Review

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Cerebral palsy — brain repair with stem cells

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Abstract: Cerebral palsy, the most common disability in childhood, is a devastating non-progressive ailment of the infants' brain with lifelong sequelae, e.g., spastic paresis, chronic pain, inability to walk, intellectual disability, behavioral disorders, for which there is no cure at present. CP may develop after pediatric brain damage caused, e.g., by hypoxic-ischemia, periventricular leukomalacia, intracranial, hypoxic-ischemic encephalopathy, trauma, stroke, and infection. About 17 million people worldwide live with cerebral palsy as a result of pediatric brain damage. This reflects both the magnitude of the personal, medical, and socioeconomic global burden of this brain disorder and the overt unmet therapeutic needs of the pediatric population. This review will focus on recent preclinical, clinical, and regulatory developments in cell therapy for infantile cerebral palsy by transplantation of cord blood derived mononuclear cells from bench to bedside. The body of evidence suggests that cord blood cell therapy of cerebral palsy in the autologous setting is feasible, effective, and safe, however, adequately powered phase 3 trials are overdue.

Keywords: autologous cord blood; cerebral palsy; mononuclear cells; neuro-regeneration; pediatric brain damage.

Dedicated to Prof. Dr. med. W. Künzel, Gießen, Germany.

Introduction

Cerebral palsy (CP), the most common disability in childhood, is a devastating non-progressive ailment of the infants' brain with lifelong sequelae (e.g., spastic paresis, chronic pain, inability to walk, intellectual disability, behavioural disorders) for which there is no cure at present (Figure 1).

CP may develop after pediatric brain damage caused, e.g., by hypoxic-ischemia, periventricular leukomalacia, intracranial hemorrhage, hypoxic-ischemic encephalopathy, trauma, stroke, and infection. About 17 million people worldwide live with cerebral palsy as a result of pediatric brain damage. This reflects both the magnitude of the personal, medical, and socioeconomic global burden of this brain disorder and the overt unmet therapeutic needs of the pediatric population [1–4].

There is no causative treatment for cerebral palsy beyond symptomatic and supportive care. However, numerous symptomatic medications, including the following, may relieve the movement difficulties associated with cerebral palsy [5]: Botulinum toxin with or without casting [6–10]. Botulinum toxin (Botox) type A may reduce spasticity for 3–6 months and may be considered for children with cerebral palsy with spasticity. Phenol intramuscular neurolysis can be used for some large muscles or when several muscles are treated, but phenol therapy is permanent.

Although antiparkinsonian drugs (e.g., anticholinergic and dopaminergic drugs) and antispasticity agents (e.g., baclofen) have primarily been used in the management of dystonia, anticonvulsants, antidopaminergic drugs, and antidepressants have also been tried.

Surgical treatments used in patients with cerebral palsy include intrathecal baclofen pump insertion to treat spasticity and/or dystonia [11], selective dorsal rhizotomy to treat velocity-dependent spasticity [12, 13], stereotactic brain surgery of the basal ganglia to improve rigidity, choreoathetosis, and tremor, and orthopedic surgical intervention to treat scoliosis, joint contractures or dislocation. However, the symptomatic nature of all these interventions is unsatisfactory and the development of causative cell-based therapeutic options is overdue [4, 14–19].

Prevention of CP

Advances in neonatal neurology continue to focus on potentially modifiable factors during the neonatal period that contribute to the development of cerebral palsy. In

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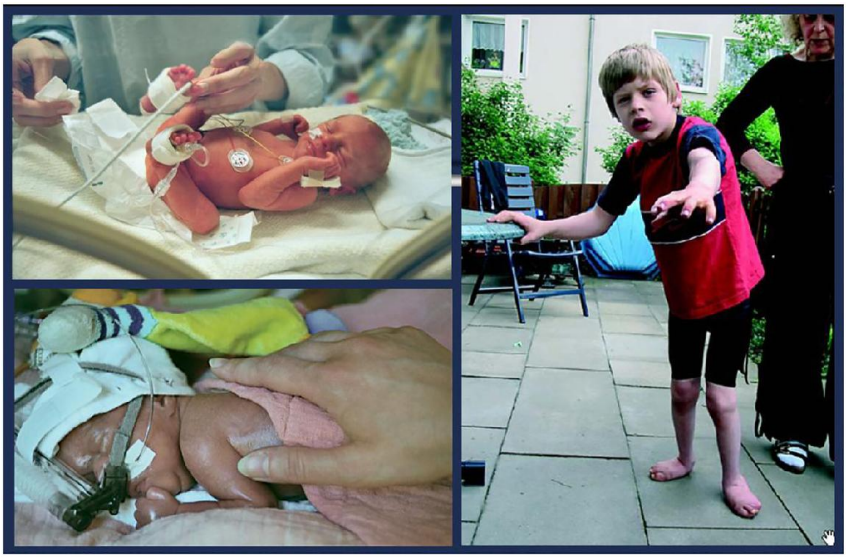

Figure 1: Preterm birth as prime risk factor for cerebral palsy. Upper left: Very preterm infant, 500 g birth weight, not ventilated, rosy complexion in a clinically healthy condition. CP risk moderate. Lower left: Very preterm infant, 500 g birth weight, ventilated, cyanotic complexion suggesting a state of circulatory centralization as sign of asphyxia and/or infection. CP risk high. Right: Boy presenting cerebral palsy with unsteady spastic gait and joint deformities [1].

recent years, several studies have shown that antenatal magnesium sulfate given to mothers at risk for preterm delivery is associated with a significant reduction in the risk of cerebral palsy [20–22]. Many other studies focus on the role of excitable amino acids and their role in neurologic injury. The hope is that more can be done in the neonatal period to prevent the permanent neurologic deficit resulting in cerebral palsy.

No set rules exist as to where or when the brain injury can occur, and injury may occur at more than one stage of fetal brain development. Additionally, causes are multiple and potentially multifactorial, including vascular insufficiency, infection, maternal factors, or underlying genetic abnormalities. Regardless of the etiology, however, the underlying brain anomaly in cerebral palsy is static, although the motor impairment and functional consequences may vary over time. By definition, cases associated with underlying disorders of a progressive or degenerative nature are excluded when diagnosing cerebral palsy.

In the United States the estimated lifetime costs to society for treatment and care for persons born in 2000 with cerebral palsy amount to US$ 11.5 billion [23].

A meta-analysis revealed that among children with cerebral palsy, 3 in 4 were in pain; 1 in 2 had an intellectual disability; 1 in 3 could not walk; 1 in 4 could not talk; 1 in 4 had epilepsy; and 1 in 10 was blind. In severe cases of cerebral palsy, 1 in 2 will die before the age of 18 [15].

Approximately 30–50% of patients with cerebral palsy have mental retardation, depending on the type [24, 25] and approximately 15–60% of children with cerebral palsy have epilepsy, being more frequent in patients with spastic quadriplegia or mental retardation.

However, even if there is no cure at present, if cerebral palsy is approached in a multidisciplinary manner, with physical, occupational, and nutritional therapy to maximize rehabilitative efforts, patients can be more fully integrated academically and socially [5].

Definition of CP

CP describes a group of permanent disorders of the development of movement and posture, causing activity limitations, attributed to non progressive disturbances that occurred in the developing fetal or infant brain. The motor disorders of cerebral palsy are often accompanied by disturbances of sensation, perception, cognition, communication and behavior, epilepsy, and by secondary musculoskeletal problems [26, 27].

Spastic CP

Spastic CP is the most common type. People will experience increased muscle tone and their movements may appear stiff or awkward. Different parts of the body can be affected [5]. Spastic hemiplegia/hemiparesis typically affects the arm, hand, and leg on one side of the body (Figure 2). Spastic diplegia/diparesis involves muscle stiffness that is predominantly in the legs. The arms may be affected to a lesser extent. Spastic quadriplegia/quadriparesis is the most severe form of CP. It is caused by widespread damage to the brain or significant brain malformations (Figure 3).

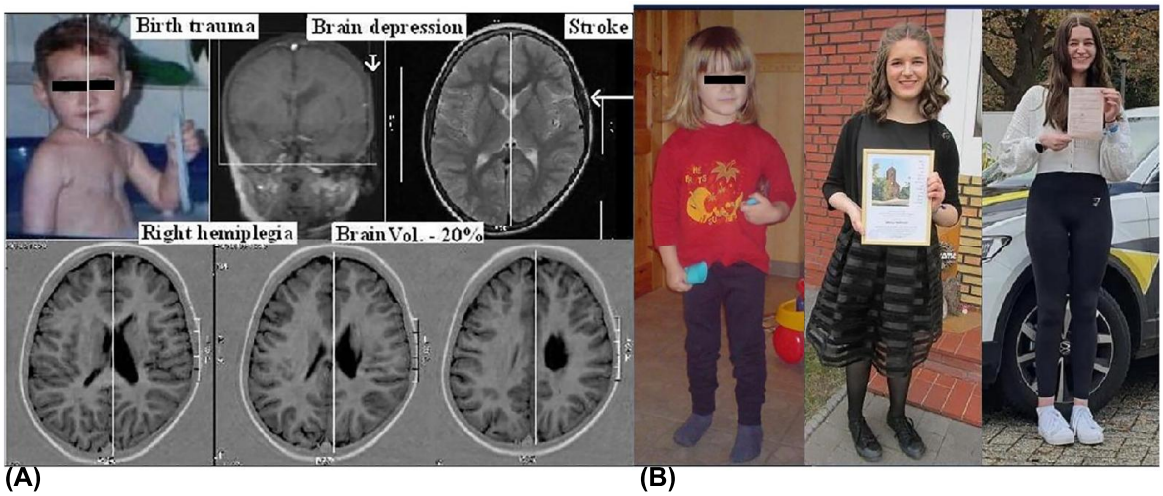

Figure 2: Trauma as risk factor for congenital cerebral palsy. (A) Traumatic fetal brain injury. An individual trial on transplantation of autologous human cord blood MNCs after neonatal arterial ischemic stroke caused by traumatic molding of the head during parturition was performed in a girl 5 years of age. The patient suffered from a unilateral white matter loss of approximately 20% resulting in hemiplegia. (B) From five years after transplantation onwards, the girl recovered from hemiplegia to such an extent, that she is now able to participate in endurance city runs, obtained the lifeguard certificate in swimming and diving (DLRG gold badge), rides two wheel bicycle, obtained a driver's licence, and plays piano using her affected right hand [28].

Dyskinetic CP

Dyskinetic motor patterns (also includes athetoid, choreoathetoid, and dystonia) are characterized by slow and uncontrollable writhing or jerky movements of the hands, feet, arms, or legs.

Ataxic CP

Ataxia affects balance and depth perception. Children with ataxia will often have poor coordination and walk unsteadily with a wide-based gait.

Etiology of CP

Cerebral palsy results from brain damage of various etiologies that can happen before, during, and after birth or during childhood, while the brain is still developing.

Cerebral palsy is caused by damage to the developing brain or abnormal development of the brain that affects a child's ability to control his or her muscles. There are several possible causes of the abnormal development or damage. There is the notion that CP is mainly caused by lack of oxygen during delivery. Now, it is considered that these causes explain only a small number of CP cases. The brain damage that leads to CP can happen before birth, during birth, within a month after birth, during the first years of a child's life or later, while the brain is still developing [32].

Congenital CP

Congenital CP is related to brain damage that happened before or during birth. The majority of CP (85–90%) is congenital, although it may not be detected until months or years later. Possible causes may include genetic abnormalities, congenital brain malformations, maternal infections/fevers, or fetal injury, e.g., traumatic arterial ischemic stroke (Figure 2) [3].

Risk factors

Low birth weight — children less than 2,500 g at birth and especially those who weigh less than 1,500 g have a greater chance of developing CP (Figure 1) [1, 33].

Premature birth — children born before the 37th week of pregnancy especially if they were born before the 32nd week of pregnancy.

Multiple births — twins, triplets, and other multiple births, especially if a twin or triplet dies before birth or shortly after birth.

Infections during pregnancy — infections lead to increases in cytokines that cause inflammation, which can lead to brain damage, e.g., fever in the mother during pregnancy or delivery, viral infections as chickenpox,

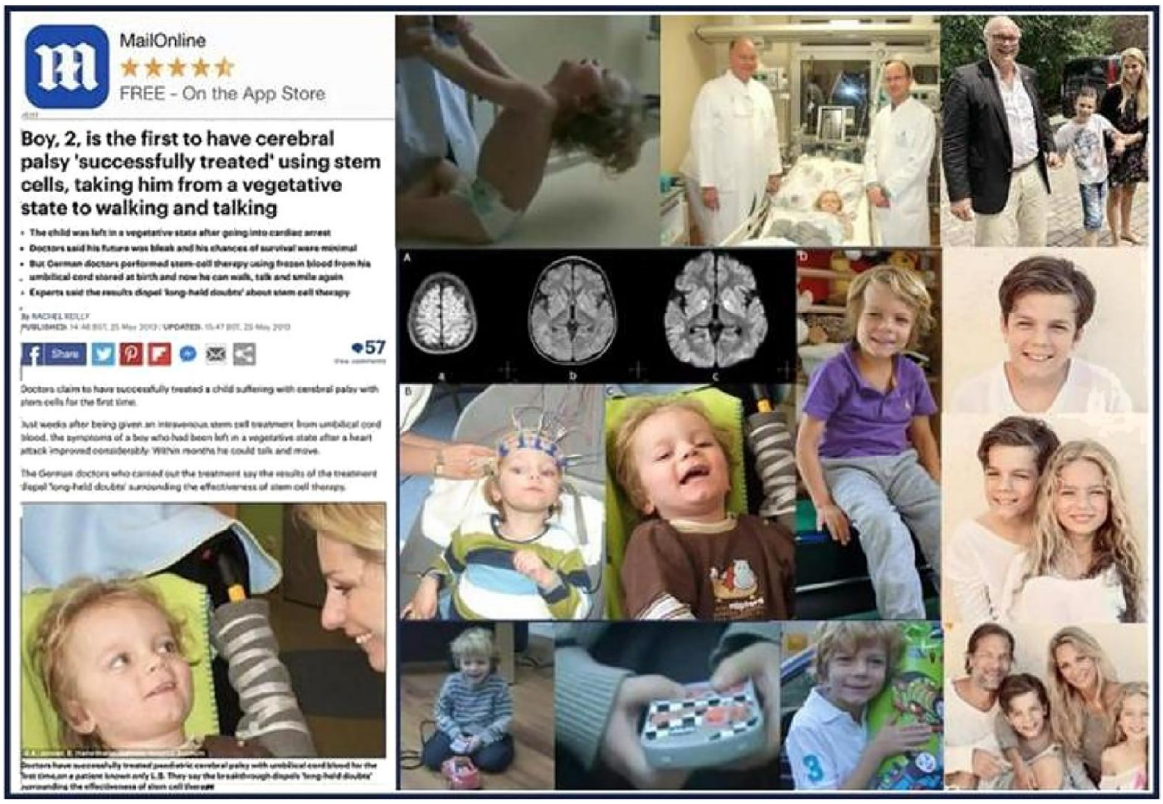

Figure 3: Acquired CP due to global hypoxic-ischemia caused by cardiac arrest. The first documented trial in man on transplantation of autologous human cord blood MNCs after global cerebral ischemia by cardiac arrest (>25 mins) was performed as individual treatment of a boy 2.8 years of age, who presented spastic quadriplegic cerebral palsy and was in a persistent vegetative state for nine weeks, on January 27, 2009 in Bochum, Germany. At 7 weeks after the stem cell treatment, EEG was normal, eyesight recovered in part, he smiled when played with, was able to sit with support, and to speak simple words. Now, at the age of 15 and 13 years after transplantation, he attends primary school, though still riding a three wheel bicycle and using a posterior gait trainer for ambulation [29–31].

rubella (german measles), cytomegalovirus (CMV), bacterial infections of the placenta or fetal membranes, or maternal pelvic infections [33].

Jaundice and kernicterus — severe jaundice and high bilirubin concentraitons in the neonate may cause kernicterus and CP due to ABO or Rh blood type incompatibility.

Medical conditions of the mother — mothers with thyroid problems, intellectual disability, or seizures also have a higher risk of having a child with CP.

Birth complications — detachment of the placenta, uterine rupture, or prolaps of the umbilical cord during birth can disrupt oxygen supply and result in CP.

Other perinatal factors: chorioamnionitis, maternal disorders of clotting (e.g., factor V Leiden deficiency), intracranial hemorrhage, newborn encephalopathy (recurrent seizures, hypotonia, coma), periventricular leukomalacia, hydrocephalus, congenital malformations [34]. Obstetrical risk factors related to brain damage as assessed by cranial ultrasound screening (n=5,618) and to psychomotor development in a matched-pair design (n=137) are displayed in Table 1 [35, 36] and Table 2 [37].

Acquired CP

A small percentage of CP is caused by brain damage that occurs more than 28 days after birth. Some causes of Acquired Cerebral Palsy include brain damage in the first few months or years of life, brain infections such as bacterial meningitis or viral encephalitis, cerebrovascular accidents, e.g., caused by cardiac arrest (Figure 3) [29–31], stroke or cerebral hemorrhage associated with coagulopathy, aneurysms, congenital heart defects, sickle cell disease, or head injury from a motor vehicle accident, a fall, or child abuse.

Risk factors

Infants are at greater risk of a brain-damaging event than older children. Neonates born preterm or at low birth weight

Table 1: Obstetrical risk factors related to brain damage (peri-/intraventricular hemorrhage I–IV; white matter damage (WMD)) according to a cranial ultrasound screening (CUS) trial on 5,618 newborns at day 1–30 in a level three perinatal center at Giessen University [35, 36].

	<=32 weeks gestation n=222	<=36 weeks gestation n=660	>=37 weeks gestation n=4,919
PIVH	43.2%	21.1%	2.1%
WMD	39.2%	18.3%	2.54%
PROM	40.1%	37.7%	13.7%
Path. CTG	39.6%	29.5%	11.4%
Twins	16.7%	20.8%	2.9%
Gestosis	14.4%	12.9%	6.8%
IUGR	8.6%	10.0%	3.2%
Haemorrhage	7.2%	7.1%	2.9%
Hypertension	6.8%	5.6%	2.8%
Mat. Fever >38 °C	5.4%	2.0%	0.8%
Amnion infection	2.7%	1.1%	0.1%
Prolonged labour	1.8%	3.3%	5.1%
Stained AF	1.8%	1.2%	5.1%
Diabetes	0.9%	3.3%	1.1%
Apgar_1<=7	76.6%	53.0%	6.4%
Apgar_5<=8	68.5%	43.9%	3.9%
Apgar_10<=8	44.1%	24.1%	0.8%
pH<=7.11	5.4%	5.6%	1.2%

PROM, premature rupture of membranes; IUGR ,intrauterine growth retardation; AF, amniotic fluid; CTG, cardiotocography, Maternal fever.

Table 2: Obstetrical risk factors related to psychomotor development of the screened infants (CUS) with and without brain damage (n=137) at 4.3 years of age as assessed by neurological examination optimality score (NOS), intelligence quotient (IQ), and Maze test (MT) in a matched-pair design [37]. The obstetrical risk factors at birth are presented by the percentage of infants suffering from moderate infantile brain dysfunction (IBD-2), defined as poor performance (<mean-1 SD) in two testing domains of NOS, IQ, or MT at 4.3 (0.8) years of age [37].

Risk factor	IBD-2
Preterm birth <=36 weeks gestation	75.0%
Asphyxia (low Apgar scores and/or low pH umb. Art)	66.7%
IUGR	50.0%
PROM, infection, chorioamnionitis, sepsis	45.8%
Path. CTG	41.7%
Twin pregnancy	41.7%
Gestosis, hypertension	33.3%
Breech presentation	25.0%
Stained amniotic fluid	12.5%
Diabetes	8.3%
Prolonged labour	8.3%

are at greater risk for acquired CP and poor psychomotor development [38]. Not getting certain vaccinations increases the risk of brain infections that can result in CP. Inadequate safety measures or lack of adult supervision can increase the risk of injury that can result in CP.

Pathophysiologic characteristics

CP is restricted to lesions of the brain only; diseases specific to the peripheral nerves of the spinal cord (e.g., spinal muscular atrophy, myelomeningocele) or to the muscles (e.g., muscular dystrophies), although causing early motor abnormalities, are not considered cerebral palsy. Major events in human brain development and their peak times of occurrence include primary neurulation, prosencephalic development, neuronal proliferation, organization, and myelination [39]: Notwithstanding prematurity of the fetal brain being a prime risk factor, cohort studies have shown an increased risk in children born slightly preterm (37–38 weeks) or post-term (42 weeks) compared with children born at 40 weeks [40].

Brain injury or abnormal brain development

Given the complexity of prenatal and neonatal brain development, injury or abnormal development may occur at any time, resulting in the varied clinical presentations of cerebral palsy (whether due to a vascular insufficiency, genetic abnormality, toxic, traumatic or infectious etiology). For example, cerebral injury before the 20th week of gestation can result in a neuronal migration deficit; injury between the 26th and 34th weeks can result in periventricular leukomalacia (foci of coagulative necrosis in the white matter adjacent to the lateral ventricles); injury between the 34th and 40th weeks can result in focal or multifocal cerebral injury [41].

Brain injury due to vascular insufficiency depends on various factors at the time of injury, including the vascular distribution to the brain, the efficiency of cerebral blood flow and regulation of blood flow, and the biochemical response of brain tissue to decreased oxygenation.

Prematurity and cerebral vasculature

The physical burden on premature infants and the immaturity of the brain and cerebral vasculature explain in

part why prematurity is a significant risk factor for cerebral palsy (Figure 1). Before term, the distribution of fetal circulation to the brain results in the tendency for hypoperfusion to the periventricular white matter [42–44].

Hypoperfusion of the juvenile brain (and the choroid plexus) can result in periventricular leukomalacia, germinal matrix hemorrhages, hypoxic-ischemic encephalopathy, and/or stroke (Figure 2) [3, 45].

Periventricular leukomalacia

Between weeks 26 and 34 of gestation, the periventricular white matter areas near the lateral ventricles are most susceptible to injury (internal capsule) [46]. Because these areas contain fibers responsible for the motor control and muscle tone of the legs, injury can result in spastic diplegia (i.e., predominant spasticity and weakness of the legs, with or without arm involvement of a lesser degree) [47, 48].

When larger lesions extend past the area of descending fibers from the motor cortex to involve the centrum semiovale and corona radiata, both the lower and upper extremities may be involved. Periventricular leukomalacia is generally symmetric and thought to be due to ischemic white matter injury in premature infants. Asymmetric injury to the periventricular white matter can result in one side of the body being more affected than the other. The germinal matrix capillaries in the periventricular region are particularly vulnerable to hypoxic-ischemic injury because of their location at a vascular border zone between the end zones of the striate and thalamic arteries. In addition, because they are brain capillaries, they have a high requirement for oxidative metabolism. Of note, human cord blood mononuclear cells given after the insult have been shown to bear therapeutic capacity for neuroregeneration [49].

Hypoxic-ischemic encephalopathy (HIE)

In HIE, birth asphyxia in the late premature and term neonate is the most common cause, resulting in oxydative stress, inflammatory response of microglia and macroglia, excitotoxicity, and cell death. In the term neonate with ischemic brain injury, certain neurons in the deep gray nuclei and perirolandic cortex are most likely to be injured, whereas other cells, such as neurons expressing nitric oxide synthase, seem to be resistant to ischemic injury. Within the basal ganglia, neurons expressing nitric oxide synthase participate in processes of oxidative stress and excitotoxicity that lead to the death of neighboring cells. Recent data suggest that apoptosis plays a prominent role in the evolution of hypoxic–ischemic injury in the neonatal brain and may be more important than necrosis after injury (see [50] for review). Interestingly, human cord blood mononuclear cells given after the insult also have been shown to ameliorate the neurologic sequelae of HIE [51].

Periventricular hemorrhage (PIVH) – intraventricular hemorrhage (IVH)

Pathogenesis of PIVH/IVH is primarily ascribed to (a) inherent fragility of the germinal matrix vasculature, (b) disturbance in the cerebral blood flow (CBF) and (c) platelet and coagulation disorders. A number of risk factors including vaginal delivery, low Apgar score, severe respiratory distress syndrome, pneumothorax, hypoxia, hypercapnia, seizures, patent ductus arteriosus, thrombocytopenia, infection, and others predispose to the development of PIVH/IVH. These risk factors appear to induce PIVH/IVH primarily by disturbing the cerebral blood flow. However, thrombocytopenia contributes to PIVH/IVH by causing haemostatic failure. Of note, a large cranial ultrasound screening study including newborns of all gestational ages, i.e., 23–43 weeks, revealed that PIVH is a fairly frequent condition (3.6%; 191/5,286) [35].

Many experts grade the severity of periventricular hemorrhage – intraventricular hemorrhage (PIVH/IVH) using a classification system originally described by Papile et al. 1978 [52]:

Grade I – Subependymal and/or germinal matrix hemorrhage.

Grade II – Subependymal hemorrhage with extension into the lateral ventricles without ventricular enlargement.

Grade III – Subependymal hemorrhage with extension into the lateral ventricles with ventricular enlargement.

Grade IV – A germinal matrix hemorrhage that dissects and extends into the adjacent brain parenchyma, irrespective of the presence or absence of intraventricular hemorrhage, is also referred to as an intraparenchymal hemorrhage when found elsewhere in the parenchyma. Hemorrhage extending into the periventricular white matter in association with an ipsilateral germinal matrix hemorrhage/intraventricular hemorrhage is termed a periventricular hemorrhagic venous infarction.

Term cerebral vascular and hypoperfusion injuries

At term, when circulation to the brain most resembles adult cerebral circulation, vascular injuries at this time tend to occur most often in the distribution of the middle cerebral artery, resulting in a spastic hemiplegic cerebral palsy (Figure 2). However, the term and toddler brain is also susceptible to hypoperfusion, which mostly targets watershed areas of the cortex (e.g., end zones of the major cerebral arteries), resulting in spastic quadriplegic cerebral palsy (Figure 3) [42]. The basal ganglia also can be affected, resulting in extrapyramidal or dyskinetic cerebral palsy.

Disturbance in cerebral blood flow

Fluctuating CBF is associated with the development of IVH. Doppler technique has been employed to measure CBF velocity in the premature infants with respiratory distress syndrome, who are on mechanical ventilator, on the first day of life. Two patterns of CBF velocity are delineated: a stable or a fluctuating CBF pattern. Stable CBF pattern consists of equal peak and trough of systolic and diastolic flow velocity, while fluctuating CBF pattern comprises continuous alteration in systolic and diastolic blood flow velocity. The neonates with fluctuating CBF velocity have higher incidence of IVH compared to infants with stable CBF pattern [53].

Pressure passivity of cerebral blood flow

Cerebral autoregulation is an ability of cerebral vessels to maintain a relatively constant CBF despite fluctuation in arterial blood pressure. The popular notion prevails that the sick premature infants are not able to sustain constant CBF at autoregulatory plateau, thus exhibiting pressure passivity of cerebral circulation. Cerebral autoregulation has been assessed by xenon clearance in earlier studies and more recently by Doppler, near-infrared spectroscopy (NIRS) or spatially resolved spectroscopy (SRS). The pressure passivity of CBF directly correlates with lower gestational age and birth weight, and is more frequently seen in sick, ventilated, and clinically unstable premature infants compared to clinically stable infants. Thus, impaired autoregulation and the subsequent development of IVH appear to be related [53].

Neonatal/perinatal ischemic stroke

In neonatal/perinatal ischemic stroke the most common cause of arterial occlusion is embolization, with sepsis and disseminated intravascular coagulation playing a major role [54]. However, also traumatic molding of the skull during parturition resulting in neonatal arterial ischemic stroke has recently been described [3, 28] (Figure 2).

Hemorrhagic cerebral infarction

Hemorrhagic cerebral infarction results when the injured capillaries in an ischemic infarct are ruptured by release of an arterial obstruction (e.g., embolus) or an increase in venous pressure, or when small amounts of bleeding from injured capillaries are not controlled by an intact clotting system. Thus, hemorrhagic infarction is observed in the newborn primarily with (1) embolic arterial occlusion because of distal movement of an embolus, (2) venous thrombosis because of the increase in venous pressure proximally, and (3) arterial thrombosis (or perhaps vasospasm) that is partial (or intermittent) or accompanied by a disturbance of coagulation. Indeed, most examples of "intracerebral hemorrhage" or "grade IV" intraventricular hemorrhage (PIVH) in the term newborn probably represent hemorrhagic venous infarction [41].

Sinus venous thrombosis (SVT)

SVT in children affects primarily neonates and results in neurologic impairment or death in approximately half of the cases. SVT is diagnosed when venous blood flow in at least one major sinus is impaired. Parenchymal infarction secondary to impaired venous drainage, and therefore not in an arterial distribution, results in approximately 60% of the cases. Perinatal complications (51%), e.g., hypoxic encephalopathy, dehydration (30%), and prothrombotic state (20%) are most common [3, 55].

Inflammation, infection

There is a growing body of evidence from clinical and epidemiologic studies that *in utero* exposure to infection plays an important role in the genesis of fetal and neonatal brain injury leading to cerebral palsy. Thus, after e.g., chorioamnionitis the incidence of periventricular white

matter damage and periventricular or intraventricular hemorrhage is significantly increased [56–58].

Many studies have shown a potentiation of hypoxic-ischemic brain injury after pretreatment with bacterial lipopolysaccharides (LPS), which leads to both increased levels of cytokines in the brain and impaired fetal cardiovascular control, in that the cardiac output is redistributed away from placenta and brain towards peripheral organs [57, 58].

Cell therapeutic approach

Over the past 15 years, 77 clinical studies have been conducted, treating 2,427 people with CP (primarily children) with stem cells [4]. The proof of principle that human cord blood mononuclear cells transplanted systemically 'home' to the damaged brain region and reduce spastic paresis has first been demonstrated in a pilot chronically prepared fetal sheep model of cerebral hypoxic-ischemia using umbilical venous transplantation of MNCs [14, 59] and was then studied extensively in a hypoxic-ischemia model in newborn rats at PN7 (the neurodevelopment of the rat at PN7 is equivalent to that of the human brain at birth) (Figure 4) [60]. In the newborn rat model we used intraperitoneal transplantation of human cord blood MNCs to demonstrate the long distance migration of cord blood derived MNCs after appropriate signals (SDF-1) [61] have been released (Figure 5) [60]. Protein antibody microarray studies *in vitro* showed the enormous capacity of human cord blood MNCs to release anti-inflammatory cytokines, growth factors, and chemokines [62]. Important information on the mode of action could be gathered *in vivo* in that the principal chemokine (SDF-1) involved in the 'homing' process was identified by antibody blocking experiments (Figure 6) [61]. Also, transplanted human MNCs from cord blood reduced glial activation along with reduced Gap-junction proteins (CX43), thus reducing glial scar formation, a prerequisite for brain plasticity and functional neuro-regeneration [64]. This leads to reduced spastic paresis, recovery of gross motor function, fine motor coordination, and muscle strength and to a recovery of neuroprocessing in the primary somatosensory cortex by normalizing inhibitory and excitatory processes (Figure 5) [60, 65]. Finally, evidence for beneficial effects on apoptosis, angiogenesis, and neuronal survival after transplantation of human cord blood MNCs was produced that explain in part the significant degree of functional neuro-regeneration in this model (Figure 6) [66].

Clinical aspects

The first documented trial on transplantation of autologous human cord blood MNCs after global cerebral ischemia by cardiac arrest (>25 min) was performed as individual treatment of a boy 2.8 years of age, who was in a persistent vegetative state for nine weeks, on January 27, 2009 in Bochum, Germany (Figure 3) [29–31]. On the day of transplantation, this boy presented quadriplegic spastic cerebral palsy, cortical blindness, and deafness. Four weeks after treatment, he executed simple tasks on demand, spasticity was largely reduced, and motor control improved. At 7 weeks, EEG was normal, eyesight recovered in part, he smiled when played with, was able to sit with support, and to speak simple words. At 1 year, spastic paresis was further reduced, free sitting and walking with support were possible. At 2 years, there was independent eating, crawling, passive standing, and walking in a gait trainer. Fine motor control had improved to such an extent that the boy managed to steer a remote control car (see video [31] supplementary material). At 3 years, receptive and expressive speech competence also improved (four-word sentences, 200 words), and there was broad understanding. Now, at the age of 15 and 13 years after transplantation, he attends primary school, though still riding a three wheel bicycle and using a posterior gait trainer for ambulation (Figure 3). Thus, autologous cord blood therapy using MNCs to treat infantile cerebral palsy has been taken from bench to bedside (Figure 7) [14].

A second individual trial on transplantation of washed autologous human cord blood MNCs after neonatal arterial ischemic stroke caused by traumatic molding of the head during parturition was performed in a girl 5 years of age, i.e., 5 years after the insult (Figure 2). The patient suffered from a unilateral white matter loss of approximately 20% resulting in hemiplegia. At five years after transplantation, the girl recovered from hemiplegia to such an extent, that she is now able to participate in endurance city runs, obtained the lifeguard certificate in swimming and diving (DLRG gold badge), rides two wheel bicycle, obtained a driver's licence, and plays piano using her affected right hand [28].

Papadopoulos (2011) [67] reported on 2 toddlers with diagnosed cerebral palsy (level III in Gross Motor Function Classification System (GMFCS)) that received a combination of autologous cord blood, low dose subcutaneous granulocyte colony stimulating factor (G-CSF) injection before and/or after cord blood transfusion, and hyperbaric oxygen therapy. In both cases significant

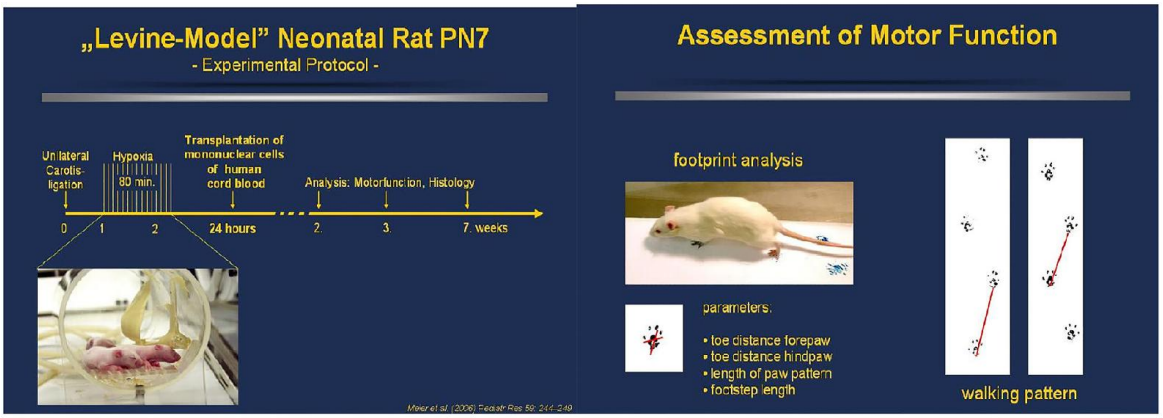

Figure 4: Levine-model for cerebral hypoxic-ischemia in neonatal rats. (A) Due to the long gestation in sheep of 140 days, we changed to the Levine-model of newborn rats in which HI was induced at PN 7 by unilateral carotid artery occlusion combined with breathing hypoxic (8% O^2) ambient air for 80 min in a perspex chamber. Human cord blood derived MNCs were injected 24 h after the insult into the peritoneal cavity of the newborn rats at PN8 [60]. (B) Motor function was assessed by footprint and walking pattern analysis. Note, reduced toe distance and step length after HI as compared with control [60].

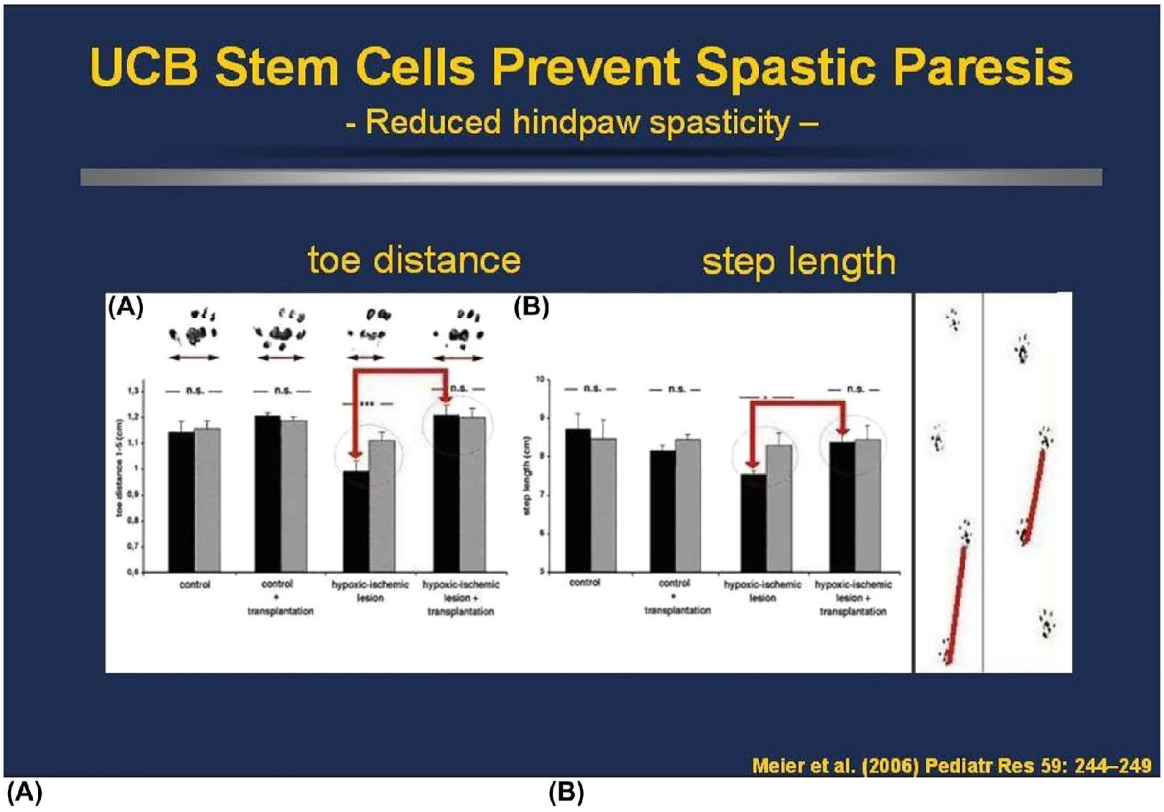

Figure 5: Human cord blood MNCs prevent spastic paresis in a newborn rat model at PN7. (A) The analysis of motor function as well as the cytochemistry of the brain (s. Figure 6) brought novel results to light. For the first time in science, perinatal spastic paresis was prevented by human MNCs given 24 h after the insult. The width of the hind paws in the group of neonates with Hypocic-ischemia plus MNCs treatment was identical to that in the control groups without HI (marked in red). (B) The same was true for the step length [60].

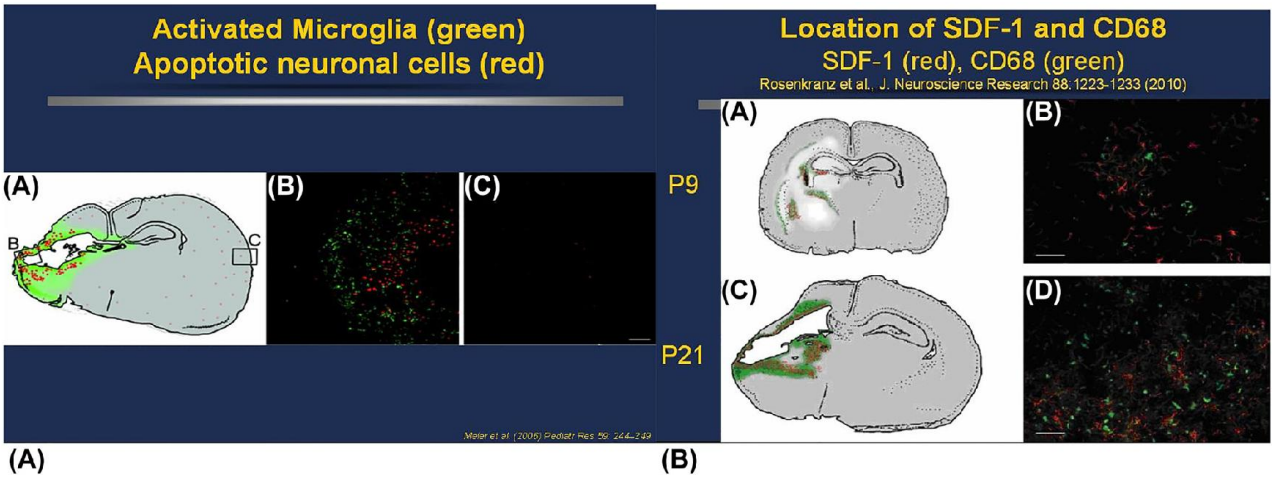

Figure 6: Activated microglia and SDF-1 expression after HI in neonatal rats at PN9 and PN21. (A) Effects of hypoxic-ischemia. In the sketch on the left, the area of activated microglia (green) contains apoptotic cells (red) equivalent to the histological slide (B-middle panel) as opposed to the control hemisphere (C-right panel). (B) Sketches (A-upper panel at P9; C-panel at P21) from histological slides (B-panel at P9; D-panel at 21) demonstrate expression of stromal derived factor –1 (SDF-1) stained in red, a chemokine causing the 'homing' of human umbilical cord blood MNCs into the damaged brain area [61] exactly in the area of activated microglia (green, expression of specific marker CD68). The surprising fact is that this SDF-1 chemokine leaves the brain area through the damaged Blood-Brain-Barrier into the systemic circulation [63] in which the human MNCs are waiting for the protein SDF-1 to dock on to the their surface receptors (CXCR4). What follows is the long-distance migration of the SDF-1+MNC complex against the concentration gradient right into the damaged brain area where the SDF-1 protein originated. This high precision 'Homing' mechanism starts the healing process by release of anti-inflammatory cytokines, nerve growth factors, and chemokines to initiate neuro-regeneration [61].

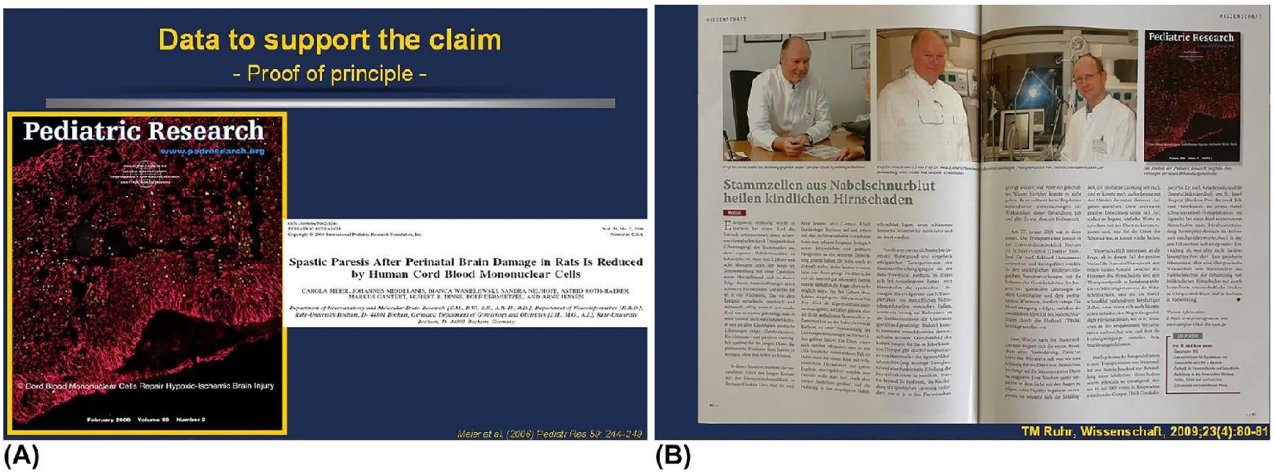

Figure 7: Publications on first experimental and first clinical evidence for cord blood MNCs being a potential cure for CP. (A) The novel observation that spastic paresis can be prevented by cord blood stem cells was highlighted on the title page of the Journal 'Pediatric Research' with the editorial comment "Cord blood Mononuclear Cells Repair Hypoxic-ischemic Brain-Injury". Of note, as depicted, the green human MNCs 'homed' right into the astroglial scaffold of the damaged rat brain [60]. (B) Two years later, the parents of a young boy aged 2.5 years suffering from massive brain damage and cerebral palsy after cardiac arrest for more than 25 min asked for help. The Ethic Committee at the Ruhr-University Bochum granted permission so that on January 27, 2009, an autologous cord blood transplantation was performed in that boy who was in a vegetative state, blind, deaf, and quadriplegic with rigid wide open pupils in spite of bright light from the ceiling (Figure 3) [29, 31]. After transplantation, he gradually recovered over the years to such an extent that he now attends primary school, rides a three-wheel bicycle, and needs a posterior gait trainer for ambulation [31].

improvements in motor ability were noted at 7 weeks and at 36 months with reduced spasticity bilaterally, and re-classification to GMFCS level I was possible.

A cohort of autologous cord blood transplantations for cerebral palsy has been published by Lee and coworkers (2012) [68], who reported on an uncontrolled single arm pilot study of 20 children with cerebral palsy to assess the safety and feasibility of the procedure as well as its potential treatment efficacy. In this carefully documented study, employing an array of neurodevelopmental examinations

and imaging techniques, including brain perfusion SPECT analysis and MRI-DTI (FA values) before and during follow-up, there were overall neurologic improvements in 5/20 children, ranging from 23 to 91 months of age, weighing 7.2–21.4 kg. From these 5 children, MRI FA values were evaluated to assess white matter integrity in 26 regions of interest (ROI). In 3 regions (temporal right, corpus callosum, and right posterior periventricular white matter) significant changes after treatment were noted when compared with pretreatment values. The authors conclude that intravenous cord blood infusion seems to be practical and safe and has yielded potential benefits in children with cerebral palsy [68]. Interestingly, though age at treatment did not show any significant differences in global outcome (n=20), it is noteworthy that within the subgroup of hypoxic-ischemic encephalopathy (HIE, n=8), the non-responders were significantly older (65.2 ± 23.1 SD months,) than the responders who showed improvements after treatment (37.3 ± 6.0 SD months) [14]. Also, the majority (15/20) of conditions possibly related to cerebral palsy did not respond to treatment [68].

An open-label study on hypoxic-ischemic encephalopathy, therapeutic cooling, and fresh autologous cord blood MNCs in neonates has been published recently [69]. To assess feasibility and safety of providing autologous umbilical cord blood (UCB) cells to neonates with hypoxic-ischemic encephalopathy (HIE), infants were enrolled in the intensive care nursery who were cooled for HIE and had available UCB in an open-label study of non-cryopreserved autologous volume- and red blood cell-reduced UCB cells (up to 4 doses adjusted for volume and red blood cell content, 10–50 million cells/dose). UCB collection and cell infusion characteristics were recorded and pre-and post-infusion vital signs. As exploratory analyses, cell recipients' hospital outcomes (mortality, oral feeds at discharge) and 1-year survival with Bayley Scales of Infant and Toddler Development (3rd edition) scores ≥85 in 3 domains (cognitive, language, and motor development) with cooled infants who did not have available cells were compared. Twenty-three infants were cooled and received cells. Median collection and infusion volumes were 36 and 4.3 mL, respectively. Vital signs including oxygen saturation were similar before and after infusions in the first 48 postnatal hours [69]. Cell recipients and concurrent cooled infants had similar hospital outcomes. Thirteen of 18 (74%) cell recipients and 19 of 46 (41%) concurrent cooled infants with known 1-year outcomes survived with scores >85. Collection, preparation, and infusion of fresh autologous UCB cells for use in infants with HIE is feasible [69].

The first double-blind, randomized, placebo-controlled trial on (allogeneic) umbilical cord blood therapy potentiated with erythropoietin and without for children with cerebral palsy has been performed in South Korea [70, 71]. Allogeneic umbilical cord blood (UCB) has therapeutic potential for cerebral palsy (CP) and concomitant administration of recombinant human erythropoietin (rhEPO) may boost the efficacy of UCB, as it has neurotrophic effects. The objectives of this study were to assess the safety and efficacy of allogeneic UCB potentiated with rhEPO in children with CP. Children with CP were randomly assigned to one of three parallel groups: the pUCB group, which received allogeneic UCB potentiated with rhEPO; the EPO group, which received rhEPO and placebo UCB; and the Control group, which received placebo UCB and placebo rhEPO. All participants received rehabilitation therapy. The main outcomes were changes in scores on the following measures during the 6 months treatment period: the gross motor performance measure (GMPM), gross motor function measure, and Bayley scales of infant development-II (BSID-II) Mental and Motor scales. F-fluorodeoxyglucose positron emission tomography (18F-FDG-PET/CT) and diffusion tensor images (DTI) were acquired at baseline and followed up to detect changes in the brain. In total, 96 subjects completed the study. Compared with the EPO (n=33) and Control (n=32) groups, the pUCB (n=31) group had significantly higher scores on the GMPM and BSID-II Mental and Motor scales at 6 months. DTI revealed significant correlations between the GMPM increment and changes in fractional anisotropy in the pUCB group. 18F-FDG-PET/CT showed differential activation and deactivation patterns between the three groups. The incidence of serious adverse events did not differ between groups. UCB treatment ameliorated motor and cognitive dysfunction in children with CP undergoing active rehabilitation, accompanied by structural and metabolic changes in the brain [70, 71].

Recently, a Cerebral Palsy-Autologous Cord Blood (CP-AC) study was designed to assess the efficacy of a single intravenous infusion of autologous CB in young children with spastic CP [72, 73].

The CP-AC study was a prospective, randomized, double blind, placebo-controlled crossover study of a single intravenous infusion of autologous CB in children ages 1–6 years with spastic CP. The Gross Motor Classification System (GMFCS) was utilized to classify the level of motor function at study entry and follow-up. Children were eligible if they were GMFCS level 2–4 or GMFCS level 1 with hemiplegia if they used their affected hand as an assist only. Children with known genetic conditions, intractable seizures, or severe microcephaly were ineligible. Autologous CB units had to

have a documented pre-cryopreservation total cell dose of ≥1 × 10^7/kg, negative sterility culture, negative maternal infectious disease screening, and confirmed identity through HLA typing of the subject and a segment attached to the CB unit. Subjects were evaluated at baseline, one year, and two years with functional evaluations (Gross Motor Function Measure-66 [GMFM-66], Peabody Developmental Motor Scales-2, Assisting Hand Assessment, Bayley Scales of Infant Development), and brain MRI. They were randomized to the order in which they received CB and placebo infusions (given one year apart). The primary endpoint was change in GMFM-66 score at one year after the initial infusion (CB or placebo). Cryopreserved CB units were shipped to and stored at Duke University until the day of treatment when they were thawed and washed in dextran 40 + 5% human serum albumin (DA). Infusions, dosed at 1–5 × 10^7/kg based on the pre-cryopreservation total nucleated cell count (TNCC) and diluted in 1.25 mL/kg of DA, were administered at baseline and 1 year later in a masked manner through a peripheral IV catheter over 5–15 min in the outpatient setting after premedication with oral acetaminophen (10–15 mg/kg), and IV methylprednisolone (0.5 mg/kg). Subjects received IV fluids and were monitored for 2–4 h post-infusion [72, 73].

Sixty-three children were enrolled with a median age of 2 years (range: 1–6) at baseline. Median TNCC of CB infusion was 2 × 10^7/kg (range: 0.4–5 × 10^7/kg) with a median CD34 dose of 0.5 × 10^7/kg (range: 0.05–4 × 10^7/kg). Infusions of autologous CB and placebo products, both containing DMSO, were well tolerated, and there were no serious adverse events related to the infusions. Preliminary analysis of the 63 patients at one year showed no statistically significant overall difference in GMFM-66 change scores between placebo and treated groups (6.9 vs. 7.5, p=0.72). However, subjects who received pre-cryopreservation cell doses of >2.5 × 10^7/kg demonstrated statistically significant improvement in GMFM-66 change scores compared to subjects who received lower cell doses (p<0.01). The authors concluded that cord blood may improve motor function in young children with spastic CP, when adequately dosed. Improvement was observed at pre-cryopreservation cell doses (>2.5 × 10^7/kg) that correspond with the minimum cell dose used for hematopoietic reconstitution in patients undergoing allogeneic CB transplantation [73].

Clinical safety

The children treated for cerebral palsy by intravenous autologous cord blood transplantations experienced only mild adverse effects, including transient hemoglobinuria, nausea, and hypertension in one case [31], mild transient nausea in 2 cases [67], hemoglobinuria plus nausea in 3/20 cases, and hemoglobinuria plus urticaria in 2/20 additional cases [68] likely to be related to DMSO content in the cord blood unit. Recent studies were conducted with washed MNCs to eliminate debris and residual DMSO [28]. The largest retrospective study on safety and feasibility of intravenous infusion of autologous cord blood, in which 184 children with acquired neurologic disorders received 194 cord blood infusions (there are no neurological outcome data available from this study), reports on only 3 (1.5%) patients showing adverse effects [74]. These patients presented infusion reactions, all responding to medical therapy and stopping the infusion. All cord blood units were thawed and washed in dextran-albumine, and premedication with acetaminophen, diphenhydramine, and methylprednisolone was provided. Interestingly, these authors report on postthaw sterility cultures that were positive in 7.6% of the infused cord blood units without causing infections in the patients, though antibiotics were not given. Intravenous infusions lasted 15–20 min and iv-fluids were administered at twice maintenance for 2–4 h after cord blood infusion. Vital signs and pulse-oximetry were monitored every 5 min during infusion and every 30 min for 2–4 h after infusion [74]. Thus, on the basis of the available information, autologous cord blood transfusion can be considered a safe and feasible treatment.

Regulatory approval process

After first successful treatments of CP in children [3, 14, 28, 31] and on the basis of preclinical studies that demonstrated the capacity of human umbilical cord blood mononuclear cells to 'home' to the brain lesion and to ameliorate the neurological sequelae of hypoxic-ischemic brain injury [59, 60] by anti-inflammatory cytokines, neuronal growth factors, and chemokines [64] through the SDF-1 'homing' mechanism [61] and the prevention of glial scars [64], we started the regulatory process at the European Medicinal Agency (EMA) (Figure 8). This included applications for classification of our cord blood stem cell product as Advanced Therapy Medicinal Product – Tissue Engineered Product (ATMP-TEP) in 2015 [75]. Secondly, applications for Orphan Medicinal Product Designations (OMPD) were filed and EMA granted BrainRepair UG OMPDs for two indications, i.e., Periventricular leukomalacia (PVL) [49] and Newborn encephalopathy (NE) in 2016 [51]. Finally, the first Paediatric Investigation Plan (PIP) for the pivotal trial (REGAIN) on efficacy and safety of human autologous mononuclear cells derived from cord blood to treat PVL

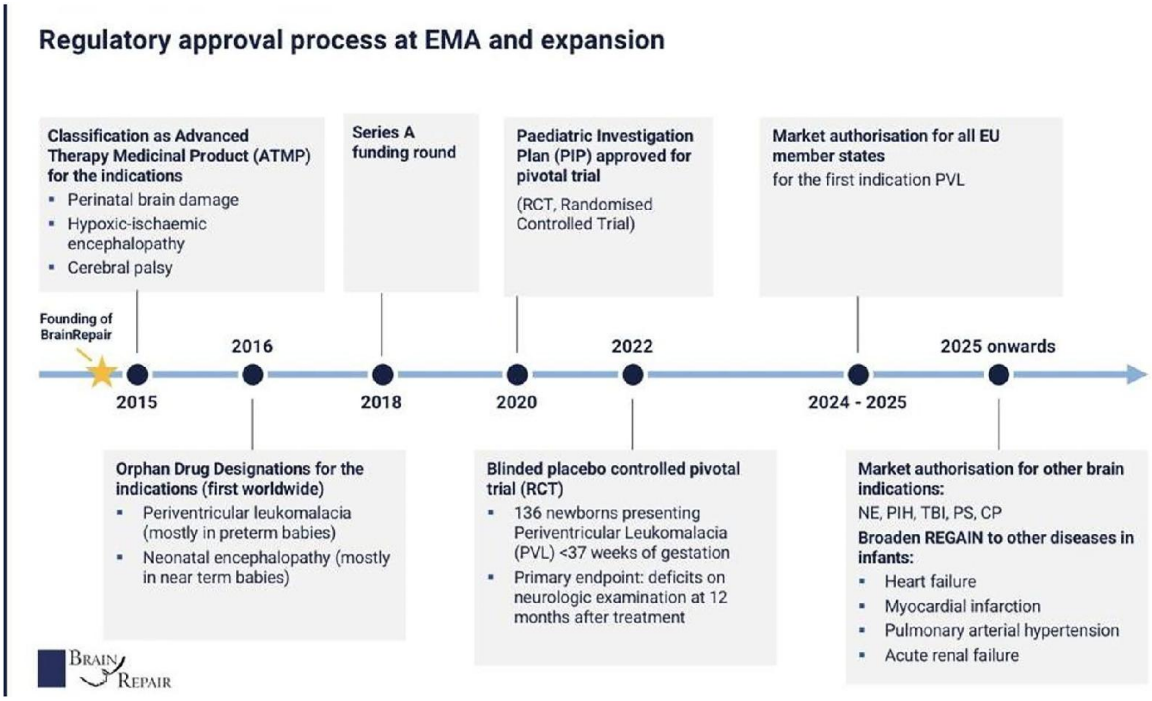

Figure 8: Regulatory approval process at the European Medicinal Agency (EMA). BrainRepair UG was founded in 2015 and the stem cell product was classified as Advanced Therapy Medicinal Product-Tissue Engineered Product (ATMP-TEP) in the same year [75]. In 2016, EMA granted the company 'Orphan Medicinal Product Designations' (OMPD) for 2 indications, i.e., Periventricular leukomalacia (PVL) and Newborn encephalopathy (NE) [49, 51]. This was followed by an agreed 'Paediatric Investigation Plan (PIP)' for the pivotal trial in 2020. Now BrainRepair UG is planning the pivotal trial as prerequisite to apply for market authorization [76].

was agreed upon in 2020 [76]. Now the pivotal trial, a prerequisite to obtain market authorisation, can be started since the Series B funding round has been finalized (Figure 8).

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Ethical approval: The local Institutional Review Board deemed the study exempt from review.

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