Title of abstract: A sequential cell therapy to recover the ependymal cells in posthemorrhagic hydrocephalus.

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Background
Germinal matrix haemorrhages and intraventricular haemorrhages (GMH/IVH) lead to posthemorrhagic hydrocephalus (PHH), a relevant cause of morbidity and mortality in the premature neonatal population. The ependyma constitutes a relevant cell barrier between the brain parenchyma and the ventricle cerebrospinal fluid (CSF). Ependyma disruption is a consequence of the GMH/IVH, which affect CSF circulation and physiology. Thus, ependyma recovery can be one of the key targets in the PHH treatment. Current hydrocephalus treatments are surgical and restricted to alleviating ventricular pressure by drainage of CSF. However, no actual therapy is aimed to help ependymal recovery.

Materials and Methods
Postnatal day 2 mice brains were used to dissect the developing germinal matrix. Tissue was gentle disaggregated, and cells were cultured in specific media to promote ependymal progenitors and ependymal differentiation. After one week, cell culture was carried out in different situations to simulate the development of ependyma in the neuroinflammatory conditions present in the GMH/IVH. In these conditions, we have tested the effect of different cell therapies in the development of ependyma. The different cell types present in the cell culture were analyzed by immunofluorescence using laser confocal microscopy.

Results
1) Ependymal progenitors under neuroinflammatory conditions present disrupted ependymal maturation with no multiciliated ependyma.
2) Sequential stem cell therapy promotes the surveillance of the ependymal progenitors but not the differentiation of multiciliated ependymal cells.

Conclusions
The molecular environment present in GMH/IVH disrupts the final differentiation of the ependyma. Sequential cell therapy is a useful approach to increase surveillance and differentiation of ependymal cells in a model of experimental posthemorrhagic hydrocephalus.

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