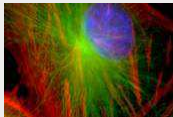


Clinical Applications of Stem Cells II

Rostock, 19.01.2012

Anne-Katrin Giese

Email: anne-katrin.giese@med.uni-rostock.de



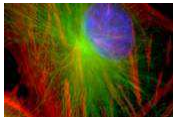
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Clinical Applications of Stem Cells II

2

- ❖ *Clinical Application of Stem Cells*
- ❖ *Diseases targeted by stem cell research*
 - ❖ *Parkinson´s Disease*
 - ❖ *Chorea Huntington*
 - ❖ *Alzheimer´s Disease*
 - ❖ *Lysosomal Storage Disorders*
 - ❖ *Multiple Sclerosis*
 - ❖ *Amyotrophic Lateral Sclerosis*
- ❖ *Considerations for the future*



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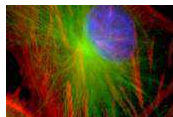
How Far Are We – Stem Cell Based Disease Models

Table 1 Stem cell-based cell therapy in experimental neurological disease models

Cell type	Disease models	Cell number	Route	Follow-up (weeks)	Functional outcome	Ref.
ESC/ESC-derived cells	MCAO (rats)	1.0×10^5	IC	8	Enhanced functional recovery	[13]
	PD (monkeys)	$6.0 \times 10^5 - 1.2 \times 10^6$	IC	14	Attenuated neurological symptoms	[14]
NSC	MCAO (rats)	5.0×10^6	IV	77	Improved functional recovery	[17]
	HD (rats)	6.0×10^6	IV	12	Improved memory function recovery	[18]
	AD (rats)	5.0×10^6	IC	9	Decreased striatal atrophy, improved functional recovery	[19]
NPC	AD (rats)	5.0×10^4	IC	1	Attenuated inflammatory reactivity and neuronal loss	[24]
	MS (mice)	2.5×10^3	ICV	2	Enhanced endogenous myelin regeneration	[25]
	SCI (rats)	4.0×10^5	IS	2	Promoted remyelination and functional neurological recovery	[26]
BMSC	SCI (rats)	5×10^5	IS	8	Promoted tissue repair and axonal outgrowth, improved locomotion	[33]
	PD (rats)	1×10^7	IV	4	Increased DA release and ameliorated behaviorally.	[46]
	AD (rats)	3.0×10^5	IC	2	Improved cognitive ability	[42]
IPSC	PD (rats)	1.0×10^6	IC	6	Promoted functional recovery	[54]
	MCAO (rats)	1.0×10^6	IC, SD	4	Improved functional recovery	[55]
	SCI (mice)	5×10^5	IS	6	Promoted locomotor function recovery	[58]

ESC embryonic stem cell, *NSC* neural stem cell, *NPC* neural progenitor cell, *BMSC* bone marrow stem cell, *IPSC* induced pluripotent stem cell, *MCAO* middle cerebral artery occlusion, *PD* Parkinson's disease, *SCI* spinal cord injury, *AD* Alzheimer's disease, *HD* Huntington's disease, *MS* multiple sclerosis, *IC* intracerebral, *IS* intraspinal, *ICV* intracerebroventricular, *IV* intravenous, *SD* subdural, *DA* dopaminergic

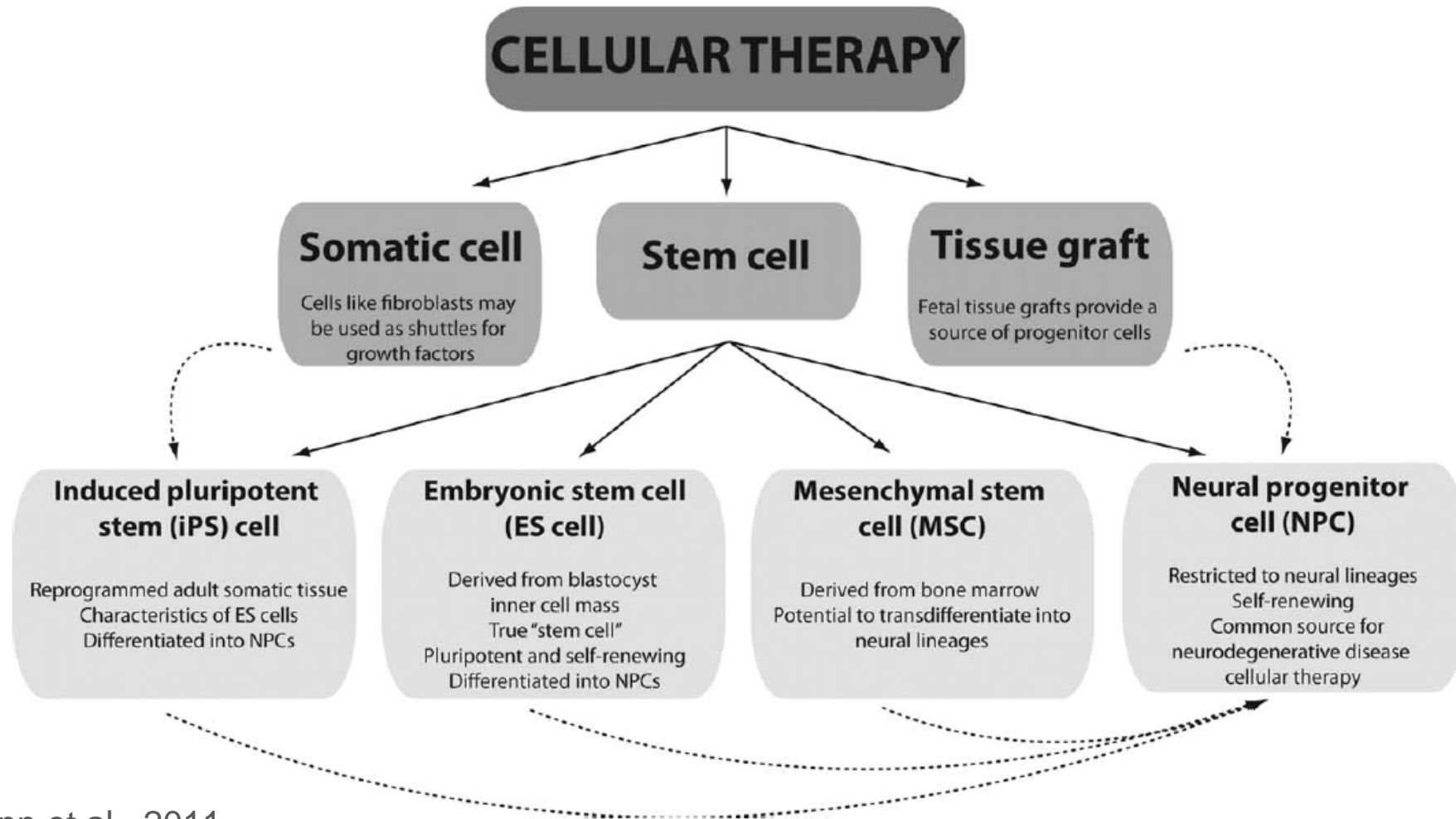
Wang et al., 2011



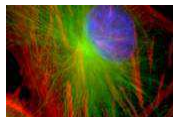
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Role of Stem Cells in Therapy



Lunn et al., 2011



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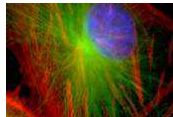
Clinical Applications of Stem Cells

5

- **Animal model vs. Human**
- Experimental vs. therapeutic
- Cell type being used
- Type of disease
- Technique of transplantation

Which neurological diseases can be addressed?

What are their key features?



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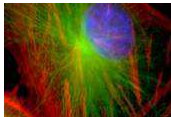


Neurologic Diseases

6

Classification of Diseases by Etiology

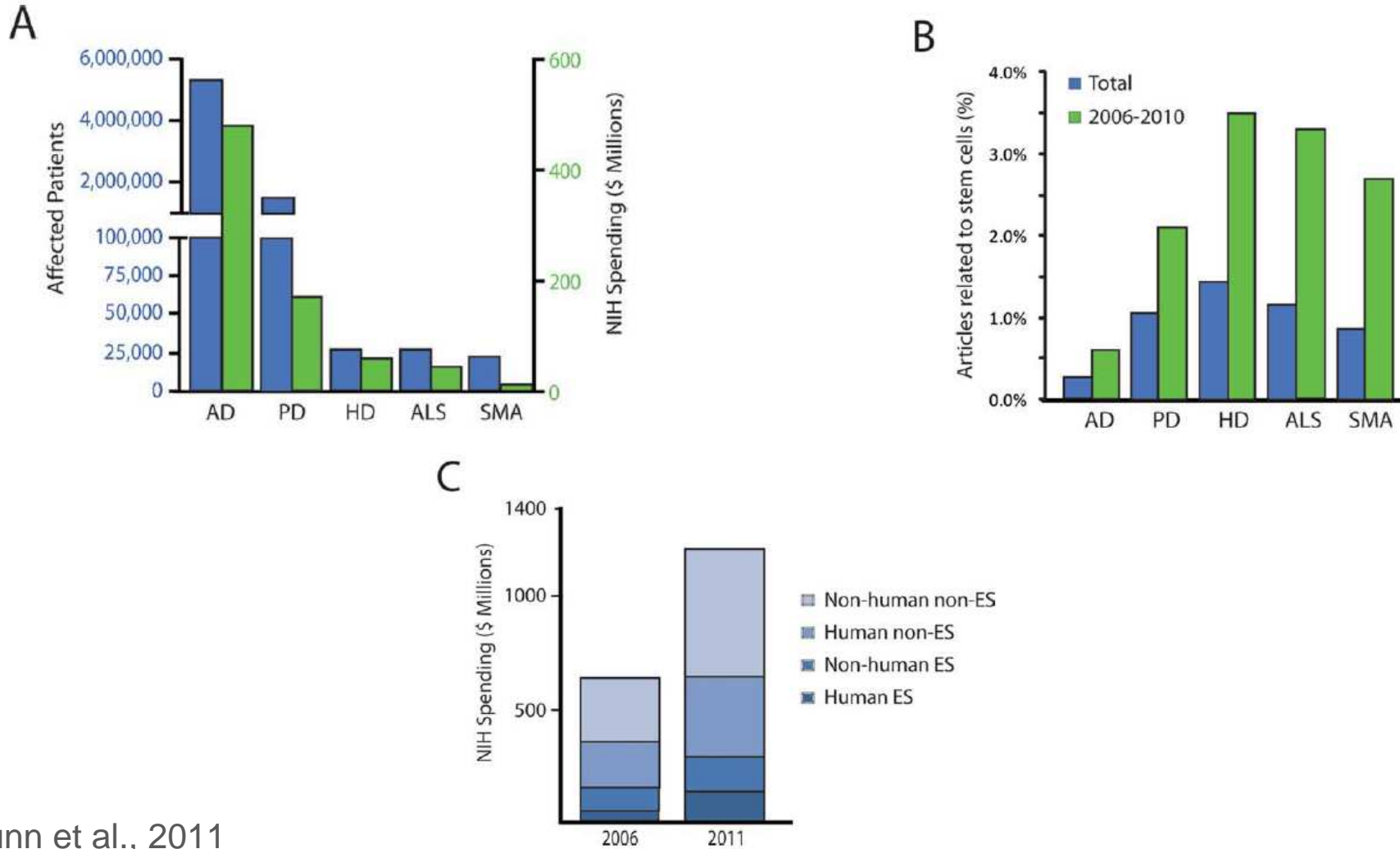
- *Inflammatory/Infectious diseases:*
→ viral, bacterial, mycotic, parasitic, autoimmune
- *Vascular diseases:*
→ ischemic stroke, hemorrhagic stroke, vascular malformation
- *Traumatic injury*
- *Metabolic-toxic damage:*
→ deficiency of nutrients, vitamins, micronutrients, toxic substances
- *Neoplasia*
- *Degenerative diseases*
- *Hereditary diseases*
- *Idiopathic diseases*



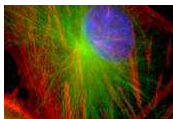
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Stem Cell Technology for Neurodegenerative Diseases



Lunn et al., 2011



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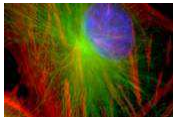
Degenerative Diseases

- Parkinson´s disease
- Chorea Huntington
- Amyotrophic lateral sclerosis
- Hereditary ataxias

→ **Movement disorders**

- Alzheimer´s Disease

→ **Dementia**



Motor System

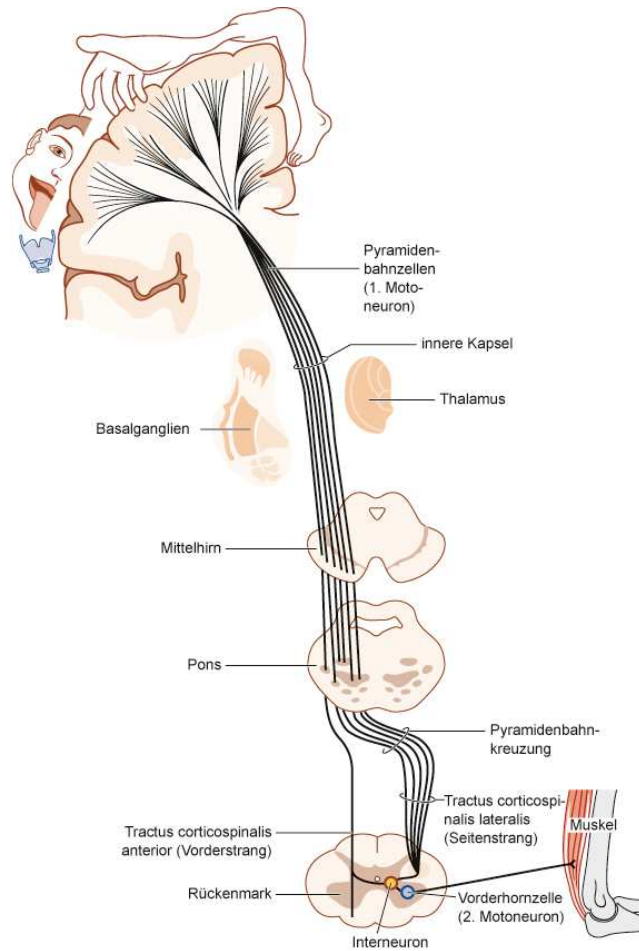


Abb. 1.14

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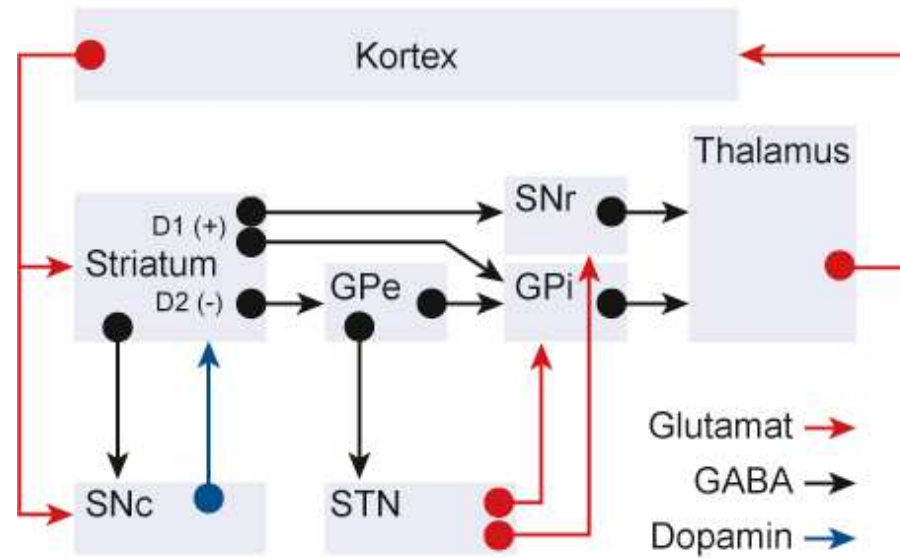
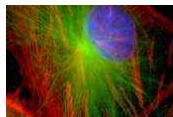


Abb. 9.1

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Extrapyramidal Movement Disorders

Extrapyramidal System:

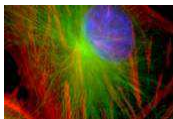
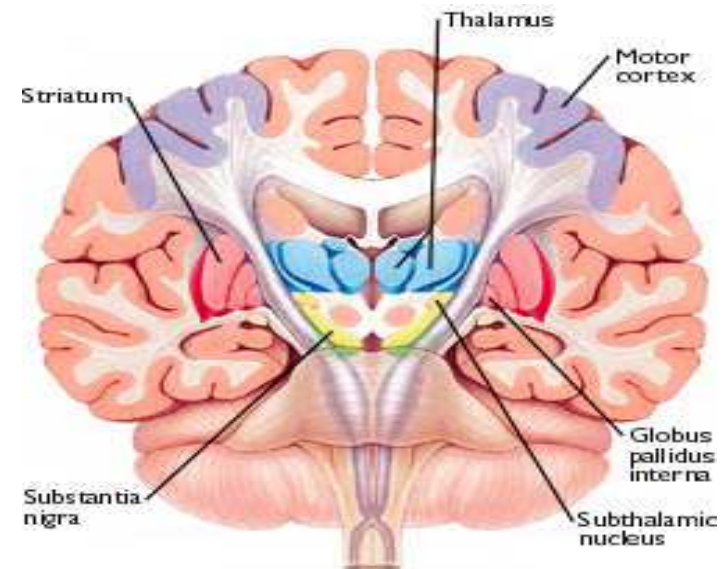
→ Basal ganglia: involuntary movement
steering of intentional movements

Parkinson's Disease

→ **hypokinetic-rigid** syndrome

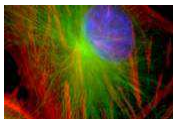
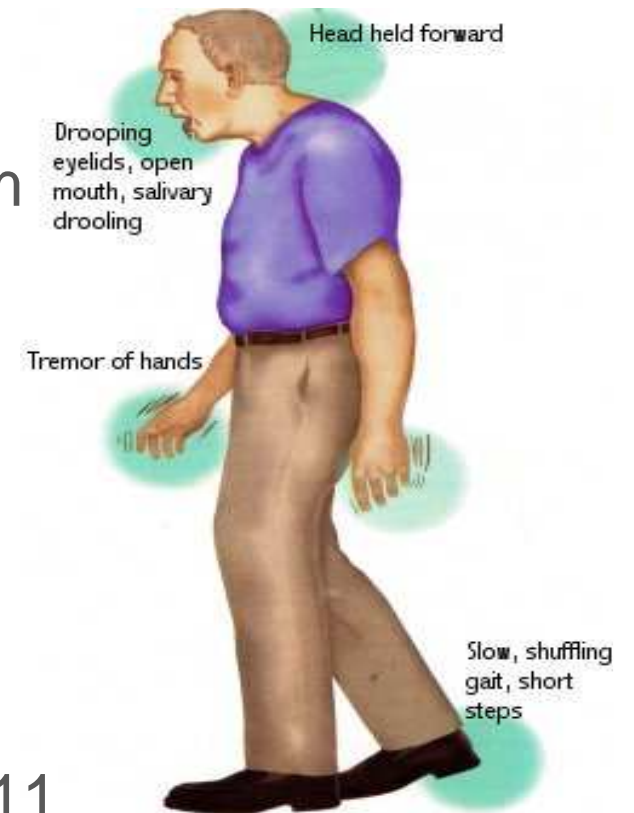
Chorea Huntington

→ **hyperkinetic-hypotonic** syndrome



Parkinson's Disease

- Degeneration of melanin-containing cells in the **Substantia nigra**
 - Loss of nigrostriatal, dopaminergic tracts
 - Histopathology: Lewy bodies in brain stem and basal ganglia
 - Rigor, tremor, akinesia/hypokinesia
- Age at manifestation: 40-60 years
- Men more often affected than females
- Prevalence: 1% of the 60-year olds
3% of the 80-year old
- Rare familial form, gene locus: Park1-Park11

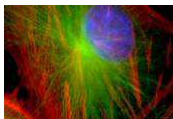


Parkinson's Disease: What Does The Patient Say?

12

- “Everything is rigid and stiff and my muscles are weaker.”
- “My feet are attached to the floor, I shuffle and scurry, I am moving insecure.”
- “I am afraid of falling and I fall more often.”
- “I have a coarse voice without having a cold.”
- “My writing is different, it is smaller and shaky.”
- “I am sad, feeling down and I cannot feel happy.”
- “I have pain, especially in the neck/shoulder region, but also in the arms and legs.”

Neurologie, Gehlen and Delank, Thieme 2010



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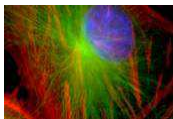


Parkinson's disease: What Does The Doctor See?

13

- Small steps, bent torso while walking
- Less or lacking arm movement while walking
- More steps while walking and turning around
- Difficulties in starting a movement or getting over a barrier
- Tendency to fall
- Resting tremor
- Hypomimia
- Low, coarse, monotonous speech

Neurologie, Gehlen and Delank, Thieme 2010



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Parkinson's Disease: Medical Approaches

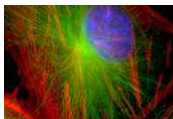
14

- Key pathology: Loss of dopaminergic neurons
Cholinergic system is overactive
- Aim of therapy: ***Increasing dopamine***
Decreasing activity of cholinergic neurons

Other therapy options:

→ Deep brain stimulation

→ Stem cell therapy? Past trials showed no benefit (Olanow et al. 2003)



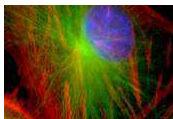
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Stem Cell Therapy for Parkinson's Disease

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- Initial stem cell therapy with fetal ventral midbrain cells (dopaminergic neurons)
- Limitations:
 - ethical concerns
 - limited availability of fetal stem cells
- Embryonic stem cells: large scale production of dopaminergic neurons
 - Ethical issues
 - Teratomas
- iPS: patient specific, but not possible for large-scale cell culture



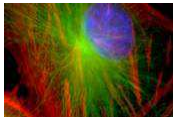
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Other Approaches For Treatment of PD

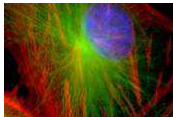
16

- Environmental enrichment:
 - support of existing dopaminergic neurons
 - slow/prevent further damage
- Growth factor therapy:
 - direct delivery
 - viral-based system
 - BDNF, VEGF, GDNF, IGF-1
 - transplantation of growth-factor producing MSC/NPC?
 - so far animal models only



Huntington's Disease

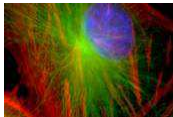
- Autosomal-dominant hereditary disorder
 - Chromosome 4, Huntington gene
 - CAG-Triplet repeats pathologically increased
 - Manifests at age 35-55
 - Prevalence 0.1‰
 - Atrophy of the Corpus striatum, later also Cortex cerebri
 - Decrease of GABA and GABA receptors
- Inhibitory GABA pathologically low
- Dis-inhibition of movements
- Hyperkinetic-hypotonic syndrome



Stem Cell Therapy for Huntington's Disease

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- Mainly medium spiny neurons damaged (MSN)
- In rodents: transplanted MSN integrated into host
- Clinical trials have used fetal-derived tissue
- Transient improvement for patients in clinical trials
- Stable period before further decline
- Problems:
 - graft over-growth
 - presence of non-neuronal cells within graft
 - ethical issue of using fetal stem cells

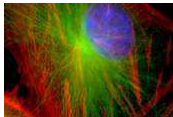


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Alzheimer's Disease

- Most frequent form of dementia
- Widespread loss of neurons
 - Cortex
 - Hippocampus
 - Amygdala
 - Basal forebrain
- Pathologic hallmarks:
 - A β -plaques
 - Neurofibrillary tangles
- Increased risk with age
- Majority: late onset (>65 years of age)

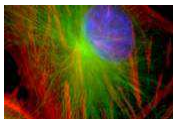


Alzheimer's Disease

- Dementia with characteristic changes in cells and tissue
- Alzheimer neurofibrills
- Senile plaques
- Cell necrosis, especially of large neurons
- Amyloidosis of blood vessels

- Loss of brain tissue
- Especially: temporally accentuated cortex and white matter atrophy

- Occasionally familial incidence: damage in Amyloid-Precursor-Protein (APP, Chromosome 21)
→ Central role of APP in disease pathology



Alzheimer's Disease

Early symptoms

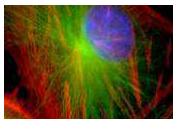
- Headaches
- Vertigo/dizziness
- Impairment of memory
- Hyposmia
- Weakness/fatigue

Late symptoms:

- Aphasia/apraxia
- Elevated muscle tonus
- Personality/character remains intact longer
- Rising restrictions in daily routine
- Final stage: severe dementia for 6-8 years

Loss of mainly cholinergic neurons

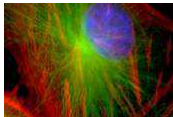
- Medication with blockers of cholinesterase and NMDA-receptors
- Modulation of neurotransmitter → Raised level of ACh



Stem Cell Therapy for Alzheimer's Disease

22

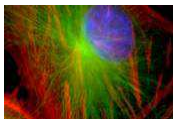
- Target of cellular therapy: hippocampus
 - Enhancement of neurogenesis
 - Replacement lost neurons
 - Delay of progression of Alzheimer's disease?
- Rodent model for transplanting NPC:
 - Increased hippocampal activity
 - Increased cognitive function
- Phase I clinical trial:
 - Genetically engineered patient fibroblasts that express NGF (nerve growth factor)



Lysosomes

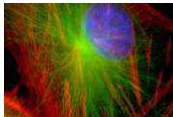
- Cell organelles
- Vesicle with single membrane
- Acidic pH
- Contain over 60 hydrolases
- Diameter: 0.1-1.1 μ m
- Degrade macromolecules into smaller compounds

- Hereditary deficiency in specific lysosomal enzymes
 - Non-degraded substances accumulate
 - Lysosomal storage disorders



Lysosomal Storage Disorders

- Approximately 40 different LSDs
- Cumulative incidence: 1:4,000
- Most common:
 - Gaucher disease
 - Fabry disease
- Other LSDs: Pompe disease, Niemann-Pick Type A/B and Type C, Hunter disease, Morquio disease
 - All rare and caused by a loss or decrease in enzyme activity of the respective enzyme



Treatment Approaches to LSDs

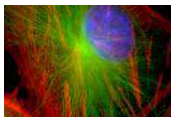
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Treatment options:

- Enzyme replacement therapy
- Substrate reduction therapy
- Pharmacological chaperones
- Stem cell therapy: transplantation of hematopoietic stem cells

- Still under research: replacement of microglia and astroglia for neuroprotection and immunomodulation?

De Filipis, 2011



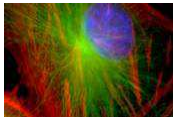
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Gaucher disease

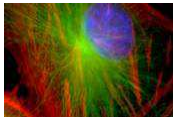
- Autosomal-recessive LSD
- Chromosome 1, β -Glucosidase impaired
- Glucocerebrosides no longer degraded
 - Accumulate
 - Mainly in macrophages
 - Release of cytokines

- Inflammation in internal organs and skeleton



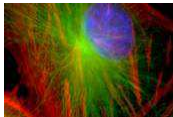
Gaucher Disease

- Adult type (I):
 - Visceral symptoms
 - Hepatomegalie, splenomegalie, anemia
 - Bone pain, osteolysis/osteonecrosis
- Acute-neuronopathic infantile type (II) and subacute-neuronopathic type (III)
 - additionally neurologic symptoms



Gaucher disease

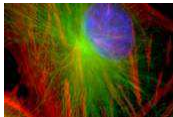
- Diagnosis:
 - Measurement of enzyme activity
 - Detection of mutation in GBA gene
- Therapy:
 - Enzyme replacement therapy
 - Substrate reduction therapy



Fabry disease

- X-linked LSD
- α -Galactosidase impaired
- Globotriaosylceramide accumulates
 - mainly in endothelium

- Main focus of disease: heart, kidney and brain
 - Well perfused organs, damage to blood vessels most prominent there

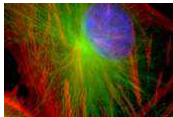


Fabry Disease

- Early symptoms:
 - Acroparesthesia
 - Hypohydrosis/Anhydrosis
 - Angiokeratomas
 - Clouding of Cornea
- Late symptoms: Stroke, heart and kidney failure
- Therapy: Enzyme replacement



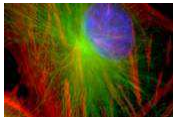
Mehta et al., 2010



Niemann-Pick Type C Disease

31

- Autosomal recessive LSD
- NPC1 or NPC2 gene affected
- Not caused by enzyme deficiency
→ Cholesterol transporter damaged
- Abnormal accumulation of cholesterol



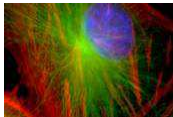
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Niemann-Pick Type C Disease

32

- Onset variable: Childhood – youth – early adulthood
- Symptoms
 - Neonatal icterus
 - Eye movement disorder
 - Cerebellar ataxia
 - Psychiatric symptoms
- Therapy: Substrate reduction therapy
 - Delays disease progression

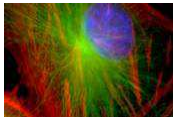


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Multiple Sclerosis

- Chronic, inflammatory, demyelinating CNS disease (“Encephalomyelitis disseminata”)
 - Loss of axons
 - Gliosis (scar tissue → “sclerosis” in “multiple” places)
 - Neurodegeneration
-
- Incidence in Central Europe: 1‰
 - Mean age: 20-45 years
 - Females affected more often than males



Multiple Sclerosis

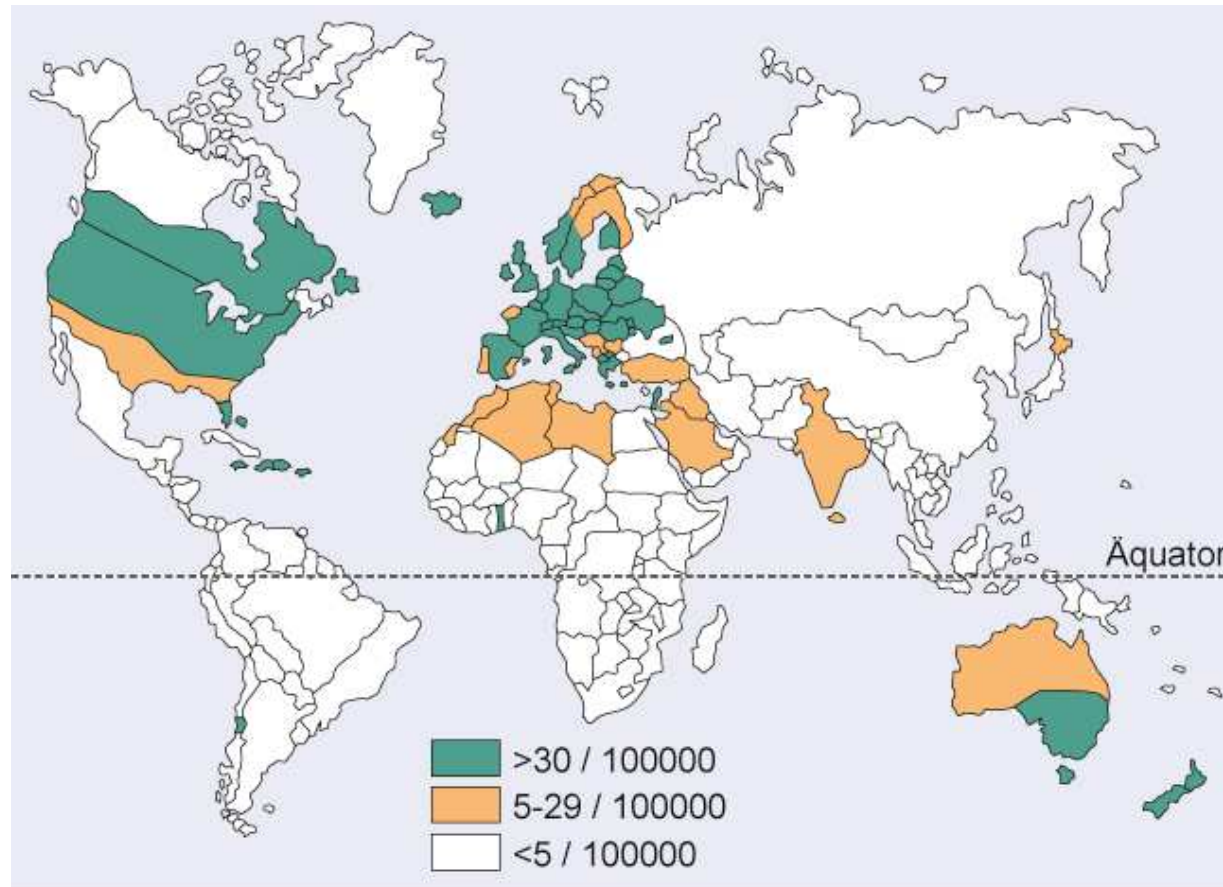
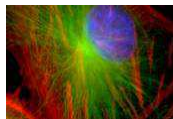


Abb. 7.1

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Multiple Sclerosis

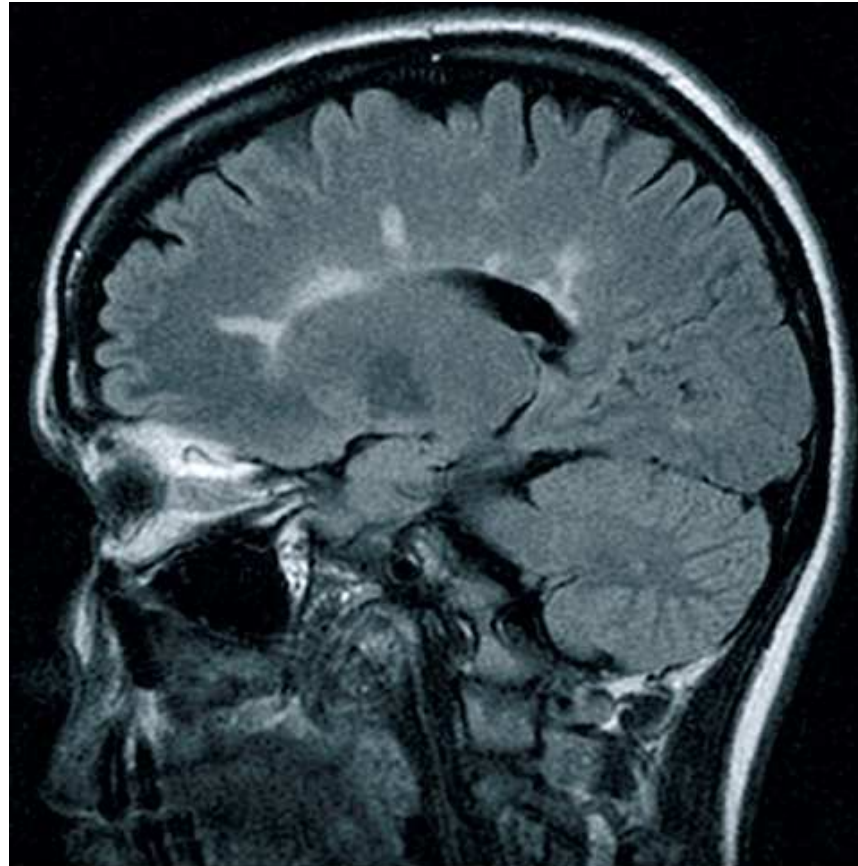
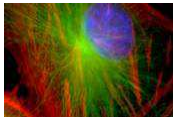


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Multiple Sclerosis

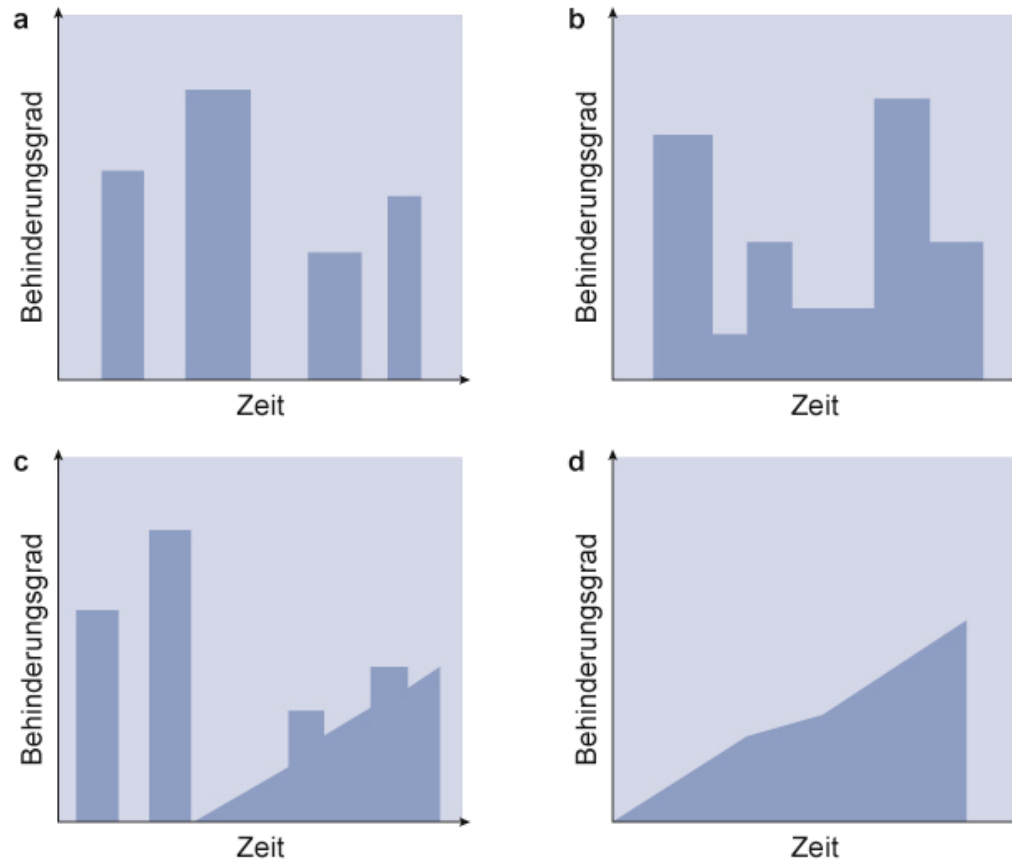
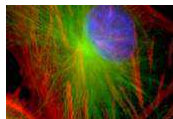


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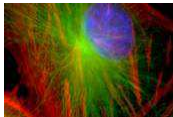
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Etiology and Pathogenesis of Multiple Sclerosis

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- MS is an autoimmune disease mediated by T-Lymphocytes
- Other factors modulating the course of the disease
 - genes, epidemiology, environment (e.g. stress)
- Infectious hypothesis: activation of T-cells during viral infection
- Autoimmune hypothesis
- Neurodegenerative hypothesis
- None of these can completely explain all aspects of the disease
- Therapy: modulation of immune system
- Transplantation of hematopoietic stem cells: large Northern American and European multicenter randomized controlled phase III clinical trial is on the way (Atkins et al., 2012)



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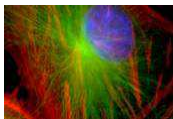


Amyotrophic Lateral Sclerosis



- Adult onset disorder
- Degeneration and loss of motor neurons
- Loss of coordination
- Loss of muscle strength
- Later: loss of complete muscle control
- Death: due to respiratory failure
- Usually: 2-5 years after diagnosis

<http://www.hawking.org.uk/uploads/8/3/0/0/8300824/1477171.jpg>



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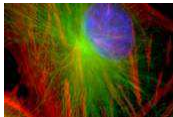
Amyotrophic Lateral Sclerosis

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- Degeneration of **BOTH 1st and 2nd** motoneuron
- Not necessarily equally affected

- Most common system atrophy of CNS
- Mainly sporadic
- Seldom familial form (5%)

- Incidence: 3:100,000
- Males more often affected than females

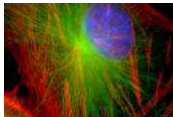


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Amyotrophic Lateral Sclerosis

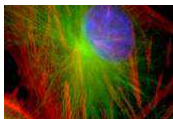
- No known cause of sporadic form
- Pathology: Glutamate appears to play a role
- Familial form: more than 12 different genes identified
(most common: Copper/Zinc-Superoxide-Dismutase (SOD1) gene)



Amyotrophic Lateral Sclerosis

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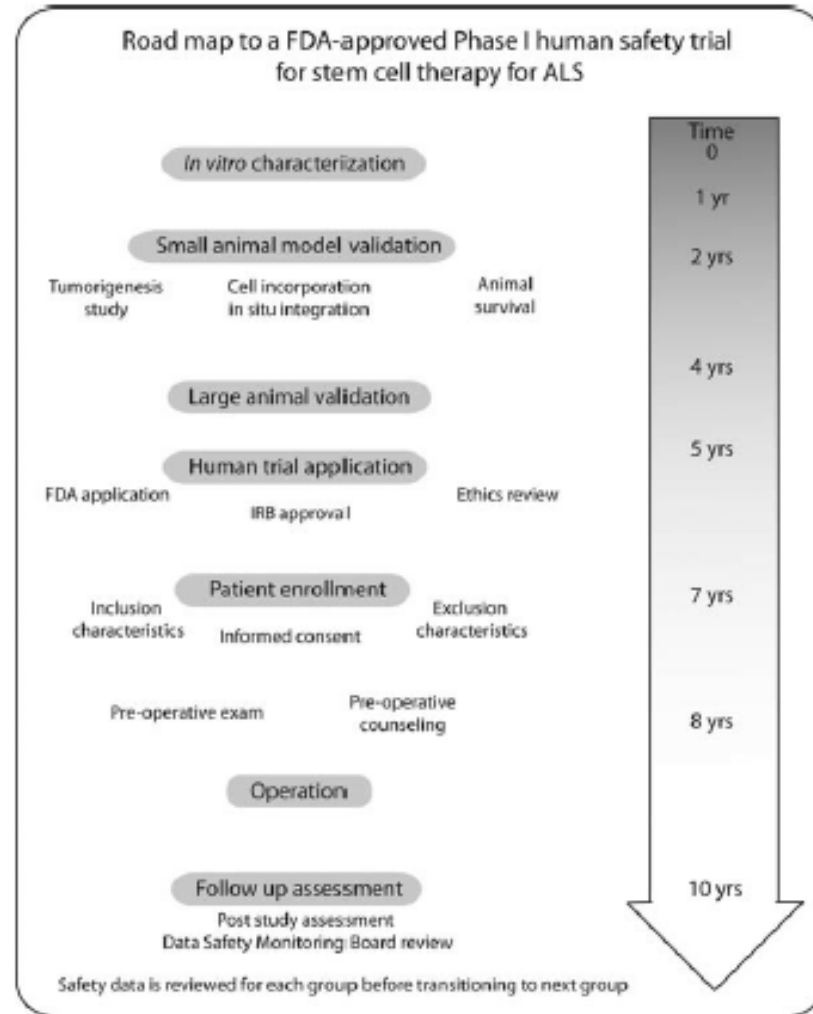
- Cellular therapies:
 - Replacement of lost neurons
 - Improvement of environment (growth factors: VEGF, BDNF)
 - Protection of motor neurons from further damage
- Rodent models:
 - Application of NPC and MSC
 - Systemic and intraspinal application
 - Delay disease progression
 - Important: Intervention BEFORE irreparable loss of motor neurons



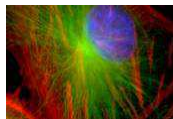
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Stem Cell Therapy for ALS



Lunn et al., 2011



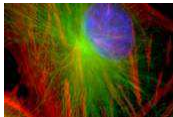
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Why Do We Need Stem Cells?

- Disease model:
 - Insight to disease pathology
 - New treatment options
 - First-line testing of new drugs
 - Drug screening

- Therapy:
 - Replacement of lost neurons
 - Modulating the environment



How Do We Track Implanted Stem Cells?

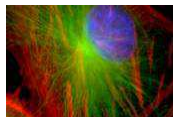
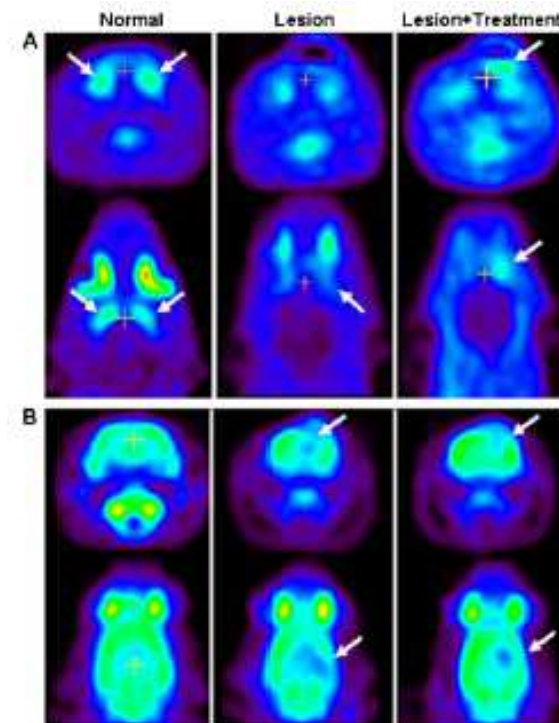
Eur J Nucl Med Mol Imaging (2011) 38:1926–1938
DOI 10.1007/s00259-011-1860-7

REVIEWARTICLE

PET molecular imaging in stem cell therapy for neurological diseases

Jiachuan Wang · Mei Tian · Hong Zhang

Fig. 1 Typical examples of ^{11}C -MMP (a) and ^{18}F -FDG (b) microPET imaging in various conditions: normal rat brain (Normal), traumatic brain injury (Lesion) and after ^{19}F -UCB-0199-positive NSC transplantation (Lesion+Treatment). Images are shown in two views: coronal and axial [14]

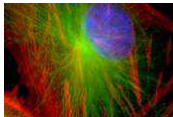


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XCell Center

- Opened in 2007 in Cologne and Düsseldorf
- Offered to inject bone marrow stem cells into:
 - brain, spinal cord and other regions
 - claimed to help recover from stroke, multiple sclerosis, ALS, spinal cord injury...
- Treatment cost up to 26,000 €
- Used on individual experimental basis („Heilversuch“)
- New legislation in 2009: „hospital exemptions“ for tissue engineering had to be applied for
 - XCell Center dis not apply for license
- 18 months transition period
- Was closed April 2011

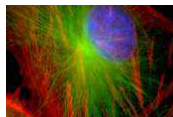


Stem Cell Therapies – Considerations for the Future

TABLE: Common Considerations When Translating Stem Cell Therapies to Neurodegenerative Disease Patients

Inclusion/exclusion criteria	Enrolling late stage patients may prevent loss of quality of life
	Late stage patients may mask any positive effects due to the intervention occurring too late in the disease course
Realistic expectation	Informed consent forms must clearly illuminate the goals of the study
	Safety trials vs efficacy trials
	Expectations of therapeutic effects based on disease state at intervention
Controlled study	Ideal study is a double-blind placebo study
	Late stage patients may mask any positive effects not observed due to the intervention occurring too late in disease
	Original PD studies offered control arm treatment after a 1-year follow-up, which confuses interpretation of efficacy
Immunosuppression	Although the brain remains an immunologically privileged site due to the blood–brain barrier, there is evidence that this barrier can be compromised in disease
	Studies of cell graft survival demonstrate that immunosuppression increases the survival of graft tissue
Potential side effects	Prevent/minimize potential side effects (ie, meningitis, fever)
	Avoid exacerbation of disease and tumor formation
	Risk vs quality of life
Safety of cellular therapy administration	Consider CNS accessibility and safety of delivery methods
	Pros/cons of systemic delivery, lumbar puncture, or stereotactic injection are important

CNS = central nervous system; PD = Parkinson disease.



Clinical Applications of Stem Cells II

