Translational Research of therapeutic angiogenesis for macrovascular complication

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Today’s my talk

1. Recent Progress of Gene Therapy
2. Therapeutic angiogenesis by hepatocyte growth factor
3. Translational research of novel angiogenic peptide
4. New concept of vascular calcification
Worldwide gene therapy clinical trials

Number of Gene Therapy Clinical Trials
Approved Worldwide 1989 - 2012

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www.wiley.co.uk/genmed/clinical
uniQure’s Glybera® First Gene Therapy Approved by European Commission

- Glybera becomes the first gene therapy approved by regulatory authorities in the Western world
- First medication approved for patients with rare metabolic disorder Lipoprotein Lipase Deficiency
- Commercial roll-out to begin second half of 2013
- Validates uniQure’s unique AAV-based gene therapy platform

Amsterdam, The Netherlands – November 2, 2012 – uniQure announced today it has received approval from the European Commission for the gene therapy Glybera® (alipogene tiparvovec), a treatment for patients with lipoprotein lipase deficiency (LPLD, also called familial hyperchylomicronemia) suffering from recurring acute pancreatitis. Patients with LPLD, a very rare, inherited disease, are unable to metabolize the fat particles carried in their blood, which leads to inflammation of the pancreas (pancreatitis), an extremely serious, painful, and potentially lethal condition. The approval makes Glybera the first gene therapy approved by regulatory authorities in the Western world.
Gene therapy clinical trials in Asia

Geographical Distribution of Gene Therapy Clinical Trials (by Country)

- Multi-country 3.9% (n=74)
- USA 63% (n=1199)
- UK 10.4% (n=198)
- Germany 4.3% (n=81)
- Switzerland 2.6% (n=49)
- France 2.4% (n=45)
- Netherlands 1.7% (n=32)
- Australia 1.6% (n=30)
- China 1.5% (n=29)
- Belgium 1.2% (n=22)
- Canada 1.2% (n=22)
- Other countries 6.4% (n=121)

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## Gene therapy clinical trials in Asia

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<tr>
<th>Country</th>
<th>Gene Therapy Clinical Trials Number</th>
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<tr>
<td>Canada</td>
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<td><strong>Total</strong></td>
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Indications of gene therapy clinical trials

Indications Addressed by Gene Therapy Clinical Trials

- Cancer diseases 64.4% (n=1186)
- Monogenic diseases 8.7% (n=161)
- Cardiovascular diseases 8.4% (n=155)
- Infectious diseases 8% (n=147)
- Neurological diseases 2% (n=36)
- Ocular diseases 1.5% (n=28)
- Inflammatory diseases 0.7% (n=13)
- Other diseases 1.4% (n=25)
- Gene marking 2.7% (n=50)
- Healthy volunteers 2.3% (n=42)
Therapeutic Angiogenesis by Growth Factor
Angiogenesis by Growth Factor

Dissociation of Pericytes

Degradation of ECM

Tip cell

Migration & Proliferation of EC

Migration of Pericytes

Maturation of Blood Vessels

Tubular Formation by EC
VEGF Gene Therapy to Treat PAD

Therapeutic Angiogenesis Induced by HGF

Recombinant HGF

ASO model
- rat (Hypertension 1999)
- rabbit (Circulation 2000)

HGF Gene Therapy

ASO model
- rat (Gene Therapy 2001)
- rabbit (Gene Therapy 2001; FASEB J 2003)

Diabetes + ASO model
- rat (Circulation 2002)

Hyperlipidemia + ASO model
- high Lp(a) Transgenic Mice (Circulation 2002)
Therapeutic Angiogenesis by HGF Gene Transfer

*Rabbit ASO Model*

control

HGF

neovascularization
Inclusion criteria

A) Sex Male or Female

B) Age over 40 years old

C) Clinical symptom Fontaine IIb/III/IV

D) Non-invasive All patients must be clarified:
* Ankle-brachial index (ABI)<0.60
* Exercise testing: >10% ABI decrease

Exclusion criteria

1. Cancer including past history
2. Diabetic retinopathy
3. Alcohol or drug-dependent within 3 months
4. Severe liver dysfunction
5. Remaining life will be <1 year due to complication
6. Attend to other clinical trials
TREAT-HGF

Stage 1 (Safety; n = 6)

4W 2W 4W 8W
HGF plasmid (0.4 mg) HGF plasmid (2 mg) HGF plasmid (2 mg) Evaluation

Stage 2 (Safety; n = 16)

4W 4W 8W
HGF plasmid (2 or 4 mg) HGF plasmid (2 or 4 mg) Evaluation
# Efficacy

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<th>Functional Endpoint</th>
<th>Clinical Endpoint</th>
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<td>Fontaine IIb</td>
<td>ABI (&gt;0.1)</td>
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<td>Fontaine III</td>
<td>ABI (&gt;0.1)</td>
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<tr>
<td>Fontaine IV</td>
<td>ABI (&gt;0.1)</td>
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- **Max Walking Distance (>25%)**
- **VAS visual analog scale (>2cm)**
- **Ulcer Size (>25%)**

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<th>1 EP</th>
<th>2 E.P.</th>
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<td>Fontaine IIb</td>
<td>1</td>
<td>4</td>
<td>2</td>
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<tr>
<td>Fontaine III</td>
<td>1</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Fontaine IV</td>
<td>2</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>total</td>
<td>4</td>
<td>10</td>
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18/22 Patients demonstrated 1 Endpoint Improvement.
1 Year Follow-up of HGF Gene Therapy

Changes in ABI
n=17

Changes in rest pain (VAS)
n=13
1 Year Follow-up of HGF Gene Therapy

Changes in Ulcer Size

Changes in Ulcer Size over time:
- Pre
- 2M
- 3M
- 6M
- 12M

Statistical significance:
- NS
- p<0.05

Sample size: n=11
Phase III trial

PAD; double-blinded trial, 41 patients

AMG0001 (HGF plasmid)

Follow up

4mg ↓ 4mg ↓

4 weeks 4 weeks 8 weeks

(Pre trial) (Evaluation & Key open)

12 weeks
Study Design

Stage 1 (Double-blinded trial)

Key open at 2 months after trans-gene

41 patients

- 13 patients, 1/3 Placebo
  - Improvement: 30.8% (4/13)

- 28 patients, 2/3 HGF plasmid
  - Improvement: 70.4% (19/27)

Stage 2 (Open-labeled trial)

Follow up

Follow up
Translational Research

*Therapeutic angiogenesis*

To identify the original therapeutic molecule

Phase I

Phase II

Phase III

Phase IV or Mega trial

preclinical

Basic
The candidate peptide forms an $\alpha$-helix structure, similar to antimicrobial peptide LL-37.

AG-30 possesses both angiogenic and anti-bacterial action.

Helical wheel drawing

Antimicrobial peptide
Cathelicidins (LL-37)


Cationic
Hydrophobic
AG-30 possesses both angiogenic and anti-bacterial action.

The process of wound healing requires both actions.

Toward clinical application
- Modify AG-30 with strong action

**Summary and Aim**

**Basic research**
- Seeds compound
- Functional analysis

**FS study**
- Leads compound
- Leads modification

**Pre-clinical study**
- Preclinical study
- Clinical study

- Bacterial infection
- Macro angiopathy
Plans of Clinical Trials

**Target Patients**: Severe Skin Ulcer of patients with Diabetes, Peripheral Arterial Diseases, Burger Diseases.

**Inclusion Criteria**: Not recovery for one month Carring (not infection) MRSA in skin ulcer

**Study Design**: Open-label (no control)

**Patients number**: 6 (study period 2 years)

**Primary End point**: Safety (because of first-in-human trial)

**Secondary End Point**: The size of skin ulcer Quantification of bacterial culture


**Ethical Committee of Osaka Univ. Hospital**: Approve (2012. Dec.)

Start ! From 2013. January
New Concepts in Vascular Calcification

Osteocalcin
Bone sialoprotein
Bone morphogenetic protein -2 and -4
Osteopontin
Osteonectin

Early lesion

Calcification

Osteogenic pathway in action

MGP    BMP-2

VSMC

Cbfa1, MSX2

Osteocalcin (OC), Osteopontin (OPN), Alkaline phosphatase (ALP),

Aortic Calcification (% / year)

Bone Density (% / year)

1st
N=39
(70±9.9)

2nd
N=39
(71±8.1)

3rd
N=39
(68±8.0)

4th
N=40
(68±9.2)

Schulz, E. J Clin Endocrinol Metab 2004 89: 4246-4253
Osteoprotegerin (OPG) KO mouse shows both osteoporosis and aortic calcification.

**Diagram:**
- RANKL (receptor activator of NF-κB Ligand)
- OPG
- RANK (receptor activator of NF-κB)
- TRAF6
- cSrc
- Ras
- ERK
- p38
- NFAT
- NFκB
- PI3K/Akt

**Pathways:**
- Osteoblast → Osteoclast
- Pre-Osteoclast → Osteoclast
- Osteoclast → Apoptosis
- RANKL → Osteoclast
- OPG → Osteoclast

**Cells:**
- Osteoblast
- Pre-Osteoclast
- Osteoclast
- Apoptosis
Basal Vascular Expression of RANKL System

RANK is expressed in both endothelial cells and smooth muscle cells.

**A**)

<table>
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<tr>
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<th>HAEC</th>
<th>HASMC</th>
<th>OB</th>
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**B**)

HAEC: human endothelial cell
HASMC: human aortic smooth muscle cell
OB: human osteoblast
RANKL induces BMP-2 expression

Stimulation with RANKL increased the calcification inducer BMP-2 expression only in HAECs.

A) BMP-2 expression in HAEC, HASMC, THP1, and Mac. cells.

B) Graph showing the effect of RANKL on BMP-2 mRNA and protein levels with and without anti-OPG Ab. * P < 0.01 vs No treat.

C) Diagram illustrating the effect of RANKL and anti-OPG Ab on BMP-2 and β-actin expression.
BMP-2 is a potent osteogenic inducer in HASMC:

BMP-2 is the main osteogenic protein induced by RANKL
Hypothesis

Under estrogen deficiency, RANKL leads to an imbalance of pro- and anti-calcification factors in vasculature.

Vascular Calcification:

- **Inhibitor**: MGP
- **Inducer**: BMP-2

☑ Bone morphogenetic protein (BMP-2) induces aortic calcification. (J Clin Inves. 2005; 115:1210-1220)

☑ VSMC produces high level of matrix Gla Protein (MGP), and the knockout mice shows severe vascular calcification (Nature. 1997; 386:78-81)

Estrogen Deficiency:

- **OPG**
- **RANKL**

Calcium binding/clearance

BMP-2 entrapment
RANKL increases calcification of HASMC

RANKL decreases MGP and induces bone-related protein expression in HASMC:

- Dexamethasone, β-Glycerophosphate, Ascorbate...
- Alizarin Red staining

* P < 0.05 vs No treat

Bone markers (osteogenesis)
Novel Animal Model for Aortic Calcification

ApoE KO mice with ovariectomy

OVX/High Fat Diet
ApoE−/−
3 months

20 μg/kg/day
17β-Estradiol (E2) by osmotic pump

High Fat Diet + E2

Sample collection

1 2 3 months
Estrogen deficiency increases vascular calcification and RANKL

- Atherosclerosis
- Calcification

**Oil Red**

- Sham
- OVX
- OVX/E2

**Alizarin Red**

- Sham
- OVX
- OVX/E2

**RANKL/18S mRNA**

- Sham
- OVX
- OVX/E2

**OPG/18S mRNA**

- Sham
- OVX
- OVX/E2
Summary 1

**RANKL in the Bone x Artery Paradox**

- **Endothelial Cell**
  - BMP2
  - Estrogen

- **RANKL**
  - Osteoblast-like cell (cbfa1, osterix, OC, ALP, OPN…)
    - Estrogen
    - MGP

- **Pre-Osteoclast**
  - Estrogen

- **Osteoclast**
  - Osteoporosis

- **Vascular Calcification**

Estrogen plays a crucial role in modulating bone remodeling and calcification processes, highlighting the complex interactions between bone and artery health.
Aortic arch calcification was independently associated with older age, current smoking, and hypertension in both men and women.
Hypothesis:
Vascular RAS is the trigger of RANKL system activation.

Ang II $\rightarrow$ RANK- RANKL $\rightarrow$ Vascular Calcification

**Alizarin Red Staining**

Dexamethasone, β-Glycerophosphate, Ascorbate...

**Graphs:***

- **C)**
  - RANK / 18S mRNA
  - RANKL / 18S mRNA

**Images:**
- Control
- RANKL
- Ang II
- RANKL + Ang II

**Bar Graph:**
- RANKL Ang II
  - -
  - +
  - -
  - +
  - +

**Images:**
- RANKL
- cbfa1
- β-actin

**Anti-RANKL**

$\star$
ApoE KO mice with ovariectomy

♀ ApoE−/− mice under OVX

1. No treat
2. Ang II (100 ng/kg/min)
3. High Fat diet
4. High fat diet + Ang II (100 ng/kg/min)
5. High fat diet + Olmesartan (3 mg/kg/day)

Von Kossa Staining

Control | Ang II | HF | HF + Ang II | HF + ARB

RANKL / 18S mRNA

RANK / 18S mRNA

OPN / 18S mRNA

OVX:
High Fat Diet
ApoE−/−
3 months

Control
Ang II
HF
HF + Ang II
HF + ARB

Von Kossa Staining
**Effect of AngII in OPG KO mice**

Unopposed RANK-RANKL signaling +AngII

- WT, OPG-/-, AT1R-/-/OPG-/- mice
  1. WT
  2. WT + Ang II (100 ng/kg/min)
  3. OPG-/-
  4. OPG-/- + Ang II (100 ng/kg/min)
  5. OPG-/-
  6. OPG-/- + Ang II (100 ng/kg/min)
  7. AT1R-/- /OPG-/-

**Von Kossa Staining**

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<th>WT</th>
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<th>OPG-/-</th>
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<th>AT1R-/-/OPG-/-</th>
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[Graphs showing quantitative data for different groups]
Summary

RAS activates RANKL system, and conversely, unopposed RANKL stimulation activates local RAS, and this vicious cycle aggravates vascular calcification.

**RANKL**

- **Angiotensin II**
- **AT1R**
- **ACE**
- **Estrogen**
- **BMP2**
- **MGP**
- **Endothelial Cell**
- **Osteoblast-like cell** (cbfa1, osterix, OC, ALP, OPN...)

**Vascular Calcification**
Biomarkers of the Osteoprotegerin Pathway
Clinical Correlates, Subclinical Disease, Incident Cardiovascular Disease, and Mortality


Raggi P. et al, Journal of Women’s Health 13; 3 2004
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Miyako-Messe, Kyoto

April 2014
4/14(mon)~17(thu)

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