Tamoxifen for the Treatment of Batten Disease

Chiara Soldati[1], Sandro Montefusco[1], Valentina Bouché[1], Diego Luis Medina[1,2*]

[1]Department of Genetics and Medicine, Telethon Institute of Genetics and Medicine (TIGEM), Pozzuoli, 80078 Naples, Italy;
[2]Department of Medical and Translational Science, Federico II University, 80131 Naples, Italy

ABSTRACT

Batten Diseases (BDs) are a group of lysosomal storage disorders characterized by seizure, visual loss, and cognitive and motor deterioration, and represent the most common forms of neurodegeneration in children. By phenotypic screening, we recently discovered a novel accumulation of Globotriaosylceramide (Gb3) in cellular and murine models of two subtypes of BD, namely CLN3 and CLN7 diseases, which together account for almost 60% of all BD patients. We used fluorescent-conjugated bacterial toxins to label Gb3 and developed a cell-based High Content Imaging (HCI) screening assay that resulted in the repurposing of the FDA-approved hit tamoxifen. Interestingly, tamoxifen showed *in vitro* and *in vivo* the activity to ameliorate BD phenotypes through a mechanism that, at least in part, involves the activation of the Transcription Factor EB (TFEB), a master gene of lysosomal function and autophagy. Together, these results might represent the first small molecule therapy to treat BDs and, if supported by accurate preclinical studies and clinical trials to determine dose and safety, tamoxifen might offer an unprecedented opportunity for BD-affected children.

Keywords: Batten Disease (BD); Globotriaosylceramide; Tamoxifen; Neuronal Ceroid Lipofuscinoses (NCL)

INTRODUCTION

The Neuronal Ceroid Lipofuscinoses (NCLs), commonly known as Batten disease (BD), are a group of recessively inherited fatal diseases of the nervous system which typically arise in childhood. Neurodegenerative disorders in children are rare, with BD being the most frequent; the incidence rates of the entire group of 13 genetically distinct NCLs varies between 1:14,000 and 1:67,000 [1-3]. NCLs are rare conditions with no cure, as treatment options that delay or stop disease progression are still missing, and the disease management remains primarily palliative and targeted at controlling the symptoms rather than curing the disease.

CLN3 disease (MIM # 204200) represents the most common form of NCL worldwide, whereas CLN7 disease (MIM # 610951) is one of the most prevalent BD in southern and Mediterranean Europe. CLN3 and CLN7 are both severe diseases characterized by neurologic involvement, with rapid and devastating progression and early mortality. Especially in CLN7, progression is rapid with mental and motor regression, loss of expressive language and seizures developing in the majority of patients early in the disease course. First seizures and further decline of motor and language function appear by age 4½ years (range of 3 to 7 years), in about one year patients are wheelchair-bound and suffer from complete loss of expressive language. Soon after blindness occurs together with loss of swallowing function (tube-fed) and patients are completely dependent on families (range of 9 to 11 years) [4]. The disease leads to a premature death at a mean age 11.5 years (range 6.5 to 18 years). The prognosis is poor with most children becoming severely disabled by mid-childhood and not surviving to reach adulthood. This has devastating consequences both on patients and caregivers, in terms of quality of life, physical and emotional costs, and on society and healthcare systems, in terms of financial burden. Unfortunately, the onset of this devastating disease cannot be prevented as it is not included in any newborn screening program for metabolic diseases.

Current management strategies focus on symptom relief and palliative care, which can reduce some symptoms but cannot eliminate the progressively worsening effects caused by neurodegeneration. Moreover, due to disease rarity, many clinicians lack experience treating individuals with this disease. Among BDs, only CLN2 relies on an Enzyme Replacement Therapy (ERT) approved by FDA and EMA, based on the

intraventricular administration of the lacking enzyme cerliponase alfa [5]. However, except CLN2, all NCLs are currently incurable diseases, with patients relying only on palliative care to ameliorate symptoms. For CLN7, first experimental treatment approaches are being evaluated for safety and efficacy in pre-clinical and ongoing clinical studies. These include antisense oligonucleotide [6], and a pharmacological treatment with MK2206 mycophenolate, an Akt inhibitor, that seems to enhance *in vitro* the cellular clearance of ceroid lipopigment deposits in a TFEB-dependent manner [7]. Also, there is a currently ongoing phase 1 open-label clinical trial which recently started the recruitment of patients, based on a single-administration of the gene therapy agent AAV9/CLN7, that will be administered intrathecally into the lumbar spinal cord region of paediatric CLN7 patients (Clinicaltrials.gov ID: NCT04737460).

In this scenario, future therapeutic strategies for all types of BD will need to target beyond the brain and the eye, and to address the likely existence of functionally distinct transcript variants. Hence, the systemic administration of small molecule therapeutics could lead or complement ERT and gene therapy approaches by treating both common and disease-specific effects. In particular, the repurposing of previously approved drugs, rather than an *ex-novo* testing of novel compounds, represents a recognized and rapid translational opportunity for incurable rare genetic diseases.

By integrating cell-based phenotypic screening and repurposing FDA-approved drugs, we have recently identified tamoxifen (Figure 1), a drug that showed activity ameliorating *in vitro* and *in vivo* pathological hallmarks of two different BDs, CLN3 and CLN7 [8]. We found that tamoxifen significantly reduces the intracellular pathological accumulation of Gb3 and the subunit c of the Mitochondrial ATP Synthase (SCMAS) in CLN3 and CLN7 cellular models. Importantly, despite its activity as a Selective Estrogen Receptor Modulator (SERM), we found that tamoxifen is able to ameliorate BD phenotypes through a mechanism that is independent of Estrogen Receptors (ERs), but involves activation of the Transcription Factor EB (TFEB), a master gene of global lysosomal function and autophagy [9]. We found that tamoxifen-mediated induction of TFEB was triggered by lysosomotropic-mediated inhibition of mTORC1. Furthermore, *in vivo* administration of tamoxifen significantly rescued $Cln7^{\Delta ex2}$ mutant mice from brain cortex Gb3 accumulation, reducing SCMAS storage, hindlimb clasping, and motor discoordination.

Importantly, our data strongly suggest that tamoxifen may be a suitable drug to treat most subtypes of NCLs rather than be specific toward a single disease form, thus widening the plethora of patients that could potentially be eligible for this therapeutic route. In this review, we discuss important clinical aspects that need to be considered to move forward with tamoxifen as a suitable drug for the treatment of multiple BDs.
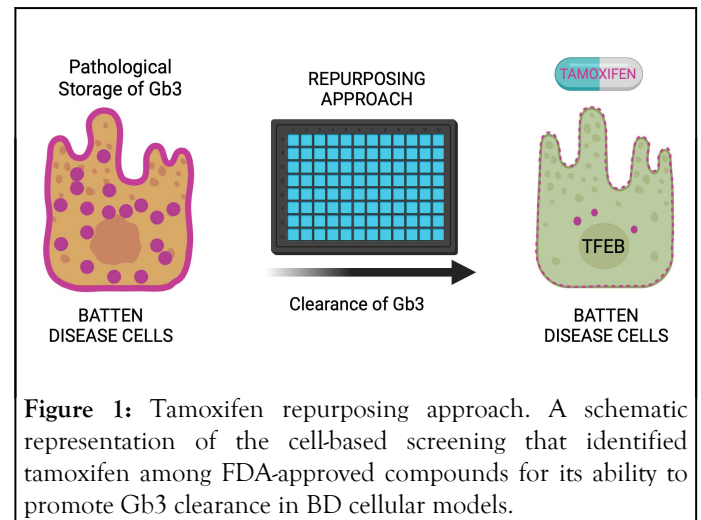

Figure 1: Tamoxifen repurposing approach. A schematic representation of the cell-based screening that identified tamoxifen among FDA-approved compounds for its ability to promote Gb3 clearance in BD cellular models.

LITERATURE REVIEW

Tamoxifen clinical aspects

Tamoxifen citrate (hereafter called tamoxifen) is a nonsteroidal SERM, that belongs to the class of organic compounds containing a 1,2-diphenylethylene moiety, known as stilbenes. Tamoxifen is a readily available EMA-and FDA-approved compound, indicated for the treatment of mainly breast cancer in a variety of settings, especially for patients with ER-positive tumors. FDA-approved indications include (i) treatment of breast cancer in both female and male patients [10], (ii) adjuvant treatment of breast cancer in patients who completed a primary treatment with surgery and radiation [11], (iii) treatment of ductal carcinoma *in situ* (non-invasive breast cancer) in female patients after surgery and radiation to reduce the risk of invasive breast cancer [12], and (iv) breast cancer risk reduction in certain patients with overt predisposition [13]. Tamoxifen also has many off-labelled uses, alone or in combination with other drugs, as shown in Table 1.

Indications	References
Treatment of progressive or recurrent desmoid tumors in combination with sulindac	Hansmann et al., 2004
Treatment of endometrioid histologies that are recurrent, metastatic, or at high-risk	Fiorica et al., 2004, Thigpen et al., 2001
Treatment of primary or secondary gynecomastia along with breast pain associated with it	Boccardo et al., 2005
Induction of ovulation in the treatment of infertility	Steiner et al., 2005
Treatment of oligospermia in combination with testosterone	Adamopoulos et al., 2003

Prophylaxis of coronary arteriosclerosis in men with a triple vessel	Clarke et al., 2001
Treatment of advanced or recurrent ovarian cancer	Hatch et al., 1991
Treatment of bladder cancer	Dellagrammaticas et al., 2001
Treatment of lung cancer in addition to initial chemotherapy treatment	Yang et al., 1999
Treatment of precocious puberty due to McCune-Albright syndrome in females	Eugster et al., 2003
Treatment of metastatic malignant melanoma	Beguerie et al., 2010
Treatment of benign mammary dysplasia	Ricciardi and Ianniruberto, 1979
Treatment of bone metastasis	Spooner and Evans, 1979
Treatment of carcinoid tumor	Moertel et al., 1984
Treatment of cutaneous polyarteritis nodosa	Cvancara et al., 1998
Treatment of hypertrophy of uterus	Magos, 1990
Treatment of meningioma	Markwalder et al., 1985
Treatment of primary breast pain, premenstrual mastodynia, or breast pain that originated from liver cirrhosis	Fentiman et al., 1988, Li et al., 2000, Serels and Melman, 1998
Prophylaxis of postmenopausal osteoporosis	Kristensen et al., 1994
Improvement of length and quality of life in patients with retinoblastoma in addition to treatment protocols	Taçyildiz et al., 2003
Treatment of Riedel's thyroiditis	De et al., 2001
Treatment of solid tumor secondary malignant neoplasms	Lissoni et al., 1996

Note: Adapted from Farrar and Jacobs, 2021

Table 1: Non-FDA-approved indications (off-label).

Pharmacokinetics

Human pharmacokinetics of tamoxifen has been extensively reviewed by Morello, et al. [14]. Briefly, upon oral administration tamoxifen is rapidly and extensively absorbed from the gastrointestinal tract [14], peak levels occur after 3 to 7 hours, and steady state is reached typically after 3 to 4 weeks. In terms of distribution, tamoxifen is a highly lipophilic agent, resulting in extensive plasma protein binding (especially to albumin). A human study of tamoxifen distribution showed high concentrations of tamoxifen in liver, lung, pancreas, brain, ovaries and breast tissue [15]. Tamoxifen is metabolized in the liver by the cytochrome P450 enzymes (primarily by CYP3A4, CYP2C9, but also CYP2D6 is involved) into its active metabolites (i.e., endoxifen and afimoxifene) [16]. Tamoxifen is then excreted in bile and mainly eliminated in faeces (only small amounts are found in urine), and has a long elimination half-life of typically 5 to 7 days [16], which is attributed to its high plasma protein binding as well as to enterohepatic recirculation.

Pharmacodynamics

As a nonsteroidal SERM, in tumour cells and other tissue targets, tamoxifen binds to ER in a competitive manner with its endogenous agonist, namely estrogen, which results in the formation of a nuclear complex that reduces DNA synthesis and suppresses estrogen effects. Such anti-estrogenic activity allows tamoxifen to inhibit growth by blocking cells in the G0-G1 phases of the cell cycle. However, depending on the tissue, it can have mixed estrogenic (i.e., uterus and liver) and anti-estrogenic (i.e., breast tissue) activity. As ER agonist, tamoxifen has been associated to increasing incidence of uterine malignancies [17,18], whereas in bone tissue it prevents bone loss, namely osteoporosis, by mimicking the effects of estrogen in osteoclasts [19]. Additionally, it has been described that tamoxifen can promote apoptosis in both ER-positive and ER-negative tumours through the activation of caspases [20-22]. Tamoxifen is considered a long-acting medication since its active metabolite, endoxifen, has a half-life of about 2 weeks. However, it has a narrow therapeutic index, though, as greater doses can cause seizures or difficulties breathing.

DISCUSSION

Dose-response studies and main non-oncologic clinical studies

Tamoxifen has been tested in a number of clinical trials as a potential therapeutic treatment for non-oncologic conditions (Table 2). For example, a double-blind randomised clinical trial in another neurodegenerative disease, Amyotrophic Lateral Sclerosis (ALS), has tested tamoxifen in 18 patients [23]. Participants were followed up at 1, 3, 6, and 12 months. In this case, the primary endpoint was patient survival or dependence on mechanical ventilation, while secondary endpoints were decline of the revised ALS Functional Rating Scale (ALSFRS-R) score and pulmonary function measured by Forced Vital Capacity (FVC). Results showed that tamoxifen exerted only a modest effect on attenuation of progression for 6 months in this small trial, therefore additional larger scale studies should be necessary to investigate whether, in view of our recent findings in BD, enhancing autophagy through TFEB activation can attenuate ALS progression.

In addition, the use of tamoxifen in Duchenne Muscular Dystrophy (DMD) children has shown encouraging results in a completed phase 1 trial, in terms of a protective effect on dystrophic muscle from contraction-induced damage, and, importantly, with no adverse effects [24]. Also, tamoxifen demonstrates promising effects in psychiatric disorders, like bipolar disorder, where its therapeutic action may be independent of interaction with ERs through the inhibition of the Protein Kinase C (PKC), a known molecular target in these disorders [25].

Clinicaltrials.gov ID	Title
NCT02166944	Tamoxifen treatment in patients with motor neuron disease
NCT02835079	Treatment effect of tamoxifen on patients with Duchenne's Muscular Dystrophy (DMD)
NCT00206544	Anti-Estrogens-A potential treatment for bipolar affective disorder in women?
NCT00026585	Examination of tamoxifen in acute mania in patients with bipolar I disorder
NCT03354039	Tamoxifen in duchenne muscular dystrophy
NCT00784940	ATAC-Bone density sub-protocol
NCT00411203	Tamoxifen in treatment of acute mania
NCT00687102	Cognition in the study of tamoxifen and raloxifene
NCT00214110	Tamoxifen therapy in Amyotrophic Lateral Sclerosis (ALS)
NCT01257581	Safety and efficacy study of creatine and tamoxifen in volunteers With Amyotrophic Lateral Sclerosis (ALS)

Table 2: Relevant non-oncologic clinical trials.

Safety

Tamoxifen's safety for paediatric use in BD remains to be fully established. However, certain clinical studies have demonstrated the safety and effectiveness of tamoxifen in young patients. For instance, a patient who took tamoxifen (10 mg TID) for over 3 years was able to successfully delay the premature puberty associated with McCune-Albright Syndrome (MAS) [26]. Additionally, it was discovered that tamoxifen treatment (20 mg QD) for 6 months in adolescents with pubertal gynecomastia was a safe, well-tolerated, and efficient treatment option, as an alternative to other medical treatments [27]. However,

prospective and controlled clinical research is required to examine the effectiveness and safety of tamoxifen in young patients affected by lysosomal storage diseases like BD.

The anti-inflammatory and anti-fibrotic properties of tamoxifen led to promising results in DMD patients [24]. The Israeli team has shown that a 6-month compassionate treatment with tamoxifen in 3 boys with DMD improved their condition. Another trial with tamoxifen on DMD was conducted on (i) 79 boys with DMD (ages 6.5 to 12) with the ability to walk and under stable treatment with glucocorticoids, and (ii) on a second group of up to 20 DMD boys (ages 10 to 16), but unable to walk and not under glucocorticoid treatment. Participants were randomly assigned to 20 mg of tamoxifen or placebo QD for 48 weeks. Preliminary data from the trial and its follow-up Open Label Extension (OLE) study confirmed that tamoxifen was safe and generally well tolerated [28]. In addition, a study in a mouse model of muscular dystrophy showed that tamoxifen and raloxifene, also a breast cancer treatment, improved cardiac, respiratory, and skeletal muscle functions, and increased bone density [29]. Overall, adverse effects in these populations have been rare, and tamoxifen seems to have an excellent safety profile. Together with our data, therefore, tamoxifen might represent a novel small molecule therapeutic for at least two types of BD, namely CLN3 and CLN7 diseases.
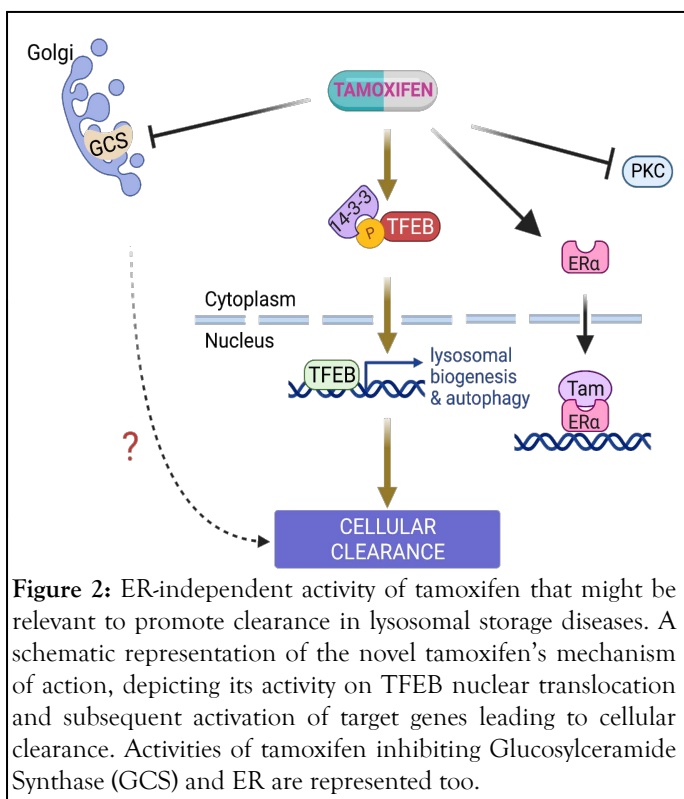
TFEB activation as strategy to tackle Lysosomal Storage Diseases (LSDs) and neurodegeneration

Lysosomal biogenesis and autophagy are transcriptionally regulated by a gene network and by its master gene TFEB, a member of the basic Helix-Loop-Helix Leucine Zipper (bHLH-Zip) transcription factors [9,30,31]. TFEB activity responds to environmental cues and is regulated by mTORC1-mediated phosphorylation, which occurs on the lysosomal surface [32]. By siRNA-mediated HCL screening, we discovered that calcineurin, a Ca^{2+}-modulated phosphatase, de-phosphorylates TFEB, thus promoting its nuclear translocation. Interestingly, we also found that calcineurin is activated by a lysosomal calcium signalling mechanism mediated by the lysosomal calcium channel Mucolipin 1 (MCOLN1) [33]. Together these results revealed, for the first time, the presence of lysosome-to-nucleus signaling mechanisms and changed the view of the lysosome from a "suicide bag" to a dynamic organelle that responds to environmental cues. The identification of a global transcriptional regulation of lysosomal function was exploited, first by us and then by other groups, to boost lysosomal function in mouse models of a variety of disease conditions [34-38]. We found that the overexpression of TFEB on cellular models of LSDs promotes the clearance of pathologic lysosomal storage. Also, we found that TFEB induces clearance through the activation of lysosomal exocytosis, by inducing both the pre-docking of lysosomes close to the plasma membrane and the release of lysosomal calcium to promote lysosomal fusion. Viral-mediated TFEB gene transfer resulted in the clearance of accumulating substrates in cells and tissues from mouse models of several types of LSDs [34]. Therefore, the possibility of modulating lysosomal function by acting on TFEB network may lead to a novel therapeutic strategy with potential applicability to

more that 50 LSDs as well as more common neurodegenerative diseases. An obvious therapeutic application of these discoveries is the identification of small molecules to activate TFEB pathway in disease conditions. In addition to tamoxifen [8], we have identified and characterized various molecules that regulate TFEB and reduce storage in LSDs, such as the natural product genistein [39,40], and more recently the CNS drug fluoxetine [41].

Mechanisms of action of tamoxifen

Although designed to be a specific ER antagonist, tamoxifen presents many off-target activities [42], including the inhibition of PKC, Calmodulin, and P-Glycoproteins [43]. Thus, in recent years there has been increasing interest within the literature for potential novel "off-target" effects of tamoxifen and its metabolites independent of their anti-ER mechanisms [44]. We found that the therapeutic effect on BD is exerted by tamoxifen through an ER-independent mechanism and discovered that it is actually mediated by the activation of TFEB (Figure 2). In addition, observations on the chemical nature of the compound suggest that the effects of tamoxifen on inducing TFEB activation and reducing intracellular Gb3 storage in CLN3 and CLN7 cell models are due to its weak-base property. Mechanistically, we observed that tamoxifen induced TFEB nuclear translocation by specifically impairing mTORC1-mediated phosphorylation of TFEB without affecting mTORC1 activity toward its canonical substrates, such as S6K, 4EBP and ULK1. Giving the recent observations that RagC/D GTPase activity can mediate selective phosphorylation of mTORC1 substrates [45], we postulated that lysosomotropic properties of tamoxifen specifically affect RagC/D activity leading to the dephosphorylation of TFEB.

Figure 2: ER-independent activity of tamoxifen that might be relevant to promote clearance in lysosomal storage diseases. A schematic representation of the novel tamoxifen's mechanism of action, depicting its activity on TFEB nuclear translocation and subsequent activation of target genes leading to cellular clearance. Activities of tamoxifen inhibiting Glucosylceramide Synthase (GCS) and ER are represented too.

Whether tamoxifen induces clearance through TFEB-mediated lysosomal exocytosis or other mechanisms still needs to be addressed in future studies. Interestingly, a few reports suggest that tamoxifen can alter glycosphingolipid metabolism in cancer cells [42,46]. Thus, future studies are needed to determine whether the reported effects of tamoxifen on glycosphingolipid regulation may contribute to Gb3 clearance and whether the activation of TFEB is involved (Figure 2).

Tamoxifen-TFEB axis to treat CNS disorders

Tamoxifen already showed a neuroprotective effect in rat ischemia models protecting brain tissue from ischemic injury [47,48]. Similarly, using a canine model of anterior circulation infarction (stroke) to mimic the human clinical condition, Boulos and colleagues were able to consistently show that tamoxifen was effective at significantly improving the canine neurological deficits and reducing the size of the stroke. Interestingly, it has been shown that the activation of TFEB confers neuroprotection in several animal models [49-51]. Consistently, another SERM, raloxifene [52], has shown neuroprotection and immunomodulatory effects in a mouse model of Parkinson's Disease (PD) [53]. On the other hand, TFEB overexpression studies have suggested it to be a promising therapeutic target to prevent neurodegeneration in various disease models including LSDs and more common neurodegenerative diseases. In PD, AAV-mediated overexpression of TFEB prevented degeneration of dopaminergic neurons in a rat model of α-synuclein induced toxicity [54]. Overexpression of TFEB in both MAPT/Tau and APP-PSEN1/PS1 mice models ameliorated Tau pathology by reducing biochemical markers such as APP and Aβ production, and rescuing behavioural phenotypes [55,56]. Similarly, TFEB activation cleared huntingtin protein aggregates and reduced neurotoxicity in Huntington's Disease (HD) transgenic mice and cellular models [57].

Together with our discovery that tamoxifen ameliorates pathological hallmarks in BD, these observations support the potential benefits of repurposing tamoxifen (and tamoxifen analogs) to treat LSDs and more common neurodegenerative disorders through the activation of TFEB pathway. Additionally, since most of the approved CNS-penetrant drugs are lysosomotropic, future studies are needed to elucidate whether all these compounds can promote clearance of pathological storage through the activation of TFEB [47-51].

CONCLUSION

Tamoxifen administration already showed a neuroprotective effect. The identification that tamoxifen, an orally administered compound that crosses the blood-brain barrier and ameliorates two subtypes of neurodegenerative BD *in vitro* and *in vivo*, may represent the first small molecule treatment suitable to tackle multiple BD. Future preclinical studies are required to better understand the mechanisms of action of tamoxifen to promote cellular clearance, which might include TFEB activation and glycosphingolipid synthesis inhibition. We envisage that clinical studies may support the translation of these findings into clinics, thus benefiting a number of paediatric patients currently lacking a therapeutic opportunity.

ACKNOWLEDGMENT

REFERENCES

1. Haltia M, Goebel HH. The neuronal ceroid-lipofuscinoses: A historical introduction. Biochim Biophys Acta. 2013;1832(11): 1795-1800.

2. Kauss V, Dambrova M, Medina DL. Pharmacological approaches to tackle NCLs. Biochim Biophys Acta Mol Basis Dis. 2020;1866(9): 165553.

3. Mole SE, Anderson G, Band HA, Berkovic SF, Cooper JD, Holthaus SM, et al. Clinical challenges and future therapeutic approaches for neuronal ceroid lipofuscinosis. Lancet Neurol. 2019;18(1):107-116.

4. Gardner E, Mole SE. The genetic basis of phenotypic heterogeneity in the neuronal ceroid lipofuscinoses. Front Neurol. 2021;12:754045.

5. Schulz A, Ajayi T, Specchio N, de Los Reyes E, Gissen P, Ballon D, et al. Study of intraventricular cerliponase alfa for CLN2 disease. N Engl J Med. 2018;378(20):1898-1907.

6. Kim J, Hu C, Moufawad El Achkar C, Black LE, Douville J, Larson A, et al. Patient-customized oligonucleotide therapy for a rare genetic disease. N Engl J Med. 2019;381(17):1644-1652.

7. Palmieri M, Pal R, Nelvagal HR, Lotfi P, Stinnett GR, Seymour ML, et al. mTORC1-independent TFEB activation *via* Akt inhibition promotes cellular clearance in neurodegenerative storage diseases. Nat Commun. 2017;8(1):1-9.

8. Soldati C, Lopez-Fabuel I, Wanderlingh LG, Garcia-Macia M, Monfregola J, Esposito A, et al. Repurposing of tamoxifen ameliorates CLN3 and CLN7 disease phenotype. EMBO Mol Med. 2021;13(10):e13742.

9. Sardiello M, Palmieri M, di Ronza A, Medina DL, Valenza M, Gennarino VA, et al. A gene network regulating lysosomal biogenesis and function. Science. 2009;325(5939):473-477.

10. Eggemann H, Altmann U, Costa SD, Ignatov A. Survival benefit of tamoxifen and aromatase inhibitor in male and female breast cancer. J Cancer Res Clin Oncol. 2018;144(2):337-341.

11. Gradishar W, Salerno KE. NCCN guidelines update: Breast cancer. J Natl Compr Canc Netw. 2016;14(5S):641-644.

12. Staley H, McCallum I, Bruce J. Postoperative tamoxifen for ductal carcinoma *in situ*: Cochrane systematic review and meta-analysis. Breast. 2014;23(5):546-551.

13. Sauter ER. Breast cancer prevention: Current approaches and future directions. Eur J Breast Health. 2018;14(2):64-71.

14. Morello KC, Wurz GT, DeGregorio MW. Pharmacokinetics of selective estrogen receptor modulators. Clin Pharmacokinet. 2003;42(4):361-372.

15. Lien EA, Wester K, Lønning PE, Solheim E, Ueland PM. Distribution of tamoxifen and metabolites into brain tissue and brain metastases in breast cancer patients. Br J Cancer. 1991;63(4): 641-645.

16. Sanchez-Spitman A, Dezentjé V, Swen J, Moes DJ, Bohringer S, Batman E, et al. Tamoxifen pharmacogenetics and metabolism: Results from the prospective CYPTAM study. J Clin Oncol. 2019;37(8):636-646.

17. Fisher B, Costantino JP, Redmond CK, Fisher ER, Wickerham DL, Cronin WM, et al. Endometrial cancer in tamoxifen-treated breast cancer patients: Findings from the National Surgical Adjuvant Breast and Bowel Project (NSABP) B-14. J Natl Cancer Inst. 1994;86(7):527-537.

18. Magriples U, Naftolin F, Schwartz PE, Carcangiu ML. High-grade endometrial carcinoma in tamoxifen-treated breast cancer patients. J Clin Oncol. 1993;11(3):485-490.

19. Nakamura T, Imai Y, Matsumoto T, Sato S, Takeuchi K, Igarashi K, et al. Estrogen prevents bone loss *via* estrogen receptor α and induction of fas ligand in osteoclasts. Cell. 2007;130(5):811-823.

20. Jordan VC. Fourteenth Gaddum Memorial Lecture. A current view of tamoxifen for the treatment and prevention of breast cancer. Br J Pharmacol. 1993;110(2):507-517.

21. Radin DP, Patel P. Delineating the molecular mechanisms of tamoxifen's oncolytic actions in estrogen receptor-negative cancers. Eur Pharmacol. 2016;781:173-180.

22. Abd-Alhaseeb MM, Massoud SM, Elsayed F, Omran GA, Salahuddin A. Evening primrose oil enhances tamoxifen's anticancer activity against breast cancer cells by inducing apoptosis, inhibiting angiogenesis, and arresting the cell cycle. Molecules. 2022;27(8): 2391.

23. Chen PC, Hsieh YC, Huang CC, Hu CJ. Tamoxifen for amyotrophic lateral sclerosis: A randomized double-blind clinical trial. Medicine. 2020;99(22):e20423.

24. Tsabari R, Simchovitz E, Lavi E, Eliav O, Avrahami R, Ben-Sasson S, et al. Safety and clinical outcome of tamoxifen in Duchenne muscular dystrophy. Neuromuscul Disord. 2021;31(9):803-813.

25. Novick AM, Scott AT, Epperson CN, Schneck CD. Neuropsychiatric effects of tamoxifen: Challenges and opportunities. Front Neuroendocrinol. 2020;59:100869.

26. Eugster EA, Shankar R, Feezle LK, Pescovitz OH. Tamoxifen treatment of progressive precocious puberty in a patient with McCune-Albright syndrome. J Pediatr Endocrinol Metab. 1999;12(5): 681-686.

27. Zehetner A. Tamoxifen to treat male pubertal gynaecomastia. Int J Pediatr Adolesc Med. 2015;2(3-4):152-156.

28. Nagy S, Hafner P, Schmidt S, Rubino-Nacht D, Schädelin S, Bieri O, et al. Tamoxifen in Duchenne Muscular Dystrophy (TAMDMD): Study protocol for a multicenter, randomized, placebo-controlled, double-blind phase 3 trial. Trials. 2019;20(1):637.

29. Wu B, Shah SN, Lu P, Bollinger LE, Blaeser A, Sparks S, et al. Long-term treatment of tamoxifen and raloxifene alleviates dystrophic phenotype and enhances muscle functions of FKRP dystroglycanopathy. Am J Pathol. 2018;188(4):1069-1080.

30. Settembre C, Di Malta C, Polito VA, Arencibia MG, Vetrini F, Erdin S, et al. TFEB links autophagy to lysosomal biogenesis. Science. 2011;332(6036):1429-1433.

31. Settembre C, de Cegli R, Mansueto G, Saha PK, Vetrini F, Visvikis O, et al. TFEB controls cellular lipid metabolism through a starvation-induced autoregulatory loop. Nat Cell Biol. 2013;15(6): 647-658.

32. Settembre C, Zoncu R, Medina DL, Vetrini F, Erdin S, Erdin S, et al. A lysosome-to-nucleus signalling mechanism senses and regulates the lysosome via mTOR and TFEB. The EMBO J. 2012;31(5): 1095-1108.

33. Medina DL, Di Paola S, Peluso I, Armani A, de Stefani D, Venditti R, et al. Lysosomal calcium signalling regulates autophagy through calcineurin and TFEB. Nat Cell Biol. 2015;17(3):288-299.

34. Medina DL, Fraldi A, Bouche V, Annunziata F, Mansueto G, Spampanato C, et al. Transcriptional activation of lysosomal exocytosis promotes cellular clearance. Dev Cell. 2011;21(3):421-430.

35. Spampanato C, Feeney E, Li L, Cardone M, Lim JA, Annunziata F, et al. Transcription Factor EB (TFEB) is a new therapeutic target for Pompe disease. EMBO Mol Med. 2013;5(5):691-706.

36. Song JX, Sun YR, Peluso I, Zeng Y, Yu X, Lu JH, et al. A novel curcumin analog binds to and activates TFEB *in vitro* and *in vivo* independent of MTOR inhibition. Autophagy. 2016;12(8): 1372-1389.

37. Rega LR, Polishchuk E, Montefusco S, Napolitano G, Tozzi G, Zhang J, et al. Activation of the transcription factor EB rescues lysosomal abnormalities in cystinotic kidney cells. Kidney Int. 2016;89(4):862-873.

38. Napolitano G, Ballabio A. TFEB at a glance. J Cell Sci. 2016;129(13):2475-2481.

39. Moskot M, Montefusco S, Jakóbkiewicz-Banecka J, Mozolewski P, Węgrzyn A, di Bernardo D, et al. The phytoestrogen genistein modulates lysosomal metabolism and Transcription Factor EB (TFEB) activation. J Biol Chem. 2014;289(24):17054-17069.

40. Settembre C, Medina DL. TFEB and the CLEAR network. Methods Cell Biol. 2015;126:45-62.

41. Capuozzo A, Montefusco S, Cacace V, Sofia M, Esposito A, Napolitano G, et al. Fluoxetine ameliorates mucopolysaccharidosis type IIIA. Mol Ther. 2022;30(4):1432-1450.

42. Morad SA, Cabot MC. Tamoxifen regulation of sphingolipid metabolism-therapeutic implications. Biochim Biophys Acta. 2015;1851(9):1134-1145.

43. Kellen JA. Genomic effects of tamoxifen. Anticancer Res. 1996;16(6B):3537-3541.

44. Clifford RE, Bowden D, Blower E, Kirwan CC, Vimalachandran D. Does tamoxifen have a therapeutic role outside of breast cancer? A systematic review of the evidence. Surg Oncol. 2020;33:100-107.

45. Napolitano G, di Malta C, Esposito A, de Araujo ME, Pece S, Bertalot G, et al. A substrate-specific mTORC1 pathway underlies Birt-Hogg-Dubé syndrome. Nature. 2020;585(7826):597-602.

46. Lavie Y, Cao HT, Volner A, Lucci A, Han TY, Geffen V, et al. Agents that reverse multidrug resistance, tamoxifen, verapamil, and cyclosporin A, block glycosphingolipid metabolism by inhibiting ceramide glycosylation in human cancer cells. J Biol Chem. 1997;272(3):1682-1687.

47. Kimelberg HK, Jin Y, Charniga C, Feustel PJ. Neuroprotective activity of tamoxifen in permanent focal ischemia. J Neurosurg. 2003;99(1):138-142.

48. Kimelberg HK. Tamoxifen as a powerful neuroprotectant in experimental stroke and implications for human stroke therapy. Recent Pat CNS Drug Discov. 2008;3(2):104-108.

49. Forte M, Marchitti S, Cotugno M, di Nonno F, Stanzione R, Bianchi F, et al. Trehalose, a natural disaccharide, reduces stroke occurrence in the stroke-prone spontaneously hypertensive rat. Pharmacol Res. 2021;173:105875.

50. Wu Z, Zhang Y, Liu Y, Chen X, Huang Z, Zhao X, et al. Melibiose confers a neuroprotection against cerebral ischemia/reperfusion injury by ameliorating autophagy flux *via* facilitation of TFEB nuclear translocation in neurons. Life. 2021;11(9):948.

51. Zhang Y, Wu Z, Huang Z, Liu Y, Chen X, Zhao X, et al. GSK-3β inhibition elicits a neuroprotection by restoring lysosomal dysfunction in neurons *via* facilitation of TFEB nuclear translocation after ischemic stroke. Brain Res. 2022;1778:147768.

52. Selyunin AS, Hutchens S, McHardy SF, Mukhopadhyay S. Tamoxifen blocks retrograde trafficking of Shiga toxin 1 and 2 and protects against lethal toxicosis. Life Sci Alliance. 2019;2(3):e201900439.

53. Poirier AA, Côté M, Bourque M, Morissette M, di Paolo T, Soulet D. Neuroprotective and immunomodulatory effects of raloxifene in the myenteric plexus of a mouse model of Parkinson's disease. Neurobiol Aging. 2016;48:61-71.

54. Decressac M, Mattsson B, Weikop P, Lundblad M, Jakobsson J, Björklund A. TFEB-mediated autophagy rescues midbrain dopamine neurons from α-synuclein toxicity. Pro Natl Acad Sci. 2013;110(19):E1817-E1826.

55. Polito VA, Li H, Martini-Stoica H, Wang B, Yang LI, Xu Y, et al. Selective clearance of aberrant tau proteins and rescue of neurotoxicity by transcription factor EB. EMBO Mol Med. 2014;6(9):1142-1160.

56. Xiao Q, Yan P, Ma X, Liu H, Perez R, Zhu A, et al. Neuronal-targeted TFEB accelerates lysosomal degradation of APP, reducing Aβ generation and amyloid plaque pathogenesis. J Neurosci. 2015;35(35):12137-12151.

57. Tsunemi T, Ashe TD, Morrison BE, Soriano KR, Au J, Roque RA, et al. PGC-1α rescues Huntington's disease proteotoxicity by preventing oxidative stress and promoting TFEB function. Sci Transl Med. 2012;4(142):142ra97.