What does it take to translate?
Lessons learned in Regenerative Medicine

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Charité - Universitätsmedizin Berlin
1860  Medical training at the Charité
1890  Foundation 1st Clinic for Orthopaedic Surgery at the Charité
1892  Most relevant orthopaedic publication:
      "The Law of Transformation of Bone"
      ("Das Gesetz der Transformation der Knochen")
1902  Initiation of the German Orthopaedic Society
2008  Foundation of the Julius Wolff Institute
Regeneration - complete

I can regrow my leg! What about you?

Stem Cells: Hopes, Fears and Reality
PhD Symposium in Berlin
December 2010
Regeneration - conserved
Clinical relevance of bone healing research

- Up to 10% of fx patients experience delayed or non-union
- Real delayed healing ratio higher but unknown
- With aging population, fx numbers will increase
- In elderly patients, delayed or unsatisfactory fracture healing outcome is rising

Image adapted from Schmidt-Bleek et al., 2015, CGFR
New, globally accepted treatment concepts based on results from KFO 102, SFB 760, FOR 2165

Translation requires crossing borders
medical need – mechanistic knowledge – technology innovation – dissemination – acceptance

Translation is…
first in patient?
What are we known for…?

- **New, globally accepted treatment concepts**
  based on results from KFO 102, SFB 760, FOR 2165

- **Translation requires crossing borders**
  medical need – mechanistic knowledge – technology innovation – dissemination – acceptance

- **Translation is…**
  reimbursement established?
What are we known for…?

• **New, globally accepted treatment concepts**
  based on results from KFO 102, SFB 760, FOR 2165

• **Translation requires crossing borders**
  medical need – mechanistic knowledge – technology innovation – dissemination – acceptance

• **Translation is…**
  establish a new standard of care?
What are we known for…?

**Hip Joint**

10 patients (8m/2w)  age: 50 - 68 years

**Knee Joint**

9 patients (6m/3w)  age: 60 - 75 years
What are we known for…?

Globally used reference https://orthoload.com/
Basis for pre-clinical assessments of any new device (ASTM, ISO) and failure analyses
Lessons learned...

• Academy is thrilled by the new and unknown
• Industry is eager in novelty but with clear de-risking strategy
  • reliable information/knowledge/technology
  • that easily integrates into existing processes

• new knowledge: Helps to compensate or reduce existing risks
• new product: Substantially progresses towards reduced risk
• New, globally accepted treatment concepts
  based on results from KFO 102, SFB 760, FOR 2165

• Translation requires crossing borders
  medical need – mechanistic knowledge – technology innovation – dissemination – acceptance

• Translation is…
  establish a new standard of care?
What are we known for…?

Expert Tibial Nail PROtect
Enhance your first line of defense
What are we known for…?

- **Start 1998**
  adapting a concept established in stents
- **Preclinical studies** (DFG funded)
  - PoC in small and large animal models
- **Patent filed** (release of growth factors)
  - Company licenced
- **Initially: local release of proteins**
  - Freedom of operation?
  - BMP or TGF-β/IGF-1 each $60 Mio
- **Hand over Charité to DePuySynthes**
  - Upscale production
  - FDA approval (20m², $20 Mio) each
What are we known for…?

**Intended use**

70% of open fractures are contaminated with environmental bacteria. The Expert Tibial Nail PROtect is intended to be used for the surgical treatment and stabilization of fractures of the tibia.

**Expert Tibial Nail PROtect**

Enhance your first line of defense

DePuy Synthes

PART OF THE JOHNSON & JOHNSON FAMILY OF COMPANIES
What are we known for…?

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  - Upscale production
  - FDA approval (20m², $20 Mio) each
- **BUT: No prove for major claim possible**
Lessons learned...

- Definition of hypothesis is key
- Early on health economic assessment
- Opportunity check
  - definition of technologies (own IP)
  - “freedom to operate” (other IP)
  - identify stakeholders
- Clinical approval pathway(s)
• **New, globally accepted treatment concepts**
  based on results from KFO 102, SFB 760, FOR 2165

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• **Translation is…**
  establish a new standard of care?
Clinical relevance of bone healing research

Clinical relevance of bone healing research

Clinical relevance of bone healing research

Lower torsion leads to improved bone healing

axial torsion [°]

ASLS

UTN

modified thread

standard
Clinical relevance of bone healing research

Lower torsion leads to improved bone healing

Angular Stable Locking System (ASLS). For angular stable locking of intra-medullary nails.
Clinical relevance of bone healing research

Randomized controlled trial (LoE I), $N = 142$, multi-center study (8 sites in 3 countries):
No difference in healing success
Clinical relevance of bone healing research

Randomized controlled trial (LoE I), N = 142, multi-center study (8 sites in 3 countries):
No difference in healing success … but …

Symmetry Ratio of the Vertical GRF Impulse

Asymmetric

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>6 Weeks Post-OP</th>
<th>12 Weeks Post-OP</th>
<th>6 Months Post-OP</th>
<th>12 Months Post-OP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asymmetric</td>
<td></td>
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Angular Stable Locking System (ASLS). For angular stable locking of intra-medullary nails.
Randomized controlled trial (LoE I), N = 142, multi-center study (8 sites in 3 countries):
No difference in healing success

Angular Stable Locking System (ASLS). For angular stable locking of intra-medullary nails.
Lessons learned...

• Solid basic understanding
  bring novel solution based on novel concept/understanding

• Relevance of initial user group (clinical trials and beyond)
  • ensure endpoint definition and study design
  • engage potent multiplier
  • train the experts to novel concepts, ensure who is user
  • stay in the loop (ongoing learning curve)
• **New, globally accepted treatment concepts**
  based on results from KFO 102, SFB 760, FOR 2165

• **Translation requires crossing borders**
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• **Translation is…**
  establish a new standard of care?
Phase I: Biomarker Development – Mechanism - PoC

Pilot Study:

n=15

n=8

n=7

Persistent signature of CD8$^+$ TEMRA cells

Phase I: Biomarker Development – Mechanism - PoC

Phase I: Biomarker Development – Mechanism - PoC

Accumulation:
3-fold at fracture site compared to blood levels

Persistent signature of CD8+ TEMRA cells

- Delayed healing patients
- Normal healing patients
- Healthy control
Phase I: Biomarker Development – Mechanism - PoC

Persistent signature of CD8+ TEMRA cells

CD8+ TEMRA: Strong IFN-γ + TNF-α production & expression

Delayed healing patients  Normal healing patients  Healthy control
Phase I: Biomarker Development – Mechanism - PoC

Inflammation:
- Apoptosis of progenitors
- Reduced osteogenesis

Persistent signature of CD8+ TEMRA cells

![Graph showing the persistent signature of CD8+ TEMRA cells across different timepoints.](image)

- Delayed healing patients
- Normal healing patients
- Healthy control

Cytokines (e.g. IFN-γ, TNF-α)
PoC in a clinically relevant mouse model

WTexp group 21 days post-op

WTexp

CD8+ TEMRA
MSCs
Cytokines (e.g. IFN-γ/ TNF-α)
Phase I: Biomarker Development – Mechanism - PoC

PoC in a clinically relevant mouse model

WTexp

CD8+

WTexp group 21 days post-op

CD8+ group 21 days post-op

BIH Regeneration

CD8+ TEMRA

MSCs

Cytokines (e.g. IFN-γ/ TNF-α)
Phase I: Biomarker Development – Mechanism - PoC

PoC in a clinically relevant mouse model

WTexp group 21 days post-op

CD8+ group 21 days post-op

CD8- group 21 days post-op

Cytokines (e.g. IFN-γ/ TNF-α)

MSCs

CD8+ TEMRA

TEMRA
Phase II: Biomarker Transfer Clinics – Confirmation

Biomarker

Pre-surgery

Blood level

Determine CD8+TEMRA levels

Predict healing outcome

Standard

normal healing

delayed healing

early & targeted intervention
Phase II: Biomarker Transfer Clinics – Confirmation

Pre-surgery Biomarker Blood level

Determine CD8+TEMRA levels

Predict healing outcome

CD8+ TEMRA cells at all study time points
High sensitivity and specificity for predicting the outcome of fracture healing

Cut-off 30% 36%
AUC 0.88 0.91
Sensitivity 100% 100%
Specificity 75% 87.5%
Phase III: Prospektive Biomarker Validation

Pre-surgery Biomarker
Blood level

Determine CD8+TEMRA levels
Predict healing outcome

Prospective Validation

N (total) = 68
Cut-off (preOP) 38%
AUC 0.83
Sensitivity 70%
Specificity 95%

✓ Pre-analytics
✓ Method validation
✓ PoC Study
✓ Confirmation pre-defined cut-off level
✓ Health economic analysis
Phase III: Prospektive Biomarker Validation

multicenter-prospective study

Coordination: Simon Reinke / Sven Geißler

Patients-in (03/2020): 515/640
drop out rate: 13% (67)
Women: 47%
Mean Age: 53y

1. Endpoint (4.5 months):
Patients (total): 305/448 (68%)
Non-Healed: 109/305 (35%)

2. Endpoint > 9 months:
Patients (total): 259/305 (85%)
Non-Healed: 46/259 (18%)
Phase III: Prospektive Biomarker Validation

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2. Endpoint > 9 months:
Patients (total): 259/305 (85%)
Non-Healed: 46/259 (18%)

• Technology development but no market access

• Market access but no own technology

• Intended use:
This reagent is used as an aid in the differential diagnosis of patients with long bone fractures having, or suspected of being at risk of having, a biologically compromised healing capacity resulting in delayed or permanent failure of bone healing (= non-union or pseudoarthrosis).
Lessons learned...

• Idea & concept (including basic science)
• Translational partners are real partners
  • people, role in organisation, trust, long standing partnership
• Diversity in stake holders
  • technology ownership vs. market access vs. sales capabilities
  • in companies: different languages, different (sales) strategies
  • tech transfer, own IP strategy, own business development
Learn from bone healing for muscle regeneration?

- Anti-inflammation to enable regeneration
- Avoid fatty degeneration

Damm et al, Clin Biomechanics, 2019, ESB Award
Learn from bone healing for muscle regeneration?

Synthetic niche for MSCs

Clinically relevant crush trauma model

Muscle repair

Qazi et al., Biomaterials 2015
Qazi et al., J Cachexia Sarcopenia Muscle. 2019
• MSC transplantation improve muscle strength.
• GF alone not beneficial, but can stimulate MSCs signaling.

Learn from bone healing for muscle regeneration?

Pumberger et al, Biomaterials 2016
Qazi et al, Biomaterials 2017
Learn from bone healing for muscle regeneration – reduce scarring

Significant reduction in scaring with MSCs & GFs

Pumberger et al, Biomaterials 2016
Qazi et al, Biomaterials 2017
Stem cells – off the shelf?

Placenta expanded stromal cells (PLX) for supporting endogeneous regeneration - from preclinical studies to phase III multicenter clinical trials

Placenta expanded stromal cells (PLX)

ICS Intermediate cell stock

Cell Expansion 3D

Downstream Detachment, wash, formulation, freezing

Cell Expansion 2D

ICS Intermediate cell stock

PLX-R18 PLX-PAD

PLX products

Off the shelf products

(>20,000 therapeutic units/placenta)
**Stem cells – off the shelf?**

<table>
<thead>
<tr>
<th>Time</th>
<th>Event</th>
<th>Groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 0</td>
<td>Trauma</td>
<td>Sham, PLX</td>
</tr>
<tr>
<td>Day 0/7</td>
<td>Transplantation of PLX</td>
<td>Sham, PLX</td>
</tr>
<tr>
<td>Day 28</td>
<td>Force test and histologic analysis</td>
<td>Sham, PLX</td>
</tr>
</tbody>
</table>

**Graph:**

- **Delayed:**
  - Fast Twitch L/R: PLX, Sham
  - Tetany L/R: PLX, Sham

- **Immediate:**
  - Fast Twitch L/R: PLX, Sham
  - Tetany L/R: PLX, Sham
Aim at complex pathogenesis in muscle injury/ischemia: myopathy, ischemia, myofiber necrosis, inflammation

Angiogenesis

Immunomodulation: Inflammation

Muscle Regeneration

VEGF
Angiogenin
Angiopoetin 1
HGF

Osteopontin
SDF1, GDF15
MIF, TNF

Decorin
MMP1, HGF
TGFβ
Galectin1

pre THA

6 months post THA

Stem cells – off the shelf?
EMA Phase I/IIa approval study

PLX-PAD was considered to be safe (n=20 patients)
Efficacy: placebo vs. intermediate dose vs. high dose

INCREASE IN GLUTEUS MEDIUS STRENGTH

Winkler et al, J Cahexia Sarcopenia Muscle 2018
PLX induces immune modulation

- „Good“ guys are kept (CD4+) if PLX present, but the high dose catches up…

Winkler et al, J Cahexia Sarcopenia Muscle 2018
PLX induces immune modulation
- But why is 300M less good than 150M?

Winkler et al, J Calhexia Sarcopenia Muscle 2018
PLX induces immune modulation

- But why is 300M less good than 150M? Reduced postOP stress related immunological changes

Winkler et al, J Cahexia Sarcopenia Muscle 2018
EMA Phase III approval study: Multicentre clinical trial

Unmet medical need: Femoral neck fractures
- Intraoperative muscle trauma on top Fx & sarcopenia in elderly patients
- impaired mobilization + surgical stress in frail
- high mortality
EMA Phase III approval study: Multicentre clinical trial

External Advisory Board

European Commission

Horizon 2020
EMA Phase III approval study: Multicentre clinical trial

- Total hip arthroplasty (THA) or Hemiarthroplasty (HA) via lateral approach
- IP administration IM during surgery in 10 injections (1.5mL each)
- **240 patients total** (09/2018 1st patient in, 11/2019 50% patients enrolled)
Lessons learned...

"refined translation"

Volk HD et al., Sci Transl Med 2015
DFG Positionspapier "Translation" Sept 2019
“prospective, mono-center, single-blinded, randomized, controlled study to assess the safety and efficacy of applying concentrated autologous CD31+ cells to promote bone healing in patients at risk with humeral head fracture”

Hypothesis

Intra-operative CD31+ cell concentration improves biologically impaired bone healing

→ Anti-inflammatory, pro-angiogenic, pro-osteogenic
→ Pro-regenerative!
→ Cells unfavourable for regeneration, such as TEMRA cells, are CD31-
PEI Pre-Advice “Osteoheal31” as Phase I/IIa approval trial

μCT

PBMCs

CD31+

Tissue Mineral Content

mg HA

2 weeks 4 weeks 6 weeks

Histology

Histomorphometry

% of total area

Cartilage Connective tissue

* Significant to control, p≤0.05, n≥5, bar = 1mm
PEI Pre-Advice “Osteoheal31” as Phase I/IIa approval trial

Availability

Angiogenic Potential

Osteogenic Potential

% CD31+ cells

relative tube length

relative calcification

young aged
female male

young aged
female male

young aged
female male

*
PEI Pre-Advice “Osteoheal31” as Phase I/IIa approval trial

Innate Immune Response - CD14+ LPS Stimulation

Adaptive Immune Response - CD8+ TCR Stimulation

Molecular profile of hematoma of treated rats

Sass et al., J Bone Miner Res. 2017
Loeffler et al Trends Endocrinol Metab. 2018
Loeffler, Sass et al., Front Immunol. 2019
PEI Pre-Advice “Osteoheal31” as Phase I/Ila approval trial

- BMBF Call „Early clinical trial“ - OsteoHeal31

- PEI statement as minimally manipulating enrichment method

  *Enrichment method in clinical study on CMV-specific T-cells (Neunhahn et.al, 2017)*

- EMA-classification of OsteoHeal31 as non-ATMP (2018)

Subject: Osteoheal31 (product ref.: H0004981) - Scientific recommendation on classification of ATMP according to Article 17 of Regulation (EC) No. 1394/2007

Further to the submission dated 04 January 2018 of an application to determine whether the medicine you are developing is an advanced therapy medicinal product, I am pleased to inform you that the Committee for Advanced Therapies (CAT), following consultation with the European Commission, has adopted at its plenary of 16 March 2018 a scientific recommendation of the classification of Osteoheal31, according to according to Article 17 of Regulation (EC) No. 1394/2007.

The EMA/CAT considers that product Osteoheal31, *does not fall within the definition of an advanced therapy medicinal product* as provided in Article 2 of Regulation (EC) No 1394/2007.
PEI Pre-Advice “Osteoheal31” as Phase I/IIa approval trial

**TACS-Technologie**

- Strep-Tactin®
- Agarosematrix
- Fab-Antikörper
- Strep-Tag®

Legend:
- Strep-Tactin® coated agarose matrix
- Strep-tagged Fab fragment
- Target cell
- Non-target cell

**Cell Pedia**

**BIH Regeneration**

**ZIM**

**Centrum für Muskuloskeletale Chirurgie**
PEI Pre-Advice “Osteoheal31” as Phase I/IIa approval trial

Regulatory Requirements

Non-ATMP

• X gene therapy
• X tissue engineering
• X somatic cell therapy

No Investigational Medicinal Product

• √ Only administrating human blood cells

Blood product according to transfusion law

Regulatory Documents:
1. GMP-Manufacturing Allowance (LaGeSo)
2. Investigational Medicinal Product Dossiers (IMPD)
3. Information Brochure (IB)
4. Testing Schedule (research ethics committee)
5. Patient Information

6. Approval and Application for Clinical Study
Lessons learned…

- Sound idea & concept (including basic science)
- IP, identify a technology provider
- Seek advice early - with PEI/EMA or authorized bodies
  - definition of technology
  - definition of approval path
  - remaining gaps, what is really needed
  - eventually, definition of patient cohorts
Mind-Set (De-Risk)

Take a Risk
Deep dive into Prototyping, Visualization and Insight Sharing

Emotional and Subjective
Looking beyond the Field & Need Finding and Contemplation

Clinical Affairs
Verify medical needs and balance health economic opportunities (incl. health care provider)

Infrastructure

„Opportunity Check“
Check freedom of operation while defining hypothesis
Campus Regenerative Therapies

BeCAT

SIM

Cranach
Campus Regenerative Therapies

a Campus for People
for Research and Development of
Regenerative Therapies

R&D Network
BCRT - Exploration by Clinical Driven Basic Research
SI-M - Simulation employing Human Model Systems
BECAT – Application of Advanced Therapies

Facilitation of R&D
ECRT – Inspiration of New Ideas
BSRT – Education of the Next Generations
BCRT – Translation of Research into Diagnostics & Therapies

Institute Building South
Future Charité-TU SI-M Building
Future BECAT Building
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