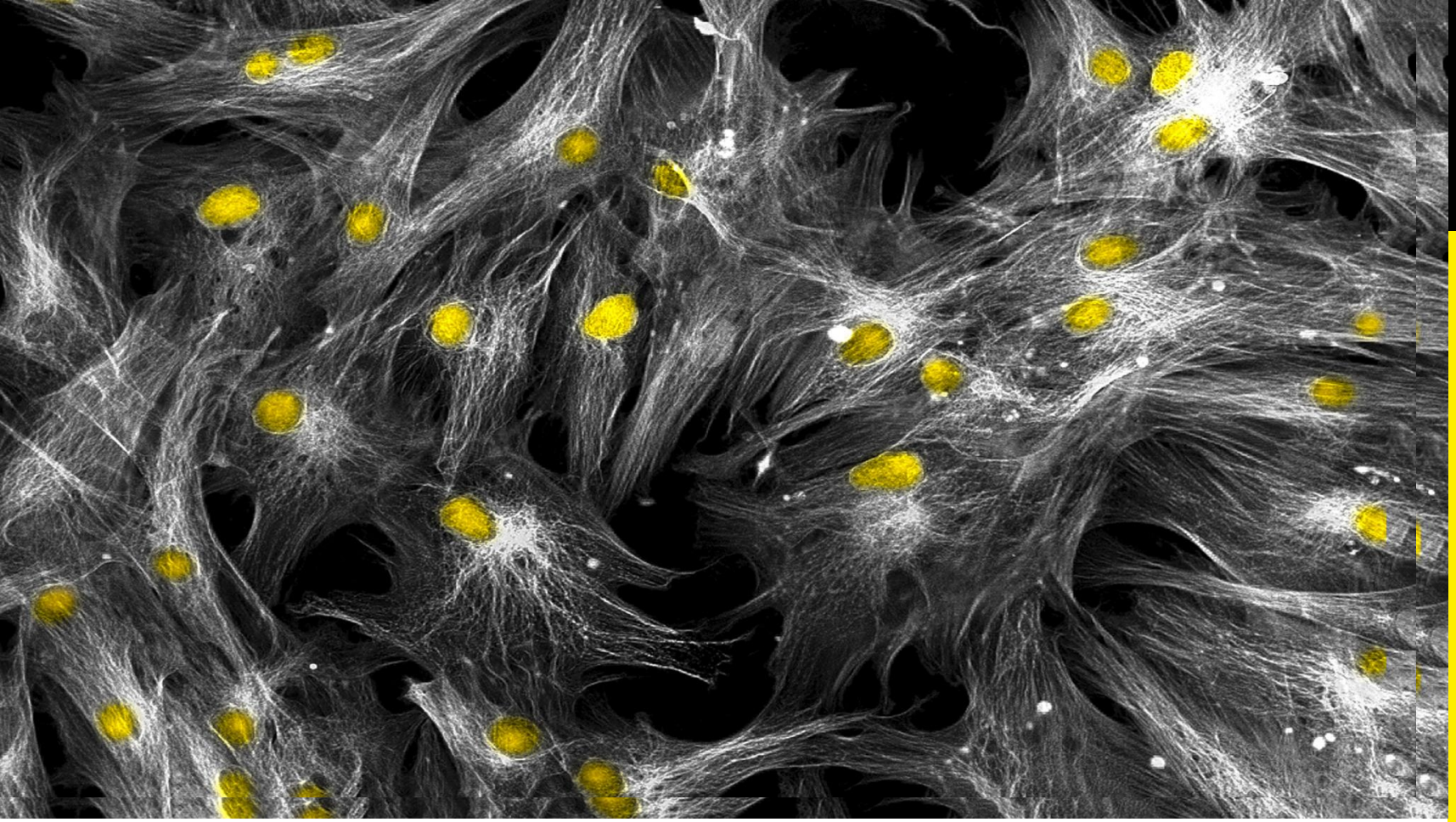

Simplifying Progress

Regulatory Considerations when Choosing Stem Cell Media

October 27, 2020

SARTORIUS

Content

Considerations for Media and Manufacturer

Manufacturer Quality System Key Points

NutriStem® Family

Cell Therapy Collaborations

cGMP Manufacturing Facility

21 CFR Part 820 Quality System Regulation (QSR)

edqm

CERTIFIED IQNet MANAGEMENT SYSTEM

CERTIFIED MEDICAL DEVICES QMS SI ISO 13485: 2003 THE STANDARDS INSTITUTION OF ISRAEL

ISO 9001:2015

ISO 13485:2016

Media and Manufacturer Features

CONSIDERATIONS FOR REGENERATIVE MEDICINE

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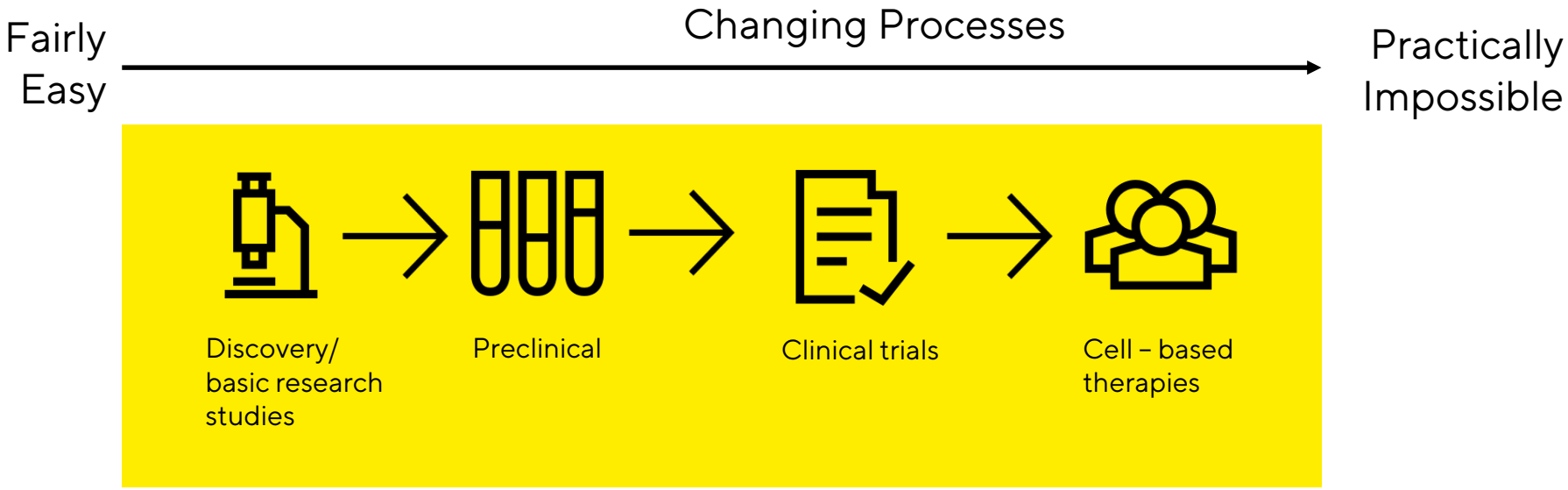
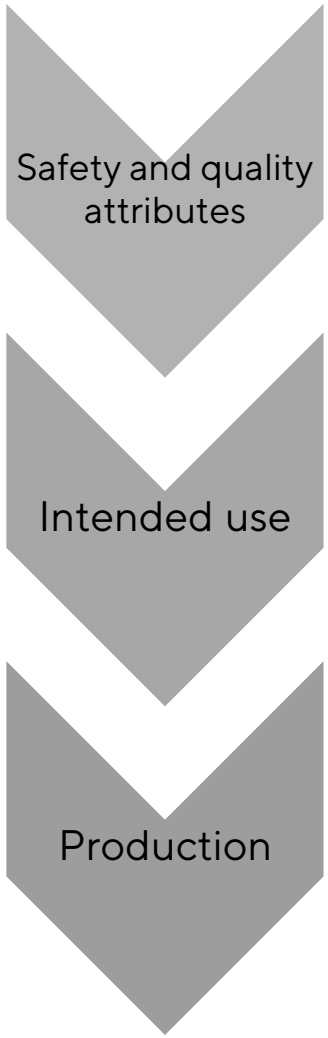
Begin troubleshooting early

- Establishing optimal cell culture systems
- Suitable reagent supplier selection
- Eliminate downstream laborious and time-consuming modifications



Media Features



Safety and quality attributes of media components:

Biological components- human/animal or recombinant, undefined materials (serum, lysates), Biological activity, purity/impurity profile, risk of adventitious agents (bacteria, viruses, etc.) , stability

Availability of information: Product documentation to support future IND submission (DMF/MF)

Ease of use: Protocol, media components (single bottle, supplements)

- Research use only (RUO)/IVD/MD/excipient/further manufacturing
- Ability to scale from RUO to cGMP: Is it transferable to cGMP?
- Compliant with regulation guidelines: cGMP, ancillary material
- Approved documents: Drug Master File availability, certifications, product dossier

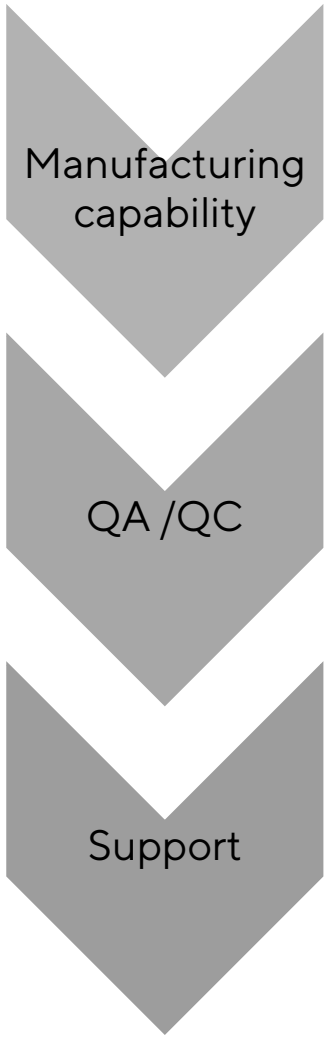
Material: **Proof of safety and traceability**

Consistency and reproducibility: lot-to-lot variation

Shelf-life and storage options

→ Confidence the supplier can support you to your goal

Manufacturer Features



- **Guarantee of supply chain:** from manufacturing to delivery
- **Consistency and reproducibility:** minimal lot-to-lot variation
- **Capacity to scale-up product:** larger lots, lot reservations, customized products
- **Production suite certifications**: Clean room levels, sterile filtration, aseptic filling

- **Quality management system:** Accreditations? Certifications?
- **Possibility for on-site audits**

- **Technical Support and overall support by professional teams (logistics, R&D, production, sales), communication strategy**
- **Available QA team:** able to collaborate with QPs and regulatory bodies
- **Available product documentation**

→ Confidence the supplier can support you to your goal

Meeting regulations and scientific quality

MANUFACTURER QUALITY SYSTEM

Guidelines to consider

- **Which Quality Standards are implemented:**
  - Implementation of QMS ISO certifications?
  - cGMP manufacturing?

BI Quality Management System

BI manufacturing operates under an established **Risk Management** based Quality Management System (QMS)

QMS comply with the international standards for Quality Management Systems as defined by ISO 13485:2016 and routinely audited by an accredited external body.

Committed to the current Good Manufacturing Practices (cGMP) comprising local and international guidelines adopted by the FDA and EMA.

Facility Certified ISO 9001:2015 and ISO 13485:2016. Our controlled-environment clean rooms are graded from ISO 8 up to ISO 5


CERTIFICATE

This is to certify that the Quality Management System of

Biological Industries Israel Beit Haemek Ltd.

Postal Service Ashrat , Beit Ha'emek , Israel

Has been audited and registered by SII-QCD as conforming to the requirements of :

ISO 9001:2015

This Certificate is Applicable to

Design, development, manufacturing, marketing and sale of animal and human culture products whithin biological and clinical research. Manufacturing and scaling-up custom media and contract manufacturing media.

Certificate No.: 86069 Initial Certification Date: 01/02/1995

Certificate Issue Date: 20/04/2017 Certification Expiry Date: 28/11/2018

Pinhas Shahar Director General

Eli Cohen-Kagan Director, Quality & Certification Division

Our Vision: To Enhance Both Global Competitiveness of our Services, with our Uncompromised Quality and Integrity

CERTIFICATE

This is to certify that the Quality Management System of

Biological Industries Israel Beit Haemek Ltd.

Postal Service Ashrat , Beit Ha'emek , Israel

Has been audited and registered by SII-QCD as conforming to the requirements of

ISO 13485:2003

This Certificate is Applicable to

1. Design, manufacture and distribution of: a.Research products for cell culture, cell biology and molecular biology. b. Medical devices including in-vitro diagnostic products. c.Cell culture reagents for industrial bio- processing. 2. Contract manufacturing of research products and aseptic sterile medical devices including in vitro diagnostic products.

Certificate No.: 80940 Initial Certification Date: 16/04/2006

Certificate Issue Date: 02/02/2016 Certification Expiry Date: 28/11/2018

Eli Stein Director General

Eli Cohen-Kagan Director, Quality & Certification Division

Our Vision: To Enhance Both Global Competitiveness of our Services, with our Uncompromised Quality and Integrity

Our products are divided into three groups:

Research Use Only

In Vitro Diagnostics Medical Devices

Ancillary materials for cell-based therapy

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What are Ancillary Materials?

International Regulations – Products used in the processing of the cell product

Raw materials for cell-based therapies:

Cells, ancillary materials, excipients, packaging

US

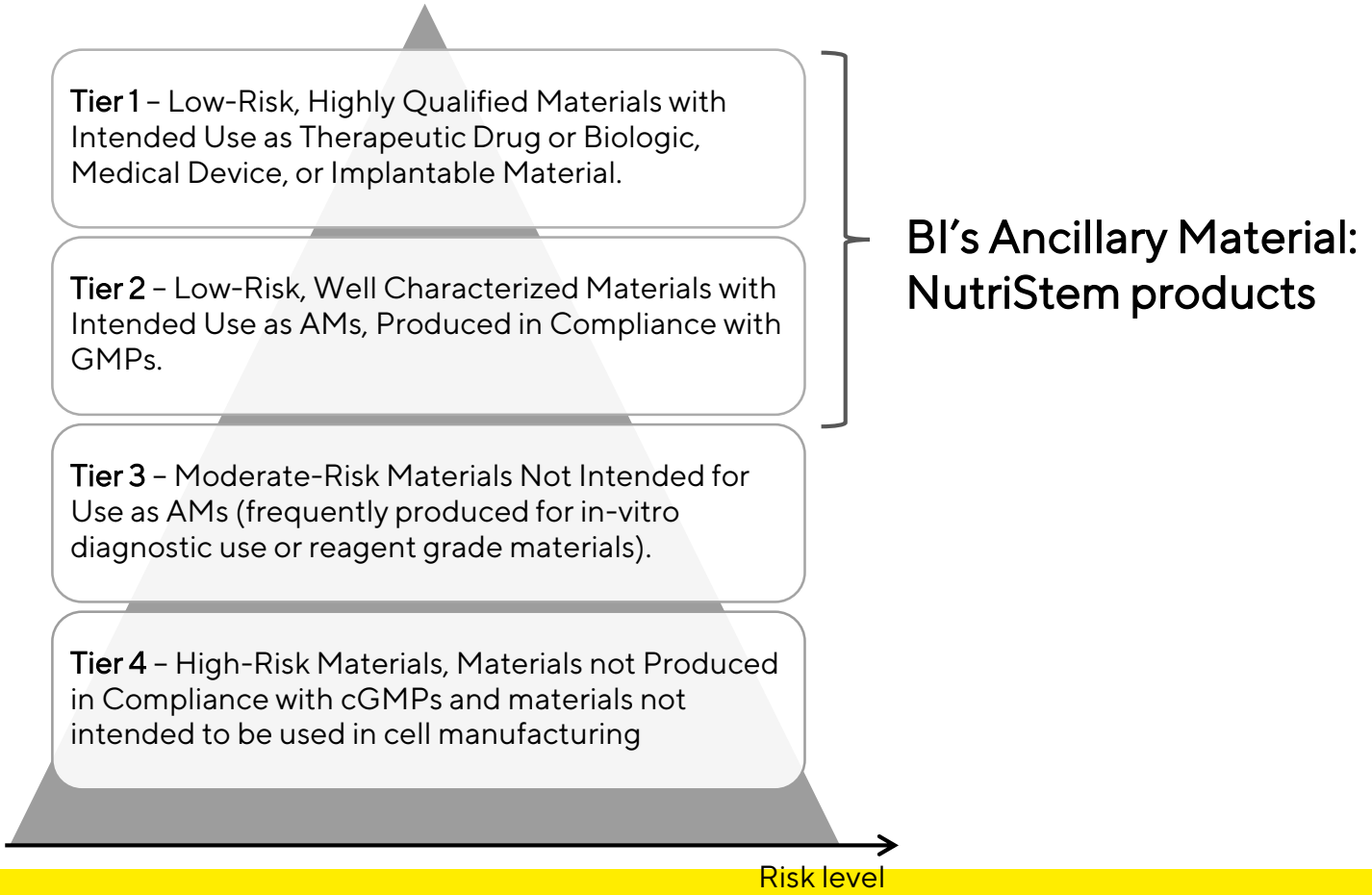
USP <1403> Ancillary Materials for Cell-,Gene-, and Tissue-Engineered Products" defines: Ancillary materials are synonymous with " processing materials that were defined in 21 CFR 1271.

EU

Regarding raw materials to be used in the process of cell-based product manufacturing the European mainly refers to the risks of biologicals derived materials **under Ph. EUR. 5-2-12, and media is considered excipient**

The HCT*/ATMP* manufacturer needs to address the ancillary materials with a risk-based approach, and for this purpose the USP defines 4 risks levels:

*HCT: Human Cells, Tissues
*ATMP: Advanced Therapy Medicinal Products

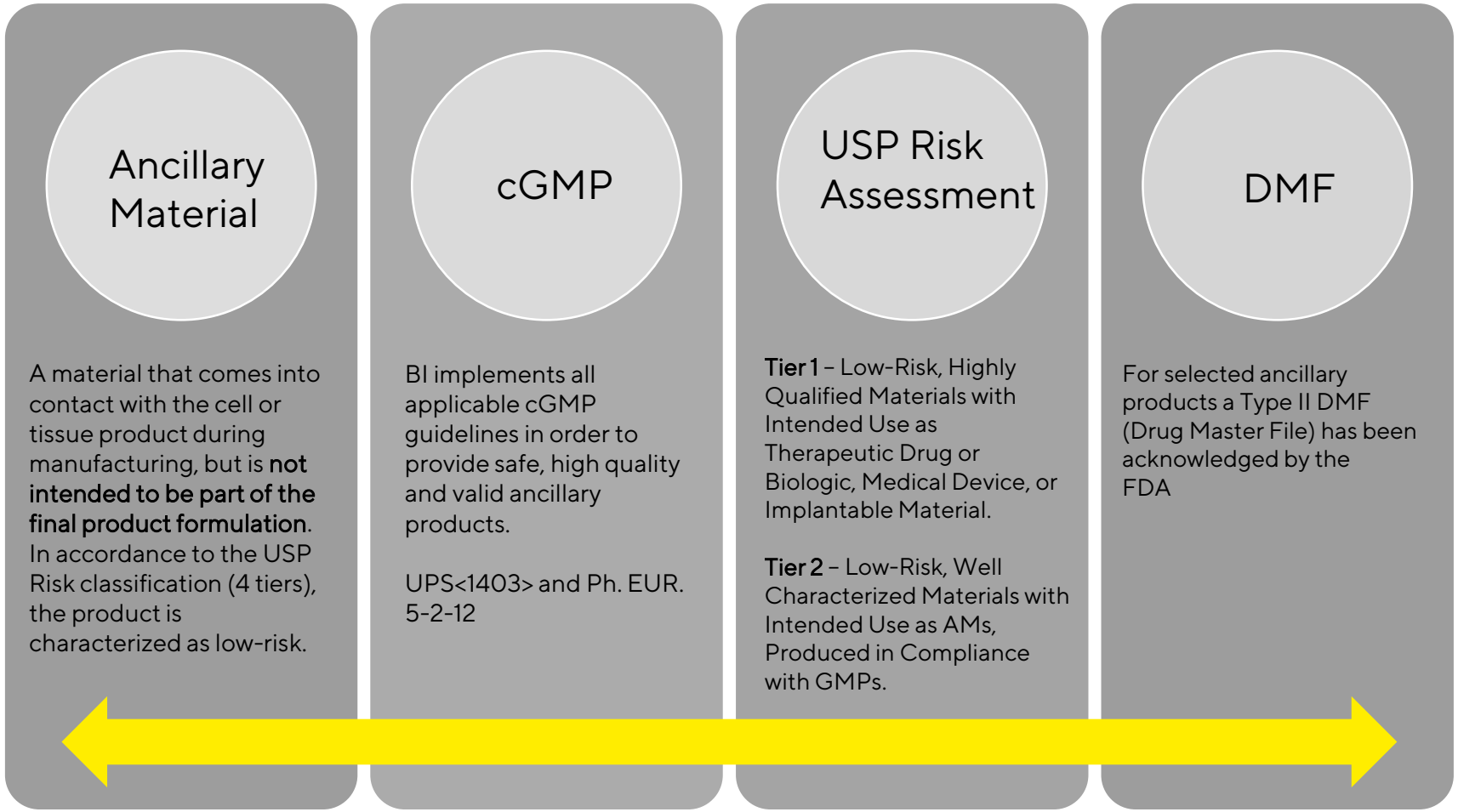


BI's Ancillary Material: NutriStem products

Ancilliary Materials at BI

- Products intended for cell based therapy classified as **Ancillary Materials /Excipients**

- **Nutristem products for hPSC and hMSC**
- NutriFreez® D10 Cryopreservation Medium



NutriStem® Family

CELL THERAPY REAGENTS AT SARTORIUS ADVANCED THERAPIES

Stem cell research and regenerative medicine applications require high quality and reliable media and auxiliary reagents.

- Defined
- Serum-free
- Xeno-free, without any non-human ingredients

  - Eliminate lot-to-lot variability (serum/ PL- batch/source)
  - Eliminate contagious health risk: viral agents, mycoplasma and prions
  - Ensure reliable performance

Serum-free, xeno-free system for human **mesenchymal stromal cells** of multiple sources

MSC NUTRISTEM® XF

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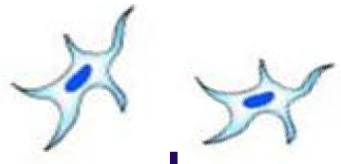
Standard MSC workflow:

MSC NutriStem

SOURCE
Bone Marrow
Adipose Tissue
Umbilical Cord
Amniotic Fluid
Placenta

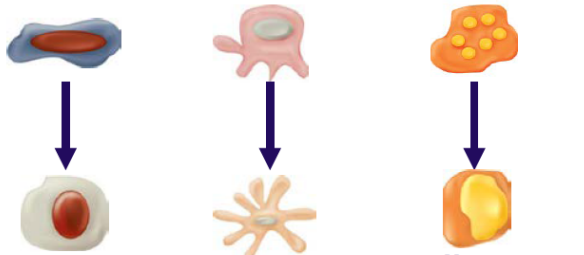
→ **ISOLATION** → **EXPANSION** →

APPLICATIONS
Basic Biology
Cancer Research
Drug Discovery
Cell Therapy
Tissue engineering



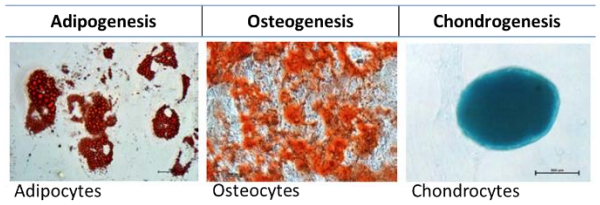
TRANS DIFFERENTIATION
Neural
Hepatic
Endothelial

MESODERM DIFFERENTIATION



Chondrocytes (cartilage) | Osteocytes (bone) | Adipocytes (fat)

MSCgo™ Differentiation Medium

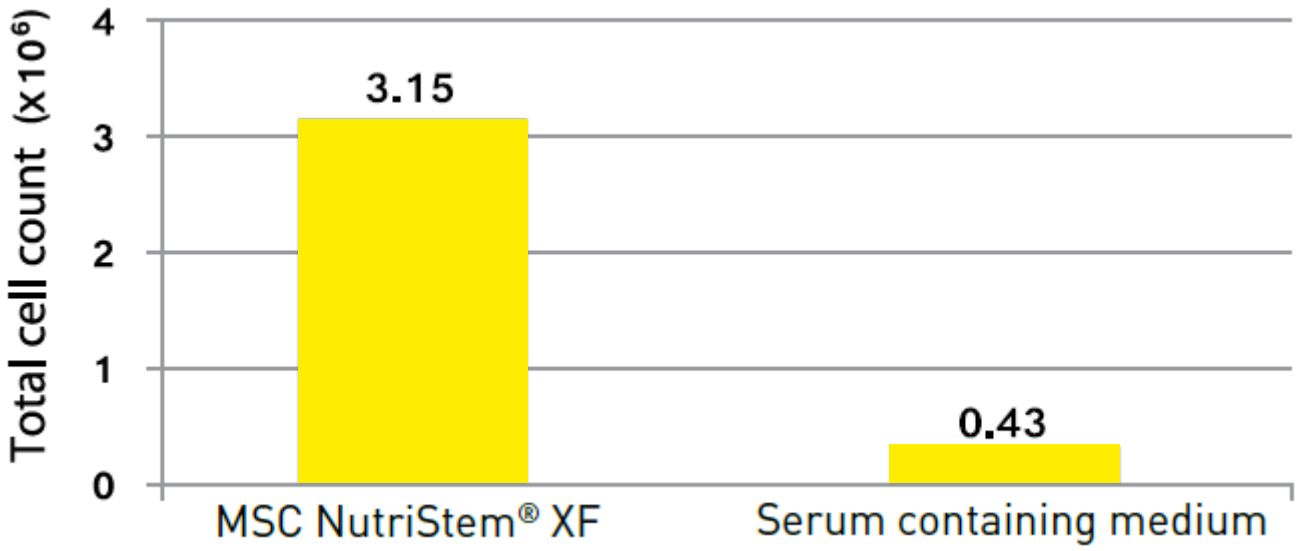


MSC isolation from multiple tissue sources (BM, AT, PL, WJ, DP)

MSC NUTRISTEM® XF

Evaluation of hMSC-BM isolation using MSC NutriStem® XF vs. FBS

hMSC were isolated from bone marrow under SF, XF culture conditions using MSC NutriStem® XF on pre-coated plates with MSC Attachment Solution,**w/o supplementation of human AB serum** and in FBS-containing medium.

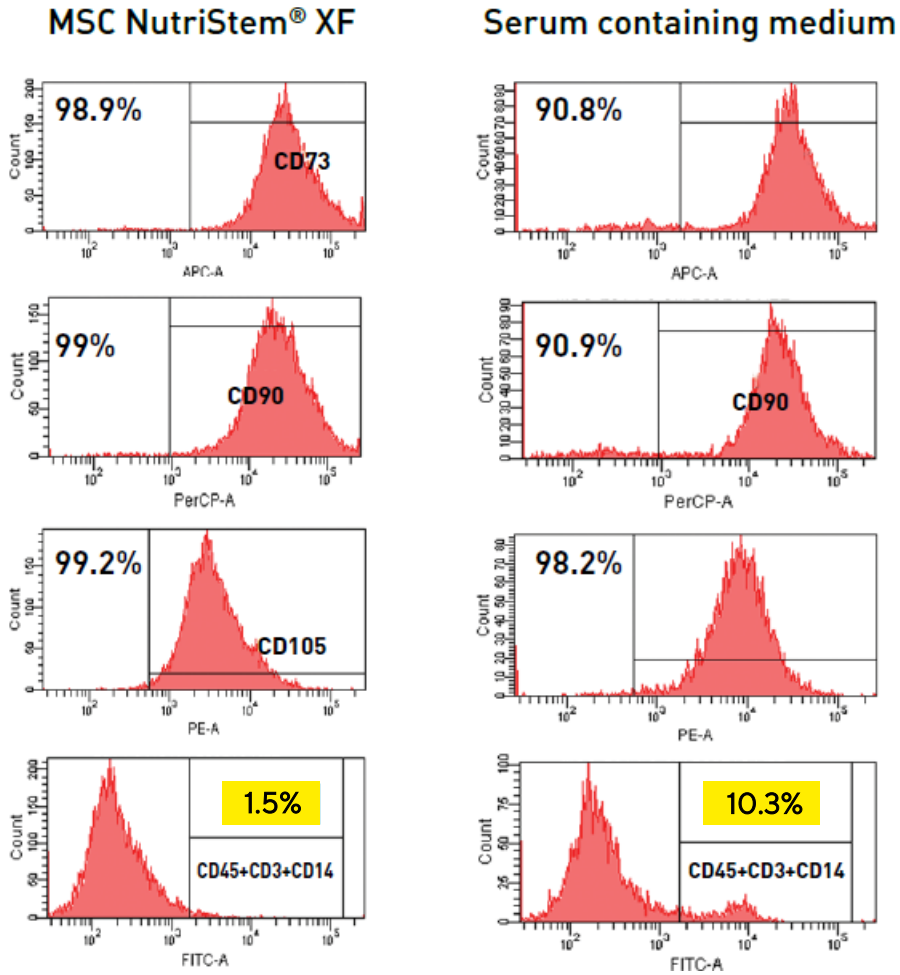


- ✓ Higher number of viable cells isolated

- Number of viable cells, measured by trypan blue exclusion assay
- 11days post initial isolation (P0).

Immunophenotyping using FACS analysis (P0)

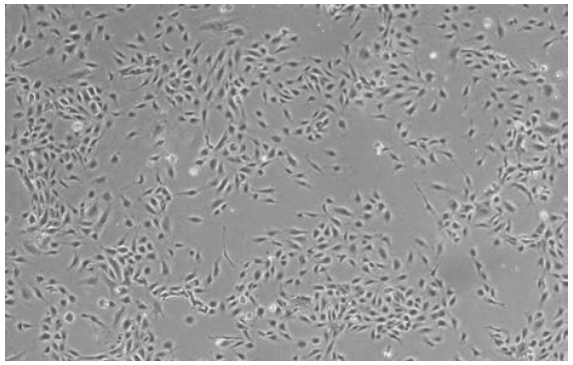
hMSC-BM



✓ High population purity, low hematopoietic contamination

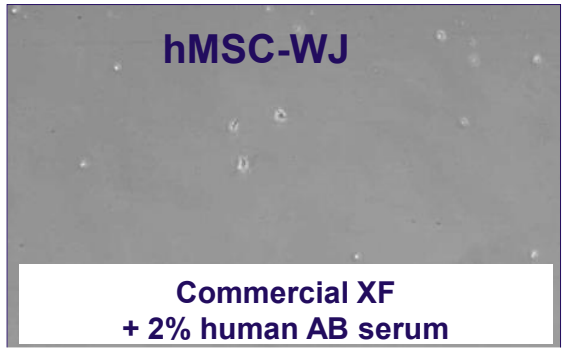
Successful isolation using MSC NutriStem®

hMSC-WJ

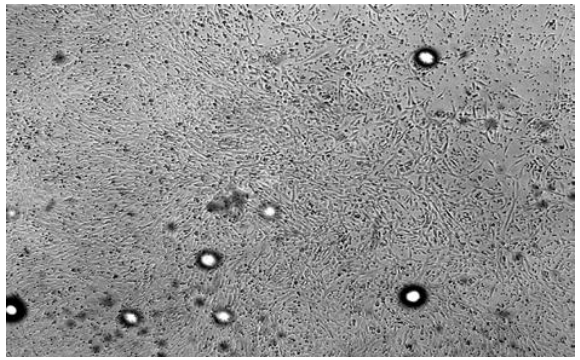


6 days post initial isolation (P0)

- **+ supplementation of 2% human AB serum**
- **on pre-coated plates** (MSC Attachment Solution)

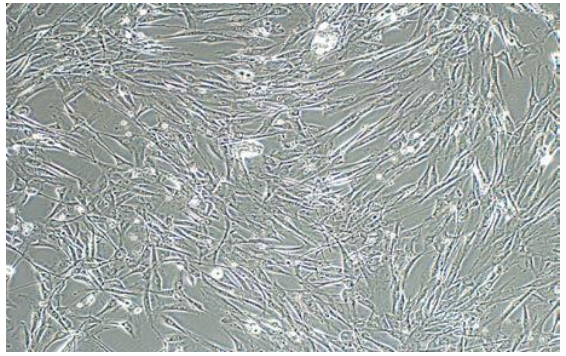


hMSC-AT



4 days post initial isolation (P0)

hMSC-PL



11 days post initial isolation (P0)

- **w/o human AB serum**
- **on pre-coated plates**
  (MSC Attachment Solution)

Isolation of hMSC from various tissues is achievable under XF (SF?) culture system using MSC NutriStem® XF

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MSC expansion and characterization from multiple tissue sources (BM, AT, PL, WJ, DP)

MSC NUTRISTEM® XF

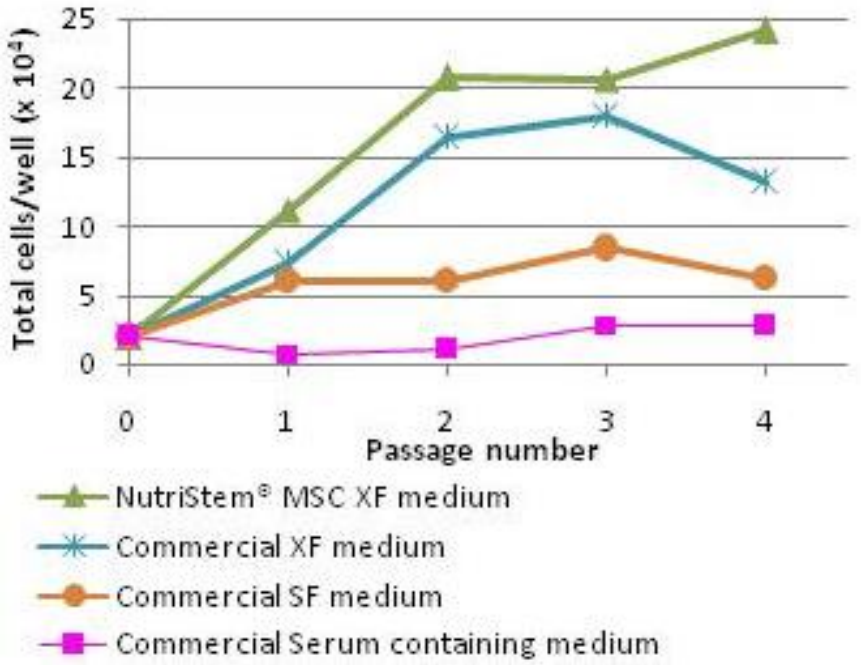
Expansion with MSC NutriStem® XF

- The culture plates were pre-coated with
- MSC Attachment Solution

- Seeding density: $2x10^4$ cells/well (12 well-plate)

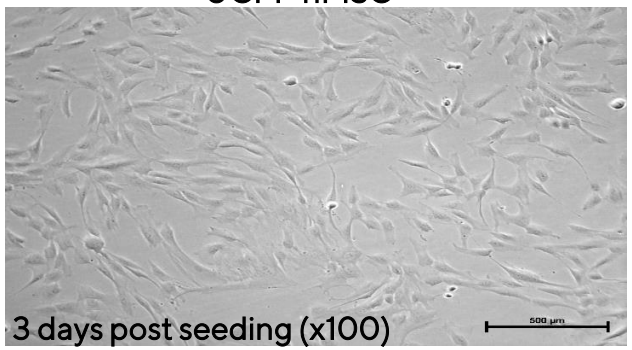
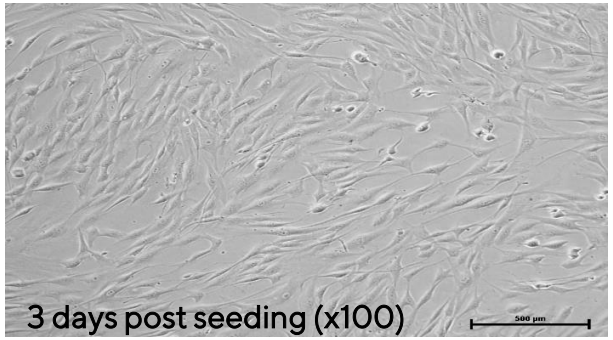
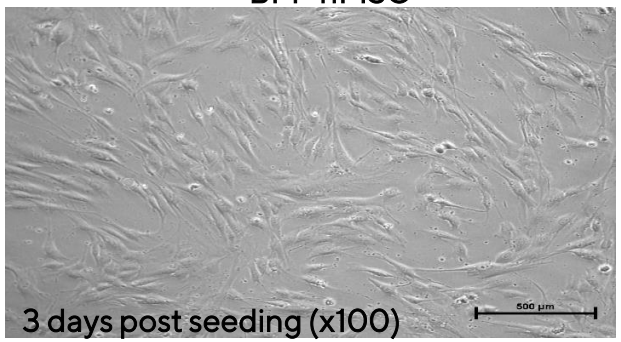
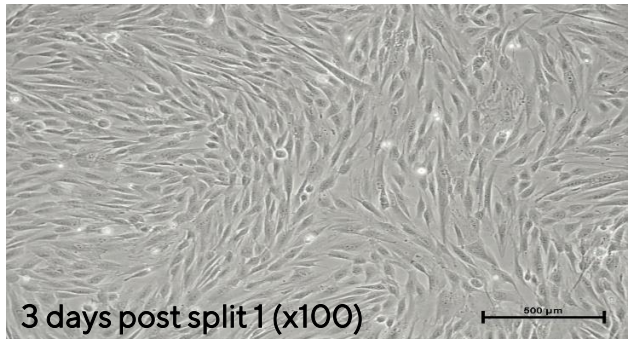
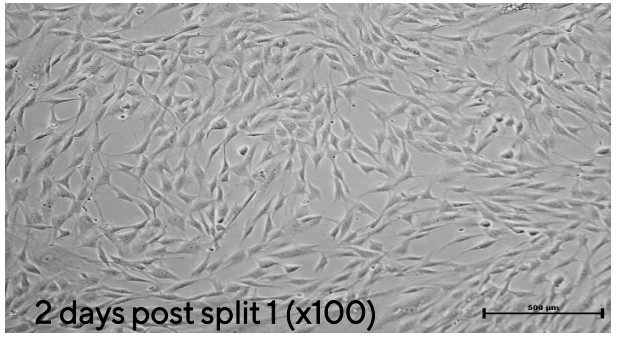
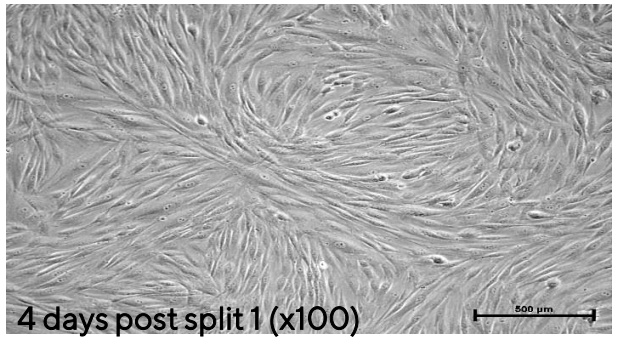
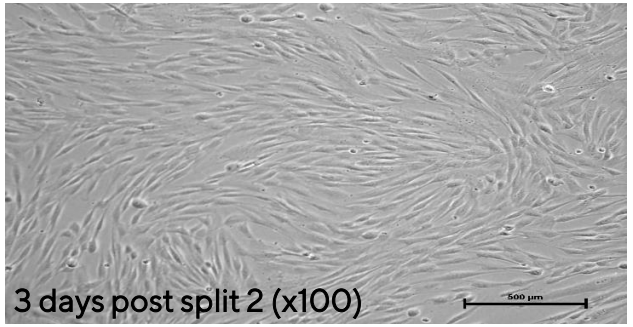
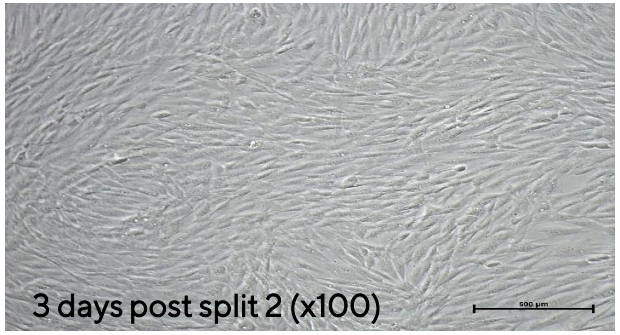
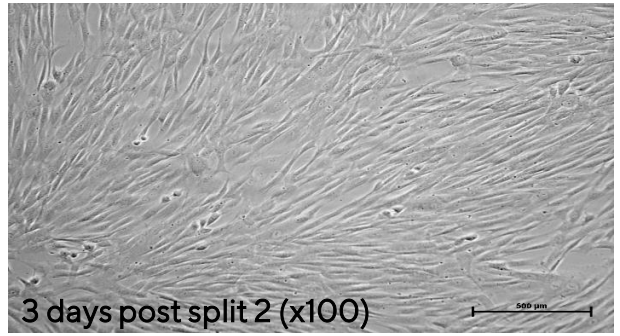
- Passage frequency of 3 days.

Expansion of hMSC-AT in various serum-free media



- hMSC-AT cultured in MSC NutriStem® XF exhibit **superior proliferation** in comparison to serum-containing medium and commercially available SF and XF media.

MSC NutriStem® XF promotes proliferation of hMSC from a variety of tissues

	UCM -hMSC	AT -hMSC	BM -hMSC
Passage 1			
Passage 2			
Passage 3			

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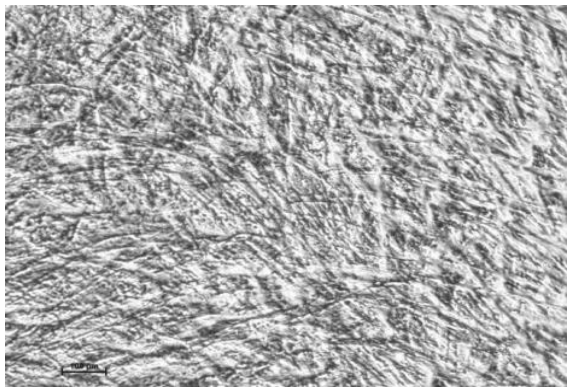
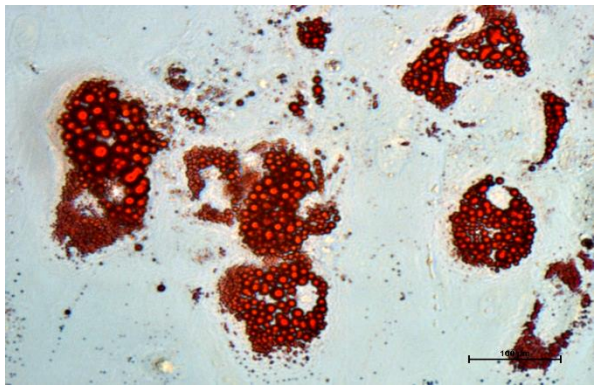
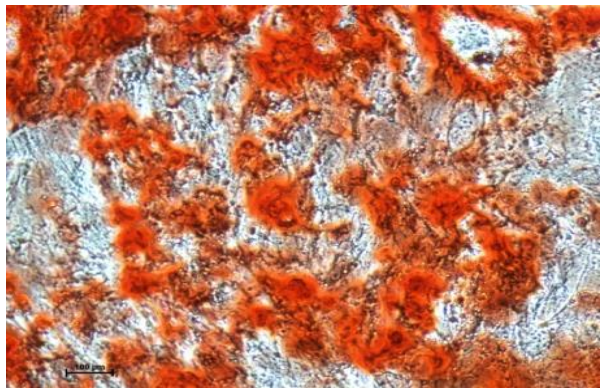
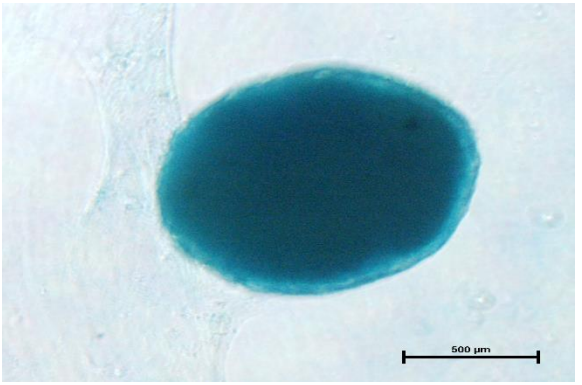
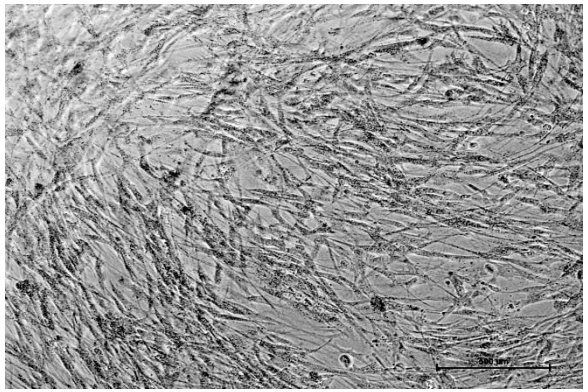
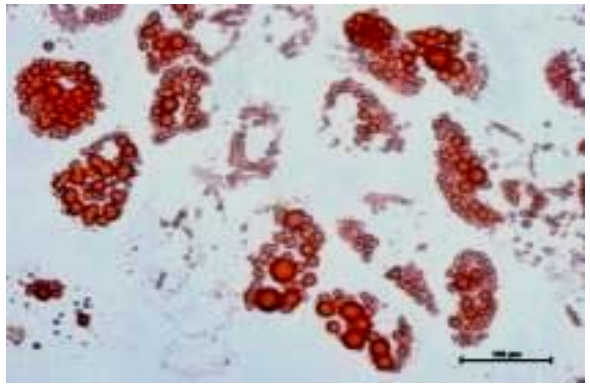
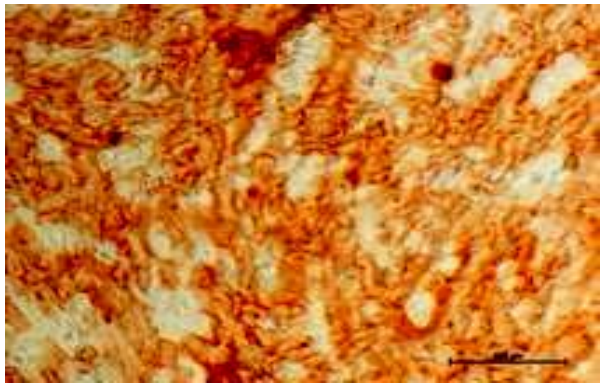
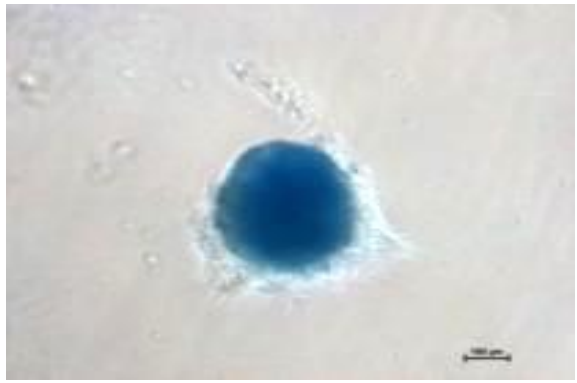
hMSC multi-lineage differentiation potential

hMSC -BM

hMSC -AT

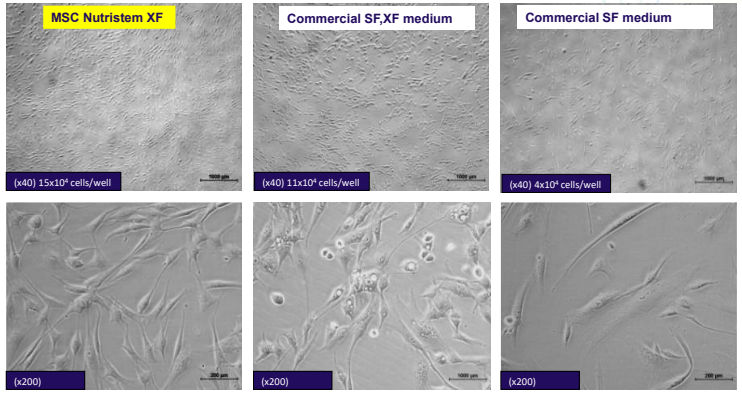





Control | Adipocytes - Oil red O | Osteoblasts - Alizarin red | Chondrocytes - Alcian blue

- ✓ hMSC cultured (3-5P) in MSC NutriStem® XF
  - Maintained their multi - lineage differentiation potential
  - With no background differentiation

Performance validation

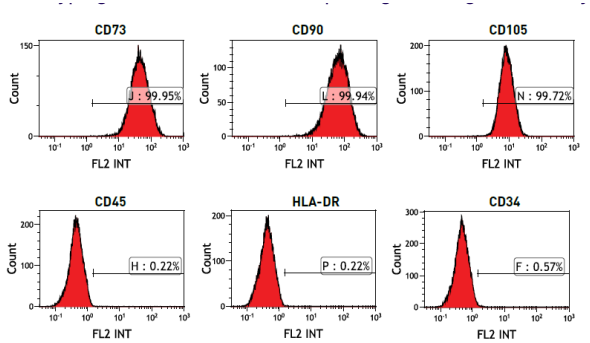
MSC NutriStem®XF: normal morphology



hMSC-AT expansion in MSC Nutristem® XF & commercially SF available media

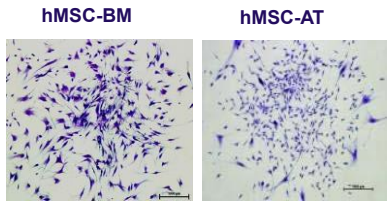
Profile markers

Immunophenotyping results of hMSC-AT at passage 2 using FACS analysis



hMSC self-renewal potential

Colony Forming Unit-Fibroblast (CFU-F) assay was used to evaluate hMSC self renewal potential



Representative colonies of hMSC-BM and hMSC-AT.
CFU-F assay was performed after 3-5 passages in MSC NutriStem® XF.
Colonies were stained with 0.5% Crystal violet.

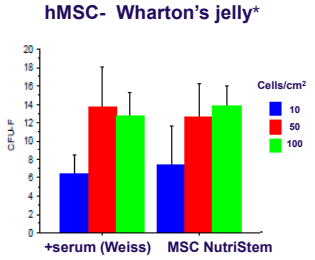


*Image kindly provided by Prof. Mark L Weiss.

Karyotypic stability

G-banding karyotype analysis of hMSC-BM and hMSC-AT cultured for 4-6 passages in MSC NutriStem®XF

✓ Achieved Performance

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MSC NutriStem® XF

Serum-free (SF), xeno-free (XF) medium specially developed for the isolation and expansion of hMSC

Provides

Defined culture system

Multiple sources: Adipose Tissue, Bone Marrow, Placenta, Wharton's Jelly, Dental Pulp

---

Features

- Isolation: higher cell count, low hematopoietic contamination
- Expansion: High proliferation, long-term culture with stable karyotype. Maintenance of MSC features.
- Suitable for clinical applications, **DMF filed with FDA**. cGMP manufacturing, for clinical trial, cell banks
- Customized packaging available

Used with MSC Attachment Solution

Serum-free, xeno-free culture system for derivation and expansion of **hESC and iPSC**

NUTRISTEM® HPSC XF

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Classical morphology of hPSC

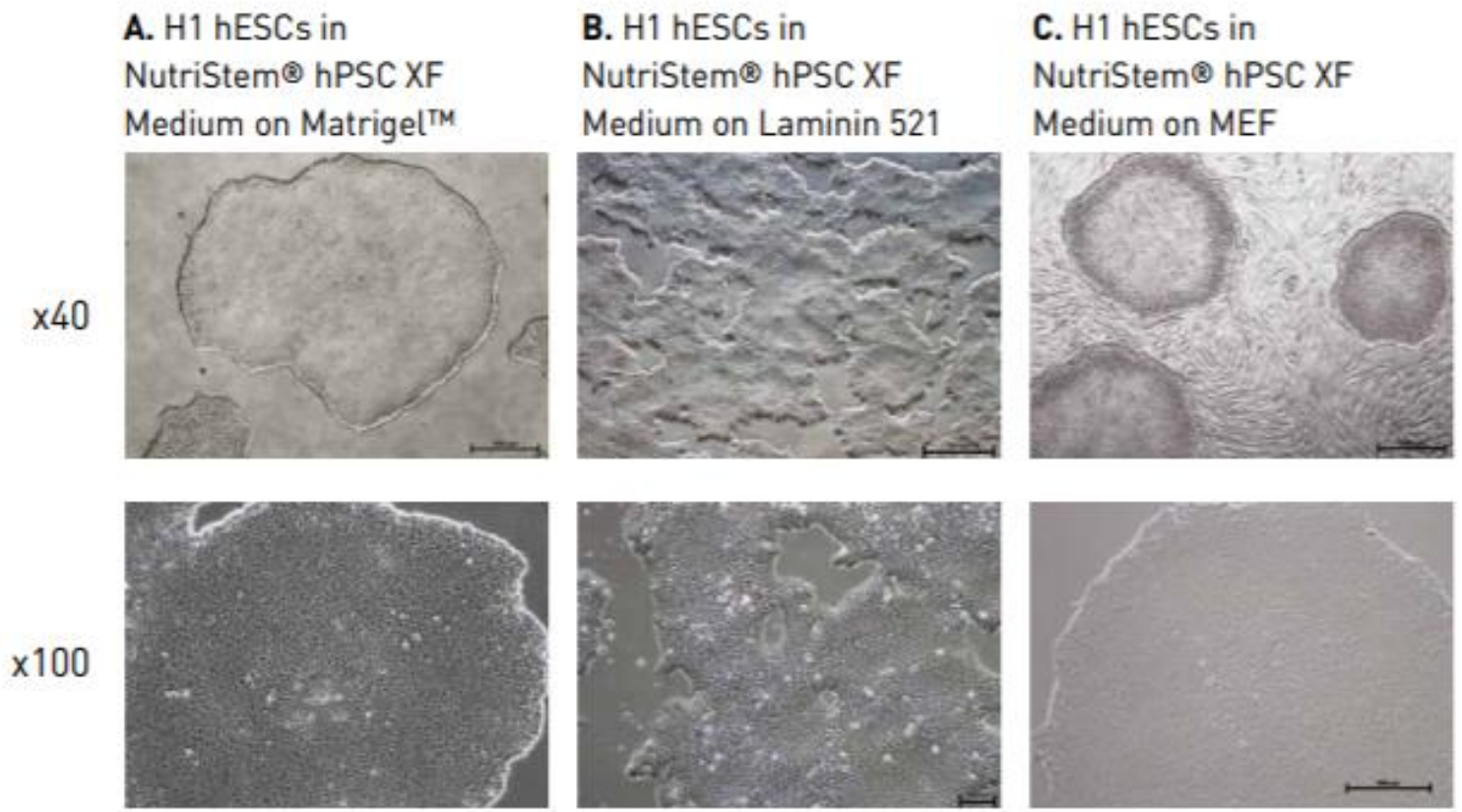

Figure 1: Classical H1 hESC (P64) morphology cultured in NutriStem® hPSC XF medium.

"The nice thing about NutriStem hPSC Medium is that **it wasn't developed with a specific matrix**," giving the lab the freedom and versatility to derive and culture new lines on a variety of substrates.

- Dr. Zoe Hewitt, University of Sheffield

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Rapid expansion and recovery after split

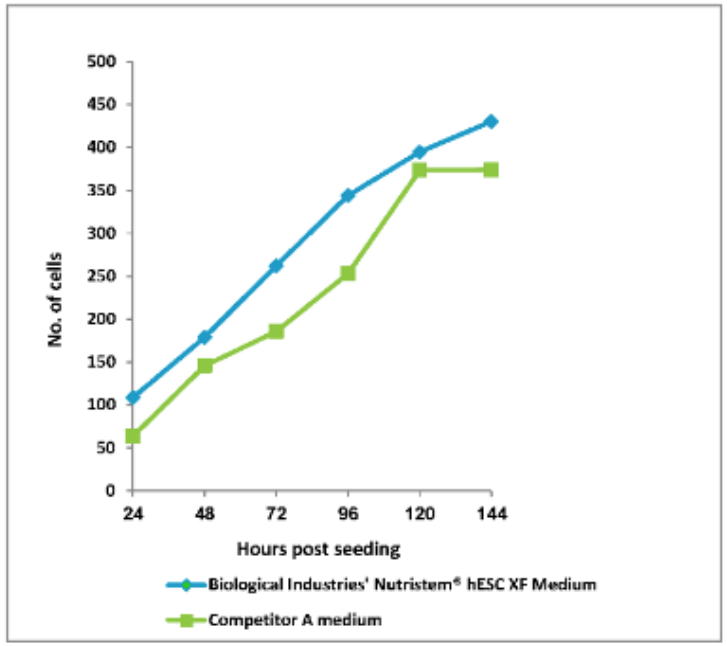
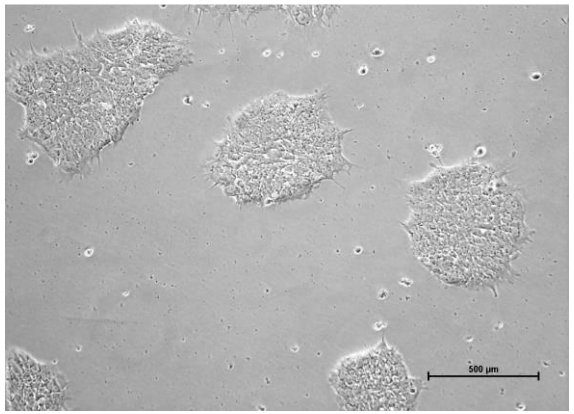
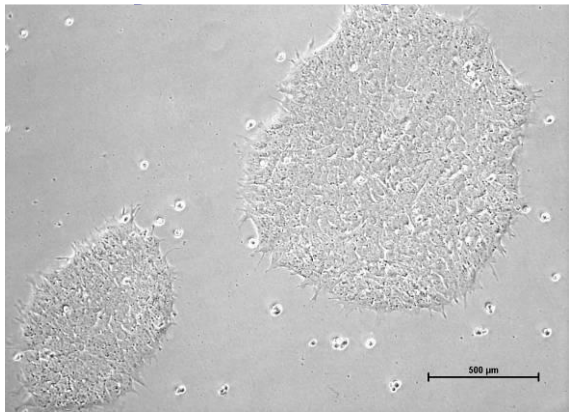


Figure: Human Embryonic Stem Cells (H1, passage 6) were seeded in 96-well plates (Matrigel coated) in BI's Nutristem® and competitor's media. Stem cell media were changed every 24 hours. Number of cells was determined using CyQuant™ cell proliferation assay kit.

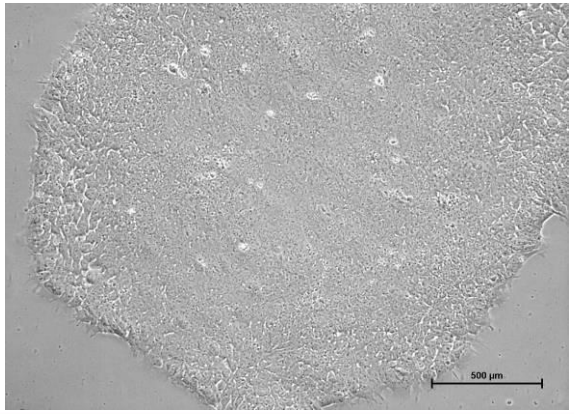
Day 1 after split



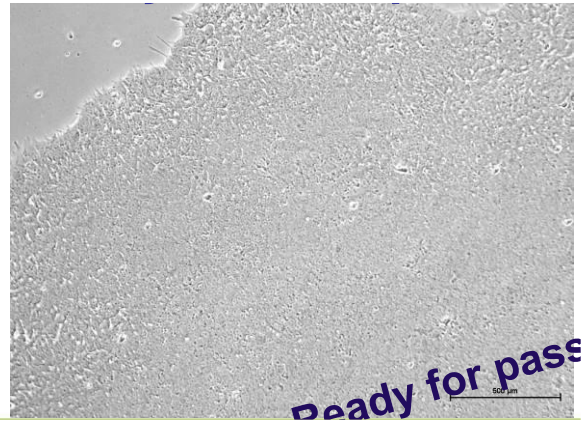
Day 2 after split



Day 3 after split



Day 4 after split



H1 hESC cultured in NutriStem after passaging using collagenase IV

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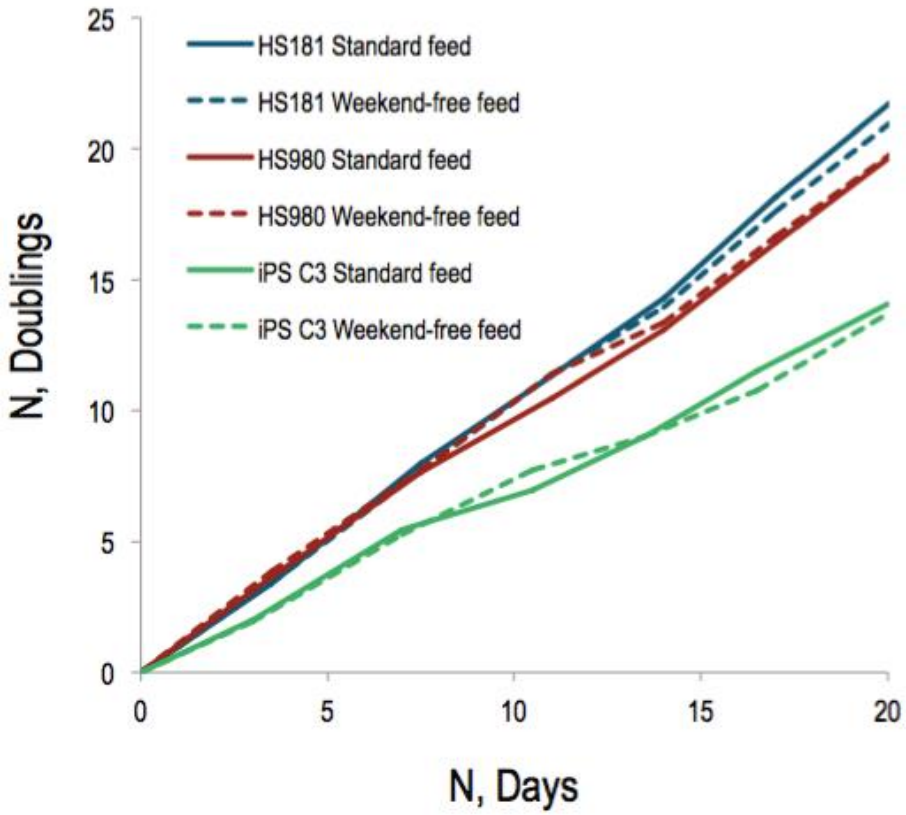
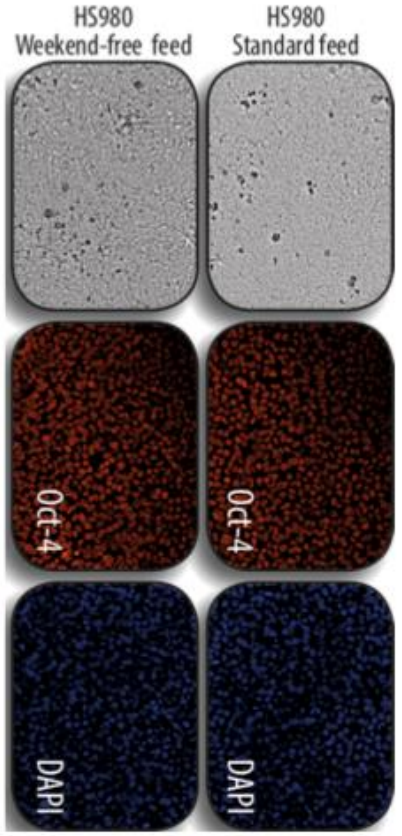
Weekend-free feeding

A.



B.



A. HS181 and HS980 hESC or iPSC were maintained in Nutristem under standard every day-feeding (filled lines) or weekend free feeding (dashed lines) for 6 passages. Fold expansion at each passage was determined by comparing the number of cells generated to the amount seeded.

B. B. Retain pluripotent phenotype.

Courtesy of Biolamina

NutriStem® hPSC XF

NutriStem® hPSC XF is a defined, XF, SF optimized for the derivation and expansion of hESC and iPSC.

Provides	Ready-to-use, one bottle formulation Physiological levels of growth factors (bFGF, TGFb) Modifiable formulations: GF-free, albumin-free
Features	▪ **Rapid expansion** hPSC: standard split 3-5 days, of 1:4-1:8 splitting ratio. ▪ **Flexible culture systems** in feeder dependant (MEF, HFF) and feeder-free culture (Matrigel™, Laminin, GelTrex) ▪ Suitable for clinical applications, **DMF filed with FDA**. cGMP manufacturing. ▪ **Weekend-free feeding** ▪ Validated performance: maintenance of hPSC characteristics

In collaboration with

Technion
Faculty of Medicine
Stem Cells Research Center
Human Embryonic Stem Cells laboratory
Technion-Israel Institute of Technology
Haifa, Israel

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Worldwide academic excellence, clinical trials and cell banking

CELL THERAPY COLLABORATIONS

Academic Excellence

The Nobel Prize in Chemistry 2004

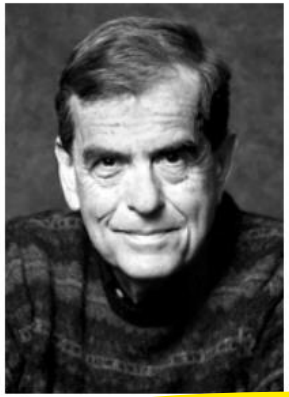
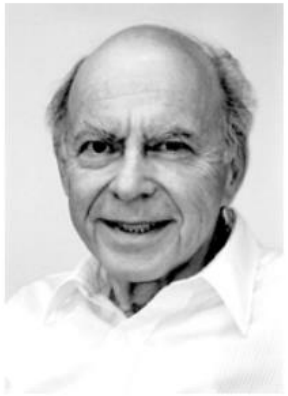


Photo: D. Porges
Aaron Ciechanover
Prize share: 1/3

Photo: D. Porges
Avram Hershko
Prize share: 1/3



Irwin Rose
Prize share: 1/3

The Nobel Prize in Chemistry 2004 was awarded jointly to Aaron Ciechanover, Avram Hershko and Irwin Rose *"for the discovery of ubiquitin-mediated protein degradation"*.

Photos: Copyright © The Nobel Foundation

Prize motivation: "for the discovery of ubiquitin-mediated protein degradation"

The Nobel Prize in Chemistry 2009

Photo: U. Montan
Venkatraman Ramakrishnan
Prize share: 1/3

Photo: U. Montan
Thomas A. Steitz
Prize share: 1/3

Photo: U. Montan
Ada E. Yonath
Prize share: 1/3

The Nobel Prize in Chemistry 2009 was awarded jointly to Venkatraman Ramakrishnan, Thomas A. Steitz and Ada E. Yonath *"for studies of the structure and function of the ribosome"*.

Photos: Copyright © The Nobel Foundation

Prize motivation: "for studies of the structure and function of the ribosome"

Clinical Trial: Septic Shock

Ottawa man survives deadly infection after taking part in world-first stem cell trial on humans

https://ottawa.ctvnews.ca/ottawa-man-survives-deadly-infection-after-taking-part-in-world-first-stem-cell-trial-on-humans-1.2840434

https://clinicaltrials.gov/ct2/show/NCT03369275

Name: Cellular Immunotherapy for Septic Shock (CISS2)

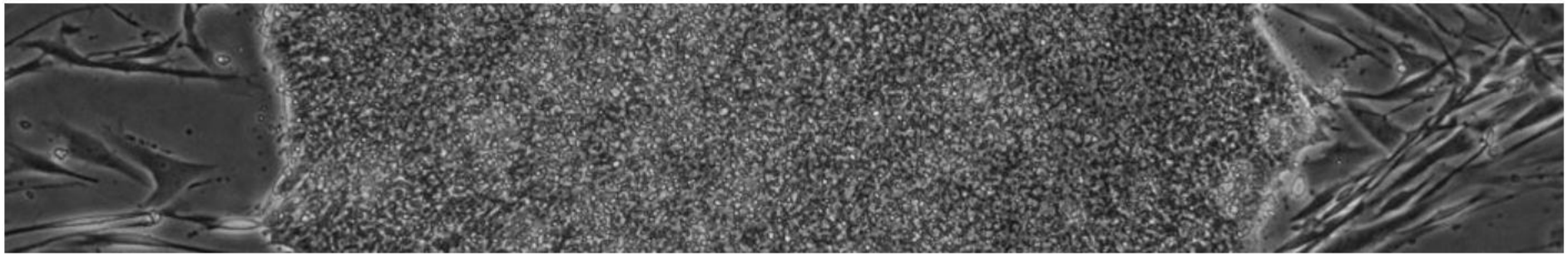
Conditions: Septic Shock, Sepsis, Pathologic Processes, Shock, Infection, Systemic Inflammatory Response Syndrome, Inflammation

Phase: Phase 2

Country: Canada

Cell Banking: First EUTCD-grade hESC Line

Supplier media to researchers at University of Sheffield



EUTCD-Grade Human Embryonic Stem Cell Lines

EUTCD-Grade stem cell lines have been derived and banked under conditions which allow them to be supplied as starting material for the development of cellular therapies. In partnership with our depositors, the EUTCD-Grade cell lines meet the requirements of the European Tissue and Cells Directives (EUTCD) and have been banked in a facility licenced by the Human Tissue Authority (HTA).

"In order to get a cell product to the end (clinic), you need to start with material that you know is going to go from start to end," she explained. The risk being taken when switching from research-grade cells, media, or other materials to a clinical-grade version is clear. "To do a lot of research on the research-grade ES cells and then move to the clinical-grade version, it could be that the protocol that you just spent 10 years developing didn't work." – **Dr. Zoe Hewitt**

http://www.nibsc.org/science_and_research/advanced_therapies/uk_stem_cell_bank/eutcd_grade_.aspx

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Cell Banking: Therapeutic Genetically Modified Cell Bank

ISCT 2017: Making the World's Largest Therapeutic GM Cell Bank University College London, United Kingdom

- Appreciation of scaling challenges for clinical manufacture and an overview of process considerations.
- Methodologies behind the translation of research to GMP manufacture.
- An understanding of clinical assay design and implementation.

http://isct2017.com/wp-content/uploads/2017/04/ISCT2017FullProgram-RevisedApr21.pdf

Contact

Ranit Lobel, PhD, MBA

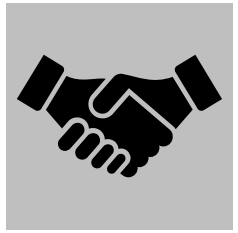
Head of Technical Support

Niva Shraga Heled, PhD

Technical Support Professional

Technical Support

BI_Support@Sartorius.com



Oren Ben-Yosef, PhD

Product Manager, Stem cells

Oren.Ben-Yosef@Sartorius.com

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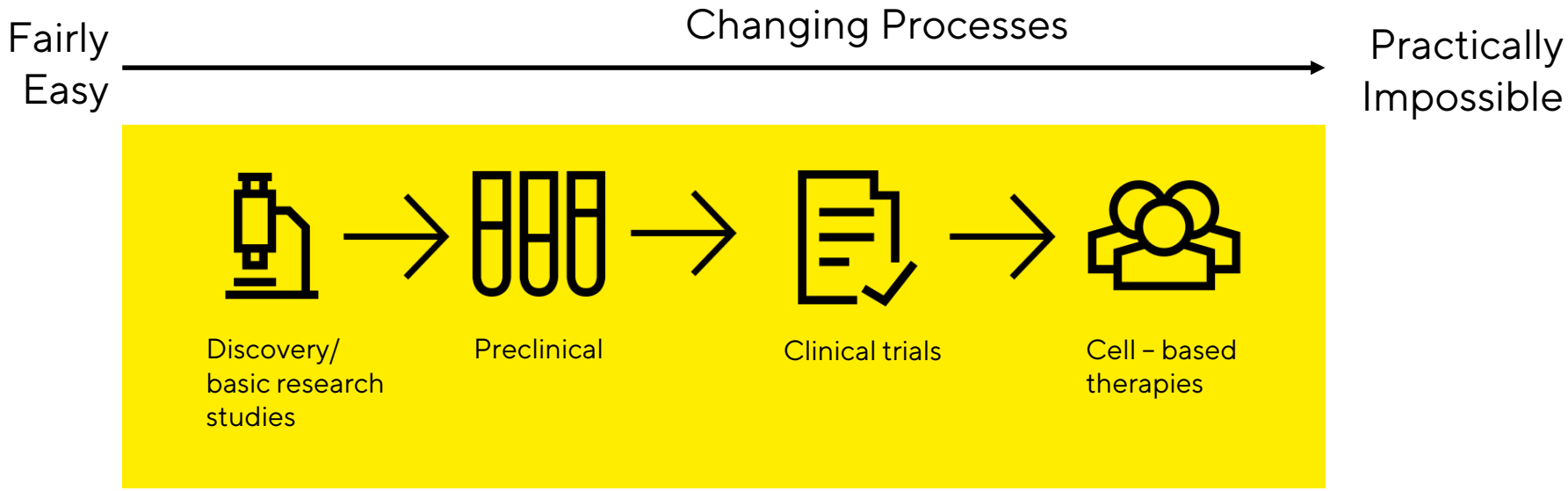
Begin troubleshooting early

- Establishing optimal cell culture systems
- Suitable reagent supplier selection
- Eliminate downstream laborious and time-consuming modifications



Thank you.

Oren Ben-Yosef, PhD
Product Manager, stem cells
Biological Industries Ltd.
Oren.Ben-Yosef@Sartoius.com

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