Translational neonatal seizure research - a reality check

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Seizures present both diagnostic and therapeutic challenges in neonates admitted to neonatal intensive care unts (NICU) worldwide. Neonatal encephalopathy, following a hypoxic-ischaemic insult around the time of birth (hypoxic-ischaemic encephalopathy or HIE), is the most common cause of seizures in term neonates and as a result, most information is available about seizure characteristics in this group.[1] [2] Many neonatal seizures are electrographic-only but seizures in moderate to severe HIE exhibit the highest rates of electroclinical uncoupling and thus continuous EEG (electroencephalography) monitoring is essential to detect and treat seizures effectively. There is now evidence that higher electrographic seizure burdens are associated with poor outcomes. [3-5]

Evidence is also emerging showing that earlier treatment is associated with better seizure control and reduced overall seizures burden.[6-8] However, earlier treatment is only possible if continuous EEG monitoring is available to identify the exact onset of seizures.[6] [9-11] Unfortunately, multicentre studies have clearly shown that there can be long gaps between the onset of seizures and the administration of first treatment and the reasons for this are multifactorial.[1] Even though the use of EEG monitoring in NICUs has increased considerably over the last 10 years, the availability of experts to interpret the EEG 24 hours a day, seven days a week is low and as a result, many seizures, particularly those that are electrographic-only are missed and may not be treated for hours after onset, if at all. Amplitude integrated EEG (aEEG) is more widely available for clinical management, but 20-40% of seizures will be missed in addition to a significant risk of false positive detections. [12] It is not recommended for clinical trials of neonatal seizure treatment [13]

What do we know about seizures in HIE?

In HIE, electrographic-only seizures predominate, making diagnosis challenging.[14] [15] Following the primary hypoxic ischaemic insult, the EEG becomes isoelectric and the time needed for the EEG to recover depends on the severity of the primary injury. What is clear from clinical studies, is that seizures in HIE do not happen in the immediate post injury period i.e., the latent phase. Neonates can exhibit abnormal movements such as automatisms, irregular myoclonic jerking, spasm like movements, back arching and hyperexcitability in the immediate post injury period i.e., during the latent phase but these movements are not considered to be seizures as they are not associated with EEG changes and can be triggered by external stimulation. Similar movements have been described in preclinical studies.[16]

Electrographic seizures emerge during the secondary injury phase (Figure 1) which starts around 6 hours after injury.[17] Studies using continuous EEG monitoring have shown that the median time to seizure onset after birth is approximately 13 hours in moderate HIE and 15 hours in severe HIE. Of course there is variability as seizure onset depends on the time of the primary injury.[18] Seizures in HIE have a typical evolution with a peak in seizure burden within the first 30 hours. This peak is reached relatively quickly in moderate HIE, at a median time of 15 hours after birth but a slower evolution is seen in severe HIE with a median peak around 27 hours.[19 20]

How do seizures respond to treatment?

For over 50 years. phenobarbital has been the most common first line treatment for neonatal seizures worldwide, despite the development of multiple new anti-seizure treatments for paediatric and adult populations.[2] Systematic reviews indicated that around 50% of neonatal seizures respond to phenobarbital.[21] Recent evidence suggests that phenobarbital may work better than previously thought if given promptly after seizure onset.[7 22] This was especially evident in the multi-centre, randomised, blinded, controlled NEOLEV2 trial which used remote EEG monitoring to help identify and treat seizures promptly. In this study the efficacy and safety of levetiracetam was compared with phenobarbital as a first-line treatment of seizures in term and near-term infants. Primary outcome (seizure freedom at 24 hr) was met in 80% of the phenobarbital group (n=24/30) compared to 28% (n=15/53) of the levetiracetam group.[23] Nevertheless, there is still a need for new drugs to treat neonatal seizures that do not respond to phenobarbital. In addition, there are concerns about the acute and long-term effects of phenobarbital. Acute adverse effects are dose dependant and include lethargy, feeding difficulties, respiratory insufficiency leading to ventilator dependency, and the need for cardiovascular support, while there are some indications that phenobarbital may have an impact on later neurodevelopmental outcome due to excess apoptosis.[24] However, studies on long term effects were done in healthy animals and it is unclear if this effect is seen in infants and if it outweighs the effect of seizure burden reduction in the neonatal period.

The views expressed by the researchers in recent commentaries[25 26] and published in this issue are very important. These investigators are striving to develop better treatments for neonatal seizures and for these efforts they must be congratulated. As in all preclinical research, it is difficult to replicate the exact clinical scenario, and this is especially the case for the neonate in the intensive care unit. Therefore, it is not surprising that there are contrasting results and opinions expressed in the results of the studies described by these researchers.

Neonates with seizures, particularly those due to HIE, have a seizure onset within 24 hours of birth. Hypoxic ischaemic brain injury, when it happens, is an unexpected event and requires emergency intervention from both obstetric and neonatal teams. The subsequent hours after birth are critical. Interventions usually start in the delivery room where every minute counts and where the neonatal team resuscitates (giving cardiorespiratory support) and stabilises the newborn for transfer to the neonatal unit for further management. Unfortunately, complications at delivery can happen in any setting and infants with HIE born outside of a cooling centre will also require urgent transfer (by ambulance or air transport) to a tertiary unit for intensive care management and therapeutic hypothermia. Moderate and severe hypoxic ischaemic injury usually manifests as multiorgan dysfunction, and infants might need respiratory support (i.e. mechanical ventilation), cardiovascular support, management of electrolyte and acid-base imbalances , correction of coagulation disturbances (transfusions) sedation and pain management. All infants with encephalopathy secondary to a hypoxic ischaemic event are closely monitored with serial neurological examinations and continuous monitoring of all vital signs (respiration, cardiovascular, temperature), as well as with neuromonitoring (aEEG/cEEG, NIRS), and neuroimaging (serial cranial ultrasounds, brain MRI) as available. Within a very short window (6 hours from birth), before the onset of secondary energy failure (figure 1), the clinical team has to decide if therapeutic hypothermia is indicated. In level III and IV neonatal units, the aEEG/EEG monitoring takes place early, often within 6 hours of age, but in other settings it may not be possible until the second day, and consequently, seizure onset may be missed. However, even when seizures are diagnosed quickly, delay in treatment may still happen because of the time required for anti-seizure medication to be prescribed, prepared and slowly administered. This can take up to 1-2 hours in a busy clinical setting.[7 22] All of this may be happening in the middle of the night when staff resources are at a minimum. It is perhaps not surprising that sometimes, it can take many

hours from seizure onset to seizure treatment. As in all critical care situations, it is not always possible to plan for all scenarios. Consequently, timelines in the real-life clinical setting are very different to the carefully prepared and orchestrated preclinical situation, which may go some way in helping to explain the conflicting results presented by the research teams working in this field.

What are the differences between human and animal studies?

Drug adverse effects are different in humans compared to animals and different in neonates compared to older children. In the NICU, neonates may be on multiple other drugs which is not the situation in controlled animal studies. This difficulty in translating preclinical findings to the clinic was evident in studies that have used bumetanide to treat neonatal seizures. The multicentre NEMO trial was terminated prematurely because of possible additive effects of bumetanide and aminoglycosides on the inner ear of neonates, which is already compromised due to hypoxia.[27] In the Boston study, this effect was not reported but may have been due to the fact that a single dose of bumetanide was used.[28] Clinical trials of drugs in neonates are extremely challenging and due to medical, regulatory and ethical reasons, there can be no tolerance for any side effects that may be associated with a trial drug. As a result, studies that are very feasible in a preclinical setting are simply impossible in the clinical situation.

The timing of drug administration is also very important and differs in clinical studies compared to controlled animal studies. Most invitro studies examined if an intervention would block the generation and propagation of induced seizures[29 30], which is the equivalent to a prophylactic study. In the preclinical in-vivo studies in question, the antiseizure drugs were given at different time points and often prophylactically: for example in Johne et al 2021[16] the drug intervention was given before intermittent asphyxia; in Johne et al 2021[31] the drug intervention was given before and after asphyxia (but before onset of seizures). In only very few studies the intervention was initiated after seizure onset, but even then, this was immediately after the onset.[32] Neither prophylactic treatment nor such precise timing of drug intervention is possible in humans. Only around half of all neonates with HIE develop seizures and it is unclear which neonates are most at risk. Neonatal seizure prediction models using

early background EEG analysis and clinical information are advancing rapidly to try and help address this question and the addition of machine learning may further help this effort. [33-35]

In one study by Hall et al in 1998,[36] Phenobarbital, was administered in a dose of 40 mg/kg intravenously soon after birth in term, severely asphyxiated newborn infants and appeared to be safe. It was associated with a 27% reduction in the incidence of seizures and a significant improvement in neurologic outcome at 3 years of age. However, this study needs to be interpreted with caution as EEG was not used to assess seizure burden and seizures were identified using clinical assessment alone, which we now know to be very inaccurate. Today, in NICUs all over the world, seizure treatment is only started when there is a strong clinical suspicion of seizures and/or there is confirmatory EEG evidence. In the preclinical studies included in this discussion, antiseizure drugs were given at different times (see figure 1) which is not the case in the NICU and this point may need to be examined in future preclinical models.

Most seizures in neonates are acute provoked seizures; the primary cause is hypoxia ischaemia, which can be acute due to a sentinel event (e.g., cord prolapse, placental abruption, profound fetal bradycardia) or chronic due to prolonged or intermittent hypoxia ischaemia. This needs to be considered in preclinical study designs, as animal models using hypoxia-only to provoke seizures may not truly represent the nature of the seizure provoking injury seen in neonates in the NICU.

Despite all of these challenges, there is an urgent need to develop animal models that truly mimic the clinical scenario for neonates in the NICU. The only way to really advance this field is for greater collaboration between both preclinical and clinical teams. The teams that have published commentaries in this issue are leading the way to advance research in neonatal seizure treatments and they have made huge strides so far. This is happening in parallel with huge advances in neonatal neurocritical care in recent years and in monitoring of seizures. Maybe now is the right time to check in with clinical researchers in the field and work collaboratively to develop truly translational models that will benefit infants worldwide.

List of Abbreviations

HIE: hypoxic-ischaemic encephalopathy

EEG: electroencephalography

aEEG: amplitude integrated electroencephalography

NICU: neonatal intensive care unts

NIRS: Near-infrared spectroscopy

MRI: magnetic resonance imaging

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Competing Interests

RMP is an investigator for studies with UCB and does consultancy work for Kephala, Ireland. She served as a Speaker and/or on Advisory Boards for Natus, GW, Esai, and UCB. G.B.B. has a consultancy with UCB and Nihon Kohden to provide advice on neonatal EEG monitoring. She is a co-founder of a start-up company Kephala LTD, which provides EEG reviewing services for industry and academia.

Ethical Publication Statement

We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

References

1. Rennie JM, de Vries LS, Blennow M, et al. Characterisation of neonatal seizures and their treatment using continuous EEG monitoring: a multicentre experience. *Arch Dis Child Fetal Neonatal Ed* 2019;104(5):F493-f501. doi: 10.1136/archdischild-2018-315624 [published Online First: 2018/11/26]
2. Glass HC, Shellhaas RA, Wusthoff CJ, et al. Contemporary Profile of Seizures in Neonates: A Prospective Cohort Study. *Journal of Pediatrics* 2016;174:98-103.e1. doi: http://dx.doi.org/10.1016/j.jpeds.2016.03.035
3. Payne ET, Zhao XY, Frndova H, et al. Seizure burden is independently associated with short term outcome in critically ill children. *Brain* 2014;137(Pt 5):1429-38. doi: https://dx.doi.org/10.1093/brain/awu042
4. Kharoshankaya L, Stevenson NJ, Livingstone V, et al. Seizure burden and neurodevelopmental outcome in neonates with hypoxic-ischemic encephalopathy. *Developmental Medicine and Child Neurology* 2016;58(12):1242-48. doi: http://dx.doi.org/10.1111/dmcn.13215
5. Fitzgerald MP, Massey SL, Fung FW, et al. High electroencephalographic seizure exposure is associated with unfavorable outcomes in neonates with hypoxic-ischemic encephalopathy. *Seizure* 2018;61:221-26. doi: 10.1016/j.seizure.2018.09.003 [published Online First: 2018/09/19]
6. Wusthoff CJ, Sundaram V, Abend NS, et al. Seizure Control in Neonates Undergoing Screening vs Confirmatory EEG Monitoring. *Neurology* 2021 doi: 10.1212/wnl.0000000000012293 [published Online First: 2021/06/04]
7. Pavel AM, Rennie JM, de Vries LS, et al. Neonatal Seizure Management: Is the Timing of Treatment Critical? *J Pediatr* 2021 doi: 10.1016/j.jpeds.2021.09.058 [published Online First: 2021/10/10]
8. Vanhatalo S, Stevenson NJ, Pressler RM, et al. Why monitor the neonatal brain-that is the important question. *Pediatr Res* 2022 doi: 10.1038/s41390-022-02040-9 [published Online First: 2022/04/03]
9. Wietstock SO, Bonifacio SL, McCulloch CE, et al. Neonatal neurocritical care service is associated with decreased administration of seizure medication. *Journal of Child Neurology* 2015;30(9):1135-41. doi: http://dx.doi.org/10.1177/0883073814553799
10. Bashir RA, Espinoza L, Vayalthrikkovil S, et al. Implementation of a Neurocritical Care Program: Improved Seizure Detection and Decreased Antiseizure Medication at Discharge in Neonates With Hypoxic-Ischemic Encephalopathy. *Pediatric Neurology* 2016;64:38-43. doi: http://dx.doi.org/10.1016/j.pediatrneurol.2016.07.007
11. Harris ML, Malloy KM, Lawson SN, et al. Standardized Treatment of Neonatal Status Epilepticus Improves Outcome. *Journal of Child Neurology* 2016;31(14):1546-54. doi: http://dx.doi.org/10.1177/0883073816664670
12. Rakshasbhuvankar A, Paul S, Nagarajan L, et al. Amplitude-integrated EEG for detection of neonatal seizures: A systematic review. *Seizure* 2015;33:90-98. doi: http://dx.doi.org/10.1016/j.seizure.2015.09.014
13. Soul JS, Pressler R, Allen M, et al. Recommendations for the design of therapeutic trials for neonatal seizures. *Pediatr Res* 2019;85(7):943-54. doi: 10.1038/s41390-018-0242-2 [published Online First: 2018/12/26]
14. Nash KB, Bonifacio SL, Glass HC, et al. Video-EEG monitoring in newborns with hypoxic-ischemic encephalopathy treated with hypothermia. *Neurology* 2011;76(6):556-62. doi: http://dx.doi.org/10.1212/WNL.0b013e31820af91a
15. Nunes ML, Yozawitz EG, Zuberi S, et al. Neonatal seizures: Is there a relationship between ictal electroclinical features and etiology? A critical appraisal based on a systematic literature review. *Epilepsia open* 2019;4(1):10-29. doi: 10.1002/epi4.12298 [published Online First: 2019/03/15]

16. Johne M, Käufer C, Römermann K, et al. A combination of phenobarbital and the bumetanide derivative bumepamine prevents neonatal seizures and subsequent hippocampal neurodegeneration in a rat model of birth asphyxia. *Epilepsia* 2021;62(6):1460-71. doi: 10.1111/epi.16912 [published Online First: 2021/05/07]
17. Hassell KJ, Ezzati M, Alonso-Alconada D, et al. New horizons for newborn brain protection: enhancing endogenous neuroprotection. *Arch Dis Child Fetal Neonatal Ed* 2015;100(6):F541-52. doi: 10.1136/archdischild-2014-306284 [published Online First: 2015/06/13]
18. Gunn AJ, Thoresen M. Neonatal encephalopathy and hypoxic-ischemic encephalopathy. *Handb Clin Neurol* 2019;162:217-37. doi: 10.1016/b978-0-444-64029-1.00010-2 [published Online First: 2019/07/22]
19. Lynch NE, Stevenson NJ, Livingstone V, et al. The temporal characteristics of seizures in neonatal hypoxic ischemic encephalopathy treated with hypothermia. *Seizure* 2015;33:60-65. doi: http://dx.doi.org/10.1016/j.seizure.2015.10.007
20. Lynch NE, Stevenson NJ, Livingstone V, et al. The temporal evolution of electrographic seizure burden in neonatal hypoxic ischemic encephalopathy. *Epilepsia* 2012;53(3):549-57. doi: https://dx.doi.org/10.1111/j.1528-1167.2011.03401.x
21. WHO. Guidelines on Neonatal Seizures. Geneva: World Health Organization 2011.
22. Apers WMJ, de Vries LS, Groenendaal F, et al. Delay in Treatment of Neonatal Seizures: A Retrospective Cohort Study. *Neonatology* 2020;117(5):599-605. doi: 10.1159/000509282 [published Online First: 2020/08/20]
23. Sharpe C, Reiner GE, Davis SL, et al. Levetiracetam Versus Phenobarbital for Neonatal Seizures: A Randomized Controlled Trial. *Pediatrics* 2020;145(6) doi: 10.1542/peds.2019-3182 [published Online First: 2020/05/10]
24. Bittigau P, Sifringer M, Ikonomidou C. Antiepileptic drugs and apoptosis in the developing brain. *Annals of the New York Academy of Sciences* 2003;993:103-14; discussion 23-4. doi: 10.1111/j.1749-6632.2003.tb07517.x [published Online First: 2003/07/11]
25. Ben-Ari Y, Delpire E. Phenobarbital, midazolam, bumetanide, and neonatal seizures: The devil is in the details. *Epilepsia* 2021;62(4):935-40. doi: 10.1111/epi.16830 [published Online First: 2021/02/04]
26. Löscher W, Kaila K. Reply to the commentary by Ben-Ari and Delpire: Bumetanide and neonatal seizures: Fiction versus reality. *Epilepsia* 2021;62(4):941-46. doi: 10.1111/epi.16866 [published Online First: 2021/03/26]
27. Pressler RM, Boylan GB, Marlow N, et al. Bumetanide for the treatment of seizures in newborn babies with hypoxic ischaemic encephalopathy (NEMO): An open-label, dose finding, and feasibility phase 1/2 trial. *The Lancet Neurology* 2015;14(5):469-77. doi: http://dx.doi.org/10.1016/S1474-4422%2814%2970303-5
28. Soul JS, Bergin AM, Stopp C, et al. A Pilot Randomized, Controlled, Double-Blind Trial of Bumetanide to Treat Neonatal Seizures. *Ann Neurol* 2021;89(2):327-40. doi: 10.1002/ana.25959 [published Online First: 2020/11/18]
29. Nardou R, Ben-Ari Y, Khalilov I. Bumetanide, an NKCC1 antagonist, does not prevent formation of epileptogenic focus but blocks epileptic focus seizures in immature rat hippocampus. *J Neurophysiol* 2009;101(6):2878-88. doi: 10.1152/jn.90761.2008 [published Online First: 2009/03/20]
30. Nardou R, Yamamoto S, Chazal G, et al. Neuronal chloride accumulation and excitatory GABA underlie aggravation of neonatal epileptiform activities by phenobarbital. *Brain* 2011;134(Pt 4):987-1002. doi: 10.1093/brain/awr041 [published Online First: 2011/03/26]
31. Johne M, Römermann K, Hampel P, et al. Phenobarbital and midazolam suppress neonatal seizures in a noninvasive rat model of birth asphyxia, whereas bumetanide is ineffective. *Epilepsia* 2021;62(4):920-34. doi: 10.1111/epi.16778 [published Online First: 2020/12/02]
32. Kang SK, Markowitz GJ, Kim ST, et al. Age- and sex-dependent susceptibility to phenobarbital-resistant neonatal seizures: role of chloride co-transporters. *Frontiers in cellular*

neuroscience 2015;9:173. doi: 10.3389/fncel.2015.00173 [published Online First: 2015/06/02]
33. Rothman SM, Glass HC, Chang T, et al. Risk factors for EEG seizures in neonates treated with hypothermia: A multicenter cohort study. *Neurology* 2014;83(19):1773-74.
34. Sansevere AJ, Kapur K, Peters JM, et al. Seizure Prediction Models in the Neonatal Intensive Care Unit. *J Clin Neurophysiol* 2019;36(3):186-94. doi: 10.1097/wnp.0000000000000574 [published Online First: 2019/03/19]
35. Macdonald-Laurs E, Sharpe C, Nespeca M, et al. Does the first hour of continuous electroencephalography predict neonatal seizures? *Arch Dis Child Fetal Neonatal Ed* 2021;106(2):162-67. doi: 10.1136/archdischild-2020-318985 [published Online First: 2020/09/16]
36. Hall RT, Hall FK, Daily DK. High-dose phenobarbital therapy in term newborn infants with severe perinatal asphyxia: a randomized, prospective study with three-year follow-up. *J Pediatr* 1998;132(2):345-8. [published Online First: 1998/03/20]
37. Douglas-Escobar M, Weiss MD. Hypoxic-ischemic encephalopathy: a review for the clinician. *JAMA pediatrics* 2015;169(4):397-403. doi: 10.1001/jamapediatrics.2014.3269 [published Online First: 2015/02/17]

Figure 1

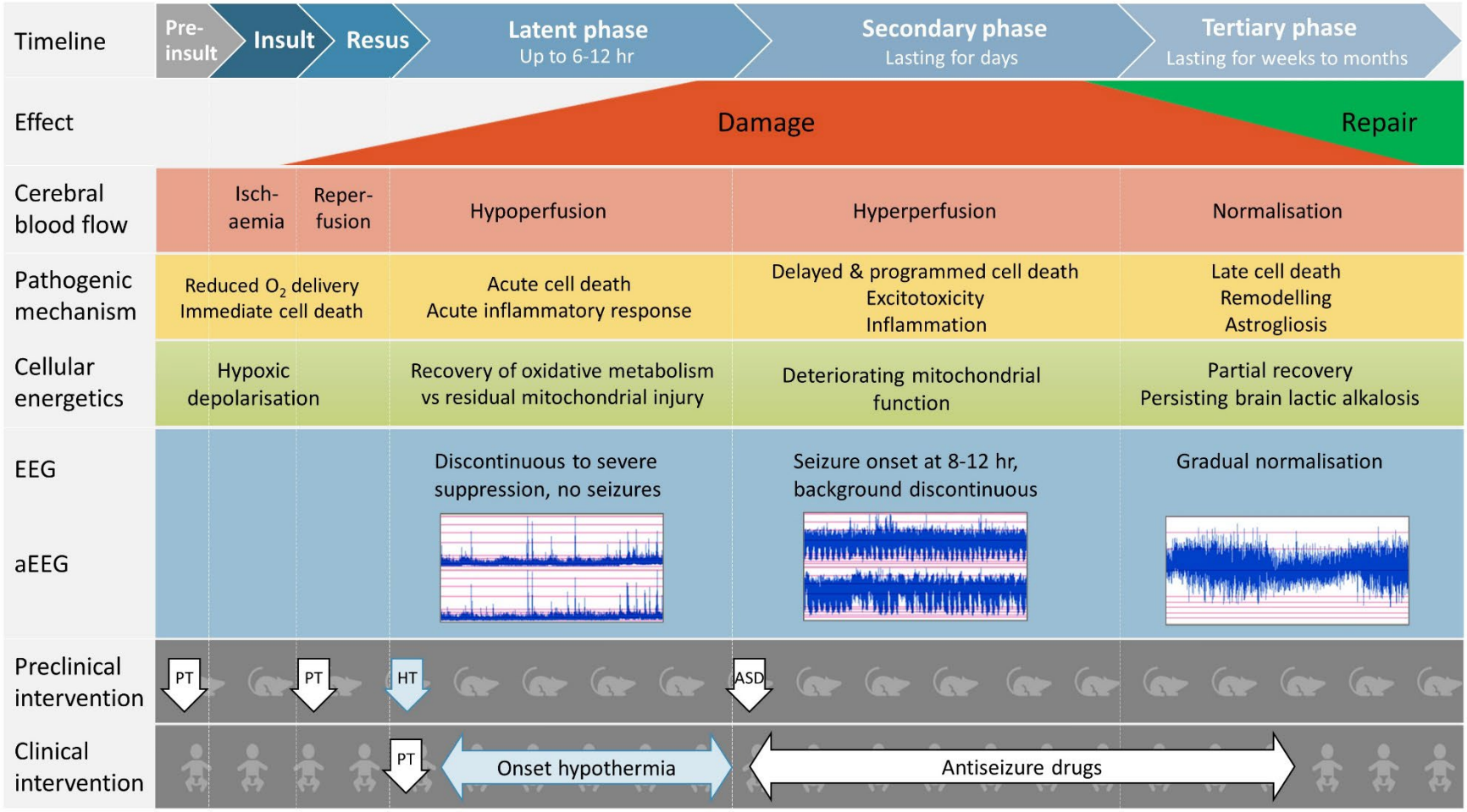
Timeline
Pre-insult
Insult
Resus
Latent phase
Up to 6-12 hr
Secondary phase
Lasting for days
Tertiary phase
Lasting for weeks to months
Effect
Damage
Repair
Cerebral blood flow
Isch-aemia
Reper-fusion
Hypoperfusion
Hyperperfusion
Normalisation
Pathogenic mechanism
Reduced O_2 delivery
Immediate cell death
Acute cell death
Acute inflammatory response
Delayed & programmed cell death
Excitotoxicity
Inflammation
Late cell death
Remodelling
Astrogliosis
Cellular energetics
Hypoxic depolarisation
Recovery of oxidative metabolism vs residual mitochondrial injury
Deteriorating mitochondrial function
Partial recovery
Persisting brain lactic alkalosis
EEG
Discontinuous to severe suppression, no seizures
Seizure onset at 8-12 hr, background discontinuous
Gradual normalisation
aEEG
Preclinical intervention
PT
PT
HT
ASD
Clinical intervention
PT
Onset hypothermia
Antiseizure drugs

Figure legend

Figure 1: Graphic representation of the pathomechanism of hypoxic ischemic encephalopathy in newborns in relationship to timing of interventions in preclinical and clinical setting. [17 37] Acute cell death starts with the initial insult but damage is maximal in the secondary phase, persisting into the tertiary phase. The EEG becomes suppressed following the primary injury and gradually recovers, first with discontinuous activity. Seizures may emerge in the secondary phase. At the bottom of the graph, timing of interventions in preclinical studies are compared with the timing of clinical trials in the NICU or in a real world setting where the start of interventions often varies by hours or even days. Many preclinical studies are in fact prophylactic interventions while in humans there is no evidence that prophylactic treatment is beneficial and consequently is not part of clinical practice.

hr: hours, PT: prophylactic therapeutics, HT therapeutic hypothermia, ASD antiseizure drugs