Stem Cells for bronchopulmonary Dysplasia: Reality or Future?
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Neonatus 2016, Poznan; Poland

1. Very preterm neonates and bronchopulmonary Dysplasia
2. Experimental Data: BPD and Stem Cells
3. First Clinical Data of Stem Cells in Neonates
4. Summary

Disclosure: Nothing related to the Topic

Data from 2003: Zeitlin et al. Pediatrics; 2008

Data from GNN Network – Causes of Mortality in VLBW Infants


Cohort: VLBW- Preterms <1500 g BW; n = 2659
Mortality ~ 10%. Pulmonary disorders account for ~ 30% of deaths up to discharge.
Rate of BPD in Europe: MOSAIC versus EPICE

In the 2003 cohort of preterms < 32 weeks of gestation, the rate of BPD was 15%. N= 4,950 preterms.

Main risk factors:
- Immaturity
- SGA-status
- Male gender
- Postnatal asphyxia and asphyxia

Apart from these data, pronounced regional effects were observed with lowest rates in Lazio/Italy and highest rates in both UK regions (Northern and Trent regions).

In the 2011/12 cohort of preterms with identical inclusion criteria, we observed a rate of 14% (unpublished data).

Analysis of risk factors etc is ongoing!

Definition of BPD was identical in both studies:
- Oxygen requirements or CPAP/mechanical ventilation at 36 weeks postmenstrual age

Analysis of risk factors for BPD in the MOSAIC Cohort (n=4950 very preterms)

<table>
<thead>
<tr>
<th>GA (weeks)</th>
<th>OR (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 26</td>
<td>36.8 (23.8-56.7)</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>26</td>
<td>22.4 (14.8-34.2)</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>27</td>
<td>11.5 (7.6-17.3)</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>28</td>
<td>7.8 (5.3-11.6)</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>29</td>
<td>3.0 (2.0-4.6)</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>30</td>
<td>2.2 (1.5-3.6)</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>31</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>m</td>
<td>1.5 (1.2-1.8)</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>w</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>PIH</td>
<td>1.6 (1.1-2.4)</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>PROM</td>
<td>0.8 (0.7-1.0)</td>
<td>n.s.</td>
</tr>
<tr>
<td>SGA (P10)</td>
<td>4.4 (3.3-6.0)</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>Apgar &lt; 7</td>
<td>1.8 (1.4-2.3)</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>IT Lazio</td>
<td>0.5 (0.3-0.9)</td>
<td>&lt;.05</td>
</tr>
<tr>
<td>FR Ile-de-France</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>UK Trent</td>
<td>1.9 (1.4-2.7)</td>
<td>&lt;.1</td>
</tr>
<tr>
<td>UK Northern</td>
<td>1.9 (1.3-2.9)</td>
<td>&lt;.1</td>
</tr>
</tbody>
</table>

PIH = pregnancy induced hypertension; PPROM = preterm premature rupture of fetal membrane; ANC = antenatal corticosteroids; IUGR = intrauterine growth restriction; Gortner L et al Neonatology 2011.
Radiographics from RDS and BPD: Classic versus new Form of BPD

What is “new” type of BPD?

1. Histological characteristic features
   a) Rarefaction of the pulmonary vascular bed
   b) Reduced alveolarization
   c) Less dominant: interstitial form, bronchial metaplasia

"new type BPD"  "classical BPD"

(Hussain NA, Hum Pathol, 1998)
Pathogenesis of BPD – schematic view of an old problem: Reaction of the preterm lung to various nocuous insults – RDS and others

Prenatal Factors
- Inflammation; tobacco exposure
- Intrauterine growth restriction

Postnatal Factors
- Mechanical ventilation
- Infections
- O₂-toxicity, bioruma
- PDA, path. flow patterns

Immature Lung
Genetic susceptibility
Stem Cell Repair

Reduced alveolarization
- Impaired collagen-elastin metabolism

Pathologic vascularization
(VEGF, NO-signal transduction)

Animal models of BPD- can we improve outcome using models for experimental Therapy?


- Problem: Does not reflect all typical pathogenic mechanisms of BPD since there is no combined prenatal and postnatal injury. Hyperoxia is not the only mechanism of lung alteration. Lack of prenatal lung insult (LPS or IUGR) and postnatal hyperoxia lead to less septal changes plus more signs of inflammation.

- Double hit: prenatal hypoxia in pregnant mice (4 days, 10% O₂) to obtain growth retarded pubs + postnatal hyperoxia (14 days, 78% O₂) to induce BPD like morphological changes (Gortner et al. 2013)

- Advantage: Mimics the clinical situation better and the mouse developmental pulmonary pathways are close to those in humans. Further aspects: it should provide a pronounced loss of alveolarization without significant fibrosis and inflammation (Levine et al. PNAS 2005)
**Stem Cells: Glossar – Focus Lung**

- **Embryonic stem cells**: Origin from the inner site from blastocyste with pluripotent properties; in Germany no option for research with this cell type and/or therapeutic use.
- **Adult stem cells**: Cells from different tissues, a.o. from bone marrow, fat tissue, etc.
- **Special situation - Umbilical cord blood**: All stem cell fractions have special properties in between the above-mentioned phenotypes; special properties include an increased viability and differentiating properties. Increased paracrine activity; Similar: Cells from the umbilical cord tissue.
- **Further: Mesenchymal stem cells**, which can be differentiated depending on physical or chemical stimulants into various tissues: Chondrocytes, adipocytes and osteocytes among others.
- **Adult tissue-specific** stem cells – nisch cells: Organ-specific stem cells, with a single specific direction of differentiation: e.g. pulmonary bronchiolo-alveolar nisch cells, which differentiate on the alveolar level into type II cells, which in part further develop into type I cells – alveolar layer cells.
- **Bronchiolar nisch cells**: toxinresistant CCSP expressing subgroup of pulmonary nisch cell – differentiation into Clara cells.

**Stem cells in experimental BPD I**

Aslam M et al., Am J Respir Crit Care Med 2009

![Stem cells in experimental BPD I diagram](image-url)
Stem Cells and BPD - what is going on?

![Diagram showing lung development and stem cell interaction](image)

**Fig. 1**: Hypothesis of the MSCs action in BPD. Exogenous MSCs (yellow) interact with the immature lung tissue (gray lung schema). Diseased lung (light blue). Thick menenchymal layer forming the primary septa, containing two layers of capillaries (red, distantly from the epithelium) and secreting enzymes and cytokines and transfer mitochondria (left dashed line). This process results in lung development, driving the restoration of the monolayer with healthy endogenous MSCs (blue). Formation of secondary septa and thinning of the blood-air barrier (darker lung schema).

Möbius and Rüdiger: Molecular and Cellular Pediatrics, 2016

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### Experimental BPD – An overview on stem cell use

#### Tab. 1: Stammzellen bei experimenteller bronchopulmonaler Dysplasie (BPD) in Nagen

<table>
<thead>
<tr>
<th>Species</th>
<th>Aalam et al. [3]</th>
<th>Van Haften et al. [25]</th>
<th>Zhang et al. [29]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse</td>
<td>Ratten</td>
<td>Ratten</td>
<td>Mäuse</td>
</tr>
<tr>
<td>Potenzial Alter (P)</td>
<td>P 1, O2-Exposition</td>
<td>P 1, O2-Exposition</td>
<td>P 1, O2-Exposition</td>
</tr>
<tr>
<td>BPD, induziert durch Intervention</td>
<td>5% Nages O₂ – P 1-14</td>
<td>5% Nages O₂ – P 1-14</td>
<td>5% Nages O₂ – P 1-14</td>
</tr>
<tr>
<td>Stammzellpräparat</td>
<td>P 4: systemische Aplication MSC</td>
<td>P 4: intratracheale Aplication MSC</td>
<td>P 5: intratracheale 2-10 Zellen oder intraperitoneal 2-10 Zellen</td>
</tr>
<tr>
<td>Kontrolle (Raumluft)</td>
<td>Kontrolle (Raumluft)</td>
<td>Kontrolle (Raumluft)</td>
<td>Kontrolle (Raumluft)</td>
</tr>
<tr>
<td>Resulte</td>
<td>KD</td>
<td>MSC</td>
<td>KD 75%</td>
</tr>
<tr>
<td>- Histologie</td>
<td>nl</td>
<td>nl</td>
<td>BPD</td>
</tr>
<tr>
<td>- Immunhistochemie</td>
<td>(++)</td>
<td>+++</td>
<td>(++)</td>
</tr>
<tr>
<td>- Immunhistochemie (Inflammation)</td>
<td>TGF-β</td>
<td>TGF-β</td>
<td>TGF-β+++</td>
</tr>
</tbody>
</table>

BPD: bronchopulmonale Dysplasie; Exp: Expression; KD: Kontrolle; 0,75%, 0,65% Versuchstiere mit entsprechendem Karotisschnitt O2- exposition ohne Stammzellen MSC, menschliche Stammzellen, SP-C: polymere-sulfat-reaction (Parylene-Clene-sulfat-reaction); BPD: bronchopulmonaler Dysplasie; RAC: radiologische Atemfehler

Gortner L et al. Monatschr Kinderheilkd 2014
BPD - Double Hit – Procedure

Variables: Growth, lung morphometry, EM-work up and molecular and protein markers

Mating

Day E14

Day E18

Day P 5: 0.5 Mio mononuclear Cells i.p. in Group 3

Normoxia

Hypoxia

Hyperoxia

Normoxia

Hypoxia

Hyperoxia

Normoxia

Hypoxia

Hyperoxia

Mating

prenatal

postnatal

Monz D et al. : PLOS 1, 2013

Lung Histology: LM-EM

Moxia

Double-hit

Double-hit with MNC

Electron Microscopy:

Li : Double Hit without MNC
Re: Double Hit with MNC

Double-hit without mononuclear cells: reduced alveolarisation; reversibility with mononuclear cells (MNCs)

Monz D et al. : PLOS 1, 2013
**Pulmonary mRNA-Expression (ΔΔCT-method)**

*Surfactantproteins: SP-B, SP-C*

*Retinol: RAR-α, -β, -γ, CRABP-1, RBP-1*

*TGF: TGFβ-1, -2, -3*

**Growth factors:** Elastin, IGF-1, HIF-1α, VEGF, mTOR

![Diagram showing gene expression changes]

Monz D et al. : PLOS 1, 2013

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**What do models of BPD mean to clinical practice?**

A potential for translation?

- If BPD starts in utero, an early postnatal therapy seems mandatory in order to stop the pathogenic process. Starting therapy by the end of the 1st week seems most promising.
- However, this approach will limit the fractions of stem cells - given the autologous way will be gone; MSCs are not feasible! CD 34 cells? CD 34/CD133 cells?
- What is the optimum number of the respective stem cell preparation? Can it be obtained from umbilical cord blood (amniotic membrane and/or Wharton jelly?). In animal experiments ca. 0.05 - 0.5 Mio MSCs were used. See below!
- What is the mechanism of MSCs under experimental condition? Paracrine growth effects, modulation of local inflammation, effects on niche cells or cell replacement during the early neonatal period. Which cells are best?
- Allogeneous therapy Do we need cells really or are growth factors adequate, e.g. EPO?
- Risk of allogeneous stem cell administration - GvHD in case of allogeneous therapy; neoplasms?
- Optimum way of cell administration – timing?
- Effect on pulmonary vascular system?
Vascular Changes in a very preterm neonate with impaired intrauterine growth and consecutive BPD – most we target improved vascular development? Goal: Reverse remodelling!

Regenerative therapies in neonatology - The future?
Allogogenous administration of MSCs in threatening BPD

Dose-escalation study in preterms < 28 weeks using allogenous MSCs for prophylaxis of BPD. Intratracheal administration of 10⁶ - 10⁷ MSCs on day 1-4. Endpoint Survival without BPD without differences between both groups. In the same issue, a 2nd paper has been published on autologous stem cells in HIE combined with hypothermia

MSCs for Prevention of BPD; Chang YS e al. J Pediatr 2014
Outcome after human MSCs in threatening BPD

Chang Y et al. J Pediatrics 2014

Open questions which must be addressed before going into clinical translation after Corean Trial: Lung

- Allogenic or autologous approach?
- Long-term risks of allogenic transplants? Graft versus host? Malignomas?
- Which type of cell? MSCs, Endothelial progenitor cells? CD34 cells?
- Optimum number of cells? Perhaps > 10 (7); (Chang Y et al 2014)
- Which way of administration in case of BPD treatment: right into the lung or systemic intravenous administration?
- Timing of treatment? 1st, 2nd week or later?
- Follow up data of the Corean trial not yet published
- What does mean the 1st clinical pilote trial?
- Other indications for stem cells in lung disorders – e.g. congenital anomalies of the surfactant system – SP-B deficiency (Gortner L, Klin Pad 2015)
- We need more preclinical data to answer these questions!
Summary

- Stem cell therapy might become an option in the future, given that further experimental data are worked out to answer open questions. These findings may be translated into clinical practice.
- These include data with respect to timing, type of cell preparation, number of cells and the route of cell administration among others.
- Ongoing clinical trials need a complete follow-up to avoid any potential long term risks, especially in allogeneic therapy.
- Further open questions: Can we optimize response by co-administration of growth factors or components of growth media? For example microvesicals.

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Project coordinator: J. Zeitlin, MA, France

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  all Homburg/Germany; HOMFOR 2011
- Prof. Ochs, Anatomy MH Hannover/Germany

NeoVitaA – Study: DFG ME144;
CD34-Cells in preterms and term neonates I - an option for autologeous therapy?

- Perinatal basics of study infants

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Preterm newborns (n=30)</th>
<th>Term newborns (n=30)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age [years]</td>
<td>32.73 ± 7.09 (32)</td>
<td>34.03 ± 6.48 (33.5)</td>
<td>0.403</td>
</tr>
<tr>
<td>No. of Gravidity</td>
<td>2.20 ± 2.79 (1)</td>
<td>2.17 ± 0.98 (2)</td>
<td>0.189</td>
</tr>
<tr>
<td>No. of Parity</td>
<td>1.57 ± 0.81 (1)</td>
<td>1.8 ± 0.84 (2)</td>
<td>0.619</td>
</tr>
<tr>
<td>Maternal diabetes</td>
<td>3 (10%)</td>
<td>5 (16.7%)</td>
<td>0.706</td>
</tr>
<tr>
<td>Smoking</td>
<td>1 (3.3%)</td>
<td>4 (13.3%)</td>
<td>0.353</td>
</tr>
<tr>
<td>Preeclampsia</td>
<td>4 (13.3%)</td>
<td>0 (0%)</td>
<td>0.112</td>
</tr>
<tr>
<td>Chorioamnionitis</td>
<td>5 (16.7%)</td>
<td>0 (0%)</td>
<td>0.052</td>
</tr>
<tr>
<td>Gestational age [wks]</td>
<td>29.63 ± 2.70 (30.14)</td>
<td>39.16 ± 1.17 (38.92)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Birth weight [g]</td>
<td>1354.77 ± 553.80 (1318)</td>
<td>3392.77 ± 489.06 (3360)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

CD34 cells in preterms II

**Results:**

<table>
<thead>
<tr>
<th></th>
<th>PCB (n=30)</th>
<th>TCB (n=30)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of WBC (x 10⁶/ml)</td>
<td>6.5 ± 3.1</td>
<td>9.4 ± 2.8</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Number of CD34+ (x 10⁶/ml)</td>
<td>4.3 ± 3.1</td>
<td>2.3 ± 1.1</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Percent of CD34+/CD38+/CD133+ (%)</td>
<td>62.18 ± 10.21</td>
<td>73.14 ± 5.68</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Percent of CD34+/CD38-/CD133- (%)</td>
<td>37.82 ± 10.21</td>
<td>26.86 ± 5.68</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Percent of CD34+/CD38- (%)</td>
<td>17.08 ± 6.52</td>
<td>12.78 ± 6.18</td>
<td>0.008</td>
</tr>
<tr>
<td>Percent of CD34-/CD38-/CD133+ (%)</td>
<td>80.32 ± 12.10</td>
<td>70.38 ± 22.76</td>
<td>0.051</td>
</tr>
<tr>
<td>Percent of CD34-/CD38-/CD133- (%)</td>
<td>19.68 ± 12.10</td>
<td>29.62 ± 22.76</td>
<td>0.051</td>
</tr>
</tbody>
</table>

PCB - Pretermcord blood ; TCB -Term cord blood
Normoxie 21%  Hypoxie 10%  Hyperoxie 75%

Normoxie 21%  Hypoxie 10%  Hyperoxie 75%

Normoxie 21%  Normoxie 21%

pränatal  postnatal

Double-hit behandlung
Double-hit
Normoxie