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The potential of MLC901 (NeuroAiD II™), a traditional Chinese medicine

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ABSTRACT: Stroke, also known as cerebral ischemia, is a common neurological disease. The therapeutic potential of MLC901 (NeuroAiD II™) has been reported in clinical trials on traumatic brain injury as well as in animal and cell models. MLC901 reduced the infarction size, ischemia-induced neurological deficits and pro-inflammatory infiltration of phagocyte. It also inhibited the ischemia-induced expression of pro-inflammatory mediators and Prx6, TLR4 signalling, and phosphorylation of NFκB. We found that the beneficial effects of MLC901 are in coherent with studies performed on the individual active ingredient. MLC901 may develop its efficacy through a synergistic effect via nine herbal extracts. MLC901 is a multifaceted traditional Chinese medicine. A cocktail of herbs provides a broader spectrum of targets. This may surpass single-target drug treatment in terms of side effect and therapeutic efficacy. MLC901 leads to various potential research directions on the development or improvement of a feasible, effective and promising herbal formulation for treating stroke patients.

Keywords: MLC901; cerebral ischemia; traditional Chinese medicine;

Stroke, also known as cerebral ischemia, is a common neurological disease with cognitive function impairment and long-term disability [1,2]. In 2010, there were 5.9 million deaths and 102 million disability-adjusted life-years were lost because of stroke, making stroke the second leading cause of death [3]. Stroke is a clinical manifestation resulted from occlusion in the blood vessels that leads to the lack of oxygen and nutrient in the infarcted brain [3]. Its representative symptoms include sudden unilateral weakness, numbness, visual loss, diplopia, altered speech, and non-orthostatic vertigo [4].

Nowadays, the standard stroke treatment is based on the use of a thrombolytic agent such as tissue plasminogen activator (tPA) within 4.5 hours after a stroke incidence [5]. Aspirin and clopidogrel are the common post-stroke treatment used on stroke patients [6]. However, there is limited effective treatment for post-stroke recovery. Scientists have been working on developing a better alternative for post-stroke treatment such as N-methyl-D-aspartate (NMDA) receptor antagonists [7] and low molecular weight (LMW) heparin [8]. These drugs succeed in improving post-stroke outcome based on the animal study but not in clinical trials [7,8]. This "bench-to-bed" approach seems not promising. Recently, Widmann *et al.* (2018) studied MLC901 [9], also known as NeuroAiD II™, is one of the traditional Chinese medicine with a combination of nine herbal extracts [10]. It is the second generation of NeuroAiD™ (MLC601) with a more straightforward formula [11]. MLC901 has remarkedly improved post-traumatic brain injury recovery in clinical trial phase two [11]. However, a little is known about how it works. Therefore, the study done by Widmann *et al.* (2018) [9] provides a credible research direction with a feasible target, signalling pathway, and assessment parameter to develop a potential therapeutic medicine for the stroke patient.

Widmann *et al*. (2018) demonstrated that (1) MLC901 reduced the infarction size and ischemia-induced neurological deficits, which were measured by neurological Bederson scores in the treated group [9]. (2) 24-hour post-ischemia, MLC901 significantly attenuated the stroke-induced pro-inflammatory infiltration of phagocytes. (3) MLC901 attenuated the increasing stroke-induced expression of pro-inflammatory mediators (IL11, IL1β, IL6, and TNFα) in the brain. (4) MLC901 remarkedly diminished the middle cerebral artery occlusion (MCAO)-induced expression of peroxiredoxin 6 (Prx6), Toll-like receptor 4 (TLR4) signalling and phosphorylation of NFκB [9]. Prx6 is a significant contributor to immunomodulation and neuroinflammation after ischemic stroke [12]. TLR4 has been linked to inflammation and the pathological progression of cerebral ischemia [13-16]. The NFκB activation is the primary signalling system associated with TLR4, and the NFκB signalling pathway has been implicated in immune responses in the ischemic brain [17,18]. In summary, MLC901 is a potent therapeutic agent for stroke treatment that significantly improves post-stroke recovery via the suppression of ischemia-induced inflammation processes.

The MLC901 consists of the extracts from nine herbs, which are *Radix astragali, Radix salvia miltiorrhizae*, *Radix paeoniae rubra*, *Rhizoma chuanxiong*, *Radix angelicae sinensis*, *Carthamus tinctorius*, *Prunus persica*, *Radix polygalae*, and *Rhizoma acori tatarinowii* [19,20]. This extract cocktail is rich in active ingredients such as astragaloside IV (AST-IV), salvianolic acid B (SAB), tanshinone IIB (TSB), tetramethylpyrazine (TMP), ferulic acid, ligustilide and butylidenephtalide, β-asarone, hydroxyl safflower yellow A (HSYA), total paeony glycoside (TPG), and presenegenin [9,11,20]. The improvement of post-stroke recovery from MLC901 treatment most likely due to the synergistic effect of these nine herbal extracts and their active compounds as each of them may target a different mechanism. Some of them, such as AST-IV, ferulic acid, TSB, and β-asarone, have been demonstrated to have a significant therapeutic effect on cerebral ischemia models. These active compounds were shown to improve neurological deficits [21], reduce infarct volume [22], reduce blood-brain barrier (BBB) permeability [23] and reduce nerve damage on the cerebral ischemic models [24-26]. Besides, AST-IV showed a neuroprotective effect by inhibiting oxygen and glucose deprivation (OGD) induced mitochondrial dysfunction [27]. Besides, HSYA showed anti-thrombotic [28], anti-inflammatory [29] and anti-oxidation effects [30] on ischemic stroke models. These observations suggest that MLC901 treatment encompasses a broader range of mechanisms and pathway which contributed to the positive outcome.

Inhibition on inflammation and apoptotic pathways are the highlighted effect of these herbs and active compounds on cerebral ischemic models. In terms of neuroinflammation that followed after the cerebral

ischemia, TMP plays a role in neuroprotection by inhibiting the activation, migration and aggregation of microglia cells, astrocytes [25] and other inflammatory cells [31]. Salvianolic acid B, which extracted from *Radix salvia miltiorrhizae* showed neuroprotective effect via the inhibition of the TLR4/MyD88/TRAF6 signalling pathway in MCAO-induced ischemic rat brain [32]. The blocking of TLR4 by SAB also restrained NFκB transcriptional activity and pro-inflammatory cytokine responses (IL-1β, IL-6, and TNF-α) [32]. Ferulic acid has also been shown that could reduce the phosphorylation of NFκB in the infarcted brain [33]. Mitochondrial-related caspase-3 apoptotic pathway is one of the apoptotic pathways targeted by MLC901. Z-ligustilide (LIG) significantly decreased Bax and caspase-3 protein expression in the ischemic cortex [34]. *Rhizoma chuanxiong, Radix Paeoniae Rubra* and their combination reduced the infarct size in the brain of MCAO rats [35]. Treatment using the combination remarkedly decreased the levels of IFN-γ, IL-1β, IL-6 and IL-12 in serum and brain tissues of MCAO rats. It also downregulated the expression of caspase-3 and caspase-12 genes as well as decreased IL-1β and IL-6 mRNA levels in MCAO brain tissue [36]. At the same time, a study also showed that β-asarone reduced cerebral autophagy induced by ischemia-reperfusion in rats via modulating c-jun n-terminal kinases (JNK), phospho-JNK (p-JNK), Bcl-2 and Beclin 1 [37]. TPG recovers energy metabolism via increasing the activity of Na^+-K^+-ATP and Ca^{2+}-ATP enzymes in the cerebral ischemia-reperfusion injured rat [38]. There is limited information about *Prunus persica* in MLC901. A study showed that the carotenoids and polyphenols in *Prunus persica* have antioxidant effects, and down-regulation of chemokine ligand 4 (CCL-4) that led to the inhibition of TNF-α, IL-1β, RAGE and NFκB expression in carbon tetrachloride (CCl4)-induced inflammation rat model [39]. In summary, the result of MLC901 shown by Widmann *et al.* (2018) [9] is coherent with the reported outcome of studies performed on the individual herb or active compound.

Potent angiogenic factor, such as vascular endothelium growth factor (VEGF) is also one of the potential targets by MLC901. Dl-3n-butylphthalide (NBP) can be found in both *Radix angelicae sinensis* and *Rhizoma chuanxiong*, and it rescued brain tissue by regulating the expression of VEGF and HIF-1 alpha during ischemic stroke [40]. This shows that MLC901 is a multifaceted candidate that potentially targets multiple pathways ranging from the anti-inflammatory pathway to promoting angiogenesis in cerebral ischemic models.

Interestingly, Widmann *et al.* (2018) reported that MLC901 inhibited the infiltration of neutrophils but not on the T-lymphocytes in the 24-hour study [9]. Studies revealed that regulatory T cells are likely to protect ischemic stroke by suppressing peripheral neutrophil-derived metallopeptidase-9 production that causes BBB leakage [41,42] and the activation of CD39/CD73 signalling [43]. Thus, it may be worth to study why and how MLC901 acts on neutrophils and T-lymphocytes. These mechanisms probably will provide new methods for targeted therapy in the future.

Overall, Widmann *et al.* (2018) [9] strengthen the idea of using a combination of multiple herbal constituents, both the herbal extract or the active ingredient as an approach to target multiple pathways. Single-target drug treatments for stroke such as NMDA receptor antagonists [7] and LMW heparin certoparin [44] have not been promising. It is known that synaptic transmission mediated by the NMDA receptor is critical for neuronal survival and blocking of NMDA receptors triggers apoptosis in the developing brains. Thus the clinical trial of NMDA receptor antagonists has failed due to the difficulty in determining the optimal antagonistic level for stroke patients and improper clinical trial design [7]. In the clinical trial of acute stroke patients, the failure of LMW heparin certoparin attributed to its side-effect, an increased risk for severe bleeding [44]. Normal or increased dose of LMW heparin certoparin did not show any improvement of functional outcome in ischemic stroke patients, while the highest dose group may lead to severe bleeding. Single-targeted drug treatment may disturb the homeostasis in the human body system and potentially exerting severe side-effects. For examples, glutamate

could damage [45,46] and protect neurons [47] under different circumstances. In general, it is hard to find a balance or determines the optimal dosage when using single-targeted drug treatment. MLC901 includes a variety of active compounds, has been demonstrated that it has a therapeutic effect on stroke. In this case, it seems that herbal extract cocktail may be better than single-targeted drug treatment.

It is undeniable that MLC901 has significant anti-inflammatory activity in MCAO ischemic mouse model. MLC901 treatment has significantly improved intricate attention and executive functioning on adults with traumatic brain injury (phase two clinical trial) [11]. Even so, we could not help to ignore the failure of MLC601 (NeuroAid™) phase three clinical trial. MLC601 that contained an additional of five animal components did not significantly improve post-stroke recovery in acute ischemic stroke patients (mean age of 61.4±11.3 years) [48]. Three months of MLC601 treatment is not statistically better than the placebo group. On the contrary, MLC601 treatment has a persistent beneficial effect up to 24 months in the acute stroke patients from the Philippines (mean age 60.2±11.1) [49]. These indicate that MLC601 may need a longer duration to improve post-stroke recovery in elder stroke patients. Looking back at MLC901, Widmann *et al.* (2018) [9] demonstrated its positive outcomes on the acute cerebral ischemia modelled young adult mice. Perhaps we could have a better and thorough understanding of the MLC901 post-stroke recovery in a spatiotemporal manner if they prolong the study or experimenting on ageing mice. This understanding may potentially prevent MLC901 study from repeating the failure observed in MLC601 phase three clinical trial.

Traditional Chinese medicine has been utilised for thousands of years in Asia, and it usually employs a mixture of herbs and extracts with different herbal formulation for different diseases. In China, there is more than 100 iconic traditional Chinese medicine such as Danqi Piantang Jiaonang (DJ) and Buchang Naoxintong Jiaonang (BNJ), which have been approved by the Chinese National Drug Administration to treat stroke clinically [50]. MLC901 contains nine herbs with more than nine effective ingredients making it a potent medicine to treat stroke patients. The key is to confirm the effective combination of herbs and their interaction. In summary, this provides a potential research direction with a feasible, effective herbal formula to develop or improve a therapeutic intervention for stroke patients.

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References

1. Kalaria RN, Akinyemi R, Ihara M. Stroke injury, cognitive impairment and vascular dementia *Biochimica Et Biophysica Acta.* 2016; 1862(5):915-925. https://doi.org/10.1016/j.bbadis.2016.01.015
2. Mijajlović MD, Pavlović A, Brainin M, Heiss W-D, Quinn TJ, Ihle-Hansen HB, et al. Post-stroke dementia – a comprehensive review. *Bmc Medicine.* 2017; 15(1):11. https://doi.org/10.1186/s12916-017-0779-7
3. Hankey GJ. Stroke. *The Lancet.* 2017; 389(10069):641-654. https://doi.org/10.1016/S0140-6736(16)30962-X
4. Hankey GJ, Blacker DJ. Is it a stroke? *BMJ.* 2015; 350(jan15 1):h56-h56. https://doi.org/10.1136/bmj.h56
5. Cheng NT, Kim AS. Intravenous Thrombolysis for Acute Ischemic Stroke Within 3 Hours Versus Between 3 and 4.5 Hours of Symptom Onset. *The Neurohospitalist.* 2015; 5(3):101-109. https://doi.org/10.1177/1941874415583116
6. Leslie J, Nigel S, Debra R. Reducing the risk of adverse thrombotic events - The role of aspirin and clopidogrel. *Australian Family Physician.* 2008; 37(9):725-726

7. Ikonomidou C, Turski L. Why did NMDA receptor antagonists fail clinical trials for stroke and traumatic brain injury? *Lancet Neurology.* 2002; 1(6):383-386. https://doi.org/10.1016/S1474-4422(02)00164-3
8. Diener HC, Ringelstein EB, von Kummer R, Langohr HD, Bewermeyer H, Landgraf H, et al. Treatment of acute ischemic stroke with the low-molecular-weight heparin certoparin: results of the TOPAS trial. Therapy of Patients With Acute Stroke (TOPAS) Investigators. *Stroke.* 2001; 32(1):22-29. https://doi.org/10.1161/01.str.32.1.22
9. Widmann C, Gandin C, Petit-Paitel A, Lazdunski M, Heurteaux C. The Traditional Chinese Medicine MLC901 inhibits inflammation processes after focal cerebral ischemia. *Scientific Reports.* 2018; 8(1):18062. https://doi.org/10.1038/s41598-018-36138-0
10. Heurteaux C, Gandin C, Borsotto M, Widmann C, Brau F, Lhuillier M, et al. Neuroprotective and neuroproliferative activities of NeuroAid (MLC601, MLC901), a Chinese medicine, in vitro and in vivo. *Neuropharmacology.* 2010; 58(7):987-1001. https://doi.org/10.1016/j.neuropharm.2010.01.001
11. Theadom A, Barker-Collo S, Jones KM, Parmar P, Bhattacharjee R, Feigin VL. MLC901 (NeuroAiD II™) for cognition after traumatic brain injury: a pilot randomized clinical trial. *European journal of neurology.* 2018; 25(8):1055-e1082. https://doi.org/10.1111/ene.13653
12. Shichita T, Hasegawa E, Kimura A, Morita R, Sakaguchi R, Takada I, et al. Peroxiredoxin family proteins are key initiators of post-ischemic inflammation in the brain. *Nature Medicine.* 2012; 18(6):911. https://doi.org/10.1038/nm.2749
13. Richard M, Carine A, Olivier T, Brigitte LM, Gilles D, Ulrich D, et al. Stroke and the immune system: from pathophysiology to new therapeutic strategies. *Lancet Neurology.* 2011; 10(5):471-480. https://doi.org/10.1016/S1474-4422(11)70066-7
14. Iadecola C, Anrather J. The immunology of stroke: from mechanisms to translation. *Nature Medicine.* 2011; 17:796. https://doi.org/10.1038/nm.2399
15. Caso JR, Pradillo JM, Hurtado O, Lorenzo P, Moro MA, Lizasoain I. Toll-Like Receptor 4 Is Involved in Brain Damage and Inflammation After Experimental Stroke. *Circulation.* 2007; 115(12):1599-1608. https://doi.org/10.1161/CIRCULATIONAHA.106.603431
16. Marsh B, Stevens SL, Packard AEB, Gopalan B, Hunter B, Leung PY, et al. Systemic lipopolysaccharide protects the brain from ischemic injury by reprogramming the response of the brain to stroke: a critical role for IRF3. *The Journal of neuroscience : the official journal of the Society for Neuroscience.* 2009; 29(31):9839-9849. https://doi.org/10.1523/JNEUROSCI.2496-09.2009
17. Harari OA, Liao JK. NF-κB and innate immunity in ischemic stroke. *Annals of the New York Academy of Sciences.* 2010; 1207:32-40. https://doi.org/10.1111/j.1749-6632.2010.05735.x
18. Yi J-H, Park S-W, Kapadia R, Vemuganti R. Role of transcription factors in mediating post-ischemic cerebral inflammation and brain damage. *Neurochemistry international.* 2007; 50(7-8):1014-1027. https://doi.org/10.1016/j.neuint.2007.04.019
19. Widmann C, Gandin C, Petit-Paitel A, Lazdunski M, Heurteaux C. The Traditional Chinese Medicine MLC901 inhibits inflammation processes after focal cerebral ischemia. *Sci Rep.* 2018; 8(1):18062. https://doi.org/10.1038/s41598-018-36138-0
20. Heurteaux C, Gandin CM, Widmann C, Brau F, Lhuillier M, Onteniente B, et al. Neuroprotective and neuroproliferative activities of NeuroAid (MLC601, MLC901), a Chinese medicine, in vitro and in vivo. *Neuropharmacology.* 2010; 58(7):987-1001. https://doi.org/10.1016/j.neuropharm.2010.01.001
21. Yang YX, Chen YT, Zhou XJ, Hong CL, Li CY, Guo JY. Beta-asarone, a major component of Acorus tatarinowii Schott, attenuates focal cerebral ischemia induced by middle cerebral artery occlusion in rats. *Bmc Complementary & Alternative Medicine.* 2013; 13(1):236
22. Chowbay B, Sheu FS. Tanshinone Ⅱ B,a primary active constituent from Salvia miltiorrhza,exhibits neuro-protective activity in experimentally stroked rats. *Neuroscience Letters.* 2007; 417(3):261-265. https://doi.org/10.1016/j.neulet.2007.02.079

23. You ZQ, Min L, Yan LZ, Zhen WZ, Xiao YW, Jin PL, et al. Astragaloside IV attenuates cerebral ischemia–reperfusion-induced increase in permeability of the blood-brain barrier in rats. *European Journal of Pharmacology.* 2009; 606(1):137-141. https://doi.org/10.1016/j.ejphar.2009.01.022
24. Kao TK, Ou YC, Kuo JS, Chen WY, Liao SL, Wu CW, et al. Neuroprotection by tetramethylpyrazine against ischemic brain injury in rats. *Neurochemistry International.* 2006; 48(3):166-176. https://doi.org/10.1016/j.neuint.2005.10.008
25. Liao SL, Kao TK, Chen WY, Lin YS, Chen SY, Raung SL, et al. Tetramethylpyrazine reduces ischemic brain injury in rats. *Neuroscience Letters.* 2004; 372(1):40-45. https://doi.org/10.1016/j.neulet.2004.09.013
26. Wang HL, Zhou QH, Xu MB, Zhou XL, Zheng GQ. Astragaloside IV for Experimental Focal Cerebral Ischemia: Preclinical Evidence and Possible Mechanisms. *Oxidative Medicine & Cellular Longevity.* 2017; 2017(1):8424326. https://doi.org/10.1155/2017/8424326
27. Xue B, Huang J, Ma B, Yang B, Chang D, Liu J. Astragaloside IV Protects Primary Cerebral Cortical Neurons from Oxygen and Glucose Deprivation/Reoxygenation by Activating the PKA/CREB Pathway. *Neuroscience.* 2019; 404:326-337. https://doi.org/10.1016/j.neuroscience.2019.01.040
28. Hai-Bo Z, Ling Z, Zheng-Hua W, Jing-Wei T, Feng-Hua F, Ke L, et al. Therapeutic effects of hydroxysafflor yellow A on focal cerebral ischemic injury in rats and its primary mechanisms. *Journal of Asian Natural Products Research.* 2005; 7(4):607-613. https://doi.org/10.1080/10286020310001625120
29. Sheng-Ying Y, Wen-Yuan G. Hydroxysafflor yellow A protects neuron against hypoxia injury and suppresses inflammatory responses following focal ischemia reperfusion in rats. *Archives of Pharmacal Research.* 2008; 31(8):1010-1015. https://doi.org/10.1007/s12272-001-1261-y
30. Sun L, Yang L, Xu YW, Liang H, Han J, Zhao RJ, et al. Neuroprotection of hydroxysafflor yellow A in the transient focal ischemia: Inhibition of protein oxidation/nitration, 12/15-lipoxygenase and blood–brain barrier disruption. *Brain Research.* 2012; 1473(6):227-235. https://doi.org/10.1016/j.brainres.2012.07.047
31. Yi C, George H, Seu-Hwa C, Yi-Cheng C, Jiing-Han L, Kuang-Hung L, et al. Tetramethylpyrazine suppresses HIF-1alpha, TNF-alpha, and activated caspase-3 expression in middle cerebral artery occlusion-induced brain ischemia in rats. *Acta Pharmacologica Sinica.* 2007; 28(3):327-333. https://doi.org/10.1111/j.1745-7254.2007.00514.x
32. Wang Y, Chen G, Yu X, Li Y, Zhang L, He Z, et al. Salvianolic Acid B Ameliorates Cerebral Ischemia/Reperfusion Injury Through Inhibiting TLR4/MyD88 Signaling Pathway. *Inflammation.* 2016; 39(4):1-11. https://doi.org/10.1007/s10753-016-0384-5
33. Cheng CY, Ho TY, Lee EJ, Su SY, Tang NY, Hsieh CL. Ferulic Acid Reduces Cerebral Infarct Through Its Antioxidative and Anti-Inflammatory Effects Following Transient Focal Cerebral Ischemia in Rats. *American Journal of Chinese Medicine.* 2008; 36(06):1105-1119. https://doi.org/10.1142/S0192415X08006570
34. Kuang X, Yao Y, Du JR, Liu YX, Wang CY, Qian ZM. Neuroprotective role of Z-ligustilide against forebrain ischemic injury in ICR mice. *Brain Research.* 2006; 1102(1):145-153. https://doi.org/10.1016/j.brainres.2006.04.110
35. Gu J, Chen J, Yang N, Hou X, Wang J, Tan X, et al. Combination of Ligusticum chuanxiong and Radix Paeoniae ameliorate focal cerebral ischemic in MCAO rats via endoplasmic reticulum stress-dependent apoptotic signaling pathway. *Journal of Ethnopharmacology.* 2016; 187:313-324. https://doi.org/10.1016/j.jep.2016.04.024
36. Gu J, Su S, Guo J, Zhu Y, Zhao M, Duan JA. Anti-inflammatory and anti-apoptotic effects of the combination of Ligusticum chuanxiong and Radix Paeoniae against focal cerebral ischaemia via TLR4/MyD88/MAPK/NF-kappaB signalling pathway in MCAO rats. *The Journal of pharmacy and pharmacology.* 2018; 70(2):268-277. https://doi.org/10.1111/jphp.12841
37. Liu L, Fang YQ, Xue ZF, He YP, Fang RM, Li L. Beta-asarone attenuates ischemia–reperfusion-induced autophagy in rat brains via modulating JNK, p-JNK, Bcl-2 and Beclin 1. *European Journal of Pharmacology.* 2012; 680(1-3):34-40. https://doi.org/10.1016/j.ejphar.2012.01.016
38. He YL, Zhe D, Dong LY: *Protective effects of total paeny glycoside against focal cerebral ischemia/reperfusion injury in rats*. 2006; vol. 10.

39. Gasparotto J, Somensi N, Bortolin RC, Girardi CS, Kunzler A, Rabelo TK, et al. Preventive supplementation with fresh and preserved peach attenuates CCl4-induced oxidative stress, inflammation and tissue damage. *The Journal of nutritional biochemistry.* 2014; 25(12):1282-1295. https://doi.org/10.1016/j.jnutbio.2014.07.004
40. Liao SJ, Lin JW, Pei Z, Liu CL, Zeng JS, Huang RX. Enhanced angiogenesis with dl -3n-butylphthalide treatment after focal cerebral ischemia in RHRSP. *Brain Research.* 2009; 1289(1289):69-78. https://doi.org/10.1016/j.brainres.2009.06.018
41. Li P, Gan Y, Sun BL, Zhang F, Lu B, Gao Y, et al. Adoptive regulatory T-cell therapy protects against cerebral ischemia. *Annals of neurology.* 2013; 74(3):458-471. https://doi.org/10.1002/ana.23815
42. Li P, Mao L, Zhou G, Leak RK, Sun BL, Chen J, et al. Adoptive regulatory T-cell therapy preserves systemic immune homeostasis after cerebral ischemia. *Stroke.* 2013; 44(12):3509-3515. https://doi.org/10.1161/strokeaha.113.002637
43. Zhang H, Xia Y, Ye Q, Yu F, Zhu W, Li P, et al. In Vivo Expansion of Regulatory T Cells with IL-2/IL-2 Antibody Complex Protects against Transient Ischemic Stroke. *The Journal of neuroscience : the official journal of the Society for Neuroscience.* 2018; 38(47):10168-10179. https://doi.org/10.1523/jneurosci.3411-17.2018
44. Diener HC, Ringelstein EB, Kummer R, Von, Langohr HD, Bewermeyer H, ., Landgraf H, ., et al. Treatment of acute ischemic stroke with the low-molecular-weight heparin certoparin: results of the TOPAS trial. Therapy of Patients With Acute Stroke (TOPAS) Investigators. *Stroke; a journal of cerebral circulation.* 2001; 32(1):22
45. D W Choi a, Rothman SM. The Role of Glutamate Neurotoxicity in Hypoxic-Ischemic Neuronal Death. *Annual Review of Neuroscience.* 1990; 13(1):171-182. https://doi.org/10.1146/annurev.ne.13.030190.001131
46. Ikonomidou C, Stefovska V, Turski L. Neuronal death enhanced by N-methyl-D-aspartate antagonists. *Proceedings of the National Academy of Sciences of the United States of America.* 2000; 97(23):12885-12890. https://doi.org/10.1073/pnas.220412197
47. Arvidsson A, Kokaia Z, Lindvall O. N-methyl-d-aspartate receptor-mediated increase of neurogenesis in adult rat dentate gyrus following stroke. *European Journal of Neuroscience.* 2001; 14(1):10-18. https://doi.org/10.1046/j.0953-816x.2001.01611.x
48. Chen CLH, Young SHY, Gan HH, Singh R, Lao AY, Baroque AC, et al. Chinese Medicine Neuroaid Efficacy on Stroke Recovery. *Stroke.* 2013; 44(8):2093-2100. https://doi.org/doi:10.1161/STROKEAHA.113.002055
49. Navarro JC, Chen CL, Lee CF, Gan HH, Lao AY, Baroque AC, et al. Durability of the beneficial effect of MLC601 (NeuroAiD™) on functional recovery among stroke patients from the Philippines in the CHIMES and CHIMES-E studies. *International Journal of Stroke.* 2017; 12(3):285-291. https://doi.org/10.1177/1747493016676615
50. Christopher C, Venketasubramanian N, Gan RN, Caroline L, David P, Chan BPL, et al. Danqi Piantang Jiaonang (DJ), a traditional Chinese medicine, in poststroke recovery. *Stroke.* 2009; 40(3):859-863. https://doi.org/10.1161/STROKEAHA.108.531616