新興產品應用與發展

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前言

- 以目前的先進醫療技術，有很多疾病仍未能得到滿意的治療。因此病人仍處於持續的生病與死亡威脅中

前言

1. **冠狀動脈阻塞**。有15%-20%病人嚴重瀰漫性冠狀動脈疾病不適合行冠狀動脈介入治療
2. **急性缺血性腦中風**。只能10%左右病人能適合積極性介入治療
3. **慢性腎臟病**: 而每年洗腎人口約有六千名人數增加
4. Severe ARDS/Sepsis: 住院死亡率達40%--60%。台灣每年有6000人以上因此病而死亡
5. 難醫治的**糖尿病傷口/大範圍燒傷**
6. 難醫治的autoimmune disease

前言

- 因此，要突破這些醫療的瓶頸仍需全人類的努力。同時，研發新的治療方向/方法是人類的共識和努力。

幹細胞治療的潛能

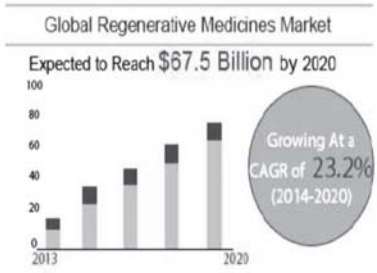
- 幹細胞的發現為醫療、科學界帶來重大的突破與希望，已成為當代最熱門的研究領域之一。幹細胞為一群尚未分化的細胞，具備自我再生能力，以及能分化為不同細胞的潛能。在發育過程中，幹細胞扮演著舉足輕重的角色，待發育成熟後，幹細胞則負責器官及組織細胞更新及修復等。

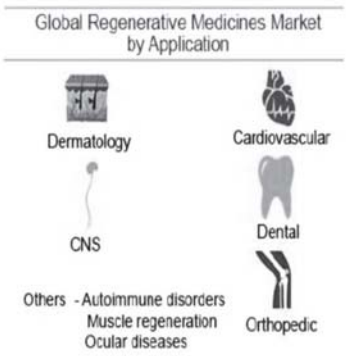
未來醫學：幹細胞當家

新興的再生醫學領域，可能會徹底、改變心臟疾病和神經退化性疾病的治療方法，不但可以解決捐贈器官不足的問題，還能讓受損肌肉和肌腱組織等完全復原。

Global Regenerative Medicines Market
Size & Forecast (2013-2020)

- The global regenerative medicine market will reach $67.6 billion by 2020, up from $16.4 billion in 2013

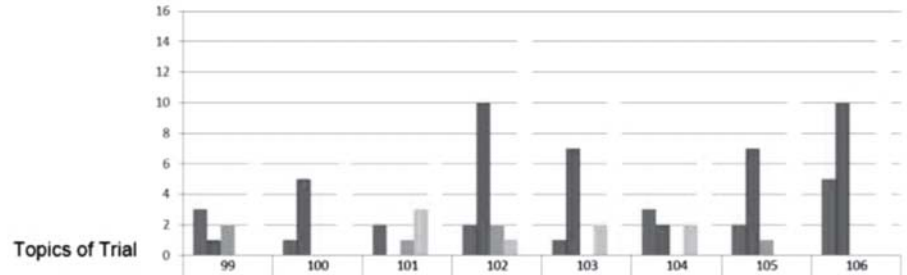




資料來源:
Allied Market Research Report Jun 2014

Cell Therapy in Taiwan
GTP符合性訪查現況

- 99~106年度，共接獲75案GTP訪查案
  - 申請案來自21家醫院、3家藥商/CRO
  - 64%委託外部實驗室進行細胞處理，13家生技公司/法人參與
  - 其中，106年度計執行11案(17場次)GTP訪查，64%委外

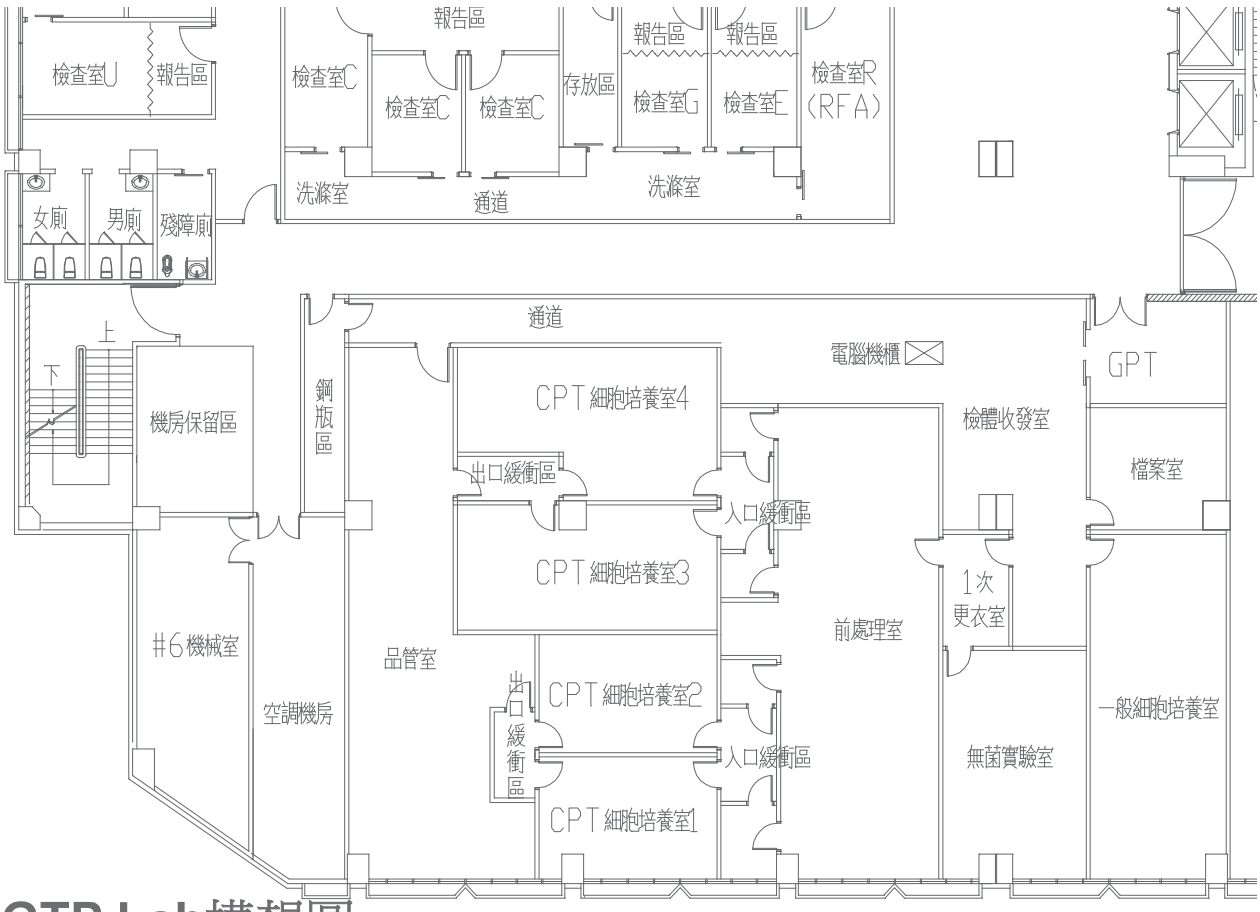


	99	100	101	102	103	104	105	106
■免疫細胞	3	1	2	2	1	3	2	5
■幹細胞	1	5	0	10	7	2	7	10
■皮膚	2	0	1	2	0	0	1	0
■其他	0	0	3	1	2	2	0	0
總計	6	6	6	15	10	7	10	15

衛生福利部 食品藥物管理署 FDA Food and Drug Administration

Modified from 傅永卿

我們的夢想
GTP Lab的構想



GTP Lab構想圖

- 林口GTP工程費用 (高雄長庚經管組推算)
  - 100坪:工程費用3600萬(含4間GTP實驗室)、儀器費用5700萬、耗材費300萬，合計9600萬
  - 改至桃園空間300坪:工程費用7200萬(含6間GTP實驗室)、儀器及耗材費用仍在估算(會再成長)
- 高雄GTP工程費用
  - 200坪:以林口4間GTP實驗室費用預估9600萬
  - 每年維持費用：人事費300萬(1博3碩)、耗材費300萬(比照林口)、電費200萬、工程費折舊360萬、儀器費折舊700萬，合計1860萬元
- 高雄GTP工程完工後每位病患成本
  - 以每年收治100位病患來計算，每次療程成本約8萬元(含醫事人力、住院費、材料及手術費用，葉醫師有明細)、GTP工程及儀器折舊費用10.6萬元、 GTP人事及耗材及電費費用8萬元、平均每一個病患負擔成本26.6萬元
- 病患存放細胞成本(QC用)
  - 液態氮桶加液態氮每年成本約20萬元，每個液態氮桶可放500個病患細胞，平均每個病患液態氮桶成本約400元，人事成本約600元(以組織銀行存放一盒檢體之人事成本計算)，合計成本1000元

我們對GTP實驗中心建構經費預估

- 耗材費用：
  - 耗材費用以每間GTP Lab 每年約一百萬的耗材費用，
  - 預估每年耗材費用 4,000,000元 (400萬)
- 電費：
  - GTP Lab 開始運轉後，預估每間月電費約 5 萬元，共計 4間GTP Lab，
  - 預估每月總電費約 20 萬元
  - 預估每年總電費約 200,000 x 12 = 2,400,000 元 (240萬)
- 總費用：
  - 工程費用及設備費用約 70,000,000 + 73,542,500 = 143,542,500 元 (14,350萬)
  - 每年支出人事，耗材，電費共計約 3,000,000 + 4,000,000 + 2,400,000 = 9,400,000 元 (940萬)

臨床醫師

為什麼期待建構GTP LAB---目的?

設立GTP Lab 的重要意義:

- 目前全球各大醫學中心正如火如荼 (in full swing)進行各類疾病的幹細胞治療。因為它有:**“醫機”及“商機”**
- 因為幹細胞研究、開發有很大商機、因此、有很多生技公司投資非常大的資金加入幹細胞開發的行列
- 跟著世界的潮流，**臺灣**幹細胞治療許多的規範也一步一步地放寬

幹細胞可治療的疾病

- Ischemic heart disease
- PAOD
- Ischemic stroke
- CKD
- ARDS, severe sepsis
- Autoimmune disease
- Skeletal-muscular disease
- Congenital newborn disease
- transplantation immune rejection
- AVN

區域GTP實驗中心的必要性 (1)

- 病患對於醫療團隊的**熟悉與信賴**為其選擇治療單位的重點考量之一
- 因此在進行幹細胞相關治療時，除其對原先治療團隊的熟悉外，安全及**便利**的自體幹**細胞抽取製備環境**、穩定的異體幹細胞來源、完善的培養環境、**即時細胞品質監控**、**高配合度治療時間**等，皆影響病患對治療團隊的選擇
- 此外，**額外的運輸**除增加運輸成本外，亦將增加幹細胞治療成效之不穩定性及風險，因此區域性GTP實驗室之設立有其必要性

區域GTP實驗中心的必要性 (2)

- 除急迫性或因病患本身細胞狀況無法選擇之幹細胞治療外，自體幹細胞為安全性及接受度最高之細胞來源。目前研究亦指出，健康且年輕來源之幹細胞具有較佳之治療效應，因此幹細胞**預防性儲存**無論在醫療成效或是營運效益上皆是值得推行之策略。因此，區域性GTP實驗室的設立，除提供該院區病患及健檢客戶的細胞儲存外，於將來需進行幹細胞治療時，亦能在其熟悉的醫療團隊及**方便的家庭照護**下進行。此外，自體幹細胞培養及分化衍生產物，如細胞激素或生長因子等，可作為醫美相關之安全且有效材料來源，如有區域性GTP實驗室設立，可進行此高附加價值之服務。

區域GTP實驗中心的必要性 (3)

- 幹細胞治療團隊可與該院區其他治療團隊建立良好的互轉機制，病患如**不適用幹細胞**治療時，亦可**轉介至其他治療**模式。因此如有一高知名度及高信任度之幹細胞治療團隊及完善之**GTP實驗中心**，除幹細胞治療本身外，**亦可帶動其他治療團隊之營運**業績

區域GTP實驗中心的必要性 (4)

- 高雄長庚再生醫學中心與**GTP實驗室**之設立將以**穩定的幹細胞生產**、**儲存**、及**便利與安全**的細胞治療為訴求，將以經營及醫療效應為目標，研究及發表將為其次要目標，其研究之主軸將為試驗成果之分析及**治療策略之改進**

區域GTP實驗中心的必要性 (5)

- 高雄長庚再生醫學與GTP實驗中心**可與國際醫療及尖端醫療共同整合**，進行高價值之特色醫療
- **有了區域GTP實驗中心更有立足點與吸引力與國際醫療及尖端醫療單位共同合作**

幹細胞治療國際合作尊家

Professor Losordo

Takayuki Asahara

營運策略

- 需求者/健康者幹細胞儲存用於將來生病所需

1.來源:健診

2.門診

3.國際醫療

4.其它醫院轉診

GPT實驗中心給病人及醫院的回饋

- 病人方面---可得到治療後疾病的改善、提高生活品質、減低家庭、社會負擔
- 醫院方面分兩部份:

1.帶動並提昇研究風氣、研究水準、論文發表質與量、以及醫院的國際知名度

2.若clinical trial完成後而衛福部同意使用幹細胞療法，可進行自費醫療(包含國際醫療)，增加醫院收入

Milestone (1)

- 希望在院內建立完整性 GTP**實驗中心**
- 目的: (1)帶動學術研究，特別是提升長庚醫院臨床研究特色。(2)提供安全有品質保證的幹細胞給院內病人使用。
- 產學/商機：有了GTP**實驗中心**。我們可量產 MSC/EPC for clinical use (費用可由病人或院外計劃支負)。也可以賣給其它醫院使用。因此、提升醫院的醫療競爭力。

Milestone (2)
(我們將來的期待)

- 建立Stem Cell Center for Regenerative Medical Research & treatment in Critical Health Care (重症醫學再生研究暨幹細胞治療中心)

What is current Good Tissue Practices (cGTP)

Subpart D—Current Good Tissue Practice

§ 1271.145 Prevention of the introduction, transmission, or spread of communicable diseases.

You must recover, process, store, label, package, and distribute HCT/Ps, and screen and test cell and tissue donors, in a way that prevents the introduction, transmission, or spread of communicable diseases.

GTP Core Requirements

(1) Requirements relating to **facilities**

(2) Requirements relating to **environmental control**

(3) Requirements relating to **equipment**

(4) Requirements relating to **supplies and reagents**

(5) Requirements relating to **recovery**

(6) Requirements relating to **processing and process controls**

(7) Requirements relating to **labeling controls**

(8) Requirements relating to **storage**

(9) Requirements relating to **receipt, pre-distribution shipment, and distribution of an HCT/P**

(10) Requirements relating to **donor eligibility determinations, donor screening, and donor testing**

The Facility

There is not one solution to build a lab,
and it depends on the purpose!

What is the purpose of the GTP lab?

Production of clinical grade cells?
Who is going to use it?
How many different protocols (cell types, sources)?
How many equipment (including installations)?

Who is going to maintain the GTP lab?

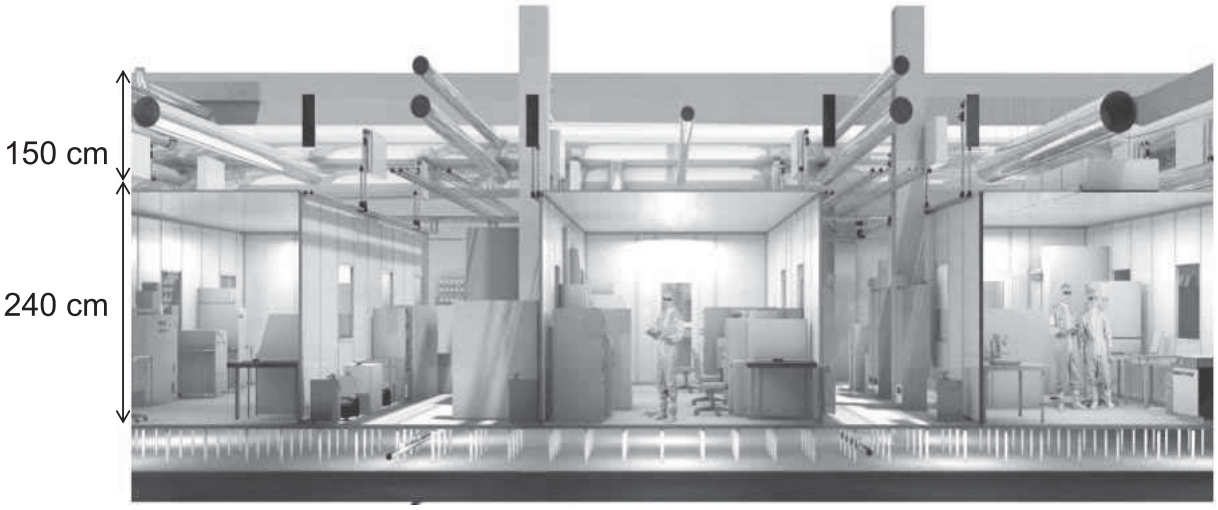
A GTP compliant facility consists of two parts

Infrastructure

Quality System

Space requirements

Space above cleanroom for placing airsupply, air handling units etc, and access for maintenance;
Enough space for placing equipment and operations;



Facility Layout

Definition of workflows

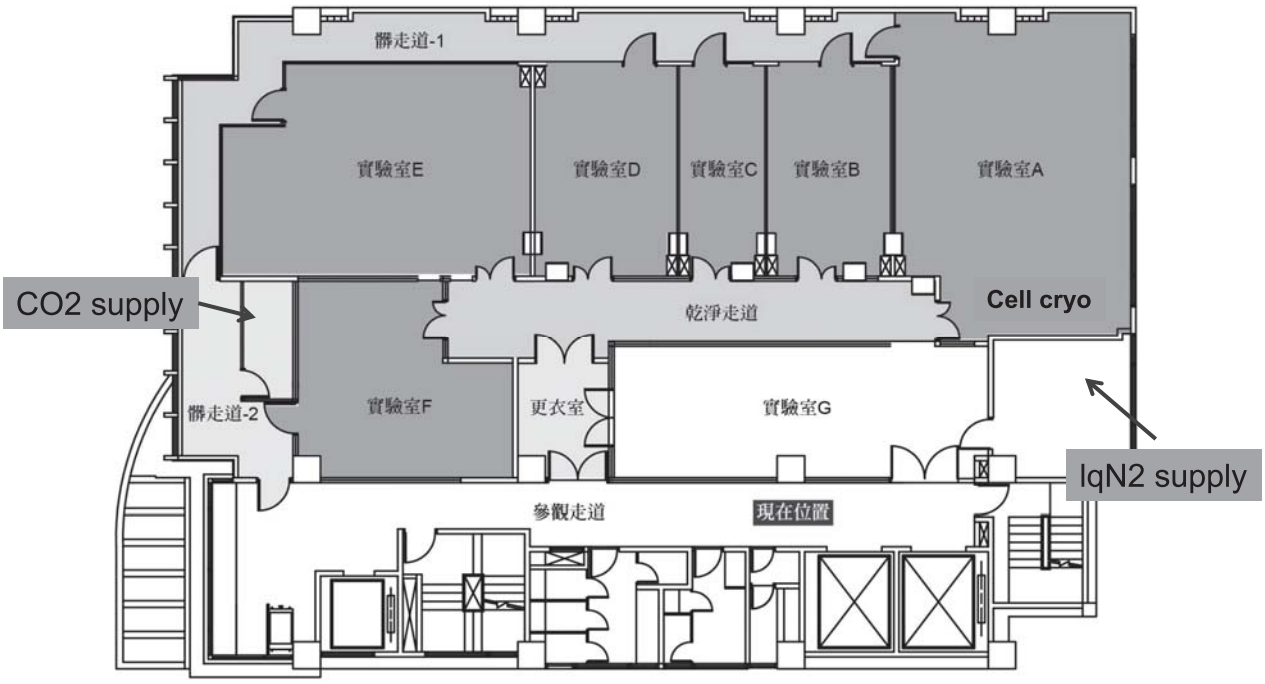
Personnel: where do staffs change clothes, go inside the cleanroom area, and how do they exit?

Material: how is material and reagents brought into the working areas?

Waste: how is the (bio)waste removed?

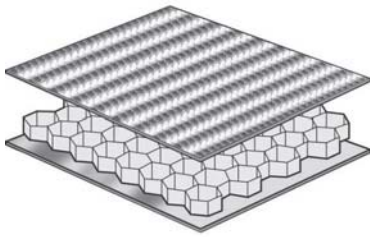
Equipment: how can equipment be moved in and out?

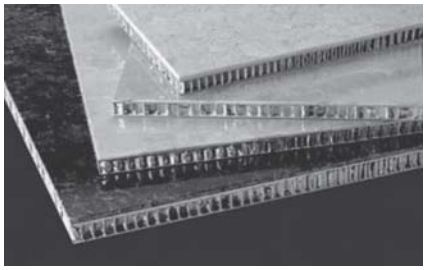
Facility Layout

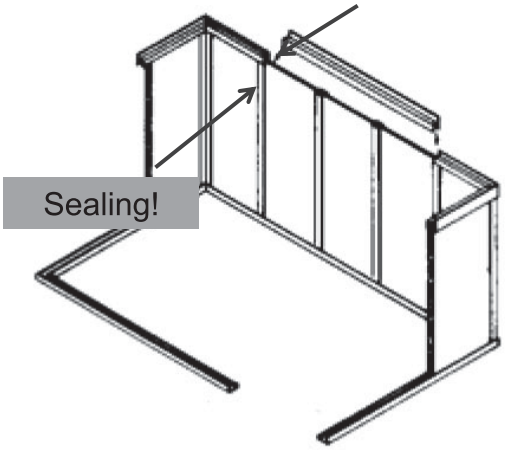


Walls

- aluminum honeycomb wall panels
- epoxy-painted walls

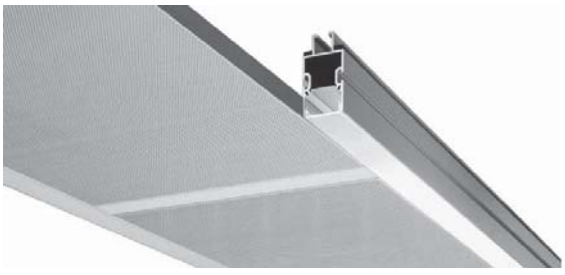


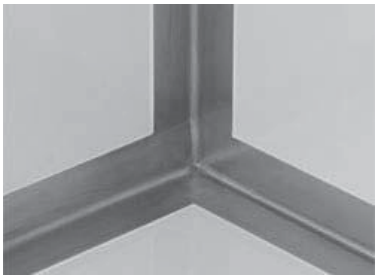




Ceiling

- **2-in. T-bar ceilings**
- **“flush-grid” systems**





vs

Floor

- **Polyurethanes feature high abrasion and impact resistance**
- **Vinyl flooring is a cost effective solution**
- **Epoxy coating: excellent Impact & Abrasion Resistance, easy clean and maintain**

Round corner
=> Less dust accumulation
=> Easier cleaning

Facility Features

- **Independent air-conditioning system**
- **HEPA filtration**
- **Pressured areas**
- **Power supply / UPS**
- **Facility monitoring and control**
- **Fire extinguishing system**
- **Backup systems**

All equipment should have a backup equipment!

e.g. power supply, air handling units

HEPA filtration

HEPA (high-efficiency particulate air)
Clean room class
10’000

Class	maximum particles/ft^3					ISO equivalent
	≥0.1 µm	≥0.2 µm	≥0.3 µm	≥0.5 µm	≥5 µm	
1	35	7.5	3	1	0.007	ISO 3
10	350	75	30	10	0.07	ISO 4
100	3,500	750	300	100	0.7	ISO 5
1,000	35,000	7,500	3000	1,000	7	ISO 6
10,000	350,000	75,000	30,000	10,000	70	ISO 7
100,000	3.5×10^6	750,000	300,000	100,000	700	ISO 8

Facility Validation

Facility (Clean rooms) must be tested and validated

Monitoring of

Temperature

Humidity

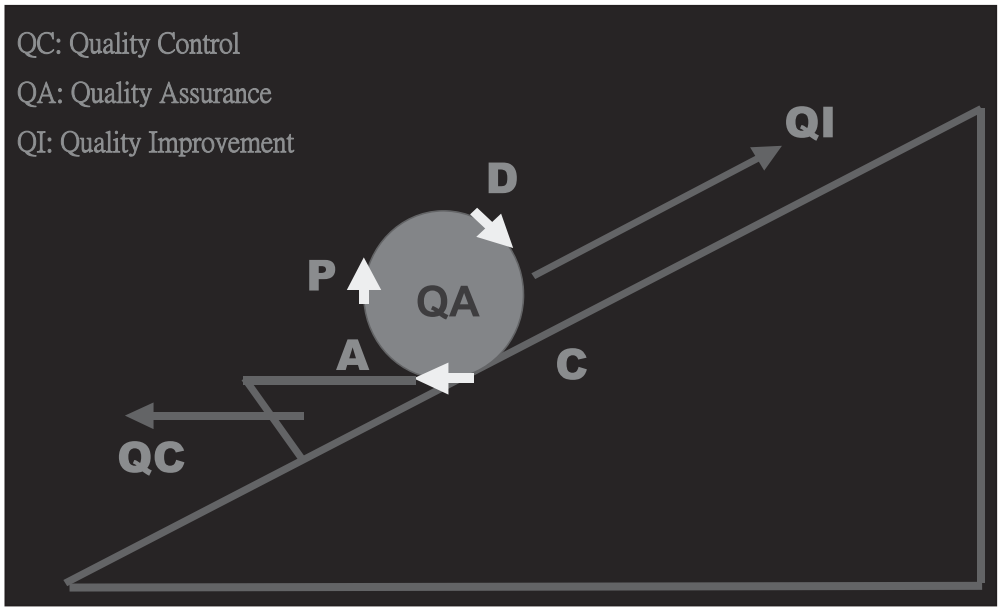
Differential Pressure

Flow Velocities

Particulate Contamination

Audit

Internal assessment: internal audit at defined time intervals
External assessment (independent)



Personnel

- Training program (on job training, and assessment)
- Proficiency testing (practical and written tests)
- Continuous education

- Job description

Who, What , How, How often must be defined in SOP

Personnel criteria Job scope Testing type Frequency of tests

Facility Maintenance

Monitoring:

- Daily routine checks,
- Monthly functionality tests for e.g emergency showers, fire extinguisher
- Microbial monitoring: settle plate (static or dynamic)

Cleaning:

- Cleaning schedules, daily weekly, monthly
- Facility testing (annual particle testing, HEPA functionality/leakage)

Equipment Maintenance

Monitoring

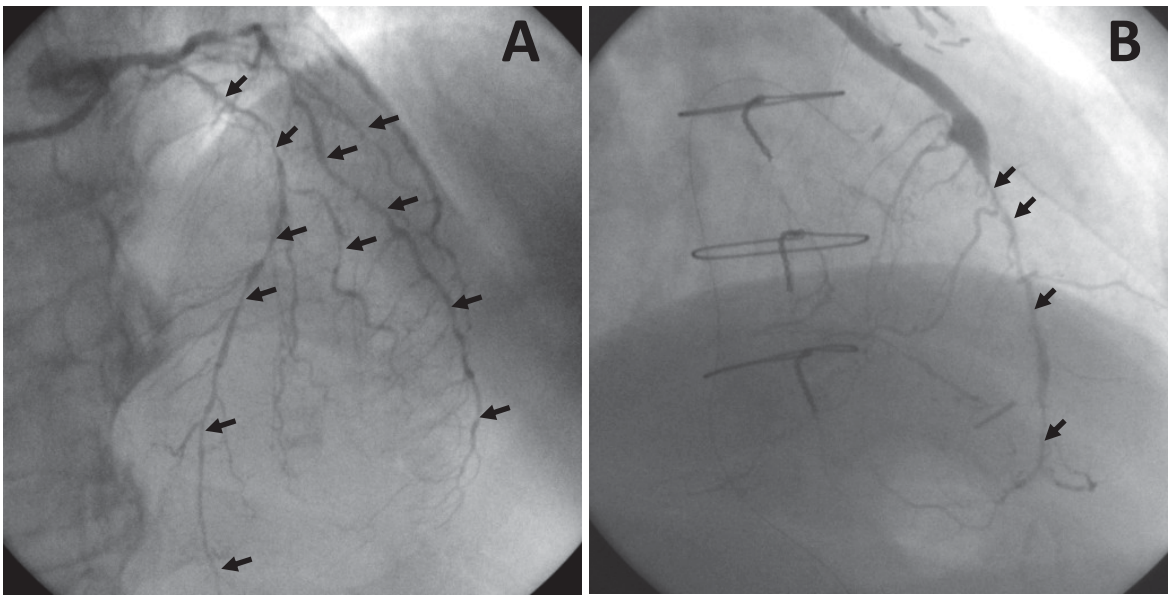
- Daily check
- Equipment alarm testing
- Microbial monitoring: e.g. swab tests for incubators

Cleaning

- Cleaning schedules, daily, monthly
- Scheduled calibration

Part II

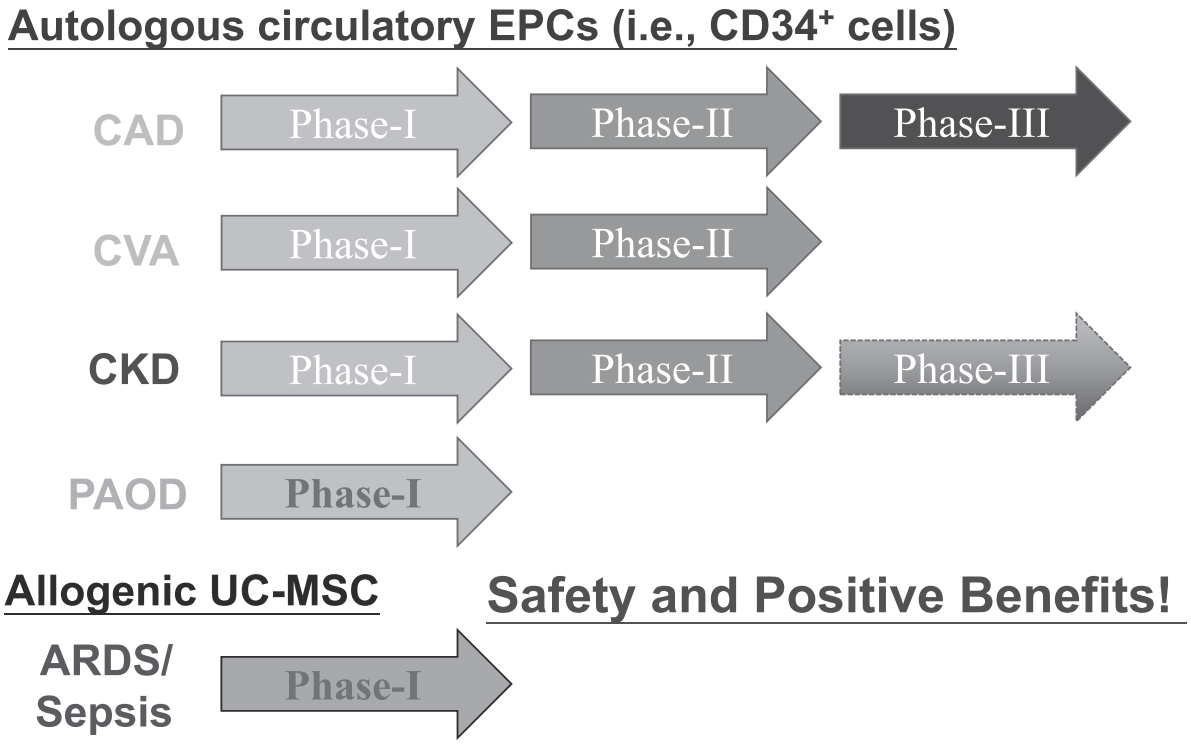
Clinical Application of stem cells for different disease entities—our experiences



Terminal CAD

1) Diffuse obstructive CAD;
2) Large reversible ischemia on thallium scan;
3) Recurrent angina despite optimal medication,
4) Repeated admissions

Current status of our clinical trials of stem cell therapy

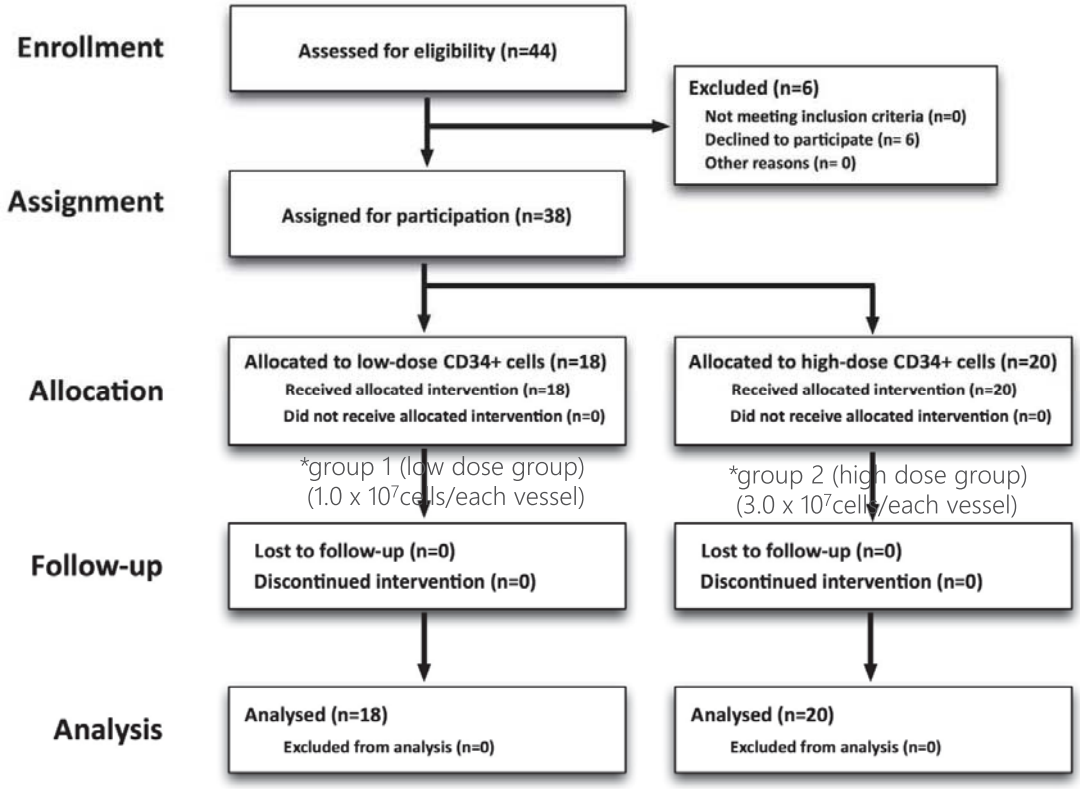


Outline--1

- Intracoronary Administration of Circulatory Derived Autologous CD34+ Cells for Patients with Severe Diffuse Coronary Artery Disease—**Phase 1, 2, & 3** Clinical Trials

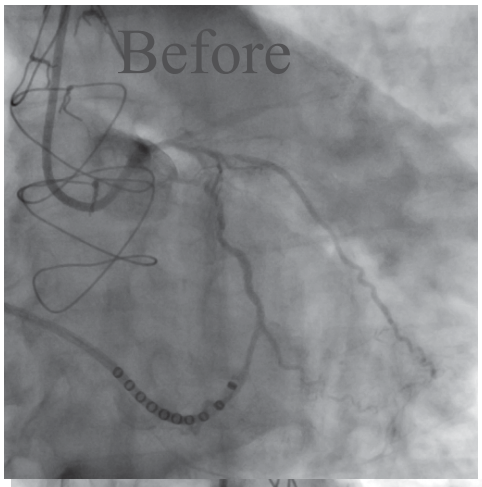
Phase I Study: End-stage CAD

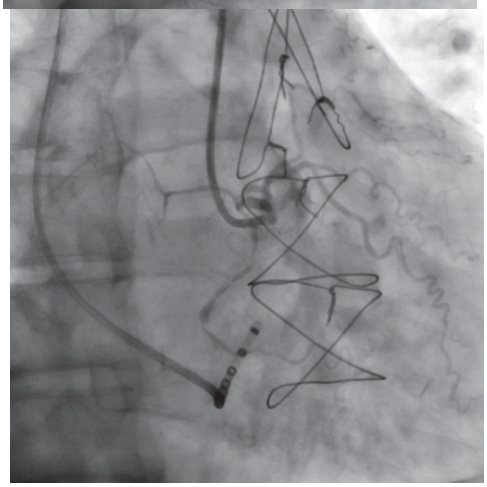
Prospective, randomized, double-blinded , single center facility
Intracoronary implantation Post G-CSF stimulation, circulatory derived autologous CD34+ cells

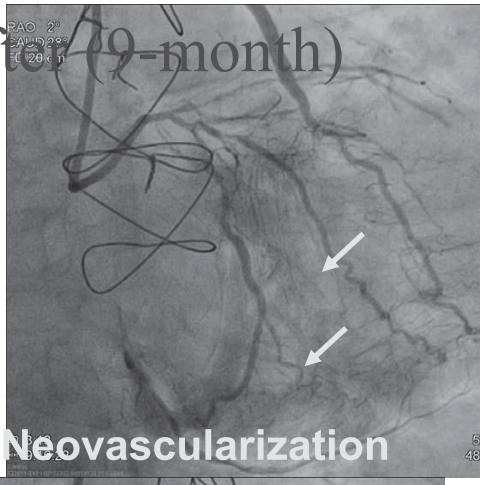


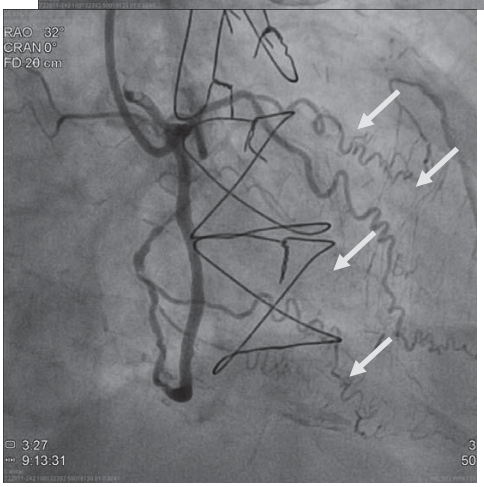
Results

One-Year follow-up outcome

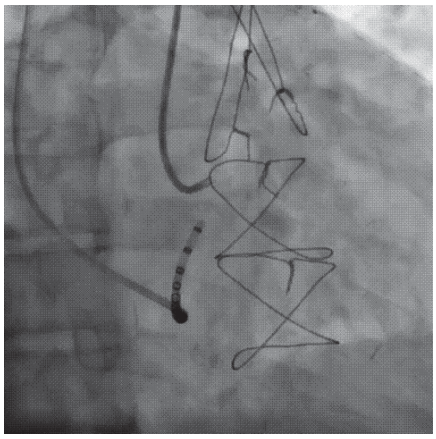


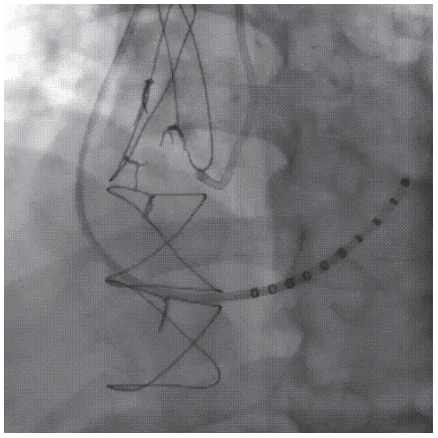




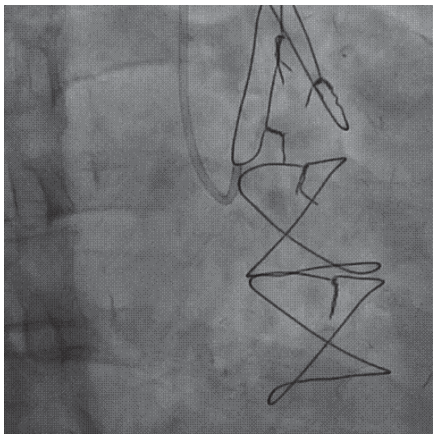


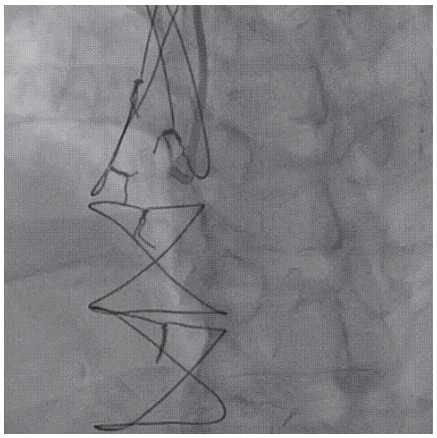
Before





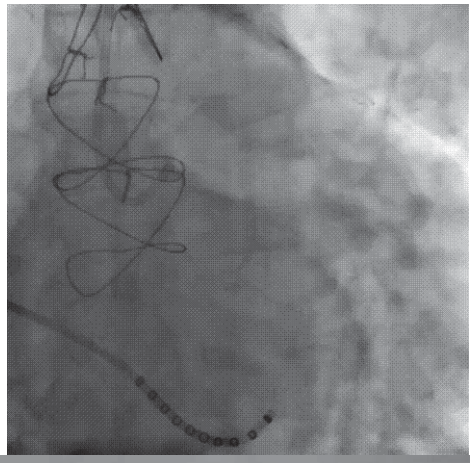
After (9-month)

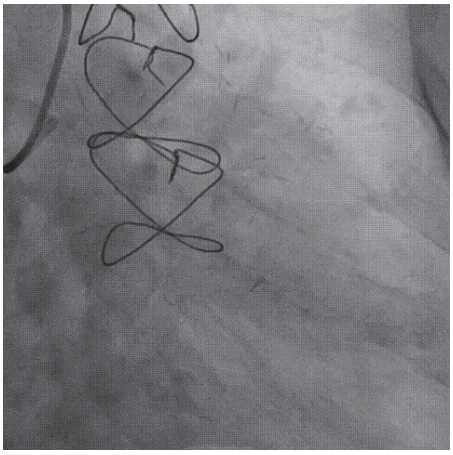




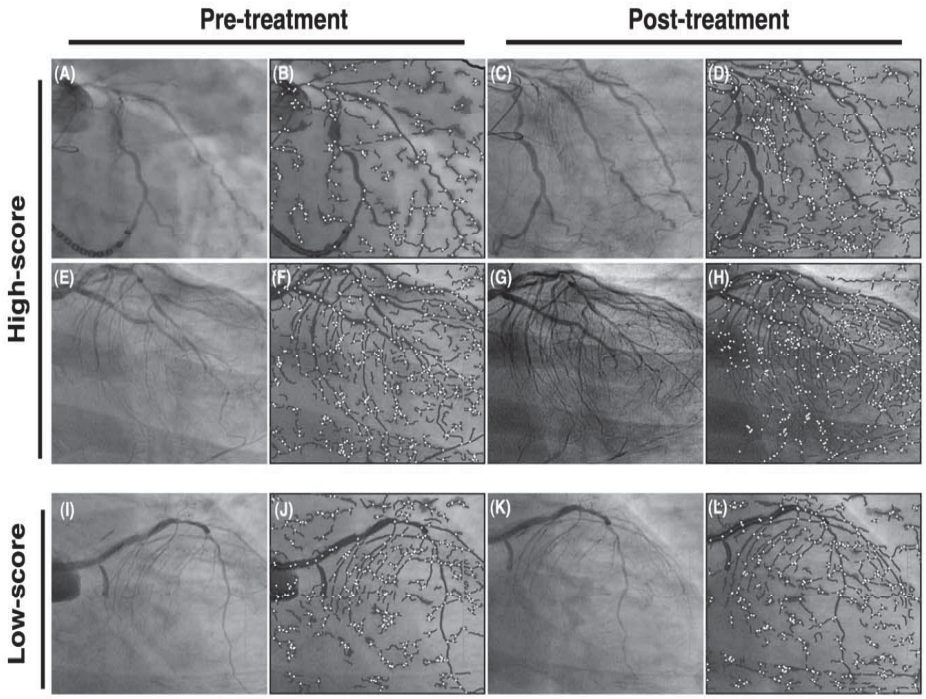
Before

After (9-month)

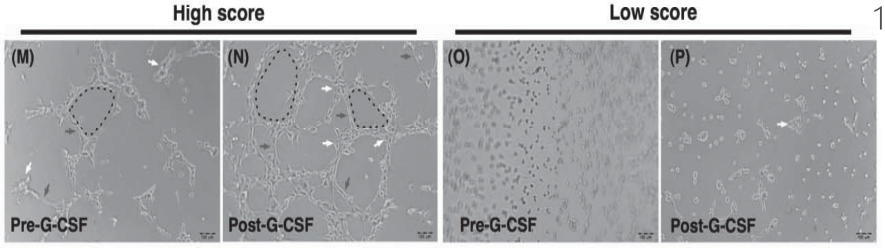




Wimasis Image Analysis

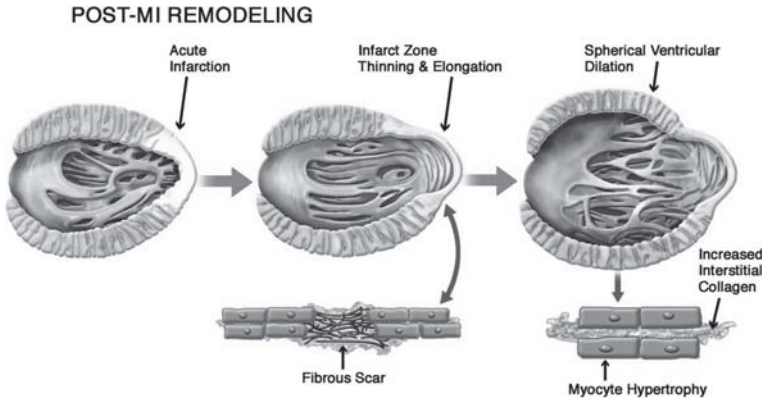


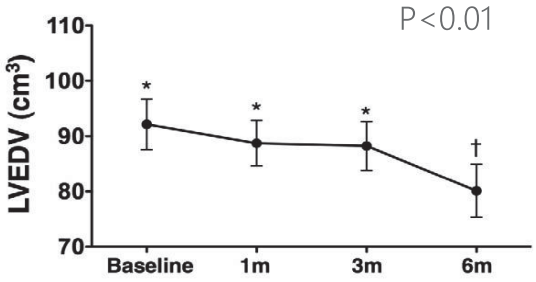
26 cases

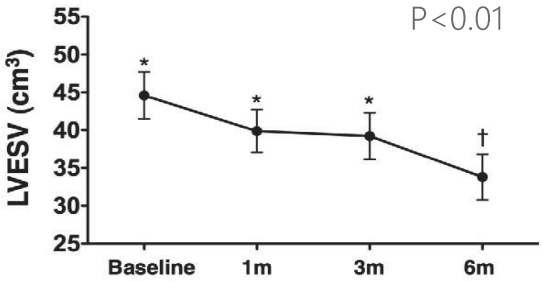


12 cases

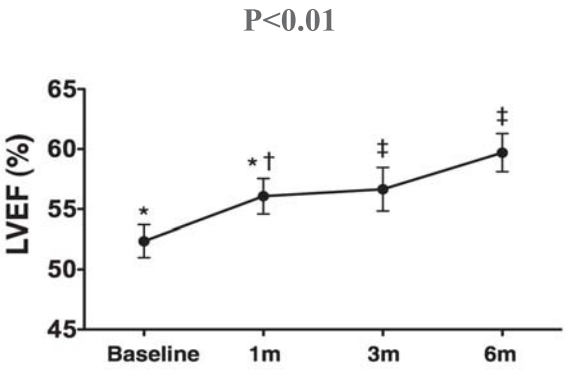
Reverse LV remodeling Continuously in 6 months post EPCs (3D echo)

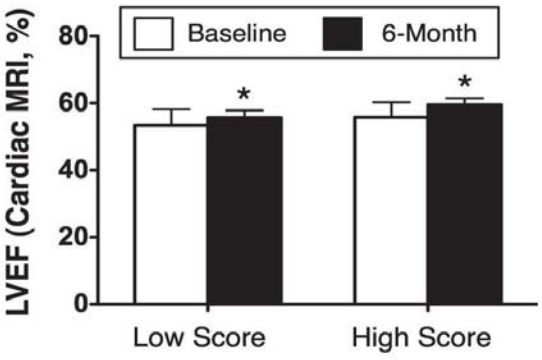


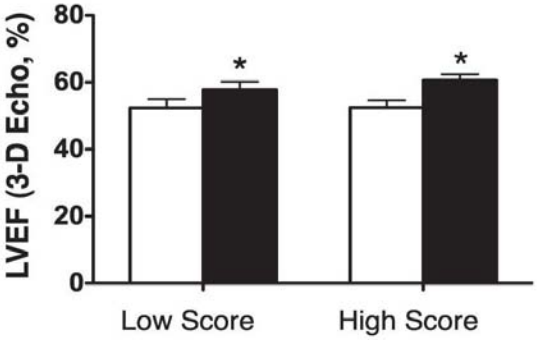


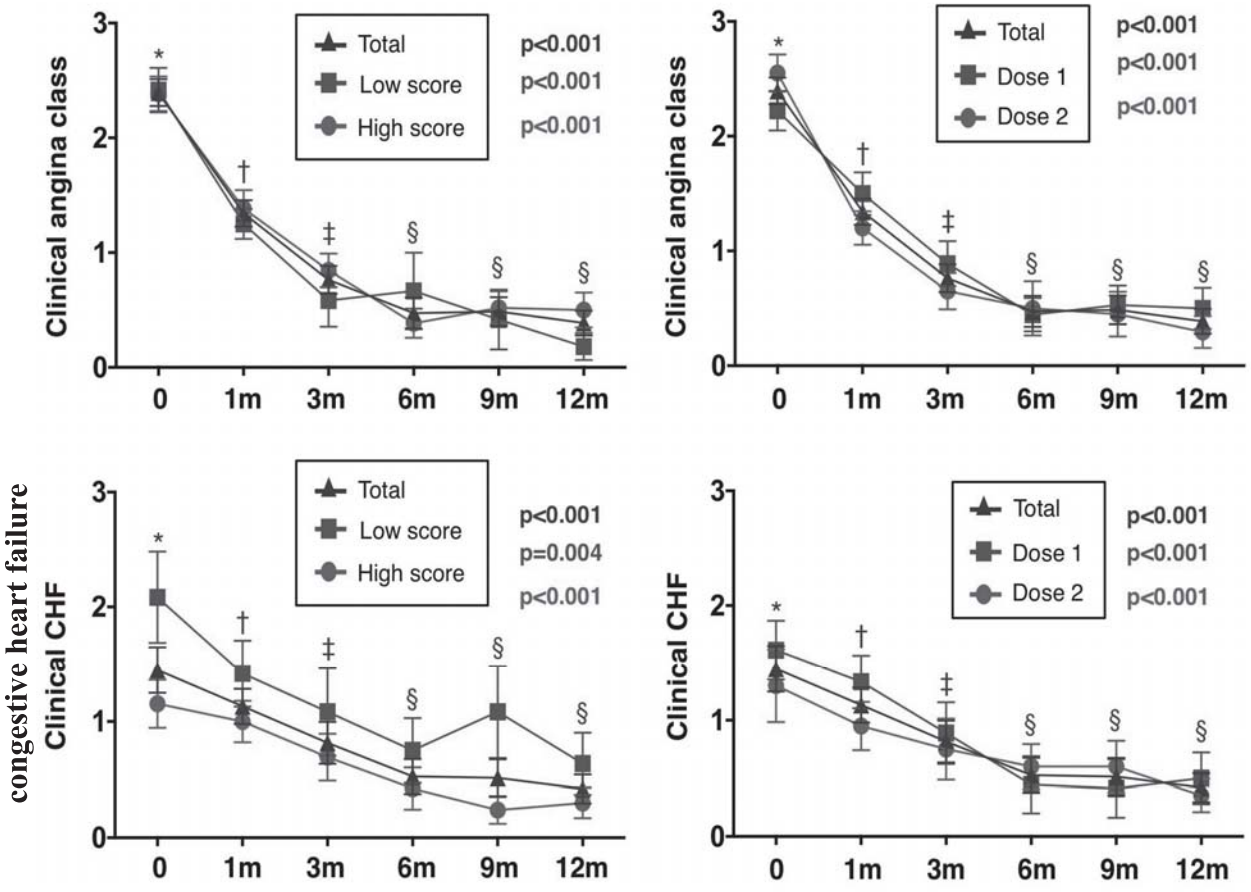


Better LV systolic function at 6 months post in both (high & low angiogenesis score) groups









Clinical outcomes

- 100% successful procedure
- One-year survival 97.4% (37/38)
- One patient (79 yrs. old) falling down to ground at bath room with ICH and death at 9-months after CD34+ cell therapy

Conclusion

- The present study, by utilizing the imaging studies, clinical and angiographic follow- up as well as the basic research, has provided more scientific data to prove that the intra-coronary transfusion of autologous CD34+ cell therapy was safe and effective for improving heart function, angina and CHF as well as a favorable clinical outcome in terminal CAD patients who were refractory to optimal medication and non candidates for coronary interventions

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Intracoronary Transfusion of Circulation-Derived CD34+ Cells Improves Left Ventricular Function in Patients With End-Stage Diffuse Coronary Artery Disease Unsuitable for Coronary Intervention*

Fan-Yen Lee, MD[1]; Yung-Lung Chen, MD[2]; Pei-Hsun Sung, MD[2]; Ming-Chun Ma, MD[3]; Sung-Nan Pei, MD[3]; Chiung-Jen Wu, MD[2]; Cheng-Hsu Yang, MD[3]; Morgan Fu, MD[3]; Sheung-Fat Ko, MD[4]; Steve Leu, PhD[5]; Hon-Kan Yip, MD[2,5,6,7]

Crit Care Med. 2015 Oct;43(10):2117-32.
doi: 10.1097/CCM.0000000000001138.

Result of five year follow-up & outcome

Table 2. Five-year clinical outcomes of 38 study patients

Variables	Patient number (n = 38)	Percentage (%)
Death from any cause	8	21.1
Non-CV death	5	13.2
MACCE	14	36.8
CV death	3	7.9
Acute myocardial infarction	3	7.9
Hospitalization for ADHF	10	26.3
Heart failure with reduced LVEF	2	5.3
Ischemic stroke	2	5.3
Target vessel revascularization	12	31.6
Newly onset atrial fibrillation	1	2.6
Sepsis	5	13.2
Gastrointestinal bleeding	2	5.3

Abbreviations: CAD, coronary artery disease; CV, cardiovascular; MACCE, major adverse cardiac and cerebrovascular event; ADHF, acute decompensated heart failure; LVEF, left ventricular ejection fraction

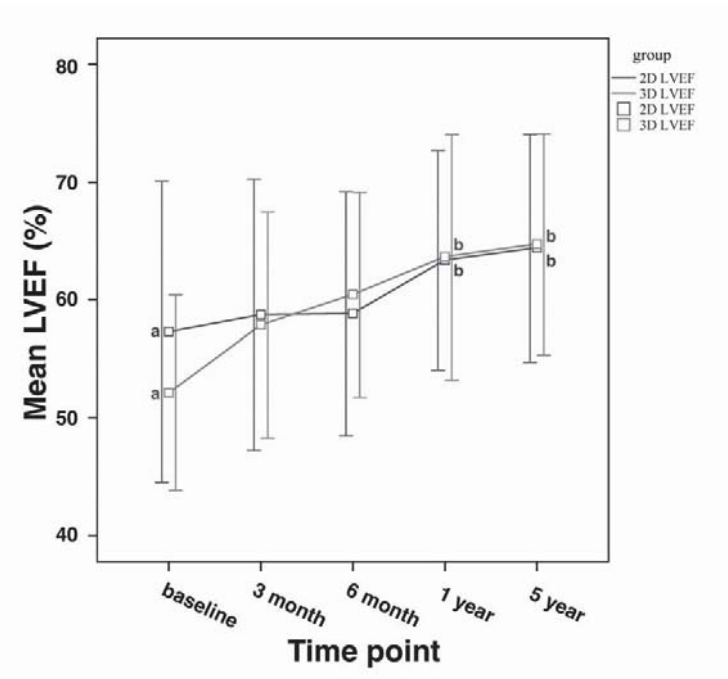
Table 3. Time courses of clinical, functional & imaging results in 30 survival patients

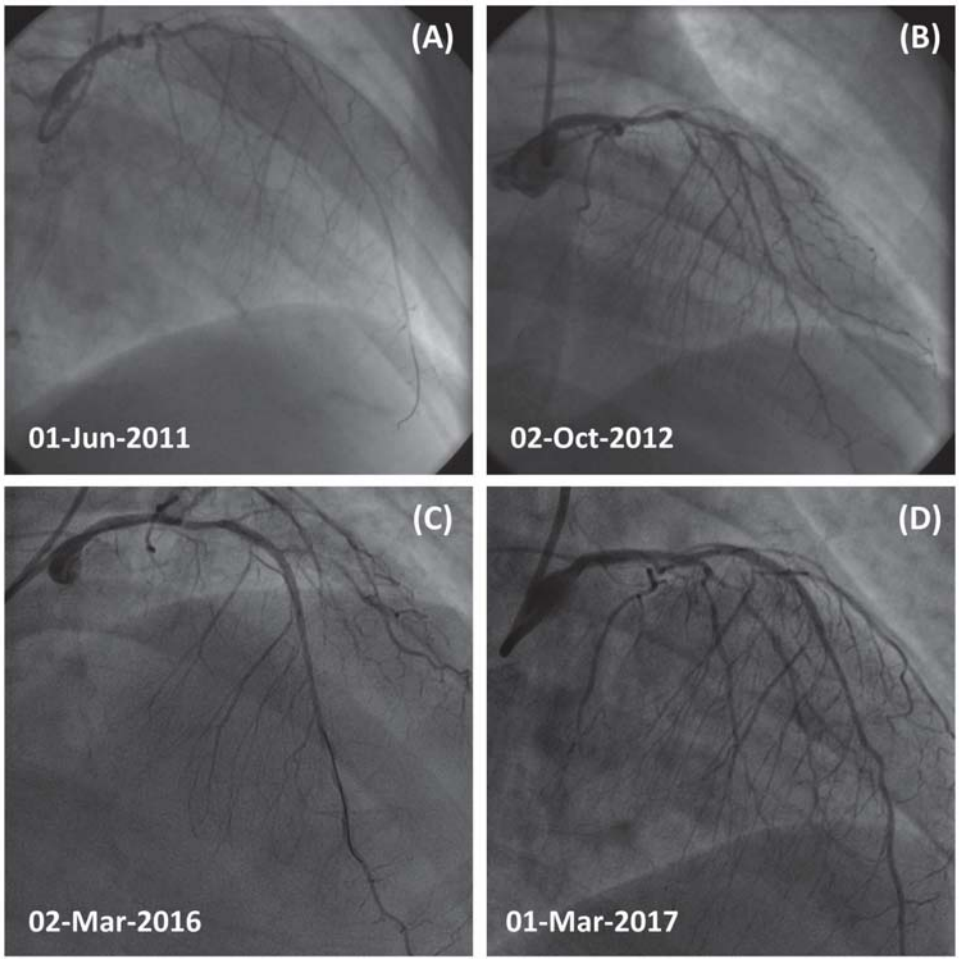
Variables	Baseline	1 year	5 years	P-value
Clinical and laboratory evaluation				
CCS angina score	2.4 ± 0.7[a]	0.2 ± 0.5[b]	0.7 ± 0.9[b]	<.001
NYHA class of HF	1.3 ± 1.2[a]	0.3 ± 0.5[b]	0.8 ± 1.1[c]	<.001
METs on CPET	4.8 ± 1.1	5.4 ± 1.6	---	0.046
Serum creatinine (mg/dL)	1.2 ± 0.4	1.4 ± 0.5	1.3 ± 0.6	0.394
Ccr (ml/min)	61.1 ± 20.6	55.2 ± 23.8	54.9 ± 23.9	0.089
Myocardial ischemia				
ST depression ≥1.0 mm on ECG†	33.3% (10/30)	13.3% (4/30)	16.7% (5/30)	0.109
Ischemia segments on thallium scan	3.5 ± 2.9	3.2 ± 2.7	---	0.285
LV chamber size				
2D LV EDD (mm)	52.1 ± 9.2	55.1 ± 6.6	53.8 ± 8.8	0.354
2D LV ESD (mm)	36.3 ± 9.6	35.7 ± 6.1	34.8 ± 8.5	0.230
3D LV EDV (mL)	91.1 ± 28.6[a]	74.8 ± 20.3[b]	68.3 ± 21.0[b]	<.001
3D LV ESV (mL)	44.3 ± 19.3[a]	27.9 ± 13.6[b]	25.0 ± 12.5[b]	<.001

Improvement in LV remodeling

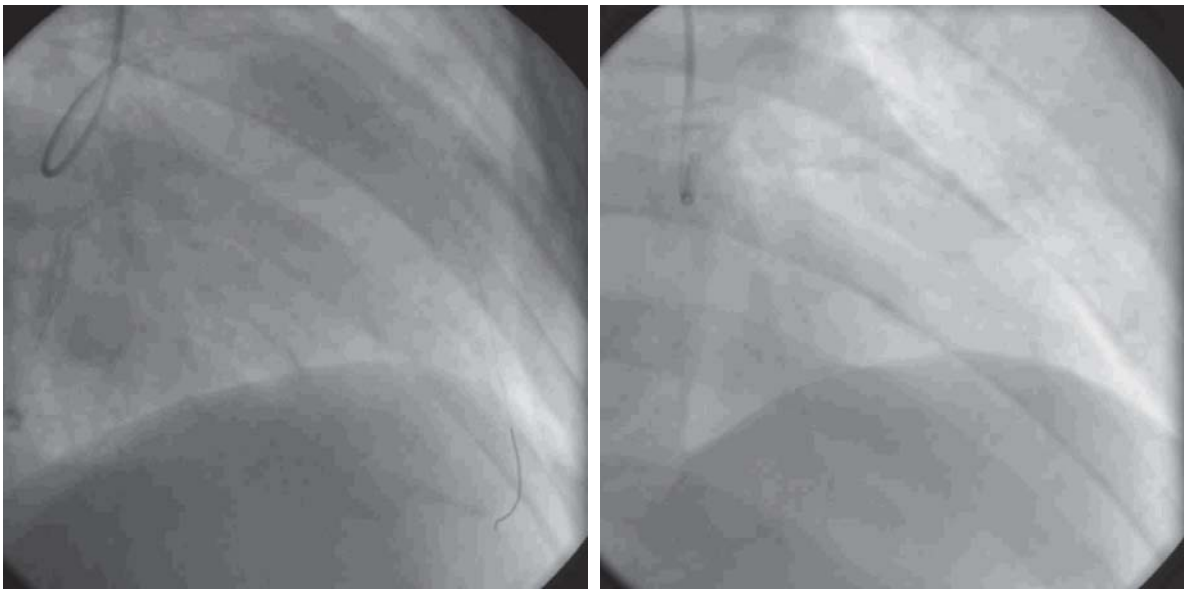
Table 3. Time courses of clinical, functional & imaging results in 30 survival patients

Variables	Baseline	1 year	5 years	P-value
LV function				
LVEF (%) measu by 2D	57.3 ± 12.8[a]	63.4 ± 9.3[b]	64.4 ± 9.7[b]	0.001
LVEF (%) measu by 3D	52.1 ± 9.1[a]	63.6 ± 10.4[b]	64.7 ± 9.4[b]	<.001



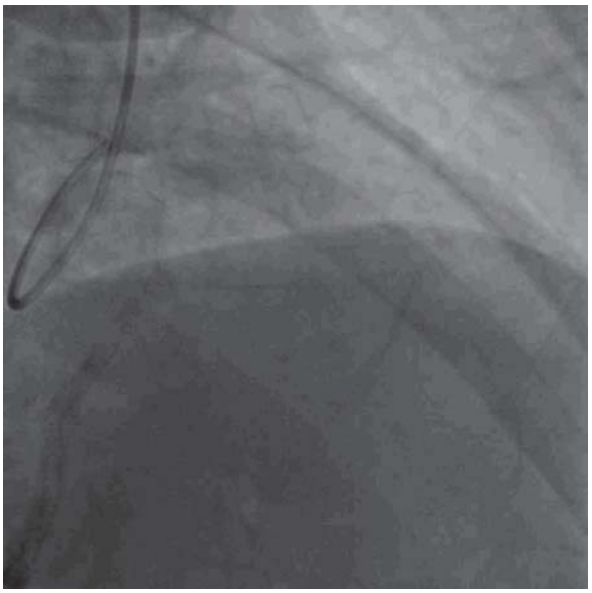


Baseline & at one-year F/U

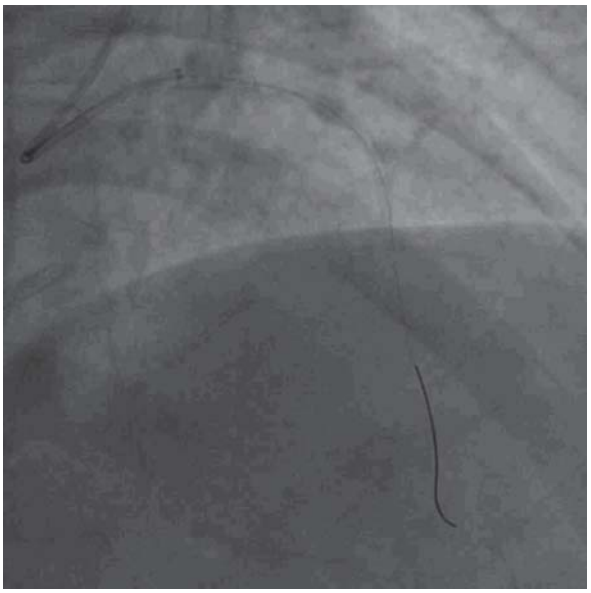


2011-06-01 2012-10-22

5th Year angiographic F/U & stenting

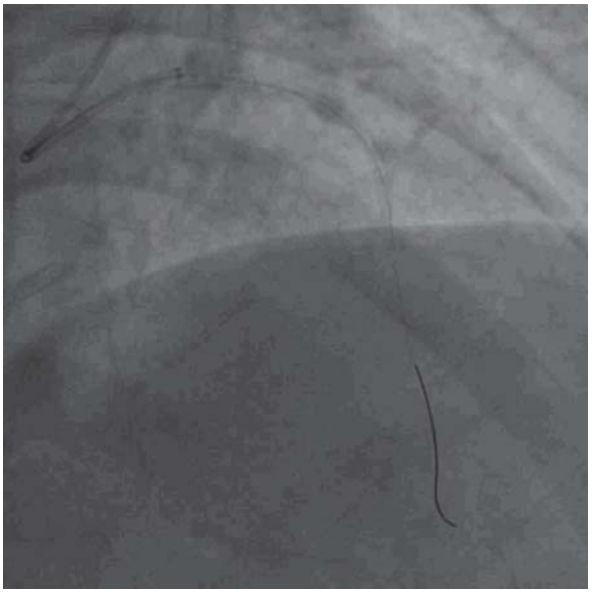


2016-03-02 Before stenting



2016-03-02 After stenting

5th & 6th year's Angiographic F/U, respectively



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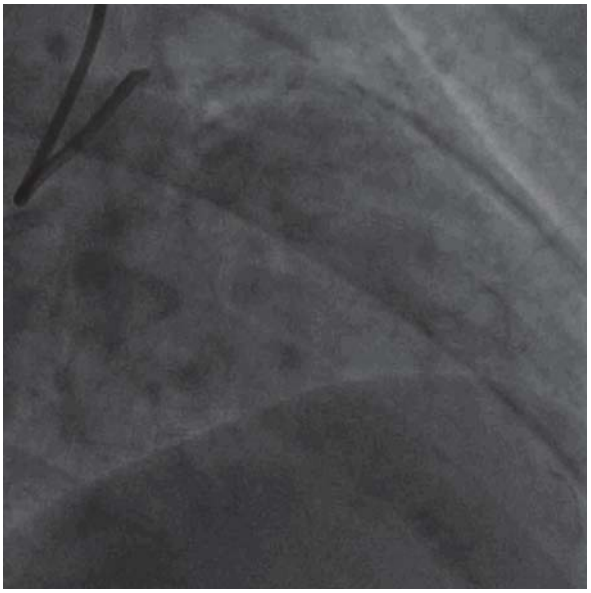


2017-03-01

Conclusion

- The results of long-term follow-up of phase I clinical with CD34+ cell therapy for diffuse CAD patients demonstrated that this therapy persistently improved heart function and frequency of angina, and reduced LV remodeling and heart failure with a favorable long-term prognostic outcome mainly through enhancing angiogenesis

Critical Care Medicine
Society of Critical Care Medicine

The Five-Year Clinical and Angiographic Follow-Up Outcomes of Intracoronary Transfusion of Circulation-Derived CD34+ Cells for Patients With End-Stage Diffuse Coronary Artery Disease Unsuitable for Coronary Intervention—Phase I Clinical Trial

Sung, Pei-Hsun, MD[1,2]; Lee, Fan-Yen, MD[3]; Tong, Meng-Shen, MD[1]; Chiang, John, Y., PhD[4,5]; Pei, Sung-Nan, MD[6]; Ma, Ming-Chun, MD[6]; Li, Yi-Chen, PhD[1]; Chen, Yung-Lung, MD[1]; Wu, Chiung-Jen, MD[1]; Sheu, Jiunn-Jye, MD[3]; Lee, Mel, S., MD, PhD[7]; Yip, Hon-Kan, MD[1,2,8,9,10]

Critical Care Medicine: May 2018 - Volume 46 - Issue 5 - p e411–e418
doi: 10.1097/CCM.0000000000003051
Online Clinical Investigations

Diffuse CAD—$CD34^+$ treatment

- **Phase II---**clinical trial—just finished and continuous F/U for one and five years
- **Phase III----**clinical trials– is just ongoing
- the first one of phase III clinical trial to be performed wordwild

Outline---2

- Intra-Carotid Arterial Transfusion of Autologous Circulatory Derived CD34+ Cells for **Ischemic Stroke Patients**—Phase I & II Clinical Trials

Stroke
2008 Jan;39(1):69-74. Epub 2007 Dec 6.

Level and Value of Circulating Endothelial Progenitor Cells in Patients After Acute Ischemic Stroke

Hon-Kan Yip, MD; Li-Teh Chang, PhD; Wen-Neng Chang, MD; Cheng-Hsien Lu, MD; Chia-Wei Liou, MD; Min-Yu Lan, MD; Josef S. Liu, MD; Ali A. Youssef, MD; Hsueh-Wen Chang, PhD

Table 1. Baseline Characteristics of Stroke, At-Risk Control, and Normal Control Groups

Variables	Study Patients (n=138)	At-Risk Control (n=40)	Normal Control (n=20)	P
Age, y (mean±SD)	66.3±10.4	66.5±9.5	67.1±9.3	0.945
Male, % (n)	65.9% (91)	62.5% (25)	60.0% (12)	0.831
Hypertension, % (n)	71.7% (99)	67.5% (27)	...	0.804
Diabetes mellitus, % (n)	42.8% (59)	42.5% (17)	...	1.0
Current smoking, % (n)	23.9% (33)	22.5% (9)	...	1.0
Hypercholesterolemia, % (n)	42.1% (58)	45.0% (18)	...	0.847
Body mass index	24.7±2.7	24.9±2.9	...	0.675
Previous stroke by history, % (n)	37.0% (51)	...	...	
Previous stroke by MRI, % (n)	50.7% (70)	...	...	
Significant ECCA stenosis	13.8% (19)	...	...	
Creatinine (mg/dL)	1.0±0.3	1.1±0.2	1.1±0.2	0.062
White blood cell count (×10³/mL)*	8.0±2.4†	6.2±1.0‡	5.9±0.6‡	<0.0001
NIHSS at 48 hours ≥12, % (n)	50.0% (69)	...	...	
Circulating level of EPCs				
CD31/CD34 (%)*	2.25±2.17†	1.34±0.93‡	1.56±0.78†‡	0.035
CD62E/CD34 (%)*	2.25±2.20†	1.25±0.99‡	1.55±0.76†‡	0.028
KDR/CD34 (%)*	2.17±1.94†	1.35±0.95‡	1.38±0.90†‡	0.025
Statin therapy	45.7% (63)	50.0% (20)	...	0.627
ACEI therapy	34.1% (47)	32.5% (13)	...	0.854
Recurrent stroke % (n)	2.2% (3)	...	...	
90-day mortality % (n)	8.0% (11)	...	...	

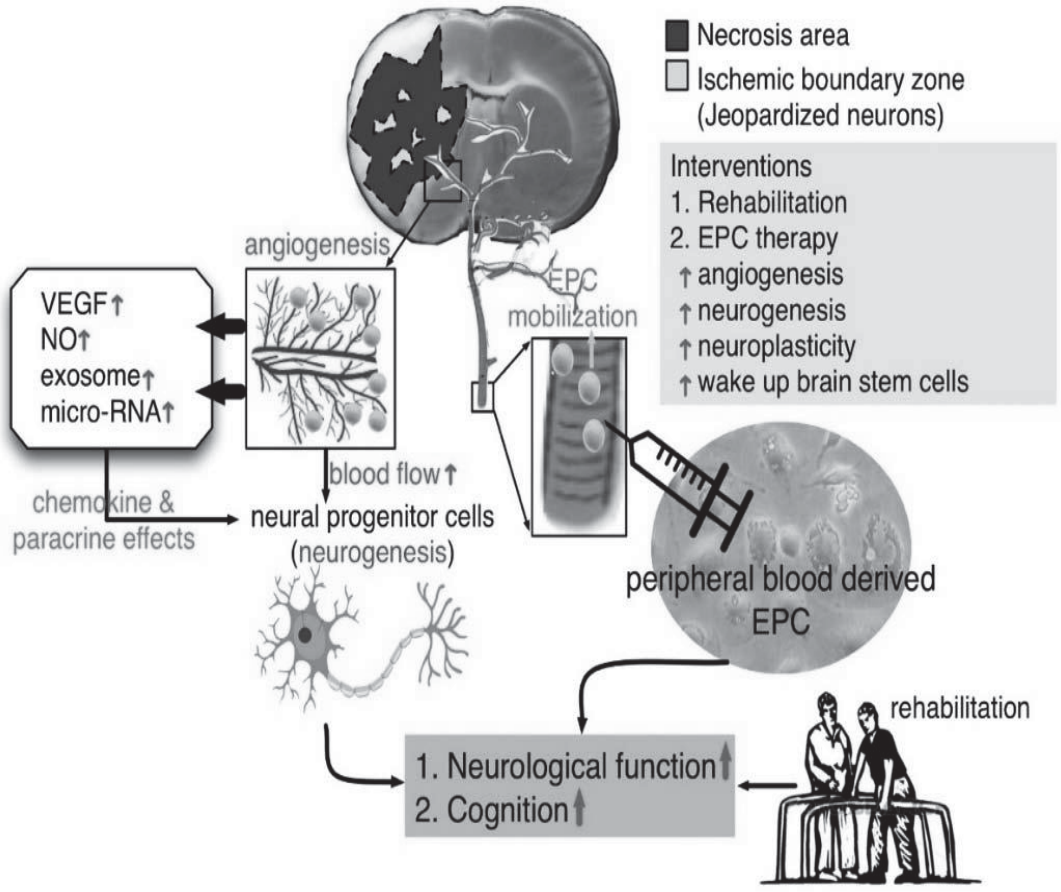
Data are expressed as mean±SD or percent (no.) of patients.
*Arsine transformation of EPC expression and log transformation of white blood cell count were used to improve the normality for statistical analysis.
†,‡Significant difference (at 0.05 level) by Tukey multiple comparison procedure.
ECCA indicates extracranial carotid artery; ACEI, angiotensin-converting enzyme inhibitor.

Table 2. Comparison of Baseline Characteristics Between Patients with NIHSS ≥12 and Patients With NIHSS <12 at 48 Hours After Ischemic Stroke

Variables	NIHSS ≥12 (n=69)	NIHSS <12 (n=69)	P
Age, y (mean±SD)	65.8±11.0	66.8±9.9	0.597
Male, % (n)	68.1% (47)	63.8% (44)	0.590
Hypertension, % (n)	69.6% (48)	73.9% (51)	0.571
Diabetes mellitus, % (n)	42.0% (29)	43.5% (30)	1.0
Current smoking, % (n)	23.2% (16)	23.0% 20	0.438
Hypercholesterolemia, % (n)	42.0% (29)	42.0% (29)	0.865
Body mass index	24.7±2.7	24.9±2.9	0.675
Previous stroke by history, % (n)	37.7% (26)	36.2% (25)	0.86
Previous stroke by MRI, % (n)	47.8% (33)	53.6% (37)	0.496
Significant ECCA stenosis	17.4% (12)	10.1% (7)	0.217
Creatinine (mg/dL)	0.97±0.26	1.01±0.26	0.327
White blood cell count (×10³/mL)	8.7±2.7	7.3±1.9	0.001
Stain therapy	43.5% (30)	47.8% (33)	0.608
ACEI therapy	34.8% (24)	33.3% (23)	0.857
Circulating level of EPCs			
CD31/CD34 (%)	1.54±1.26	2.95±2.62	0.0002
CD62E/CD34 (%)	1.55±1.52	2.94±2.55	0.0003
KDR/CD34 (%)	1.53±1.29	2.81±2.25	0.0002

Data are expressed as mean±SD or percent (no.) of patients.
ECCA indicates extracranial carotid artery; ACEI, angiotensin-converting enzyme inhibitor.

The increase in circulating EPC was strongly associated with favorable clinical outcome after IS.



This phase I clinical trial:

- To address the **safety** of CD34+ cell therapy for **old ischemic stroke (IS)** (IS>6 months, 8.6±6.4 years) patients (n=9)
- To evaluate the neurological function after the therapy.

Table 1. Baseline characteristics of 9 old ischemic stroke patients

Variables	% (n) or mean ± SD
Age (yrs.) (mean ± SD)	62.8 ± 10.6
Male gender (%)	88.9% (8)
Current smoking (%)	33.3% (3)
Hypertension (%)	100% (9)
Dyslipidemia (%)	33.3% (3)
Diabetes mellitus (%)	22.2% (2)
Mean ischemic stroke period prior to cell therapy (yrs.)	8.6 ± 6.4
History of old myocardial infarction (%)	0% (0)
Body mass index (mean ± SD)	23.7 ± 2.3
Atrial fibrillation (%)	11.1% (1)
Obstructive coronary artery disease (≥50% stenosis) (%)	88.9% (8)
History of coronary artery intervention (%)	88.9% (8)
Medication (%)	
Antiplatelet agent (%)	100% (9)
ACEI/ARB (%)	33.3% (3)
Beta-blocker agent	44.4% (4)
Statin (%)	77.8% (7)
New oral coagulant agent (%)	11.1% (1)
Calcium channel blocker (%)	11.1% (1)
Laboratory parameters	
Red blood cell count ($x10^6$)	4.53 ± 0.65
White blood cell count ($x10^3$)	6.6 ± 1.5
Platelet count ($x\ 10^3$)	214.5 ± 48.8
Hemoglobin (g/dL)	13.8 ± 1.6
Creatinine (mg/dL)	1.31 ± 0.51
Creatinine clearance rate (CCr) (ml/min)	63.3 ± 24.5

ACEI = angiotensin converting enzyme inhibitor; ARB = angiotensin II type I receptor blocker.

Table 2. Clinical outcomes of 9 patients after circulatory autologous CD34+ cell therapy

Patient list (No)	NIHSS	MRS	Barthel index	Clinical F/U[M]†	CASI score
No. 1	8‡	x	x	24 (S)*	
Cell therapy[0D]	3	4	50 ↑		66.3 ↑
Cell therapy[6M]	3	3	65		78.5
No. 2	10‡	x	x	24 (S)*	
Cell therapy[0D]	6	2	95 ↑		82.9 ↑
Cell therapy[6M]	6	2	100		90.7
No. 3	13‡	x	x	21 (S)*	
Cell therapy[0D]	5	3	35 ↑		72.2 ↑
Cell therapy[6M]	5	4	45		74.4
No. 4	10‡	x	x	21 (S)*	
Cell therapy[0D]	3	2	100		86.2 ↑
Cell therapy[6M]	3	2	100		97.5
No. 5	9‡	x	x	18 (S)*	
Cell therapy[0D]	6	2	95 ↑		91 ↑
Cell therapy[6M]	6	2	100		96.1
No. 6	9‡	x	x	15 (S)*	
Cell therapy[0D]	0	2	100		96
Cell therapy[6M]	1	2	100		89
No. 7	9‡	x	x	10 (S)*	
Cell therapy[0D]	5	4	35 **4/9= 44 %**		76.8 **5/9= 56 %**
Cell therapy[6M]	5	4	35		61.3
No. 8	10‡	x	x	9 (S)*	
Cell therapy[0D]	9	4	25		87.4
Cell therapy[6M]	9	4	25		
No. 9	9‡	x	x	7 (S)*	
Cell therapy[0D]	5	1	100		83.3
Cell therapy[6M]	4	1	100		

Table 3. Comparison of standard deviation of Tc-99m ECD brain perfusion SPECT study before (baseline) and after (at six-month) CD34+ cell therapy in 9 study patients

Variables	Median SD (Q1, Q3) (day 0)	Median SD (Q1, Q3) (6 M)	Median difference (Q1, Q3)	P*
Basal ganglia (L)	0.5 (-2.1, 1.7)	0.5 (-2.2, 1.8)	0 (-0.1, 0.1)	0.766
Basal ganglia (R)	0 (-2.5, 0.4)	0.1 (-2.2, 1.3)	-0.3 (-0.7, 0.2)	0.172†
Central region (L)	-0.5 (-2.0, 2.2)	-0.5 (-1.2, 2.6)	-0.2 (-0.8, 0)	0.188†
Central region (R)	-0.8 (-2.3, 0.3)	-0.5 (-2.2, 1.1)	-0.2 (-0.7, 0.4)	0.406
Cingulate & paracingulate gyri (L)	-0.1 (-1.4, 1.2)	0.1 (-0.8, 1.5)	-0.3 (-0.5, 0.3)	0.438
Cingulate & paracingulate gyri(R)	0.4 (-2.2, 0.8)	-1.6 (-2.5, 0.4)	-0.3 (-0.7, 0)	0.250
Frontal lobe (L)	-0.6 (-1.4, 2.8)	0.4 (-0.9, 2.5)	-0.9 (-1.0, -0.5)	0.008†
Frontal lobe (R)	-1.3 (-1.7, 0.3)	-0.8 (-1.2, 1.0)	-0.5 (-0.7, -0.3)	0.094†
Occipital lobe (L)	0.7 (-0.8, 1.9)	1.1 (-0.1, 2.0)	-0.5 (-0.7, -0.63)	0.109†
Occipital lobe (R)	0.3 (0, 1.8)	1.0 (0.6, 1.9)	-0.6 (-0.7, -0.5)	0.078†
Parietal lobe (L)	-0.4 (-0.9, 2.0)	0.1 (-0.2, 1.5)	0 (-0.2, 0.3)	0.945
Parietal lobe (R)	-0.1 (-0.6, 1.4)	0.6 (-0.5, 2.0)	-0.2 (-1.2, 0.1)	0.313
Temporal lobe (L)	0.1 (-0.5, 1.8)	0.2 (0, 1.4)	-0.3 (-0.8, 0.4)	0.590
Temporal lobe (R)	-0.4 (-0.4, 1.5)	0.9 (-0.1, 1.5)	-0.3 (-1.3, 0.3)	0.320

*: by Wilcoxon Sign-rank test for paired data.

L = left; R = right, M = month; SD = standard deviation.

† Great response: defined as the p value<0.2

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Conclusion

- The therapy was **100% safe** and all of our study patients survive without recurrent IS and are still followed up at outpatient department.
- The **6-month neurological function (i.e., Barthel index)** was notably improved up to more than **40%** of the study patients after receiving CD34+ cell therapy.
- Consistent with neurological function test, **the 6-month neuro-psychological test (i.e., CASI) was also notably improved** among these patients with the cell therapy.

→Intra-carotid artery administration of CD34+ cells is safe & may offer some potential benefit for old IS patients

Phase II clinical trial of $CD34^+$ treatment for acute ischemic stroke

IS on going!!!

Outline---3

- Peripheral blood-derived stem cell/progenitor cell ($CD34^+$) therapy on **chronic kidney disease**--Phase 1 and 2 clinical trials

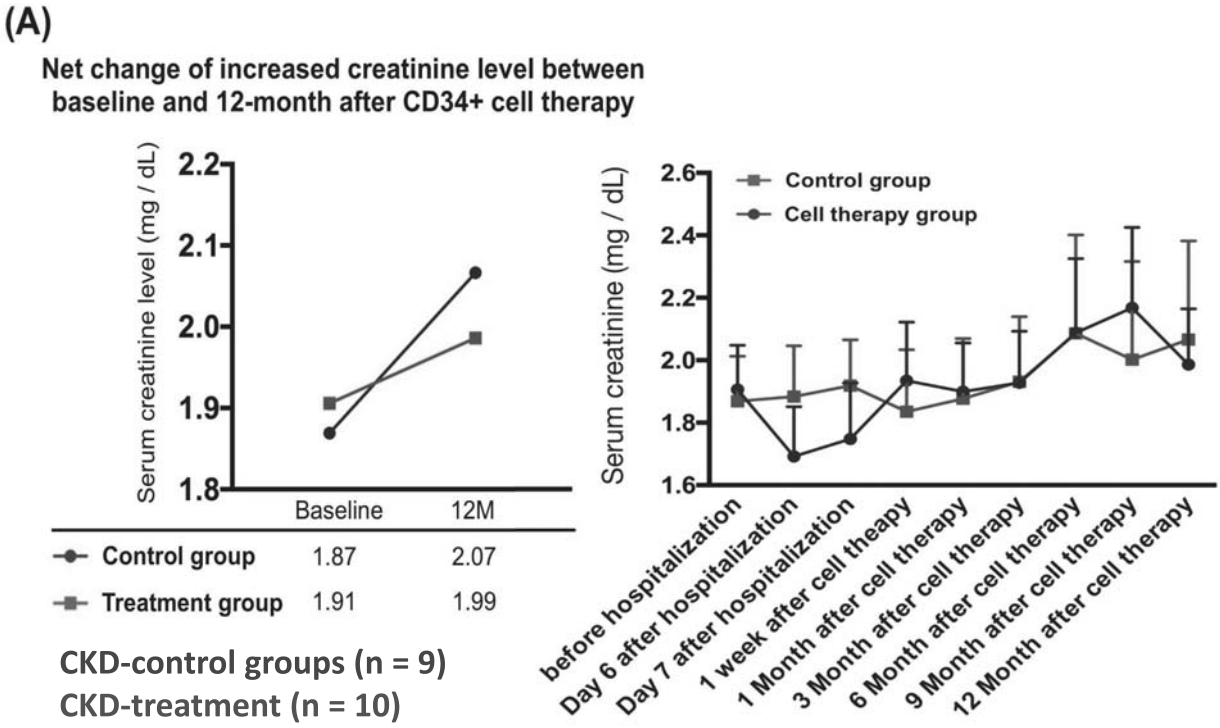
Enrollment and Allocation

November 2014 and October 2015 (1-year follow-up)
27 eligible subjects with advanced CKD

CKD-treatment group (n=10) → G-CSF s.c. injection (5μg/kg twice daily for 4 days)
CD34+ cells **($5.0x10^7$)** into **R't renal artery**

CKD-control group (n=9) → Screening failure (i.e., refused EPC Tx)
in the initial state of eligibility assessment

Serial changes of creatinine levels between CKD-control and CKD-treatment groups during one-year follow-up



Clinical follow-up

HTN 100%, DM 70%, dyslipidemia 80%, CAD 70%/MVD.
100% RAAS-blockade use.

- One-year survival rate was 100%.
- However, two patients underwent PCI for acute NSTEMI at about 8 months after CD34+ cell therapy (Killip-1 in one patient and Killip-3 in the other patient, respectively)
- The NSTEMI patient with Killip-3 upon presentation at the time interval after 12 months of CD34+ cell therapy developed ESRD on regular H/D after PCI mainly due to contrast-induced nephrotoxicity.
- These two patients remain regular follow-up at outpatient department.

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Research Paper

Investigated the safety of intra-renal arterial transfusion of autologous CD34+ cells and time courses of creatinine levels, endothelial dysfunction biomarkers and micro-RNAs in chronic kidney disease patients—phase I clinical trial

Mel S. Lee[1,*], Fan-Yen Lee[2,*], Yung-Lung Chen[3], Pei-Hsun Sung[3], Hsin-Ju Chiang[4], Kuan-Hung Chen[5], Tien-Hung Huang[3], Yi-Ling Chen[3], John Y. Chiang[6,7], Tsung-Cheng Yin[1], Hsueh-Wen Chang[8], Hon-Kan Yip[3,9,10,11,12]

[1]

Conclusion

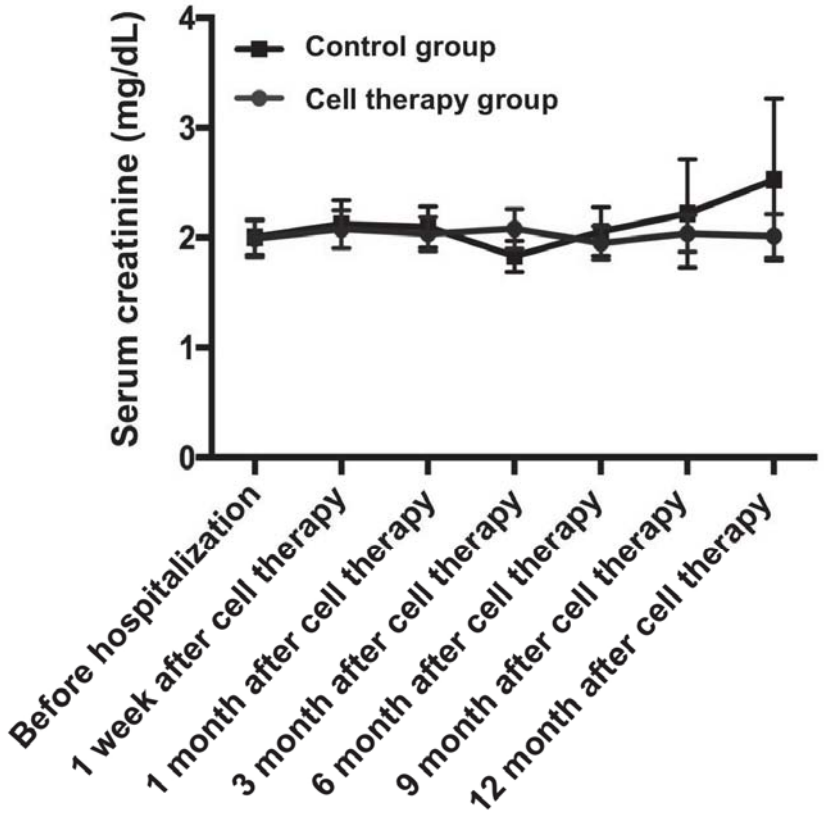
- In conclusion, CD34+ cell therapy was **safe** and maintained the renal function in stationary state at the end of study period.

- Based upon the safety of this phase I clinical trial, we are now going on the phase II clinical trial with the same therapeutic management for advanced CKD.

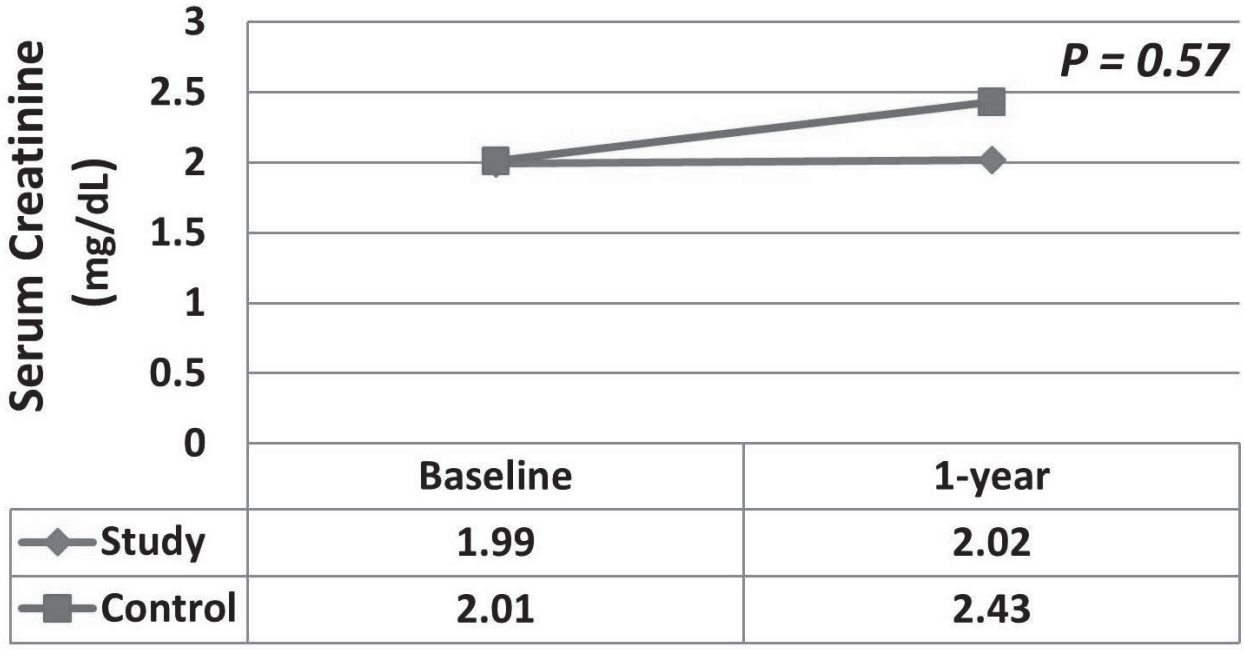
Result of **Phase 2** CKD Clinical Trial

Table 3. Comparison of renal function, albuminuria, and proteinuria at different time points							
Variables	**Baseline**	**1-month**	**3-month**	**6-month**	**9-month**	**12-month**	**P-value**
CrCl (mL/min)						*P = 0.974	
Study	39.4 ± 12.6	37.7 ± 11.3	39.3 ± 14.0	39.0 ± 12.2	37.5 ± 11.6	39.1 ± 12.5	0.385
Control	38.7 ± 12.4	37.6 ± 12.9	41.5 ± 10.9	37.9 ± 12.7	38.0 ± 10.4	39.0 ± 13.7	0.338
BUN (mg/dL)						*P = 0.555	
Study	27.4 ± 6.8	28.1 ± 9.2	29.4 ± 11.1	29.1 ± 7.5	31.2 ± 11.0	30.1 ± 8.4	0.170
Control	33.7 ± 17.2	32.9 ± 15.7	28.8 ± 11.5	31.6 ± 17.7	37.1 ± 27.8	33.5 ± 19.9	0.758
Serum Creatinine (mg/dL)						*P = 0.609	
Study	2.0 ± 0.8	2.0 ± 0.7	2.1 ± 0.8	2.0 ± 0.7	2.0 ± 0.8	2.0 ± 0.7	0.328
Control	2.0 ± 0.7	2.1 ± 0.8	1.9 ± 0.6	2.0 ± 0.8	2.2 ± 1.6	2.3 ± 1.5	0.103
Urine ACR						*P = 0.220	
Study	1101.8	966.7	1180.0	1481.8	1857.4	1999.9	0.214
Control	931.5	960.9	1035.0	828.7	782.2	879.5	0.756
Urine PCR						*P = 0.207	
Study	643.0	563.4	615.4	736.8	1149.2	1151.6	0.303
Control	441.1	476.1	529.8	385.1	266.5	448.2	0.948

*P indicates comparison between study and control groups with independent t test.



Serial Change of Serum Creatinine



	Baseline	1-year
Study	1.99	2.02
Control	2.01	2.43

Conclusions

- In phase 2 Clinical Trial with CD34+ treatment for CKD
- It seems to be negative results in this clinical trial

1. Still not yet to complete F/U,
2. Small sample size distort the statistical significance
3. The deteriorated creatinine level is slowly. So, it needs more time to F/U

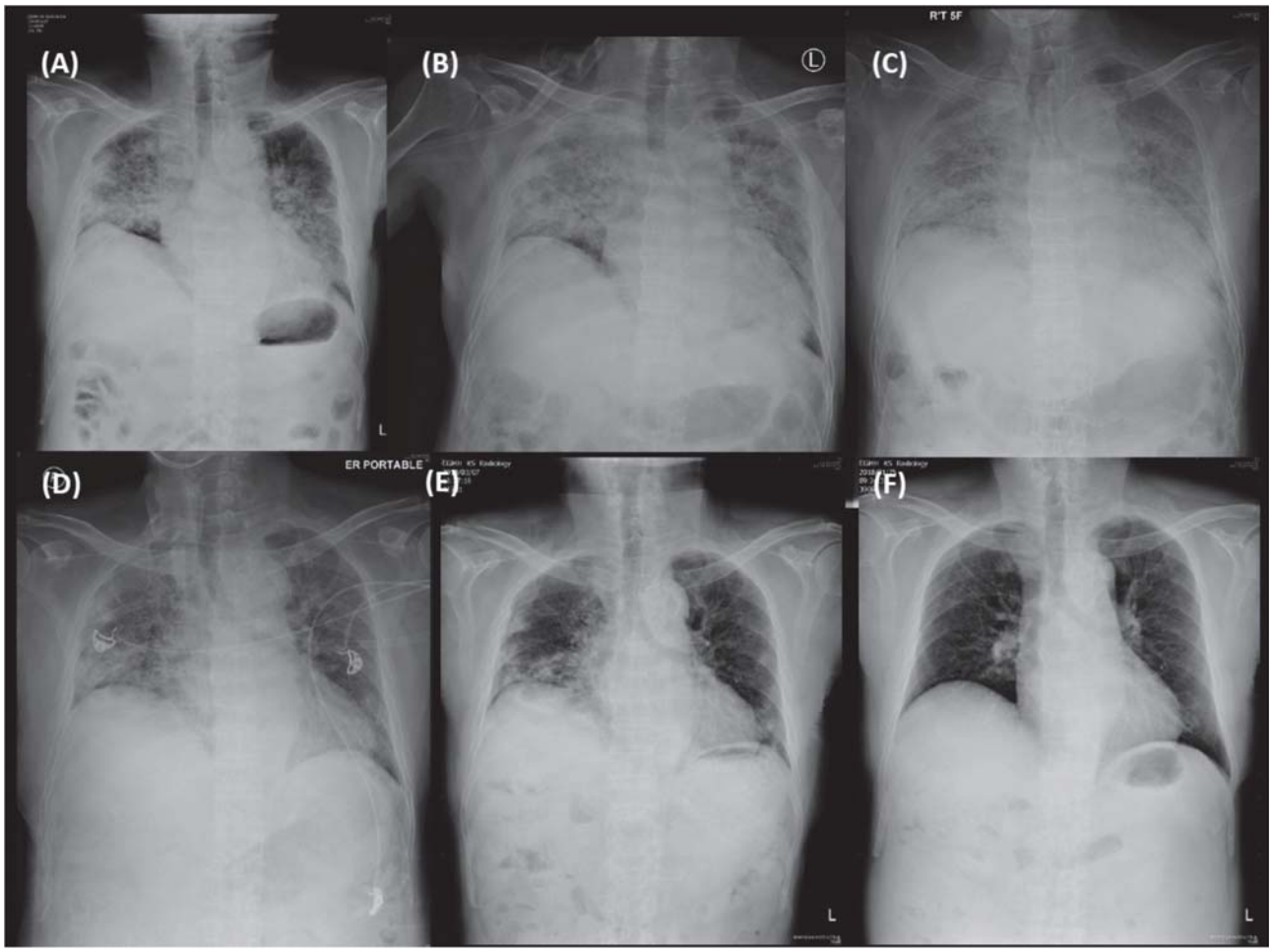
Outline---4

- **Combination Autologous Peripheral blood-derived stem cell/progenitor cell (CD34+) + Stem Cells & Hyperbaric Oxygen Therapy for Severe Limb Ischemia: investigate the safety and efficacy**

1. PAOD—Phase I clinical trial
2. Just is on going

Outline---5

- **Application of Human Umbilical Cord-Derived Mesenchymal Stem Cells for Severe Acute Respiratory Distress Syndrome and Patients with Profound septic shock complicated with multiple organ failure—Phase I clinical trial**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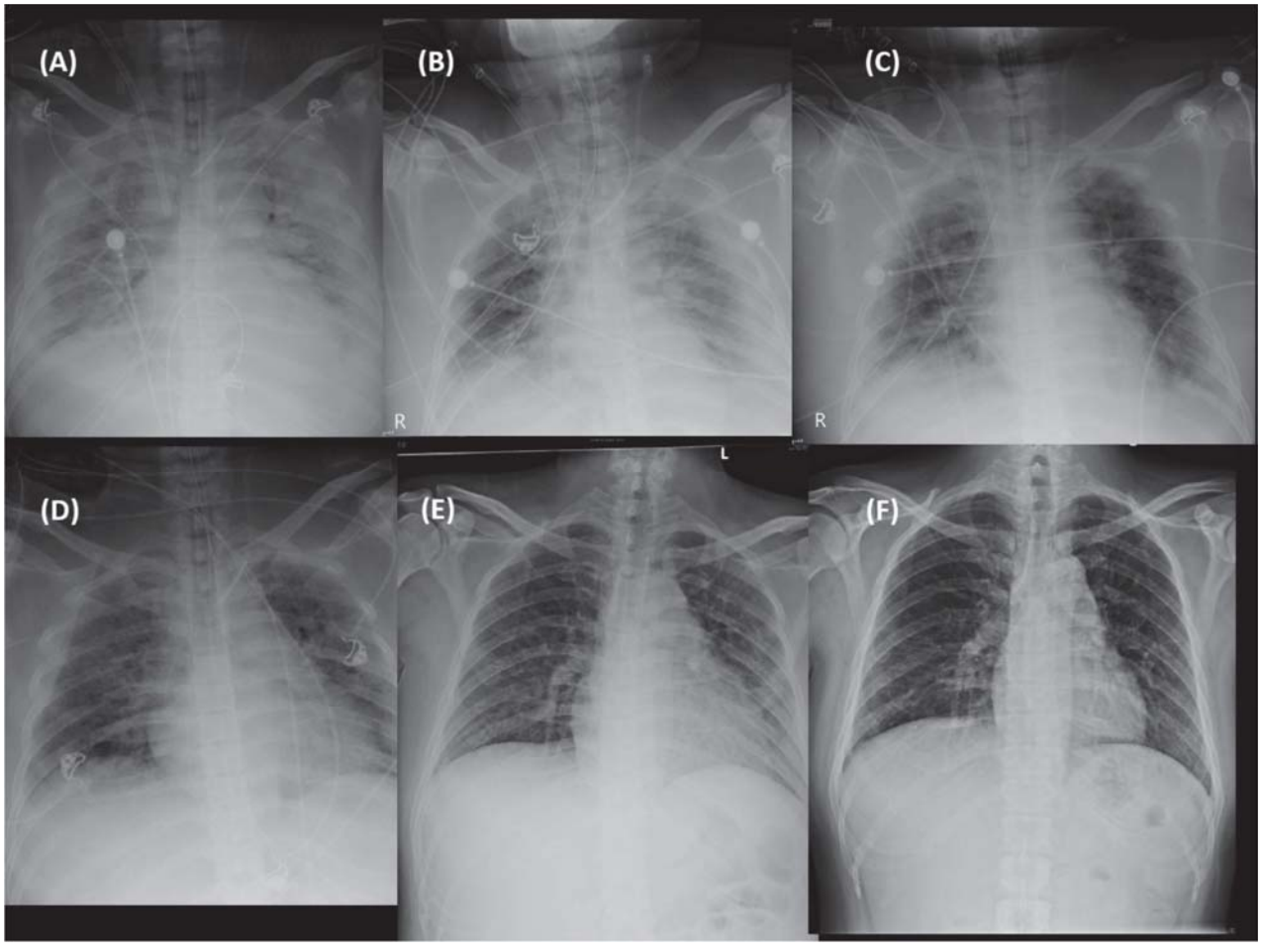
(A)
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Is still on going now,

- 5 very severe ARDS/Sepsis patients were prospectively enrolled
- 2 died and 3 survived & uneventfully be discharged as well as at OPD F/U
- Conclusion: Allogenic MSC may have therapeutic potential for those of critical ARDS/sepsis patients

Acknowledgement

Clinical Trial Division