Autologous Multi- Lineage Pluripotent Cells (AMPCs)

A NOVELTY INVENTION

Company Introduction

- Autologous Stem Cell Technology (ASCT) is an Australian stem cell company established in July 2011
- First in patenting a method of producing multi-lineage potential stem cells without genetic manipulation
- Autologous Multilineage Potential Cells (AMPCs) can differentiate (transform) into any cell type of the body
- In 2011, the Australian Therapeutic Good Administration (TGA) deemed AMPCs as goods exempt from regulation under the Therapeutics Goods Order No. 1 of 2011
- AMPCs are used in over 400 treatments for various degenerative diseases
- AMPC are the only stem cell product in Australia to receive insurance protection. Lloyd's of London provides insurance coverage and no claims have been made since 2011.

Company Introduction

- **TGA Compliant**
  - Compliant with Therapeutic Goods (Excluded Goods) Order No. 1 of 2011 (Image_1)
  - Must be collected from a patient who is under the clinical care and treatment of a medical practitioner registered under a law of a State or an internal Territory
  - Must be manufactured by medical practitioner or by a person under the professional supervision of that medical practitioner
  - Must be used in a single indication and in a single course of treatment of that patient by the same medical practitioner
  - Over 300 cases of reinfusion with regulatory compliance

Australian Government
Department of Health and Ageing
Therapeutic Goods Administration

Dr Teresa Schafer
Partner, Piper Alderman
Level 23
Governor Macquarie Tower
Sydney NSW 2000

R12/66229

Dear Dr Schafer

Subject: Application of Therapeutic Goods (Excluded Goods) Order No. 1 of 2011

Thank you for your enquiry regarding the application of specific provisions in paragraph 4(q) of the Therapeutic Goods (Excluded Goods) Order No. 1 of 2011 (the Order).

You will appreciate that it is not possible for the TGA to provide definitive advice about how the Order might apply in any particular case. However I am happy to provide the following information.

As you are aware, for the exclusion to apply to cells under paragraph 4(q) of the Order, the patient from whom the cells are collected must be under the clinical care of a registered medical practitioner and that *same* medical practitioner must perform, or professionally supervise the person performing, each step of manufacture. This professional oversight must extend to all aspects of manufacture of the cells. This would be expected to involve, for instance, being professionally responsible for the protocols within the laboratory in relation to the manufacture of the product and also performing, or professionally supervising, the re-infusion of the product. In such a situation the practitioner would be ultimately professionally responsible for the clinical care of the patient and any outcomes of the treatment.

By specifying that the cells must be collected for a therapeutic application in the treatment of a single indication and course of treatment, paragraph 4(q) makes it clear that the patient from whom the cells are collected must have at that time the condition that the autologous procedure is intended to treat. This would not cover the collection and storage of a patient's cells or tissues for no specific indication to be used at a later date.

You make the statement in your letter that if mononuclear stem cells derived from peripheral blood for autologous use as described are not therapeutic goods "... it follows that there would be no regulatory requirements for similar use of such stems cells in the treatment of any type of patient, including terminally ill patients or other patients with life-threatening conditions, so long as treatment was for a single indication and single course of treatment in the same patient". Whether or not particular cells are covered by paragraph 4(q) will depend on whether the circumstances in which they are collected and used satisfy all the conditions

PO Box 100 Woden ACT 2606 ABN 40 939 406 804
Phone: 02 6232 8444 Fax: 02 6232 8605 Email: info@tga.gov.au www.tga.gov.au

TGA Health Safety Regulation

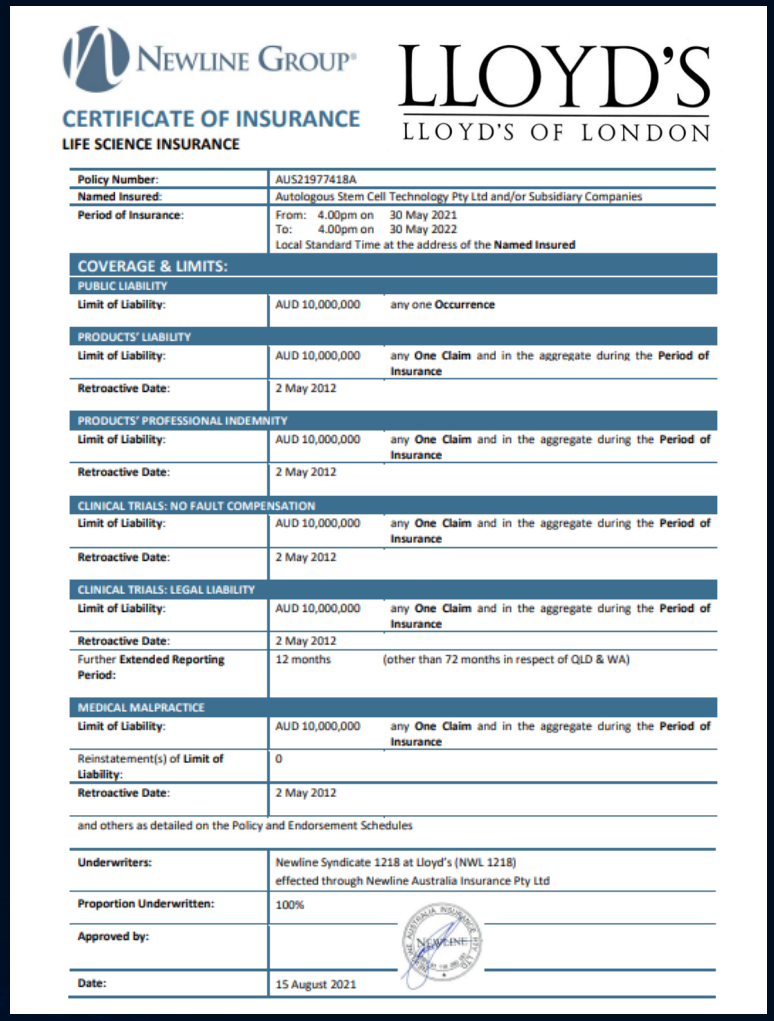
Image_1

Company Introduction

- **Medical Insurance Coverage**
  - By Lloyd's of London for up to AUD 20 million dollars
  - No claims have been made (Image_2)

- **Patented Intellectual Property**
  - ASCT's proprietary technology is currently registered

Region/Type	Patent/Application Number
PCT	PCT/AU2018/05123
Australian provisional	2018902168
Australian national entry	2018428450
US national entry	16/217,335
Europe entry	18923493.3



NEWLINE GROUP®

LLOYD'S
LLOYD'S OF LONDON

CERTIFICATE OF INSURANCE
LIFE SCIENCE INSURANCE

Policy Number:	AUS21977418A
Named Insured:	Autologous Stem Cell Technology Pty Ltd and/or Subsidiary Companies
Period of Insurance:	From: 4.00pm on 30 May 2021 To: 4.00pm on 30 May 2022 Local Standard Time at the address of the **Named Insured**

COVERAGE & LIMITS:

PUBLIC LIABILITY		
Limit of Liability:	AUD 10,000,000	any one **Occurrence**
PRODUCTS' LIABILITY		
Limit of Liability:	AUD 10,000,000	any **One Claim** and in the aggregate during the **Period of Insurance**
Retroactive Date:	2 May 2012	
PRODUCTS' PROFESSIONAL INDEMNITY		
Limit of Liability:	AUD 10,000,000	any **One Claim** and in the aggregate during the **Period of Insurance**
Retroactive Date:	2 May 2012	
CLINICAL TRIALS: NO FAULT COMPENSATION		
Limit of Liability:	AUD 10,000,000	any **One Claim** and in the aggregate during the **Period of Insurance**
Retroactive Date:	2 May 2012	
CLINICAL TRIALS: LEGAL LIABILITY		
Limit of Liability:	AUD 10,000,000	any **One Claim** and in the aggregate during the **Period of Insurance**
Retroactive Date:	2 May 2012	
Further **Extended Reporting Period:**	12 months	(other than 72 months in respect of QLD & WA)
MEDICAL MALPRACTICE		
Limit of Liability:	AUD 10,000,000	any **One Claim** and in the aggregate during the **Period of Insurance**
Reinstatement(s) of **Limit of Liability:**	0	
Retroactive Date:	2 May 2012	

and others as detailed on the Policy and Endorsement Schedules

Underwriters:	Newline Syndicate 1218 at Lloyd's (NWL 1218) effected through Newline Australia Insurance Pty Ltd
Proportion Underwritten:	100%
Approved by:	
Date:	15 August 2021

Image_2

Why AMPCs

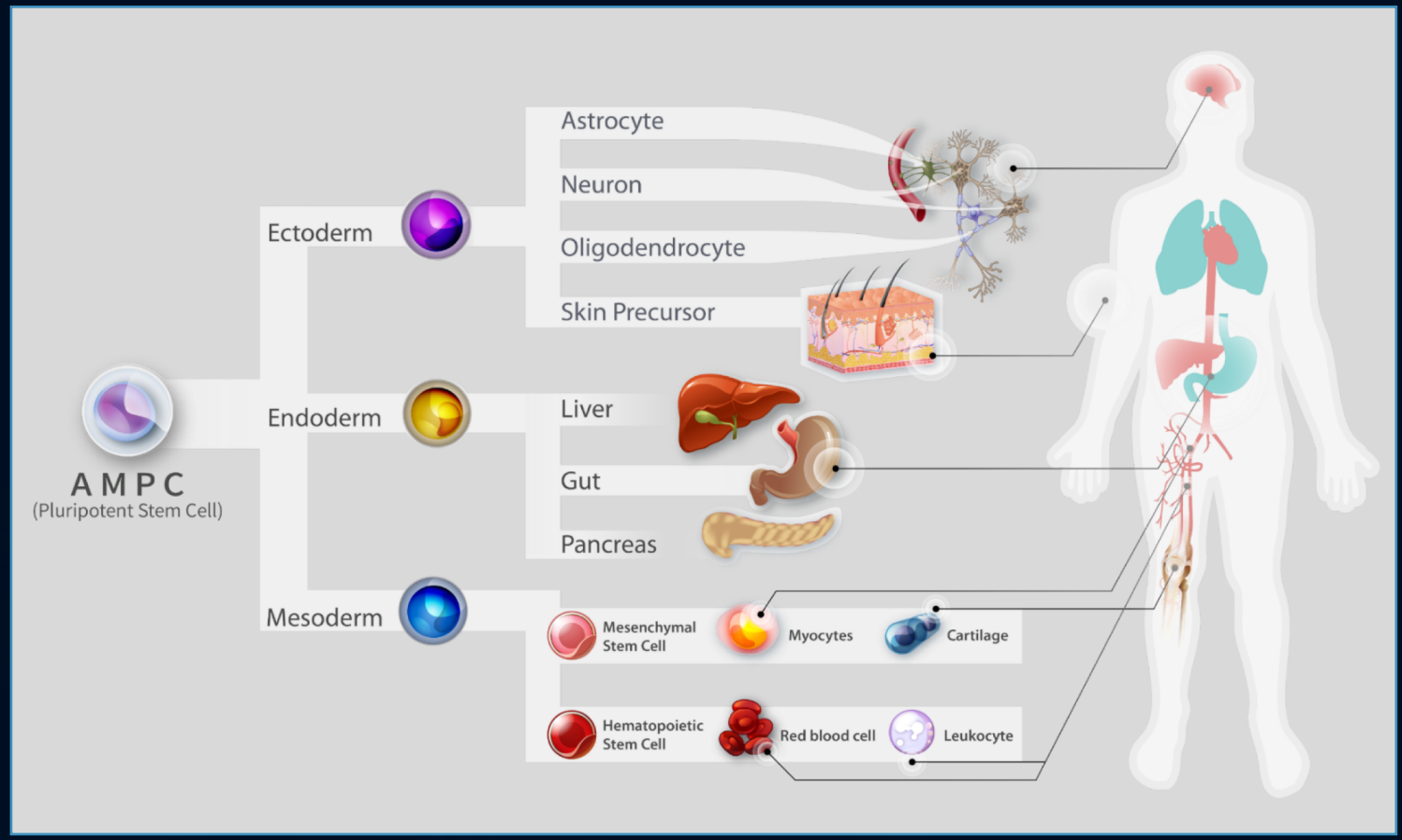
- What are AMPCs?
- Core Competency
- Why sets AMPCs apart?
- How AMPCs functions?

Why AMPCs

- **What are AMPCs?**
  - Self-renewing cells with potential multi-lineage differentiations
  - Culture from individual's own blood and differentiate from leukocytes
  - Differentiate into all the three germ layers: ectoderm, endoderm, and mesoderm
- **Core Competency**
  - Able to be cultured a high amount of CD_{34}
  - Able to differentiate into any type of cells regarding ectoderm, endoderm, and mesoderm features
  - Have good distribution capability to penetrate into all of organs
  - Able to suppress tumor cells growth
- **Why sets AMPCs apart?**
  - Wide range of differentiation allows therapeutic effect for large scope of degenerative diseases, including arthritis, leukaemia, cardiovascular disease, chronic kidney diseases, liver diseases, and certain autoimmune diseases
  - Homing effect identifies priority sites for regenerative effects
  - Insurance coverage offers comprehensive protection for patients and medical practitioners. No claims have been made to date.

Why AMPCs

- **How AMPCs functions?**
  - Differentiate into various cell groups, , but confined to differentiate into only one cell group, such as blood cells.
  - Demonstrate ability to differentiate into different specialised body cells
    Examples：
    - Neurons (Ectoderm)
    - Liver cells (Endoderm)
    - Osteoblasts (Mesoderm)
    - Cardiac cells (Mesoderm)
  - Receptive to the body's chemical signals, thus recognising sites that require stem cell regenerative effects for bodily repair (homing effects).



AMPCs Safety

- Main Features
- AMPCs Culture Steps (Closed-system reduces contamination risk)
- Safety Standards
- No Tumour Formation Concern
- High AMPCs counts achieved without genetic expansion
- Supress Tumour Growth
- Tumourigenicity studies conducted

AMPCs Safety

- **Main Features**
  - Cultured from autologous blood and free from genetic manipulation
  - Autologous cells eliminate risk of rejection and graft versus host disease
  - Uninvolved genetic manipulation during culture-process
  - Nil mutational concerns due to non-somatic induced pluripotent stem cells
  - Nil ethical concerns due to non-collection embryonic stem cells
  - Short term safety therapy, about 6-7 days
  - Legitimated patent and high amount of liability insurance

AMPCs Safety

AMPCs Culture Steps

STEP_1: Blood Collection

- Collect venous blood about 250-400mls
- Adjust for weight and health conditions

STEP_2: Blood Centrifuged

- Cell separation
- The number of leukocyte suspensions ranges from $1x10^8$~$1x10^9$

STEP_3: Cell Culture

Blood centrifuged

- Cell separation
- The number of leukocyte suspensions ranges from $1x10^8$~$1x10^9$

STEP_4: Safety and Quality Assurance

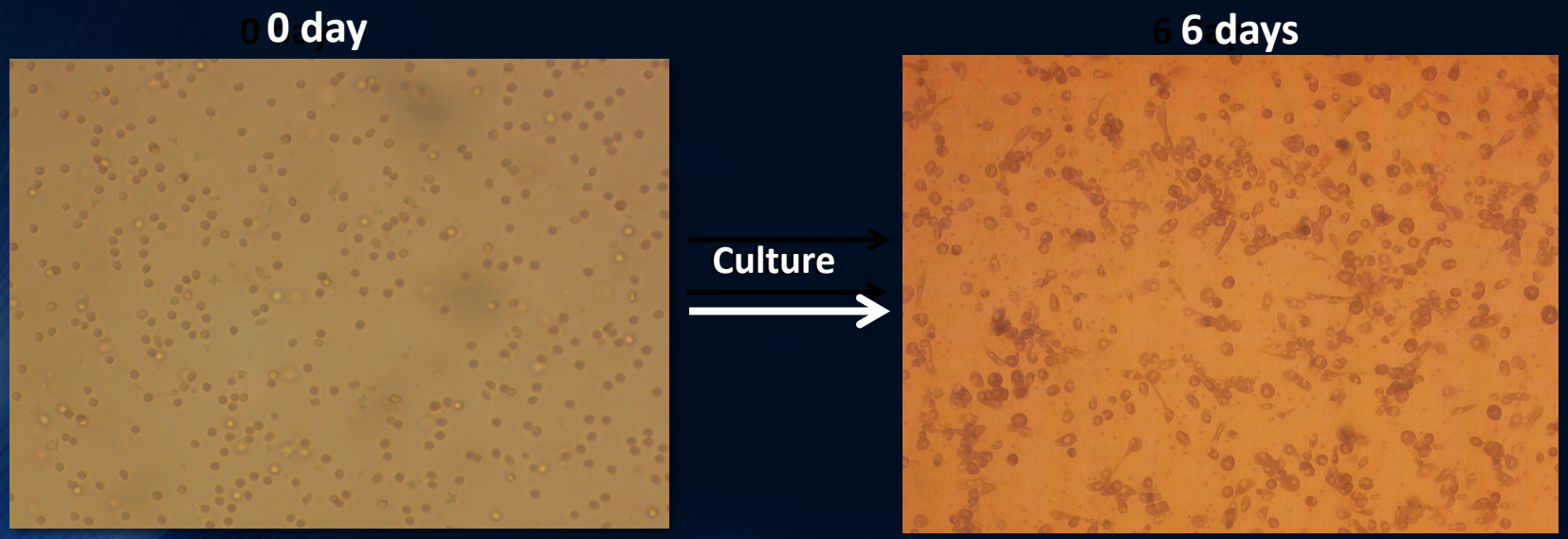
- Samples are analyzed by external laboratory accredited by the Nation Association of Testing Authorities (NATA) for endotoxins, bioburdens, mycoplasma, and microbials.

STEP_5: Reinfusion

- Subcutaneous allergy test with 1 ml of sample
- Intravenous reinfusion
- Injections into allocated parts (e.g. knee or skin)

AMPCs Safety

- **Cultured AMPCs**
  - Autologous
  - Closed-system
  - No genetic manipulation
  - Multi-lineage Potential



AMPCs Safety

- **Safety Standards**
- Recognised by the Australian TGA Ordered Goods Exemption in 2011
- Human therapeutic use of the product may only be conducted by Australian-registered medical practitioners
- Production laboratory is compliant with ISO9001:2008 standards for quality assurance
- All samples must be tested by contamination testing laboratories accredited by the NATA
- Regulatory compliance allows medical insurance coverage by Lloyd’s of London for up to AUD20 million.

AMPCs Safety

AMPCs Distribution

- The distribution of AMPC in a mice model observed under IVIS imaging (Image_3).
- AMPC were labelled with fluorescence dye DiRTM (AMPC-DiR cells), then injected into nude mice by tail vein.
- Tumour formation was not observed after 7 days in any organ (Image_4).

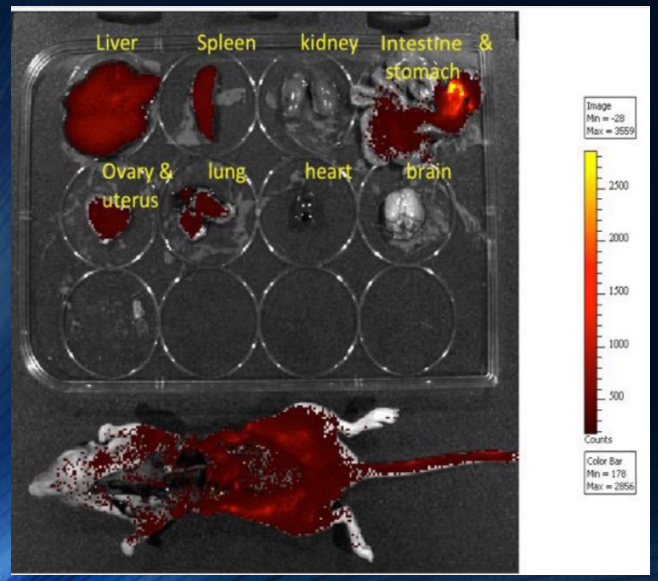


Image_4

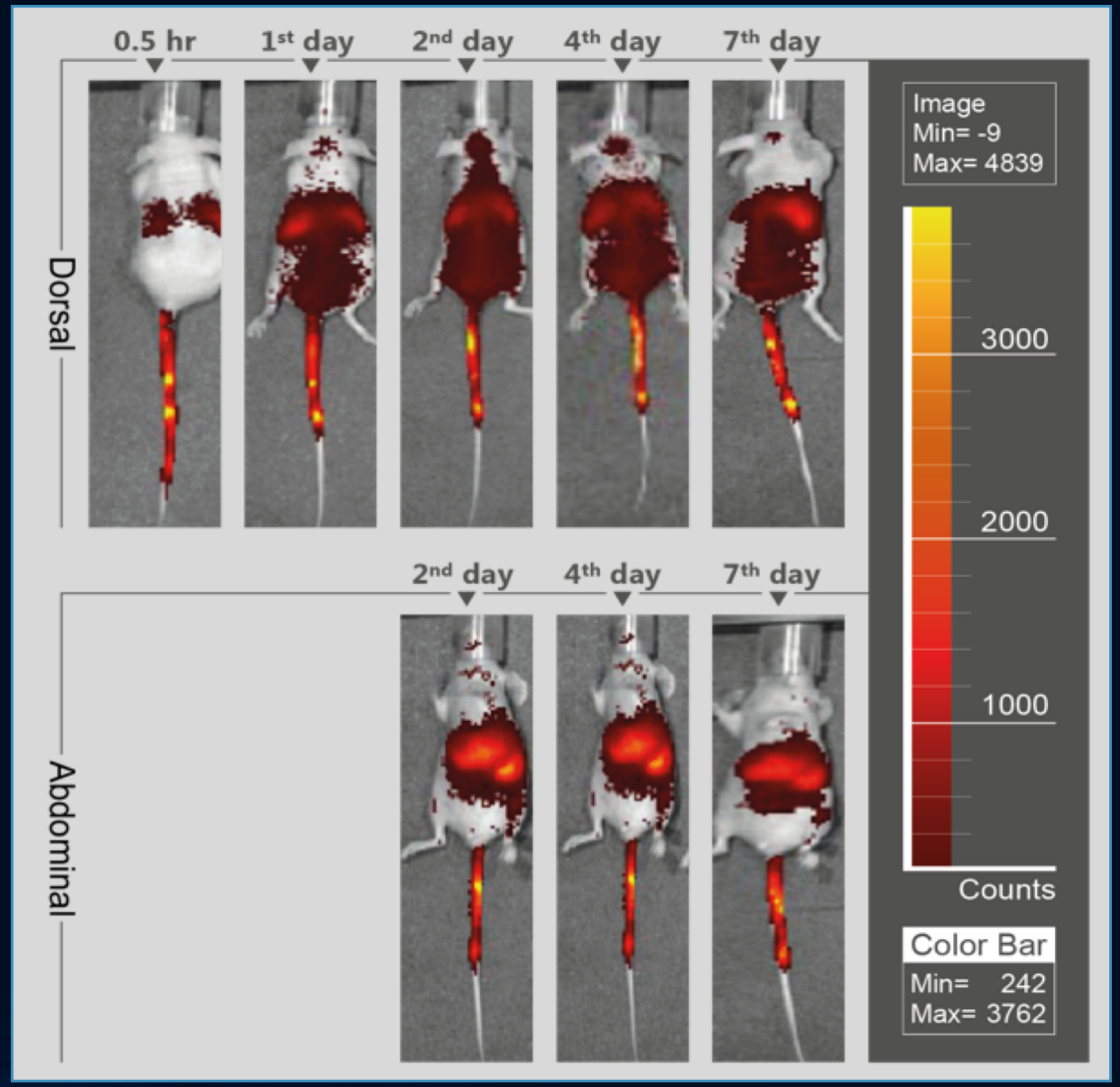


Image_3

AMPCs Safety

- **Effects of AMPCs Escalating Doses**
  - 3 different AMPCs doses were injected into mice to determine toxicity (Table 1)
  - Mice sacrificed at 14 days for histopathological analysis (Table 2)

AMPC (Table 1)	
Cells/20 g	Cells/kg
2.00E+05	1.00E+07
1.00E+06	5.00E+07
2.00E+06	1.00E+08

Table 2 Severity grading of histopathological findings.

	Acute toxicity study (BALB/c mice)											
Dose of AMPC (cells/20 g)	0 (Control)			2E+05			1E+06			2E+06		
Mouse number	28	29	30	25	26	27	22	23	24	19	20	21
Brain												
: vacuolation and glial change	0	0	0	0	0	0	0	0	0	0	0	0
Heart												
: epicardial mineralisation	0	0	3	3	0	0	0	0	2	3	0	0
: epicardial inflammation	0	0	3	3	0	0	0	2	2	3	0	0
Lung												
: neutrophilic inflammation	0	0	0	0	0	1	0	0	0	0	0	0
: macrophage accumulation	0	0	0	0	0	0	0	0	0	0	0	0
Liver:												
: inflammation	1	1	1	1	1	1	1	1	1	1	1	0
: hepatopathy	0	0	0	0	0	0	0	0	0	0	0	0
Kidney												
: lymphocytic inflammation	1	0	1	1	0	0	0	0	0	0	1	0
Spleen												
: lymphoid depletion	0	0	0	0	0	0	0	0	0	0	0	0

AMPCs Safety

- **Suppress Tumour Growth**
  - Tumors tagged with D-Luciferin are introduced into mouse models prior to day 0 and left to develop. AMPC introduction occurs on day 0 (13 days after cancer introduction) and slower cancer progression is observed in the mouse injected with AMPC (left) using IVIS imaging of D-Luciferin (Image_5).
  - Cancer cells with AMPC (right) are injected at the same time in the experimental model. The model with AMPC (right) showed significantly slower tumors growth compared to the control model (left) (Image_6).

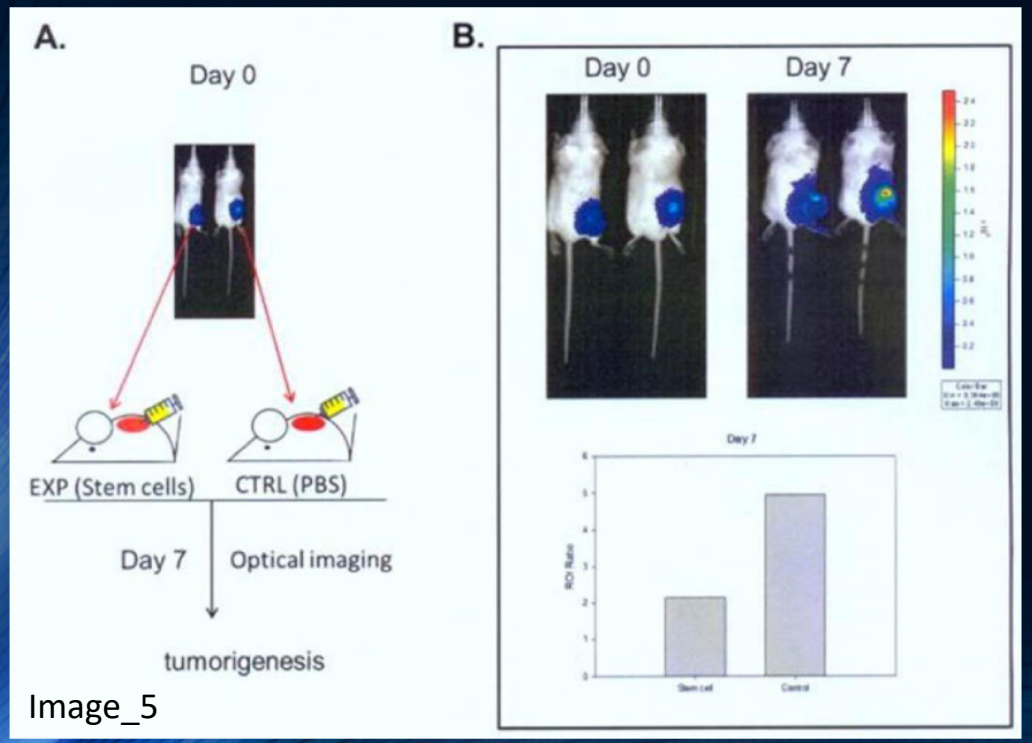


Image_5

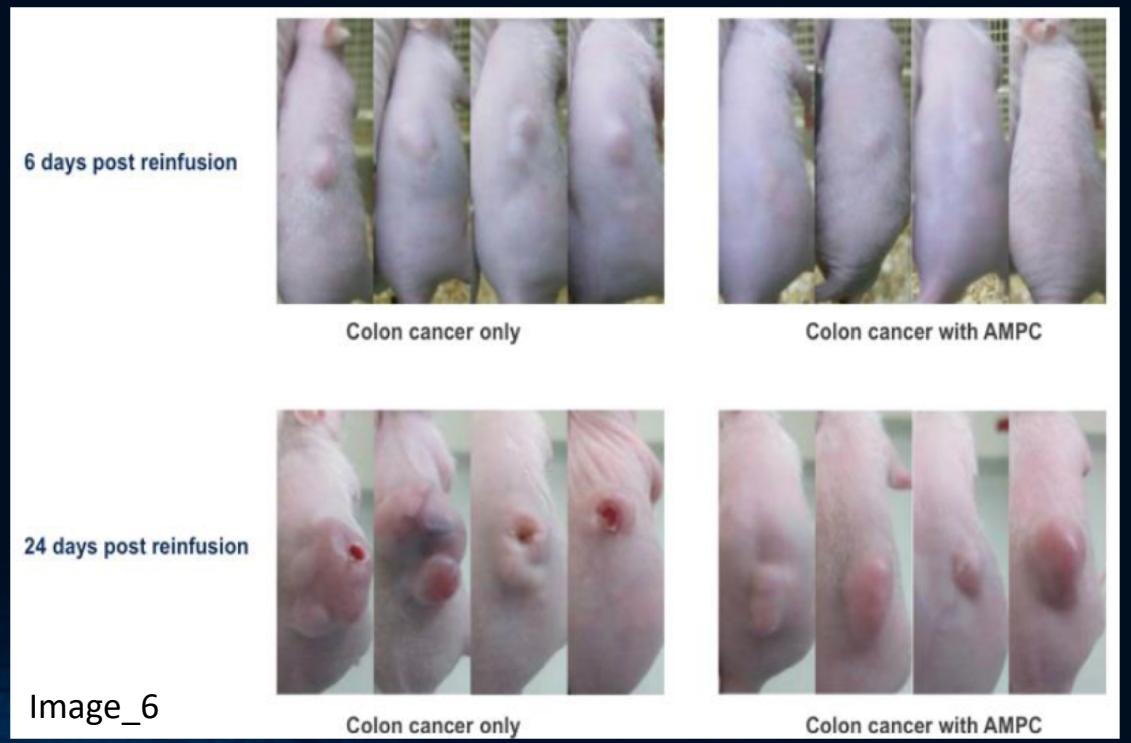


Image_6

AMPCs Safety

Tumourigenicity Studies

- Long-term tumourigenicity studies on immune-compromised mice.
- A high number of AMPCs introduced to mice with congenital immune deficiency, through subcutaneous implantation. The result indicated that:
  - No tumors were observed after 6 months.
  - No significant abnormalities were observed that were related to AMPC introduction
  - High AMPCs counts achieved without genetic expansion

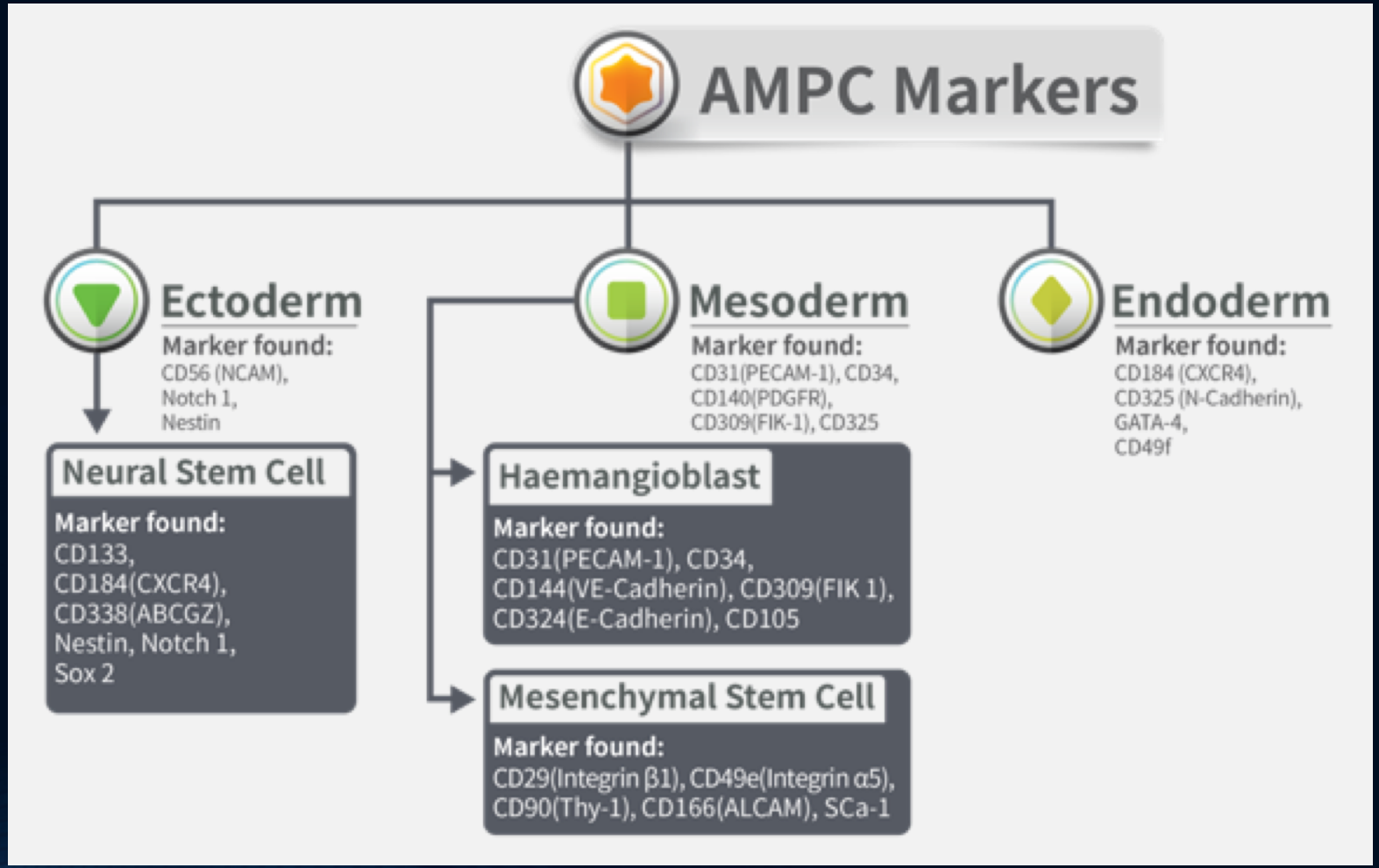
Group	Female - Control			Female - AMPC							Male - Control			Male - AMPC						
Mouse number	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70
Brain																				
: lymphocytic inflammation, meninges	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	2	0	0
: granulomatous inflammation, cerebellum	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0
Heart	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lung																				
: macrophage accumulation	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0
Liver:																				
: mononuclear or mixed cell inflammation	1	3	1	1	1	2	0	0	3	0	0	1	1	0	0	1	0	1	0	1
: neutrophilic inflammmation	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
: subcapsular hepatocellular fatty change	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0
: foci of hepatocellular alteration	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3
Kidney																				
: lymphocytic inflammation	1	1	0	1	1	0	0	2	0	1	1	1	2	1	0	1	1	1	1	0
: cortical tubular hyperplasia	0	0	1	1	1	0	1	0	1	1	3	1	1	1	1	1	1	1	1	1
: medullary tubular mineralisation	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	1	0
Spleen																				
: marginal zone increase	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Stomach (non-glandular)																				
: epithelial hyperplasia	0	0	0	0	0	0	0	0	3	0	0	2	0	0	0	0	0	0	0	0
: mixed cell inflammation	0	0	0	0	0	0	0	0	3	0	0	1	0	0	0	0	0	0	0	0
Stomach (glandular)																				
: mixed cell inflammation	0	0	3	0	1	1	0	0	1	2	0	2	0	NP	0	0	0	0	0	1
Small intestine	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Large intestine	0	0	0	0	0	0	0	0	0	NP	0	0	0	0	0	0	0	0	0	0
Ovary	0	0	0	0	0	0	0	0	0	0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Testicle	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	0	0	0	0	0	0	0	0	0

Functional Analysis of AMPCs

- Multi-Lineage Differentiation Potential
- AMPCs Differentiations

Functional Analysis of AMPCs

- **Multi-Lineage Differentiation Potential**

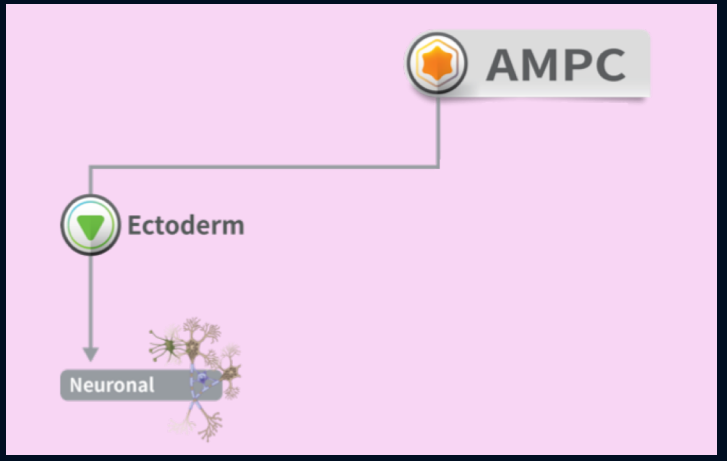


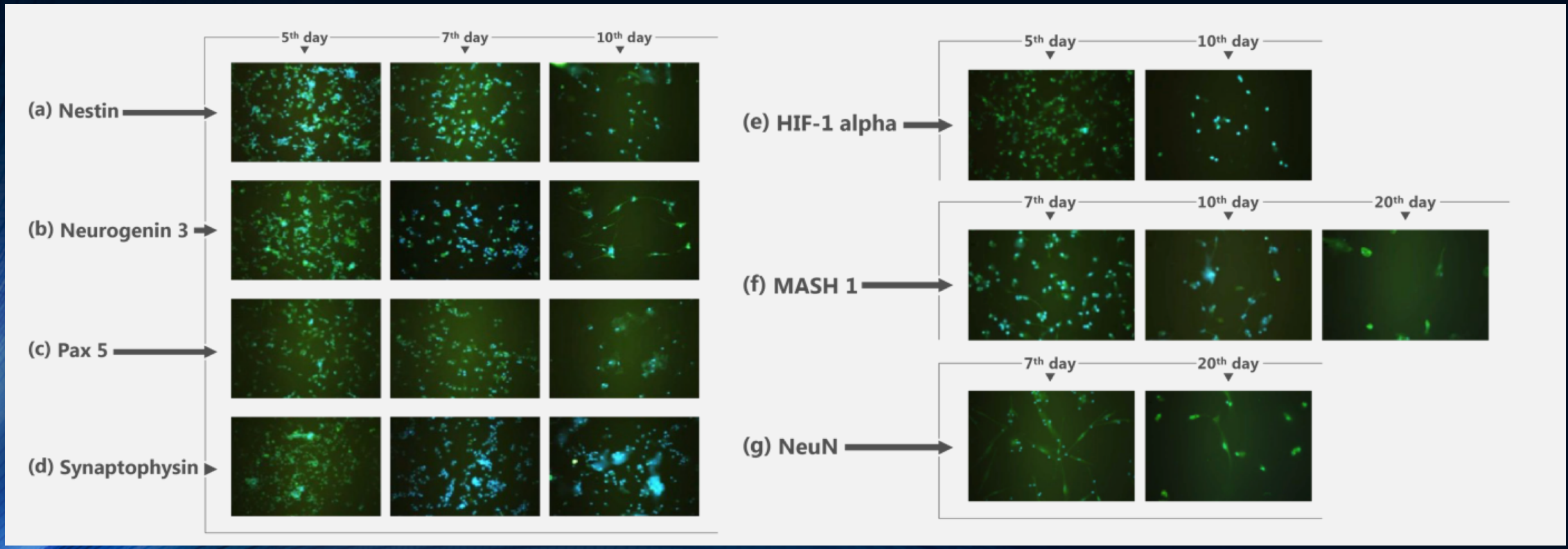
- The three germ layer lineages of embryonic germ layers: Ectoderm, Mesoderm, Endoderm.
- Cell markers are dynamic and their expression changes depending on stages of differentiation.

Functional Analysis of AMPCs

Neuronal Differentiation from AMPCs

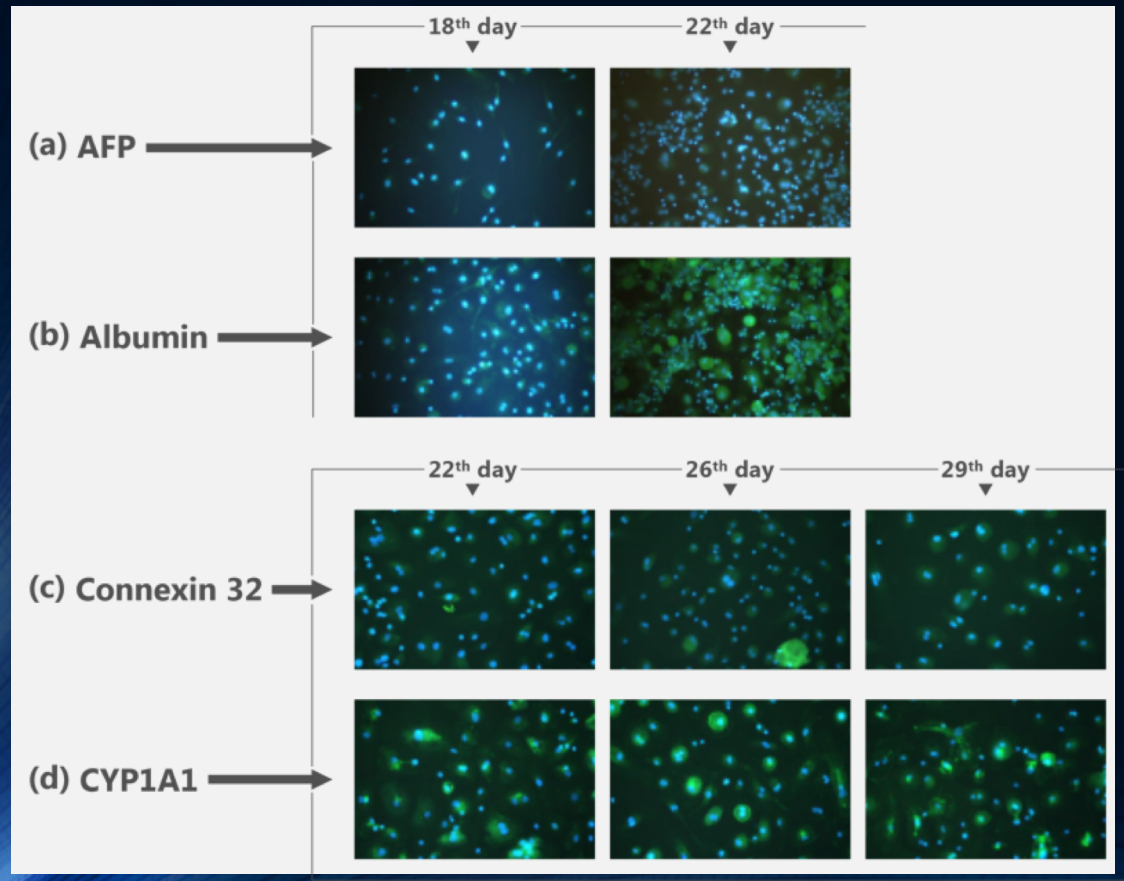
- The protein markers expressed in neuronal differentiation of AMPC.
- The differentiated cells were observed to express Nestin, Neurogenin 3, Pax5, HIF-1 alpha, MASH1, NeuN, and Synaptophysin markers. These markers indicate AMPC differentiation into neurons.

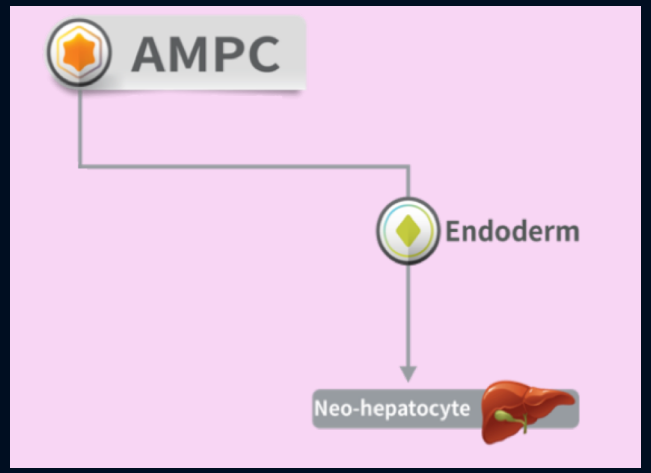




Functional Analysis of AMPCs

- **Neo-hepatocyte (Liver) Differentiation from AMPCs**
  - The protein marker expression of AMPC neo-hepatocyte differentiation.

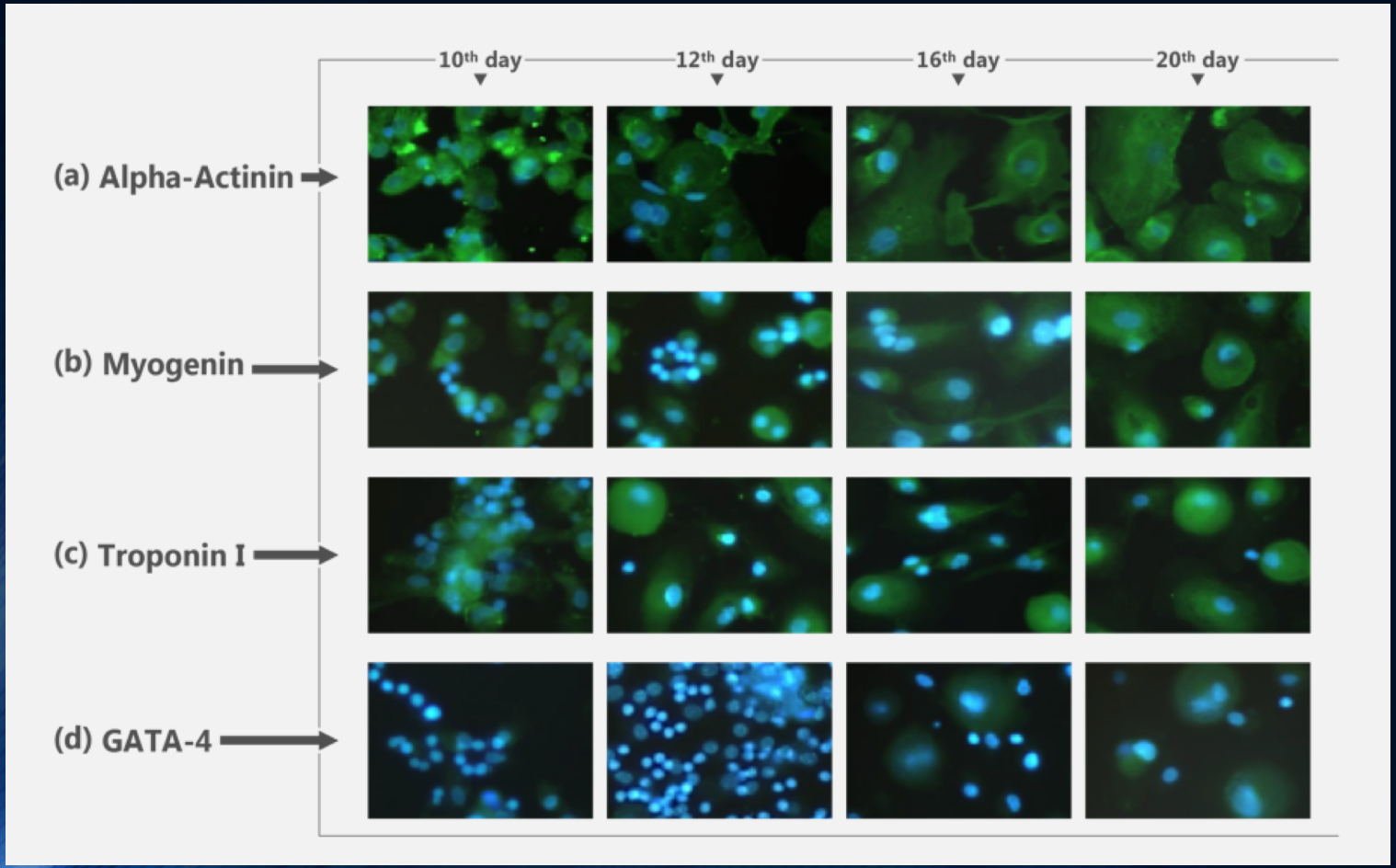


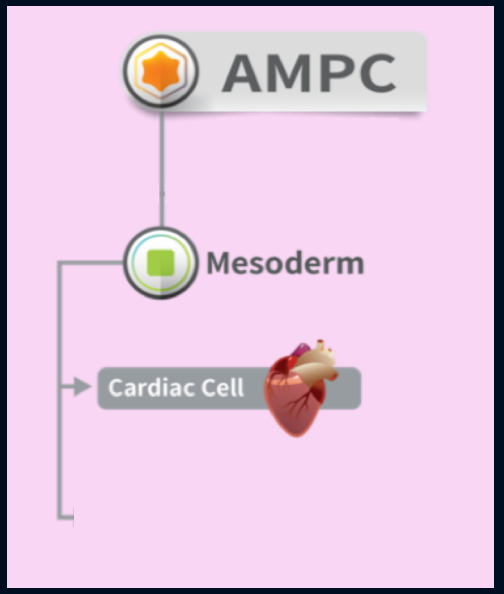


- The differentiated cells were observed to express Albumin, Connexin 32, AFP, and CYP1A1 markers. These markers indicate AMPC differentiation into liver cells

Functional Analysis of AMPCs

- **Cardiac Cell (Heart) Differentiation from AMPCs**
  - The protein marker expression of AMPC neo-hepatocyte differentiation.



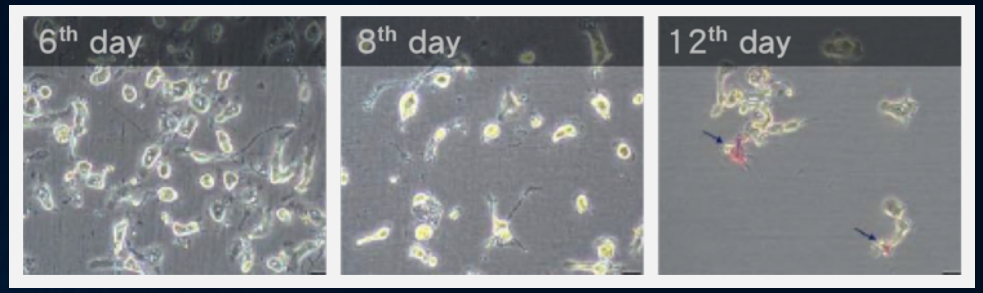


- The differentiated cells were observed to express Alpha Actinin, Myogenin, GATA-4, and Troponin I marker. These markers indicate AMPC differentiation into cardiac cells.

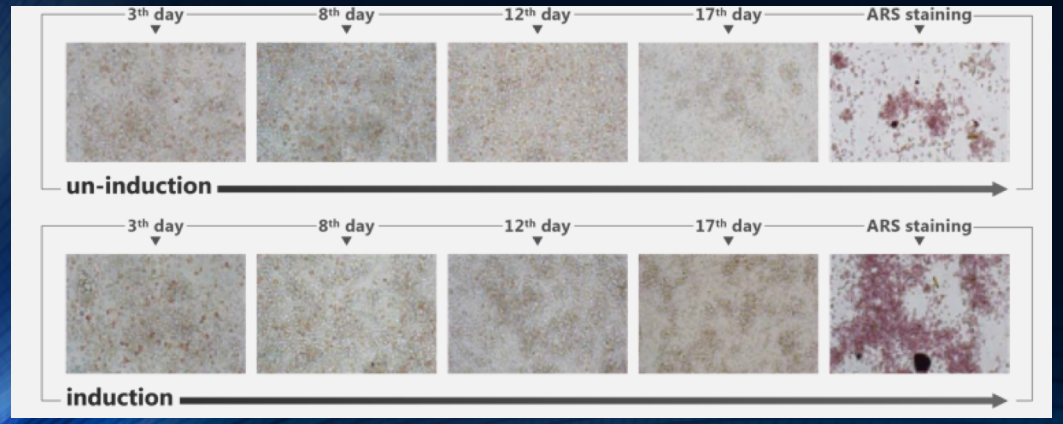
Functional Analysis of AMPCs

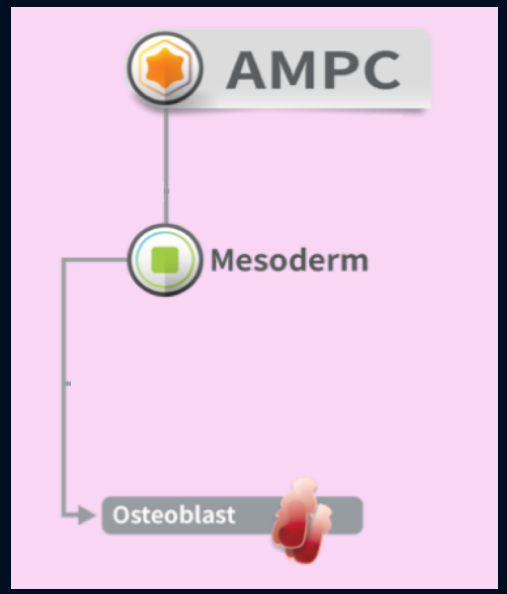
Cardiac Cell (Heart) Differentiation from AMPCs

- The expression of ALP in AMPC differentiation into osteoblasts.



- ARS staining of AMPC osteoblast differentiation showing increasing calcium accumulation. Spontaneous differentiation was observed in the un-induced group.





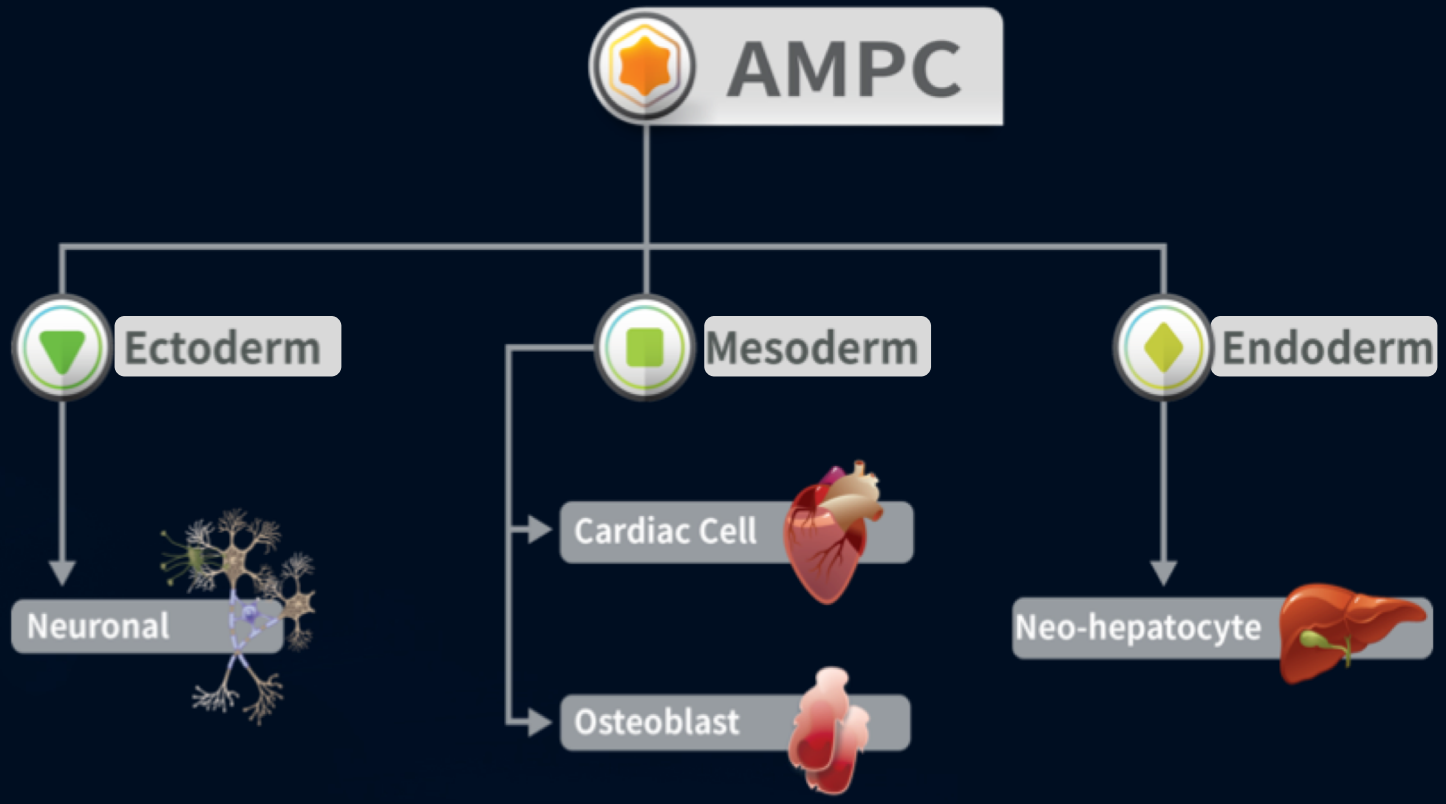
- The differentiated cells were then observed to express ALP marker, and to accumulate calcium. These characteristics suggest that AMPCs have differentiated into osteoblasts.

Molecular Analysis of AMPCs

- Pluripotency Indications
- Classical Stem Cell Marker CD34+
- Classical Stem Cell Marker CD45+
- Pluripotency Gene Expression
- Expression of Pluripotency-related Genes
- Analysis of Embryonic Stem Cell Markers

Molecular Analysis of AMPCs

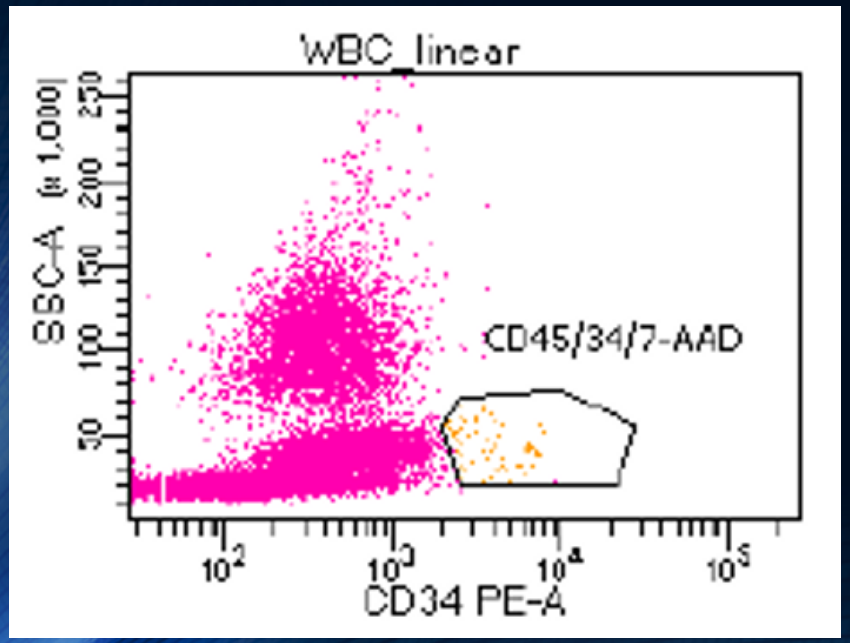
- **Pluripotency Indications**
  - Pluripotent differentiation potential is the ability to differentiate into all cell types of the body.
  - Goal of molecular analysis is to determine degree of similarity to ESC.
  - Yet molecular markers do not strictly define expected functional development of pluripotent stem cells. (Singh *et al.* 2016)



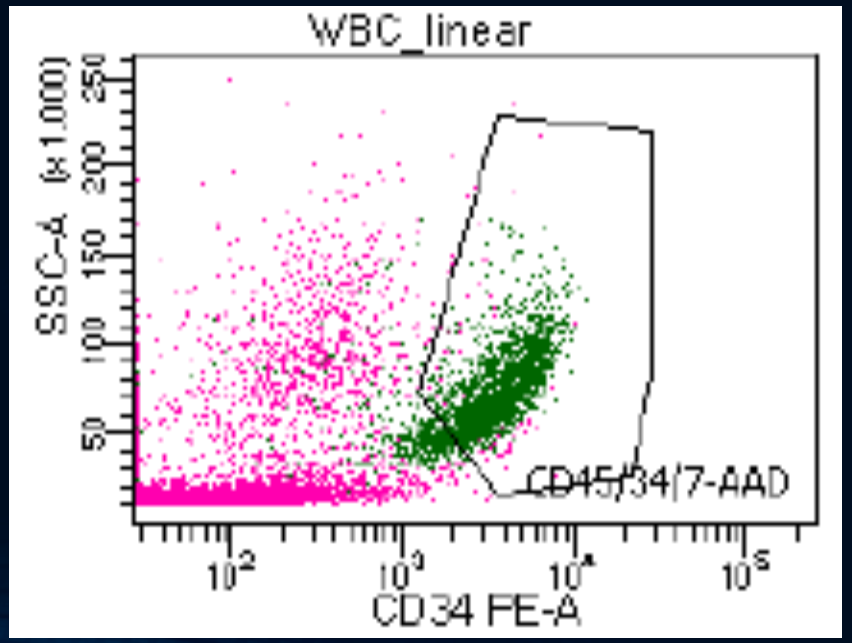
Molecular Analysis of AMPCs

- **Classical Stem Cell Marker CD34+**
  - CD34+ cells are classically haematopoietic stem cell markers
  - Also markers for endothelial progenitor cells
  - Have been used in CKD treatment studies (Lee et al 2017, Choi et al 2004)
  - Posited therapeutic mechanism: in *vivo* angiogenesis in kidneys

CD34 = 1.6 x 10^6



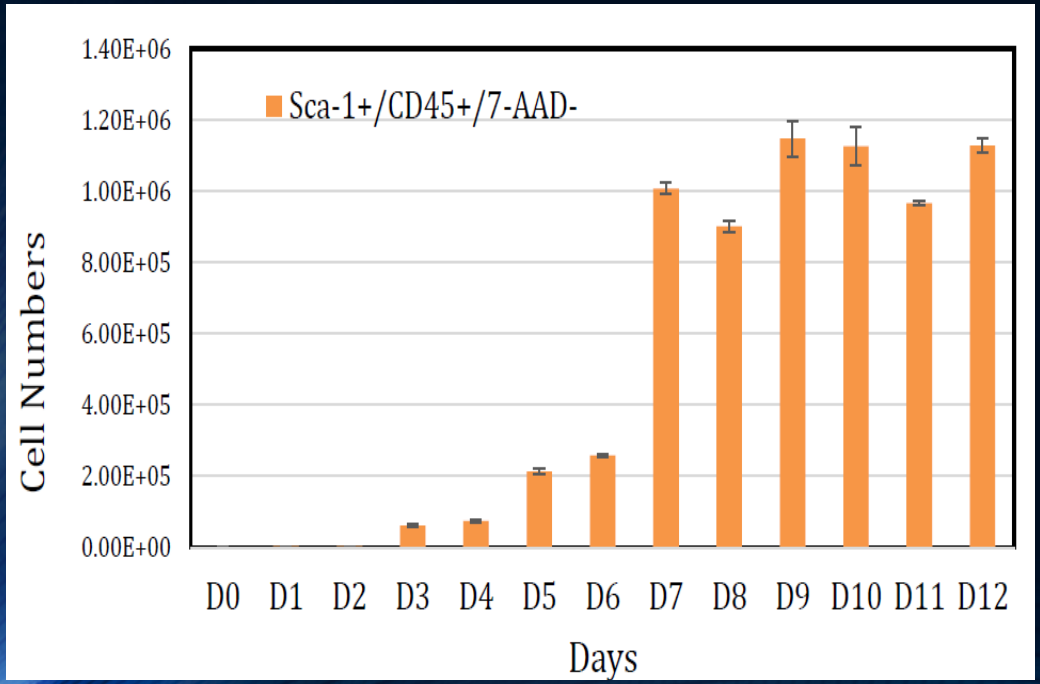
CD34 = 1.2 x 10^7



Molecular Analysis of AMPCs

Classical Stem Cell Marker CD45+

- AMPCs are cultured from leukocytes by reverse engineering
- CD34+ cells are leukocyte markers, cells were calculated on day 4 and 5 of culture as total cell counts.
  - Increase in cell count proportions could be attributed to lymphocyte proliferation or AMPCs self-renewal.
  - Self-renewal could be intensified after introduction into the body due to body's signaling proteins.

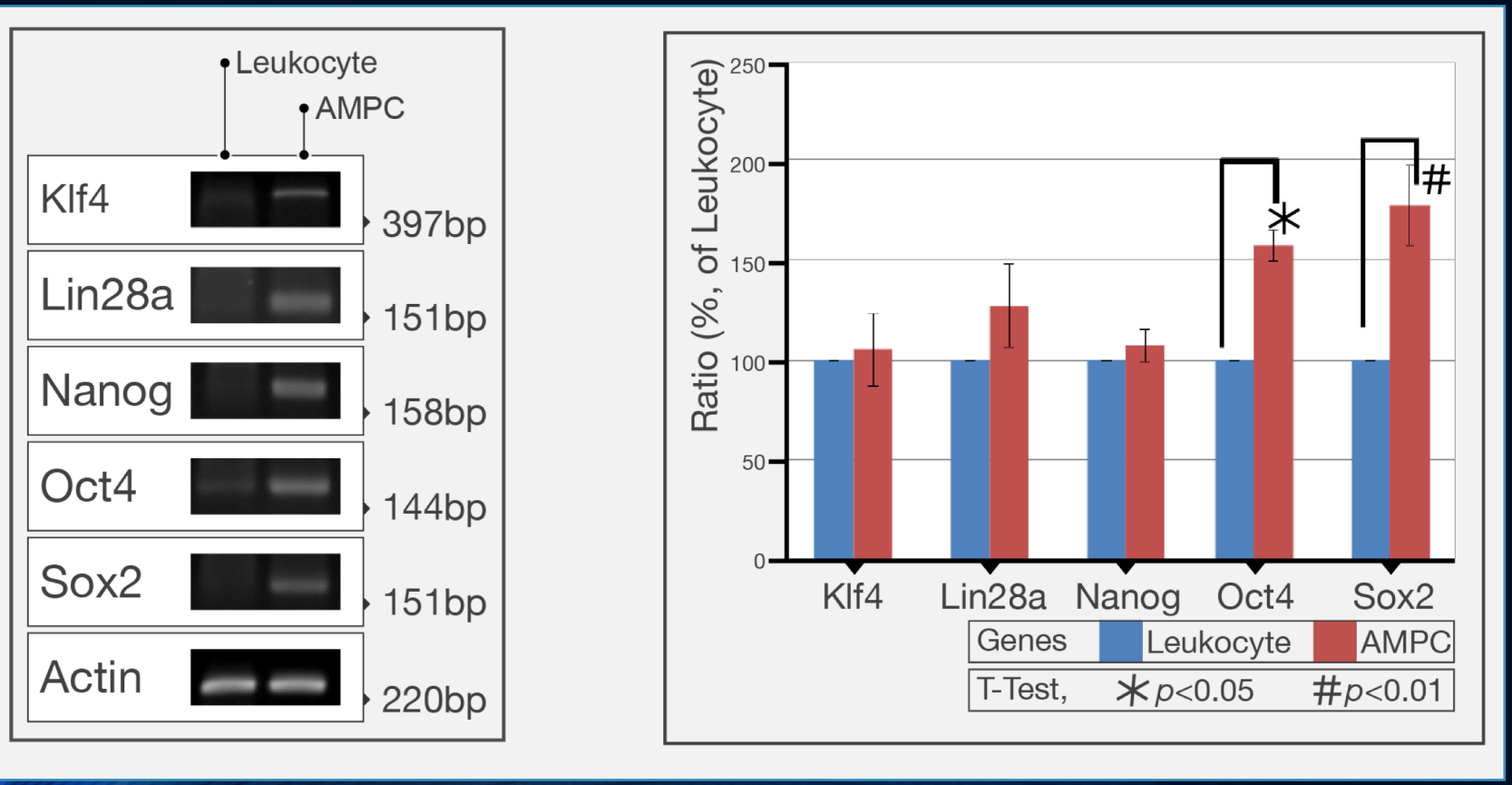


	Day0	Day4	Day5
CD45+	$3.8x10^8$	$4.2x10^8$ (110.5%)	$4.6x10^8$(121.1%)

- Changes in Sca-1 expression against CD45+ expression of leukocytes during the AMPC culture period.

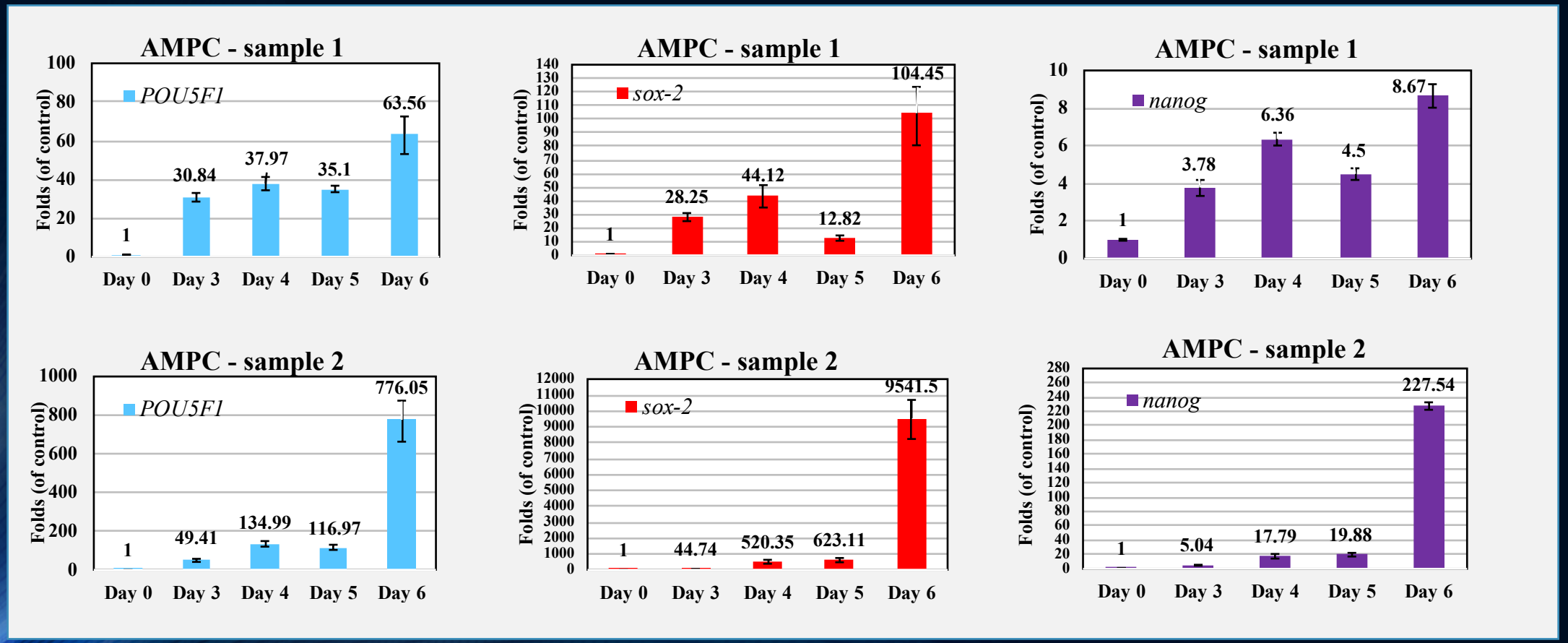
Molecular Analysis of AMPCs

- **Pluripotency Gene Expression: *oct4, sox2, nanog*(OSN)**
  - *oct4, sox2, and nanog* are considered to be key pluripotency maintenance genes.
  - Decrease in expression of either one will result in a decrease in expression of the other 2 (Fong *et al*. 2006).



Molecular Analysis of AMPCs

- **Expression of Pluripotency-related Genes**
  - *oct4, sox2*, and *nanog* (OSN genes) are genes associated with pluripotency.

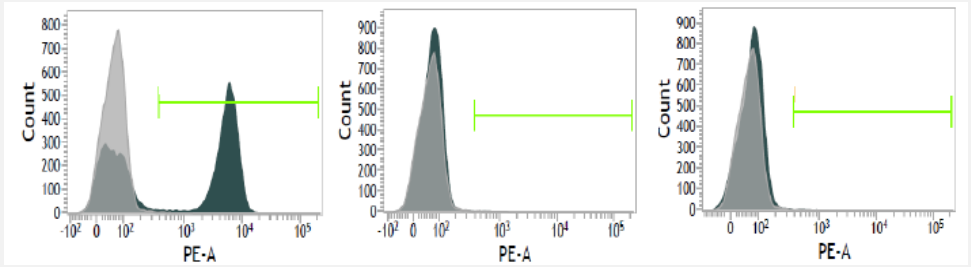


Molecular Analysis of AMPCs

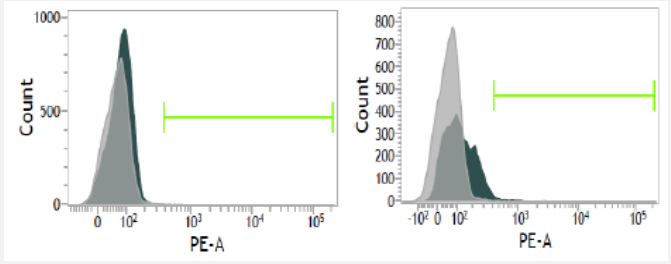
- **Analysis of Embryonic Stem Cell Markers**
  - SSEA-1 increases as cells differentiate
  - Evidence suggest SSEA-3 and SSEA-4 are not involved in pluripotency maintenance (Brimble *et al* 2006).
  - TRA-1-60 and TRA-1-81 are also found in human carcinoma in addition to ESC (Zhao *et al* 2012).

Leukocytes:

SSEA-1 SSEA-3 SSEA-4

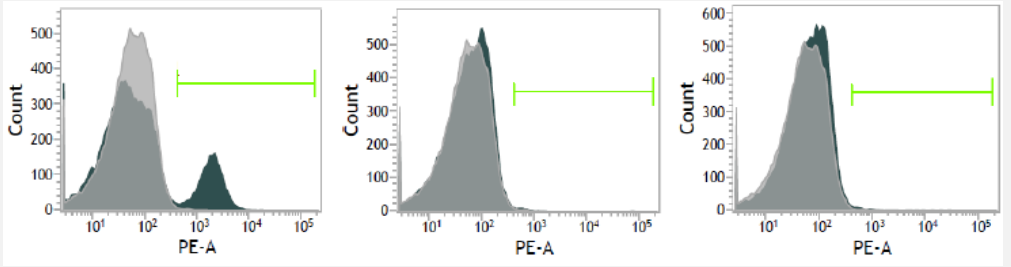


TRA-1-60 TRA-1-81

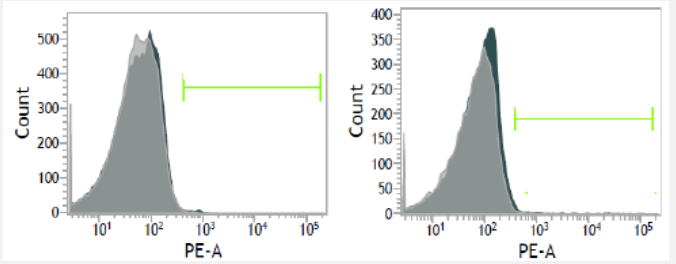


AMPC:

SSEA-1 SSEA-3 SSEA-4



TRA-1-60 TRA-1-81



AMPCs Characters

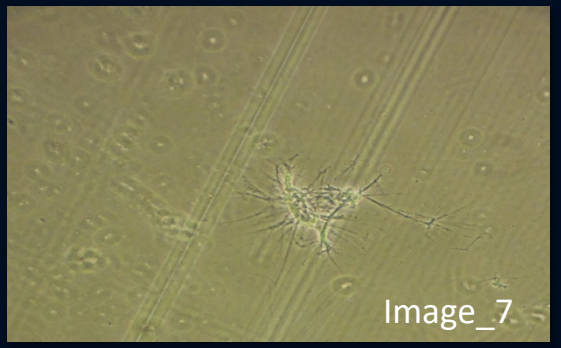
- Homing effect
- Self Renewal

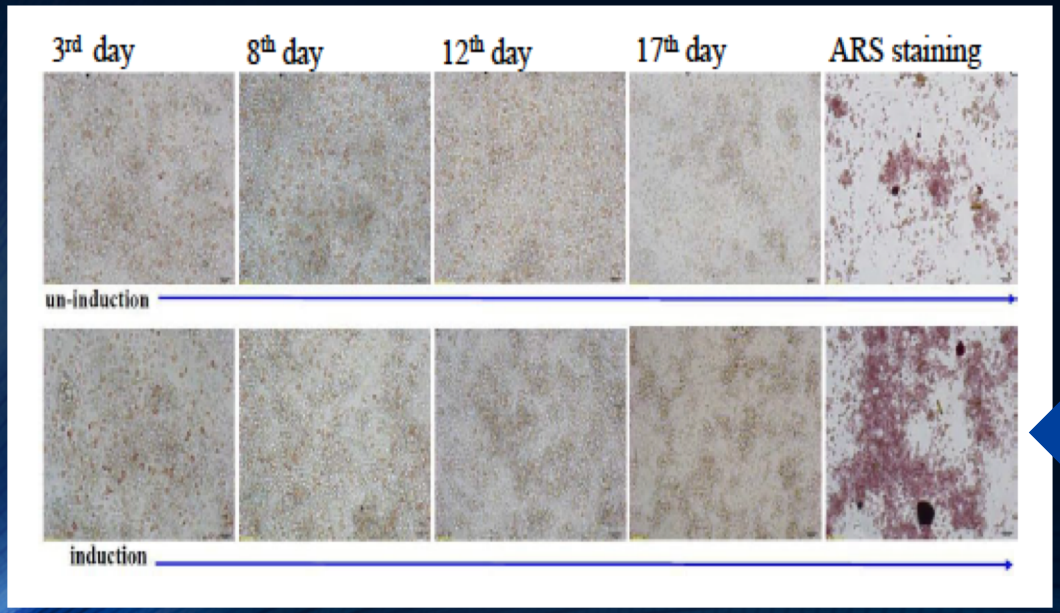
AMPCs Characters

- **Homing effect**

  Refer to the ability of circulating stem cell or exogenously administered stem cell to locate and enter an environmental niche.

  - Dendritic cells (Image_7) are antigen-presenting cells. Once activated, they move to the lymph tissue to interact with to interact with T cells and B cells and help shape the adaptive immune response, such as controlling cancers.
  - Dendritic cells are successfully observed from AMPCs dedifferentiation culture.





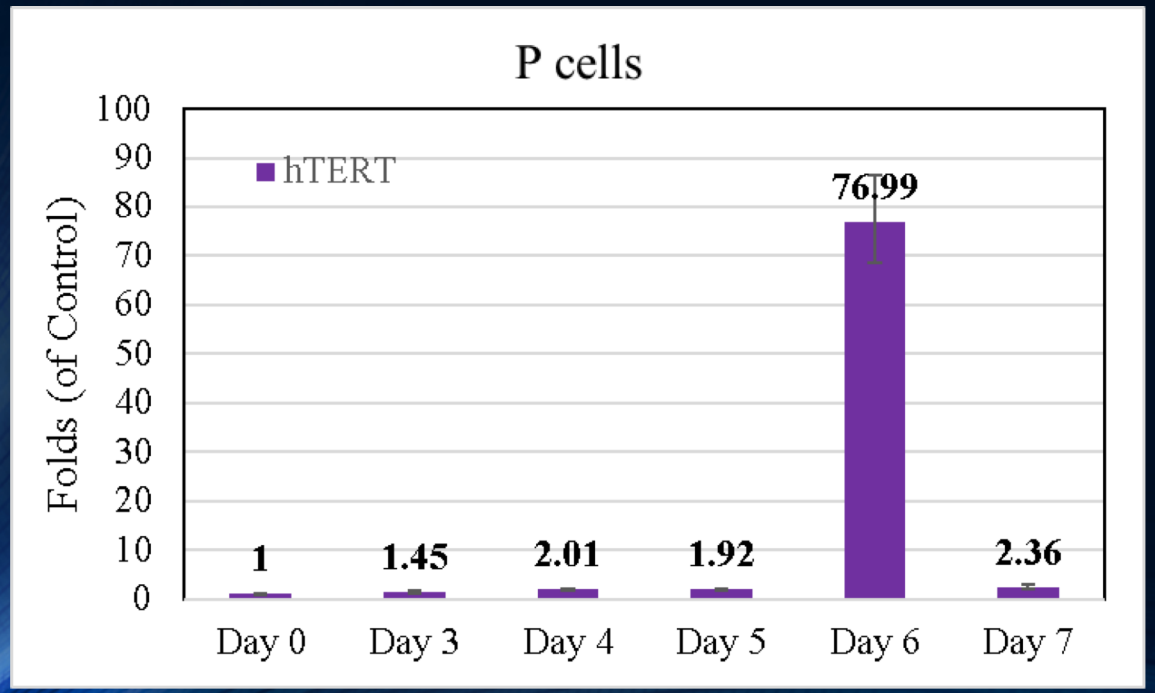
- Uninduced AMPC demonstrated spontaneous osteoblast differentiation.
- Leukocytes collected from post-cancer patient showed emergence of dendritic cells post-culture.

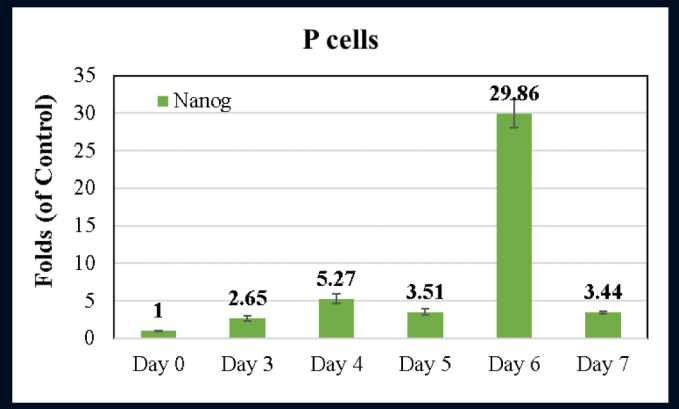
AMPCs Characters

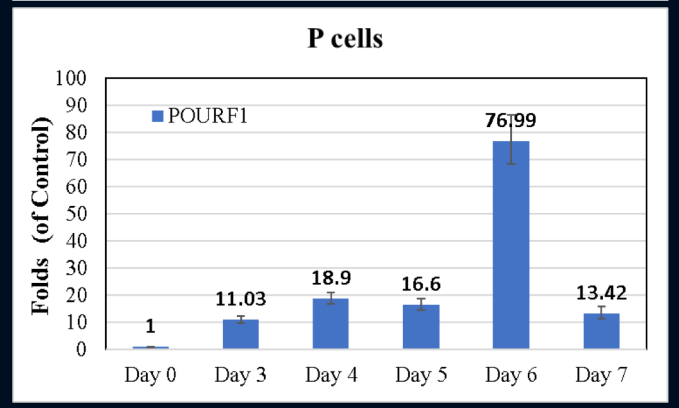
- **Self Renewal**

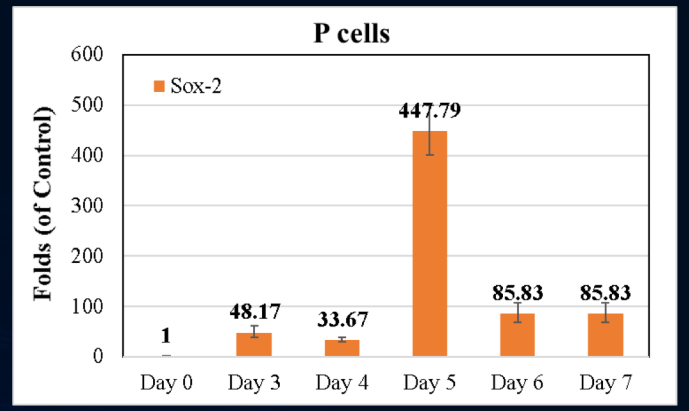
  Refer to AMPCs promoting self renewal and regeneration ability

  - Upregulation of *hTERT* self-renewal gene expression over the AMPC culture period.
  - In line with upregulation of pluripotency genes *oct4, sox2, and nanog*.









AMPCs Characters

Self Renewal

- Self renewal cells, Sca-1 and CD117, significant increased after introducing AMPCs.

Day	$CD117^{+}$ /CD45+/7-AAD^{-} ($\times 10^4$ / ml)	Sca-1+ /CD45+/7-AAD^{-} ($\times 10^4$ / ml)
Day 0	0.14 ± 0.01	0.16 ± 0.01
Day 1	0.82 ± 0.06	0.87 ± 0.05
Day 2	3.12 ± 0.49	3.50 ± 0.48
Day 3	4.85 ± 0.49	6.47 ± 0.52
Day 4	6.46 ± 0.01	13.20 ± 0.14

- Sca-1, full name stem Cells ANTI-1, is a cell membrane protein (GPI-AP) belonging to LY6 gene family, which can affect the generation of hematopoietic stem cells and repair and regenerate the heart.
- CD117 is a cytokine receptor expressed on the surface of hematopoietic stem cells and belongs to receptor Tyrosine kinase TYPE III. When this receptor binds to stem cell factors, the formation of protein dimer will activate its intrinsic tyrosine kinase activity. It phosphorylates and activates signal transduction molecules in cells, further regulating cell survival, proliferation and differentiation.

Comparison between Stem Cells

Comparison between Stem Cells

Stem Cell Marker	Leukocytes (AMPC pre-culture)	AMPC (5 day culture)	ESC[1,2,3,4]	iPSC[5]	STAP[6]
Oct4	Little to no expression. Shown by RT-PCR.	Yes. Significant increase shown by RT-PCR	Yes	Yes. Significant increase shown by RT-PCR, western blot, DNA microarray.	Yes. Significant expression shown by GFP fluorescence and Q-PCR.
Nanog	Little to no expression. Shown by RT-PCR.	Yes. Slight increase shown by RT-PCR	Yes	Yes. Significant increase shown by RT-PCR, western blot, DNA microarray, immunocytochemistry.	Yes. Significant expression shown by Q-PCR.
Sox2	Little to no expression. Shown by RT-PCR.	Yes. Significant increase shown by RT-PCR	Yes	Yes. Significant increase shown by RT-PCR, western blot, DNA microarray.	Yes. Significant expression shown by Q-PCR.
SSEA-1	Yes. Slight expression shown by flow cytometry.	Yes. Though expression decreased from pre-culture shown by flow cytometry.	No. Increases after differentiation.	No. Shown via immunocytochemistry.	Yes. Shown via immunostaining
SSEA-3	Little to no expression. Shown by flow cytometry.	Little to no expression. Shown by flow cytometry.	Yes	Present. Shown via immunocytochemistry.	N/A
SSEA-4	Little to no expression. Shown by flow cytometry.	Little to no expression. Shown by flow cytometry.	Yes	Present. Shown via immunocytochemistry.	N/A
TRA-1-60	Little to no expression. Shown by flow cytometry.	Little to no expression. Shown by flow cytometer.	Yes	Present. Shown via immunocytochemistry.	N/A
TRA-1-81	Little to no expression. Shown by flow cytometry.	Little to no expression. Shown by flow cytometry.	Yes	Present. Shown via immunocytochemistry.	N/A
Lin28	Little to no expression. Shown by RT-PCR.	Yes. Slight increase shown by RT-PCR	Yes	Yes. Shown by western blot.	N/A
Klf4	Little to no expression. Shown by RT-PCR.	Yes. Slight increase shown by RT-PCR	Yes	Yes. Slight increase shown by Q-PCR, RT-PCR, western blot.	N/A
c-MYC	N/A	N/A	Yes	Yes. Slight increase shown by Q-PCR, RT-PCR, western blot.	N/A

Comparison between Stem Cells

Stem Cell Marker	Leukocytes (AMPC pre-culture)	AMPC (5 day culture)
Oct4	Little to no expression. Shown by RT-PCR.	Yes. Significant increase shown by RT-PCR
Nanog	Little to no expression. Shown by RT-PCR.	Yes. Slight increase shown by RT-PCR
Sox2	Little to no expression. Shown by RT-PCR.	Yes. Significant increase shown by RT-PCR
SSEA-1	Yes. Slight expression shown by flow cytometry.	Yes. Though expression decreased from pre-culture shown by flow cytometry.
SSEA-3	Little to no expression. Shown by flow cytometry.	Little to no expression. Shown by flow cytometry.
SSEA-4	Little to no expression. Shown by flow cytometry.	Little to no expression. Shown by flow cytometry.
TRA-1-60	Little to no expression. Shown by flow cytometry.	Little to no expression. Shown by flow cytometer.
TRA-1-81	Little to no expression. Shown by flow cytometry.	Little to no expression. Shown by flow cytometry.
Lin28	Little to no expression. Shown by RT-PCR.	Yes. Slight increase shown by RT-PCR
Klf4	Little to no expression. Shown by RT-PCR.	Yes. Slight increase shown by RT-PCR
c-MYC	N/A	N/A

Comparison between Stem Cells

Stem Cell Marker	AMPC (5 day culture)	ESC
Oct4	Yes. Significant increase shown by RT-PCR	Yes
Nanog	Yes. Slight increase shown by RT-PCR	Yes
Sox2	Yes. Significant increase shown by RT-PCR	Yes
SSEA-1	Yes. Though expression decreased from pre-culture shown by flow cytometry.	No. Increases after differentiation.
SSEA-3	Little to no expression. Shown by flow cytometry.	Yes
SSEA-4	Little to no expression. Shown by flow cytometry.	Yes
TRA-1-60	Little to no expression. Shown by flow cytometer.	Yes
TRA-1-81	Little to no expression. Shown by flow cytometry.	Yes
Lin28	Yes. Slight increase shown by RT-PCR	Yes
Klf4	Yes. Slight increase shown by RT-PCR	Yes
c-MYC	N/A	Yes

Comparison between Stem Cells

Stem Cell Marker	AMPC (5 day culture)	iPSC[5]
Oct4	Yes. Significant increase shown by RT-PCR	Yes. Significant increase shown by RT-PCR, western blot, DNA microarray.
Nanog	Yes. Slight increase shown by RT-PCR	Yes. Significant increase shown by RT-PCR, western blot, DNA microarray, immunocytochemistry.
Sox2	Yes. Significant increase shown by RT-PCR	Yes. Significant increase shown by RT-PCR, western blot, DNA microarray.
SSEA-1	Yes. Though expression decreased from pre-culture shown by flow cytometry.	No. Shown via immunocytochemistry.
SSEA-3	Little to no expression. Shown by flow cytometry.	Present. Shown via immunocytochemistry.
SSEA-4	Little to no expression. Shown by flow cytometry.	Present. Shown via immunocytochemistry.
TRA-1-60	Little to no expression. Shown by flow cytometer.	Present. Shown via immunocytochemistry.
TRA-1-81	Little to no expression. Shown by flow cytometry.	Present. Shown via immunocytochemistry.
Lin28	Yes. Slight increase shown by RT-PCR	Yes. Shown by western blot.
Klf4	Yes. Slight increase shown by RT-PCR	Yes. Slight increase shown by Q-PCR, RT-PCR, western blot.
c-MYC	N/A	Yes. Slight increase shown by Q-PCR, RT-PCR, western blot.

Comparison between Stem Cells

Stem Cell Marker	AMPC (5 day culture)	STAP[6]
Oct4	Yes. Significant increase shown by RT-PCR	Yes. Significant expression shown by GFP fluorescence and Q-PCR.
Nanog	Yes. Slight increase shown by RT-PCR	Yes. Significant expression shown by Q-PCR.
Sox2	Yes. Significant increase shown by RT-PCR	Yes. Significant expression shown by Q-PCR.
SSEA-1	Yes. Though expression decreased from pre-culture shown by flow cytometry.	Yes. Shown via immunostaining
SSEA-3	Little to no expression. Shown by flow cytometry.	N/A
SSEA-4	Little to no expression. Shown by flow cytometry.	N/A
TRA-1-60	Little to no expression. Shown by flow cytometer.	N/A
TRA-1-81	Little to no expression. Shown by flow cytometry.	N/A
Lin28	Yes. Slight increase shown by RT-PCR	N/A
Klf4	Yes. Slight increase shown by RT-PCR	N/A
c-MYC	N/A	N/A

Proof of Concept

Proof of Concept

- **Chronic Kidney Disease (CKD)**

Estimated Glomerular Filtration Rate (eGFR)

- Indicates the ability of kidneys to filter waste from blood and measures overall kidney function
- Deteriorates as they age
- eGFR values lower than 15 require dialysis

CKD Stages	eGFR （mL/min/m²)
Stage 1: Mild kidney damage with normal kidney function	≧90
Stage 2 ： Kidney damage with mild loss of kidney function	60-89
Stage 3: Mild to moderate kidney damage with equal loss of kidney function	30-59
Stage 4 Severe loss of kidney function	15-29
Stage 5: Kidney failure	＜15

Proof of Concept

- **Case_CKD_Stage3**

  Male, 75-year-old

  - Intravenous Infusion
  - Elevated eGFR levels after AMPCs reinfusion
  - Improved energy levels

Date	Event	Estimate eGFR	Stage of CKD
1 May 2013	eGFR Measurement	**52.5**	**Stage 3**
7 September 2013	**AMPCs Intervention**	**-**	**-**
28 October 2013	eGFR Measurement	**57.0**	**Stage 3**
31 December 2013	eGFR Measurement	**57.0**	**Stage 3**
14 April 2014	**AMPCs Intervention**	**-**	**-**
10 July 2014	eGFR Measurement	**56.9**	**Stage 3**
28 January 2015	eGFR Measurement	**56.9**	**Stage 3**
24 April 2015	eGFR Measurement	**62.4**	**Stage 2**

Proof of Concept

- **Case_CKD_Stage4**

  Female, 55-year-old

  - Renal Artery Injection
  - Elevated eGFR levels after treatments

Date	Event	Estimate eGFR	Stage of CKD
21 March 2016	eGFR Measurement	**19.3**	**Stage 4**
20 May 2016	**Renal artery injection**	**-**	**-**
15 July 2016	eGFR Measurement	**25.6**	**Stage 4**
18 November 2016	**Renal artery injection**	**-**	
15 January 2017	eGFR Measurement	**31.7**	**Stage 3**
10 March 2017	**Renal artery injection**	**-**	**-**
10 April 2017	eGFR Measurement	**34.6**	**Stage 3**
Note: Patient received Kidney Transplant after the last test date.			

Proof of Concept

- **Case_CKD_Stage4**

  Female, 58-year-old

  - Renal Artery Injection
  - Elevated eGFR levels after treatments

Date	Event	Estimate eGFR	Stage of CKD
20 September 2017	eGFR Measurement	**27.8**	**Stage 4**
22 September 2017	**Renal artery injection**	**-**	**-**
11 November 2017	eGFR Measurement	**35**	**Stage 3**
19 January 2018	**Renal artery injection**	**-**	
27 February 2018	eGFR Measurement	**38**	**Stage 3**
13 July 2018	**Renal artery injection**	**-**	**-**
20 July 2018	eGFR Measurement	**36**	**Stage 3**
17 September 2018		**40**	**Stage 3**
10 December 2018		**45**	**Stage 3**

Proof of Concept

- **Case_CKD_Stage5**

  Male, 47-year-old

  - Intravenous Infusion
  - Elevated eGFR levels after AMPCs reinfusion

Date	Event	Estimate eGFR	Stage of CKD
15 January 2018	eGFR Measurement	**4.7**	**Stage 5**
19 January 2018	**AMPCs Intervention**	**-**	**-**
26 February 2018	eGFR Measurement	**14.6**	**Stage 5**
18 May 2018	**AMPCs Intervention**	**-**	**Stage 5**
25 June 2018	eGFR Measurement	**15.7**	**Stage 4**
17 September 2018	eGFR Measurement	**14.3**	**Stage 5**
26 October 2018	**AMPCs Intervention**	**-**	
27 November 2018	eGFR Measurement	**22.49**	**Stage 4**

Proof of Concept

- **Case_CKD_Stage5**

  Male, 44-year-old

  - Intravenous Infusion
  - Elevated eGFR after treatments

Date	Event	Estimate eGFR	Stage of CKD
14 May 2018	eGFR Measurement	**4.02**	**Stage 5**
25 May 2018	**AMPCs Intervention**	**-**	**-**
29 June 2018	eGFR Measurement	**4.60**	**Stage 5**
20 August 2018	**AMPCs Intervention**	**-**	
24 September 2018	eGFR Measurement	**8.06**	**Stage 5**
5 October 2018	**AMPCs Intervention**	**-**	**-**
20 November 2018	eGFR Measurement	**13.16**	**Stage 5**
-	-	-	-

Proof of Concept

- **Case_CKD_Stage5**

  Male, 52-year-old

  - 2x Renal Artery Injection
    1x Intravenous Infusion
  - Elevated eGFR levels after treatments

Date	Event	Estimate eGFR	Stage of CKD
1 May 2018	eGFR Measurement	**8.00**	**Stage 5**
25 May 2018	**Intravenous Infusion**	**-**	**-**
26 June 2018	eGFR Measurement	**6.06**	**Stage 5**
20 August 2018	**Renal artery injection**	**-**	
3 October 2018	eGFR Measurement	**19.35**	**Stage 4**
5 October 2018	**Renal artery injection**	**-**	**-**
26 November 2018	eGFR Measurement	**18.01**	**Stage 4**

Proof of Concept

- **Case_CKD_Stage5**

  Male, 46-year-old

  - Intravenous Infusion
  - Elevated eGFR levels after treatments

Date	Event	Estimate eGFR	Stage of CKD
12 January 2018	eGFR Measurement	**9.03**	**Stage 5**
19 January 2018	**Intravenous Infusion**	**-**	**-**
27 February2018	eGFR Measurement	**8.80**	**Stage 5**
18 May 2018	**Intravenous Infusion**	**-**	**-**
21 May 2018	eGFR Measurement	**13.70**	**Stage 5**
22 June 2018	eGFR Measuremen	**12.40**	**Stage 5**
17 September 2018	eGFR Measurement	**15.60**	**Stage 4**
26 October 2018	**Intravenous Infusion**	**-**	**-**
28 November 2019	eGFR Measuremen	**17.46**	**Stage 4**

Proof of Concept

- **Case_CKD_Stage5**

  Female, 60-year-old

  - 1x Renal Artery Injection
    2x Intravenous Infusion
  - Elevated eGFR levels after treatments

Date	Event	Estimate eGFR	Stage of CKD
18 June 2018	eGFR Measurement	**6.49**	**Stage 5**
6 July 2018	**Intravenous Infusion**	**-**	**-**
17 August 2018	eGFR Measurement	**7.08**	**Stage 5**
7 September 2018	**Intravenous Infusion**	**-**	
16 October 2018	eGFR Measurement	**6.64**	**Stage 5**
11 January 2019	**Renal artery injection**	**-**	**-**
13 January 2019	eGFR Measurement	**36.40**	**Stage 3**

Proof of Concept

- **Post-cancer recovery**

Male, 36-year-old • Intravenous Infusion

- Increased in lymphocyte counts
- Decreased myelocyte counts

Date	Results				
2013 Apr 10		Platelet Count		365 x10⁹/L	(150-450)
	++	White Cell Count		23.7 x10⁹/L	(4.0-11.0)
	+++	Neutrophils	85 %	20.1 x10⁹/L	(2.0-7.5)
	-	Lymphocytes	3 %	0.7 x10⁹/L	(1.1-4.0)
	++	Monocytes	8 %	1.9 x10⁹/L	(0.2-1.0)
	+	Eosinophils	2 %	0.47 x10⁹/L	(0.04-0.40)
	+	Basophils	1 %	0.24 x10⁹/L	(< 0.21)
	+	Myelocytes	1 %	0.24 x10⁹/L	
2013 Apr 13	AMPC intervention				
2013 Apr 19		Platelet Count		366 x10⁹/L	(150-450)
	++	White Cell Count		26.3 x10⁹/L	(4.0-11.0)
	++	Neutrophils	79 %	20.8 x10⁹/L	(2.0-7.5)
		Lymphocytes	10 %	2.6 x10⁹/L	(1.1-4.0)
	++	Monocytes	9 %	2.4 x10⁹/L	(0.2-1.0)
		Eosinophils	1 %	0.26 x10⁹/L	(0.04-0.40)
	-	Basophils	1 %	0.26 x10⁹/L	(< 0.21)

Proof of Concept

- **Acute myeloid leukaemia**

  Female, 57-year-old • AMPCs reinfusion on 11/11/2013

11/10/2013

RBC	紅血球	397	↓	萬/cumm	男460～620女400～60
HEMATOCRIT	血球容積比	38.1		%	男40～54,女38～47
M.C.V.	平均紅血球值	95.8		fL	83～101
HEMOGLOBIN	血色素	12.9		gm%	男14～18,女12～16
WBC	白血球	3500	↓	/cumm	5000～10000
PLATELET	血小板	16.1		萬/cumm	15～45萬
WBC. D.C	白血球分類:				
NEUTRO-SEG.	中性球	43	↓	%	55～75
LYMPHOCYTE	淋巴球	42	↑	%	25～35
MONOCYTE	單核球	3		%	0～6
EOSINOPHIL	嗜酸性白血球	1		%	0～4
Atypical LYM.	非典型淋巴球	11		%	0～20
M.C.H.	平均血色素量	32.4		pg	27～34
M.C.H.C.	平均色素濃度	33.8		%	30～36
Prothrombin Time		9.9(INR0.93)		Sec.	9.6～12.0
aP.T.T		25.9		Sec.	24～36.8

17/02/2014

Test items		Test value	Unit	H / L Reference Value
白血球	WBC	6.4	1000/Ul	M3.9~10.6 F3.5-10
紅血球	RBC	4.18	MILON/Ul	M4.5-5.9 F4.0-5.5
血色素	HGB	13.3	g/dL	M13.5-17.5 F12-17
血中紅血球百分比	HCT	38.3	%	M41-53 F36-46
紅血球平均容積	MCV	91.6	UMM	80-100
紅血球色素	MCH	31.9	pg/Cell	26-34
紅血球色素濃度	MCHC	34.8	g/dL	31-37
紅血球分佈變異數	RDW	12.8	%	11.5-14.5
血小板	PLT	201	1000/uL	150-400
SEGMENT	SEGMENT	57.0	%	42-74
嗜伊紅性球	EOS	2.0	%	0-5
MONO	MONO	8.0	%	0-12
淋巴球	LYM	33.0	%	20-56

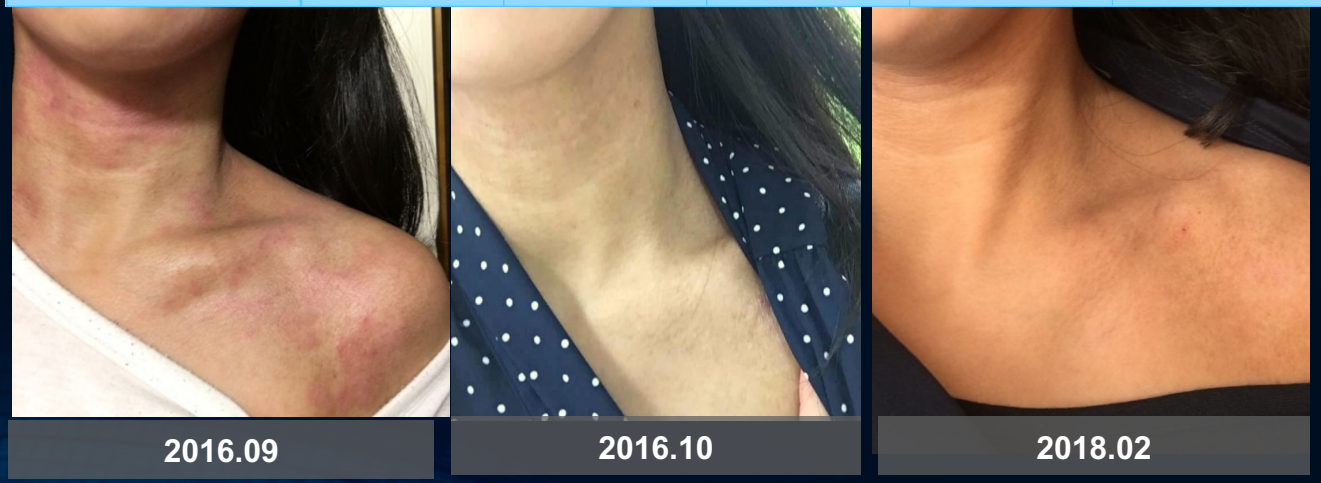
Proof of Concept

- **Atopic dermatitis**

  Female, 23-year-old

  - Allergy immune protein IgE and eosinophils lowered
  - Lowered frequency of episodes
  - Improved skin healing

Test Items	20160729	20161208	20170724	20180504	Ref. value
Blood Count					
WBC	6690	6130	6970	7060	4000-11000 cumm
Neutrophils	54	52.1	54.4	64.0	40-75 %
Lymphocytes	29.6	27.4	27.5	21.2	20-45 %
Monocytes	5.5	5.9	7.6	7.6	2-10 %
Eosinophils	10.3	13.5	8.9	6.2	0-6 %
Basophils	0.6	1.1	1.6	1.0	0-1 %
Other					
IgE	1669.8	1039	1067	718	Ad:<250 kU/L

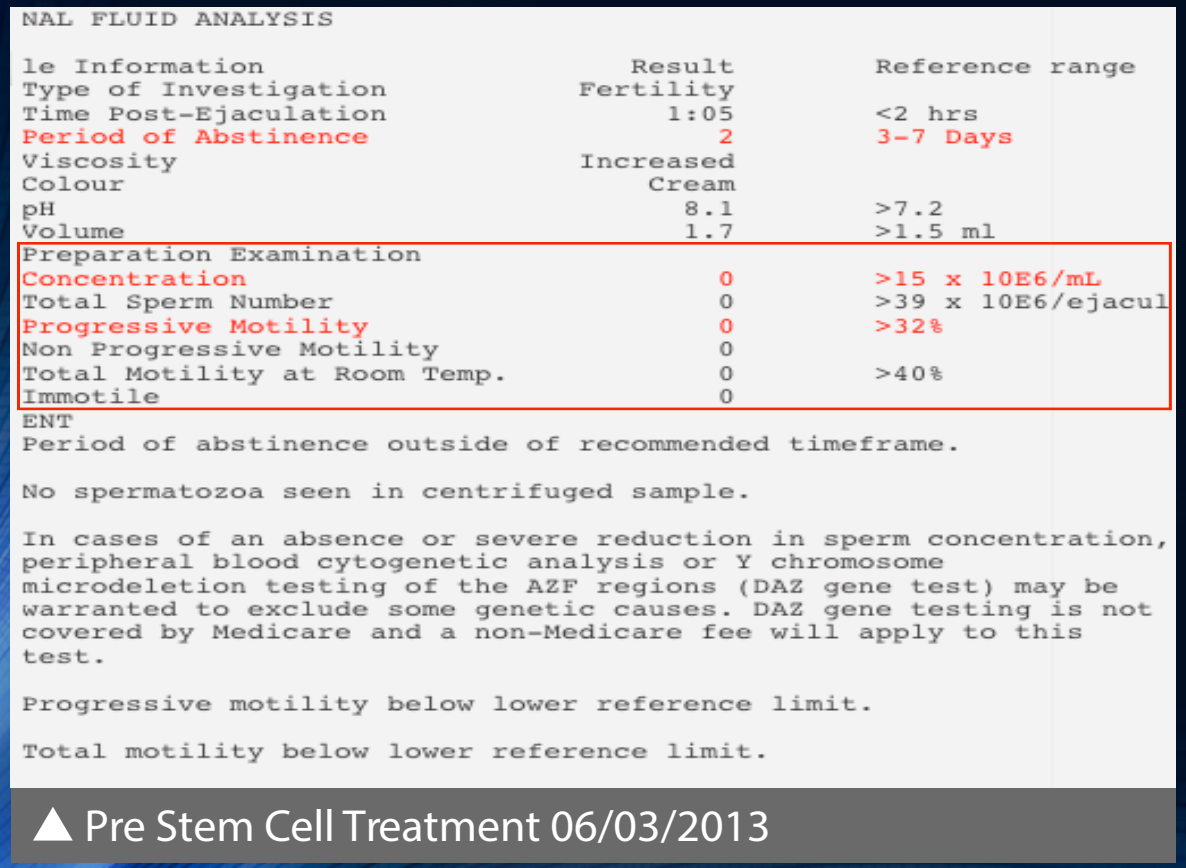


Proof of Concept

- **Infertility**

  Male, 45-year-old

  - AMPCs reinfusion on 01/06/2013
  - Slight improved in sperm concentration and motility.



NAL FLUID ANALYSIS

le Information	Result	Reference range
Type of Investigation	Fertility	
Time Post-Ejaculation	1:05	<2 hrs
Period of Abstinence	2	3-7 Days
Viscosity	Increased	
Colour	Cream	
pH	8.1	>7.2
Volume	1.7	>1.5 ml
Preparation Examination		
Concentration	0	>15 x 10E6/mL
Total Sperm Number	0	>39 x 10E6/ejacul
Progressive Motility	0	>32%
Non Progressive Motility	0	
Total Motility at Room Temp.	0	>40%
Immotile	0	

ENT
Period of abstinence outside of recommended timeframe.

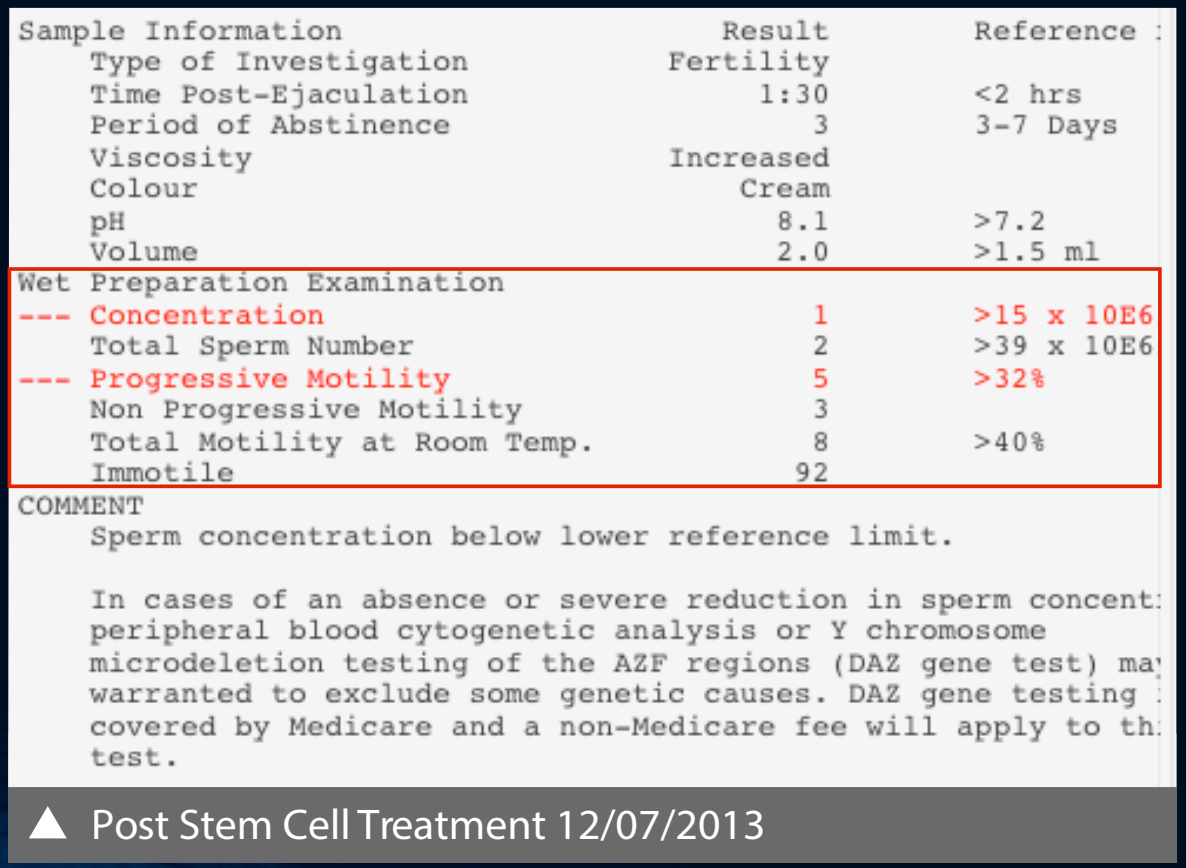
No spermatozoa seen in centrifuged sample.

In cases of an absence or severe reduction in sperm concentration, peripheral blood cytogenetic analysis or Y chromosome microdeletion testing of the AZF regions (DAZ gene test) may be warranted to exclude some genetic causes. DAZ gene testing is not covered by Medicare and a non-Medicare fee will apply to this test.

Progressive motility below lower reference limit.

Total motility below lower reference limit.

▲ Pre Stem Cell Treatment 06/03/2013



Sample Information	Result	Reference :
Type of Investigation	Fertility	
Time Post-Ejaculation	1:30	<2 hrs
Period of Abstinence	3	3-7 Days
Viscosity	Increased	
Colour	Cream	
pH	8.1	>7.2
Volume	2.0	>1.5 ml
Wet Preparation Examination		
--- Concentration	1	>15 x 10E6
Total Sperm Number	2	>39 x 10E6
--- Progressive Motility	5	>32%
Non Progressive Motility	3	
Total Motility at Room Temp.	8	>40%
Immotile	92	

COMMENT
Sperm concentration below lower reference limit.

In cases of an absence or severe reduction in sperm concent: peripheral blood cytogenetic analysis or Y chromosome microdeletion testing of the AZF regions (DAZ gene test) ma warranted to exclude some genetic causes. DAZ gene testing covered by Medicare and a non-Medicare fee will apply to th test.

▲ Post Stem Cell Treatment 12/07/2013

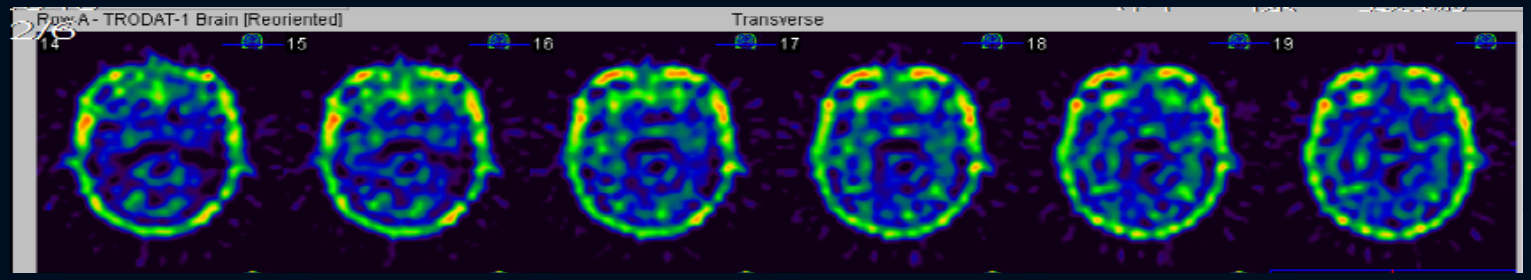
Proof of Concept

- **Parkinson's Disease**

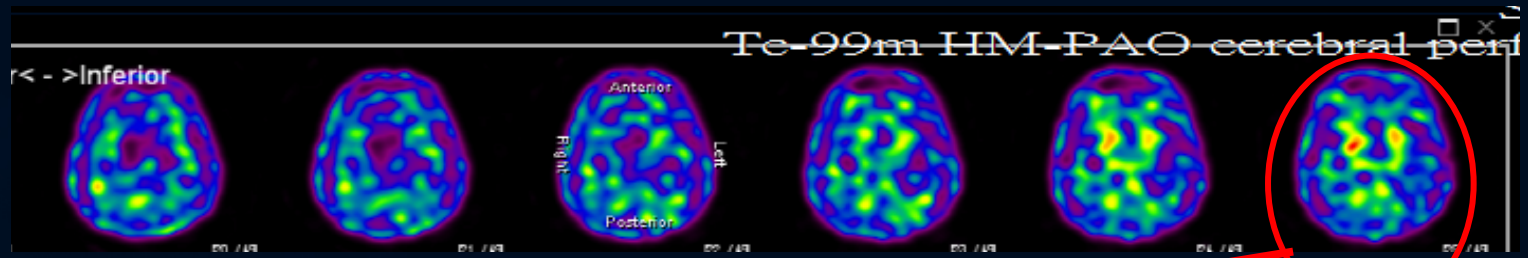
  Male,56-year-old

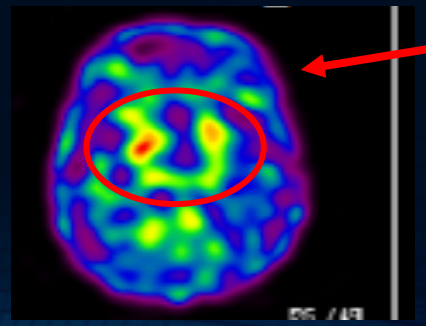
  - AMPCs reinfusion
  - Slight improvement in dopamine levels.
  - Self-reported improved mobility and quality of life.

2013/1/11 **Before**



2014/5/17 **After**





Tc-99m-Trodat-1 Dopamine Transporter B rain SPECT imaging

Proof of Concept

- **Multiple Sclerosis**

  Female,48-year-old

  - Diagnosed with multiple sclerosis for 5 years.
  - Late stage of disease
  - Full body paralysis.
  - Improvements in mobility after two treatments.
  - Score on the expanded disability status scale (EDSS) improved from 9 to 7.5.

Proof of Concept

- **Post-stroke recovery**

  Male,81-year-old

  - Semi-paralysed for up to 1 year after incidence of stroke
  - Improved mobility in lower body after treatment