c-KIT, platelet derived growth factor receptor -α, platelet derived growth factor receptor-β and discoidin domain receptors)	Plexiform neurofibroma and MPNST	Nilotinib inhibited proliferation and viability of Schwann cell lines and MPNST cell lines	Efficacy of nilotinib is being examined *in vivo* in immunodeficient mice	The highly selective antitumor effect make it a very exciting therapeutic agent pending wider animal study results

(*Contd*...)

Table 1: (*Continued*)

Sl. No.	Article	Type of research	Agent used	Indication	Results	Inference and future	Comments
9	Endo *et al*.[31]	Preclinical (MPNST cell lines from excised human MPNST)	Everolimus (mTOR inhibitor)	MPNST	Everolimus inhibited cell proliferation, cell motility and invasion of cell lines derived from sporadic and neurofibromatosis type 1related MPNST	Further *in vitro* and *in vivo* studies are needed to evaluate its efficacy either singly or in combination	Everolimus represents an effective treatment option for MPNSTs and since it is already in use for some cancers, its introduction for MPNST treatment is probably not too far away
10	Watson *et al*.[35]	Preclinical (MPNST cell lines)	Combination of Everolimus (inhibitor of mTOR) and PD0325901 (inhibitor of MEK)	MPNST	The combination therapy was noted to inhibit cell growth and induces apoptosis in MPNST cell lines	This combination was found to be effective in improving survival in mouse models. It may be beneficial for human use in the future	More research is needed to know the effectiveness of this agent
11	Deyle *et al*.[36]	Preclinical (human MPNST cell lines)	Four different oncolytic viruses were used - measles virus, a vaccinia vector, a vesicular stomatitis virus and a recombinant vesicular stomatitis virus	MPNST	The measles and vaccinia oncolytic viruses significantly reduced the number of viable cells, when examined at 48 h and 72 h post inoculation	Further mouse xenograft trials are underway.	This study represents great potential since it is a novel treatment modality

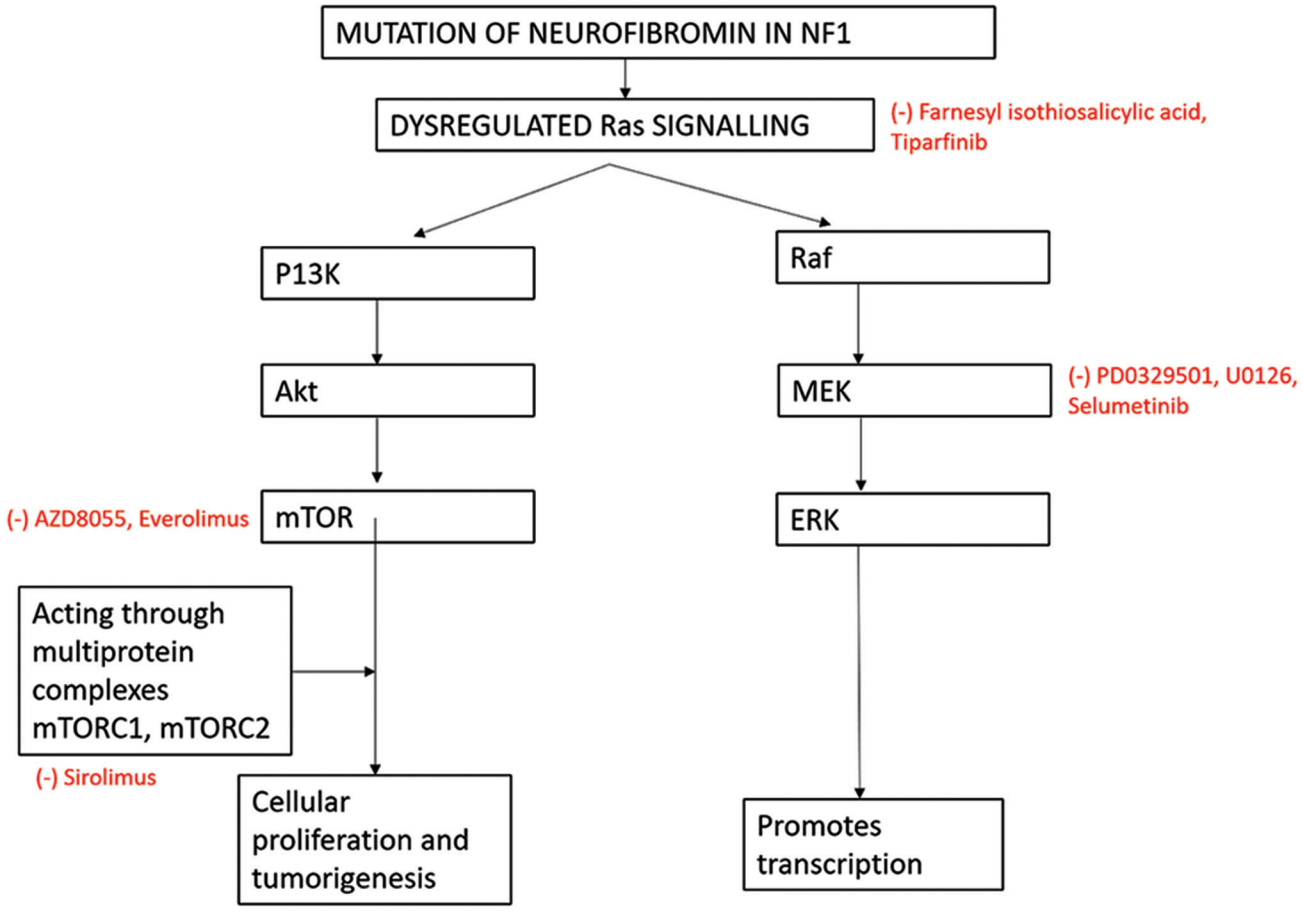

Figure 2: Pathways involved in pathogenesis of neurofibromatosis and drugs acting on the same

Table 2: Studies related to plexiform neurofibroma

Sl. No.	Name of research	Type of research	Agent/intervention used	Indication	Results	Inference and future	Comments
1	Demestre *et al*.[18]	Preclinical (plexiform neurofibroma derived Schwann cells and xenograft plexiform neurofibroma tumor fragment in mice)	Imatinib, a receptor tyrosine kinase inhibitor for four weeks	plexiform neurofibroma	There was a reduced Schwann cell viability *in vitro*. There was a reduction in the size of transplanted neurofibromas	The authors recommend further studies and research into the molecular mechanisms by which Imatinib acts	Since it is a cell line study, subsequent animal studies to document the molecule's action are needed
2	Robertson *et al*.[19]	Phase II clinical trial on neurofibromatosis type 1 patients having clinically significant plexiform neurofibroma)	Oral Imatinib in a dose of 800 mg twice daily X six months for adults and 440 mg/m^2/day for children	plexiform neurofibroma	Regression of plexiform neurofibroma was noted in 26% of patients. Common adverse effects were reversible skin Rash and edema	A larger multi institutional clinical trial of patients with plexiform neurofibromas is recommended by the authors	The study population was relatively small in number (23). Larger clinical trials could help in rapid introduction of this agent in the market
3	Jiang *et al*.[30]	Experimental design (plexiform neurofibroma derived Schwann cells and MPNST cell lines)	Nilotinib (receptor tyrosine kinase inhibitor)	Plexiform neurofibroma (plexiform neurofibroma) and MPNST	Nilotinib inhibited proliferation and viability of Schwann cell lines and MPNST cell lines. It was also pro apoptotic, highly selective and had anticollagenase activity	Nilotinib was found to be better tolerated and has lesser toxicity than Imatinib. Efficacy of Nilotinib is being examined *in vivo* in immunodeficient mice	Since it has a more attractive safety profile than Imatinib, more studies could help in confirming its effectiveness
4	Wei *et al*.[20]	Experimental design (plexiform neurofibroma culture cells *in vitro* and xenograft plexiform neurofibroma fragments in mice)	Nilotinib (tyrosine kinase inhibitor) vs Imatinib. Nilotinib was administered in a dose of 100 mg/kg X four weeks	Plexiform neurofibroma	Nilotinib inhibited plexiform neurofibroma cells more than Imatinib *in vitro* and suppressed the plexiform neurofibroma xenograft more strongly than Imatinib in the *in vivo* study	The authors suggest that it should be considered as a therapeutic option for plexiform neurofibroma. It was found to be better tolerated and has lesser toxicity than Imatinib	Animal studies to document efficacy and adverse effects are required
5	Jakacki *et al*.[21]	Phase II trial on plexiform neurofibroma patients	Pegylated Interferon alpha-2b one mcg/kg/week for one year, two years or as long as progression continued in patients who were asymptomatic, asymptomatic or documented progression respectively	Plexiform neurofibroma	There was a doubling in the time to progression for patients with progressive plexiform neurofibroma. A subset of patients showed clinical and radiological improvement	Interferon alpha-2b should be considered a treatment option in patients with life threatening and morbid disease during the phase of active growth	The sample size was 82, distributed across 3 strata. Larger multicentric studies are needed prior to therapeutic use
6	Widemann *et al*.[22]	Phase II trial on neurofibromatosis type 1 associated progressive plexiform neurofibroma in patients aged 3–21 years	Pirfenidone, 500 mg/m^2 three times X 28 days	plexiform neurofibroma	Pirfenidone does not significantly compared to the placebo and did not cause improvement in quality of life	Pirfenidone does not warrant further evaluation in children with plexiform neurofibroma	The sample size was 30 and since there was no improvement in quality of life, this agent was deemed unsuitable for children
7	Babovic-Vuksanovic *et al*.[23]	Phase II trial on type I Neurofibromatosis patients with inoperable, symptomatic paRaspinal neurofibromas and plexiform neurofibromas	Pirfenidone 800 three times	Inoperable paRaspinal neurofibromas and plexiform neurofibroma	29.6% of the patients demonstrated a decrease in tumor volume by 15% or more. It was well tolerated	Pirfenidone may have a role in retarding the growth of neurofibromas	The sample size of this open labelled clinical trial was 24. Larger, clinical trials are needed

(*Contd*...)

Table 2: (*Continued*)

Sl. No.	Name of research	Type of research	Agent/intervention used	Indication	Results	Inference and future	Comments
8	Dombi *et al*.[24]	Phase 1 clinical trial (children with type 1 Neurofibromatosis and inoperable plexiform neurofibroma)	Selumetinib, oral selective MEK1 and 2 inhibitor 20 to 30 mg/sq m body surface area twice daily in 28 day cycles	plexiform neurofibroma	71% of the children had upto 20% reduction in tumor volume. Common adverse effects were acneiform Rash and GI upset	The authors concluded that children with inoperable plexiform neurofibromas benefitted from treatment with Selumetinib without suffering severe adverse effects	This phase 1 trial with 24 children had encouraging results and needs follow up with phase 2 trials
9	Jousma *et al*.[26]	Experimental design (neurofibromatosis type 1 flox/flox;Dhh-Cre mouse model)	MEK inhibitor PD0325901	Neurofibromas	Prior treatment with PD0325901 delayed onset of neurofibromas and tumor shrinkage occurred in established tumors	*In vitro* studies demonstrate the effectiveness of this agent	The results of these trials could help design human trials for further evaluation
10	Weiss *et al*.[27]	Phase II study (patients with inoperable, neurofibromatosis type 1 progressive plexiform neurofibroma)	Sirolimus (allosteric inhibitor of mTOR1) administered orally in 28 day cycles in doses to achieve a minimum blood level of 10–15 ng/ml)	plexiform neurofibroma	The time to progression of Sirolimus treated plexiform neurofibroma subjects was significantly longer than controls	Given the lack of serious adverse effects, Sirolimus could be considered a treatment option in plexiform neurofibromas	This single arm phase 2 trial had subjects randomized to receive Sirolimus(n=29) or placebo (*n*=49). Randomization and blinding techniques were not mentioned. More follow up studies with larger sample size are needed
11	Widemann *et al*.[28]	Randomised cross over trial. Subjects were children or adults with neurofibromatosis type 1 associated plexiform neurofibroma and radiological evidence of progression to receive either Tipifarnib (200 mg/m^2 orally every 12 h) or placebo and crossed over at the time of tumor progression	Tipifarnib, a selective farnesyltranferase inhibitor, blocking Ras signaling proteins and inhibiting cellular proliferation	Plexiform neurofibroma	In the first phase the time to progression was 10.6 months for the placebo arm and 19.2 months for the Tipifarnib arm. No significant adverse effects were reported	Tipifarnib did not significantly prolong time to progression of plexiform neurofibromas compared to placebo	The sample size was 62. Randomization process and double blinding were mentioned
12	Harigai *et al*.[29]	Experimental design (cell culture of cells obtained from NF patients and brain tissue of mice inoculated with cells obtained from NF patients)	Tranilast- an inhibitor of epithelial-mesenchymal transition signaling	Neurofibromas in neurofibromatosis type 1	Tranilast treated cells were seen to have decreased proliferation of neurofibromatosis type 1 cells *in vitro*. It also suppresses invasion and proliferation in neurofibromatosis type 1 tumor cells in mice	The effect of Tranilast on epithelial-mesenchymal transition and angiogenesis related genes of neurofibromin deficient cells was found to be stronger *in vitro* than *in vivo*	The authors suggest further investigations for the therapeutic role of Tranilast in neurofibromas
13	Hiatt *et al*.[37]	Experimental design (neurofibromatosis type 1 mutant mice *in vivo* and *in vitro*)	GTPase activating proteins related domains of neurofibromin and Ras were transducted with the aid of retroviruses into neurofibromatosis type 1 deficient cells	Neurofibromatosis	Expression of neurofibromin GTPase activating proteins related domains in neurofibromatosis type 1 deficient cells restores normal growth and cytokine signaling	Similar effect was not seen with RAS GTPase activating proteins related domains	Further studies are needed to evaluate the roles of these two GTPase activating proteins related domains
14	Jessen *et al*.[14]	Experimental design(neurofibromatosis mouse model and n neurofibromatosis type 1 patient MPNST cell xenografts)	PD0325901 (MEK inhibitor)	MPNST and neurofibromas	Treatment with PD0325901 reduced abnormal cellular proliferation and caused shrinkage of NF tumors in 80%	Further studies are needed to extrapolate it use clinically.	More animal studies are needed to monitor for effectiveness and adverse effect profile

Table 3: Studies other than those related to malignant peripheral nerve sheath tumor and plexiform neurofibroma

Sl. No.	Name of research	Type of research	Agent/ intervention used	Indication	Results	Inference and future	Comments
1	Baht *et al*.[38]	Experimental design (cell cultures prepared from bone marrow of mice deficient in neurofibromatosis type 1 and mice deficient in neurofibromatosis type 1 in whom fractures were induced)	Nefopam- inhibits beta- catenin	Delayed and aberrant fracture healing in neurofibromatosis type 1 patients	Nefopam increases osteoblastic differentiation in neurofibromatosis type 1 deficient bone marrow cells	Nefopam enhances fracture repair in neurofibromatosis type 1 deficient mice	Nefopam could be a potential treatment option for treatment of fractures in neurofibromatosis type 1 patients, as opined by the author. Further trials are needed to document effectiveness
2	Sullivan *et al*.[39]	Experimental design (cell cultures of osteoblasts from neurofibromatosis type 1 deficient and normal mice)	SP600125- c-Jun N-terminal kinase inhibitor	Orthopedic complications of neurofibromatosis type 1	Treatment with c-Jun N-terminal kinase inhibitor increases osteogenesis in neurofibromatosis type 1 deficient mice	Activation of the c-Jun N-terminal kinase pathway is postulated to cause the defects in bone metabolism and this explains the effect of c-Jun N-terminal kinase inhibition	Further experimental studies are needed to substantiate the role of c-Jun N-terminal kinase inhibition in neurofibromatosis type 1 related bone defects
3	Daginakatte *et al*.[40]	Experimental design (neurofibromatosis type 1 deficient mice and neurofibromatosis type 1 deficient microglia cultured from these mice)	SP600125- c-Jun N-terminal kinase inhibitor	Optic nerve glioma	Administration of SP600125 resulted in reduced proliferation and motility of neurofibromatosis deficient microglia. *In vivo*, there was reduced proliferation of optic nerve glioma	The study suggests that neurofibromatosis type 1 deficient microglia play an important role in the pathogenesis of Optic nerve glioma	c-Jun N-terminal kinase inhibition demonstrates a potential site for future therapeutic intervention in the management of optic nerve gliomas, the surgical treatment of which can sometimes be an ophthalmologist's therapeutic dilemma
4	Moutal *et al*.[41]	Experimental design- CRISPR- associated 9 genome editing system was used to truncate the C terminal end of Neurofibromin of rats	(S) Lacosamide- an inhibitor of phosphorylation of cytosolic regulatory protein collapsin response mediator protein 2, which in turn down regulates the Calcium and Sodium channels	neurofibromatosis type 1 associated pain	Administration of (S) Lacosamide results in normalization and excitability of channel current densities and reduction in hyperalgesia	Collapsin response mediator protein 2 is a key point to regulate hyperalgesia in neurofibromatosis type 1.	This opens an avenue to understand the mechanism of pain and search for newer agents to alleviate pain. Further studies are needed
5	Omrani *et al*.[42]	Experimental design (using neurofibromatosis type 1 mouse models)	Lamotrigine- a hyperpolarization-activated cyclic nucleotide-gated channel agonist	Cognitive impairment in neurofibromatosis type 1	Lamotrigine restored the electrophysiological depression and the cognitive impairment in mouse models of neurofibromatosis type 1	This research provides provides a target for future drug development	

in combination with surgical excision in the treatment of these tumors.[32] Compound 21, a pro-apoptotic agent, reduced tumor burden and acted preferentially on malignant peripheral nerve sheath tumor cells. An additive effect was noted when compound 21 was combined with inhibitors of the P13K pathway.[33] However, more studies are required for refining the dosage and studing adverse effect profiles for its clinical use. One of the most promising agents appears to be AZD8055, an inhibitor of both mammalian target of rapamycin C1 and mammalian target of rapamycin C2, it was noted to be superior to rapamycin and showed synergistic action with MEK inhibitors and bromodomain and extra-terminal bromodomain inhibitors and could be considered a new effective therapy for malignant peripheral nerve sheath tumors.[34] However, it also awaits clinical trials. Two drug combinations were studied in the trials for malignant peripheral nerve sheath tumor – the first a combination of everolimus and MEK inhibitor and the second a combination of PLX3397, a selective c-Fms (macrophage colony-stimulating factor receptor) and receptor tyrosine kinase inhibitor and TORC1 inhibitor, rapamycin.[11,35] Both of them showed better therapeutic response than the individual agents. A mode of action utilizing targeted gene therapy by using the oncolytic measles virus to selectively destroy malignant peripheral nerve sheath tumor cells, which showed antiproliferative action, has also been utilized. Mice xenograft trials are underway and this trial represents great potential.[36] Thus, although there are a number of studies of targeted genetic treatment of malignant peripheral nerve sheath tumor, all are in preclinical stages. Further studies and clinical trials are needed before any of these agents could be applied for human use.

Regarding plexiform neurofibromas, studies with receptor tyrosine kinase inhibitors showed efficacy and nilotinib was found to have a lesser adverse effect profile than Imatinib. However, no clinical trials of nilotinib in plexiform neurofibroma patients were available.[11,18-20] Phase II trials of peg Interferon alpha-2b, which acts by causing inhibition of transcription and secretion of antiangiogenic factors, showed doubling of time to progression; however further clinical trials were suggested.[21] The antifibrotic agent pirfenidone, which acts by modulating the expression of growth factors and cytokines, showed efficacy in reducing tumor size in adults, but was not found to have the desired efficacy in childhood plexiform neurofibromas, in two phase II trials.[22,23] Selumetinib, a MEK inhibitor, in a Phase I trial showed encouraging results and was suggested as a therapeutic option in inoperable cases.[24] The FDA approved the use of selumetinib for pediatric, inoperable plexiform neurofibromas on April 10, 2020.[25] The mammalian target of rapamycin C1 inhibitor, sirolimus, showed efficacy in phase II clinical trials and the MEK inhibitor PD0325901 also showed promise.[26,27] The randomized crossover trial of Tipifarnib, which acts by Ras inhibition, was not found to increase time to progression significantly.[28] Tranilast, an inhibitor of epithelial-mesenchymal transition signaling, which is a factor involved in downregulation of E-cadherin and extracellular matrix production was noted to have antiproliferative action in preclinical trials. As it is a therapeutic agent already in use, clinical trials could help in rapidly utilizing this medication for use in patients.[29] A highly exciting treatment option is transduction of GTPase activating proteins related domains of neurofibromin into neurofibromatosis type 1 deficient cells using retroviruses, thereby restoring normal growth and cytokine signaling in these cells.[37]

Abnormal bone healing is another morbidity associated with neurofibromatosis type 1. Nefopam, which inhibits beta catenin was found to improve fracture healing in neurofibromatosis type 1 deficient mice and improve osteoblastic differentiation in neurofibromatosis type 1 deficient marrow cells.[38] Patients with neurofibromatosis type 1 have increased activation of the Ras/MAPK signaling pathway, which causes elevated beta catenin levels, which, in turn, causes undifferentiated mesenchymal stem cells to differentiate to a fibrous phenotype, causing delayed fracture healing and increased fibrous tissue growth at fracture sites. This action was found to be reversed by Nefopam and since Nefopam is an agent already in use, clinical trials would help accelerate its use in patients. The c-Jun N-terminal kinase inhibitor, SP600125, is another agent which was found to increase osteogenesis in neurofibromatosis type 1 deficient osteoblast cultures.[39] neurofibromatosis type 1 leads to the inhibition of the Ras-Raf-1/MEK/ERK axis, which can as a downstream effect cause activation of the c-Jun N-terminal kinase pathway, which is postulated to cause the defects in bone metabolism and this action can be reversed by c-Jun N-terminal kinase inhibition.

c-Jun N-terminal kinase inhibitor, SP600125, has also been utilized in preclinical trials of optic pathway gliomas and there was reduced proliferation and motility of neurofibromatosis deficient microglia and reduced size of glioma.[40] This presents a potential site for future therapeutic interventions in optic nerve gliomas.

Chronic idiopathic pain is a known association of neurofibromatosis type 1.[41] In a study by Moutal *et al*., clustered regularly interspaced short palindromic repeats -associated nine genome editing system was used to truncate the C terminal end of neurofibromin as seen in many patients with neurofibromatosis type 1. Truncation of neurofibromin is associated with increased activation of the voltage gated calcium and sodium channels in the dorsal root ganglia, which may be associated with hyperalgesia. (S) Lacosamide – an inhibitor of phosphorylation of cytosolic regulatory protein collapsin response mediator protein 2, which in turn down regulates the calcium and sodium channels was, noted to be effective in normalization of channel, activation and excitability and reduce hyperalgesia following clustered regularly interspaced short palindromic repeats shortening of neurofibromin in a rat model.[41] This could open an avenue to understand the mechanism of pain and search for newer agents to alleviate pain.

Cognitive impairment and learning disability were other features of neurofibromatosis type 1. Omrani *et al*. used lamotrigine – a hyperpolarization-activated cyclic nucleotide-gated channel agonist.[42] Inhibition of hyperpolarization-activated cyclic nucleotide-gated channels in neurofibromatosis type 1 mutants is thought to be the cause of neuronal inhibition, which in turn leads to cognitive impairment. Lamotrigine restored the electrophysiological inhibition and cognitive impairment in mouse models. This could provide a roadmap for future drug development.

Conclusion

Although there are a number of trials for providing therapeutic options at the genetic and molecular level for the various physical and psychological morbidities associated with neurofibromatosis type 1, most of them are in the preclinical stage. Transition to clinical trials and randomized controlled trials could help accelerate market introduction of these agents for patient use. The FDA approval of selumetinib for pediatric plexiform neurofibromatosis is an exciting step for gene based therapy and it gives hope for acceptance of other gene and molecular targeted therapy in this field. Neurofibromatosis type 1 is the commonest phakomatoses, with a relatively high prevalence worldwide, targeted genetic and molecular therapy could help ameliorate the various associated disabilities and disorders with precision and minimum disturbance to the other systems and the general health of the individual. More effort and resources should be diverted to this type of intervention and it most likely represents the future direction of management of not only neurofibromatosis type 1, but many other diseases.

Declaration of patient consent

Patient's consent not required as there are no patients in this study.

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Conflicts of interest

There are no conflicts of interest.

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