

Transforming Curry Extract to Liposomal Curcumin (Lipocurc™) in Parkinson Disease (PD) Therapeutics Landscape: Emerging Role of Epigenetics Signaling and Nanotechnology

Simon S Chiu^{1,2*}, Kristen Terpstra³, Michel Woodbury-Farina⁴, Ram Mishra^{5,6}, Vladimir Badmaev⁷, Joshua T Varghese⁸, Hana Raheb¹, Ed Lui⁹, Zack cernovsky¹, Yves Bureau¹, Mariwan Husni¹⁰, John Copen¹¹, Mujeeb Shad¹², Hou Jirui¹³, Autumn Carriere^{1,14}, Zahra Khazaeipool^{1,15}, Robbie Campbell¹ and Weam Sieffien¹⁶

¹Department of Psychiatry, Western University (University of Western Ontario), Lawson Health Research Institute, London, Ontario, Canada

²Geriatric Mental Health Program, London Health Sciences Centre, Ontario, Canada

³Neurological Unit, St Michel's Hospital Affiliated University, Toronto, Ontario, Canada

⁴Department of Psychiatry, School of Medicine, University of Puerto Rico, Puerto Rico

⁵Department of Psychiatry and Neurosciences, McMaster University School of Medicine, McMaster University Health Sciences Centre, Neuropharmacology Lab, Hamilton, Ontario, Canada

⁶Department of Medicine, School of Medicine, McMaster University Division of Neurology, Department of Clinical Neurological Sciences, McMaster University Health Sciences Centre, Hamilton, Ontario, Canada

⁷Medical Director, Medical Holdings Inc, New York, NY, USA

⁸Currently Year III Medical Student, Marian University College of Osteopathic Medicine, Indianapolis, Indiana, USA

⁹Department of Pharmacology, Western University, London, Ontario, Canada

¹⁰Department of Psychiatry, Northern Ontario Medical School, Thunderbay, Ontario, Canada

¹¹Department of Psychiatry, University of Victoria Medical Campus, University of British Columbia, Victoria, BC, Canada

¹²Department of Psychiatry, Oregon Health Sciences University, Portland, Oregon, USA

¹³Dalian Pharmaceutical Co. Natural Medicine Research and Development, Dalian Province, China

¹⁴Currently Nursing Program, Faculty Applied Sciences, Nipissing University, North Bay, ON, Canada

¹⁵Postdoctoral Research Fellow, University of Western Ontario, London, Ontario, Canada

¹⁶Currently Year III Medical Student, Faculty of Medicine, University of Toronto, Toronto, Ontario, Canada

***Corresponding Author:** Simon S Chiu, Geriatric Mental Health Program, London Health Sciences Centre, Ontario, Canada.

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Abstract

Despite advances in genetics and brain imaging, none of the currently approved drugs for treatment of Parkinson Disease (PD) ranking second to Alzheimer Dementia Alzheimer Dementia (AD) the commonest neurodegenerative disorder, can modify the course of PD in terms of progressive functional decline. There is a paucity of data on the neurobiological underpinnings of prodromal phase of non-motor symptoms (NMS) of PD. Epigenetics signaling paradigm in probing genetics-environment interface comprises three components: DNA methylation, histone modification and non-coding RNA, in regulating expression of PD related genes. In PD, epigenomics plays an emerging pivotal role in shaping the impact of downstream cascade of dopamine neuronal loss, neuro-inflammation and oxidative stress in the basal ganglia and mesolimbic and mesocortical brain regions. We briefly review the current and emerging pharmaceutical treatment options for PD and related motor and NMS complications. We review the multi-faceted pharmacology of curry extract isolated from plant *Curcuma Longata* in translational models of PD, highlighting on the interplay of curcumin's molecular footprints as epigenetics modulator interfacing differentially with alpha-Synuclein (SYN) and LRRK-2 Leucine-rich-repeat-kinase-2 (LRRK-2) mutation, the putative culprits in shaping the progressive course of PD. We briefly summarize the crucial milestones in transforming curcumin from curry extract to liposome-based curcumin: Lipocurc™ via the navigating the challenging roadmap of nanotechnology. We summarize the pharmacokinetics data from Phase I trial of Lipocurc™ exhibiting a highly favorable safety and tolerability profile of the potential PD drug lead. We conclude that both translational and clinical studies of curcumin serve as impetus for designing a transformative nanotechnology driven epigenetic-based PD therapeutics platform to the clinical arena. Accelerating biomarker-based clinical trials of Lipocurc™ may be the game-changer in PD therapeutics landscape.

Keywords: Curcumin; Parkinson Disease; Nanotechnology; Liposome; Epigenetics; Alpha-Synuclein (SYN) Leucine-Rich-Repeat-Kinase-2 (LRRK-2)

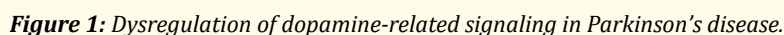
I. PD update: Prodromal and non-motor symptoms (NMS)

Parkinson Disease ranks second only to Alzheimer's Dementia (AD) as the commonest neurodegenerative disorder [1]. With the aging population, the prevalence of PD is expected to double over the next 25 years not only in USA, but also in the developing countries in Asia and South America. The core motor symptoms of PD consist of bradykinesia, resting tremor, akinesia, muscle rigidity and postural instability. For the past decades, research advances have refined the construct of PD as a multi-system neurodegenerative disorder with non-motor symptoms (NMS) emerging somewhat insidiously during the prodromal phase of PD. The cluster of NMS consists of reduced sense of smell, REM-related sleep disorder, daytime sleepiness, infrequent bowel movement, orthostatic hypotension, depression, and anxiety. With the progression of PD, the quality of life is gradually compromised by co-morbid conditions including cognitive impairment progressing to PD-Dementia (PDD). In PD, cognitive decline is best manifested as visuospatial, attentional, and executive deficits, accompanying memory deficits. Dementia in PD (PDD) is thought to be related directly to the accumulation of alpha-SYN within the inclusion body named Lewy body. Whereas dementia follows the onset of PD symptoms, Lewy body dementia (LBD) PD symptoms and may share overlapping brain pathways with PDD. The molecular footprints for the conversion to PDD and LBD remains elusive and ill-defined.

The emergence of the prodromal phase of PD is best understood in terms of the finding that overt motor symptoms appear as late as twenty years when the dopamine cell loss in zona compacta of the substantia nigra: SNc and the decline in striatal dopamine level reach the threshold of more than 40% as compared with the baseline [1,2]. Recent studies [2] have shed light on the integrative view that PD belongs to the category of alpha-synucleinopathy overlapping with atypical Parkinsonism disorders including multiple system atrophy and supranuclear palsy. Alpha-synuclein (SYN) has recently been found in the gut, calling into question as to gut-brain-motor system nexus in PD.

The composite heuristic model of PD: dysregulation of dopamine signaling in the basal ganglia is modified to embrace the neural network malfunctioning of the cortico-basal ganglia-thalamic cortical (CBGTC) loop [3]. Brain imaging studies in PD have corroborated earlier neurobiological findings in identifying abnormalities in the limbic circuitry involving the brain regions: the orbitofrontal and anterior cingulate cortices, amygdala, thalamus and ventral striatum, as the substrates underlying the pathophysiology of depression, apathy and anxiety in PD. Positron Emission Tomography (PET) has complemented MRI imaging in identifying lesions in the mesolimbic dopaminergic and serotonergic pathways as contributing towards NMS [4]. New molecular imaging advances in labelling the array of neurotransmitters in PD and atypical Parkinsonism can identify potential biomarkers for early changes to predict the clinical course of PD in order to elucidate the complexity of neural circuitry dysfunction in PD.

Advances in our understanding of the complexities of PD are translated towards new ways of targeting various signaling pathways involved in PD [1-4]. As depicted in figure 1, the pathophysiology of PD is best visualized at the synapse in the context of imbalance between pre- and post-synaptic dopamine synapses and the impact of non-dopaminergic pathways: serotonin, NMDA (N-methyl-D-aspartic acid) glutamate system. The environmental determinants: insecticide and air pollutants exposure, nutritional imbalance and metabolic disorder: Type II diabetes mellitus, interact with susceptible PD genes to unravel the epigenetics landscape in PD. At the cellular level, pharmacological targeting of the dynamics of inflammation, apoptosis, and neurogenesis, has opened new vistas towards novel PD drug development template.



Taken together, converging evidence suggests that the cluster of NMS intersect with extrapyramidal motor symptoms (EPS) at the intricate cross-roads of parallel mesolimbic and nigrostriatal dopamine systems regulating motor control and cognitive and behavioral function. The detailed cellular and molecular signatures underlying the EPS and NMS circuitry dysfunction may unlock the key towards our understanding of the progressive nature of PD. However, there is a paucity of studies to evaluate the choice of treatment options for PD-related NMS: anxiety, depression, cognitive impairment leading to dementia, and psychosis.

II. Current and emerging PD drugs

Recent systematic reviews and meta-analysis of PD treatment by international panel of experts conclude that Levo-DOPA (L-DOPA) in the presence of peripheral decarboxylase inhibitor: benserazide or carbidopa remains the mainstay for the management of PD motor symptoms [9-11]. The enthusiastic search for innovative pharmacological interventions to delay, prevent and slow the rate of progression has met with limited success. The International Parkinson and Movement Disorder Society evidence medicine review concludes that no clinically useful interventions are yet available to prevent/delay PD disease progression [9]. There appears to be significant gaps in translating advances in cellular and molecular footprints and neural network signatures driving PD course to inform PD therapeutics development. The L-DOPA sparing therapeutic strategy to minimize L-DOPA dyskinesia and motor fluctuations have not been supported by the findings from the ELLDOPA (Early versus Later Levodopa therapy in PD patients) study showing L-DOPA did not accelerate PD progression [12]. In the ELLDOPA multicenter, parallel-group, double-blind, dosage-ranging, randomized, controlled clinical trial, 361 early PD patients were randomized to three dosages of Carbidopa/levodopa 12.5/50 mg tid, 25/100 mg tid and 50/200 mg tid and placebo groups with endpoint defined as the change in the total Unified Parkinson's Disease Rating Scale (UPDRS) between baseline and Week 42. The results showed that L-DOPA may slow down the rate of PD despite the paradoxical negative impact on the dopamine transporter as measured with the percent change in striatal ⁽¹²³⁾iodine 2-beta-carboxymethoxy-3-beta-(4-iodophenyl)tropane (beta-CIT) uptake between baseline and Week 40 visits. The ELLDOPA study demonstrated that dyskinesia and L-DOPA wearing off motor complication can occur as early as 6 months, and high dosage of L-DOPA can delay or prevent gait freezing.

Converging evidence suggests that L-DOPA exerts complex multiple actions on both DA and non-DA neurons including GABAergic, glutamatergic and cholinergic neurons [13]. L-DOPA has further been found to evoke marked increase in extra-cellular DA in extra-striatal regions: nucleus accumbens, the subthalamic nucleus and the frontal cortex. Auto-oxidation of dopamine has been suggested as the mechanism for oxidative stress triggering and sustaining DA-neurodegeneration and may account for the decline in the functional activity of the pre-synaptic DA transporter. Recently, Single Photon Emission Tomography: SPECT imaging of DA transporter has gained some credibility as the sensitive biomarker for clarifying the differential diagnosis of PD and for monitoring the treatment responses in PD [14]. Currently, there is no consensus of international criteria to support the validity of diagnosis of PD at the early or prodromal phase in both motor and non-motor domains.

Refinement of DA receptor subtype classification and function and sensitivity in response to L-DOPA treatment leads to development of a series of DA agonists (bromocriptine, apomorphine, rotigotine, pramipexole and rotigotine) in early PD treatment [15], with divergent efficacy in reducing the functional impairment. Non-ergot DA agonists: pramipexole, ropinirole and transdermal rotigotine, are preferred over the ergot DA agonists due to the highly favourable safety profile. A systematic review of long-acting non-ergot DA agonists: rotigotine transdermal patch, extended-release pramipexole, and ropinirole prolonged-release, indicates that nanotechnology-assisted formulation improved the PD motor symptoms through increasing the "on-time" while reducing the "off-time" and activities of daily living (ADL) [16]. Controlled or sustained release formulations reflect highly promising approach of using nano-technology-driven delivery systems to prolong the actions of DA agonists through encapsulating the active drugs in bio-compatible carriers. However, no consistent findings of premature emergence of L-DOPA induced dyskinesia (LID) were reported. Both positive and negative findings of DA agonists on NMS: sleep disturbances and depressive symptoms for long acting DA agonists [17]. Impulse control disorders: pathological gambling has been reported with sub-chronic PD treatment with L-DOPA and DA agonists. The issue of starting PD with long acting DA agonists as monotherapy in preference over L-DOPA remains highly controversial.

The model of Stage-based clinical management paradigm in PD provides the rationale for developing bold drug delivery systems for advanced PD with severe motor fluctuations. Infusion pumps requiring surgical procedure and installation of drug-loaded pumps have been approved in Europe and USA [18]. Review of the outcome data from three double blinded randomized controlled trials in advanced

PD indicates that LCIG (l-DOPA-carbidopa intestinal gel infusion) in bypassing the gastric mucosa for duodenal installation increased the “On” time and reduced the “off” time and improved the quality of life without severe dyskinesia. Follow-up extension studies confirm the effects are sustained. Similar positive findings are reported for infusion pumps filled with apomorphine, the prototypal agonist at Dopamine D-1/D-2 receptors [19]. Apomorphine delivery system in encompassing subcutaneous route for injection or chronic infusion is more flexible with fast onset of action, but devoid of the well known side effects of nausea and vomiting associated with oral dosage. In view of the high degree of variability in pharmacokinetics and sensitivity, apomorphine dosage in PD has to be individualized. Alternative routes of delivery: intra-nasal and inhalation are being explored. Taken together, LCIG and apomorphine pumps are acceptable devices for advanced PD.

Targeting monoamine oxidase-subtype B (MAO-B) in prolonging the therapeutic actions of L-DOPA at the DA synapses through blocking the degradation of dopamine via the pathway of MAO-B inhibition has sparked interest as a disease-modifying strategy in PD [20]. In PD clinical trials, selegiline as the earliest MAO-B inhibitor with no diet restriction requirement related to lack of tyramine potentiation, exhibits consistent level of efficacy in meeting the outcome endpoints defined as the change in the Unified Parkinson’s Disease Rating Scale (UPDRS) score and serious adverse events. In the ADAGIO study in early PD using the delayed-start double blinded trial, rasagiline at daily oral dosage of 1 mg, appeared to hold promise in altering PD course [21]. However, the original claim of rasagiline as a disease-modifying agent in PD has been disputed by a subsequent 3-year open label ADAGIO follow up study. The lack of validated biomarkers to monitor PD progression may be the confounding factor for the discrepancy in interpretation of the results.

A recent network approach to analyse the relative efficacy of the three MAO-B inhibitors: selegiline, rasagiline and safinamide indicated that selegiline ranks first in efficacy when combined with l-DOPA in early PD [22]. On the other hand, Safinamide, the newest comer to the class of MAO-B inhibitors, exhibits a very favorable safety profile and is efficacious as monotherapy or combination therapy, in reducing the severity of both motor and NMD symptoms in early PD [23]. Safinamide exhibits additional neuroprotective action in inhibiting glutamate release and in blocking of voltage-dependent sodium channels, modulation of calcium channels. Further controlled studies are required to evaluate critically the role of safinamide mesylate in mid-to-late stage of PD, especially with regard to its effects in reducing or preventing the emergence of L-DOPA-induced dyskinesia (LID).

The discovery of potent inhibitors of the enzyme: Catechol-O-methyl-transferase: COMT involved in regulating the degradation of dopamine and related catecholamines at the synaptic terminal has sparked interest to increase the availability of L-DOPA formulation combined with DOPA decarboxylase inhibitors: carbidopa and benserazide [24]. COMT catalyzes the transfer of the activated methyl group: S-adenosyl methionine to endogenous catecholamines and 3-o-methyldopa is the biomarker of COMT inhibitor. The therapeutic goal of combining COMT inhibitors with L-DOPA is to slow the metabolism of L-DOPA and provide the network milieu for sustained DA modulation, while slowing auto-oxidation of dopamine at the synaptic terminals.

A recent systematic review of 14 clinical studies of adjuvant treatment with entacapone Entacapone, 1st generation COMT inhibitor in L-DOPA maintained late-advanced PD (n = 2,804) found that entacapone significantly increased the “on-time” and reduced the “off-time” of motor complications and improved the core motor symptoms as measured with the United Parkinson’s Disease Rating Scale (UPDRS) scores [25]. The adverse events rates reported to be significantly higher than placebo group included nausea, urine discoloration, gastrointestinal disorder and dyskinesia. The 2nd generation of COMT inhibitor, tolcapone exhibits dual targeting of both central and peripheral COMT. Phase II and Phase III clinical trials of tolcapone (TPC) found significant beneficial effects in improving PD motor fluctuations and hence reducing L-DOPA dosage requirements [26]. However, the rare albeit serious severe hepatotoxicity calls for more stringent monitoring of the liver function. Subsequent re-analysis of the pharmaco-surveillance data concludes that the risk is very small if hepatic monitoring guidelines are followed.

Opicapone (OPC), the prototypal 3rd generation COMT inhibitor, has recently been approved in Europe as highly efficacious and safe adjunct treatment to L-DOPA therapy in counteracting the motor fluctuations of PD [27]. COMT inhibitors modulate the pharmacokinetics of L-DOPA through the COMT-mediated methylation pathway whereby L-DOPA bioavailability is increased. The elimination half-life and minimal effective concentration of L-DOPA are both increased. Pooled analysis of the two pivotal trials of OPC: BIPARK I and BIPARK II [27] showed that OPC improved motor fluctuations, regardless of the baseline Hoehn-Yahr stage and PD duration, as early as the 1st week of treatment in reducing the “off-time” in a dose dependent manner, with the maximum effect. OPC at 50 mg daily dosage, reduced the off-time by 2.26 hr compared with 1.40 hr I the placebo control. In the 1-yr extension open label of BIPARK II trial indicated that the increase in “ON-time” occurred without marked increase in dyskinesia or worsening of the core PD symptoms. No hepatobiliary adverse events were observed. Formal application has been filed with US FDA for approval as adjunct to L-DOPA. While OPC is safe and well tolerated in PD, 4.6% of patients experienced weight loss and hallucinations.

Taken together, development of COMT inhibitors in PD is based on rational design of enzyme-substrate interactions with respect to identifying through X-ray crystal structure analysis of the active site and co-factor: magnesium involvement at the catechol and S-adenosyladenosine (SAM) sites [25-27]. The once daily oral dosage of OPC indicated for end-of-dose motor fluctuations in late or mid-stage of PD, appears to be most appealing to PD patient compliance. It remains to be seen whether OPC treatment as adjunct or first-line choice in early PD will be equally neuroprotective in delaying or preventing the motor complications.

Our therapeutic discussions of DA agonists, MAO-B inhibitors and COMT inhibitors are driven by the Stage-based individualized approach in managing “ON” and “OFF” motor fluctuations of PD. In advanced PD, LID often emerges when the daily “Off-time” is increased [28]. The negative impact of LID on the quality of life and functional impairment cannot be over-estimated, let alone the caregiver burden. Pharmacological management of LID underscore the severity of LID-related functional impairment. Amantadine, originally developed as an anti-viral drug, has rekindled interest as a drug lead for the management of L-DOPA induced dyskinesia [29]. Based on the positive findings from the pivotal trials in LID, extended release formulation of amantadine ER; becomes the first drug approved by US FDA for the treatment of LID. In the 13-week and 25-week trial of LID, amantadine ER at the oral dosage of 274 mg significantly improved LID as measured with patient-reported Unified Dyskinesia Rating Scale (UDysRS) scoring and ON/OFF times. Adverse events appeared to be mild and well tolerated. The most common treatment emergent adverse events included hallucinations, dizziness, dry mouth and peripheral oedema. Long term safety and efficacy are lacking pending completion of the Phase IV pharmacovigilance study targeting the elderly population. Reviewing the pharmacology of amantadine reveals that amantadine behaves as a putative potent NMDA non-competitive antagonine modulating the release of dopamine in a coordinated manner and regulating dopamine receptor binding at DA-2/3 receptor in both nigrostriatal and mesolimbic dopamine systems.

PD drug platforms targeting non-DA signaling in relentless search for disease modifying therapeutics continue. In view of the role of purinergic signaling in modulating DA neurotransmission in the striatum, targeting adenosine receptors: A1 and A2 with cAMP as the downstream effector, paves the way for the potential drug lead: istradefylline the putative A2a antagonist [30]. A meta-analysis [30] of four 12-week placebo-controlled clinical studies recruiting total of 1,143 participants diagnosed as PD showed that istradefylline, recently approved by US FDA as add-on treatment to L-DOPA/carbidopa in PD, is efficacious in reducing “off” periods and improving the motor symptoms in advanced PD. Whether istradefylline is beneficial L-DOPA dyskinesia (LID) remains inconclusive. However, istradefylline may exert positive benefits in NMS of cognition and depression; further controlled studies are needed to corroborate its efficacy in delaying cognitive decline and in facilitating recovery from PD-related Depressive disorder.

As discussed earlier, endoplasmic reticulum stress (ERS) has been proposed as the likely triggering event for misfold SYN conformation progressing to oligomers and aggregates. The spread of SYN through cell-to-cell transfer in a prion-like fashion can be the prime event for the devastating nature of PD. Targeting ERS can be beneficial in PD. In translational model of PD, zonisamide is unique in that it possesses anti-epileptic property while dual targeting both dual targeting ERS and MAO-B inhibition in PD models [36]. In the *in vitro* neuroblastoma cell treated with neurotoxin MPP⁺ (1-methyl-4-phenylpyridinium), zonisamide suppressed cell death. In the *in vivo* MPP⁺ model, zonisamide at 20 mg/kg po, antagonized apoptosis and inhibited ERS-induced cell death via downregulating the ERS-related factors: C/EBP homologous protein (CHOP) and caspase-3 activation within the mitochondria. A systematic review of four randomized placebo controlled trials in PD in Japan found that zonisamide significantly reduced wearing-off time and UPDRS total scores compared with placebo [36], with no negative impact on dyskinesia. Zonisamide belongs to the new class of multi-target MAO-B inhibitor in addition to its effects in regulating ERS.

Taken together, MAO-B inhibitors dampen dysregulated dopamine metabolism resulting unchecked oxidative stress and neuroinflammatory pathways. COMT inhibitors on the other hand reset the prime regulatory control of dopamine metabolism at the crucial synapses at the nigro-striatal DA-mediated pathway. Both modes of action by MAO-B inhibitors and COMT and the novel drug delivery systems of L-DOPA and DA agonists, are directly relevant to the pathophysiology of PD. In reviewing the current and pipeline PD drugs, we note that PD therapeutic approaches have not yet embraced the emerging role of the Epigenetics code in PD. In PD landscape, deciphering the Epigenetics code can be the high impact and low risk drug platform in transforming epigenetics standing at the interface of classical genetics (genetic variants, common varying number, gene mutation modes) and the vast albeit interrelated SYN-aggregation and DA neuronal loss, mediating the oxidative stress and neuro-inflammation cascade.

III. Epigenetics Signaling in PD

Five familial gene loci have been extensively studied in PD: alpha-synuclein (SYN), Parkin, PINK1, DJ-1 and L-leucine rich repeat kinase 2 (LRRK-2) [32]. Recent genome wide association studies (GWAS) have clarified that variations in the two familial PD genes: SYN and LRRK-2 confer significant risk factors for sporadic PD. The genetic variants of α -synuclein and leucine-rich repeat kinase (LRRK2) confer phenotypes in familial and sporadic PD. The hallmark of PD: Lewy bodies consisting of α -Synuclein and LRRK2 often undergo posttranslational modifications (PTMS). PTMS are key processes in regulating the stability, localization, and function of protein and are highly vulnerable towards environmental toxins: exposure to insecticides (paraquat, rotenone) [33]. Environmental factors including exposure to pesticides have only recently been increasingly recognized as contributing towards PD. Meta-analysis of longitudinal epidemiological studies of PD revealed that exposure to pesticides increased PD risk [33]. While the exact mechanistic relationship between the cumulative dose and duration of insecticide exposure and the onset and severity of PD remains to be refined, PD models based on the insecticide: rotenone, reproduces the core elements of PD. These considerations highlight the complex interplay of genes and environmental determinants to encompass the array of herbicide and insecticide exposure, metabolic events of insulin resistance, traumatic brain injury, and dietary effects in PD.

In the post-genomic era, there is growing interest to decipher the so-called epigenetics code underlying PD [34,35]. In general, epigenetics mechanisms shed new light on the mode of regulating gene expressions without any change in the primary DNA sequence. A genome wide association study examining brain and blood samples from PD patients found a distinct pattern of differential DNA methylation of PD-related genes in both brain samples and peripheral leukocytes from PD patients [36]. Both hyper- and hypo-methylation of the PD genes were reported. A recent study found evidence for aberrant epigenome using the new bioscience technology: induced pluripotent stem cell (iPSC)-derived dopaminergic neurons (nDA) generated by cell reprogramming of somatic skin cells (fibroblasts) obtained from both sporadic and LRRK-2 monogenic variants of PD patients [37]. The iPSC-DA study demonstrated for the first time

extensive DNA methylation and RNA expression changes in the two groups of PD patients. The molecular footprints emerged only when the stem cells underwent differentiation to the nDA. DNA hyper-methylation was most marked in the specific gene regulatory regions: PD DAn compared with the control cohort resulting in the down-regulation of the host of transcription factors related directly to PD. The iPSCs from PD only partially acquire fully the full epigenetic identity. The iPSC study, if replicated in larger samples and in other PD cell lines, may serve as the phenotype screening for drug discovery and for monitoring the responses of potential PD drugs in clinical trials. Whether the aberrant epigenome can be reversed by current PD therapy or pipeline PD drugs remains to be examined.

Selective DNA methylation changes have also been found. A recent study found changes in selective DNA methylation changes in PD at the alpha-synuclein gene: SNCA, but not at the LRRK2 promoter gene [38]. In leukocytes from PD clients, cytosine guanosine dinucleotides islands: abbreviated CpG-2 loci, SNCA was differentially hypo-methylated. Moreover, the 2nd, 4th and 9th CpG sites were significantly hypo-methylated in PD patients. Another independent study confirmed hypomethylation of SNCA [39]. Hypo-methylation of SNCA intron-1 was detected in peripheral blood mononuclear leukocytes: PBMCs of PD patients and DNA methylation levels were associated with Rep1 polymorphism. The shorter allele was associated with higher level of SNCA intron-1 methylation, and genotypes carrying the shorter allele showed significantly higher methylation level of SNCA intron-1 than genotypes carrying the longer allele. Taken together, methylation of SNCA has become the core feature of epigenetics landscape in PD.

Histone modification represents yet another mode of post-translation modification of the N-terminus of histone proteins which coil around DNA [34,40]. Histone acetylation at the lysine residue of the histone proteins is the commonest mode of epigenetics-mediated transcription regulation and is closely controlled by fine equilibrium between Histone histone acetyltransferases (HATs) and histone deacetylases (HDACs). In general, activation of HAT by endogenous or exogenous ligands relaxes the chromatin structure, and facilitates active transcription. On the other hand, HDACs shift the chromatin structure towards more condensed state called heterochromatin and represses transcription. It is likely that PD is characterized by a highly restricted epigenome responsive towards the interacting neurotransmitter systems: dopamine, GABA and serotonin, in the basal ganglia.

Histone modification in PD is primarily mediated through HDAC and histone acetyltransferase: HAT. For the past decade, great strides have been made in characterizing the four classes of HDAC in terms of structural and functional diversity, tissue distribution and relevance to CNS disorders [40,41]. Isoform-specific HDAC inhibitors: HDAC class I/II inhibitors have drawn much interest in drug development in PD, with mixed results. Valproate and phenylbutyrate inhibit HDAC class I isoforms: 1, 2, 3, 8 and Class IIa isoforms: 4, 5, 7, 9. It is noted that varinostat and trichostatin, oncology drug leads, hit Class I, Class IIa and Class IIb isoforms 6, and 10. Unlike the zinc-dependent Class I and Class IIa and Class IIb, Class III requires NAD⁺: nicotinamide as the co-factor and is re-named as Sirtuins: 1-7. The Class IV HDAC has isoforms 11. The structural diversity of HDACs most likely makes HDAC a highly attractive drug target for PD.

Two preliminary studies support the model of HAT/HDAC imbalance in PD [40]. A postmortem brain study found histone H-3 acetylation levels were markedly higher in midbrain DA neurons and motor cortex of PD patients compared to matched control [41]. Under these conditions, midbrains from PD showed apoptosis at a faster rate. Treatment of neuroblastoma cell line with the neurotoxin, 1-methyl-4-phenylpyridinium (MPP⁺)- results in degradation of HDACs and interferes with the function of lysosomal autophagy pathway in maintaining cellular homeostasis and cell survival. Inhibition of autophagy abolished MPP⁺-induced cell death and degradation of HDACs. We suggest that the dysregulation model of histone acetylation can be further validated in the CSF and peripheral bio-fluids of plasma and lymphocytes, in order to examine critically the emerging role of cascade of epigenetic regulation of autophagy in PD.

Non-coding RNAs (ncRNA) or microRNAs (miRNA), have recently joined the privileged class of epigenetics network as the key post-transcriptional regulator of gene expression through binding to the 3'-untranslated region (UTR) of their target mRNAs [43,44]. The net result is to de-stabilize the target mRNA of the respective genes modulating the host of biological responses in various brain regions. *In*

vivo and *in vitro* models of PD, miRNAs, also abbreviated as miRNA, can regulate oxidative stress and protect dopamine neurons from excessive insult and eventual cell death.

A systematic review of studies on miRNAs in PD [44] for the past decade reveals that differential expression of miRNAs analysis distinguished PD from health controls and between treated and untreated PD patients. The next generation small RNA sequencing (NGS) technology has been found to be very promising tool in unravelling the emerging role of miRNA in diagnosing PD and likely in therapeutics monitoring. Furthermore, the differentially expressed miRNAs detected in extracellular vesicles (EV) released from the cerebrospinal fluid to the peripheral bio-fluids: serum, and urine compartments [45]. The discovery and characterization of EVs as nanometer-sized membrane-bound vesicles known as exosomes and micro-vesicles, opens new horizon in our understanding of transcellular communication of miRNAs, proteins and lipids among different cell types. Exosome mode of transport in bio-fluids: blood, plasma, urine and CSF, can even unravel the enigmatic progressive nature neurodegenerative disorders. Serum miRNA has been found to correlate with PD severity and densities of plaques and tangles and with Lewy body pathology [45]. Controversies still exist whether Lewy Body Dementia (LBD) is overlapping yet distinct from Parkinson Disease Dementia. In LBW syndrome neurocognitive impairment precedes the onset of motor symptoms of Parkinsonism indistinguishable from PD.

Our review calls into question whether circulating miRNAs in body fluids of PD function as diagnostic biomarker of the severity and progression of PD, and whether miRNAs predict treatment response. Increased oxidative stress and chronic inflammation synergize in signaling the dynamics of neurodegeneration in PD by altering the levels of miRNAs and their target proteins. Antioxidants may provide neuroprotection by changing the levels of miRNAs and their target proteins.

IV. Pleiotropic pharmacology of curcumin in hitting epigenetics in PD

a) Translational PD studies

Despite advances in PD research, none of the drug leads target specifically on Epigenetics and LRRK-2 signaling pathways in PD. Very few pharmaceutical and biotechnology drug development platforms evolve from natural product and medicinal chemistry of spices and herbal folk medicine to High-Throughput Screening (HTS) assay stage in order to reach the pre-clinical trial stage of drug development. Recently, the turmeric spice, the century-old curry spice extract popular in India and Southeast Asia, and more recently in North America and Europe, isolated from the *Curcuma longata* (Figure 1) [46,47], has recently drawn a great deal of enthusiasm for its pleiotropic CNS effects in hitting multiple pharmacological targets in the brain. Intriguing enough, curcumin hits molecular signatures common to both cancer and brain disorders [47,48] and hence paves the way for repurposing curcumin from oncology drug lead to the new frontier of CNS drug development.

Curcumin modulates multiple cell signaling footprints shared by PD and cancer initiation and subsequent progression [49]. Cross-talks of cancer and neurodegenerative disorders like PD find unusual niche in multiple pathways regulating cellular homeostasis:

1. Cell cycle [cyclin D1 and cyclin E], apoptosis [activation of caspases and down-regulation of anti-apoptotic gene products];
2. Neuronal proliferation [(Human epidermal growth factor: HER-2, epidermal growth factor receptor (EGFR)], Activator protein 1 (AP-1), neuronal survival phosphatidylinositol 3-kinases serine/threonine-specific protein kinase (PI3K/AKT pathway);
3. Cytoskeleton: Matrix metalloproteinase (MMP)-9 (MMP-9 and adhesion molecules);
4. Angiogenesis: Vascular endothelial growth factor: (VEGF).

More significantly, curcumin hits two pivotal signal pathways directly associated with PD: neuro-inflammation (Nuclear Factor KappaB: NF-kappaB, Tumor necrosis factor: TNF, Interleukin: IL-6, cyclooxygenase (COX-2), and 5-Lipoxygenase (LOX) and alpha-synuclein (SYN) (Figure 2). The protein kinase: Akt, plays an important role in numerous cellular processes: glucose metabolism, apoptosis, cell proliferation, transcription and cell migration. On the other hand, the PI3K is activated by G-protein-coupled receptors and tyrosine kinase receptors which have drawn marked interest in PD. The MMP-9 regulates remodeling processes involving inflammation, and

fibrosis and confers signals for stimulation of blood vessels.

In a systematic review of curcumin's effects in neurotoxin-based model of PD, the results from 13 translational studies are highly consistent in reporting the protective effect of curcumin against neuronal loss in the substantia nigra and restored dopamine levels in the striatum [50]. Curcumin has neuroprotective effects in the 6-hydroxydopamine lesion model in rats as measured by decreased loss of tyrosine hydroxylase-immunoreactive fibers, decreased activation of astrocytes and microglia and sustained superoxide dismutase levels in the lesioned striatum [51]. In the MPTP model, curcumin at 80 mg/kg ip and tetrahydrocurcumin (60 mg/kg ip) significantly reversed depletion of dopamine and inhibited the monoamine oxidase (MAO)-B activity [52]. The novel pyrazole analogue of curcumin: CNB-001 attenuated the behavioral impairment (catalepsy, akinesia) and down-regulated the inflammatory and apoptotic markers (tumor necrosis factor- α , interleukin-1 β , interleukin-6, inducible nitric oxide synthase, glial fibrillary acidic protein, cyclooxygenase-2 and Bax) [53]. Furthermore CNB-001 rescued dopamine neurons and enhanced expression of dopamine transporter and vesicular monoamine transporter 2 (VMAT2) expressions and enhanced mitochondrial function through regulating the anti-oxidant stress pathway. Our research group has shown for the first time that in the Park 7 (DJ-1)-knockout rat model of Parkinson's disease, intravenous Lipocurc™ improved motor impairment [54]. We chose DJ-1 knockout model in view of the converging evidence in support of DJ-1 gene as the prime gatekeeper of oxidative stress pathway. Lipocurc™, at the dosage of 20 mg/kg iv 3x weekly for 8 weeks, enhanced locomotor activity, reduced neuronal apoptosis and stimulated dopaminergic neurons in the substantia nigra.

b) Curcumin, synuclein: SYN and epigenetics dynamics in PD

Recently, misfolded α -synuclein (SYN) localized in the Lewy bodies of dopaminergic neurons forming neurotoxic SYN oligomers is considered as the trigger of the inflammation cascade in PD [55]. PD may even be redefined as DA-mediated SYN dysregulation disorder. Targeting SYN aggregation is gaining momentum in designing high impact PD therapeutics platform. Curcumin reduces SYN toxicity by binding to preformed SYN-oligomers and fibrils [56]. In the SH-SY5Y cells overexpressed A53T SYN, curcumin potently reduced A53T SYN accumulation and restored autophagy through down-regulation of mammalian-target-of- rapamycin (mTOR)/p70 ribosomal protein S6 kinase (p70S6K) signaling [57]. Curcumin reduces SYN neurotoxicity through inhibiting the extracellular signal-regulated kinase (ERK) signaling. Curcumin can maintain integrity of DA neurons through rescuing autophagy and inhibiting SYN aggregation and neurotoxicity. Induction of autophagy and accelerating SYN clearance can be the game-changer in resigning PD therapeutics and may ultimately delay the spread of SYN and hence modifying the course of PD.

Emerging evidence indicates that SYN spreads in a prion-like manner starting from the gut and ascends upwards to the brain [58]. With the discovery of SYN in the enteric nervous system (ENS), the model that SYN spreads from ENS to the basal ganglia via the vagus nerve. Tracking Prion spread may ultimately advance our understanding of the progression of PD. Truncal vagotomy reduced the risk of PD [59]. Constipation has been shown as the likely prodromal symptom of PD [60]. Targeting Gut-Vagus-Brain (GVB) nexis with diet, pre- and pro-biotics and nutraceuticals can be the next phase of high impact and low risk PD therapeutics landscape. Curcumin has been found for the first time to behave as an anti-prion compound in a cell-free high throughput assay for characterizing the interactions of potential anti-prion fibril compounds on both the oligomer and fibril formation [61].

As discussed earlier, epigenome functioning plays an emerging role in pathophysiology of PD [32-35]. Phytochemicals may be the best bioactive agents to bridge the gap of aberrant epigenomics patterning underlying induction of autophagy and degradation of SYN in PD [62]. Curcumin is best described as a potent modulator of epigenetics signal pathways at multiple sites: curcumin is unique in interacting with multiple components of epigenetics signaling [63-66]. Curcumin suppresses DNA methyltransferase (DNA MET) and induces global genomic hypo-methylation of genes. In addition, curcumin inhibits Class I HDAC (Histone Deacetylase) isoforms 1, 3, 4, 5, 8; how-

ever, curcumin concomitantly activates Class III HDAC (Sirtuin1). Class III HDACs prefers NAD⁺ as a reactant to deacetylate acetyl lysine residues of protein substrates forming nicotinamide, the deacetylated product, and the metabolite 2'-O-acetyl-ADP-ribose.

The key epigenetics partners: HDAC and HAT, collaborate in forming close collaborative network in selectively activating PD-protective gene and silencing PD-at risk genes in a reciprocal manner [67]. The family of Sirtuins is the newcomer to the PD and preliminary evidence suggests that the epigenetics regulation of Sirtuins extends beyond brain disorders to embrace cardio-vascular diseases and metabolic disorders: diabetes mellitus and obesity [68]. Our research group has demonstrated that in the small cohort of schizophrenics treated with antipsychotics, insulin resistance correlates with the severity of neuroleptic-induced Parkinsonism and neurocognition [69]. Curcumin in activating HAT (histone acetyltransferase, and Sirtuins, is in a prime position to restore the homeostasis of HAT and HDAC activities in PD [63-66,70].

In high throughput epigenetics screening assay using HeLa nuclear extract, curcumin was found to be more potent in inhibiting HDAC than valproic acid and sodium butyrate [71,72]. The inhibition constant K_i of curcumin (539 nM) was comparable to K_i of Trichostatin A (504 nM). In the mutant SYN *Drosophila* transgenic model, diet supplementation with HDAC inhibitor, sodium butyrate, rescued locomotor impairment and even early mortality [73]. Paradoxically, flies with the genetic knockdown of HDAC activity through Sin3A loss-of-function mutation (Sin3A(1of)) were resistant to rotenone-induced locomotor impairment and early mortality. Paradoxically, curcumin activates the special class of NADH-dependent HDAC: Sirtuins SIRT1 [68,70]. The finding of curcumin's up-regulation of SIRT1 expression explains the neuroprotection of curcumin against the host of neurological disorders related to NMDA-(N-methyl-D-aspartic acid)-glutamate excitotoxicity, β -amyloid-induced cell death in cortical neurons, cerebral ischemic damage, and stroke mediated by the deacetylation of p53 and attenuation of apoptosis. We have shown the anti-apoptosis property of curcumin in the DJ-1 Knockout PD model [54].

There is preliminary evidence suggesting that histone modification and miRNA cross-talks can be the epigenetics driver affecting the course of PD. In the MPP⁺ treated *in vitro* PD model, loss of function analysis revealed that miR-494-3p expression activated SIRT3 function resulting in downstream enhancement in cell viability and reduction in apoptosis [74]. Both miRNA and sirtuins can function in a highly regulated and cooperative manner in attenuating the inflammation and oxidative stress pathway. In the human astro-glial cell culture, physiological dosages of iron and aluminum exerted synergistic effects in up-regulating reactive oxygen species (ROS): the ROS abundance correlated with the changes in Nuclear Factor- κ B (NF- κ B) DNA binding and miRNA-125b and miRNA-146a expression [75]. Emerging evidence suggests that curcumin plays the role as a putative NF- κ B inhibitor in reversing the negative effects of oxidative stress on NF- κ B signaling and the induction of miRNA-125b and miRNA-146a [63-66,75]. It is significant curcumin modulates miRNAs (miRNA-15a, miRNA-16, miRNA-21, miRNA-22, miRNA-26, miRNA-101, miRNA-146, miRNA-200, miRNA-203) and their multiple target genes [76]. Targeting brain-specific miRNA by curcumin will unravel new vista of PD therapeutics development. Further investigation of curcumin action on miRNAs in PD models will advance our understanding of miRNA-histone-coupling with regard to the respective targeting genes in PD.

In summary, curcumin and its analogues, as a pan-epigenetics regulator interacting with the epigenetics triad: DNA methylation, Histone modifications and miRNA, hit major targets relevant to PD: SYN aggregation, MAO-B (Monoamine oxidase)-B, cytokine-driven inflammation (Tumor-necrosis factor), oxidative stress network through the epigenetics signal pathway (summarized in figure 2).

c) Curcumin, epigenetics

Four of the common PD genes: Parkin, PINK1, DJ-1, LRRK2, exhibiting various modes of inheritance (autosomal dominant and autosomal recessive, early onset versus late-onset), with genetic polymorphisms, contribute to a large extent towards the pathophysiology of PD [77]. The LRRK-2 gene is linked to familial form of PD akin to the sporadic form of PD from the clinical phenotype analysis. Although

the sporadic PD is influenced by epigenetics determinants, the LRRK-2 familial PD may be impacted by the specific gene structure and function, as well as the components of the epigenetics network conferring neuronal vulnerability towards PD. The LRRK2 gene belongs to the large ROCO protein family by virtue of the Ras complex protein (Roc) domain, followed closely by the C-terminal Roc (COR) domain [78]. LRRK2 comprises 51 exons encoding 2527 amino acid residues. The genetic and structural characteristics of LRRK2 as the multi-domain protein complex has been largely elucidated and has been shown to consist of an ankyrin repeat region, a leucine-rich repeat domain, Roc domain, a COR domain, a mitogen activated kinase domain (MAPK) and a beta-propeller (WD40) domain. From the functional perspective, LRRK2 is known for LRRK-2 kinase activity mediated by the microtubule-associated-protein Tau (MAPT) gene domain and LRRK-2 GTPase activity mediated by the Roc and COR domains.

Recent research in PD has focused on the odd interplay of SYN and LRRK2 [79] as culprits in PD with regard to clinical phenotype, pathophysiology and designing pharmacological targets for PD drug development. Since SYN aggregates form the core of the Lewy body inclusions in PD, cross-talks among SYN Leucine-rich repeat kinase-2 (LRRK-2), alpha-SYN and Tau aggregates as prototypal multi-component phosphoproteins regulating phosphorylation-dephosphorylation cycle via phosphatase. Taken together, mutations of LRRK2 can have adverse effect on the rate of guanosine triphosphate (GTP) hydrolysis, enhance LRRK-2 kinase and LRRK-2 GTPase activity. The series of biochemical reactions can in turn trigger the cascade of cell death pathways including autophagic-lysosomal pathway, intracellular trafficking, mitochondrial dysfunction, and the ubiquitin-proteasome system. There is a paucity of data to compare the long term effects of LRRK-2 mutations: R-1441, G2019S in various ethnic groups across the world on the course of PD.

A recent *in vitro* study has identified for the first time curcumin as a prototypal LRRK-2 kinase inhibitor [80]. In the HEK293T cells transfected with LRRK-2 variants, hydrogen peroxide significantly increased both the wild type and G2019S- LRRK2 kinase activity as measured with *in vitro* auto-phosphorylation. Curcumin inhibited LRRK2 phosphorylation at ser-20132 site in a dose dependent manner. Curcumin at 10 microM reduced LRRK-2 kinase activity by more than 90% in both the LRRK2 immunoblotting assays and *in vitro* kinase assay using purified LRRK-2 proteins. Curcumin action in inhibiting LRRK-2 kinase parallels its effects on behavioral and dopamine neuronal loss rescue [80]. In the LRRK2 transgenic Drosophila model, curcumin appeared to induce LRRK-2 expression indices: mRNA and levels, in a time dependent manner in the rodent mesencephalic cell model *in vitro* [81]. The effect is selective, since the expression of PD-at risk genes: alpha-SYN parkin, and PTEN-induced putative kinase 1 (PINK1) were not expressed. Differential responses of normal versus diseased cell cultures may explain the discrepant findings.

Biphasic responses of DA neurons to curcumin application, may be an alternative explanation. In future, iPSC stem cells may afford a more reliable and consistent glimpse into the possible therapeutic benefits compared with adverse events. Whether curcumin confers bidirectional modulatory effects on dopamine-driven versus non-dopamine mesencephalic cells remain awaits further systemic investigation.

Very few studies have investigated whether the early stage of LRRK-2 knockout rodent resembles the prodromal phase of PD with emergence of non-motor symptoms. Our research group has reported preliminary result that in the young rats with LRRK-2 R1441G mutation, compensatory upregulation in the expression of the neurotrophic factor: cerebral dopamine neurotrophic factor (CDNF), in the hippocampus, independent of any change in the motor system [82]. The finding is relevant to our understanding of the prodromal non-motor phase of PD. Furthermore, the recently discovered neurotrophic factor: CDNF protects DA neurons from endoplasmic reticulum-stress-induced cell death through interacting ER-derived chaperones to dissolve SYN aggregates and hence reduce Lewy bodies formation in PD. Chaperones function to stabilize the conformation of proteins complexes, prevent further cell-to-cell propagation of SYN and facilitate the clearance of potentially neurotoxic SYN aggregates *in vivo*.

Despite the limited inconclusive data on the role of curcumin as a LRRK-2 kinase inhibitor, pharmacological targeting of LRRK-2 kinase with the design of small molecules as LRRK-2 kinase inhibitor has gathered momentum [83,84]. Recently the GTPase domain has emerged as a heuristic therapeutic target for counteracting the detrimental impact of LRRK-2 mutations in PD. Preliminary evidence indicates targeting LRRK-2 GTPase may soon become yet another high-impact calculated-risk frontier for PD drug development. Systematic analysis of the effect sizes of preclinical trials in *in vivo* and *in vitro* can guide the better development of Phase I/Phase II/Phase III to reduce the likelihood of off-set adverse events. Biomarker-based clinical trials with standardized a priori outcome measures. The ubiquitous localization of LRRK2 in non-neuronal tissues: kidney and lung, has raised concern regarding the development of LRRK-2 kinase inhibitor. Hitherto we are not aware of any drug leads possessing dual LRRK-2 kinase/GTPase and epigenetic modulator for PD drugs. In principle drug lead displaying dual properties will offer synergistic advantage, since LRRK-2 mutation exerts its likely neurotoxic effects on synaptic function and plasticity via shifting the epigenetics regulation towards HDAC-3 mediated deacetylation and hence compromising neuronal survival.

We propose that the mechanism of action of curcumin in PD is closely related to the concerted LRRK2 -coupled-HDAC inhibition. The model can be examined in detail by evaluating the degree of native curcumin and liposome-based curcumin in *in vitro* LRRK2 in target engagement assays [85]. In summary, curcumin has been shown to consistently hit the three key PD-related targets regulated by epigenetics mechanisms (Figure 2 and 3):

1. The anti-inflammatory phenotype exhibited by activated astrocytes: pro-inflammatory cytokines, Tumor necrosis alpha, IL-1b;
2. The anti-oxidant pathway involving glutathione and superoxide dismutase and inducible nitric oxide;
3. The anti-apoptosis signal interfering with iron deposition and melanin synthesis.

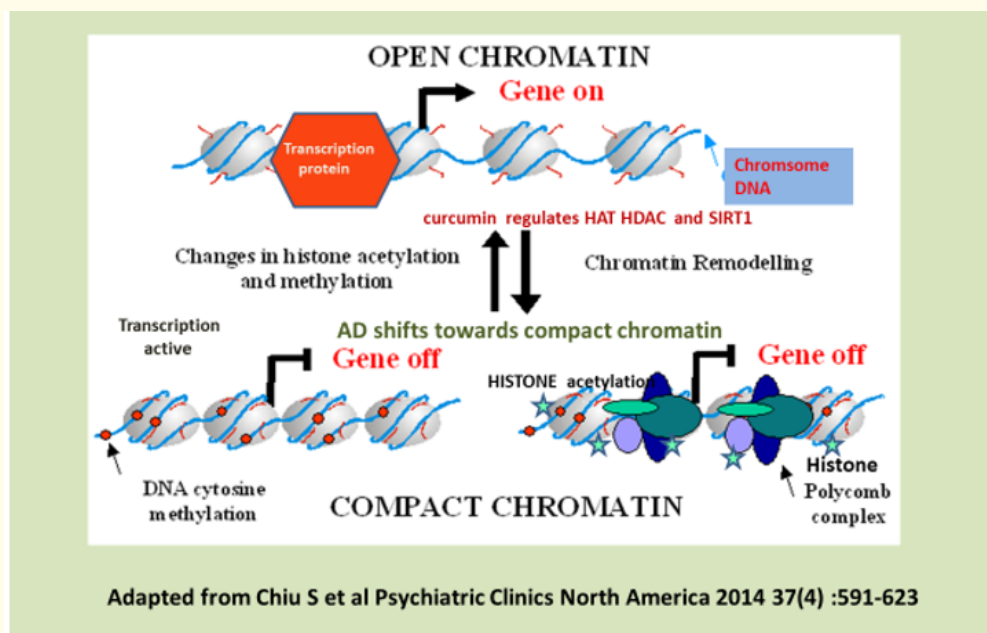


Figure 2: Curcumin modulates chromatin and genes “turned on and off” in Parkinson’s disease.

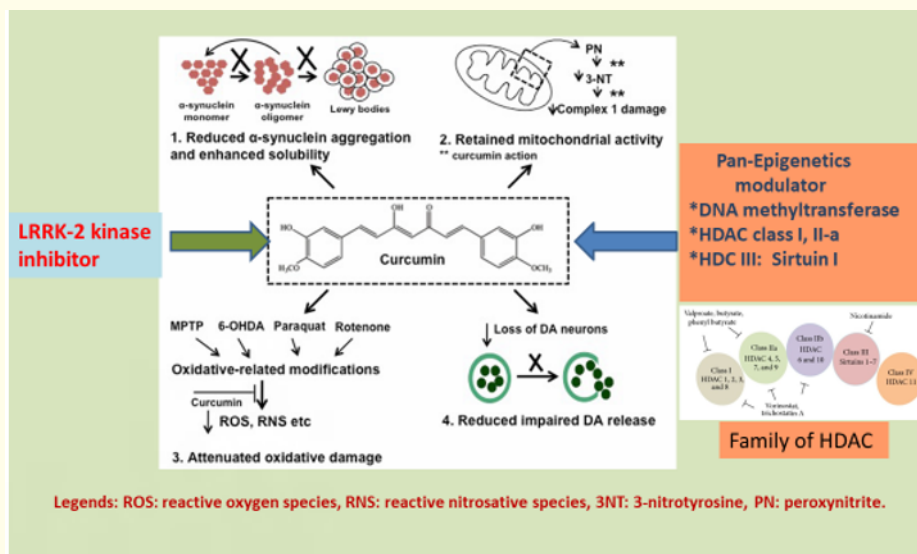


Figure 3: Molecular footprints of curcumin in Parkinson's disease synthesis of evidence.

V. Transforming curcumin to innovative PD drug

Despite positive findings of curcumin in translational models of PD, curcumin has yet to find its clinical niche above and beyond the classification of curry spice or curry extract as “dietary supplements, natural health products and wellness anti-aging platform”. The major drawback in translating the efficacy of curcumin from preclinical models to clinical arena of PD has only limited bio-availability of oral curcumin [86]. Despite preclinical studies of curcumin in PD models, no clinical study has yet been carried out in PD patients. Variability in bioavailability from the vast array of curcumin-related supplements, standardized extracts and natural health products may explain in terms of the divergent formulation standards in physico-chemical and biological stability, solubility, criteria of quality control and nature of quantitative determination of curcumin and metabolites derived from rapid first-pass metabolism. No data is available to validate the biological activities of metabolites from gut microbiota. For PD, curcumin may intercept at the gut-vagus-brain (GVB) nexus on transit to the brain. These considerations raise the important question as to the pharmacokinetics barriers and enhanced formulation requirements for pharmaceutical agents versus dietary supplements for health benefits claims when filing for therapeutic indications. For a novel drug lead with clearly defined therapeutic indications formal application has to be submitted to US FDA and equivalent drug control and approval agencies elsewhere, for a unique IND: Investigational New Drug Application number prior to designing and developing traditional Phase I/Phase II/Phase III vigorous evaluation for drug approval and release to health care providers for the prevention and treatment of diseases and medical conditions. We consider that the consistent efficacy of curcumin in translational models of PD, inform us to adopt a transformative paradigm to redesign curcumin as a potential drug by exploring nanotechnology formulation to accelerate the transition.

The curcumin component of Lipocurc was originally synthesized at Sami Labs Bangalore, India to 99.2% purity for conducting clinical trials with the original indication filed for the treatment of cancer. SignPath Pharmac. Inc (Sandy Utah USA) has taken the lead in developing curcumin as patented intravenous which has been refined and subsequently approved by US FDA has been approved by US FDA as investigational drug for clinical trials in oncology and more recently for brain disorders. The curcumin component of Lipocurc is originally synthesized at Sami Labs Bangalore to 99.2% purity for the treatment of cancer. The intravenous liposomal formulation of curcumin has been shown to penetrate across the blood-brain barrier (BBB) [86-88] (Figure 4).

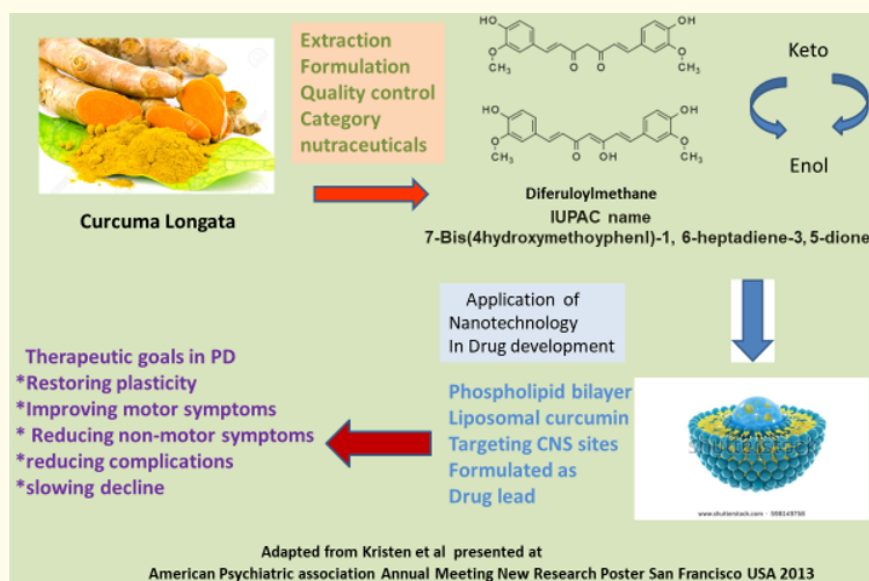


Figure 4: Transforming curcumin extract to Lipocurc as therapeutic strategy in Parkinson's disease.

For the past few years, there has been unprecedented interest in searching for brain-specific drug delivery system for CNS disorders. Nanotechnology through encapsulating the active drug inside bio-compatible materials: liposomes, polymers like hydrogel, has taken a promising strategic position in creating the new drug development platform [89]. Nanotechnology has extended its therapeutic role in oncology to brain disorders in anchoring drug development template in terms of improving the solubility, enhancing the bioavailability, reducing serious adverse events, minimizing off-target effects, and highly localizing the drug lead or nutraceuticals to the specific site of action in the brain.

Since none of the currently approved drugs can modify the course of PD, integrating nanotechnology with PD drug platform can be the cutting edge wave for PD therapeutics development [94]. Nano-formulations of potential PD drugs can bypass the faulty blood-brain-barrier (BBB) in PD through effective delivery of drug leads to the targeted brain sites through diverse mechanisms including diffusion, carrier-mediated uptake, endocytosis and internalization of the nano-drug formulations administered through non-oral routes: transdermal, intra-nasal, intravenous infusion or subcutaneous administration. Nano-formulation-based strategies consist of the systematic integration of compatible biomaterials: adjuvants, stabilizers, conjugates/polymer conjugates, lipid/liposomes, hydro/micro/nano gels, micelles, and nanoparticles to encapsulate the active drug: curcumin for accelerating the roadmap towards novel PD therapeutics. These considerations form the basis of optimizing drug delivery system with pharmacodynamics at the highly localized target to alter, delay or prevent the progression of PD, setting the initial stage for Nanotechnology-driven Personalized medicine in PD. Curcumin has benefited greatly from applying nanoparticles to prolong the pharmacological half-lives, to increase the physico-chemical stability and to prevent enzymatic degradation en route to the brain from the gut.

VI. Transforming PD therapeutics landscape with Lipocurc™

SignPath Pharma. Sandy Utah, USA has succeeded in formulating a patented liposome-based curcumin shown to be bioactive in tumor models and PD models. Pharmacokinetic studies have been completed in three species: rodents, dogs and humans [88,90-93]. In the rodent species, rats were given intravenous bolus injections three times a week for 4 weeks (empty liposomes, and 10, 20 and 40 mg/

kg Lipocurc™ [88]. For the rats, there were no deaths on the study, and no changes in the clinical signs, body weight, food consumption, clinical chemistries or organ weights, and no treatment-related adverse effects. Treatment with empty liposomes had the least effect, with a dose-response effect of the Lipocurc. In view of the wide safety margin, the No-Observed-Adverse-Effect-Level (NOAEL) for the rat was considered to be greater than 40 mg/kg. dosage. In the canine species [90,91], the beagle dogs were given 1 hour intravenous infusions at 10 mL/kg/h for 4 weeks (5% dextrose in water, empty liposomes, and 5 and 20 mg/kg Lipocurc). The beagle dogs treated with 5 mg/kg Lipocurc™, showed no signs of toxicity from overall analysis of the generated data: clinical observations, ophthalmology, ECGs, clinical pathology, gross necropsy and histopathology.

Two Phase I studies of Lipocurc in two cohorts in two cohorts; healthy volunteer subjects and patients diagnosed as cancer have been completed and published [92,93]. In the study on healthy control subjects [92] the protocol consisted of dose escalation study of single infusions of Lipocurc over 2 hours in healthy volunteers allocated to 5 subjects/group (4 active drug and 1 placebo) over 9 dosage-groups. 10, 20, 40, 80, 120, 180, 240, 320 and 400 mg/m². The results showed Lipocurc exhibited favorable tolerability and toxicity profile in normal subjects [92]. For analysis of metabolism of curcumin, serial blood collection for examining the changes in total curcumin and tetrahydrocurcumin was carried out at baseline, and during infusion at 15, 30, 90 minutes. Upon termination of the infusion, blood samples were drawn at the end of infusion and 5, 10, 15, 30, 45, minutes, and 1, 2, 4, 8, 24 and 48 hours post-infusion time intervals. Serial sampling was continued until the highest planned dose (400 mg/m²) was reached. transient echinocyte formation with no long lasting adverse effects observed. The infusion of Lipocurc resulted in rapid and dose-dependent rise of plasma levels of curcumin with Tmax values ranging from 0.9 - 1.7 hr. Cmax ranged between 42 ± 22 ng/mL and 2359 ± 412 ng/mL for 10 and 400 mg/m².

The 2nd Phase I trial found similar profile of safety and tolerability in patients with metastatic cancer [93]. The 8-week dose escalation protocol consisted of weekly intravenous infusion of Lipocurc with 100 mg/m² as the starting dosage over 8 hr. The starting dosage was increased to 300 mg/m² over 6 hr. The results showed no dose-limiting toxicity over the dosage range between 100 and 300 mg/m² was observed in the 26 patients over the dosage range between 100 and 300 mg/m². Of six patients receiving 300 mg/m² over 6 h, one patient developed hemolysis, and three other patients experienced hemoglobin decreases > 2 g/dL without signs of hemolysis. Pharmacokinetic analyses revealed stable curcumin plasma concentrations during infusion followed by rapid declines to undetectable levels after the infusion. While anti-tumor activity by RECIST V1.1 was not detected, significant tumor marker responses and transient clinical benefit were observed in two patients. As a guide to future Phase II trial in cancer patients, 300 mg/m² infusion over 6 hrs appeared to be the maximum tolerated dosage (MTD) of Liposome curcumin. For PD clinical trial, we may choose a lower conservative dosage of curcumin as the MTD to gain wider safety and tolerability margin.

In the present study, we propose to conduct Phase 1b of the patented liposome- encapsulated curcumin: Lipocurc in Parkinson disease patients to validate the multi- targeted neuroprotection of curcumin in Parkinson disease models. The overall objective is to examine whether Lipocurc is safe in PD and holds its promise in improving the motor symptoms of PD. We organize our proposed Phase Ib/ Phase II clinical trial of Lipocurc in PD using the milestone-driven drug development template for maximizing impact, minimizing risk, reducing cost and saving time approach.

In Milestone I, we will validate LRRK-2 kinase and GTPase activities and panel of zinc-dependent and NADH⁺HDAC activities as the putative pharmaco-dynamic biomarkers of Lipocurc action in PD. Previous study has shown curcumin behaves as a potent LRRK-2 kinase inhibitor [84]. We will use lymphocytes from PD patients to examine whether curcumin inhibits LRRK-2 kinase in a manner akin to the *Drosophila* model of oxidative stress. Furthermore, we will correlate LRRK-2 kinase and LRRK-2 GTPase with changes in inflammatory biomarker: Interleukin: IL-2 and IL-6 shown to be sensitive to PD disease severity.

In Milestone II, PD patients maintained on PD drugs after screening will be will receive either Lipocurc starting at 300 mg/M² over 2 hrs infusion, followed by Lipocurc 300 mg/M² every 4 days for the next 6 weeks. We will examine the effects of Lipocurc on UPDRS (United Parkinson Disease rating scale) and cluster of non-motor symptoms using the single arm Phase II futility trial protocol. We will recruit 30 subjects and define PD responders to Lipocurc as 3.0 point change in UPDRS at the end of study. If the response rate falls below 64%, the null hypothesis of Lipocurc treatment response is futile, is rejected, and Lipocurc is to be advanced to Phase III pivotal trial for accelerating Lipocurc as a novel therapeutics for treatment of PD. For pragmatic reasons, we choose the single arm futility trial design to arrive at the “GO or NOT-GO” decision point to fast track the lead compound in PD drug development.

Our study is predicated on our hypothesis is that Lipocurc functions as a LRRK-2 kinase inhibitor and HDAC modulator regulating the epigenetics-mediated inflammation signal cascade pathway in PD. The finding that asymptomatic LRRK-2 mutation carriers exhibit increase in peripheral inflammation indices, highlights LRRK2 as the prime driver in PD. We adopt the modified Bayesian adaptive approach to design the Phase Ib/II trial based upon the Bayesian model of Futility Clinical trial decision matrix and to analyse efficacy relative to adverse events to fast track Lipocurc to frontline of PD therapeutics. Our proof-of-concept Phase II protocol focusses on motor symptoms of PD as the primary outcome of PD trial.

VII. Conclusion and Future Directions

Our exploratory study paves the way to further investigate the role of LRRK2 intersecting with the Rab protein partners in PD. We have included the phosphorylated Rab10 GTPase protocol to examine changes in LRRK-2 kinase activity in PD [85]. Combining inflammation biomarkers with LRRK-2 kinase and epigenetics will chart a novel roadmap in PD. Curcumin has a unique toolbox to interact with LRRK-2 and SYN link in PD. We will design further studies to address the issue of modifying PD progression through enhancing antioxidant-mediated SYN clearance and stimulating neurogenesis. The issue whether Lipocurc™ will improve non-motor symptoms (depression, psychosis, impulse control disorder, dementia) and modify PD course will be our future research and development agenda. Curcumin, as the prime oxidative stress gatekeeper, may ultimately slow PD decline through restoring autophagy dysfunctions and stimulating neurogenesis. The vision of translating advances in delineating epigenomes in PD and in harnessing the advances in nanotechnology [94] towards formulating Lipocurc™ can open new frontier in PD therapeutics with the goal of modifying the PD, improving the symptom burden of PD and of aborting the onset of PD. We have yet to realign our approach with the global view of targeting non-motor symptoms of PD (anxiety, depression, psychosis and dementia) with Lipocurc™. Curcumin is likely to exhibit efficacy in reducing the severity of psychotic symptoms: visual hallucinations and delusions, in PD.

We conclude that disease-modifying therapeutic approach has to translate mechanisms of spread of SYN: trans-neuronal, neuron-to-glia and trans-synaptic interface to drug targets for both motor and nonmotor symptoms of PD. Facilitating cross-talks between the nigro-striatal and mesolimbic DA systems in PD may be essential. In this respect the Prion-like model of has been proposed to explain the progression of PD within the context of SYN oligomer and aggregates formation, seeding, SYN spread and autonomous SYN propagation may guide PD drug therapeutics [95,96]. Prion-like mechanisms has been proposed as the potent albeit neglected driver for the spread of SYN: from seeding to inter- and intra-cellular and inter-neuronal transfer to the final propagation. Targeting prion-like SYN spread will soon become the newest player in PD therapeutics development. PrP^C is regulated by rho-GTPase-coupled-cofilin-1 pathway, the putative mediator of cytoskeletal remodeling and synapse plasticity implicated in Prion CNS disorders [97] and epigenomics [34,35] in PD.

Misfolding and aggregation into amyloids of the prion protein (PrP) underlies the development of the fatal transmissible spongiform encephalopathy (TSE) related to Creutzfeld-Jakob disease (CJD) No drugs are currently investigated and approved for reversing the rapidly progressive course of CJD. Curcumin, in the *vitro* model of transmissible spongiform encephalopathy (TSE) related to Creutzfeld-Jakob disease (CJD), potently inhibited the accumulation of the protease-resistant prion protein (PrP-res) with an IC-50 (inhibition

constant) of 10 nM and partially blocked the conversion of PrP to PrP-res [98]. A recent *in vitro* study of curcumin in formation of prion fibrils has shown that curcumin partially binds to the intermediate complex of the amyloid configuration of PrP intermediate complex for the prion protein (PrP) to assume the final beta-stranded conformation PrP-res, the toxic aggregates in the highly infectious CJD [99]. In the *in vivo* murine model of CJD induced by intra-cerebral administration of prion protein, low dosage as contrasted to the high dosage of curcumin protected the mice from potentially fatal neurotoxic effects of prion protein with significantly longer survival time than untreated control ($p < 0.01$) [100].

Taken together, the preliminary findings of curcumin in TSE/CJD models raise the feasibility as the therapeutic potential of BBB penetrant drug lead: Lipocurc™ in CJD, PDD and LBD. The PrP and conversion to PrP-res can hold the elusive key towards unravelling the progressive relentless course of PD. We find a paucity of emerging therapeutic strategies targeting the odd trias: Epigenetics signaling pathway, LRRK-2 kinase and the cellular prion protein. We hypothesize that anti-prion drugs capable of simultaneously inhibiting SYN oligomer and fibril formation through intercepting with the epigenetics and LRRK-2 kinase and related kinase pathway can offer high impact low risk therapeutic leads in PD.

In summary, the overall objective of our Curcumin primed PD drug development has to embrace both epigenetics and nanotechnology in focusing on the inter-related themes of healthy aging encompassing, reprogramming of diseased brain network, and facilitating active trafficking across brain regions and between neurons and glia. Innovative translational studies in discovery of potential novel PD therapeutics have to be guided and instructed in a timely fashion by a solid consortium from diverse disciplines and specialties: medicinal chemistry, pharmacologists, cellular and molecular geneticists, neuroscientists and clinicians. Lipocurc™ is a unique PD drug candidate in embracing the unprecedented triad: epigenetics signaling, nanotechnology and prion protein modulation in drug therapeutic development (Figure 4). The diagnostic and prognostic utility of epigenetics targeting DNA methylation, histone modification and non-coding RNA remains to be further investigated and warrants creative academia-government-industry collaborative partnership.

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Author's Contributions

All the co-authors listed belong to the Translational Epigenetics Research group led by Simon Chiu MD PhD and Ram Mishra PhD and have participated in different aspects of PD research.

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Bibliography

1. Hye Mi Lee and Seong-Beom Koh. "Many Faces of Parkinson's Disease: Non-Motor Symptoms of Parkinson's Disease". *Journal of Movement Disorders* 8.2 (2015): 92-97.
2. Dickson DW. "Neuropathology of Parkinson disease". *Parkinsonism and Related Disorders* 46.1 (2018): S30-S33.

3. Thobois S., *et al.* "Imaging the Etiology of Apathy, Anxiety, and Depression in Parkinson's Disease: Implication for Treatment". *Current Neurology and Neuroscience Reports* 17.10 (2017): 76.
4. Strafella AP, *et al.* "Molecular imaging to track Parkinson's disease and atypical parkinsonisms: New imaging frontiers". *Movement Disorders* 32.2 (2017): 181-192.
5. Fullard ME., *et al.* "Olfactory Dysfunction as an Early Biomarker in Parkinson's Disease". *Neuroscience Bulletin* 33.5 (2017): 515-525.
6. Burré J., *et al.* "Cell Biology and Pathophysiology of α -Synuclein". *Cold Spring Harbor Perspectives in Medicine* 8.3 (2018).
7. Höglinger GU., *et al.* "A new dopaminergic nigro-olfactory projection". *Acta Neuropathologica* 130.3 (2015): 333-348.
8. Domellöf ME., *et al.* "Olfactory dysfunction and dementia in newly diagnosed patients with Parkinson's disease". *Parkinsonism and Related Disorders* 38 (2017): 41-47.
9. Fox SH., *et al.* "International Parkinson and movement disorder society evidence-based medicine review: Update on treatments for the motor symptoms of Parkinson's disease". *Movement Disorders* 33.8 (2018): 1248-1266.
10. Lang AE and Espay AJ. "Disease Modification in Parkinson's Disease: Current Approaches, Challenges, and Future Considerations". *Movement Disorders* 33.5 (2018): 660-677.
11. Connolly BS and Lang AE. "Pharmacological treatment of Parkinson disease: a review". *Journal of the American Medical Association* 311.16 (2014): 1670-1683.
12. Fahn S Parkinson Study Group. "Does levodopa slow or hasten the rate of progression of Parkinson's disease?". *Journal of Neurology* 252.4 (2005): IV37-IV42FAHN
13. De Deurwaerdère P., *et al.* "Expanding the repertoire of L-DOPA's actions: A comprehensive review of its functional neurochemistry". *Progress in Neurobiology* 151 (2017): 57-100.
14. Cova I and Priori A. "Diagnostic biomarkers for Parkinson's disease at a glance: where are we?". *Journal of Neural Transmission* 125.10 (2018): 1417-1432.
15. Zhou CQ., *et al.* "Meta-analysis of the efficacy and safety of long-acting non-ergot dopamine agonists in Parkinson's disease". *Journal of Clinical Neuroscience* 21.7 (2014): 1094-1101.
16. Schaeffer E and Berg D. "Dopaminergic Therapies for Non-motor Symptoms in Parkinson's Disease". *CNS Drugs* 31.7 (2017): 551-570.
17. Sanford M and Scott LJ. "Rotigotine transdermal patch: a review of its use in the treatment of Parkinson's disease". *CNS Drugs* 25.8 (2011): 699-719.
18. Wirdefeldt K., *et al.* "Levodopa-Carbidopa Intestinal Gel in Patients with Parkinson's Disease: A Systematic Review". *CNS Drugs* 30.5 (2016): 381-404.
19. Unti E., *et al.* "Apomorphine hydrochloride for the treatment of Parkinson's disease". *Expert Review of Neurotherapeutics* 15.7 (2015): 723-732.
20. Szökö É., *et al.* "Pharmacological aspects of the neuroprotective effects of irreversible MAO-B inhibitors, selegiline and rasagiline, in Parkinson's disease". *Journal of Neural Transmission* 125.11 (2018): 1735-1749.
21. Hoy SM and Keating GM. "Rasagiline: a review of its use in the treatment of idiopathic Parkinson's disease". *Drugs* 72.5 (2012): 643-669.

22. Binde CD., *et al.* "A multiple treatment comparison meta-analysis of monoamine oxidase type B inhibitors for Parkinson's disease". *British Journal of Clinical Pharmacology* 84.9 (2018): 1917-1927.
23. Blair HA and Dhillon S. "Safinamide: A Review in Parkinson's Disease". *CNS Drugs* 31.2 (2017): 169-176.
24. Katsaiti I and Nixon J. "Are There Benefits in Adding Catechol-O Methyltransferase Inhibitors in the Pharmacotherapy of Parkinson's Disease Patients? A Systematic Review". *Journal of Parkinson's Disease* 8.2 (2018): 217-231.
25. Li J., *et al.* "Efficacy and Safety of Adjuvant Treatment with Entacapone in Advanced Parkinson's Disease with Motor Fluctuation: A Systematic Meta-Analysis". *European Neurology* 78.3-4 (2017): 143-153.
26. Leegwater-Kim J and Waters C. "Role of tolcapone in the treatment of Parkinson's disease". *Expert Review of Neurotherapeutics* 7.12 (2007): 1649-1657.
27. Lees AJ., *et al.* "Opicapone for the management of end-of-dose motor fluctuations in patients with Parkinson's disease treated with L-DOPA". *Expert Review of Neurotherapeutics* 17.7 (2017): 649-659.
28. Müller T and Möhr JD. "Recent Clinical Advances in Pharmacotherapy for Levodopa-Induced Dyskinesia". *Drugs* 79.13 (2019): 1367-1374.
29. Paik J and Keam SJ. "Amantadine Extended-Release (GOCOVRI™): A Review in Levodopa-Induced Dyskinesia in Parkinson's Disease". *CNS Drugs* 32.8 (2018): 797-806.
30. Sako W., *et al.* "The effect of estradefylline for Parkinson's disease: A meta-analysis". *Scientific Reports* 7.1 (2017): 18018.
31. Matsunaga S., *et al.* "Combination Therapy with Zonisamide and Antiparkinson Drugs for Parkinson's Disease: A Meta-Analysis". *Journal of Alzheimer's Disease* 56.4 (2017): 1229-1239.
32. Outeiro TF., *et al.* "LRRK2, alpha-synuclein, and tau: partners in crime or unfortunate bystanders?". *Biochemical Society Transactions* 47.3 (2019): 827-838.
33. Pezzoli G and Cereda E. "Exposure to pesticides or solvents and risk of Parkinson disease". *Neurology* 80.22 (2013): 2035-2041.
34. Shane V., *et al.* "The Epigenome as a therapeutic target for Parkinson's disease". *Neural Regeneration Research* 11.11 (2016): 1735-1738.
35. Labbe C., *et al.* "Epigenetic regulation in Parkinson's disease". *Acta Neuropathologica* 132 (2016): 515-530.
36. Masliah E., *et al.* "Distinctive patterns of DNA methylation associated with Parkinson disease: identification of concordant epigenetic changes in brain and peripheral blood leukocytes". *Epigenetics* 8 (2013): 1030-1038.
37. Fernández-Santiago R., *et al.* "Aberrant epigenome in iPSC-derived dopaminergic neurons from Parkinson's disease patients". *EMBO Molecular Medicine* 7.12 (2015): 1529-1546.
38. Tan YY., *et al.* "Methylation of α -synuclein and leucine-rich repeat kinase 2 in leukocyte DNA of Parkinson's disease patients". *Parkinsonism and Related Disorders* 20.3 (2014): 308-313.
39. Ai SX., *et al.* "Hypomethylation of SNCA in blood of patients with sporadic Parkinson's disease". *Journal of the Neurological Sciences* 337.1-2 (2014): 123-128.
40. Harrison IF and Dexter DT. "Epigenetic targeting of histone deacetylase: therapeutic potential in Parkinson's disease?". *Pharmacology and Therapeutics* 140.1 (2013): 34-52.

41. Gebremedhin KG and Rademacher DJ. "Histone H3 acetylation in the postmortem Parkinson's disease primary motor cortex". *Neuroscience Letters* 627 (2016): 121-125.
42. Park G., *et al.* "Regulation of Histone Acetylation by Autophagy in Parkinson Disease". *Journal of Biological Chemistry* 291.7 (2016): 3531-3540.
43. Izadpanah M., *et al.* "Potential of Extracellular Vesicles in Neurodegenerative Diseases: Diagnostic and Therapeutic Indications". *Journal of Molecular Neuroscience* 66.2 (2018): 172-179.
44. Martinez B and Peplow PV. "MicroRNAs in Parkinson's disease and emerging therapeutic targets". *Neural Regeneration Research* 12.12 (2017): 1945-1959.
45. Burgos K., *et al.* "Profiles of extracellular miRNA in cerebrospinal fluid and serum from patients with Alzheimer's and Parkinson's diseases correlate with disease status and features of pathology". *PLoS One*. 9.5 (2014): e94839.
46. Bhat A Mahalakshmi AM., *et al.* "Benefits of curcumin in brain disorders". *BioFactors* 45.5 (2019): 666-689.
47. Ji HF and Shen L. "The multiple pharmaceutical potential of curcumin in Parkinson's disease". *CNS and Neurological Disorders - Drug Targets* 13.2 (2014): 369-373.
48. Maiti P and Dunbar GL. "Use of Curcumin, a Natural Polyphenol for Targeting Molecular Pathways in Treating Age-Related Neurodegenerative Diseases". *International Journal of Molecular Sciences* 19.6 (2018): E1637.
49. Boyanapalli SS and Tony Kong AN. "'Curcumin, the King of Spices': Epigenetic Regulatory Mechanisms in the Prevention of Cancer, Neurological, and Inflammatory Diseases". *Current Pharmacology Reports* 1.2 (2015): 129-139.
50. Wang XS., *et al.* "Neuroprotective properties of curcumin in toxin-base animal models of Parkinson's disease: a systematic experiment literatures review". *BMC Complementary and Alternative Medicine* 17.1 (2017): 412.
51. Tripanichkulwand Jaroensuppaperch E-O. "Ameliorating effects of curcumin on 6-OHDA- induced dopamine denervation, glial response and SOD1redu". *Scientific Reports* 6 (2016): 28511.
52. Rajeswari A and Sabesan M. "Inhibition of monoamine oxidase-B by the polyphenolic compound, curcumin and its metabolite tetrahydrocurcumin, in a model of Parkinson's disease induced by MPTP neurodegeneration in mice". *Inflammopharmacology* 16 (2008): 96-99.
53. Jayaraj RL., *et al.* "CNB-001 a novel curcumin derivative, guards dopamine neurons in MPTP model of Parkinson's disease". *BioMed Research International* (2014): 236182.
54. Chiu S., *et al.* "Liposomal- formulated curcumin [Lipocurc™] targeting HDAC (histone deacetylase) prevents apoptosis and improves motor deficits in Park 7 (DJ-1)-knockout rat model of Parkinson's disease: implications for epigenetics-based nanotechnology-driven drug platform". *Journal of Complementary and Integrative Medicine* (2013).
55. Mochizuki H., *et al.* "A refined concept: α -synuclein dysregulation disease". *Neurochemistry International* 119 (2018): 84-96.
56. Singh PK., *et al.* "Curcumin modulates α -synuclein aggregation and toxicity". *ACS Chemical Neuroscience* 4.3 (2013): 393-407.
57. Jiang TF., *et al.* "Curcumin ameliorates the neurodegenerative pathology in A53T α -synuclein cell model of Parkinson's disease through the downregulation of mTOR/p70S6K signaling and the recovery of macroautophagy". *Journal of Neuroimmune Pharmacology* 8.1 (2013): 356-369.

58. Borghammer P. "How does Parkinson's disease begin? Perspectives on neuroanatomical pathways, prions, and histology". *Movement Disorders Journal* 33.1 (2018): 48-57.
59. Breen DP, *et al.* "Gut-brain axis and the spread of α -synuclein pathology: Vagal highway or dead end?". *Movement Disorders* 34.3 (2019): 307-316.
60. Sharma A, *et al.* "Constipation in Parkinson's Disease: a Nuisance or Nuanced Answer to the Pathophysiological Puzzle?". *Current Gastroenterology Reports* 20.1 (2018): 1.
61. Ladner-Keay CL, *et al.* "A simple in vitro assay for assessing the efficacy, mechanisms and kinetics of anti-prion fibril compounds". *Prion* 12.5-6 (2018): 280-300.
62. Limanaqi F, *et al.* "Phytochemicals Bridging Autophagy Induction and Alpha-Synuclein Degradation in Parkinsonism". *International Journal of Molecular Science* 20.13 (2019): E3274.
63. Teiten MH, *et al.* "Curcumin as a regulator of epigenetic events". *Molecular Nutrition and Food Research* 57.9 (2013): 1619-1629.
64. Fu S and Kurzrock R. "Development of curcumin as an epigenetic agent". *Cancer* 116.20 (2010): 4670-4676.
65. Vahid F, *et al.* "The role dietary of bioactive compounds on the regulation of histone acetylases and deacetylases: a review". *Gene* 562.1 (2015): 8-15.
66. Hassan FU, *et al.* "Curcumin as an Alternative Epigenetic Modulator: Mechanism of Action and Potential Effects". *Frontiers in Genetics* 10 (2019): 514.
67. Ganai SA, *et al.* "Modulating epigenetic HAT activity for reinstating acetylation homeostasis: A promising therapeutic strategy for neurological disorders". *Pharmacology and Therapeutics* 166 (2016): 106-122.
68. Tang BL. "Sirtuins as modifiers of Parkinson's disease pathology". *Journal of Neuroscience Research* 95.4 (2017): 930-942.
69. Chiu Simon, *et al.* "Exploratory Study of Curcumin isolated from Turmeric Curcuma Longa, the Putative Histone Deacetylase Inhibitor, as added-on strategy to antipsychotics in treating negative symptoms and Neuro-cognitive deficits in Schizophrenia". *Advance Research Journal of Multi-disciplinary Discoveries* 40 (2015).
70. Zendedel E, *et al.* "Impact of curcumin on sirtuins: A review". *Journal of Cellular Biochemistry* 119.12 (2018): 10291-10300.
71. Bora-Tatar G, *et al.* "Molecular modifications on carboxylic acid derivatives as potent histone deacetylase inhibitors: activity and docking studies". *Bioorganic and Medicinal Chemistry* 17 (2007): 5219-5228.
72. Genevieve P Delcuve, *et al.* "Davie Roles of histone deacetylases in epigenetic regulation: emerging paradigms from studies with inhibitors: review". *Clinical Epigenetics* 4 (2012): 5.
73. St Laurent R, *et al.* "Sodium butyrate improves locomotor impairment and early mortality in a rotenone-induced Drosophila model of Parkinson's disease". *Neuroscience* 246 (2013): 382-390.
74. Geng L, *et al.* "miR-494-3p modulates the progression of in vitro and in vivo Parkinson's disease models by targeting SIRT3". *Neuroscience Letters* 675 (2018): 23-30.
75. Pogue, *et al.* "Up-regulation of NF-kB-sensitive miRNA-125b and miRNA-146a in metal sulfate-stressed human astroglial (HAG) primary cell cultures". *Journal of Inorganic Biochemistry* 105.11 (2011): 1434-1437.
76. Momtazi AA, *et al.* "Role of microRNAs in the Therapeutic Effects of Curcumin in Non-Cancer Diseases". *Molecular Diagnosis and Therapy* 20.4 (2016): 335-345.

77. Kluss JH., *et al.* "LRRK2 links genetic and sporadic Parkinson's disease". *Biochemical Society Transactions* 30 47.2 (2019): 651-661.
78. Chen ML and Wu RM. "LRRK 2 gene mutations in the pathophysiology of the Roco domain and therapeutic targets for Parkinson's disease: a review". *Journal of Biomedical Science* 25.1 (2018): 52.
79. Outeiro TF Harvey K., *et al.* "LRRK2, alpha-synuclein, and tau: partners in crime or unfortunate bystanders?". *Biochemical Society Transactions* 47.3 (2019): 827-838.
80. Yang D., *et al.* "LRRK2 kinase activity mediates toxic interactions between genetic mutation and oxidative stress in a Drosophila model: suppression by curcumin". *Neurobiology of Disease* 47.3 (2012): 385-392.
81. Ortiz-Ortiz MA., *et al.* "Curcumin exposure induces expression of the Parkinson's disease-associated leucine-rich repeat kinase 2 (LRRK2) in rat mesencephalic cells". *Neuroscience Letters* 468.2 (2010): 120-124.
82. Varghese Josh K., *et al.* "Differential expression of cerebral dopamine neurotrophic factor (CDNF) in the hippocampus and in the striatum of LRRK-2 mutant rat model of Parkinson's disease". *International Conference in Parkinson Disease, Movement Disorders Journal* 31 (2016).
83. Vau Nguyen AP and Moore DJ. "Understanding the GTPase Activity of LRRK2: Regulation, Function, and Neurotoxicity". *Advances in Neurobiology* 14 (2017): 71-88.
84. Han KA., *et al.* "Leucine-rich repeat kinase 2 exacerbates neuronal cytotoxicity through phosphorylation of histone deacetylase 3 and histone deacetylation". *Human Molecular Genetics* 26.1 (2017): 1-18.
85. Luerman GC., *et al.* "Phosphoproteomic evaluation of pharmacological inhibition of leucine-rich repeat kinase 2 reveals significant off-target effects of LRRK-2-IN-1". *Journal of Neurochemistry* 128.4 (2014): 561-576.
86. Jamwal R. "Bioavailable curcumin formulations: A review of pharmacokinetic studies in healthy volunteers". *Journal of Integrative Medicine* 16.6 (2018): 367-374.
87. Kunnumakkara AB., *et al.* "Is curcumin bioavailability a problem in humans: lessons from clinical trials". *Expert Opinion on Drug Metabolism and Toxicology* 15.9 (2019): 705-733.
88. Chiu S., *et al.* "Intravenous Curcumin Distribution in the Rat Brain". *Journal of Anticancer Research* 31.3 (2011): 907-912.
89. Yavarpour-Bali H., *et al.* "Curcumin-loaded nanoparticles: a novel therapeutic strategy in treatment of central nervous system disorders". *International Journal of Nanomedicine* 14 (2019): 4449-4460.
90. Helson L., *et al.* "Infusion pharmacokinetics of Lipocurc (liposomal curcumin) and its metabolite tetrahydrocurcumin in Beagle dogs". *Anticancer Research* 32.10 (2012): 4365-4370.
91. Matabudul D., *et al.* "Tissue distribution of (Lipocurc™) liposomal curcumin and tetrahydrocurcumin following two- and eight-hour infusions in Beagle dogs". *Anticancer Research* 32.10 (2012): 4359-4364.
92. Angela S., *et al.* "Safety, Tolerability and pharmacokinetics of liposomal curcumin (Lipocurc™) in healthy humans". *International Journal Clinical Pharmacology and Therapeutics* 23 (2014): 1-12.
93. Richard Greil., *et al.* "A phase 1 dose-escalation study on the safety, tolerability and activity of liposomal curcumin (Lipocurc™) in patients with locally advanced or metastatic cancer". *Cancer Chemotherapy and Pharmacology* 82.4 (2018): 695-706.
94. Trompetero A., *et al.* "Alzheimer's Disease and Parkinson's Disease: A Review of Current Treatment Adopting a Nanotechnology Approach". *Current Pharmaceutical Design* 24.1 (2018): 22-45.

95. Goedert M and Neurodegeneration. "Alzheimer's and Parkinson's diseases: The prion concept in relation to assembled A β , tau, and α -synuclein". *Science* 349.6248 (2015): 1255555.
96. Volpicelli-Daley L and Brundin P. "Prion-like propagation of pathology in Parkinson disease". *Handbook of Clinical Neurology* 153 (2018): 321-335.
97. Zhong Z., *et al.* "Prion-like protein aggregates exploit the RHO GTPase to cofilin-1 signaling pathway to enter cells". *The EMBO Journal* 37.6 (2018): e97822.
98. Caughey B., *et al.* "Inhibition of protease-resistant prion protein accumulation in vitro by curcumin". *Journal of Virology* 77.9 (2003): 5499-5502.
99. Hafner-Bratkovic I., *et al.* "Curcumin binds to the alpha-helical intermediate and to the amyloid form of prion protein - a new mechanism for the inhibition of PrP(Sc) accumulation". *Journal of Neurochemistry* 104.6 (2008): 1553-1564.
100. Riemer C., *et al.* "Evaluation of drugs for treatment of prion infections of the central nervous system". *Journal of General Virology* 89 (2008): 594-597.

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