新興生醫產品之應用
幹細胞軟骨再生

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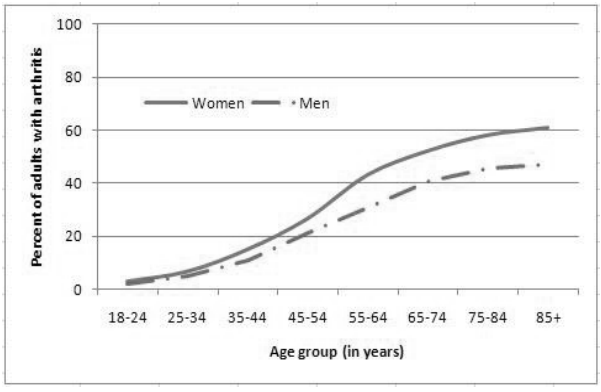
退化性關節炎（Osteoarthritis，OA）

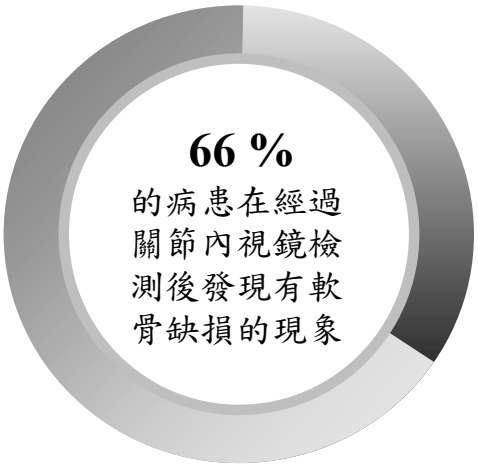
- 退化性關節炎是中老年人常見的疾病，以膝關節最為常見，因關節長期使用或外來因素的破壞，使關節軟骨產生碎片剝落，進而出現發炎、疼痛、變形等症狀。
- 全球統計，≥ 60歲的老年人中，9.6 %男性及18 %女性患有OA（約7,580萬人），平均罹患年齡逐年降低。
- OA所造成的醫療花費巨大，每年估計佔已開發國家（美英澳法等）約1-2.5 %的國民生產總值（GNP）。

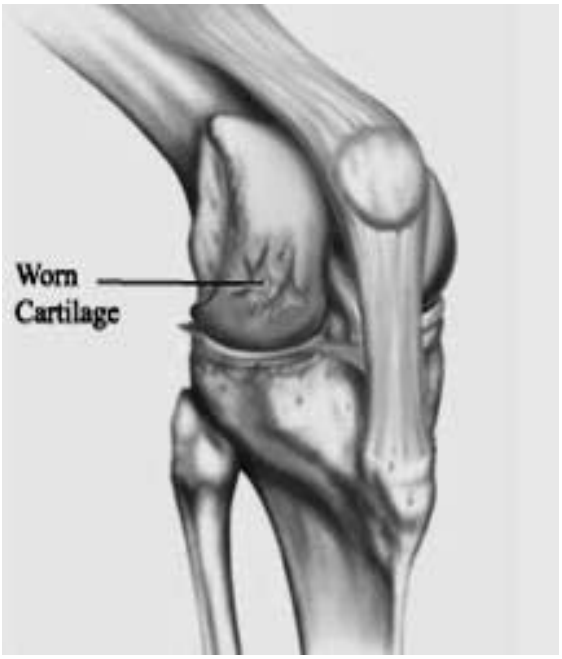


NHIS，2007-2009

March, L.M. & Bachmeier, C.J. Economics of osteoarthritis: a global perspective. Baillieres Clin Rheumatol 11, 817-834 (1997).
Wolf A.D. & Pfleger B. Burden of major musculoskeletal conditions. Bulletin of the World Health Organization, 81, 646-656 (2003).

軟骨缺損-退化性關節炎的主要病徵





- 關節軟骨本身無血管，導致
  - 缺乏血液中養分與氧氣的補給
  - 自我再生、癒合的功能不足
  - 一旦損傷，很容易引起OA

Curl WW, Krome J, Gordon ES, et al. Cartilage injuries: a review of 31,516 knee arthroscopies. Arthroscopy 1997;13:456-60.
Hjelle K, Solheim E, Strand T, et al. Articular cartilage defects in 1,000 knee arthroscopies. Arthroscopy. 2002;18:730-4.
Aroen A, Loken S, Heir S, et al. Articular cartilage lesions in 993 consecutive knee arthroscopies. Am J Sports Med. 2004;32:211-5.

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OA治療市場規模

- 全球OA治療市場：2009年為50億美金，2016年達到複合年成長率1.5 %（55億美金的規模）。
- 台灣OA健保支出：佔總支出的1.4 %（約78億新台幣），成長率6.7 %，於2013年佔總支出的第12位。
- 美國OA治療市場：2009年為26億美金，全球第一。
- 美國軟骨缺損治療市場：2007年5000-6000萬美金。
- 美國Genzyme Carticel 收益：
  - 1997 first quarter：110萬美金
  - 1998 first quarter：250萬美金
  - 1999 first quarter：400萬美金

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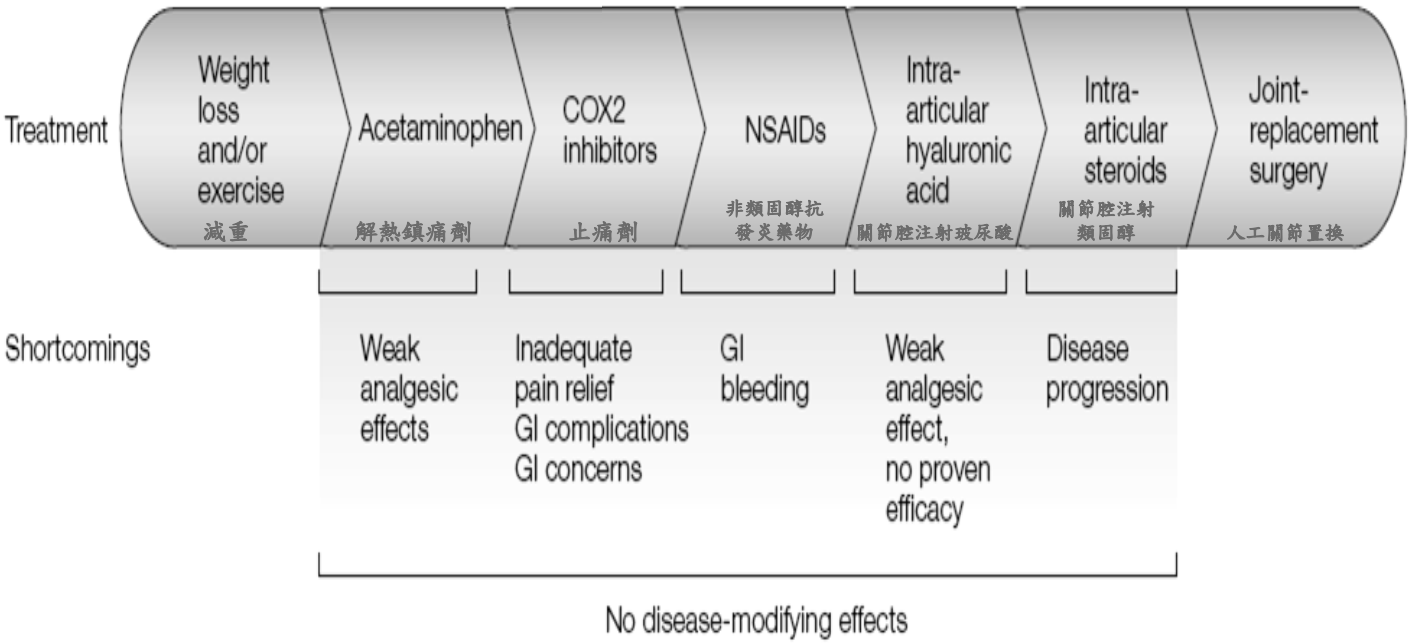
一般臨床治療流程



COX2：cyclooxygenase 2；NSAID：non-steroidal anti-inflammatory drug

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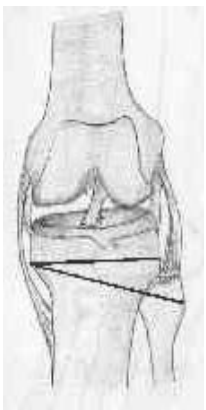
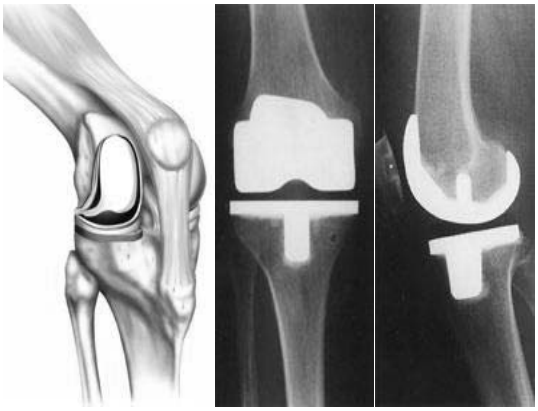
人工關節置換術



切骨術



單側或全關節人工關節置換

- 使用年限：約10-15年
- 可承重，但無法如正常關節般進行較劇烈的運動
- 年輕與喜好運動之患者的接受度低

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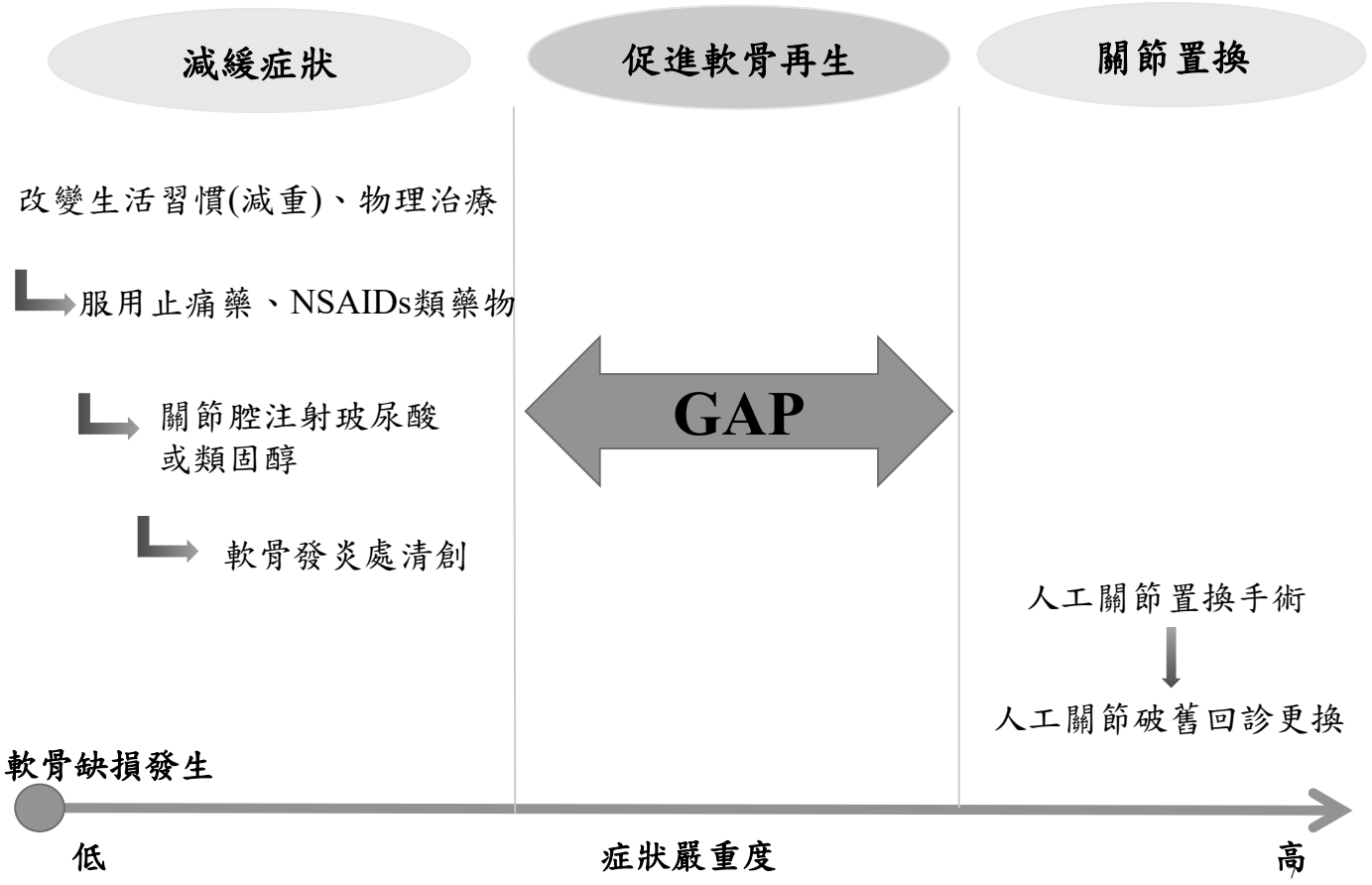
以再生醫學的方法銜接臨床治療的窘境



目前軟骨缺損的治療方法

1. 骨髓刺激術 (Marrow stimulation techniques)

- Multiple drilling
- Abrasion arthroplasty
- Microfracture

2. 自體骨軟骨柱鑲嵌移植術
(Autogenous osteochondral transplantation)

Mosaicplasty

3. 自體軟骨細胞移植術
(Autologous Chondrocyte Implantation, ACI)

理想軟骨移植物的要素

- 使用病患的自體細胞
- 有足量的軟骨細胞可用以修補大面積的缺損
- 使用正常軟骨以外的細胞來源
- 軟骨細胞能在移植處均勻分布
- 與周圍軟骨能夠緊密接合
- 能保有軟骨細胞的表現並生成透明軟骨

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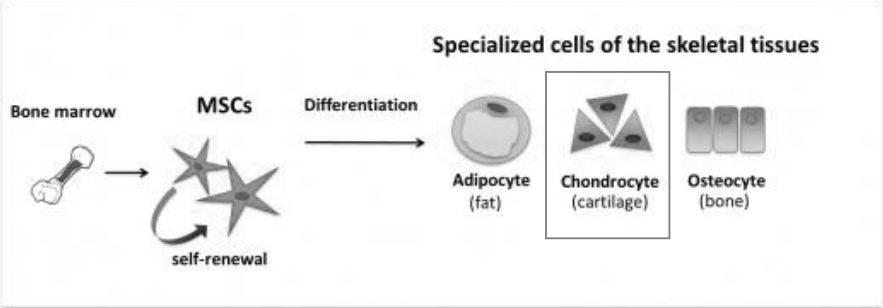
Kartigen®技術優勢-細胞來源

- 使用病患自體的間葉幹細胞（MSC）



- MSC具有再生與多元分化的性質
  - 可作為移植軟骨受損處細胞來源
  - 於移植前分化成為類軟骨細胞
- 獨有培養MSC的配方

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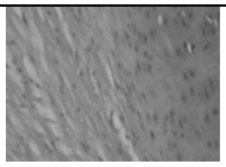
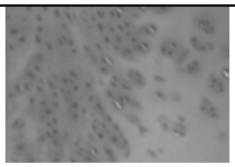
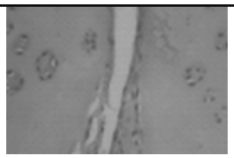
Kartigen®技術優勢-
MSC分化為類軟骨細胞後之高再生性

Pipeline

Stages	Stage 1	Stage 2	Stage 3	Stage 4
	MSCs still keep its phenotype	MSCs toward chondrogenesis but no ECM expression yet.	(Defined stage) Regulated cells started to produce ECM but no lacunar developed yet. The regulated cells in this stage were defined as chondrocyte-like cell; we may call it as immature chondrocyte as well.	MSCs were completely regulated into matured chondrocyte; where ECM and lacunar were all developed.
ECM expression	x	x	o	o
Lacunar formation	x	x	x	o
The picture of repair tissue and host tissue (the defect on swine articular cartilage)	 Before pre-chondrocyte, the MSCs still keep the pliable ability that would be differentiated into osteolinage cell and recovered as fibro-cartilage.		 If MSCs were regulated into the chondrocyte-like cell and implanted into the defect site, it would be fully recovered by hyaline cartilage.	 After MSCs comletely regulated into chondrocyte, it could not fully integrate with the host tissue.

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Kartigen®技術優勢-已榮獲多國專利

國別	專利編號	專利名稱
臺灣	I469993	修復軟骨損傷之外科移植物
美國	8,574,614	SURGICAL GRAFTS FOR REPAIRING CHONDRAL DEFECTS
歐盟*	2392358	SURGICAL GRAFTS FOR REPAIRING CHONDRAL DEFECTS
中國大陸	ZL2011 1 0034680.5	修復軟骨損傷之外科移植物
日本	特許第5764807号	軟骨欠損の修復に用いる外科用グラフト
新加坡	190660	SURGICAL GRAFTS FOR REPAIRING CHONDRAL DEFECTS

*另於歐洲八國（英國、法國、德國、波蘭、瑞士、瑞典、義大利、西班牙）也已擁有專利

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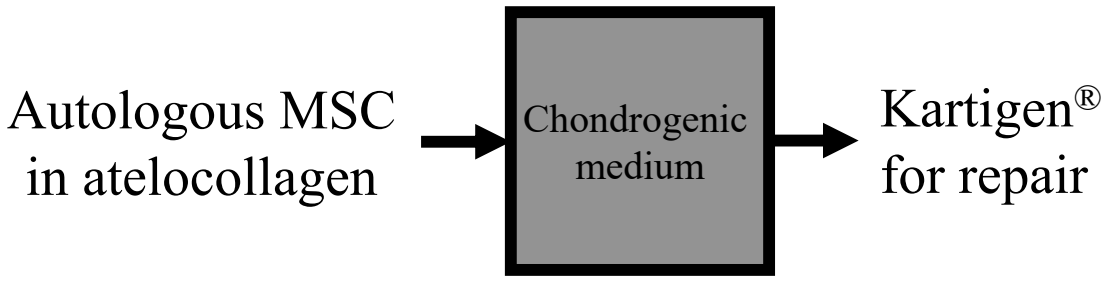
MSC-derived Chondrocyte Implantation



- One-step surgery procedure
- No need to harvest autologous chondrocyte from cartilage

(Hwa-Chang Liu et al. 2005)

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Patients' Profile

Grade IV chondral defect of osteonecrosis or osteoarthritis of the medial femoral condyle

Patient demographics	
Age (years)	66.4 (47-83)
Sex (M/F)	6/6
Knee (right/left)	7/5
Defect size (cm^2)	1.97 (0.91-3.14)

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- The non-operated knees were used as control.
- The result was analyzed by student's t test or Chi square as they could be applied.

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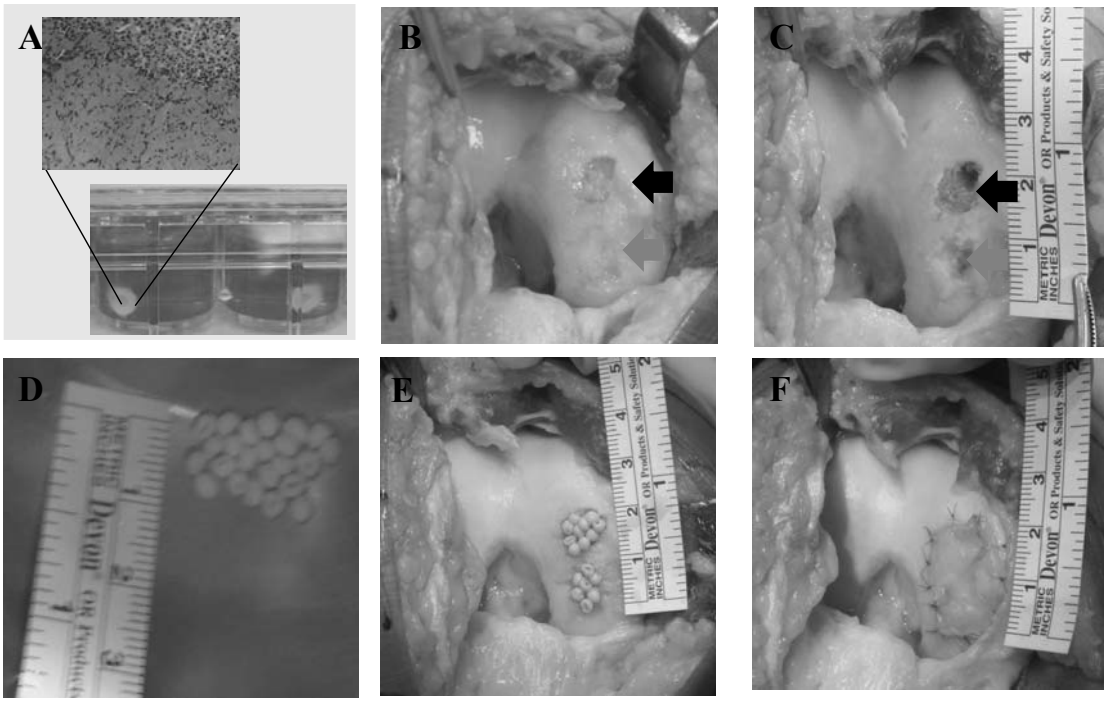
試驗流程



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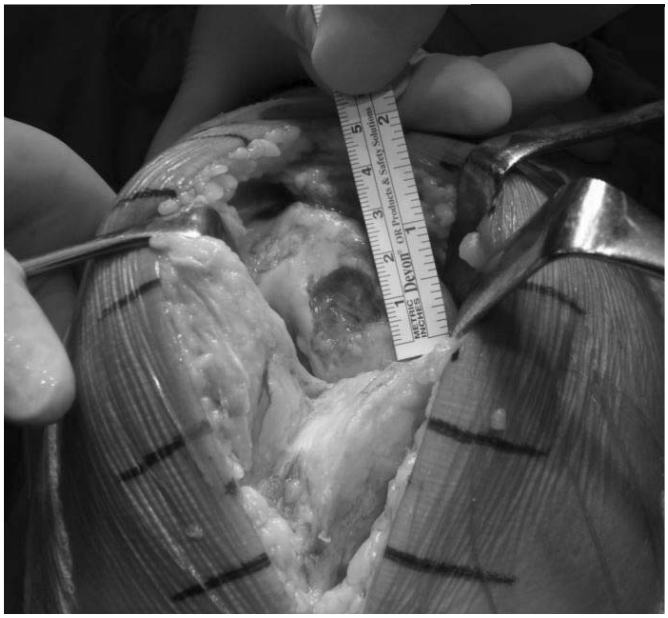
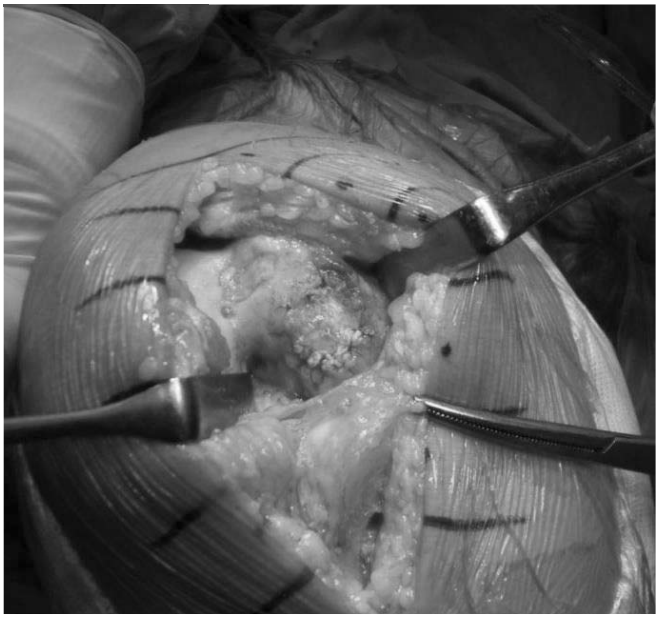
移植過程



1.2 cm * 1.8 cm大小的骨軟骨缺損於內側大腿骨髁



將軟谷原移植到缺損部位

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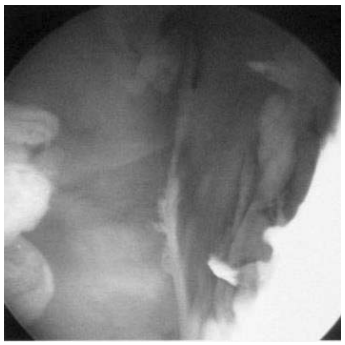
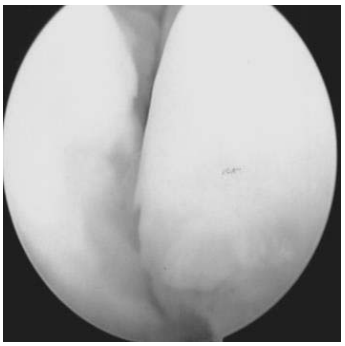
內視鏡-觀察軟骨外觀與硬度

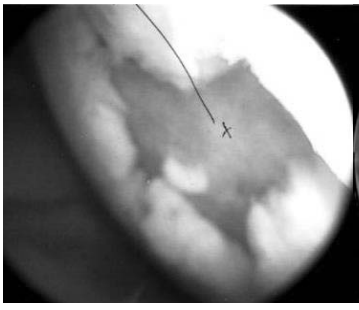
術前



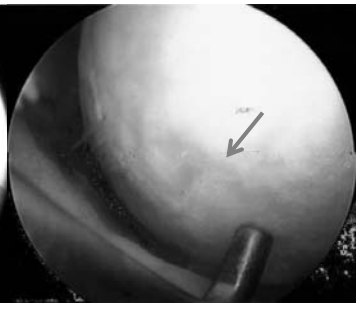
術後1.5 years



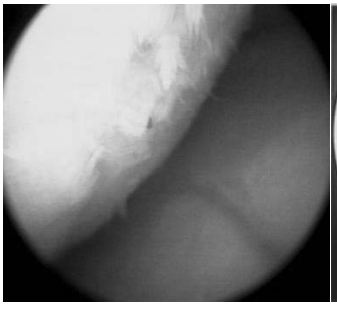
術前



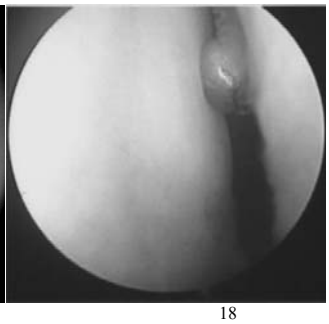
術後 1 year



術前



術後 1 year



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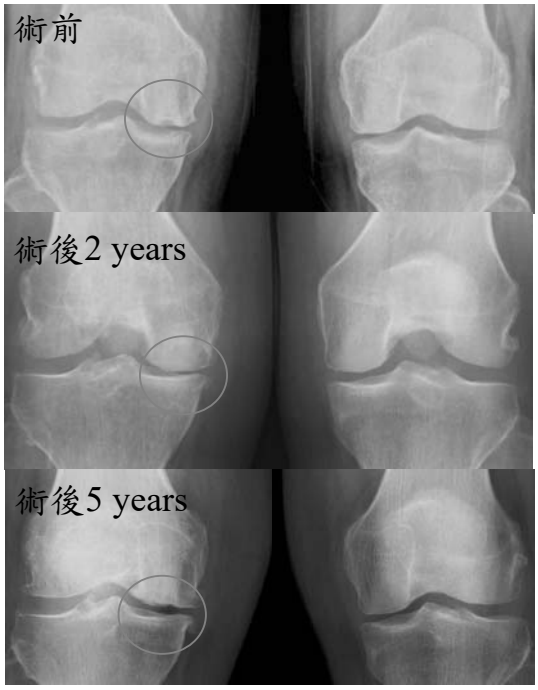
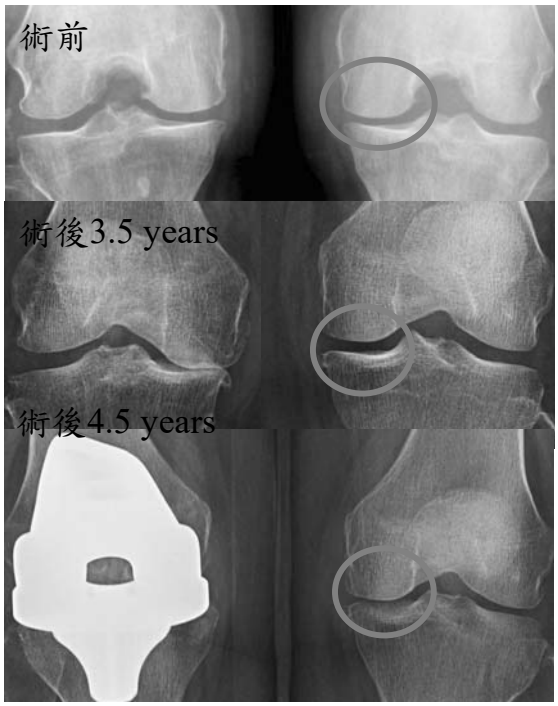
X光影像分析

修補後的膝蓋結果較未修補的膝蓋相當甚至更佳





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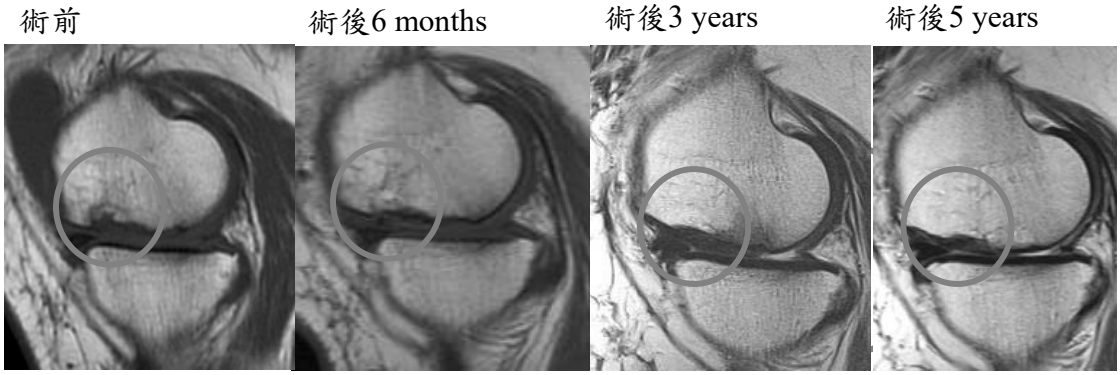
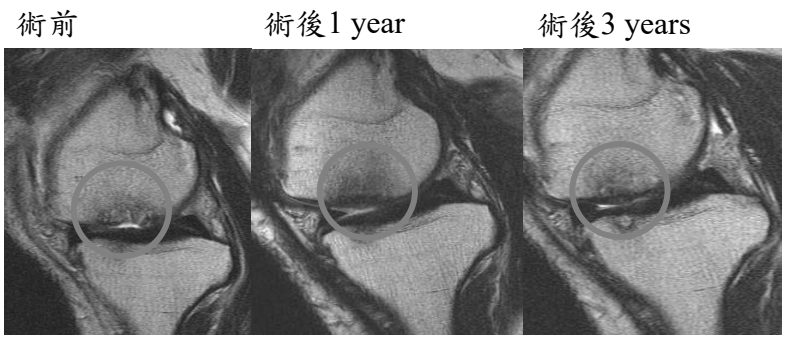
MRI影像分析





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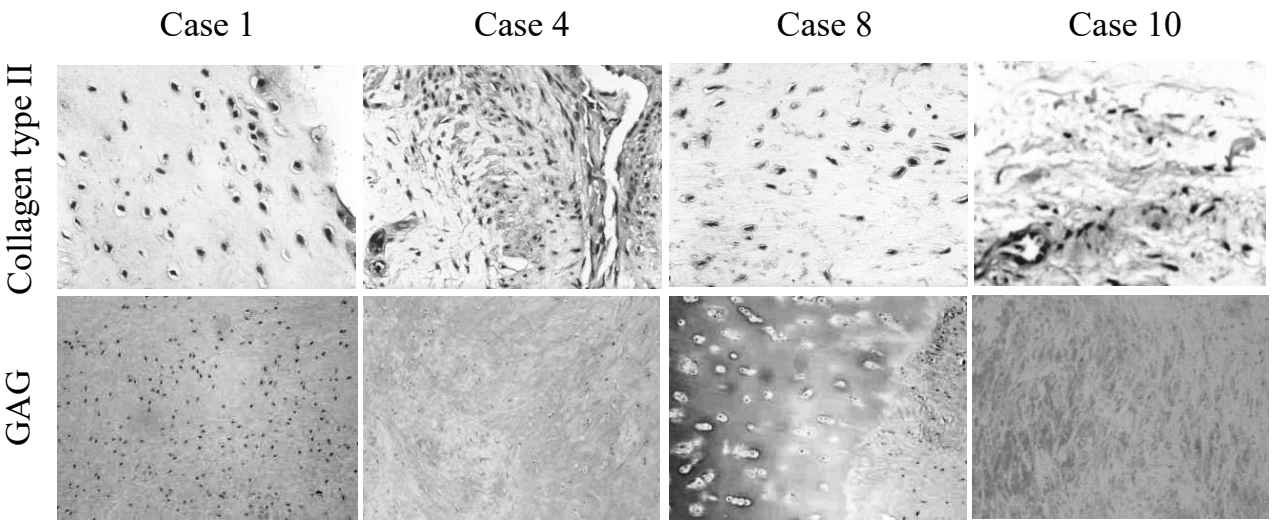
ECM of Repaired Cartilage



	Case 1	Case 4	Case 8	Case 10
GAG expression	+	+	+	+
Collagen type II	+	+	+	+

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Discussion

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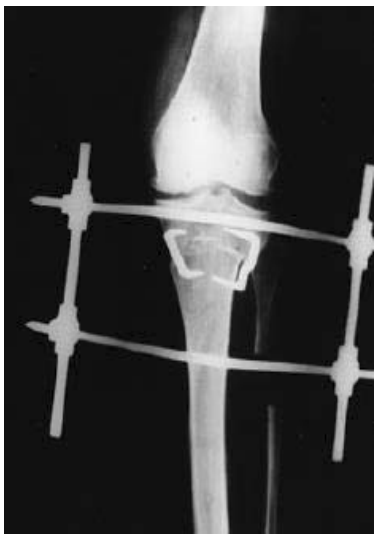
- No valgus osteotomy, nor other operative procedure was added in this study. Nevertheless, the IKDC score improved significantly at half year,1 year, 2 years and 5 years after operation, respectively.



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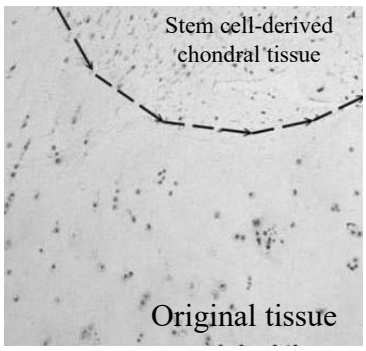
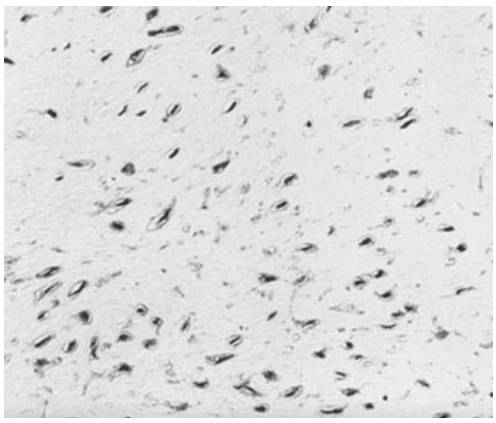
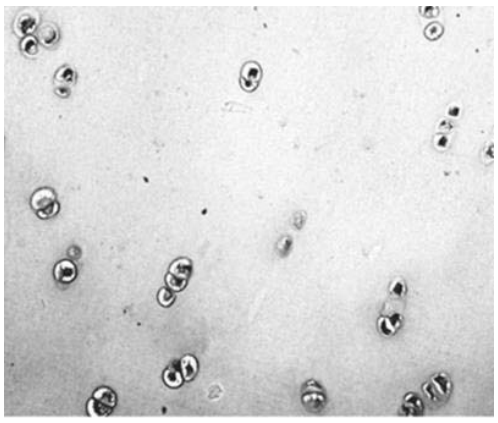
- The biopsy specimens revealed :
  - ➢high cellular density in the graft.
  - ➢The cells were smaller than the original chondrocytes
  - ➢There is no lacuna in the cells, which is commonly seen in mature chondrocytes.





Stem cell-derived chondral tissue



Original tissue

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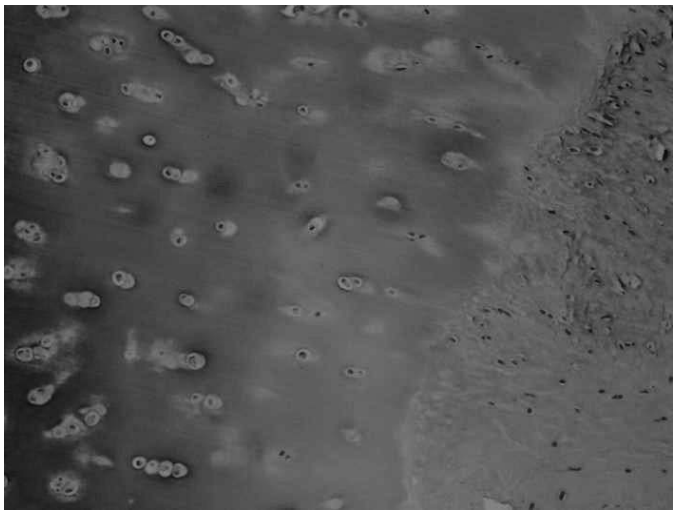
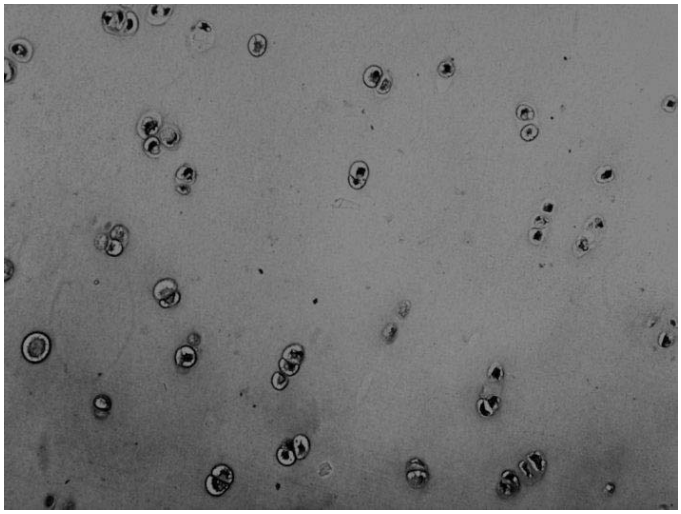
- The implanted bioproduct demonstrated the existence of GAG and collagen II



GAG stain



Collagen Type II stain

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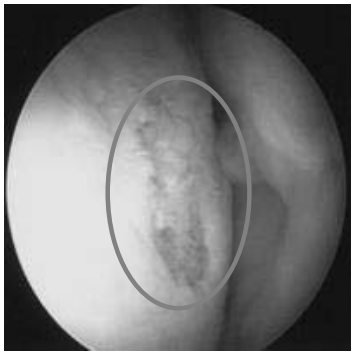
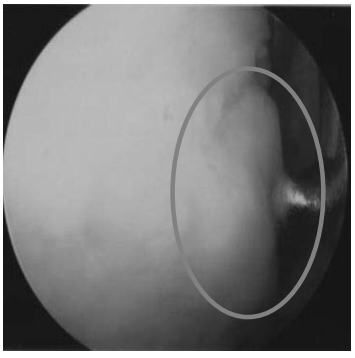
- The implanted tissue is softer than original cartilage. It has cushion effect and may become harder in the future.
- Integration of cartilage between the recipient site and chondral graft is good, there is no gap.



Pre OP



Post OP

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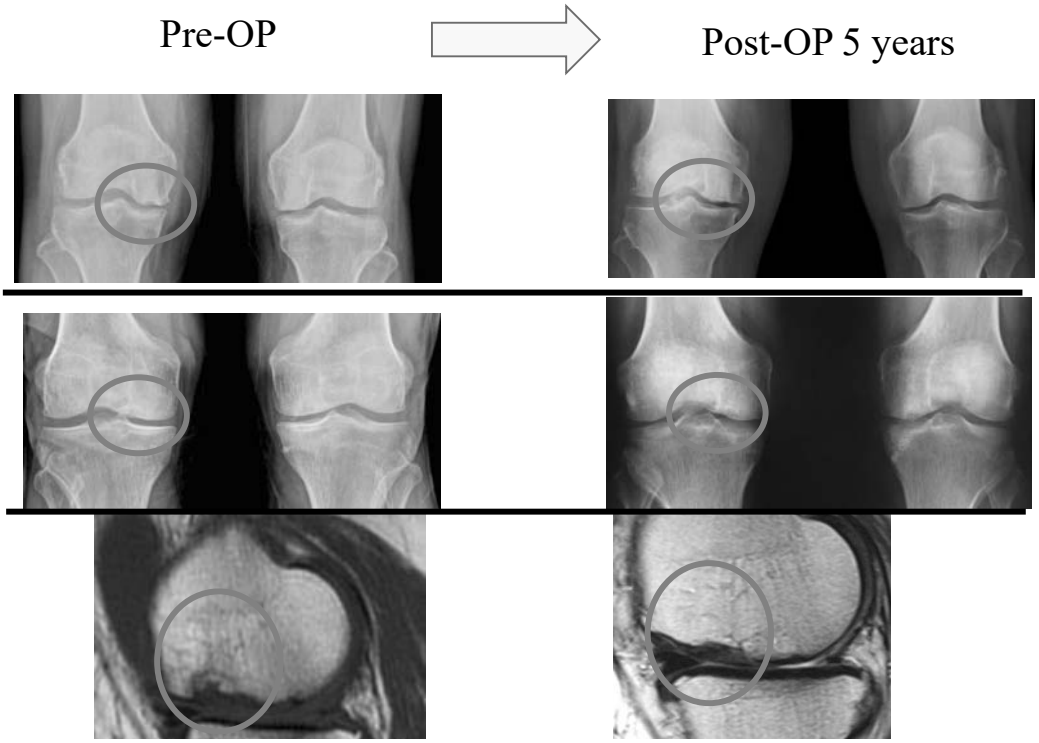
- By MRI & X-ray result, the implanted chondral tissue seemed to be able to maintain the joint space, even at 5 years after operation.



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Summary

- 12 patients with chondral defects of grade IV were treated with stem-cell-derived chondral cells. Except one patient died of malignancy, not related to the study. The remained 11 patients were followed up for 41 to 79 months (average 62.5 months).
- IKDC score at half year and 1 year after operation showed significant improvement of the knee function (from 46.12 to 68.29 and 77.35, respectively). This score was maintained in the following 2 and 5 years.
- Arthroscopy appearance of 6 patients demonstrated good recovery of cartilage along with the nearly full score of ICRS assessment.
- Biopsies of implanted tissue revealed the presence of GAGs and type II collagen productions.

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Summary

- The induced chondral cells were similar to mature chondrocytes, but without lacuna, and become layered hyaline cartilage in the follow up period.
- The induced cells were able to maintain the joint space as confirmed by radiographs and MRI analysis.
- No complications such as deep vein thrombosis, infection or tumor formation (chondrosarcoma and synovial chondromatosis) was found.
- The stem-cell-derived chondral cells seems effective in repairing full-thickness chondral defect. We name the cells Kartigen ®

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Thank you for your attention

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精進新興生醫產品
GTP符合性管理制度之研析 GTP研習會

生醫品質矯正及預防措施
(Corrective and preventive action, CAPA)

主講人 蕭學英 2019/10/21

大綱

一. CAPA矯正措施和預防措施系統

二. 品質系統法規相關要求

三. CAPA及其措施步驟

四. 相關範例

一、矯正措施和預防措施系統
CAPA System

- The role of a CAPA system is to continuously improve product and processes in the Quality system
- CAPA is a continuous Quality improvement subsystem
- CAPA is Facts and data driven
- CAPA decision making is based on risk assessment and impact assessment
- Risk assessment is performed on three levels:
  End-user, compliance and business
- CAPA系統的作用是持續改善品質體系中的產品和製程
- CAPA是持續品質改善的子系統
- CAPA是事實和數據驅動
- CAPA決策是基於風險評估和影響評估
- 風險評估分為三個級別：最終用戶、符合法規性和商業需求
- CAPA是品質管理體系的一部分，不遵守CAPA處理的規定，可能會被視為違反有關製造規範。

Purpose of the CAPA Subsystem
矯正和預防措施子系統的目的

- To collect and analyze information to identify actual and potential product and quality problems
- To investigate product and quality problems and take appropriate and effective corrective or preventive action
- To verify or validate the effectiveness of corrective and preventive actions
- To communicate corrective and preventive actions to the appropriate people
- To provide information for management review
- To document activities
- 收集和分析資料，以識別實際和潛在的產品和品質問題
- 調查產品和品質問題，並採取適當有效的矯正或預防措施
- 驗證或確效矯正和預防措施的有效性
- 向適當的人員溝通矯正和預防措施
- 提供資料供管理審核
- 完成書面記錄

Definition

矯正 Correction：

採取動作去消除檢驗到的不合格。

1. 可以與矯正措施一起進行更正。
2. 矯正是例如：重製或重新修改。

矯正措施 Corrective action ：

以消除檢測到的不合格或其他不良情況的**原因**。

1. 不合格發生後採取的行動
2. 不合格的原因可能不止一個。
3. 採取矯正措施以防止**再次**發生。
4. 矯正和矯正措施之間存在差異。

預防措施 Preventive action ：

採取行動消除潛在不合格或其他不良情況的原因

1. 發生之前採取的行動
2. 潛在的不合格可能有多個原因。
3. 採取預防措施以防止發生。

二、了解品質系統法規要求

藥品品質保證

The basic principle of quality assurance is that a drug should be produced that is fit for its intended use. This principle incorporates the understanding that the following conditions exist:

- Quality, safety, and efficacy are designed or built into the product.
- Quality cannot be adequately assured merely by in-process and finished-product inspection or testing.
- Each step of a manufacturing process is controlled to assure that the finished product meets all quality attributes including specifications.

品質保證的基本原則是應該生產出符合其**預期用途**的藥物。 該原則包含以下要件：

- **品質、安全**和**功效**已設計或內置到產品中。
- 僅通過製程中和成品的檢查或試驗是**不能充分保證**品質。
- **控管製造過程的每個步驟**，以確保最終產品符合包括規格在內的所有品質屬性。

*FDA process validation guidance

Pharmaceutical Quality System (PQS)

Pharmaceutical Quality System (PQS):

Management system to direct and control a pharmaceutical company with regard to quality.

藥品品質系統－在品質方面指導和管控製藥業的管理體系。

FDA QSR七要項：

- Management,
- Design controls,
- Production & process controls,
- Records, Records/documents/change controls,
- Material controls,
- Facility & equipment controls,
- **Corrective & Preventive actions.**

藥品品質系統(PQS)要素

3.2. Pharmaceutical quality system elements

The elements described below might be, required in part under regional GMP regulations. However, the Q10 model's intent is to enhance these elements in order to promote the lifecycle approach to product quality. These four elements are:

- Process performance and product quality monitoring system;
- **Corrective action and preventive action (CAPA) system;**
- Change management system;
- Management review of process performance and product quality.

3.2. 藥品品質系統要素

GMP法規可能會部分要求以下所述的元素。但是，Q10模型的目的是增強這些要素，以促進產品生命週期的方法提高產品品質。這四個要素是：

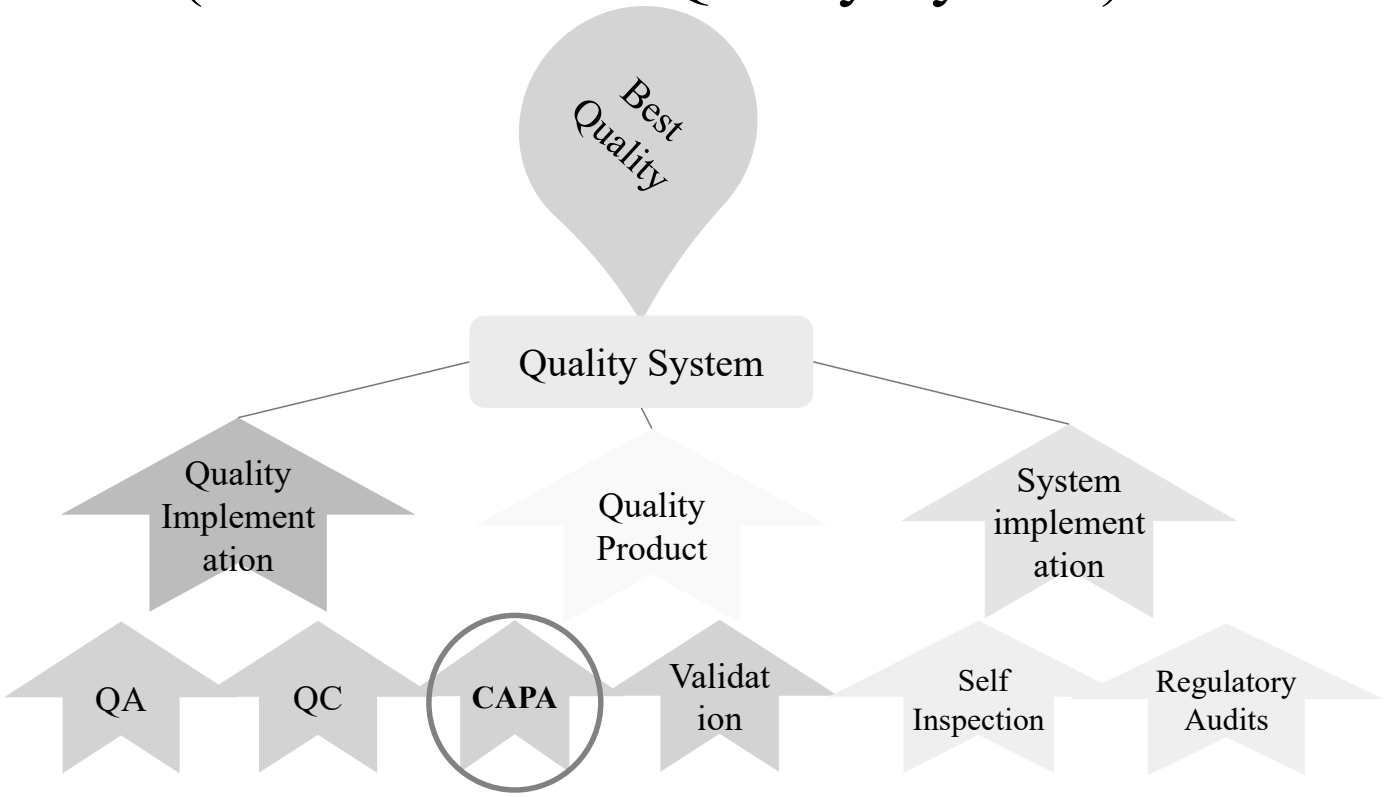
- 製程性能和產品品質監控系統；
- **矯正措施和預防措施（CAPA）系統；**
- 變更管制系統；
- 對製程性能和產品品質進行管理層審查。

*ICH guideline Q10 on pharmaceutical quality system EMA/CHMP/ICH7

FDA - The six subsystems of a modern pharmaceutical quality system(PQS) cGMP

為了有效和持續確保藥品品質，可經由**藥品生命週期**之**品質管理系統**科學邏輯思考與風險考量來實現。

藥品品質系統 (Pharmaceutical Quality System)



持續改善製程性能和產品品質
CONTINUAL IMPROVEMENT OF PROCESS PERFORMANCE AND PRODUCT QUALITY

Lifecycle Stage Goals 產品生命週期目標	Pharmaceutical Quality System Elements 藥品品質系統要素
1. Pharmaceutical Development 藥品開發 2. Technology Transfer 技術移轉 3. Commercial Manufacturing 商業化生產 4. Product Discontinuation 產品終止	1. Process Performance and Product Quality Monitoring System 製程效能和產品品質監控系統 2. Corrective Action and Preventive Action (CAPA) System 矯正和預防措施系統 3. Change Management System 變更管理系統 4. Management Review of Process Performance and Product Quality 製程性能和產品品質的管理審核

ICH Q10 Recommends a Product Lifecycle Approach
ICH Q10建議產品生命週期方法

Application of Corrective and Preventive Action System Throughout the Product Lifecycle
矯正和預防措施系統在整個產品生命週期中的應用

Pharmaceutical Development 藥品開發	Technology Transfer 技術移轉	Commercial Manufacturing 商業化生產	Product Discontinuation 產品終止
Product or process variability is explored. CAPA methodology is useful where corrective actions and preventive actions are incorporated into the iterative design and development process. 探討產品或製程的變異性。在將矯正措施和預防措施併入到反複設計和開發製程中，CAPA方法學是非常有用。	CAPA can be used as an effective system for feedback, feedforward, and continual improvement. CAPA是可用於反饋、前饋和持續改進的有效系統。	CAPA should be used, and the effectiveness of the actions should be evaluated. 應該運用CAPA，並應該評估措施的有效性。	CAPA should continue after the product is discontinued. The impact on product remaining on the market should be considered, as well as other products that might be affected. 產品停產後CAPA仍應持續。應該考慮對市場上剩餘產品的影響以及可能受影響的其他產品。
https://www.fda.gov/media/85266/download			

現今CAPA管理系統的基礎

現今，大多數CAPA都是從例外開始的，並且以

- 以製造為重點
  –偏差、不合格、年度產品審核、管理審核、投訴、風險管理和確效等。
- 以患者為中心
  –基於風險
  –努力、資源和時間表與患者風險
- 強大管理
  –矯正措施和時間表的適當性
- 管理審核
  –定義的指標計劃
  –升級製程
  –管理承諾

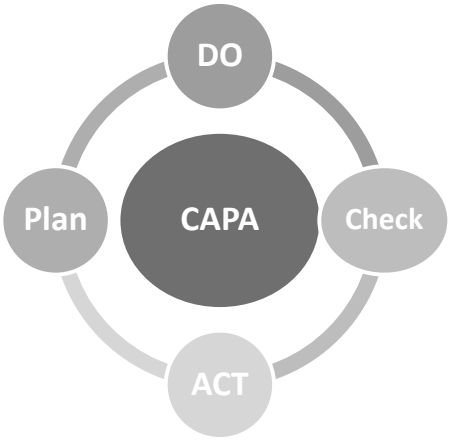
https://www.fda.gov/media/85266/download

The CAPA Life Cycle

Where to Start? Planning

Success of CAPA depends upon the planning that goes into it.
Plans should include…

1. Establishing Data Sources and Criteria
2. Measuring and Analysis of Data Sources
3. Improvement Plans
4. Input to Management



計劃應該包括.....

1. 建立數據來源和標準
2. 數據源的測量和分析
3. 改善計劃
4. 輸入至管理

建立執行計劃-培訓-驗證～後勤問題～監管問題-紀錄文檔列表

三、 CAPA及其措施步驟

1. Management System
   管理系統
2. Collect Data to Determine the Major Cause
   收集數據以確定主要原因
3. Root Cause Analysis (RCA)
   根本原因分析
4. Perform Impact and Risk Assessments
   執行影響和風險評估
5. Determining CAPAs and Document Changes
   確定CAPA和管制文檔的更改
6. Form a Conclusion
   得出結論
7. Initiate Effectiveness Checks (ECs)
   啟動有效性檢查（EC）
8. CAPA Activities for Management Review
   CAPA稽核和管理審查

CAPA及其措施步驟

1. 管理系統：部署一個良好有效的管理系統，使用系統跟踪事件以防止部門混淆，並調查工作中發生的任何事件。
2. 收集數據以確定主要原因： 應用此過程收集所需的所有數據。在沒有偏差情況下執行，以便確定問題的根本原因。收集的數據通常會根據所處理的問題或正在調查的事件而定。
3. 根本原因分析（RCA）：在收集數據後執行。當根本原因被排除時，要給出原因和理由。
4. 執行影響和風險評估： 評估將考慮問題或事件對產品的影響。
5. 確定CAPA和文檔更改：在調查過程中消除問題的根本原因，確定並執行CAPA。按照使用的單獨程序完成和管理文檔。
6. 形成結論：概述事件、影響、風險評估以及任何矯正和預防措施所強調的根本原因。
7. 啟動有效性檢查（ Effectiveness Checks ,EC）：這有助於開發內部監控，以解決調查成功可以處理的方式。在一個安全的環境中進行，遵循特定的步驟，程序，並側重於確定問題的根本原因。
8. CAPA管理審查：當出現意外問題或事件時，公司需要進行詳盡記錄的調查，通過流程變更提供補救措施或處理的程序，修改內部文檔和更改以幫助解決關鍵問題可能另外出現的問題。

1. Management System 管理系統

矯正措施和預防措施（CAPA）系統

3.2.2. Corrective action and preventive action (CAPA) system

- The pharmaceutical company should have a system for implementing corrective actions and preventive actions resulting from the investigation of complaints, product rejections, non-conformances, recalls, deviations, audits, regulatory inspections and findings, and trends from process performance and product quality monitoring.

- 製藥公司應該有一個實施矯正措施和預防措施的系統
- 該矯正措施和預防措施是由對投訴、產品拒收、不合格、召回、偏差、稽查、監管稽查和發現以及過程性能和產品品質監控趨勢調查得出。

ICH guideline Q10 on pharmaceutical quality system EMA/CHMP/ICH/214732/2007

有效的CAPA系統的推動力

ICH Q10 Require that Management have a formal process for reviewing the QS …The review should include:

(a) Measurement of achievement of pharmaceutical quality system objectives

(b) Assessment of performance indicators that can be used to monitor the effectiveness of processes within the pharmaceutical quality system, such as:

(1) Complaint, deviation, CAPA & change management……

ICH Q10 管理層需有正式流程來審核QS…審查應包括：

（a）衡量藥品品質體系目標的實現

（b）評估可用於監測藥品品質體系內流程有效性的績效指標，例如：

（1）投訴、偏差、 CAPA和變更管制……

管理的支持和審查對於有效的CAPA流程至關重要。

https://www.fda.gov/media/85266/download

2. 收集數據以確定主要原因
Collect Data to Determine the Major Cause

Analyze processes, work operations, concessions, quality audit reports, quality records, service records, complaints, returned product, and other sources of quality data to identify existing and potential causes of nonconforming product, or other quality problems.

分析製程、工作運作、妥協讓步、品質稽查報告、品質記錄、服務維修記錄、投訴、退貨和其他品質數據來源、以確定不合格產品的現有和潛在原因或其他品質問題。

- 非典型/異常/異常結果Atypical / Aberrant / Anomalous – 結果仍在規格範圍內但是意外、可疑、不規則、偏離或不正常。
- 異常/偏差/超出趨勢OOT/超出規格OOS。

範例 CAPA收集數據來源

Examples of Internal Data Sources	內部數據來源
Process Control Data	製程控制數據
Test/Inspection data	試驗/檢驗數據
Device History Records	設備歷史記錄
Internal Audits	內部稽核
Nonconforming material reports	不合格原料報告
Rework and Scrap/Yield Data	重新作業和報廢/產量之數據
Training records	培訓記錄
Examples of External Data Sources	**外部數據來源**
Adverse Event Reporting	不良事件報告
FDA	FDA
Even similar devices from competitors	來自競爭對手的類似設備
Supplier Controls	供應商管控
Customers	顧客
Complaints	投訴
Servicing repairs	維修保養

Biological Product Deviations

- The amended regulation at 21 CFR 600.14 and the new regulation at 21 CFR 606.171 require reporting of any **event** associated with the manufacturing, to include testing, processing, packing, labeling, or storage, or with the holding or distribution of a licensed biological product or a blood or a blood component, in which the safety, purity, or potency of a distributed product may be affected. A manufacturer is required to report to the ……
- FDA published a final rule ……for reporting certain **deviations** in manufacturing of Human Cells, Tissues, and Cellular and Tissue-Based Products (HCT/P)……
- …..新法規要求報告與製造相關的任何事件，包括檢驗、製程、包裝，標籤或儲存、或許可的生物產品或 血液或血液成分保存或運銷，可能會影響配送產品的安全性，純度或效力。 製造商都必須報告… 。
- FDA發布了最終規則….要求在人類細胞、組織以及基於細胞和組織的產品（HCT / P）製造中的某些偏差須報告…

https://www.fda.gov/vaccines-blood-biologics/report-problem-center-biologics-evaluation-research/biological-product-deviations

遏制 Containment

遏制是指阻止問題或潛在問題的隱含性或影響性，以使其不擴散。此階段的問題是

1.什麼是問題或潛在問題？

2.重要嗎？

3.尋找長期解決方案時，我們應該如何矯正它？

4.會影響我們的使命嗎？

5.解決的風險是什麼？

6.對產品、過程和品質管理體係有什麼影響？

遏制可能涉及以下方面：

1.停產2.召回3.停止運送4.隔離5.再加工/重製

遏制的程度或程度應適合於問題或潛在問題帶來的風險。

Approach to Data Analysis
數據分析方法

Non-statistical & Statistical Techniques

- Use a risk-based approach to rank areas, Select items with major impact, i.e. Product related or Process related. Proceed with items from high to low impact and eventually assure all areas are addressed.
- Use of Statistical Methodology; Appropriate statistical methodology shall be employed where necessary to detect recurring quality problems.

非統計和統計技術

- 使用基於風險的方法對有問題進行排序，選擇具重大影響項目，如與產品相關或製程相關。從高影響到低影響項目進行處理，並最終確保所有問題都得到解決。
- 使用統計方法; 必要時應採用適當的統計方法來檢測重複出現的品質問題。

3. Investigate to Determine Root Cause
調查確定根本原因

Root Cause, RCA 根本原因

Investigate the cause of nonconformities relating to product, processes, and the quality system.

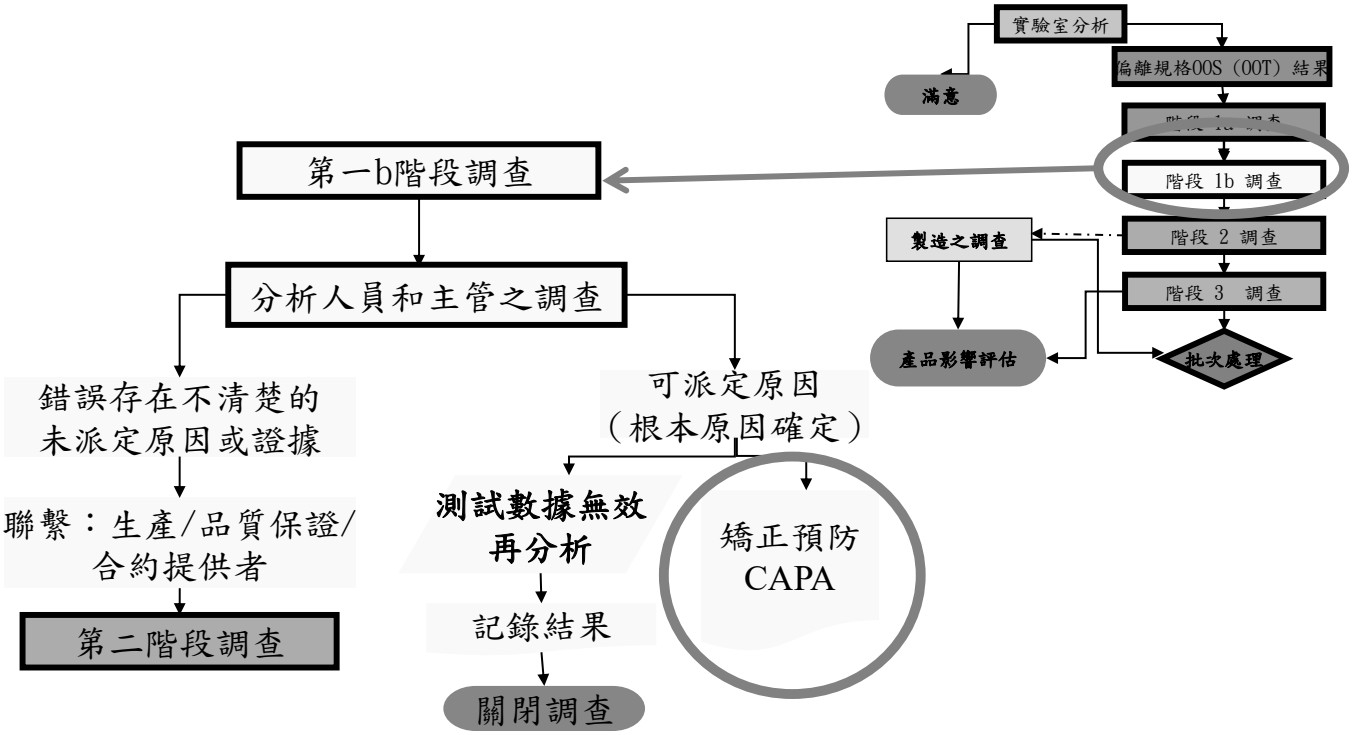
- …it requires that nonconforming product discovered before or after distribution be investigated to the degree commensurate with the significance and risk of the nonconformity.
- 調查與產品、製程和品質系統相關的不合格原因。
- 要求對運銷前或運銷後發現的不合格產品進行調查，使其與不合格的重要性和風險程度相對稱。

調查根本原因

- A structured approach to the investigation process should be used with the objective of determining the root cause.
- The level of effort, formality, and documentation of the investigation should be commensurate with the level of risk, in line with ICH Q9.
- CAPA methodology should result in product and process improvements and enhanced product and process understanding.
- 為了確定根本原因，應使用一種結構化的調查程序方法。
- 根據ICH Q9，調查的工作水準，形式和調查書面紀錄文件應與風險水平相當。
- CAPA方法應該改善產品和製程以及增強對產品和製程的了解。

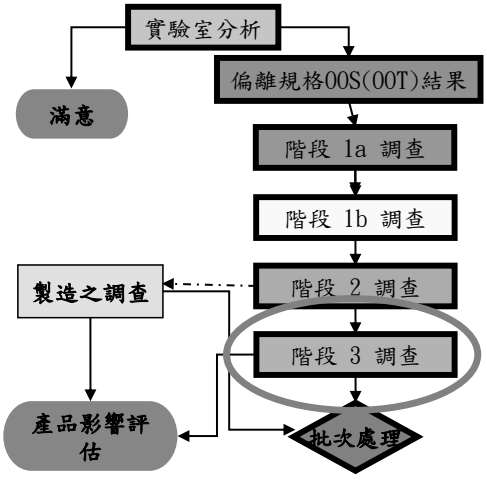
ICH guideline Q10 on pharmaceutical quality system EMA/CHMP/ICH/214732/2007

OOS 第一 b 階段調查



OOS 第三階段調查

- 必須確定批次的品質和處置決定。
- 一旦批次被拒絕，進一步測試以確定失敗原因是沒有限制，可以採取矯正措施。
- 進一步測試結果，是不可逆轉原批次拒絕的決定。
- OOS結果對於其他批次、安定性研究、確效過製程和測試程序的影響，應由品管QC和品保QA確定，並在結論中記錄，並採取適當的矯正預防措施(CAPA)。



根本原因分析調查的影響

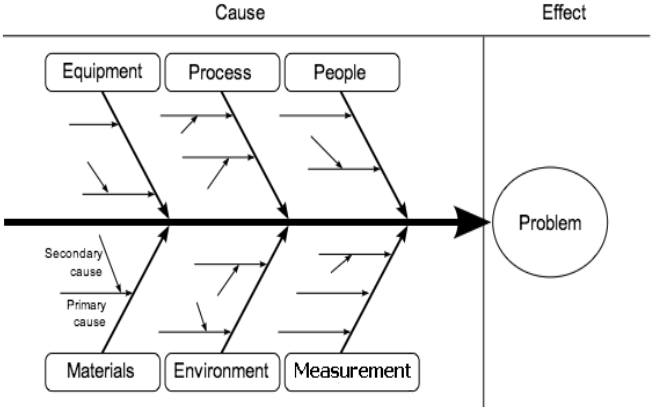
- 當未確定真正的根本原因時，將發生主要問題，針對該問題指定的CAPA將不會為真正的指定原因，並且根本無法解決。當根本原因仍未解決時，將來會再次出現相同的問題。
- 在確定實際根本原因的過程中，還確定了許多可能和次要原因。這些可能的原因有助於我們確定未來的問題。
- 確定真正根本原因常見的方法：
  - 所有參與根本原因調查的員工都須了解直接原因和根本原因。
  - 必須對員工進行深入發覺原因的培訓。
  - 5個為什麼(5 Why)? 、魚骨圖和故障樹等工具可以有效地調查一個複雜的問題。

魚骨圖

魚骨圖(因果圖)是腦力激盪工具，用於識別問題的根本原因。是一種分析工具，它提供了一種系統方式來查看影響以及造成這些影響的原因。

分析原因並消除瑣碎的想法。對原因進行排名並圈出最有可能的原因，以供進一步考慮和研究。

Components
- Head of a Fish: Problem or Effect
- Horizontal Branches: Primary Cause
- Sub – Branches: Secondary cause



預防措施 Preventive action

- 預防措施是為了解決管理系統中尚未引起產品或服務不合格的缺陷而實施的更改。
- 預防措施的提出通常來自過程中參與者的建議，是一個主動的過程，可以識別出改進的機會，而不是對已發現的問題或投訴的反應。
- 除審查操作程序外，預防措施還可能涉及數據分析，包括趨勢和風險分析以及能力驗證結果。
- 預防措施的重點是避免產生不合格事項，通常還包括提高效率。
- 預防措施可以解決與所提供的產品或服務或內部管理系統有關的技術要求。
- 在發現改進的機會或需要採取預防措施時，應制定、實施和監控行動計劃，以減少不合格事項的可能性，並利用改進的機會。
- 預防措施流程將包括控制措施的應用，確保預防措施有效。

啟動CAPA的決定

啟動CAPA的決定是基於:

1. The risk associated with the finding 與發現相關的風險
   - Regulatory risk 法規監管風險
   - Business risk 商業風險
   - Risk to the end user of your product
     對產品最終用戶的風險
2. An adverse trend exists 不利趨勢的存在
3. Impact assessment data 影響評估數據
   - Implications 歸責
   - Cost 成本

範例：啟動CAPA

1. CAPA的啟動要求由有關部門負責人向QA部門提交源文件。
2. 部門負責人應決定是否需要具有主管QA的CAPA。
3. 部門主管將獲得QA發出的CAPA表格。QA人員應在發布表格之前在表格上寫下源文件名稱和來源文件編號。
4. 部門負責人應填寫CAPA表格。
   a. CAPA啟動日期
   b. 預定完成日期
   c. 在相應的框中選擇√標記，選擇啟動CAPA的部門。
   d. 在相應的框中選擇受√標記影響的相關係統。如果除上述系統之外的任何其他系統受到影響，請在提供的空白處寫入系統。
   e. 簡要說明源文件中的CAPA描述以及矯正和預防措施細節。
   f. 部門主管應簽名和日期。
5. 部門負責人應將CAPA表格發送給QA。
6. GM QA /指定人員應在CAPA表格中分配一個編號，並在CAPA日誌中輸入相關條目。將CAPA表格轉發給相關部門。
7. CAPA應在每個部門的日曆年中按順序編號，並附有部門識別碼。

4.執行影響評估和風險評估
Perform Impact and Risk Assessments

The Risk and Degree of Corrective and Preventive Action

…the degree of corrective and preventive action taken to eliminate or minimize actual or potential nonconformities must be appropriate to the magnitude of the problem and commensurate with the risks encountered. . .

關於矯正和預防措施的風險和程度

.....為消除或盡量減少實際或潛在的不合格而採取的矯正和預防措施的程度，必須與問題的嚴重程度相對應，並與所遇到的風險相對稱。

異常/偏差/OOT/OOS等之CAPA需要與影響程度和風險相對稱。

5.確定CAPA和文檔更改
Identify Corrective and Preventive Actions

1. Identify the action(s) needed to correct and prevent recurrence of nonconforming product and other quality problems.
   確定矯正和預防不合格產品再次發生和其他品質問題所需的措施。
2. Identify Action(s) to be taken 確定要採取的措施
   - No further action necessary 無需採取進一步措施
   - Correction 矯正
   - Corrective Action 矯正措施
   - Preventative Action 預防措施

5.確定CAPA和文檔更改
Identify Corrective and Preventive Actions

- Implement and record changes in methods and procedures needed to correct and prevent identified quality problems.
- 實施並記錄矯正和預防已確定品質問題所需的方法和程序的變更

實施矯正和預防措施
Implement Corrective and Preventive Actions

Robust process

Standard methodology

Information system

Effective Training

穩健的程序(製程)

標準方法論

資訊系統

有效的培訓

溝通CAPA訊息
Communicating CAPA Information

- Disseminate information related to quality problems or nonconforming products to those directly responsible for assuring the quality of such product or the prevention of such problems.
- Submit relevant information on identified quality problems, as well as corrective and preventive actions, for management review.
- 向直接負責確保此類產品品質或預防此類問題的相關人員溝通與品質問題或不合格產品相關的訊息。
- 提交有關已確定品質問題的相關訊息，以及矯正和預防措施，以便進行管理審核。

範例：CAPA關閉和驗證

1. 措施完成後，部門主管應證明擬議的CAPA已完成並實施，並附有相關措施書面記錄。
2. QA部門應通過審查證明文件並對其進行認證來核實CAPA的實施和完成情況。
3. 由CAPA提出的變更都應通過變更管制參考SOP，並以CAPA格式提及。
4. 所有變更管制、偏差、差異，引起CAPA的事故報告應通過CAPA表格處理。
5. 所有設施升級/資本購買要求/品質體系的重大變化以及產生CAPA的監管，承諾的遵守情況，應通過CAPA表格解決。
6. 應保留每個CAPA的書面記錄。
7. 完成的CAPA副本應提供給相關部門。QA部門負責人負責編制CAPA訊息，並在GMP委員會會議/管理評審會議期間向管理層提交摘要。
8. 管理層應在「管理審查會議」中每季度審查/驗證同一季度。
9. 從內部稽查、外部/客戶審核和監管稽查中獲得的與CAPA相關的資訊和文件被視為機密信息，並且只有在獲得技術總監和QA最高負責人的批准後才能提供給監管審查。

7.啟動有效性檢查
Effectiveness Checks ,EC

驗證/驗證 矯正和預防措施
Verify/Validate Corrective and Preventive Actions

- Verify or validate the corrective and preventive action to ensure that such action is effective and does not adversely affect the finished product.

  驗證或確效矯正和預防措施以確保此類措施有效且不會對最終產品產生不利影響。

- FDA has revised Sec. 820.100(a)(4) to reflect that preventive, as well as corrective, action must be verified or validated.

  FDA修改Sec. 820.100(a)(4)，以反應預防和矯正措施必須經過驗證或確認。

Documenting Corrective Action and Preventive Action Activities

Document all activities required and their results.

書面記錄所有的CAPA措施要求及其結果

8. CAPA和內部稽核和管理審查
CAPA and Internal Audits and Mgmt Reviews

CAPA and Internal Audits and Mgmt Reviews

- …FDA has the authority to review such records and the obligation to do so to protect the public health….
- Manufacturers will be required to make this information readily available to an FDA investigator.

關於CAPA和內部稽核和管理審查

- …FDA有權也是義務審查這類記錄，以保護公眾健康.....
- 製造商被要求將此資訊隨時提供給FDA調查員。

8. CAPA措施的管理審核
CAPA Activities for Management Review

- The manufacturer's procedures should clearly define the criteria to be followed to determine what information will be considered "relevant" to the action taken and why.
- FDA emphasizes that it is always management's responsibility to ensure that all nonconformity issues are handled appropriately.
- 製造商的程序應明確定義要遵循的標準以確定哪些訊息將被視為與所採取的措施“相關”以及原因。
- FDA強調，管理階層始終有責任要確保妥善處理所有不合格的問題。

FDA Inspection

FDA Inspection

Manufacturers should consider that their Corrective Action and Preventive Action documentation can demonstrate to FDA that the manufacturer's quality system is effective and enables the manufacturer to identify problems quickly and implement effective corrective and preventive actions.

FDA監管檢查

製造商應考量其矯正措施和預防措施文件，可以向FDA證明製造商的品質體係是有效的，並且能讓製造商能夠快速發現問題，並實施有效的矯正和預防措施。

未來的應用

The Future
More CAPAs will be based on nonexception type data such as:
– Data trending and holistic data reviews
– Continuous Improvement Projects
– Industry and Regulatory Surveillance
– Cost of Quality Model
– Implement CAPA earlier in the development process
未來
更多的CAPA將基於非異常類型的數據，例如：
–數據趨勢和整體數據審查
–持續改進項目
–產業和法規監管
–品質模型的成本
–在開發過程的早期實施CAPA
https://www.fda.gov/media/85266/download

FDA WARNING LETTER

- A公司沒有在偏差日誌中記錄客戶投訴中包括分析品質數據源的要求。根據FDA的說法該公司也未能提供時間表來確定何時完成修復CAPA。
- FDA對B公司未建立程序描述將如何分析數據以檢測重複出現的品質問題。包括未能證明程序包括驗證或確認行動的要求。該公司沒有證明它有任何流程可以確保管理層對CAPA計劃的任何改進進行最終審查。
- C公司因一系列CAPA缺失但過早結束CAPA調查也是一個問題。FDA檢查員提到了許多CAPA問題，包括：未證明其已進行了必要的驗證確效和記錄即關閉CAPA記錄。
- FDA告知D公司未能調查“*無法解釋的差異或失敗*”。FDA發現對偏差和投訴的調查（根本原因分析）不足。特別是所描述的原因沒有得到充分證實，調查也沒有延伸到其他可能受影響的批次。此外，矯正和預防措施（CAPA）並未按計劃執行，其有效性沒有得到充分評估。

FDA WARNING LETTER

- 警告信上，FDA告知xxx公司未能調查“無法解釋的差異或失敗”。當局特別提到偏差和CAPA的管理。
- FDA發現對偏差和投訴的調查（根本原因分析）不足。特別是所描述的原因沒有得到充分證實，調查也沒有擴展到其他可能受影響的批次。此外，矯正和預防措施（CAPA）並未按計劃執行，其有效性還沒有得到充分評估。
- FDA希望“對整個系統進行全面，獨立的評估，以調查偏差、非典型事件、投訴、不合格結果和失敗”，包括對CAPA有效性的評估。

總結

- ✓ 記住事情總是一定會發生
- ✓ CAPA是持續改善的極有價值的工具
- ✓ 實施風險管理並首先關注重要問題
- ✓ 作為專業人員，應努力防止重大問題
- ✓ 使用指標監控績效
- ✓ 謹防意外後果
- ✓ 如何處理問題將決定我們成敗！
- ✓ 穩健的CAPA流程有助於做出明智的決定！

References

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- CAPA within the Pharmaceutical Quality System https://www.fda.gov/media/85266/download
- Corrective and Preventive Action Basics https://www.fda.gov/files/about%20fda/published/CDRH-Learn-Presenation--Corrective-and-Preventive-Action-Basics.pdf
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- Guidance for Industry Q10 Pharmaceutical Quality System https://www.fda.gov/media/71553/download
- The Medicines and Healthcare products Regulatory Agency (MHRA) Guidance Out of Specification & Out of Trend Investigations 2017

謝謝聆聽!

Q&A

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範例：偏差

1. 偏差通報
2. 立即處理措施
3. 事件登錄與編號
4. 事件說明
5. 確認調查單位
6. 事件調查與矯正
7. 偏差報告
8. 結案
9. 紀錄保存與歸檔

範例：SOP_____矯正措施和預防措施（CAPA）

1.0目的：

制定矯正和預防措施（CAPA）管理應遵循的程序，包括追蹤和報告CAPA的狀態。

2.0範圍：

本SOP適用於追蹤和追蹤未完成的CAPA以及已完成的CAPA確效。

矯正措施：已採取措施矯正，修復或矯正特定的偏差，缺陷或不良情況。

預防措施：已採取措施消除偏差，缺陷或其他不良情況的原因，以防止將來發生此類事件或類似事件。

CAPA原始文件被標識為：

- GMP調查 GMP Investigations
- 偏差 Deviations
- 實驗室（OOS）調查 Laboratory (OOS) Investigations
- 內部稽查報告 Internal Audit Reports
- 外部/客戶稽查 External / Customer Audits
- 年度產品審查 Annual Product Reviews
- 監管稽查報告Regulatory Inspection Reports
- 管理措施計劃Management Action Plans
- 法規/藥典要求的變更Changes in regulatory / Pharmacopoeia requirements
- 產品缺失Product Failures
- 投訴 Complaints
- 產品召回Product recall
- 退貨 Returned Goods
- 發生率報告 Incidence Reports
- 差異 Discrepancies

3.0責任：

所有部門負責人

4.0問責：

QA負責人

範例：SOP矯正措施和預防措施（CAPA）2/3

5.0程序：

5.1 源文件應提供建議的矯正和預防措施。建議的矯正和預防措施應在實施前由品質保證部門批准。建議的矯正和預防措施應在CAPA評估期間以形式進行驗證。

5.2 CAPA表應視為源文件中矯正和預防措施的追蹤表。

5.3 CAPA的啟動：

5.3.1在啟動範圍中提及的任何原始文件期間，部門主管應決定是否需要CAPA。

5.3.2部門負責人應獲得品質保證簽發的CAPA表格。QA人員應將原始文件名稱和原始文件編號寫在表格發行前。發行的CAPA表格的記錄應由QA人員保存。

5.3.3部門負責人應填寫CAPA表格。

5.3.3.1寫下部門名稱

5.3.3.2 CAPA發起的日期

5.3.3.3預定完成日期

5.3.4進行**根本原因分析**，並根據源文件編寫描述。

5.3.5簡要寫出源文件中的CAPA說明以及矯正和預防措施的詳細信息。

5.3.6部門負責人應在姓名上簽名並註明日期。

5.3.7部門主管應將CAPA表格發送給QA人員。

5.3.8 GMP QA/指定人應在CAPA表格中分配參考編號，並在CAPA日誌中進行相關輸入。將CAPA表格轉發到有關部門。

5.3.9在每個日曆年中，CAPA應按編號順序編號，並帶有部門識別碼。典型的CAPA表格

應編號為CAPA/XXX/YYY/Z

範例：SOP矯正措施和預防措施（CAPA）3/3

5.4 CAPA的關閉和驗證：

5.4.1措施完成後，部門主管應證明擬議的CAPA及其相關措施已完成並得到實施。

5.4.2品質檢查人員應通過審核支持文件來驗證CAPA的實施和完成並進行認證。

5.5由於CAPA提出的任何變更均應通過變更管制SOP進行。必須以CAPA格式提及相同的內容。

5.6引起CAPA的所有偏差和差異報告應通過CAPA表格處理。

5.7所有設施升級/資本購買要求/品質體系的重大變更，以符合引起CAPA的監管承諾，應通過CAPA表格解決。

5.8應保留每個CAPA的記錄。完整的CAPA副本應由QA人員提供給有關部門的負責人。CAPA表格的副本應附在原始文件上。

5.9部門主管應彙編CAPA資料，並在GMP委員會會議/管理評審會議期間將摘要提交管理層。

5.10管理層應在“管理評審會議”上每季度評審/驗證同一項目。

5.11與CAPA的內部稽核，外部/客戶審核和監管檢查相關的訊息和文件被視為機密訊息，並且只有在獲得技術總監和高級副總裁批准後才能提供給監管審查。

範例：CAPA申請表

Request No.:
Originating Department Name
Date
Present Process / Problem :
Proposed Action (If any):
Acceptance Criteria of proposed action :
Approval by Head of Originating Department : Signature/Date

範例：CAPA 格式參考表

Report No.		
Department Name :		
Ref. request No.(if any):-		
Description :		
Corrective action:	Target date of completion	Completion Date
Preventive Action :		
Acceptance Criteria :		
Approval: Head Originating department (Sign./Date)	Head Quality Assurance (Sign./Date)	
Implementation and Follow up verified by Originating department (Sign./Date)	Quality Assurance (Sign./Date)	

艾默生物醫學股份有限公司
EMO BIOMEDICINE CORP.

細胞治療產品CMC品管方法之選擇與開發

The Selection and Development of CMC QC Method for Therapeutic Cell-Based Product

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- 成立於2004年，專注於臨床醫療用細胞產品之檢測分析、製程開發、與製造。
- 委託研究、測試、及製造實驗室:
  - 細胞產品檢測與分析(Testing Lab): 2007~
  - 細胞產品製程開發與製造(GTP Compliant): 2010~
  - 藥品之人類細胞反應臨床前研究 & 臨床試驗檢體檢測(醫藥研發服務公司；CRO):2013~

細胞治療產品測試
Accredited **Testing Lab** of TAF

TAF: Taiwan Accreditation Foundation
(全國認證基金會)
Ilac-MRA: International Laboratory Accreditation Cooperation Mutual Recognition Arrangement

- Lab No: 1809
- Accredited in June **2007**
- The accreditation is in accordance with **ISO/IEC 17025:2017**
- TAF recognition is bilateral recognized by 60 economies and 73 accreditation organizations.
- The latest date to pass the renewal accreditation was June 2019.
  (effective until July 2022)

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細胞治療產品測試
Accredited **Drugs Testing Lab** of Taiwan FDA

- Accreditation no: 005
- Accredited in Nov 2009
- The accreditation is in accordance with ISO/IEC 17025:2005.
- Test item: Endotoxin (drug)
- The latest date to pass the renewal accreditation was Dec 2018
  (effective until Dec 2021)

4

	Contract Analysis/Testing Service	Brief Description	Preclinical Drug Ingredient Testing	Clinical Trial: Whole Blood	Clinical Trial: Plasma Serum	Cell-based Product	Supernatant of Cell Culture	Drugs
Safety Test	Mycoplasma Testing	PCR Method (TFDA Guidance)				●	●	
	Sterility Testing	TFDA Guidance				●	●	
	Endotoxin Testing	End-point and Kinetic Chromogenic Method (USP<85>)				●	●	●
Phenotyping (Identity) Assay	Cell Surface Markers of Immune Cells	**Whole Blood:** CD45, CD3, CD4, CD8, CD56, CD19	●	●				
		Lymphocyte: CD45, CD3, CD4, CD8, CD56, CD19, NKG2D, CD16				●		
		Dendritic Cells: CD14, CD80, CD83, CD86, HLA-DR, CCR-7				●		
		γδT cell: CD3, Vγ9 TCR, CD27, CD45RA, CD69, NKG2D		●		●		
		Others: PD-1, IDO		●		●		
	Mesenchymal Stromal Cell (MSC) Phenotype	CD29, CD73, CD90, CD105, CD11b, CD19, CD34, CD45, HLA-DR (ISCT Proposed)				●		
	Hematopoietic Progenitor ($CD34^+$) Cell Enumeration	ISHAGE Guidelines (Single-Platform)		●		●		
Biological Function Assay	Natural Killer Cell (NK) Cytotoxicity Assay	NK Cell of Human PBMCs ; NK92 Cell Line	●	●		●		
	MCF-7 Cell Cytotoxicity Assay	Determine the ability to kill MCF-7 cell line	●	●		●		
	IDO Expression and Activity Assay	Immunomodulatory Assay for MSC Cell Product: Determine IDO expression by flow and activity by kynurenine				●		
	PBMC Suppression Assay	Immunomodulatory Assay for MSC Cell Product				●		
	Cell Proliferation/Cytotoxicity Assay	Human PBMC; 14 Cell Lines (Hep3B, IMR90, CA46, Jurkat)	●	●				
	Cytokine Gene Expression Assay (Method: Real-time PCR)	IFN-γ, TNF-α, TGF-β, GM-CSF, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12, IL-13 expressed in human PBMCs	●	●		●		
		IFN-γ, IL-2, IL-8 expressed in Jurkat cell (Immunostimulant Screening)	●					
		TNF-α, IL-1β, IL-6, IL-8 expressed in U937 cell (Immunosuppressant Screening)	●					
	Cytokine Protein Expression Assay (Method: FlowCytometry or ELISA)	TNF-α, IFN-γ, IL-1β, IL-2, IL-4, IL-10, IL-12p40, IgG, IgM, IgE released from human PBMC (ELISA)	●		●	●		
		TNF-α, IFN-γ, IL-2, IL-4, IL-5, IL-10 expressed in human PBMCs (Intracellular Staining)	●	●		●		
		IFN-γ, IL-2 expressed in Jurkat cell (Immunostimulant Screening)	●					
		TNF-α, IL-1βexpressed in U937 cell (Immunosuppressant Screening)	●					

Accredited by TAF; Accredited by Taiwan FDA

2019年艾默生醫TAF認證檢測項目

2019 年艾默生醫通過 TAF 認證(ISO/IEC 17025:2017)檢測項目

	項　　目	符合規範	
細胞產品安全性試驗	黴漿菌檢測- Real-time PCR (Mycoplasma Testing- Real-time PCR) 〈偵測極限：10 cfu/mL〉	TAF 認證; 中華藥典 7009(7009.1); EP2.6.7 及 2.6.21	New!
	黴漿菌檢測- PCR 法 (Mycoplasma Testing- PCR) 〈偵測極限：10 cfu/mL〉	TAF 認證	展延
	內毒素檢測—動力量色法 (Endotoxin Testing) 〈偵測極限：0.01 EU/mL〉	TAF 認證; TFDA 認可; USP<85>	New!
	無菌試驗：待測件確效 (Sterility Testing) 〈待測件抑菌/黴菌性檢測〉	TAF 認證; 中華藥典 7001; USP<71>	展延
	無菌試驗 (Sterility Testing)	TAF 認證; 中華藥典 7001; USP<71>	展延
細胞產品特性鑑別	淋巴細胞表面標記檢測 (Lymphocytes Surface Marker Analysis)	TAF 認證	展延
	間質幹細胞表面標記檢測 (MSCs Surface Marker Analysis)	TAF 認證	展延
細胞產品功能性分析	間質幹細胞免疫抑制能力 - IDO 定量檢測 (MSC Immunosuppressive function - IDO Quantification Assay)	TAF 認證	New!
	自然殺手細胞毒殺能力檢測 (NK Cytotoxicity Assay)	TAF 認證	展延

經濟部工業局 Industrial Development Bureau, Ministry of Economic Affairs

經濟部核可「醫藥研發服務公司」：2013 年~迄今

TAF(Taiwan Accreditation Foundation)認證：2007 年~迄今

食品藥物管理署(TFDA)認證：2009 年~迄今

生技醫藥研發服務 (Customized CRO Service)

Contract Research for Biologics Development

Discovery Drug or Biosimilars

- ✓ Basic Research
- ✓ Comparability Study

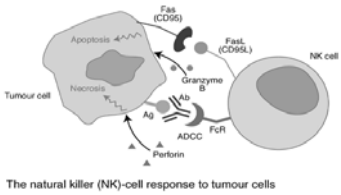
Pre-Clinical Development

Bioassay

- ✓ Development and Optimization
- ✓ Qualification &Validation

Clinical Trial

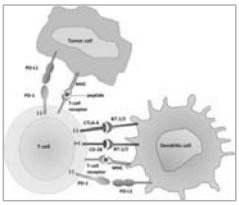
- ✓ Performing Validated Bioassay



The natural killer (NK)-cell response to tumour cells

Examples:

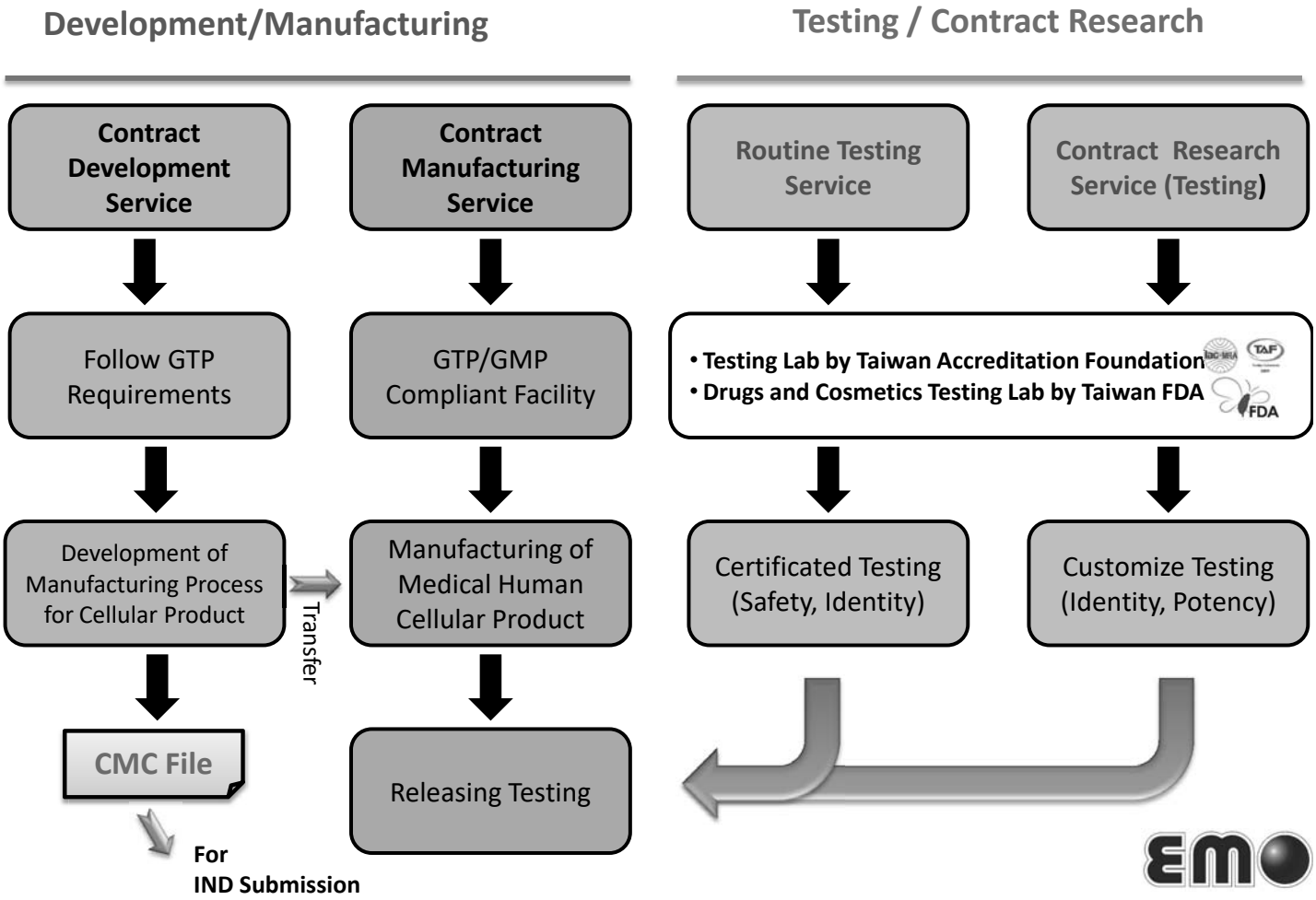
- ***ADCC***
  (Antibody-dependent Cell-mediated Cytotoxicity)
- ***Apoptosis***
- ***Cell Binding Assay***
- ***Cell Differentiation***
- ***Cell Proliferation***
- ***Immunoassays***
- ***Intracellular Staining***
- ***Ligand Binding Assay***
- ***Neutralization Assay***
- ***MLR***
  (Mixed Lymphocyte Reaction)
  Analysis of Specific CTL
  (by Flow &Tetramer)



細胞檢測分析&製備之品質管理系統(QMS)

ISO/IEC 17025:2017 & GTP/GMP

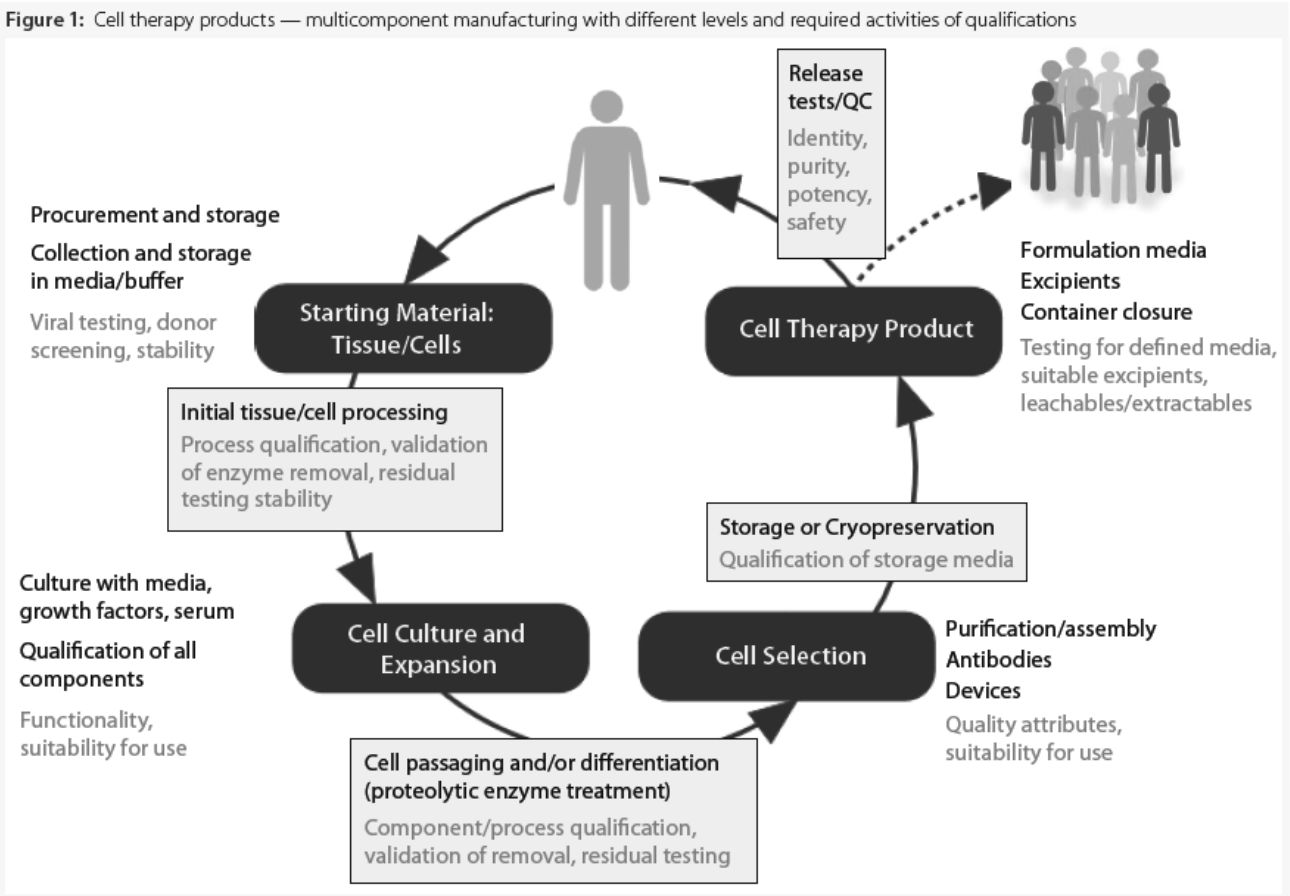
Complete Services for Cell-based Product Development



Manufacturing of Cell-Based Products

BioProcess International 11(8) Sep. 2013

Figure 1: Cell therapy products — multicomponent manufacturing with different levels and required activities of qualifications



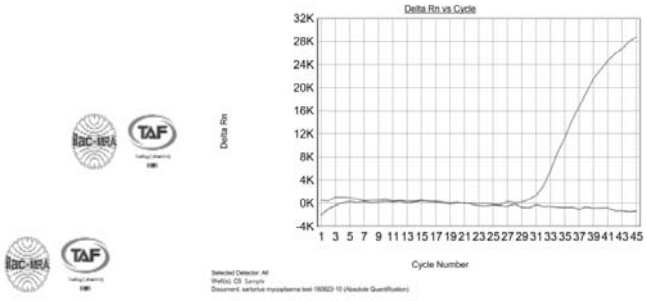
細胞治療產品之CMC品管方法

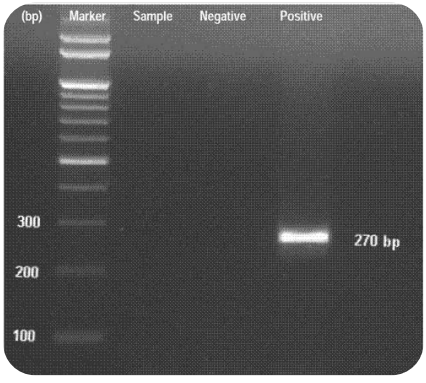
- **Safety Test** (Microbiology):
  Sterility, Mycoplasma, Endotoxin
- **Product Characterization**

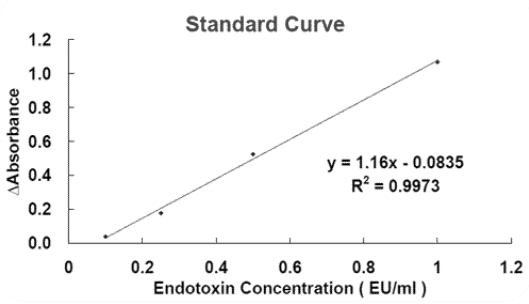
1) Physicochemical Assay
2) Biological Assay
3) Potency Assay-Quantitative & Biological Assay
4) Surrogate Biomarker
5) Matrix Assays

Safety Test of Cell-Based Product

- **Sterility testing**
- **Mycoplasma testing**
- **Endotoxin testing**
  - **Endpoint**
  - **Kinetic**







Definition of Cell Product Characterization

Cytotherapy. 2013 Jan;15(1):9-19.

Table I. Definition of key terminology for cell product characterization.

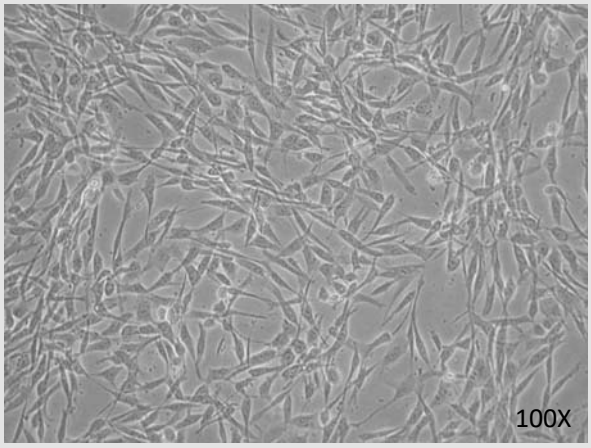
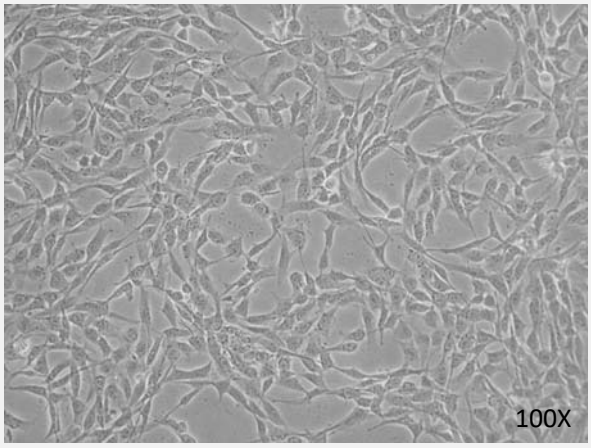
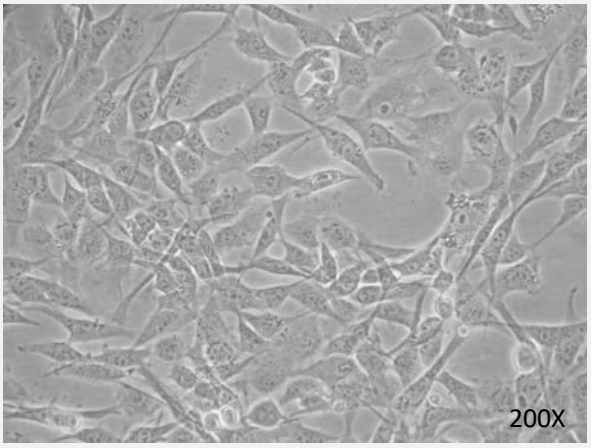
Characterization parameter	Definition
Physicochemical characterization	Refers to the use of methods that measure physical and chemical characteristics. Examples for CTP: *Physical:* size, morphology, light-scattering properties, tensile strength, cell number, confluence *Chemical:* identification of phenotypic markers and secreted substances, genotype, gene expression profile
Biologic characterization	Refers to the use of methods that measure biologic function (i.e., how the physicochemical characteristics influence biologic systems). Examples for CTP: *Biologic: in vitro* or *in vivo* measurements of cytotoxicity, cell growth, de-differentiation, proliferation, migration, tissue remodeling
Potency[a]	Quantitative measure of relevant biologic function of a CTP based on the attributes that are linked to relevant biologic properties[b]
Comparability testing	Exercise to evaluate the impact of changes to a manufacturing process on the validity of quality, non-clinical or clinical data relating to a CTP or its components
Comparable[c]	Conclusion that the product has highly similar quality attributes before and after manufacturing process changes and that no adverse impact on the safety or efficacy, including immunogenicity, of the product occurred
Biocompatibility[c]	Ability of a material to perform with an appropriate host response in a specific application
Stability testing[c]	Determination of the shelf life under storage and in use for the product and its intermediates
Stability	Duration over which the quality of the product is maintained within pre-defined parameters
Release assay	Validated test method with pre-defined acceptance criteria to which manufactured product needs to conform to be released for clinical use

[a]Adapted from (6).
[b]As a measure of relevant biologic function, potency should be based on biologic characterization. Where this is not feasible, a physico-chemical measure may be used as a surrogate for potency at release or stability, as long as it can be correlated to a measure of relevant biologic function.
[c]Adapted from (21).

細胞治療產品製程管控與放行檢測

- 關鍵製程與製程管控 (In-Process controls)
  - Compatibility studies
  - Stability studies
  - Comparability
- 最終產品的放行檢測 (Final Product Release Testing)
- 批次分析 (Batch Analysis)

Physical Characteristics -Cell Morphology

MSC-1	 100X	 More morphological consistency and more spindle-like shape. 200X
MSC-2	 100X	 200X

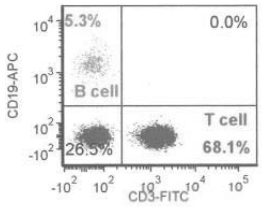
Chemical Characteristics -Cell Surface Markers

- **Whole Blood**

  CD45, CD3, CD4, CD8, CD56, CD19

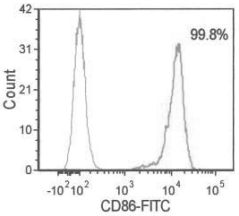
- **Immune Cell Product** (Lymphocyte)

  CD45, CD3, CD4, CD8, CD56, CD19, **NKG2D**, CD16



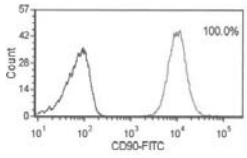
- **Dendritic Cell**

  CD14, CD80, CD83, CD86, HLA-DR, CCR-7



- **γδT Cell**

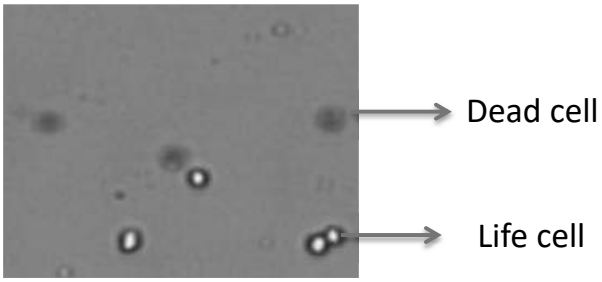
  CD3, Vγ9 TCR, CD27, CD45RA, CD69, NKG2D



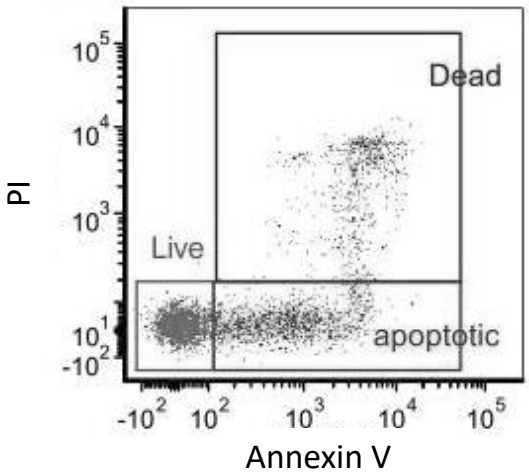
- **Mesenchymal Stromal Cell (MSC)**
- **PDL-1 (MSC ,tumor cells..)/PD-1(activated lymphocyte..)**

Selection of Assay- Cell Viability

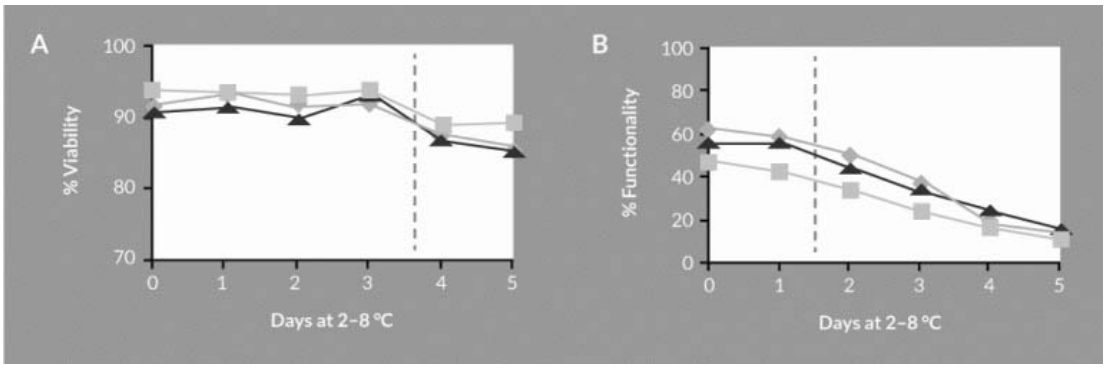
Trypan Blue Exclusion Assay



Annexin V / PI Staining



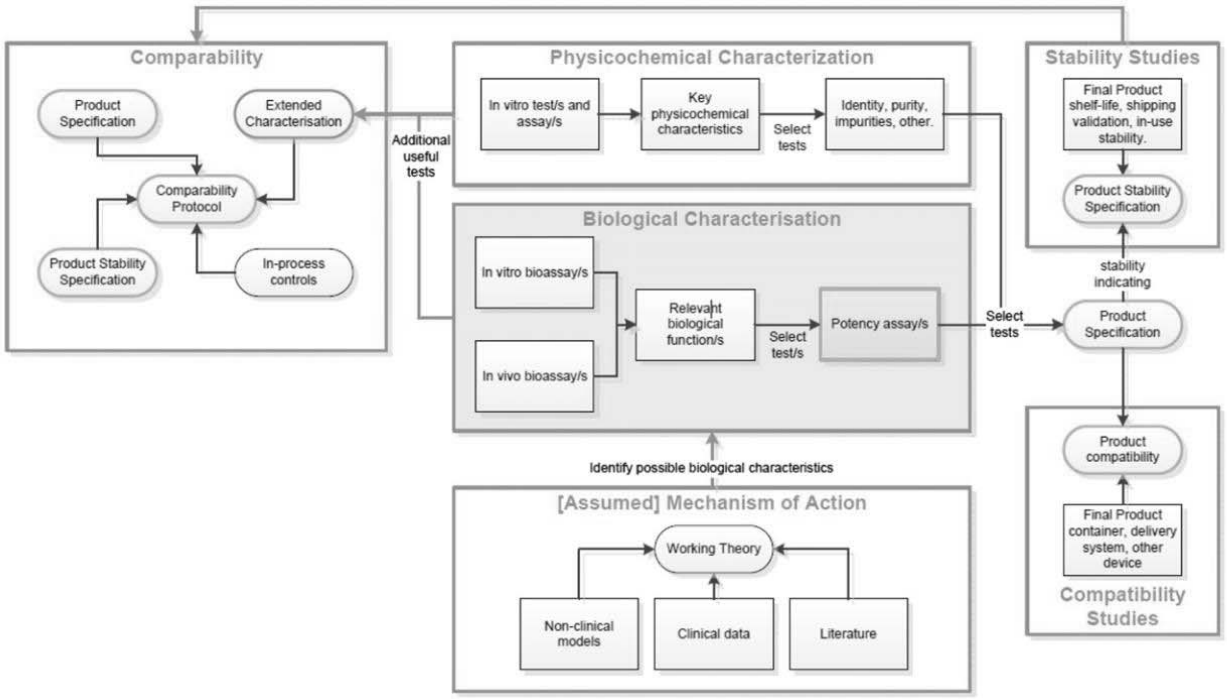
Assay Selection for Cellular Product



(A) **cell viability** and (B) **cell functionality**.
The stability of a cellular product during storage at 2–8°C was assessed using a cell viability assay (1A) and a complex co-culture cell functionality assay (1B). **Cell viability** declined after 3 days of storage. ***Cell functionality** dropped steadily after only 1 day at 2–8°C.

*In this example, **cell viability** was measured by a simple membrane integrity test. **Cell functionality**, on the other hand, was assessed using a complex co-culture method.*
*In contrast to the viability measurement, which was rapid and very precise, the **functionality assay** was lengthy, difficult to control, required careful operator training and displayed significant day-to-day variability. The validation paths for these two assays are likely very different.*
*(*Analytical considerations for cellular therapy manufacturing, *BIOINSIGHTS 2017. by Chris Wiwi, Analytical Research and Development, Celgene.)*

Central Role of Potency Assessment of Determination of CTP Quality



Potency *is central to* ***biologic*** *characterization, which, underwritten by the hypothesis for* ***MOA*** *together with a description of the* ***physicochemical*** *properties, provides the platform for* ***product specification*** *and analysis of product* ***comparability,*** *stability and* ***compatibility****.*

Cytotherapy. 2013 Jan;15(1):9-19.

細胞治療產品檢測

Cytotherapy, 2019; 21: 275–277

International Society ISCT Cell & Gene Therapy

Check for updates

Advancing cellular therapies towards standard of care: a focus on testing of cellular therapy products

PATRICK J. HANLEY[1,4] & MARK LOWDELL[2,3,4]

[1]Center for Cancer and Immunology Research, Center for Cancer and Blood Disorders, Children's Research Institute, Children's National Medical Center and The George Washington University, Washington, DC, USA,[2]Immuno-Gene Therapy Scientific Committee, International Society for Cell and Gene Therapy, Vancouver, Canada,[3]Department of Haematology, Cancer Institute, University College London, London, UK, and [4]Centre for Cell, Gene & Tissue Therapeutics, Royal Free Hospital, London, UK

Testing for Cellular Therapy Products- Cytotherapy 2019

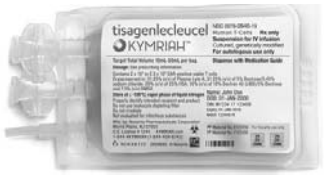
Table 1. Master testing table of suggested release criteria for each of the cell types covered in this special edition.

Cell type	Viability	Identity	Purity	Microbiology	Potency (phase 3)	Stability
CAR T cells	>70% of target cell dose by trypan blue or flow cytometry	CAR expression	>5% CAR-T transduced cells	Aerobic, anaerobic and fungal testing for ≥10 d or ≥7 d by approved rapid test (Bactec or BacT/Alert)	Cytokine release by ELISA, ICS or ELISPOT	Preserved transgene expression
		CD3 expression Flow cytometry	Absence of B cells Absence of Dynabeads Free from other contaminants (cytokines, serum, etc.)	Mycoplasma testing <5 EU/kg endotoxin		Viability Function
TAA T cells	>70% viable by trypan blue	<2% CD3-/CD83+	Lack of alloreactivity via cytotoxicity assay	BacT/Alert for ≥4 d (release) and cultured for 14 d (bacterial)/21 d (fungal)	T-cell specificity by ELISPOT or other functional assay	Vial integrity
		<2% CD19+ HLA identity between donor and T-cell product		Mycoplasma <5 EU/mL endotoxin		Sterility Viability Cytokine release
Gene-modified hematopoietic stem cells	≥70% of target cell dose by trypan blue or flow cytometry	CD34+	≥80% CD34+	Bacterial/fungal sterility testing	NGS or VCN	
		Positive for biological activity of transgene	Transduction efficiency	Mycoplasma		
Exogenous TCRs	80% of target cell dose	% T cell	Transduction efficiency >5%	Sterility using BacTec and Gram Stain	Number of genetically modified cells Gene copy number Transgene expression Product activity level	
TILs	>70% of target cell dose using Cellometer AO/PI or trypan blue	>70% CD45+/CD3+		Sterility (aerobic, anaerobic, fungal) <5 EU/kg endotoxin	>200 pg/mL IFN-γ	
NK cells	>70% of target cell dose	CD3-/CD56+ plus CD16 if the mode of action is presumed to be ADCC	Dependent on whether autologous or allogeneic, the degree of HLA-mismatch and whether replication incompetent	Aerobic, anaerobic and fungal Testing for ≥10 d or ≥7 d by approved rapid test Bactec or BacT/Alert Mycoplasma testing <5 EU/kg endotoxin	Cytotoxicity by flow cytometry or NK cell degranulation marker CD107a as a surrogate	
MSCs	≥90% of target cell dose at cryopreservation	No chromosomal abnormalities		Sterility testing (cultured for 7 d)	Immune assays	Biological characterization
	≥70% of target cell dose post-thaw	Basic ISCT criteria at a minimum but recommend function-specific markers		Mycoplasma testing	Angiogenic assays	Physiochemical assessment
	Determined by trypan blue, Cell Counter or Annexin V/PI			Endotoxin <5 EU/kg/h or <0.2 EU/kg/h for intrathecal	Detection of secreted factors	Rheological studies Morphological studies

TAA; TCR, T-cell receptor; TILs, Tumor Infiltrating Lymphocytes; NK, natural killer; ADCC, Antibody-Dependent Cellular Cytotoxicity; ELISA, enzyme-linked immunosorbent assay; ICS, Intracellular Cytokine Staining; NGS, Next Generation Sequencing; VCN, Vector Copy Number; IFN, interferon.

CAR-T

- **Manufacturer:** Novartis Pharmaceuticals Corporation
- **Proper Name:** CTL019 (tisagenlecleucel)
- **Tradename:** KYMRIAH



- **Licensed by US FDA:** 30th August 2017 ; 1st May 2018
- **Indication**
  - ① Indicated for the treatment of patients up to 25 years of age with **B-cell precursor acute lymphoblastic leukemia (ALL)** that is refractory or in second or later relapse.
  - ② Adult patients with relapsed or refractory (r/r) large B-cell lymphoma after two or more lines of systemic therapy including **diffuse large B-cell lymphoma (DLBCL)** not otherwise specified, high grade B-cell lymphoma and DLBCL arising from follicular lymphoma.

CTL019 T cells
Mode of Action

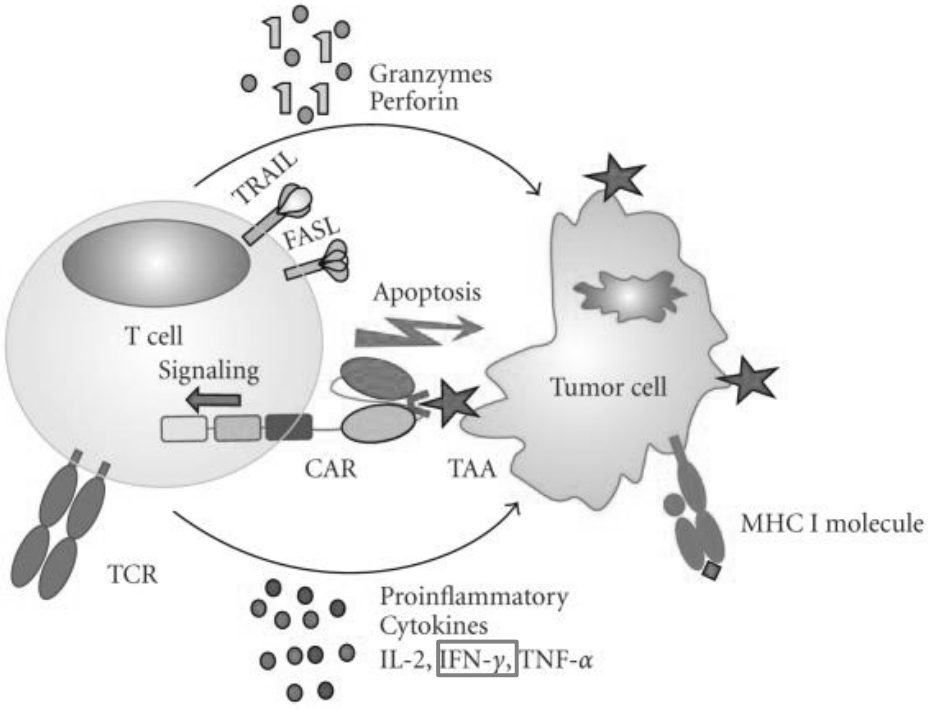
- **Recognition of a common protein (CD19) by chimeric antigen receptor (CAR)**
- **Signaling through CD3 intracellular pathway**
- **Activation of CTL responses -Expansion of the cells**

-High Cytotoxic Granule content

-Strong expression of cytotoxic agents (FasL, **IFN-g)**

-High expression potential of necessary cytokines / chemokines

CartellieriM, J Biomed Biotech 2010



CAR-T: Quality Assurance

CM-9

Quality assurance of CTL019 cell product

Clinical Site 1 → Manufacturing Facility 2 3 4 **5** → Clinical Site 6

Appearance and description
- Color

Safety
- Bacterial endotoxins
- Sterility
- Mycoplasma
- Determination of VSV-G DNA by quantitative PCR (surrogate for RCL)

Purity
- Percentage of viable T cells
- Determination of transduction efficiency by CAR quantitative PCR
- Cell viability

Impurities
- Determination of residual beads by microscopy
- Percentage of viable $CD19^+$ B cells

Identity
- Identity by CAR quantitative PCR (qPCR)

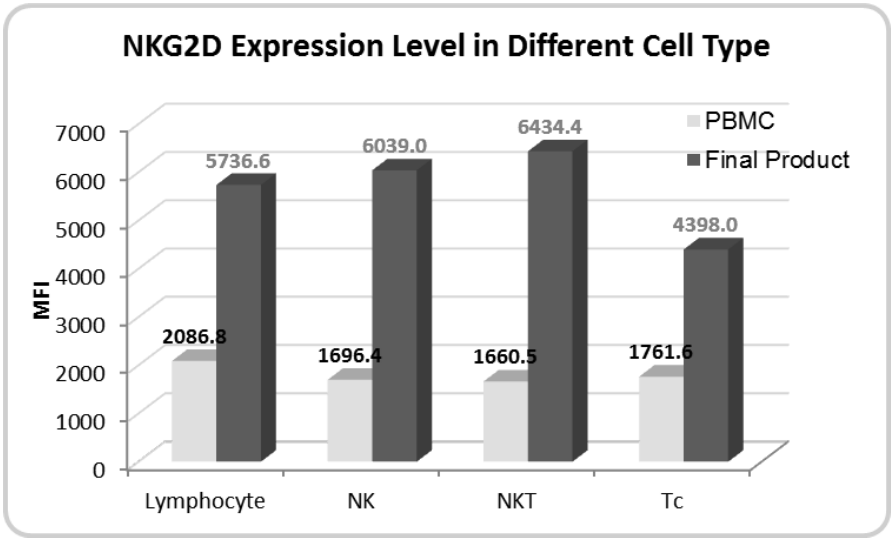
Quantity
- Total cell count
- Number of viable cells (calculated)
- Dose (calculated)

Potency
- Determination of CAR expression by flow cytometry
- Release of IFN-γ in response to CD19-expressing target cells

RCL=replication-competent lentivirus.

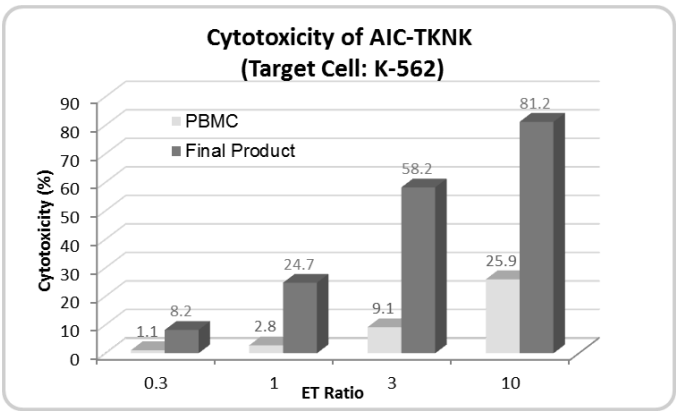
Biomarkers Analysis (NKG2D Receptor)

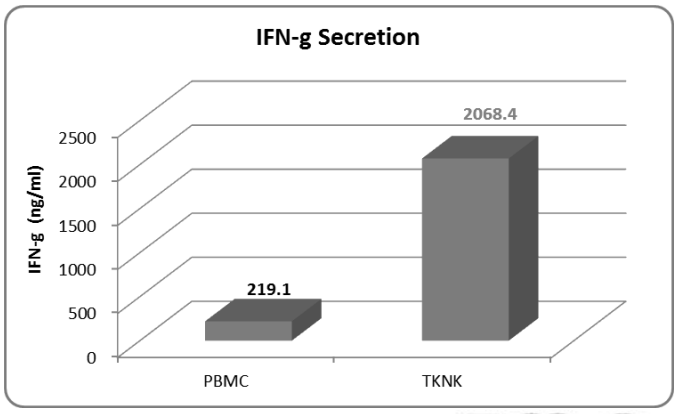
- **Characterization of Activated Cell Product**



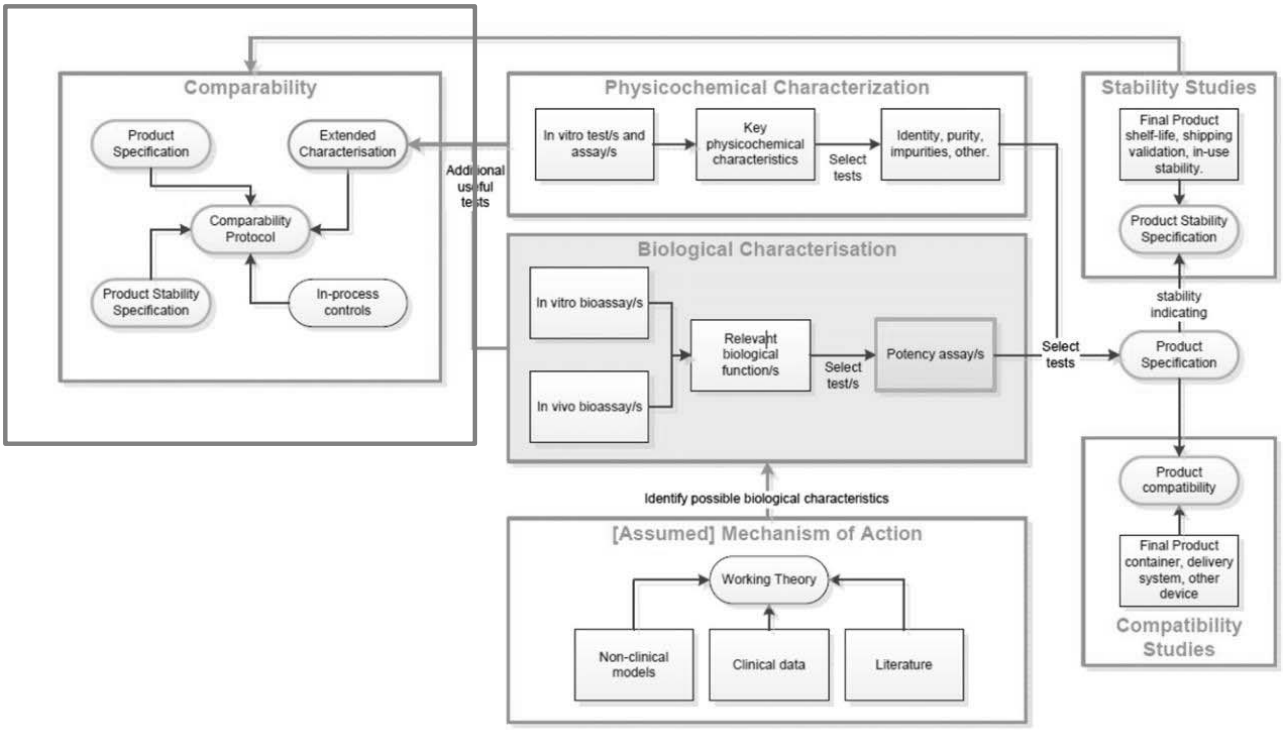
Biological Assays of T Cells Product

- **Potency of T Cell Product (**IFN-γ secretion & Cytotoxicity**)**
- **Quantitative biological method**





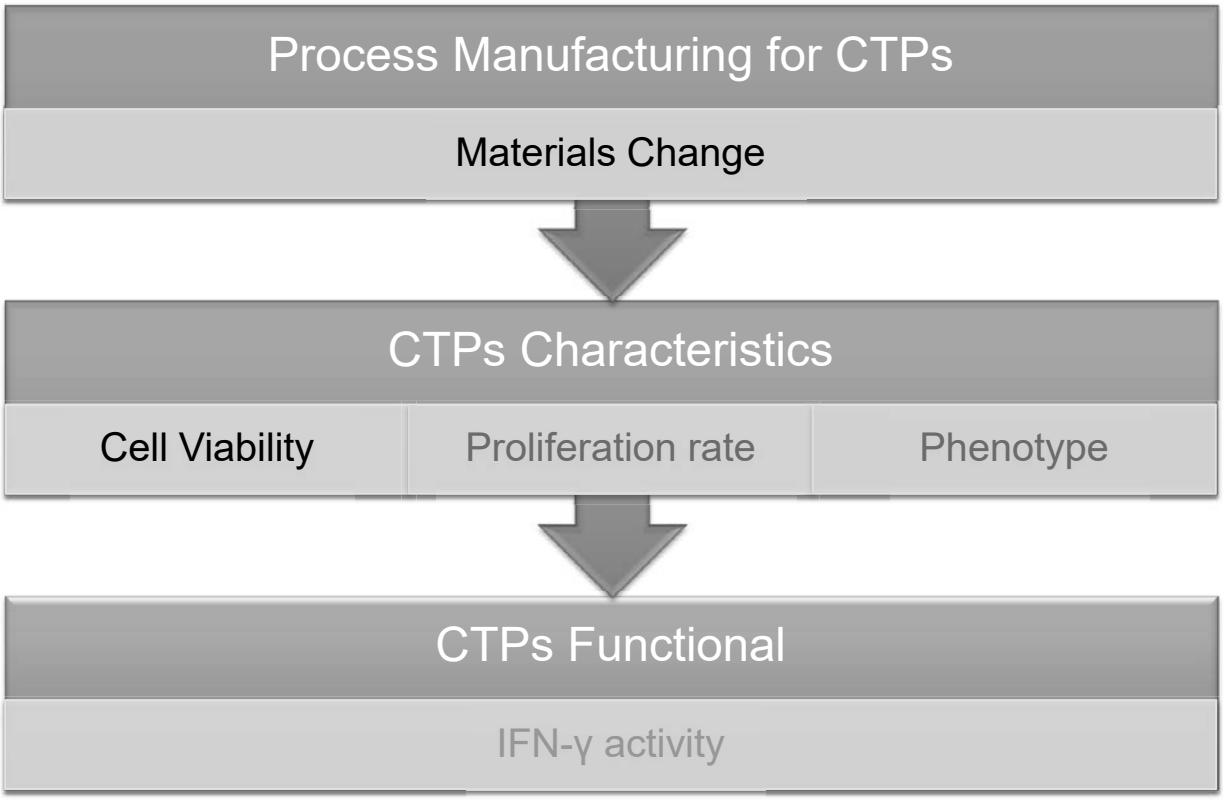
Product Characterization Testings



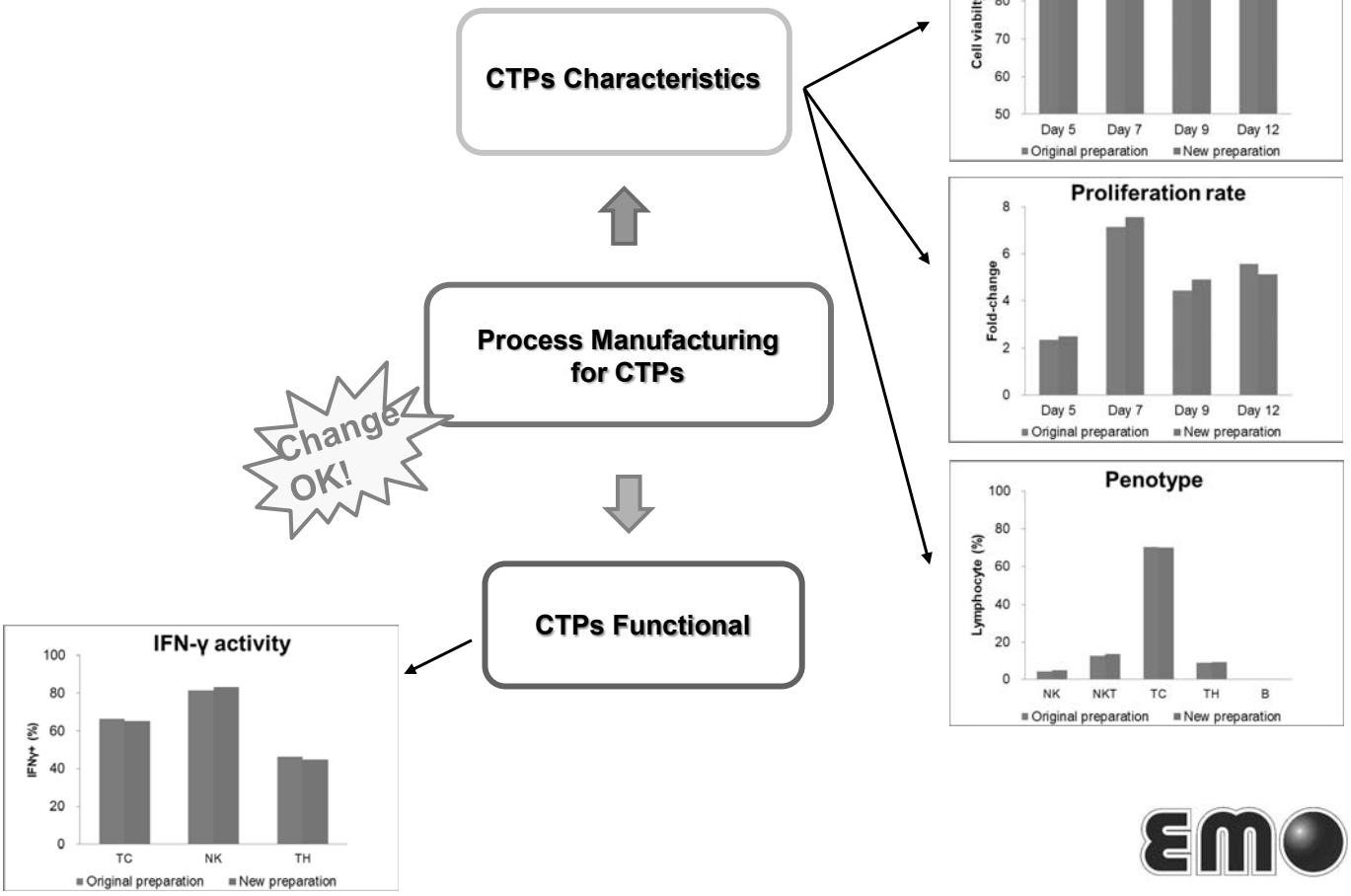
Cytotherapy. 2013 Jan;15(1):9-19. .

Comparability Studies

T cells Product

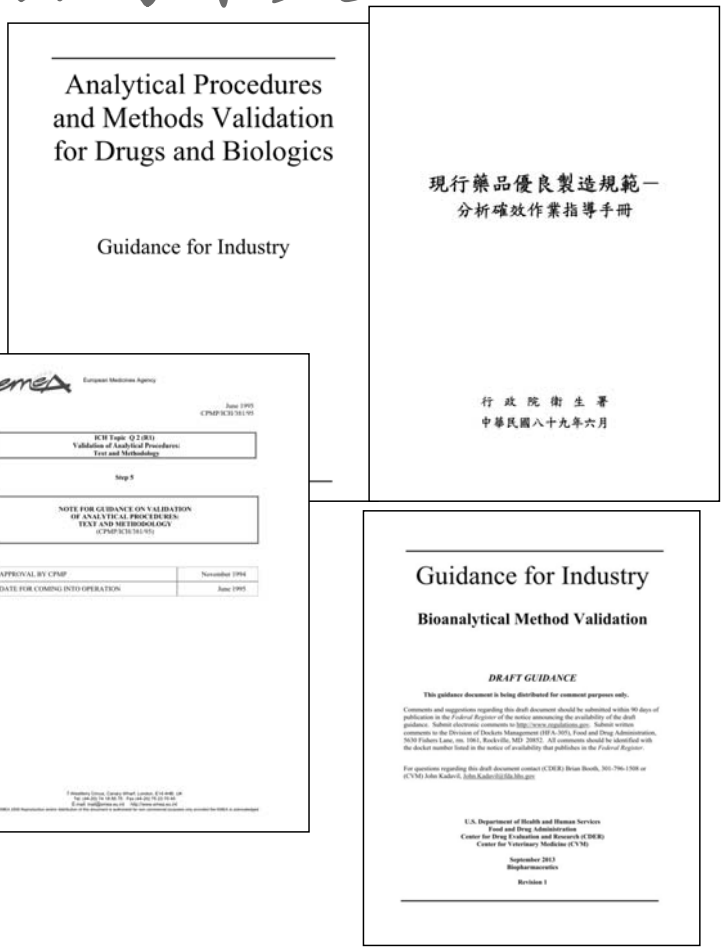


Comparability studies
T Cells Product



分析方法的確效

- **Precision**
  - Repeatability
  - Intermediate precision
  - Reproducibility
- **Accuracy**
- **Specificity**
- **Linearity and Range**
- **Robustness**



Mesenchymal Stem Cells: Time to Change the Name!

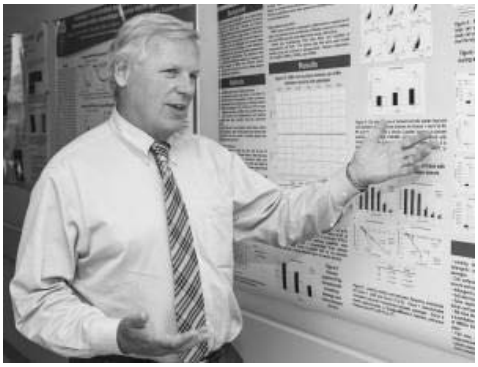
ARNOLD I. CAPLAN - STEM CELLS TRANSLATIONALMEDICINE **2017;6:1445–1451**

- SUMMARY
- Mesenchymal stem cells (MSCs) were officially named more than **25 years ago** to represent a class of cells from human and mammalian bone marrow and periosteum that could be isolated and expanded in culture while maintaining their in vitro capacity to be induced to form a variety of mesodermal phenotypes and tissues. The in vitro capacity to form bone, cartilage, fat, etc., became an assay for identifying this class of multipotent cells and around which several companies were formed in the 1990s to medically exploit the regenerative capabilities of MSCs.
- Today, there are hundreds of clinics and hundreds of **clinical trials** using human **MSCs** with **very few**, if any, **focusing on the in vitro multipotential capacities** of these cells.
- Unfortunately, the fact that MSCs are called "**stem cells**" is being used to infer that patients will receive direct medical benefit, because they **imagine** that these cells will **differentiate** into regenerating tissue producing cells. Such a stem cell treatment will presumably cure the patient of their medically relevant difficulties ranging from osteoarthritic (bone-on-bone) knees to various neurological maladies including dementia.
- I now **urge that we change the name** of **MSC**s to **Medicinal Signaling Cells** to more accurately reflect the fact that these cells **home in on sites of injury** or disease and **secrete bioactive factors** that are **immunomodulatory** and **trophic (**regenerative) meaning that these **cells make therapeutic drugs in situ** that are medicinal.
- It is, indeed, the patient's own site-specific and tissue-specific **resident stem cells** that construct the new tissue as stimulated by the **bioactive factors** secreted by the exogenously supplied MSCs.

Predicting Stem Cell Activity to Ensure Safe and Effective Therapies

March 7, 2018 By: Steven R. Bauer, Ph.D.

- *…**As of January 2018, no MSC-based clinical trials have resulted in FDA-approved treatments**. One significant challenge is ensuring that the MSCs will work together to perform the same desired function when they are administered to patients.*
- *…MSC-based therapies are not available yet. But the ability to predict **specific** functions **of different preparations of MSCs** in the lab may be a big step toward getting **safe and effective FDA-approved treatments to patients**.*



Steve Bauer, Ph.D., chief of the Cellular and Tissues Therapy Branch, Division of Cellular and Gene Therapies, in the Office of Tissues and Advanced Therapies, at CBER.

MSC Products with Regulatory Approval or in the Late-stage Clinical Trial

Table I. MSC products with regulatory approval or in late-stage clinical trials.

Company	Product	Indication	Stage	Reporting
Approvals				
Mesoblast	TEMCELL HS	Acute graft-versus-host disease	Market approval in Japan	2016
Stempeutics	Stempeucel	Critical limb ischemia (Buerger's disease)	Limited market approval in India	2017
Takeda	Alofisel	Complex perianal fistulas in adult Crohn's disease	Market approval in European Union	2018
Pipeline				
Athersys	MultiStem	Ischemic stroke	Phase 3 (specific protocol assessment)	Initiating 2018
Bone Therapeutics	PREOB	Osteonecrosis of the hip	Phase 3	Expected 2H 2018
Brainstorm	NurOwn	Amyotrophic lateral sclerosis	Phase 3	Expected late 2019
Cytori	ECCI-50	Male stress urinary incontinence	Phase 3	Anticipated 1H 2019
Mesoblast	MPC-150-IM	Moderate to severe chronic heart failure	Phase 3	Complete enrollment 2H CY 2018
	MSC-100-IV	Acute graft-versus-host disease	Phase 3	Day 180 safety data Quarter 3 CY18
	MPC-06-ID	Chronic low back pain due to disc degeneration	Phase 3	Enrollment in the trial completed in Quarter 1 2018

1H, first half of fiscal year; 2H, second half of fiscal year; CY, calendar year.

Manufacturing and Assessments of Potency for MSC Products

Cytotherapy, 2019; 21: 289–306

International Society ISCT Cell & Gene Therapy

Mesenchymal stromal cell therapy: progress in manufacturing and assessments of potency

KEVIN P. ROBB[1,2], JOAN C. FITZGERALD[3], FRANK BARRY[1,3] & SOWMYA VISWANATHAN[1,2,4,5]

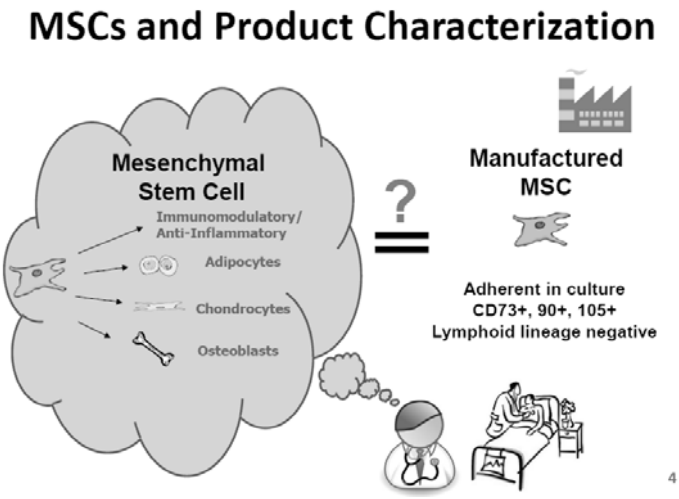
[1]*The Arthritis Program, University Health Network, Toronto, Canada;,*[2]*Institute of Biomaterials and Biomedical Engineering, University of Toronto, Toronto, Canada,*[3]*Regenerative Medicine Institute (REMEDI), National University of Ireland, Galway, Ireland,*[4]*Cell Therapy Program, University Health Network, Toronto, Canada, and* [5]*Division of Hematology, Department of Medicine, University of Toronto, Toronto, Canada*

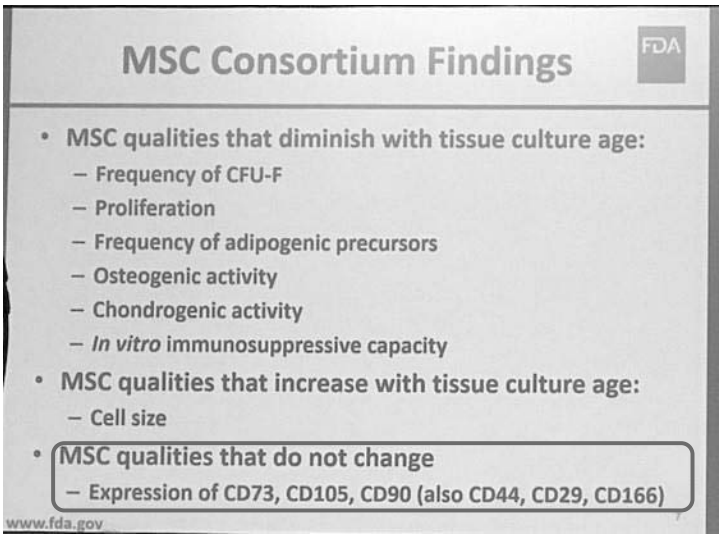
Abstract

Mesenchymal stromal cell (MSC) therapies have been pursued for a broad spectrum of indications but mixed reports on clinical efficacy have given rise to some degree of skepticism regarding the effectiveness of this approach. However, recent reports of successful clinical outcomes and regulatory approvals for graft-versus-host disease, Crohn's disease and critical limb ischemia have prompted a shift in this perspective. With hundreds of clinical trials involving MSCs currently underway and an increasing demand for large-scale manufacturing protocols, there is a critical need to develop standards that can be applied to processing methods and to establish consensus assays for both MSC processing control and MSC product release. Reference materials and validated, uniformly applied tests for quality control of MSC products are needed. Here, we review recent developments in MSC manufacturing technologies, release testing and potency assays. We conclude that, although MSCs hold considerable promise clinically, economies of scale have yet to be achieved although numerous bioreactor technologies for scalable production of MSCs exist. Additionally, rigorous disease-specific product testing and comprehensive understanding of mechanisms of action, which are linked to relevant process and product release potency assays, will be required to ensure that these therapies continue to be successful.

MSCs Product Characterization and Clinical Trial

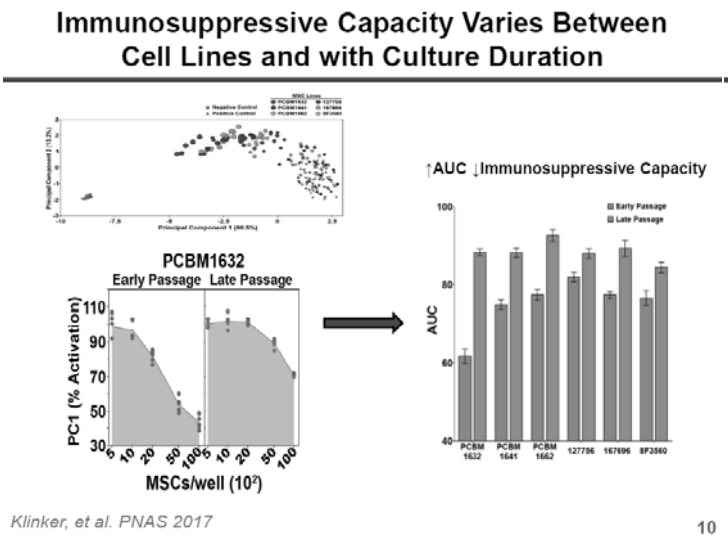
By: Steven R. Bauer, Ph.D. (FDA CBER); 2018 ISCT

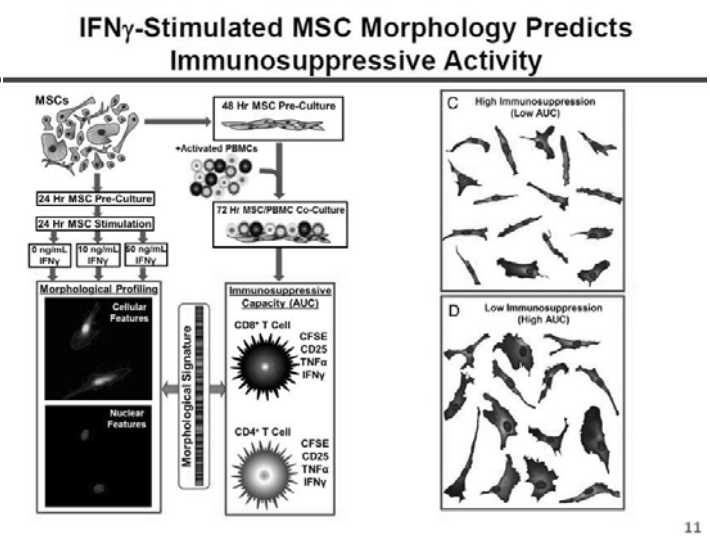




Immunosuppressive Capacity *V.S.* Culture Duration

By: Steven R. Bauer, Ph.D.





Morphological profiling using machine learning reveals emergent subpopulations of interferon-γ–stimulated mesenchymal stromal cells that predict immunosuppression

January 2019 Cytotherapy :Volume 21, Issue 1, p1-124

- **Background**
- Although a preponderance of pre-clinical data demonstrates the immunosuppressive potential of mesenchymal stromal cells (MSCs), significant heterogeneity and **lack of critical quality attributes (CQAs) based on immunosuppressive capacity** likely have contributed **to inconsistent clinical outcomes**.
- This heterogeneity exists not only between MSC lots derived from different donors, tissues and manufacturing conditions, but also within a given MSC lot in the form of functional subpopulations.
- We therefore explored the potential of **functionally relevant morphological profiling (FRMP)** to identify morphological subpopulations **predictive of the immunosuppressive capacity of MSCs** derived from **multiple donors, manufacturers** and **passages**.

- **Results**
- Multiple **IFN-γ–stimulated** subpopulations significantly correlated with the ability of MSCs **to inhibit $CD4^+$ and $CD8^+$ T-cell activation** and served as effective CQAs to predict the immunosuppressive capacity of additional manufactured MSC lots.
- We further characterized the emergence of morphological heterogeneity following IFN-γ stimulation, which provides a strategy for identifying functional subpopulations for future single-cell characterization and enrichment techniques.
- **Discussion**
- This work provides a generalizable analytical platform for assessing functional heterogeneity based on single-cell morphological responses that could be used to identify **novel CQAs** and **inform cell manufacturing decisions**.

- ROSS A. MARKLEIN, MATTHEW W. KLINKER, KATHERINE A. DRAKE, HANNAH G. POLIKOWSKY , ELIZABETH C. LESSEY-MORILLON, **STEVEN R. BAUER**

MSC細胞產品的免疫調節作用機轉

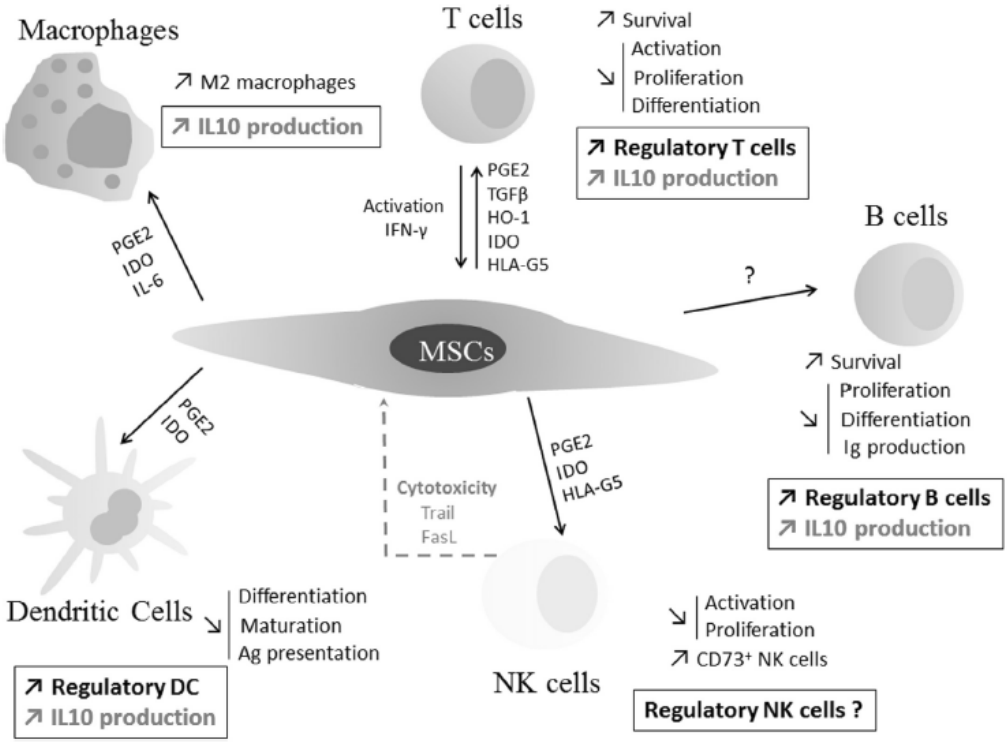


Figure 1. MSCs re-educate the immune cells to induce the generation of regulatory immune cells with tolerogenic properties. These regulatory immune cells such as Tregs, Bregs, regulatory APC and NK cells will gather to create a tolerogenic environment suitable to modulate the immune response. Multiple regulatory pathways with a central role for IL-10 could then be used by these cells to finally establish immunomodulation.

In vitro Potency Assay for MSC Used in Immunotherapy

Cytotherapy, 2017; 19: 784–797

International Society for Cellular Therapy ISCT®

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REVIEW ARTICLE

Regulatory perspective on *in vitro* potency assays for human mesenchymal stromal cells used in immunotherapy

CHARLOTTE DE WOLF, MARJA VAN DE BOVENKAMP & MARCEL HOEFNAGEL

Medicines Evaluation Board (CBG-MEB), Utrecht, The Netherlands

Abstract

Mesenchymal stromal cells (MSCs) are multipotent cells derived from various tissues that can differentiate into several cell types. MSCs are able to modulate the response of immune cells of the innate and adaptive immune system. Because of these multimodal properties, the potential use of MSCs for immunotherapies is currently explored in various clinical indications. Due to the diversity of potential MSC medicinal products at the level of cell source, manufacturing process and indication, distinct functionality tests may be needed to ensure the quality for each of the different products. In this review, we focus on *in vitro* potency assays proposed for characterization and release of different MSC medicinal products. We discuss the most used functional assays, as presented in scientific advices and literature, highlighting specific advantages and limitations of the various assays. Currently, the most proposed and accepted potency assay for release is based on *in vitro* inhibition of T cell proliferation or other functionalities. However, for some products, assays based on other MSC or responder cell properties may be more appropriate. In all cases, the biological relevance of the proposed assay for the intended clinical activity should be substantiated with appropriate product-specific (non-)clinical data. In case practical considerations prevent the use of the ideal potency assay at release, use of a surrogate marker or test could be considered if correlation with functionality has been demonstrated. Nevertheless, as the field of MSC immunology is evolving, improvements can be expected in relevant assays and consequently in guidance related to potency testing.

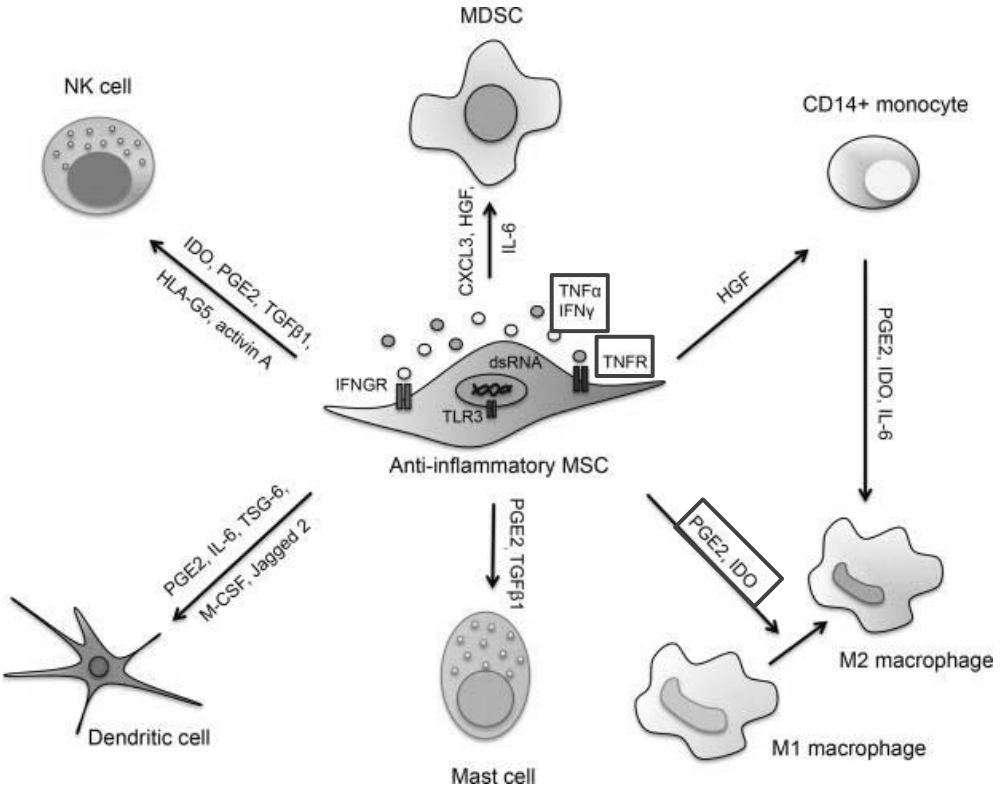
Prochymal: MOA

EMO

- Manufacturer: **Mesoblast** (Osiris Therapeutics)
- **Allogenic MSC** from **bone marrow**

- Homing to sites of injury/inflammation
- Immunomodulation: suppression of T-lymphocytes at injury/inflammation sites
- Anti-inflammatory activity: inhibition of pro-inflammatory cytokines (TNF-α and IFN-γ)
- Indication: Treatment GvHD

Prochymal: MOA



41

Immunol Lett. 2015 Dec;168(2):140-6

Potency Markers for Screening (Prochymal)

Marker	Justification for marker selection
Prostaglandin E2 (PGE_2)	PGE_2 suppresses immune response. MSCs produce PGE_2, and PGE_2 mediates MSC-induced immunosuppressive and anti-inflammatory effects *in vitro.*
Indoleamine 2,3-dioxygenase (IDO) enzyme activity	IDO is an enzyme inducible by pro-inflammatory cytokines such as IFN-γ and TNF-α. IDO inhibits immune response via depletion of tryptophan, an amino acid that is essential for immune cell activation. IDO enzyme mediates MSC-induced immunosuppression *in vitro.*
Tumor Necrosis Factor-α (TNF-α)	TNF-α is a pro-inflammatory cytokine playing an important role in GVHD. MSCs inhibit TNF-α secretion by immune cells *in vitro*.
Interferon-γ (IFN-γ)	IFN-γ is a cytokine secreted by Th1 cells that are involved in GVHD development. MSCs can inhibit secretion of IFN-γ that is beneficial for GVHD treatment
Tumor Necrosis Factor-α Receptor (TNFR)	TNFR is expressed on MSCs. TNFα is present in organs targeted by GVHD. TNF-α via TNFR up-regulates secretion of PGE_2, induces expression of IDO and stimulates MSC migration *in vitro*. TNFR is a mediator of MSC biological activities.

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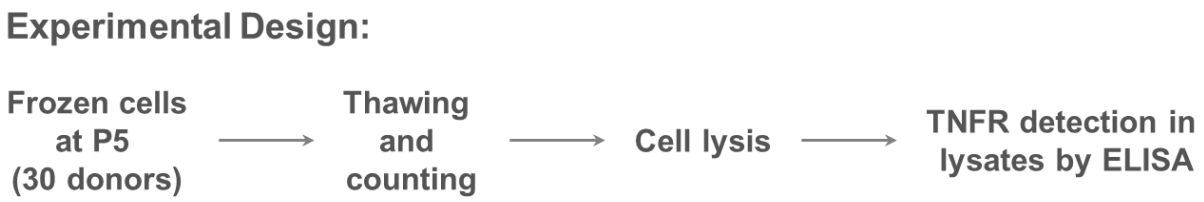
Prochymal效價檢測：TNFR I的定量

- 檢測方法



- 合適性分析
  - 可量化？
  - 可確效？ Commercially available ELISA kit
  - 與Biological activity的關聯性？

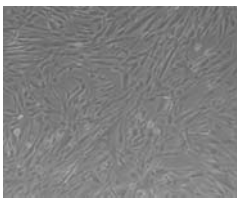
自體ADSC產品(RegStem®)治療退化性關節炎之開發及其臨床試驗

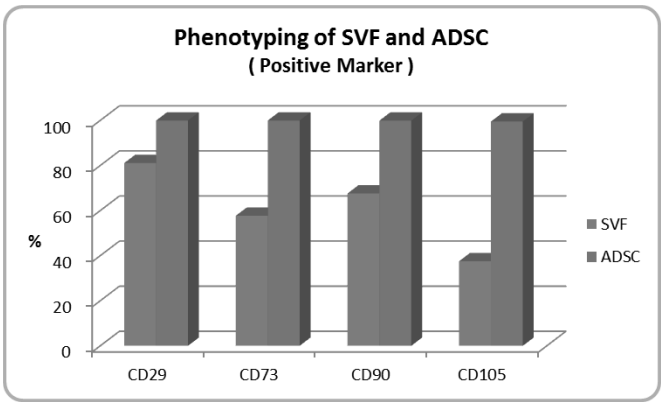
Mechanism of Action :

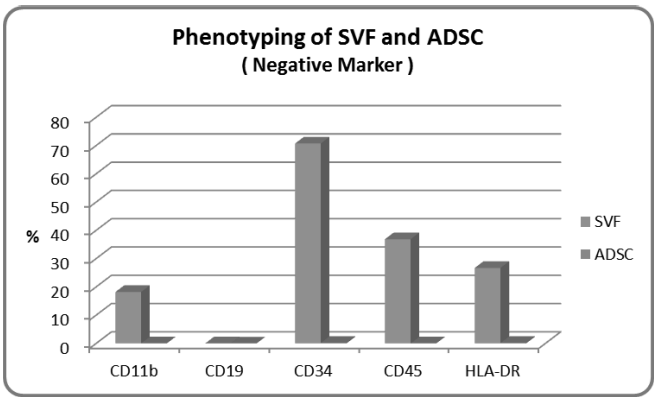
- Anti-inflammation
- Chondrogenesis

Characteristics of MSC

- **Characterization of ADSC**
  - According to Position paper of **ISCT** in **2006**, minimal criteria of MSC must be….
    - **Plastic-adherent**
    - **Express CD73, 90 and 105**, and **lack expression** of CD11b(or CD14), CD19(or CD79α), CD34, CD45 and HLA-DR.
    - Differentiate to osteoblasts, adipocytes and chondroblasts in vitro.

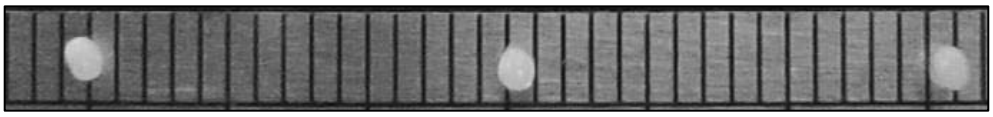


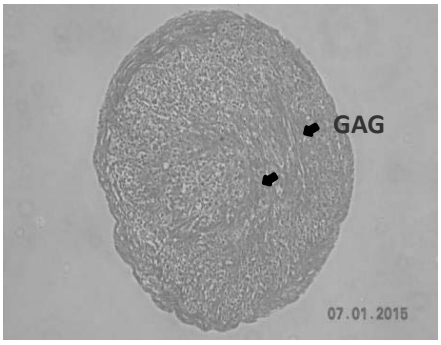




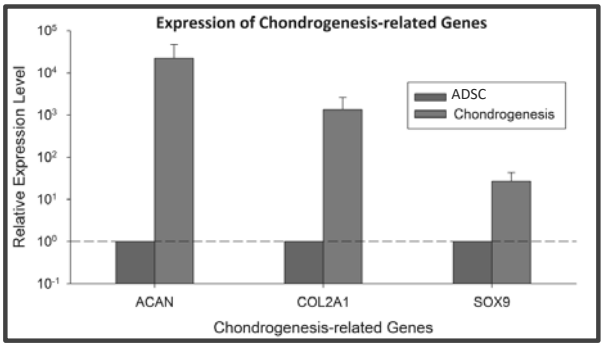
MSC Characterization

- **ADSC**
  - Chondrogenesis





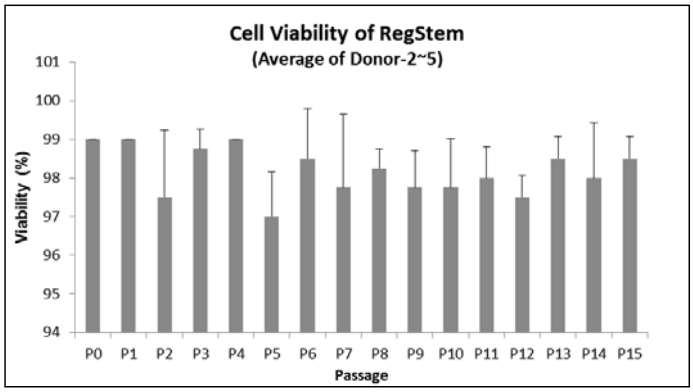
Alcian blue staining



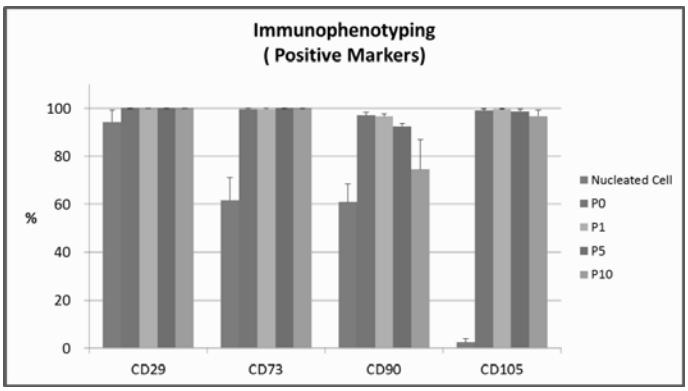
Consistency of CTP after *Ex Vivo* Expansion

(Tested by EMO Biomedicine in 2015)

Cell Viability of Different Passages of CTP after Thawing

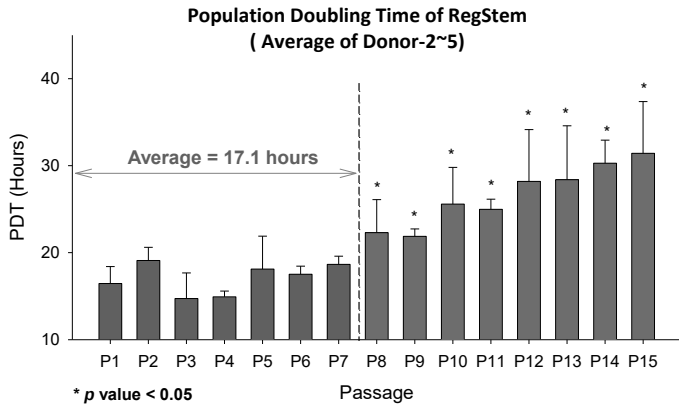


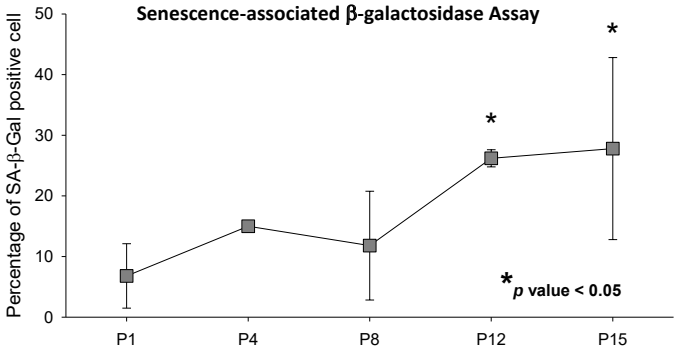
Cell Markers of Different Passages of CTP after Thawing



Cell Growth Kinetics vs. Cellular Senescence

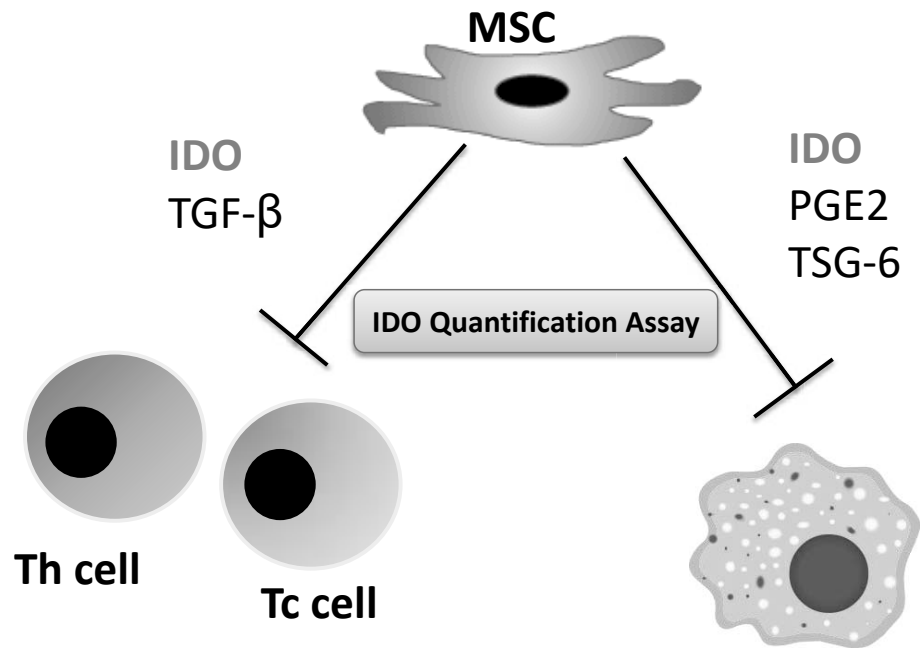
(Tested by EMO Biomedicine in 2015)





- After passage 7, population doubling time was increased significantly by passage.
- Percentage of aging cell was increased after passage 8.

Immunosuppression of MSC



Proliferation ↓
IL-2 ↓

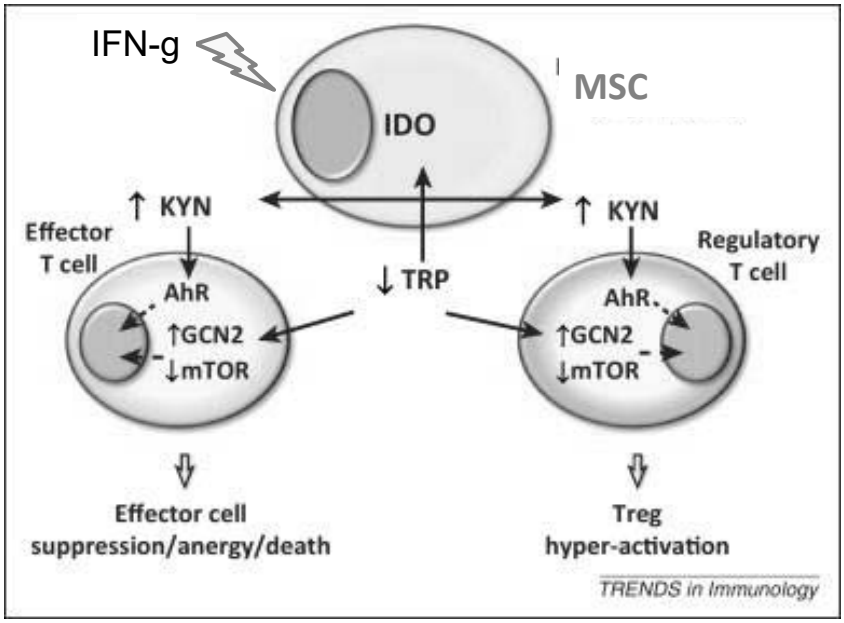
TNF-α ↓ IL-10 ↑ **(Macrophage M1→M2)**

T cell Suppression Assay

Macrophage Inhibition Assay

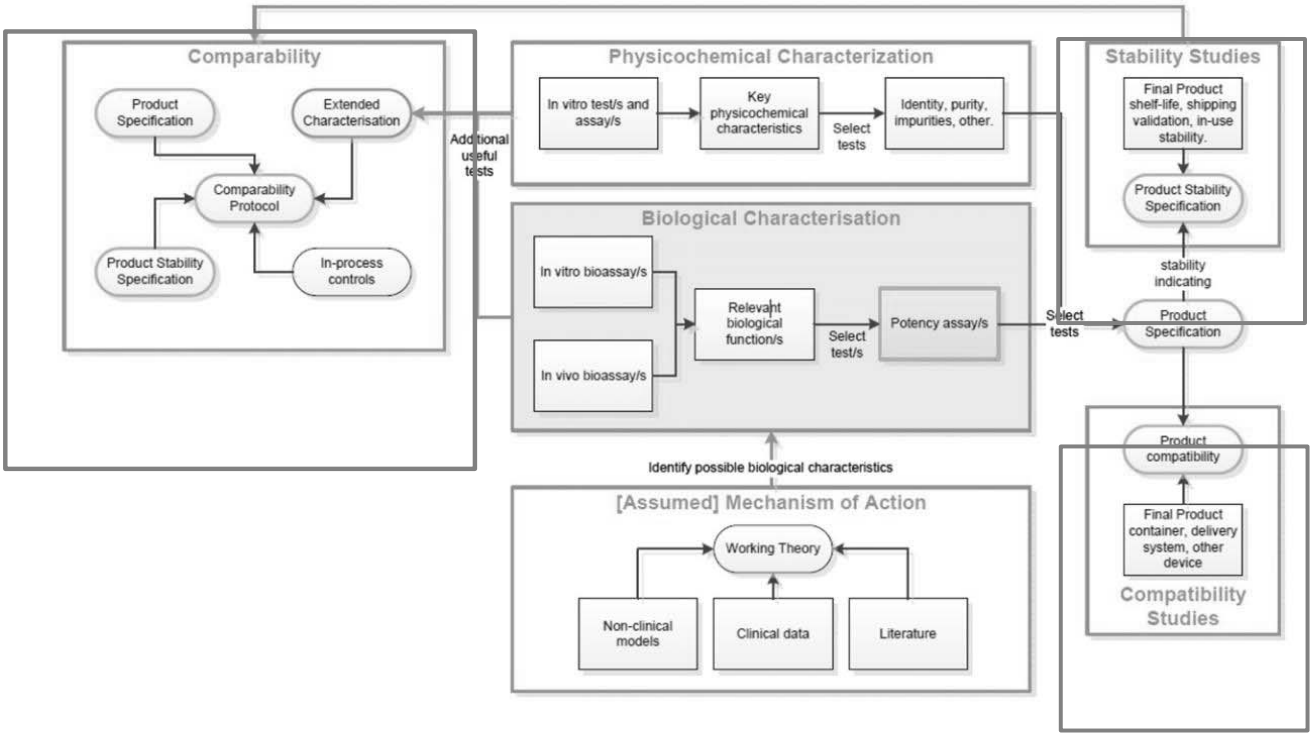
IDO Quantification Assay

IDO (Indoleamine 2,3-dioxygenase)是MSC受到發炎性細胞激素刺激後，所產生抑制免疫細胞的關鍵酵素，也是全球各研發團隊針對MSC產品免疫調節功能與效價分析，競相開發的重要標的。



- 受到IFN-g刺激的MSC會產生IDO
- IDO可使Tryptophan代謝為Kynurenine，進而抑制T細胞及活化Treg細胞
- 以流式細胞儀定量MSC產生的IDO

Indoleamine 2,3 dioxygenase and metabolic control of immune responses



IDO定量檢測和其它生物分析方法，使用於OA臨床試驗之製造流程與品質管控，以確保MSC產品的一致性與功能性。

Comparability Studies

MSC-Based Products

Comparability

Manufacturing Process Change

MSC Product Characteristics

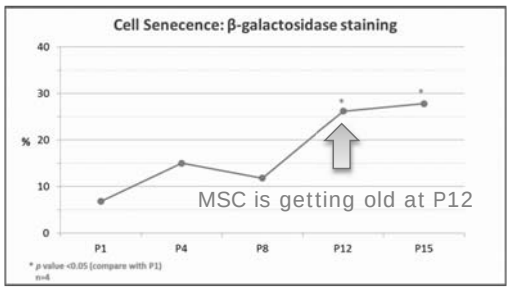
Cell Viability | Doubling Time | Phenotype

Immunosuppressive Function

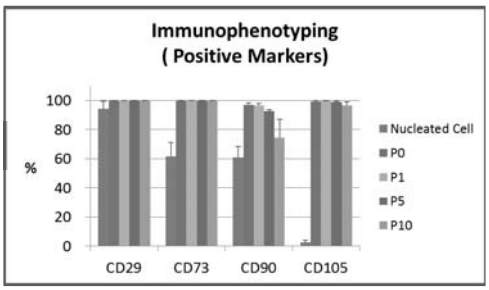
IDO assay, suppression of T cells proliferation, TNF-α inhibition,..

IDO Quantification Assay

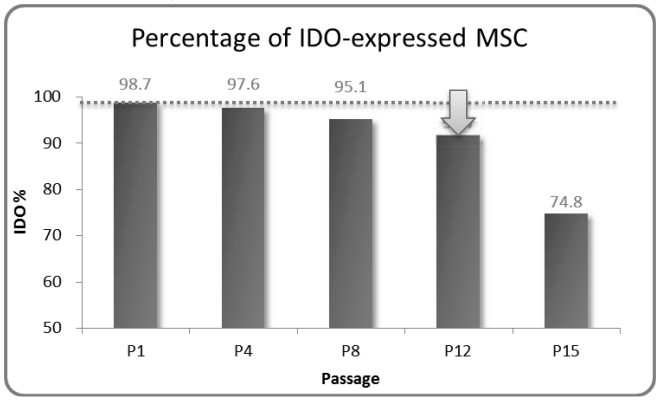
Capable to distinguish aged MSC

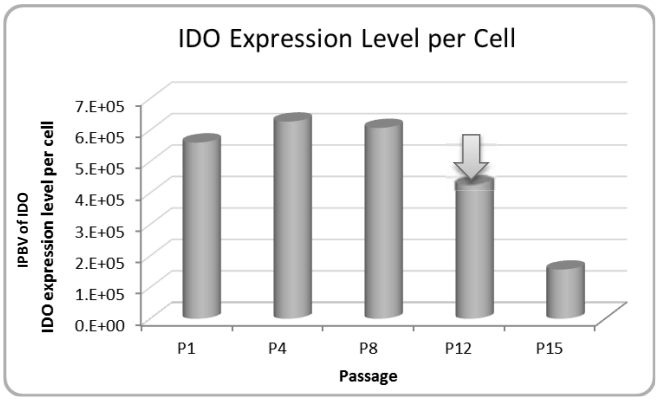


Cell senescence can't be detected by CD markers



Cell senescence can be detected by IDO quantification assay

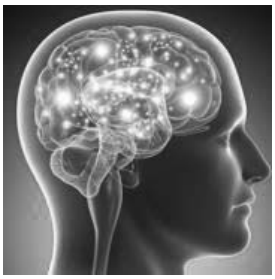


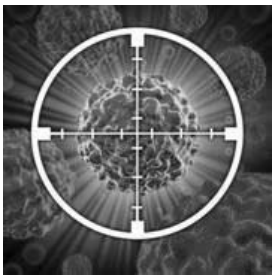


Data from EMO Biomedicine Corp.

細胞治療國際發展趨勢







長聖國際生技股份有限公司
Ever Supreme Bio Technology Co.,Ltd
主講者： 蕭秀玲 博士

2019-10-21

Number of Studies in 2019

Region Name	N
World	34,076
Africa	618
Central America	554
East Asia	4,458
Japan	661
Europe	8,624
Middle East	1,191
North America	19,575
Canada	2,478
Mexico	380
United States	18,646
North Asia	799
Pacifica	1,253
South America	852
South Asia	444
Southeast Asia	703

2019 Oct

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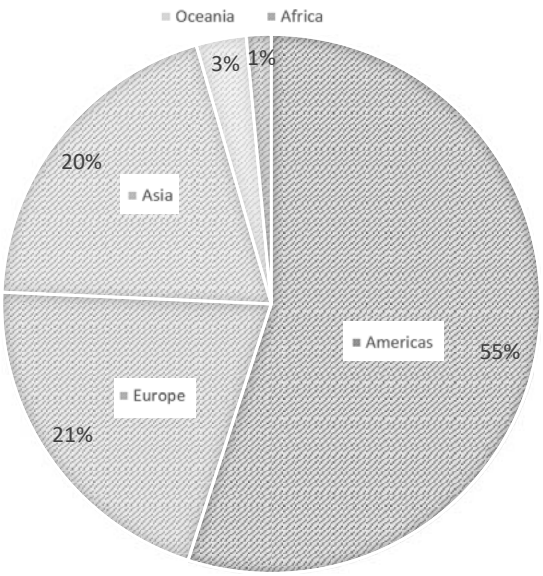
2018 Sep

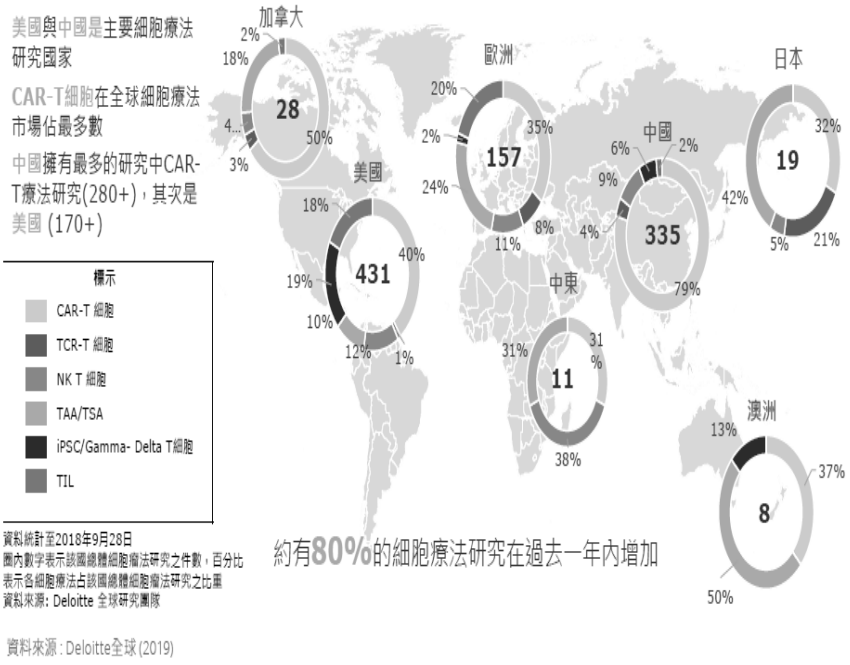
Colors indicate the number of studies with locations in that region.
Least Most
Labels give the exact number of studies.

As of Oct., 2019. ClinicalTrials.gov

細胞治療臨床試驗分布區域及件數

2019年





As of Oct., 2019. ClinicalTrials.gov

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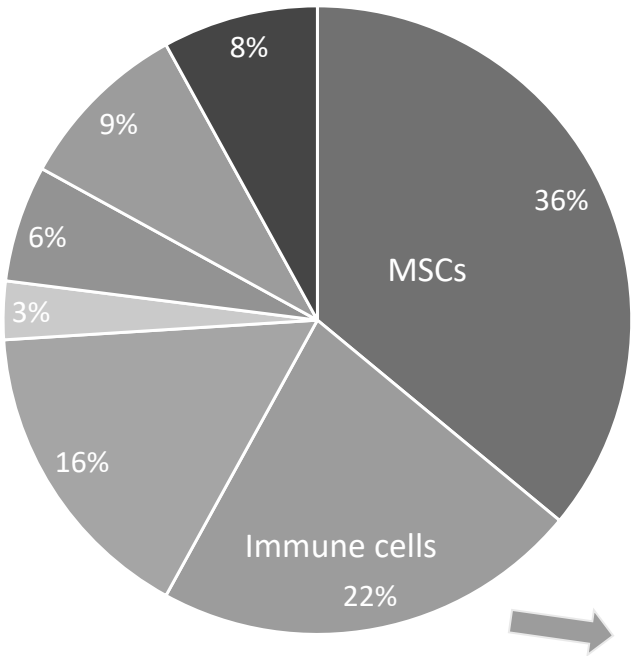
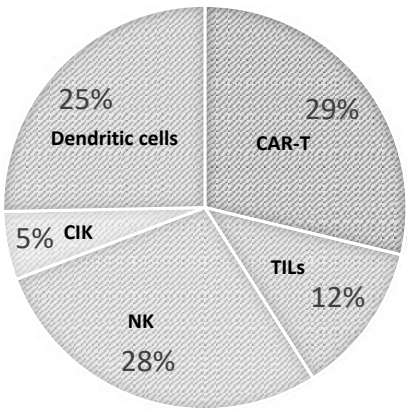
Deloitte, 2019.

全球細胞治療臨床試驗之細胞種類



- MSCs (adipose-derived, bone marrow, umbilical cord)
- Immune cells (CAR-T, TILs, NK, CIK, Dendritic cells)
- Endothelial progenitor cells
- Cord Blood Stem cells
- Fibroblat
- Epithelial cells
- Others



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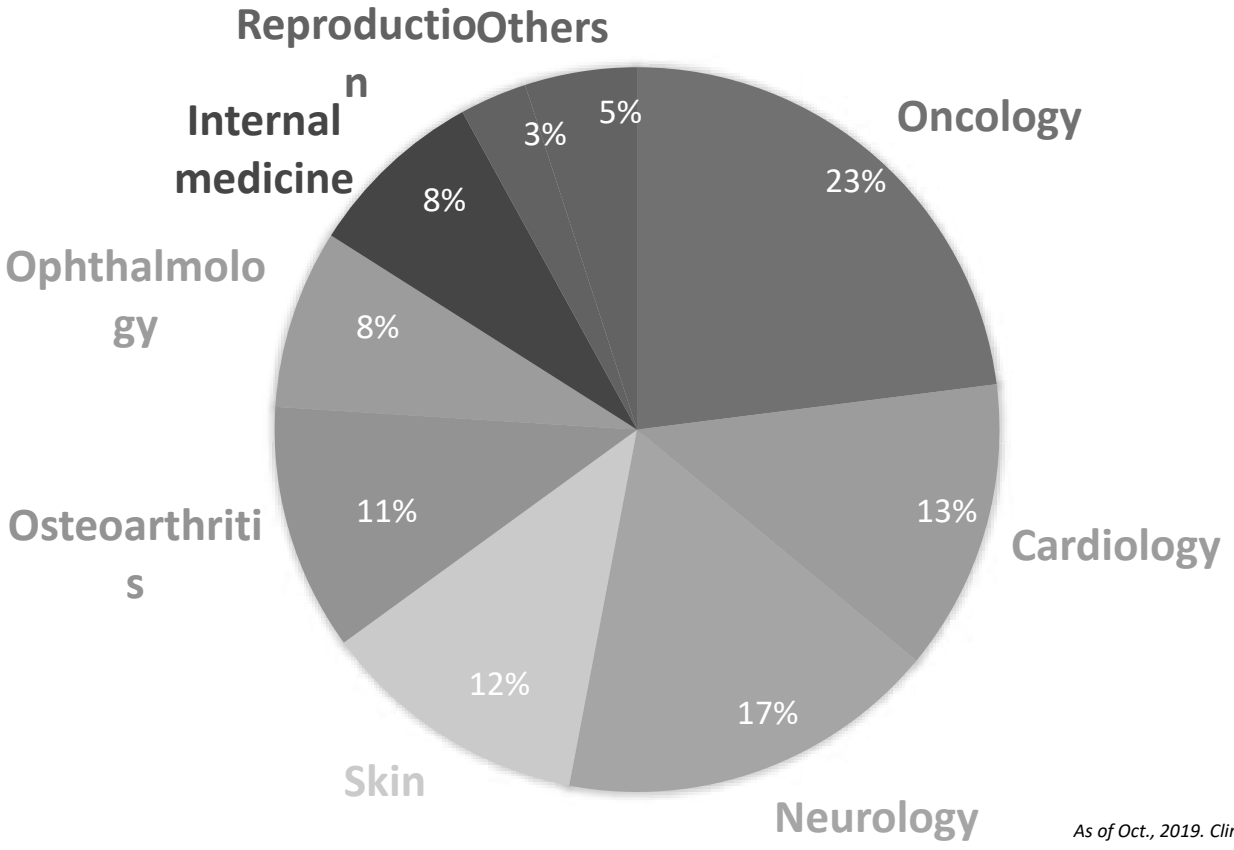
As of Oct., 2019. ClinicalTrials.gov

全球細胞治療臨床試驗之適應症



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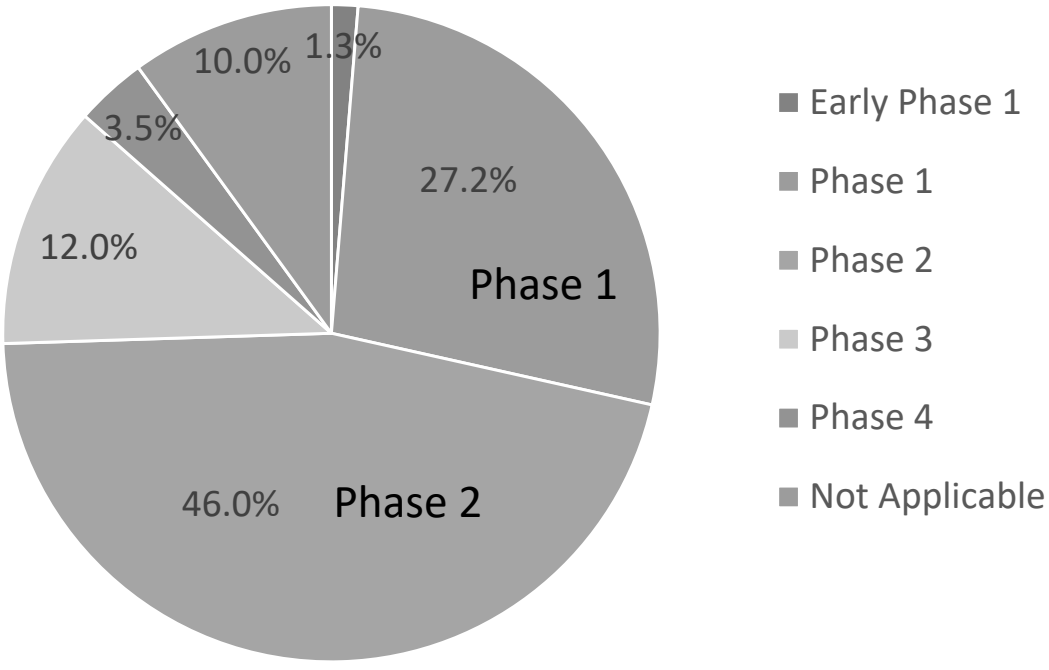
As of Oct., 2019. ClinicalTrials.gov

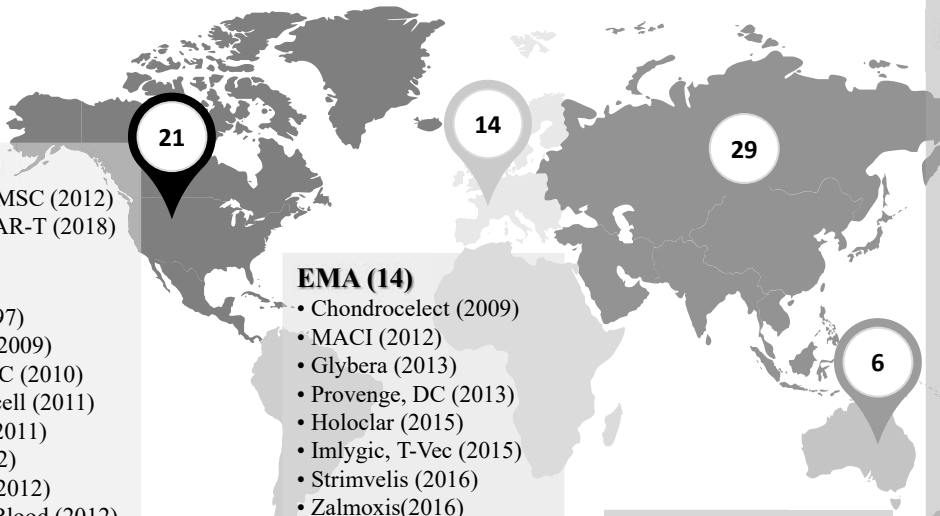
全球細胞治療臨床試驗階段



6

As of 07 Oct., 2019. ClinicalTrials.gov

國際細胞及基因治療產品上市情況



Canada (2)
- Prochymal, MSC (2012)
- Kymirah, CAR-T (2018)

US (19)
- Carticel (1997)
- Prochymal (2009)
- Provenge, DC (2010)
- Laviv, fibrocell (2011)
- Hemacord (2011)
- Gintuit (2012)
- MultiStem (2012)
- HPC, Cord Blood (2012)
- HPC, Cord Blood (2013)
- Ducord (2012)
- Allocord (2013)
- Imlygic, T-Vec (2015)
- Clevecord (2016)
- HPC, Cord Bolld (2016)
- MACI (2016)
- Kymriah(CAR-T)(2017)
- Luxturna(2017)
- Yescarta(CAR-T)(2017)
- Recell (2018)

EMA (14)
- Chondrocelect (2009)
- MACI (2012)
- Glybera (2013)
- Provenge, DC (2013)
- Holoclar (2015)
- Imlygic, T-Vec (2015)
- Strimvelis (2016)
- Zalmoxis(2016)
- Spherox (2017)
- Chondrosphere (2017)
- Alofisel (2018)
- Kymirah, CAR-T (2018)
- LUXTURNA (2018)
- Yescarta (2018)

Australia (4)
- Cartogen (2002)
- ReCell/CellSpray (2006)
- Amniofix, EpiFix (2018)
- EpiBurn (2018)

New Zealand (2)
- ReCell/CellSpray
- Prochymal, MSC (2012)

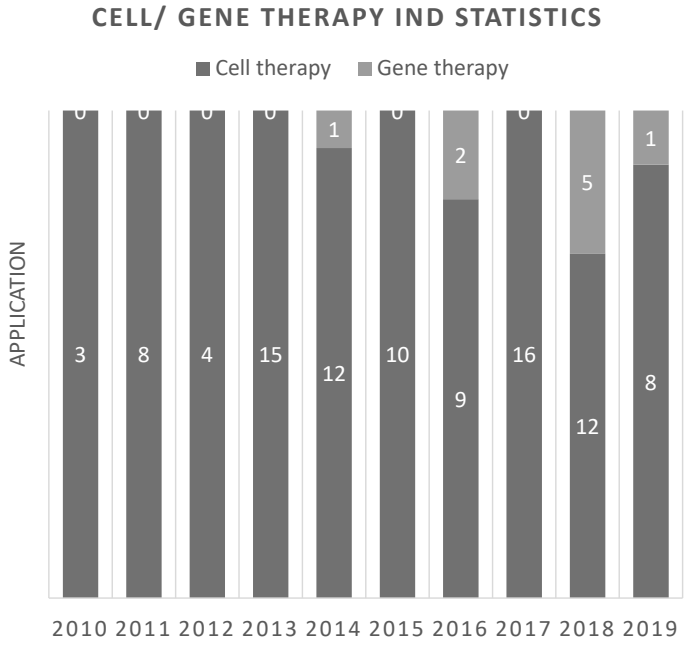
Japan (5)
- JACE (2007)
- JACC (2012)
- TEMCELL, MSC (2015)
- HearSheet (2015)
- Kymirah, CAR-T (2019)

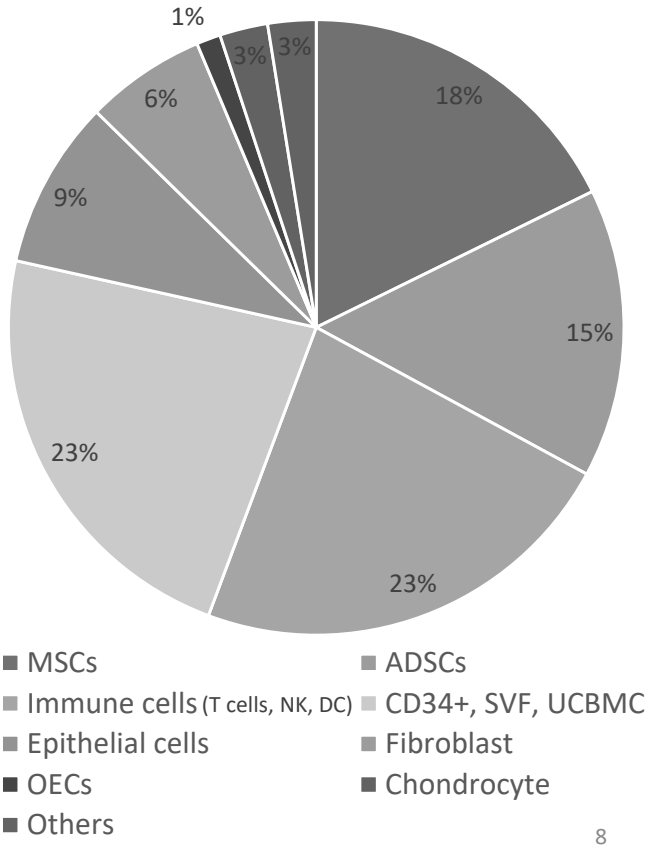
Korea (20)
- Chondron (2001)
- Holoderm (2002)
- Kaloderm (2005)
- Keraheal (2006)
- CreaVax-RCC (2007)
- Immuncell-LC (2007)
- Hyakgraft-3D (2007)
- Innolak (2007)
- Adipocell (2008)
- RMS Ossron (2009)
- QueenCell (2010)
- AutoStem (2010)
- CureSkin (2011)
- Hearticellgram-AMI (2011)
- Cartistem, MSC (2012)
- Cupistem (2012)
- Neuronata-R (2014)
- Keraheal-allo (2015)
- Rosmir (2017)
- Invossa-K (2017)

Singapore (3)
- Chondrotransplant
- ReCell/CellSpray
- Cartogen

India (1)
- APCeden (2017)

台灣細胞治療臨床試驗現況





TFDA 食品藥物管理局

8

國際再生醫療管理方式

國家	臺灣		日本		韓國	美國		歐盟
特色	雙法雙軌 (技術+產品)管理				以產品(藥品)管理為主，但設定例外情況			
權責單位	衛生福利部醫事司	食品藥物管理署(TFDA)	厚生勞働省醫政局(MHLW)	厚生勞働省醫藥食品局(PMDA)	食品藥品安全部(MFDS)	美國食品藥物管理局(US FDA)		歐洲藥物管理局(EMA)
法規	•特定醫療技術檢查檢驗 •醫療儀器施行或使用 •特管法	•藥事法 •再生醫療製劑管理條例 (草案)	再生醫療等安全性確保法	藥品醫療機器法(藥機法)	藥事法	公共衛生服務法 (PHS Act)	21世紀醫療法 (21 Century Cures Act)	•2004/23/EC號指令 •1394/2007號規則
管理	細胞治療技術(自體為主)	•細胞治療產品 •基因治療產品 •組織工程產品 •複合性產品	•自由診療 (醫療技術) •臨床研究	再生醫學製品	生物藥品	人類細胞組織物(HCT/Ps)-依風險分類	再生醫學認定及加速核准(RMAT)	先進治療產品(ATMP)
說明	•醫療機構提出申請 •客製化 •製程管控-GTP標準 •開放6項細胞治療技術	•藥商提出申請 •商品化 •製程管控- GTP GMP •取得製劑許可證可販售	•依風險分級管理，制定各管理規範及程(Class 1,2,3)。 •醫療機構可以委外部企業操作細胞培養加工。	•須執行臨床試驗(IND)、查驗登記申請(NDA) •建立「有條件期限許可」。7年內完成療效確認性臨床試驗。	•須執行臨床試驗(IND)、查驗登記申請(NDA)	PHS 361 •最小操作 •同源使用 •不與其他物質併用 •不產生系統性作用 •例如硬骨、軟骨、眼角膜等組織物 PHS 351 •非361者 •須申請臨床試驗(IND)或查驗登記(BLA)	•制定再生醫學先進療法認定機制及審查規範。 •協助加速研發再生醫學治療。	細胞處理為連續操作者(substantial manipulation)由EMA審查，其餘由各國家管理
例外	無		無		醫院執行最小操作屬醫療法管理範疇	取自特定個人後，經由同一外科手術而使用於同一個人	排除PHS361產品	Hospital exemption非常態製造、符合品質標準、在同一國內使用、由醫院特定醫生負責
名詞使用	特管法 (特定醫療技術檢查檢驗醫療儀器施行或使用管理辦法修正草案)		再生醫療等製品 (包含細胞治療及基因治療產品)		NA	Regenerative Medicine Advanced Therapy ，RMAT		Advanced Therapy Medicinal Product，ATMPs

全球細胞治療市場沿革

1993 細胞治療臨床試驗興起

1997 Carticel首先獲US FDA核准
至2019年期間，共有70種經核准上市之藥物

2017 全球細胞治療技術市場達112億美元
- 北美占主導地位，達33.8億美元
  亞太地區市場預計將實現最高複合年增長率
- 全球基因治療市場規模約45億美元
  細胞治療約40億美元

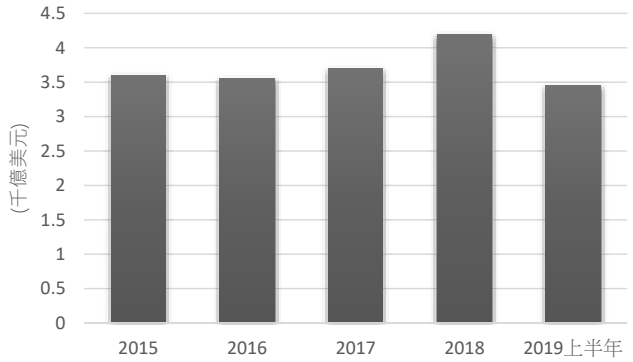
2018 2018-2025年市場複合年增長率16.81%

2019 年初，FDA預測，至2025年間，每年將會有10-20款新藥獲得批准

2025 全球細胞治療技術市場預計將超過340億美元

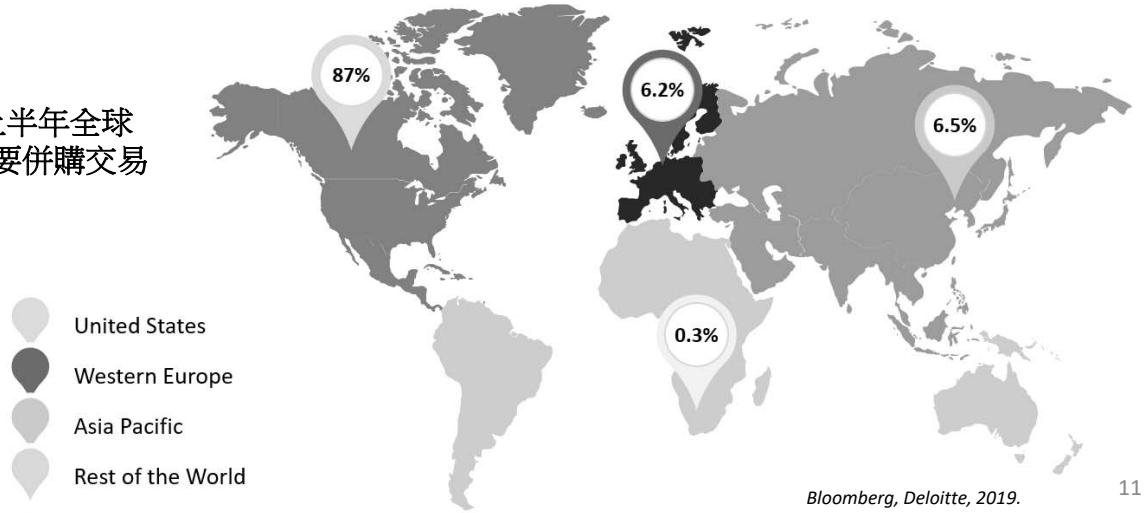
Fiormarkets,2019.; Deloitte, 2019.

全球生物醫藥併購交易趨勢



- 近年交易穩定成長
- 2018年交易額已達四千億美元
- 2019上半年竟已達2018年之80%

2019年上半年全球生技業主要併購交易



Bloomberg, Deloitte, 2019.

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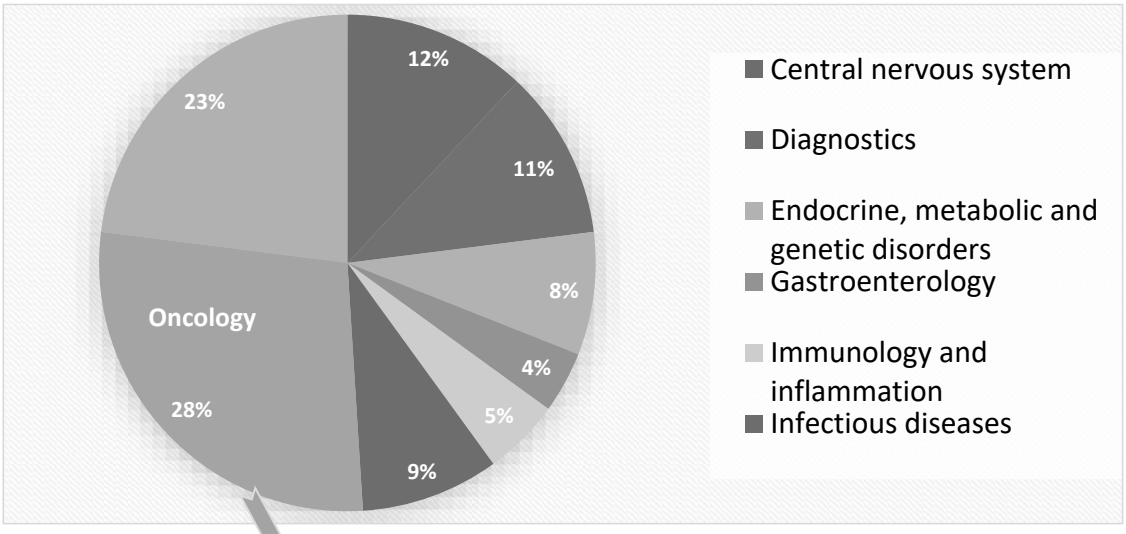
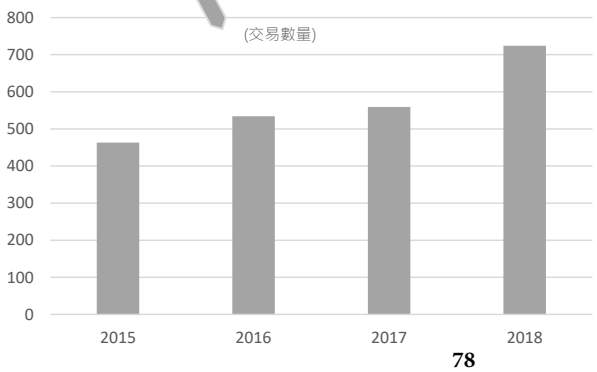
2018全球生物醫藥併購交易領域





12

Nature Reviews / drug discovery, 2019.

2008-2019 全球生技醫藥前15大併購案

交易總價 (億美元)	買家	被收購者	宣布時間
740	BMS (Bristol-Myers Squibb)	Celgene	2019.01.03 (未完成)
680	Pfizer	Wyeth	2009.01.26
660	Actavis (更名Allergan)	Allergan	2014.11.17
630	AbbVie	Allergan	2019.06.25 (未完成)
620	Tageda	Shire	2018.05.08
496	Merck	Schering-Plough	2009.03.09
468	Roche	Genentech	2008.07.21
429	Medtronic	Govidien	2014.06.16
405	Teva	Allergan (學名藥事業部)	2015.07.27
387	Novartis	Alcon	2008.04.07
320	Shire	Baxalta	2015.08.04
300	Johnson & Johnson	Actelion	2017.01.26
282	Allergan	Forest Laboratories	2014.02.24
210	AbbVie	Pharmacyclics	2015.03.04
201	Sanofi	Genzyme	2010.08.30

Genet, 2019.

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透過併購進攻再生醫學產業

2017.08

Gilead 119億美元併購 Kite Pharma

- CAR-T藥物Yescarta於2017.10通過美國FDA核准，2018.08通過歐盟EMA核准
- Gilead於2019.04公布將於美國馬里蘭州建置超過八萬平方公尺的CAR-T及TCR細胞產品製造工廠，以為其產品線準備。

2018.01

Celgene 90億美元收購 Juno

- CD19為標靶CAR-T細胞治療產品共有三種：JCAR015、JCAR017及JCAR014。
- CAR-T藥物JCAR017於2018向FDA提出申請，預計2019年獲得上市批准。
- 將該藥視為「2020年以後的重要增長動力」，潛在的銷售額高達30億美元。

2019.01

BMS 740億美元收購 Celgene

- 合併後擁有 9 種產品，年銷售額超過 10 億美元。
- 預計發布6種新藥物，包含 TYK2 和 ozanimod 等免疫學和發炎治療藥物。潛在收入為 150 億美元。

BMS以740 億美元高額併購免疫治療公司Celgene，將一舉納入Celgene於2018年收購的CAR-T公司Juno，成為繼Novartis、Gilead之後緊追而上的CAR-T競爭者。

光商時報, 2019.

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製造業投入細胞治療領域

日本化學大廠Fujifilm
- 2015年併購由iPS細胞權威James Thomson創立的CDI (Cellular Dynamics International)
- 2016年投資澳洲公司Cynata Therapeutics
- 因應細胞產品的量化需求，積極於全球佈局細胞工廠。

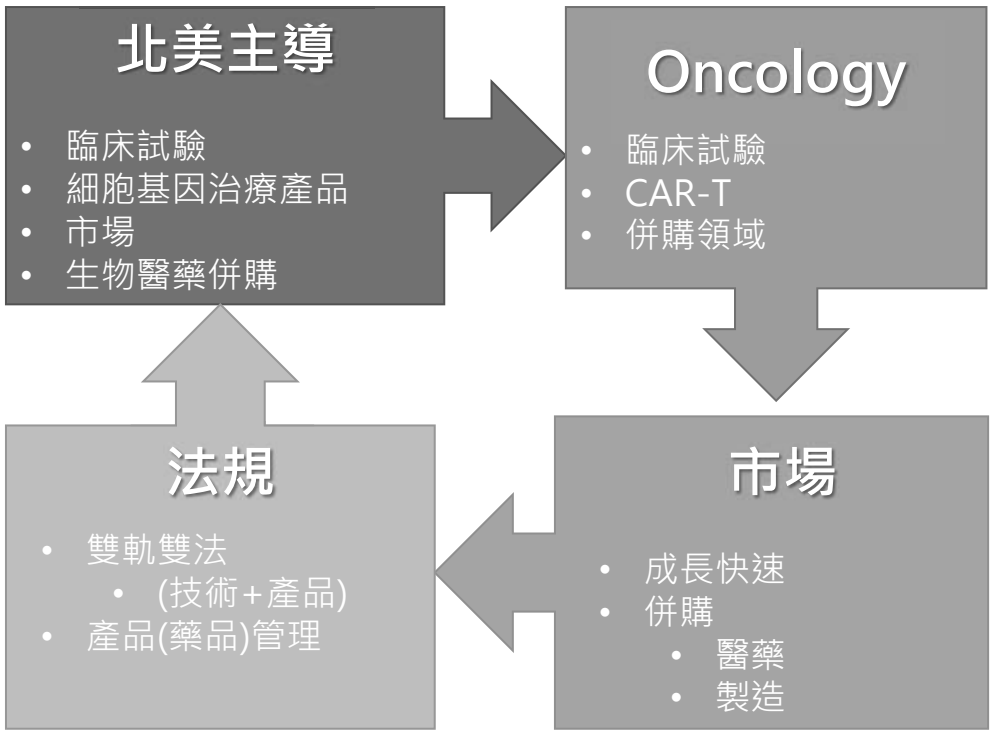
日立集團
Hitachi
- 2017年收購擁有20年生產再生醫學產品經驗的美國PCT公司
- 2019年收購德國Apceth Biopharma，該公司具有符合歐盟現行法規、BSL2和ICH指南之實驗室。

日立化成
Hitachi Chemical Company
- 2018年在橫濱建造自動化再生醫療工廠
- 2019年發表用於iPS細胞商用生產的自動培養裝置iACE2，於封閉式的環境中培養並追蹤iPS細胞。

美國儀器設備企業
Danaher
- 2019年宣布以214億美元收購美商GE Life Sciences生物製藥部門。

15
光商時報, 2019.

全球細胞治療



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感謝聆聽，敬請指教

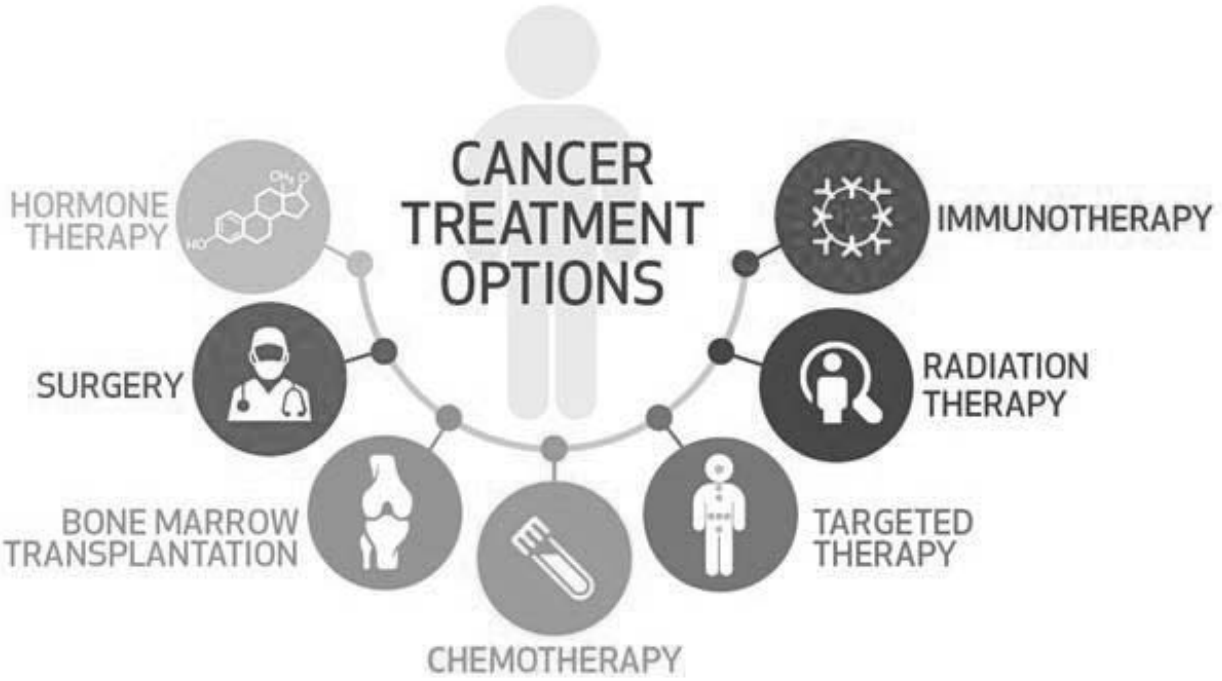
長聖生技
Ever Supreme Bio Technology

CART治療的現況

台灣細胞醫療協會 理事
沛爾生技 副總經理兼技術長
台大醫院 內科部血液科（兼任）
林建廷
October 21, 2019

Outline

- Immunotherapy briefing
- Cell therapy: Non-gene-editing vs gene-editing
- CART design & manufacturing
- Hurdles of CART treatment in Taiwan
- Summary



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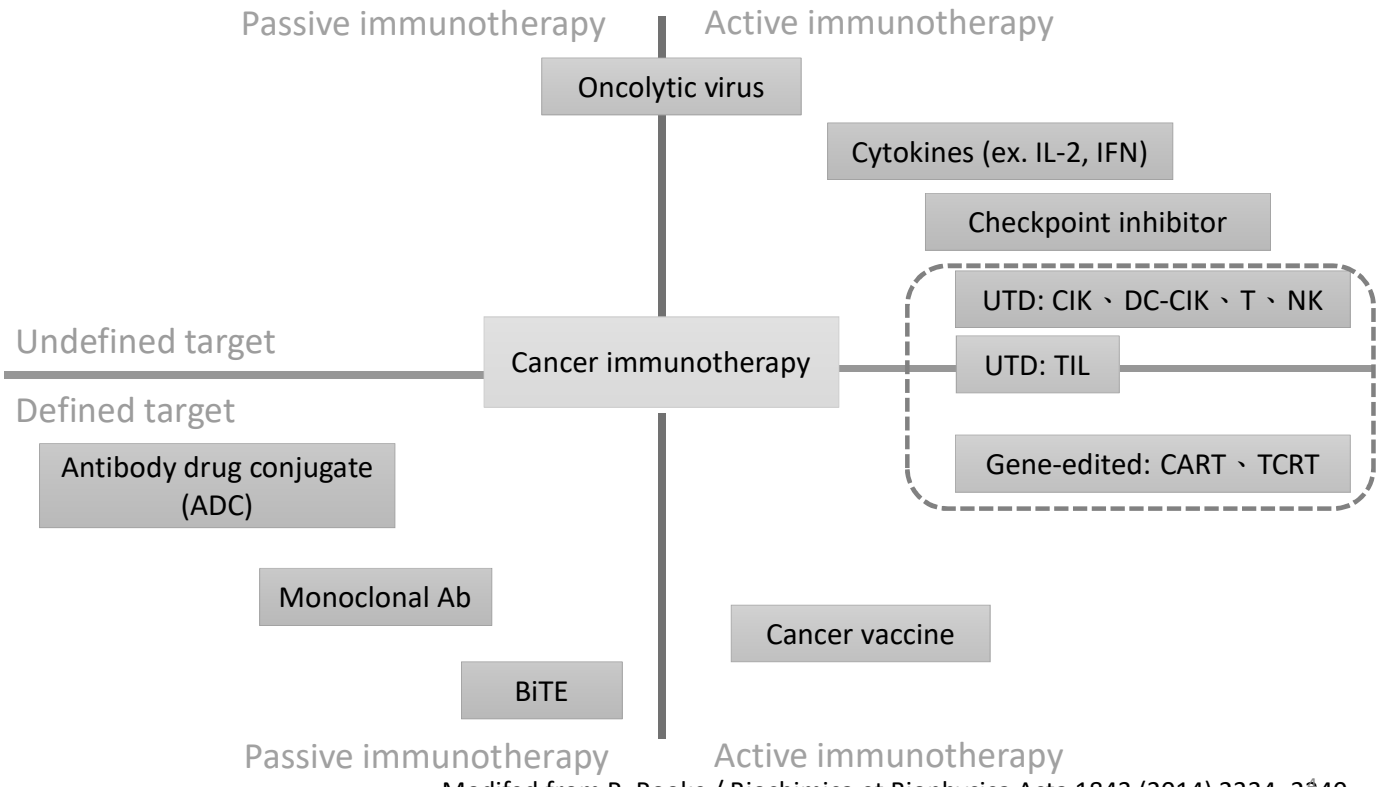
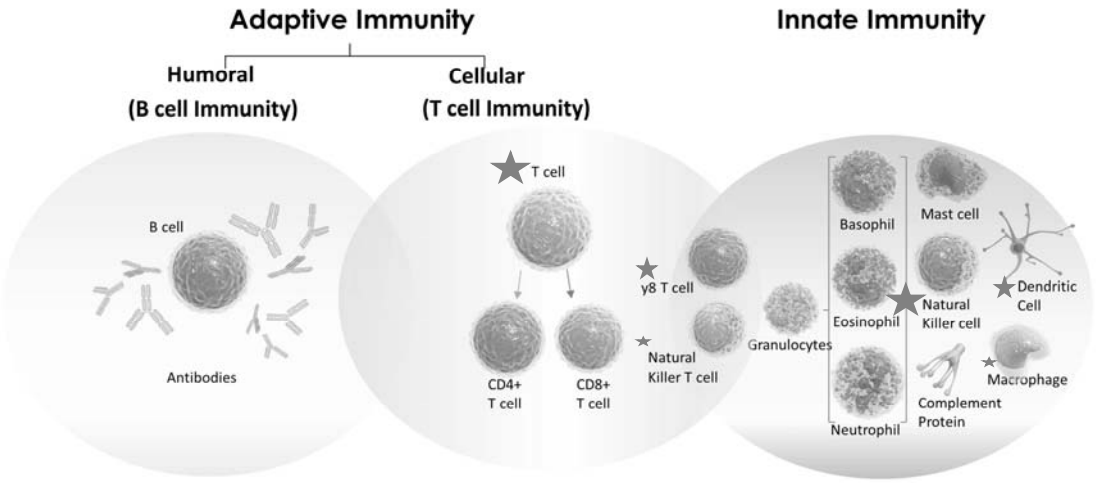


Modifed from R. Rooke / Biochimica et Biophysica Acta 1843 (2014) 2334–2340

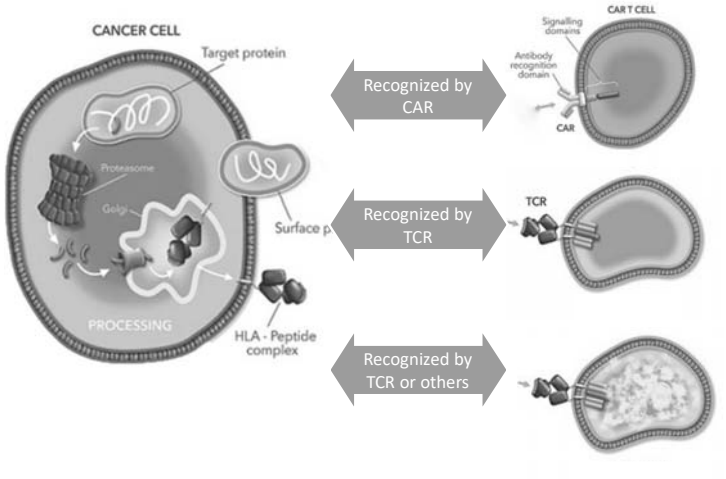
Immune system and cancer therapy



- 期望免疫細胞發揮“專一”&”非專一”攻擊癌細胞的能力
- 可以結合標靶、抗體、化療、免疫檢查電抑制劑等併用
- 部分免疫細胞具有記憶能力，可能抑制癌症的復發
- 副作用輕微，較能維持生活品質（CART細胞治療除外）

5

如何辨認癌細胞?



CART
- MHC independent
- Surface protein
- Specific killing

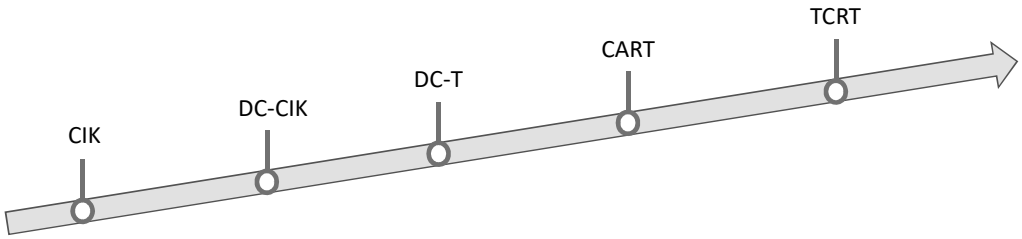
TCRT、TIL、DC-T
- MHC dependent
- Intracellular peptide
- Specific killing

NK、gdT、iNKT、CIK、DC-CIK
- MHC independent
- NKG2D、NCR、DNAM1 killing receptor
- Non-specific killing

Cell therapy may be synergistic with mAb、CPI.

6

Cell therapy: moving forward



	CIK	DC-CIK	DC-T
Culture period	16-18 days	16-18 days	30-35 days
Phenotype	CD3/CD56+ >20%	CD3/CD56+ >20%	CD3+>80% (CD8:40-70%)
Cell dose	50-500M	50-500M	50-500M
Dosing frequency	Q1-2wks *6 doses	Q1-2wks *6 doses	Q2wks *6 doses

Outline

- Immunotherapy briefing
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- CART design & manufacturing
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- Summary

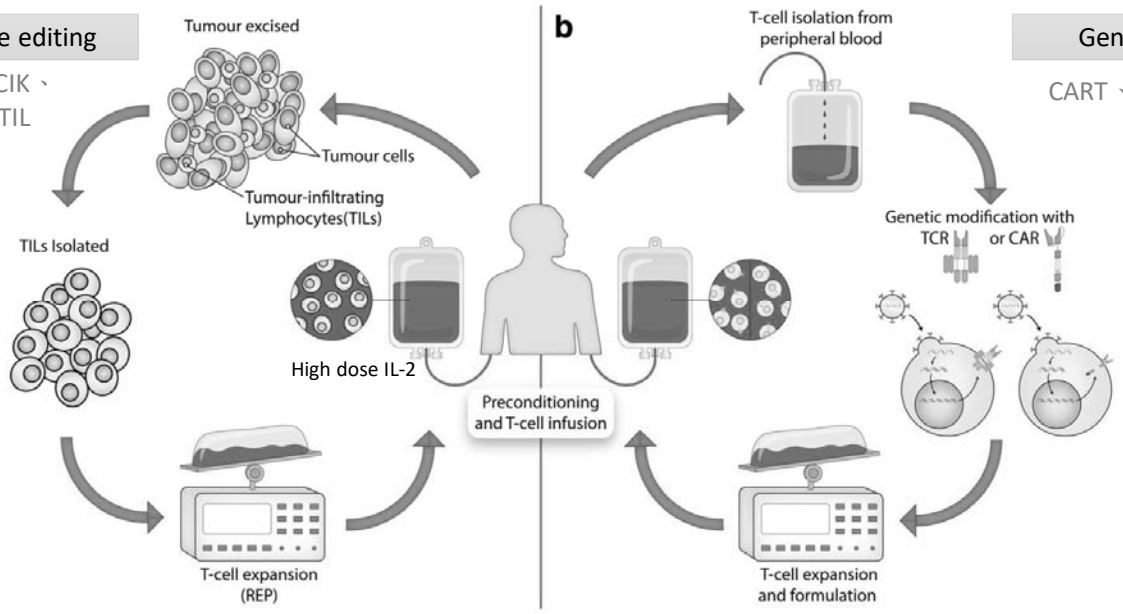
How to use T or NK cells?

Non-gene editing

CIK、DC-CIK、
T、NK、TIL

Gene-editing

CART、TCRT



Özcan Met et al, Semin Immunopathol. 2018 Sep 5. doi: 10.1007/s00281-018-0703-z

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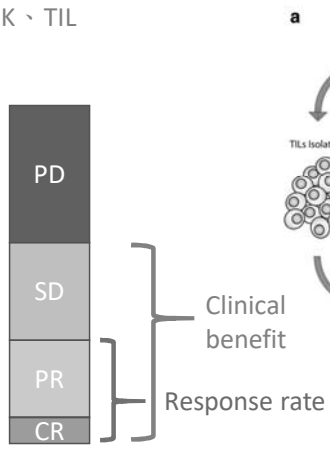
How to use T or NK cells and expectation

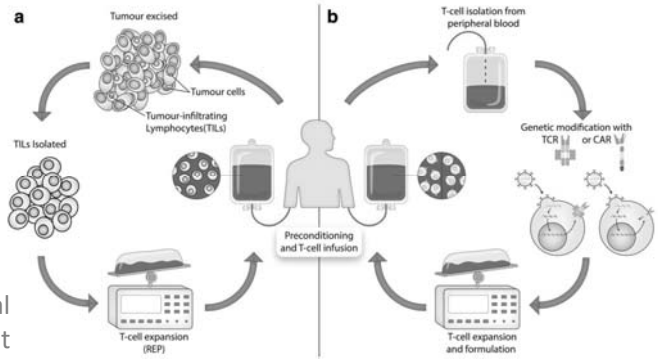
Non-gene editing

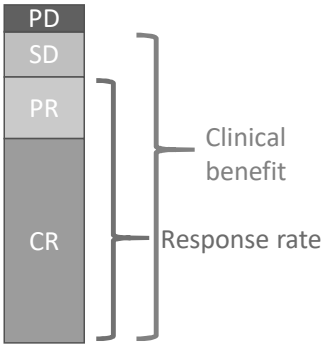
CIK、DC-CIK、
T、NK、TIL

Gene-editing

CART、TCRT







Özcan Met et al, Semin Immunopathol. 2018 Sep 5. doi: 10.1007/s00281-018-0703-z

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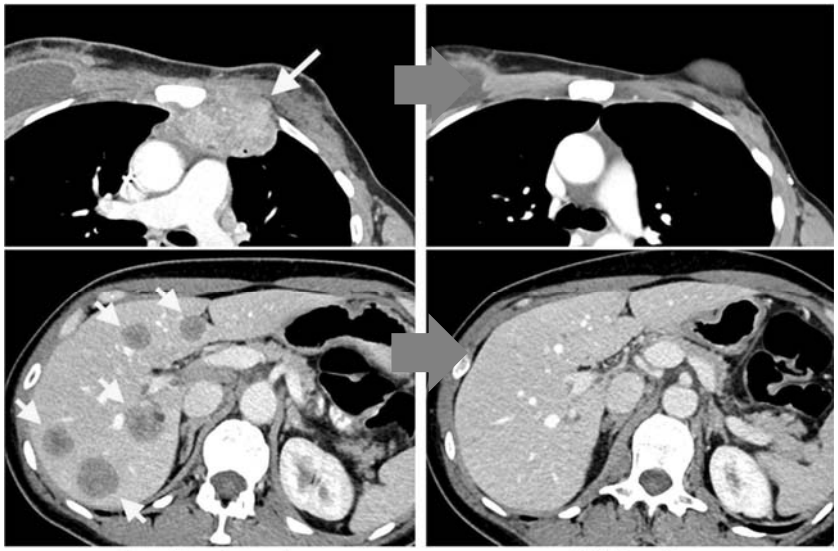
Tumor infiltrating lymphocytes (TIL) for breast cancer

Breast cancer

62 somatic mutations identified.

Received TIL reactive to mutated proteins + IL-2 + pembrolizumab.

Cancer free for 2.5+ years.

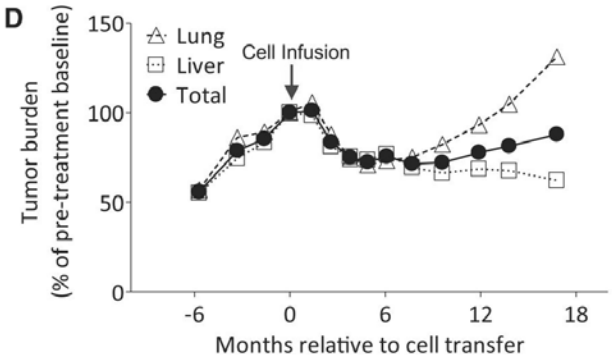


Laszlo G. Radvanyi et al, Nature Medicine, volume 24, pages703–704 (2018) [11]

Cancer Immunotherapy Based on Mutation-Specific CD4+ T Cells in a Patient with Epithelial Cancer

Eric Tran,[1] Simon Turcotte,[1]* Alena Gros,[1] Paul F. Robbins,[1] Yong-Chen Lu,[1] Mark E. Dudley,[1]† John R. Wunderlich,[1] Robert P. Somerville,[1] Katherine Hogan,[1] Christian S. Hinrichs,[1] Maria R. Parkhurst,[1] James C. Yang,[1] Steven A. Rosenberg[1]‡

Cholangiocarcinoma



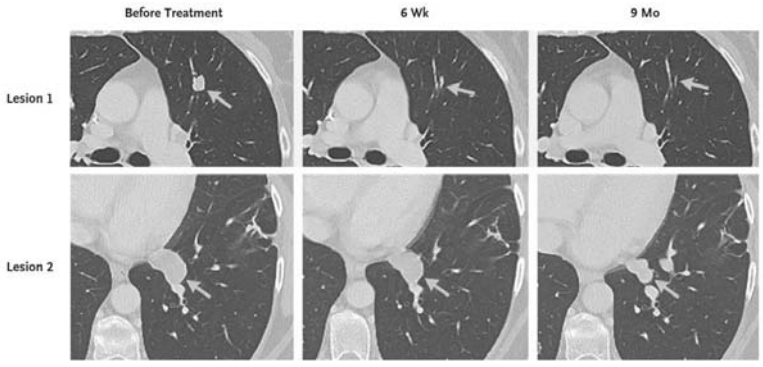
Eric Tran et al, Science 2014:344, Issue 6184, pp. 641-645

BRIEF REPORT

T-Cell Transfer Therapy Targeting Mutant KRAS in Cancer

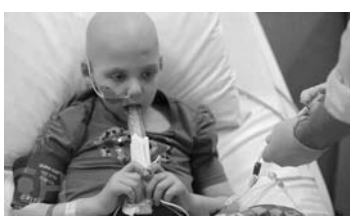
Eric Tran, Ph.D., Paul F. Robbins, Ph.D., Yong-Chen Lu, Ph.D., Todd D. Prickett, Ph.D., Jared J. Gartner, M.Sc., Li Jia, M.Sc., Anna Pasetto, Ph.D., Zhili Zheng, Ph.D., Satyajit Ray, Ph.D., Eric M. Groh, M.D., Isaac R. Kriley, M.D., and Steven A. Rosenberg, M.D., Ph.D.

Colorectal cancer



Eric Tran et al, N Engl J Med 2016;375:2255-62. [12]

Chimeric antigen receptor T cells (CART)



+

NOVARTIS

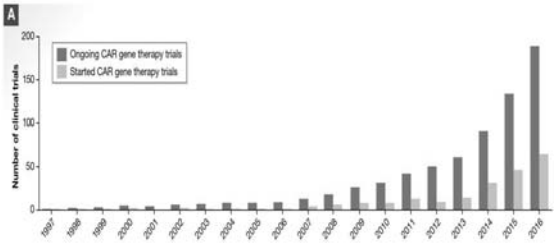
Kite Pharma

GILEAD

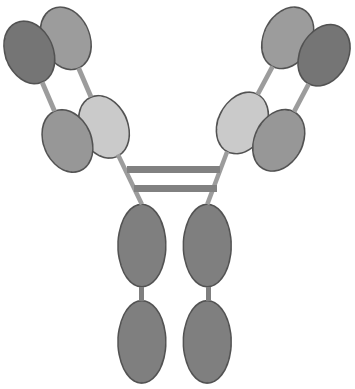
FDA APPROVED

EU APPROVED

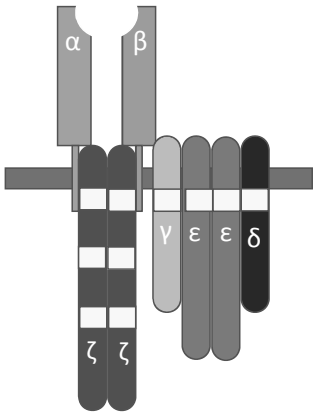
厚生労働省
Ministry of Health, Labour and Welfare



何謂CART細胞?

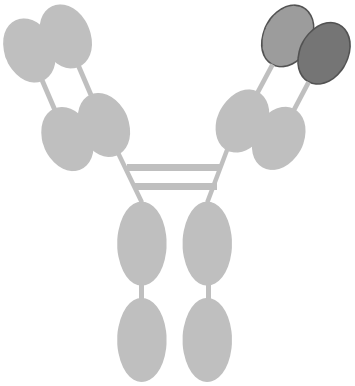


抗體
（來自B細胞）

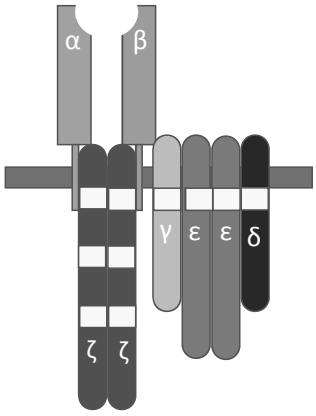


T細胞受體
（來自T細胞）

何謂CART細胞?



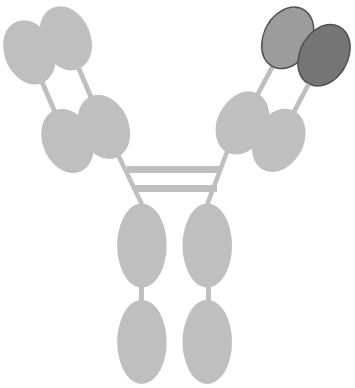
抗體
（來自B細胞）



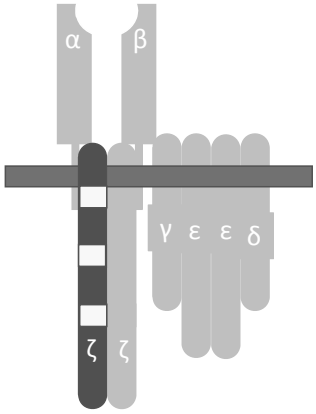
T細胞受體
（來自T細胞）

15

何謂CART細胞?



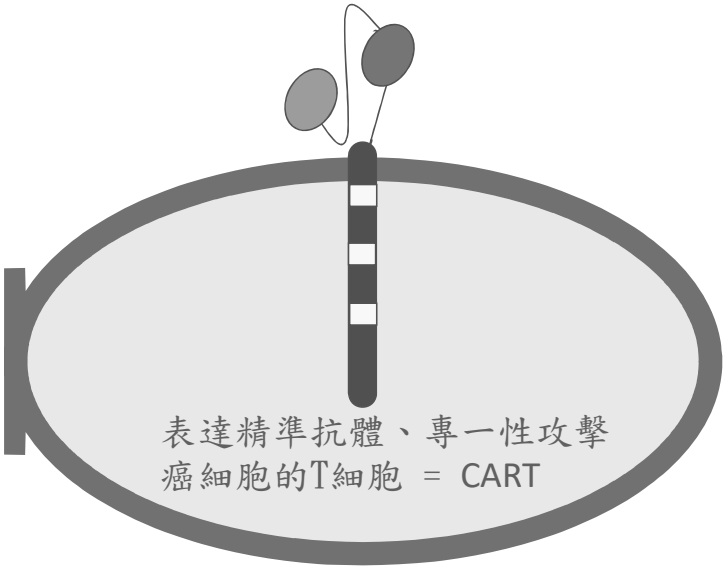
抗體
（來自B細胞）



T細胞受體
（來自T細胞）

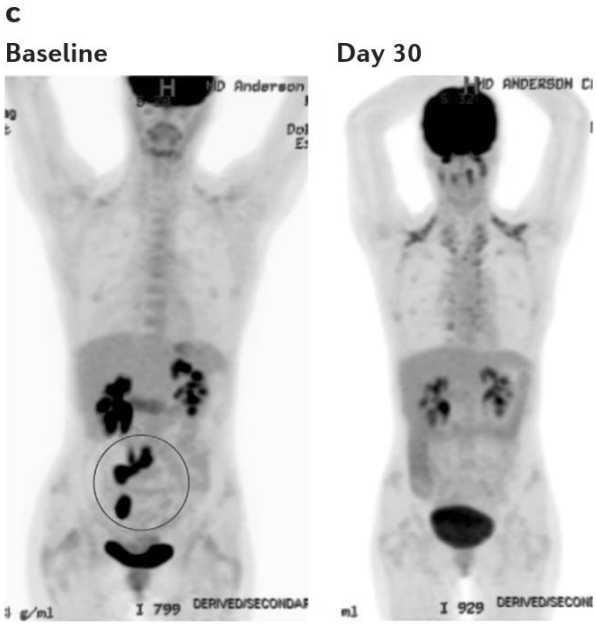
16

何謂CART細胞?



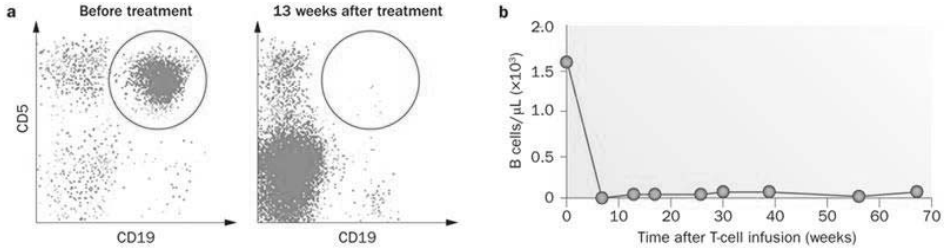
17

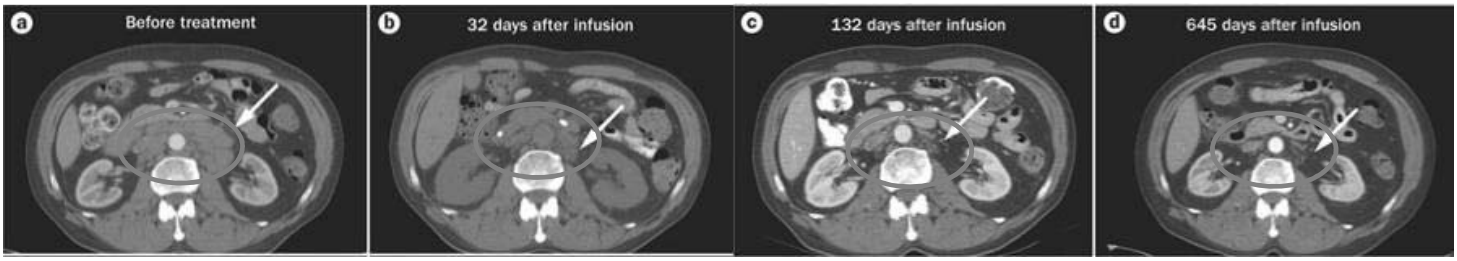
After CART19 for DLBCL, complete remission



Sattva S. Neelapu et al, Nature Reviews Clinical Oncology (2017)

After CART19 for CLL, complete remission





Kochenderfer, J. N. et al. Blood 119, 2709–2720 (2012).

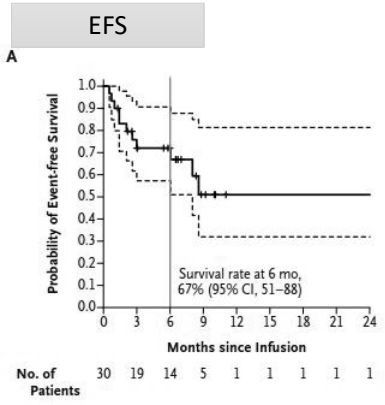
ORIGINAL ARTICLE

Chimeric Antigen Receptor T Cells for Sustained Remissions in Leukemia

Shannon L. Maude, M.D., Ph.D., Noelle Frey, M.D., Pamela A. Shaw, Ph.D., Richard Aplenc, M.D., Ph.D., David M. Barrett, M.D., Ph.D., Nancy J. Bunin, M.D., Anne Chew, Ph.D., Vanessa E. Gonzalez, M.B.A., Zhaohui Zheng, M.S., Simon F. Lacey, Ph.D., Yolanda D. Mahnke, Ph.D., Jan J. Melenhorst, Ph.D., Susan R. Rheingold, M.D., Angela Shen, M.D., David T. Teachey, M.D., Bruce L. Levine, Ph.D., Carl H. June, M.D., David L. Porter, M.D., and Stephan A. Grupp, M.D., Ph.D.

- Morphological CR@1m= 27/30 (90%)
- Flow RD negativity= 22/25 (88%)
- 2 pts with CNS blasts➔ negative

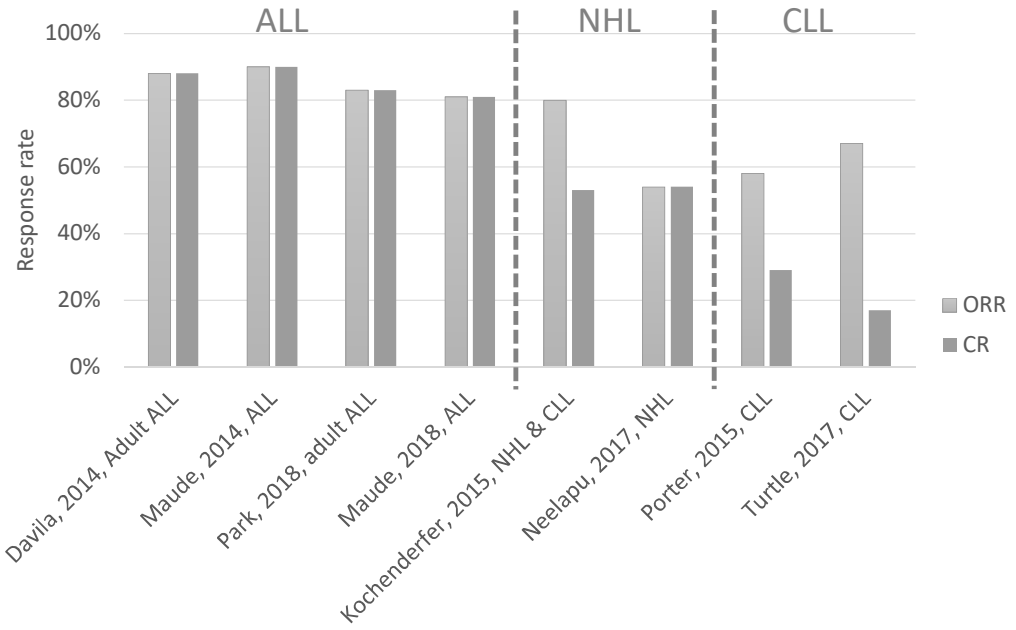
- Pts with CR (N=23)
  - 7 (30%) relapsed (6 wks~ 8.5m)



7 relapsed
3 no response
1 MDS

Maude et al, N Engl J Med 2014;371:1507-17.

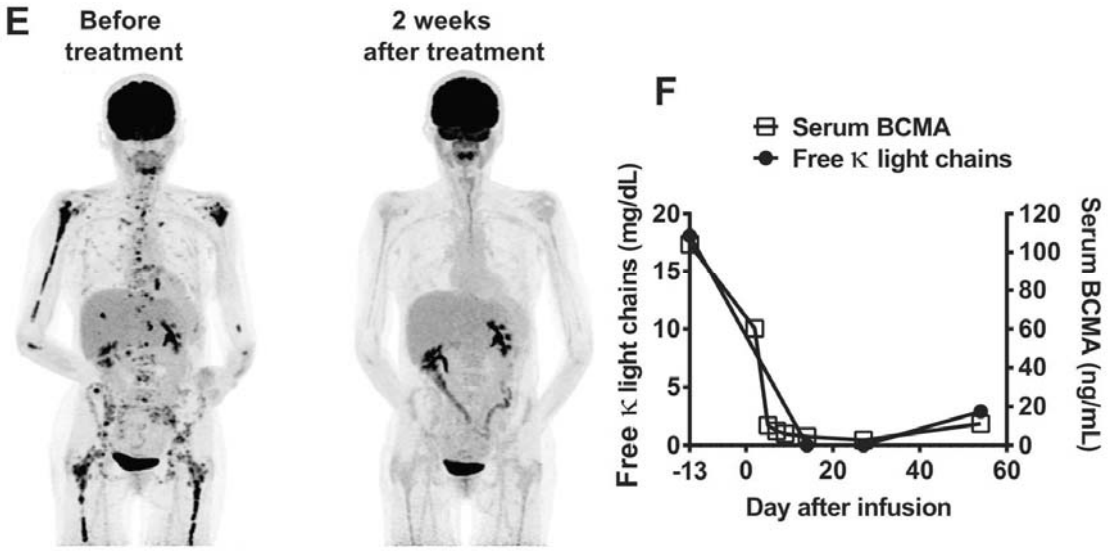
Response rate of CART19



Özcan Met et al, Semin Immunopathol. 2018 Sep 5. doi: 10.1007/s00281-018-0703-z

21

After anti-BCMA CART for MM, complete remission



Ali SA et al, Blood. 2016 Sep 29;128(13):1688-700

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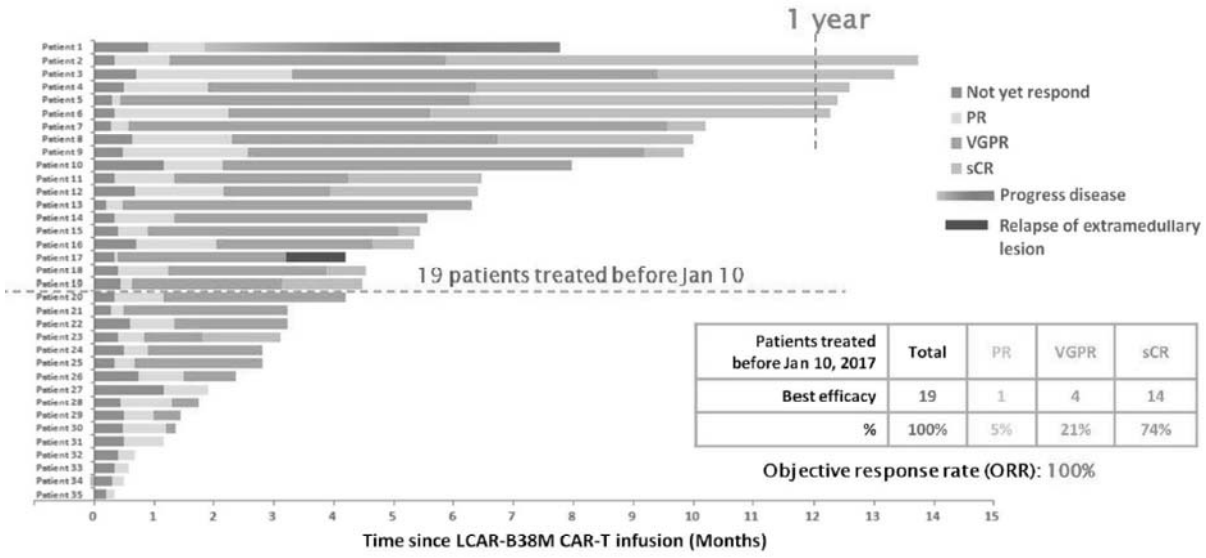
Legend Anti-BCMA CART

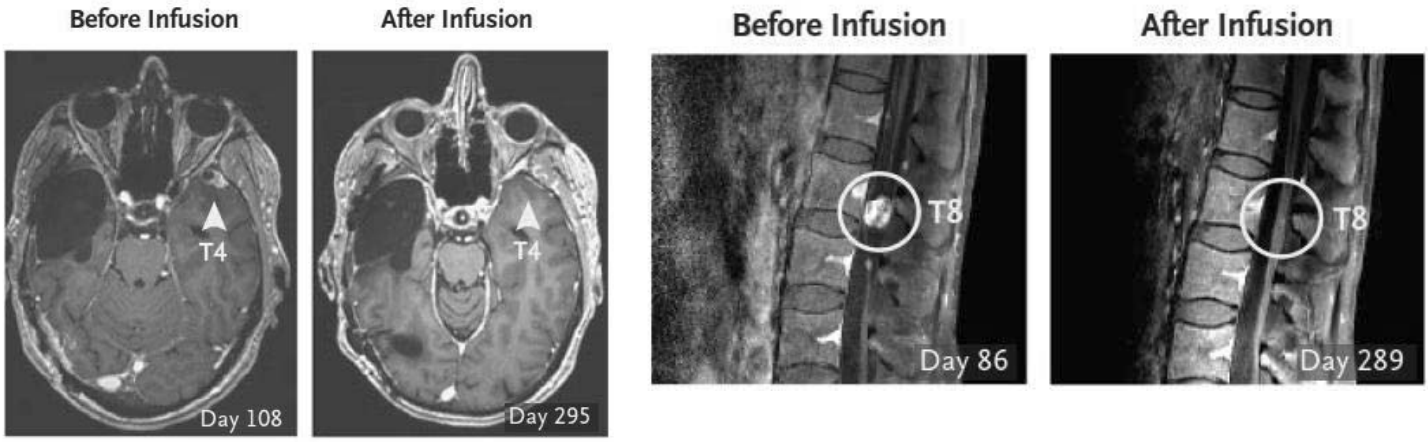


Patients treated before Jan 10, 2017	Total	PR	VGPR	sCR
Best efficacy	19	1	4	14
%	100%	5%	21%	74%

Objective response rate (ORR): 100%

Zhao W et al. ASCO 2017

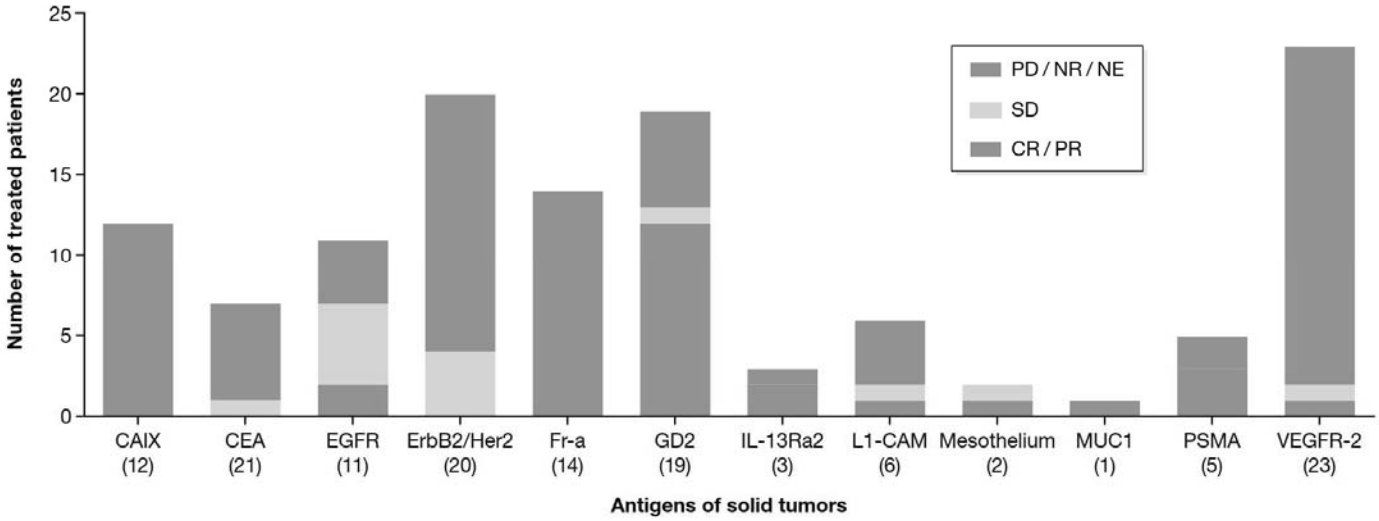
After anti-IL13Ra CART for brain tumor, complete remission



*Glioblastoma multiforme

Brown et al, N Engl J Med 375, 2561-9 (2016)

Clinical outcome in solid cancers: need more works



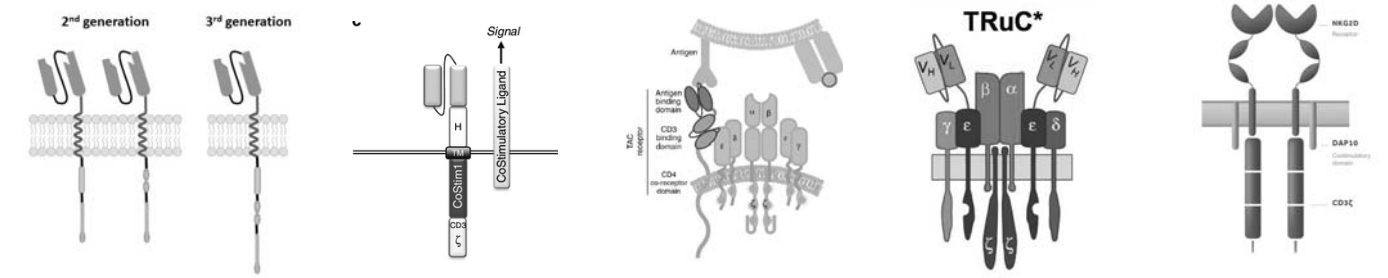
Jessica Hartmann et al, EMBO molecular medicine,DOI 10.15252/emmm.201607485

Outline

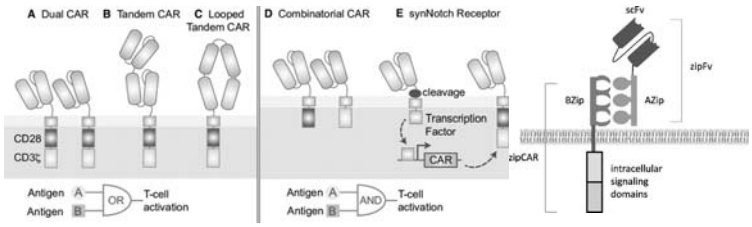
- Immunotherapy briefing
- Cell therapy: Non-gene-editing vs gene-editing
- CART design & manufacturing
- Hurdles of CART treatment in Taiwan
- Summary

Various chimeric antigen receptor T designs

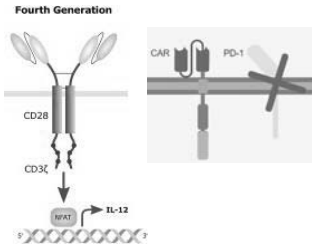
Different types of T cell activation



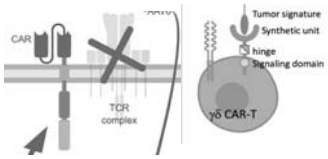
More targets



More powerful



Universal



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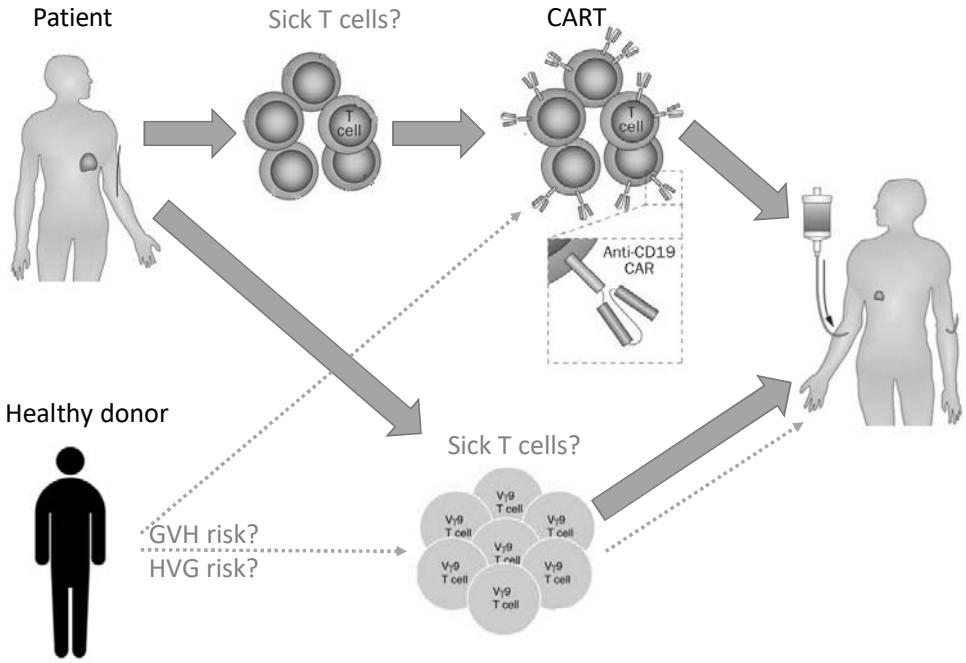
Cells origin: Autologous vs Allogeneic



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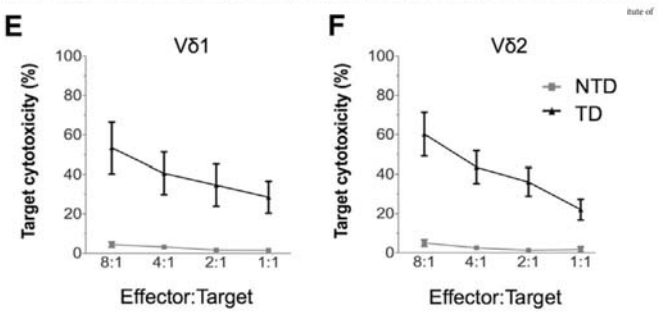
Gamma delta T cells with CAR

Molecular Therapy
Original Article

Chimeric Antigen Receptor-Engineered Human Gamma Delta T Cells: Enhanced Cytotoxicity with Retention of Cross Presentation

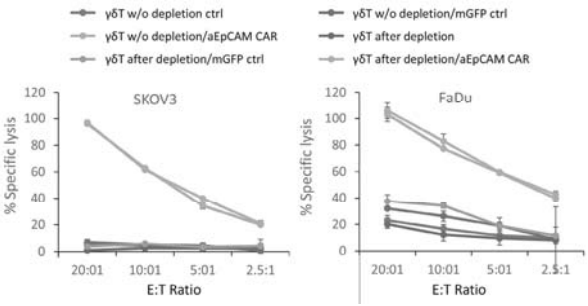
Anna Capsomidis,[1] Gabriel Benthall,[1] Heleen H. Van Acker,[1] Jonathan Fisher,[1] Anne M. Kramer,[2] Zarah Abeln,[2] Yvonne Majani,[1] Talia Gileadi,[1] Rebecca Wallace,[1] Kenth Gustafsson,[2] Barry Flutter,[1] and John Anderson[1]



Cytotherapy, 2018; ■■: ■■–■■

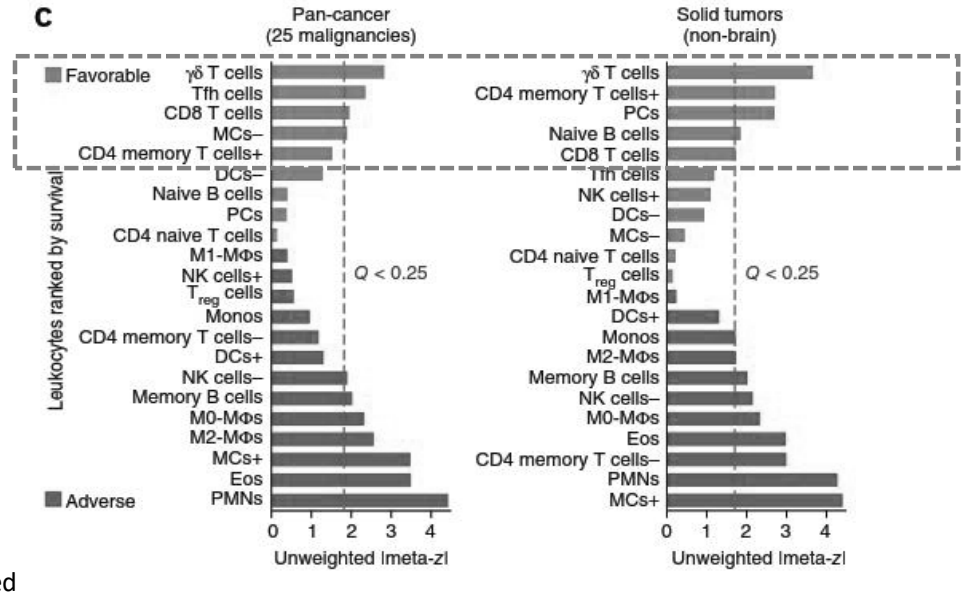
Large-scale expansion of Vγ9Vδ2 T cells with engineered K562 feeder cells in G-Rex vessels and their use as chimeric antigen receptor–modified effector cells

LIN XIAO[1,*], CAN CHEN[2,*], ZHENDONG LI[1], SUMIN ZHU[1], JOHAN CK TAY[1], XI ZHANG[1], SHIJUN ZHA[1], JIEMING ZENG[3], WEE KIAT TAN[2], XIN LIU[4], WEE JOO CHNG[4,5,6] & SHU WANG[1,3]



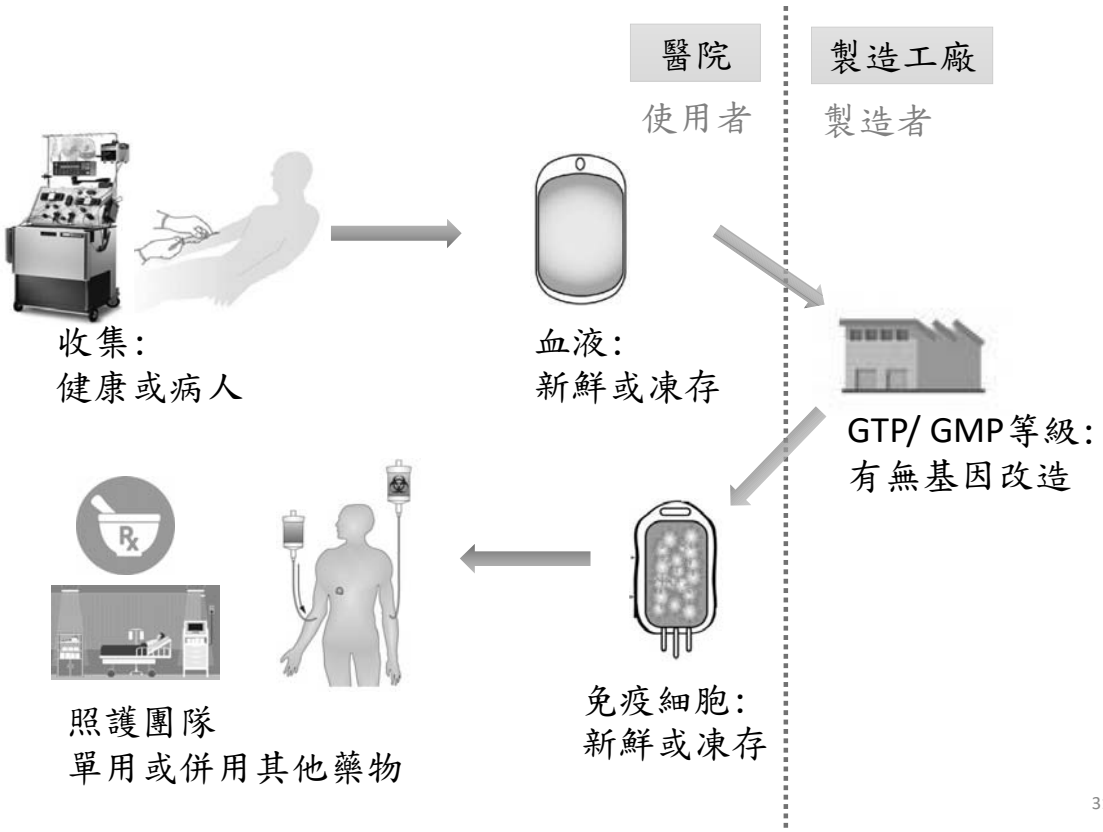
Anna Capsomidis et al, Molecular therapy, doi.org/10.1016/j.ymthe.2017.12.001
Lin Xiao et al, Cytotherapy, 2018; doi.org/10.1016/j.jcyt.2017.12.014

Gamma delta T cells is favorable



+: activated
-: resting

Gentles et al, Nature medicine 2015 (21): 938-945.



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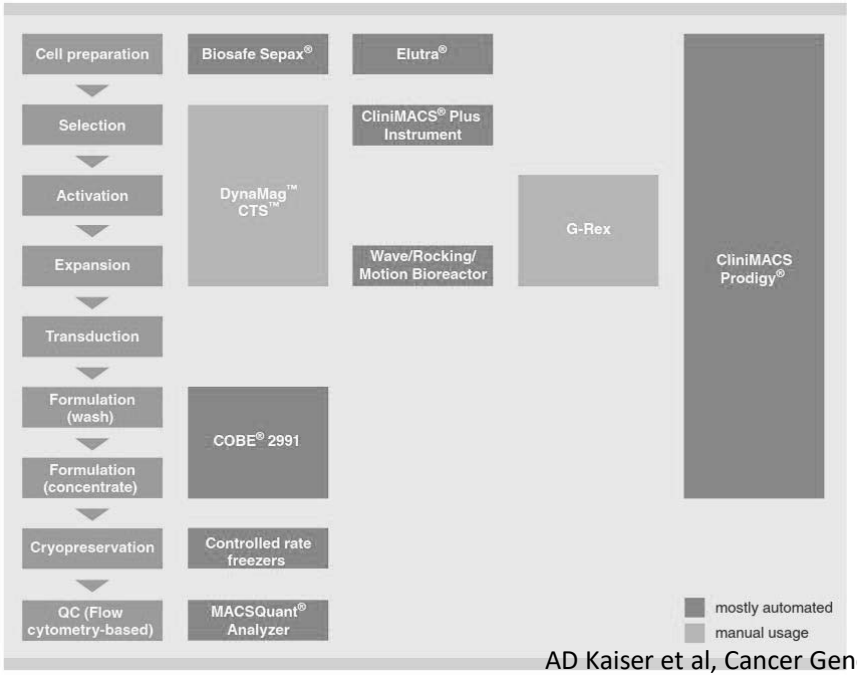
GMP-grade CART Manufacturing



AD Kaiser et al, Cancer Gene Therapy (2015) 22, 72–78

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方案一:

GMP facility

WB or leukapheresis

Options:
- By hand
- Sofia

PBMC → T cell enrichment → T cell activation → T cell transduction → T cell expansion → wash, formulation, cryo → QC check

Options:
- Overnight culture
- GMP dynabeads + dynamag CTS
- CliniMACS

Options:
- Miltenyi TransAct
- Dynabeads

Options:
- Flask
- G-Rex/ gather rex

GMP media
GMP cytokine

- Sofia
- Cell counter
- LN2-free freezer

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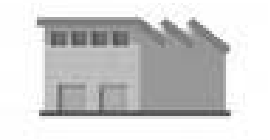
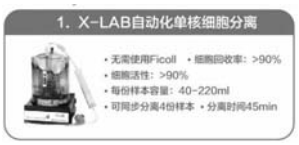
方案二: CAR-TXPress

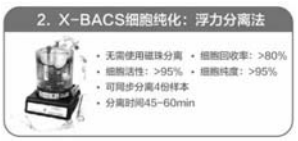
GMP facility



WB or leukapheresis



PBMC → T cell enrichment → T cell activation → T cell transduction → T cell expansion → wash, formulation, cryo → QC check



Options:
- Miltenyi TransAct
- Dynabeads

G-Rex/ gather rex

GMP media
GMP cytokine

- Cell counter

https://kknews.cc/zh-tw/science/jmql856.html

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方案三:

- 全球唯一automatic manufacturing of CART using viral system

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Outline

- Immunotherapy briefing
- Cell therapy: Non-gene-editing vs gene-editing
- CART design & manufacturing
- Hurdles of CART treatment in Taiwan
- Summary

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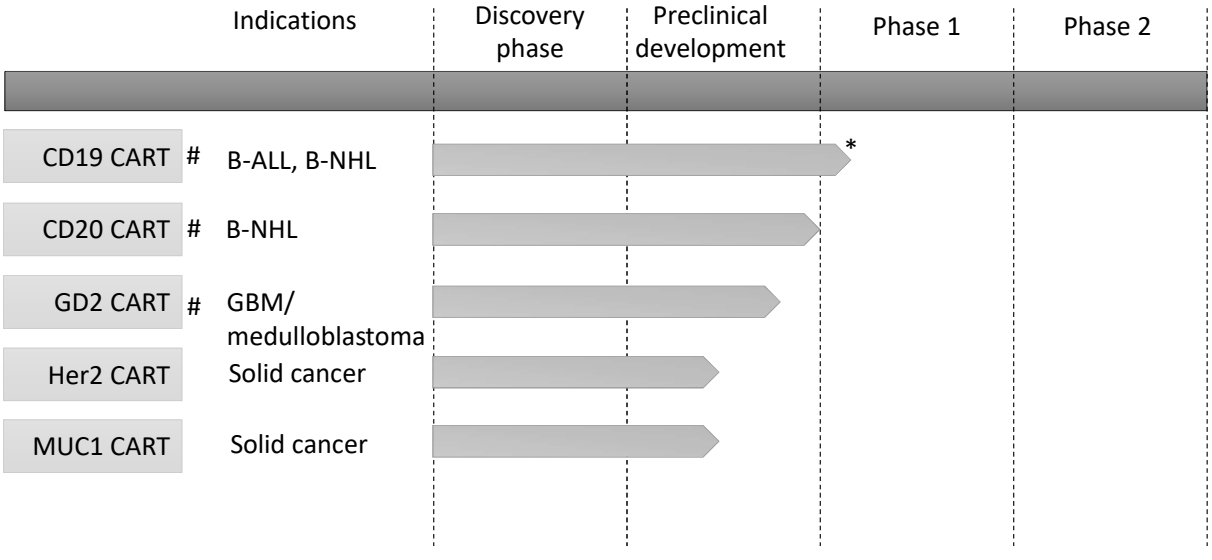
CART is under rapid evolution

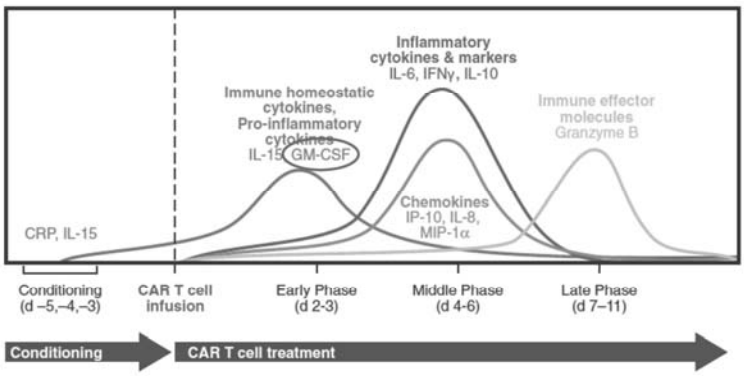
PELL is working on better efficacy/ multi-target



Compassionate CART use experience.

* Recently, we recruited people from CDE and CRA to facilitate trial submission.

CART toxicity initiator: GM-CSF



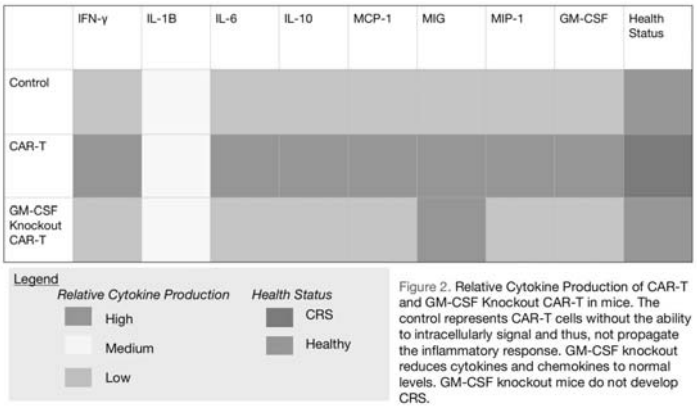


Figure 2. Relative Cytokine Production of CAR-T and GM-CSF Knockout CAR-T in mice. The control represents CAR-T cells without the ability to intracellularly signal and thus, not propagate the inflammatory response. GM-CSF knockout reduces cytokines and chemokines to normal levels. GM-CSF knockout mice do not develop CRS.

Zhenguang Wang et al, Biomarker Research20186:4

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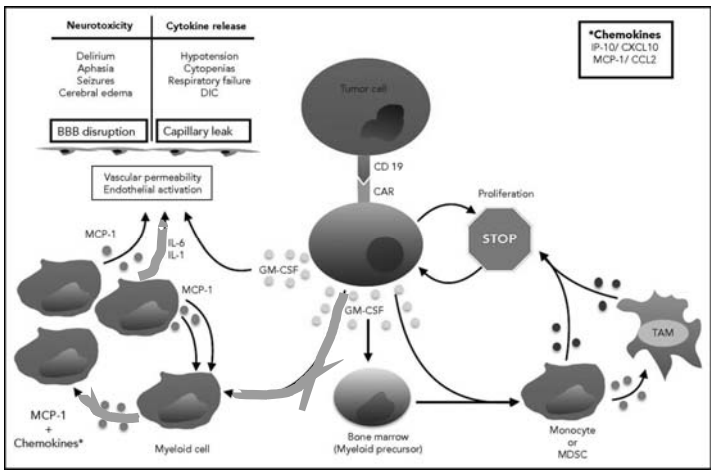
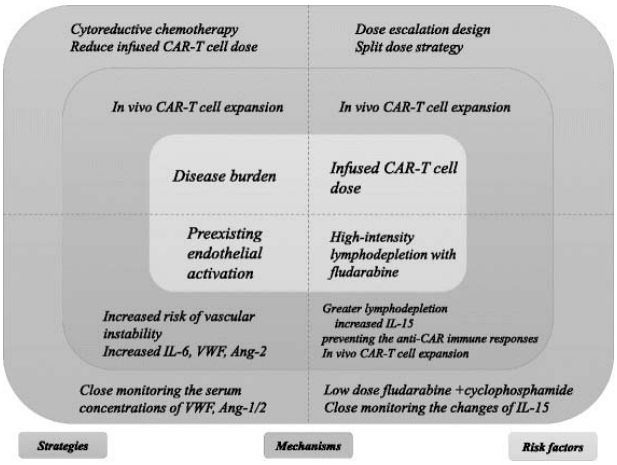
How to reduce risk factors of CART?





*Anti-IL6 prophylaxis: more CRES
*Anti-GM-CSF: lenzilumab

Omar Ahmed, Blood 2019 133:2114-2116
Zhenguang Wang et al, Biomarker Research20186:4

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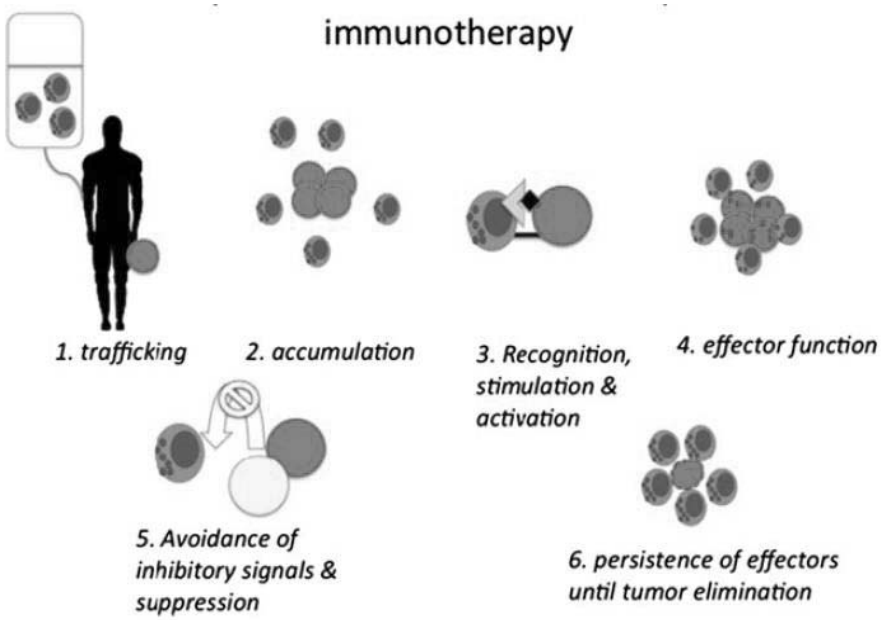
Requirements for Successful Cell Activity



Gill S, June CH. *Immunol Rev*. 2015;263(1):68-89.

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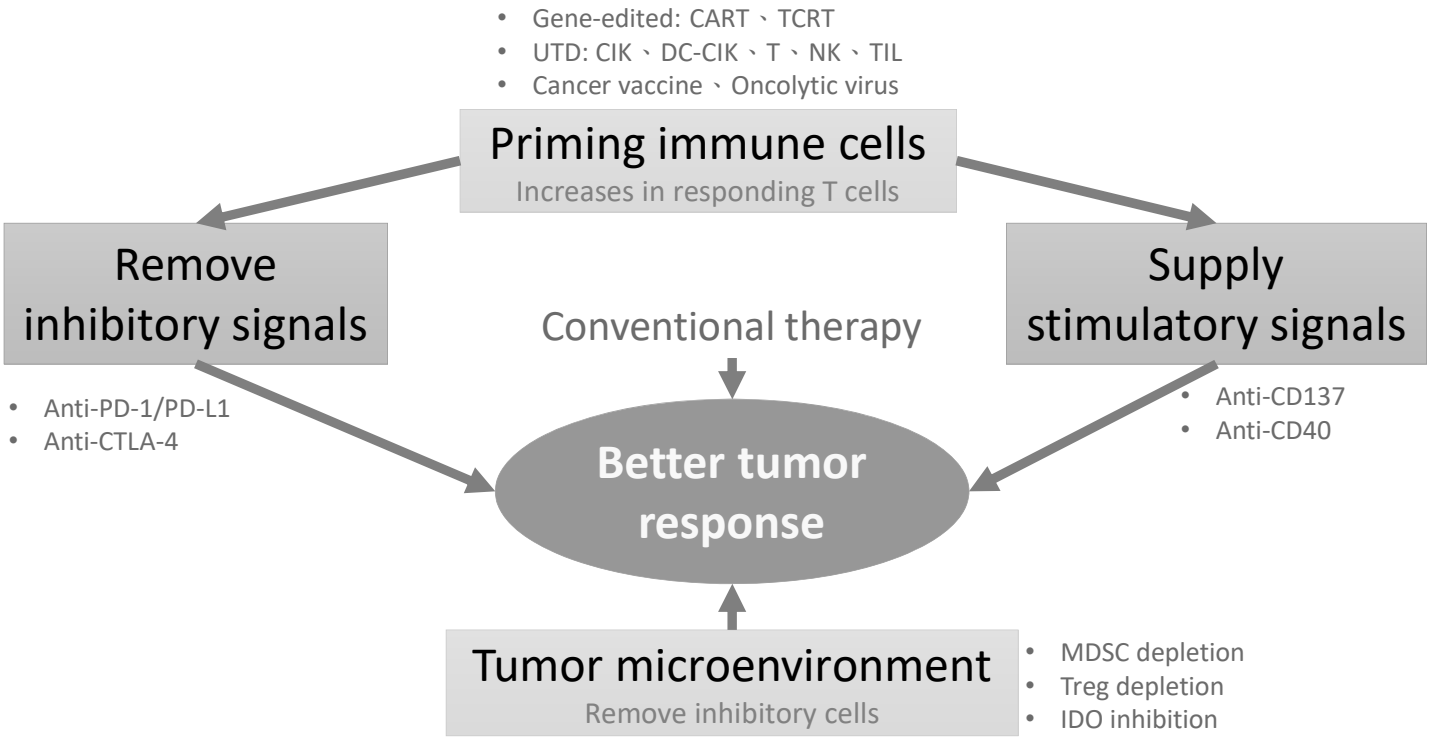

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Theresa L. Whiteside et al, Clin Cancer Res April 15 2016 (22) (8) 1845-1855

CART設計方向

- 不須使用過度複雜技術，ex.第二代技術已經足夠 (41BB or CD28)
- Humanized scFV，以確保可以反覆給予相同CART治療
- 將治療成功點，壓在併用藥物(checkpoint inhibitor, BTK inhibitor)、sequential CART、bridging to allogeneic-SCT治療上

- 著重CART設計、新技術整合
- 將治療成功點，壓在novel design CART治療上

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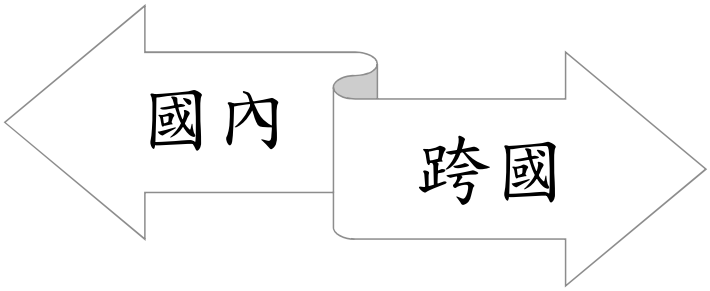
細胞運送 & 運送狀態



- 預期是fresh cells，門檻較低

- Novartis: frozen
- Gilead: fresh cells
- 國際物流公司負責通關，避免X光檢查

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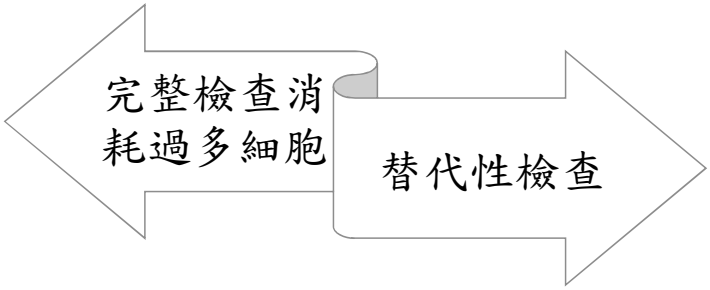
Final product放行標準



- 完整檢查除耗費時間，還耗費大量得來不易的細胞
- TFDA能接受那些替代檢查?閾值設在哪裡?

45

Summary

- CART will be an important player:
  - Definitely true for hema cancers.
  - Probably for solid cancers.
- To achieve a good efficacy:
  - Novel design
  - High quality manufacturing process
  - Good supportive care in the hospital

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