What's Important in Hypoxic Cell Culture

A Growing Trend in Cancer & Stem Cell Research

Speaker: Samantha Lee

Agenda

- **Hypoxia definition**
- **Why is Low Oxygen Important in Tissue Culture?**

  Cancer, Cancer Statistics, Stem Cells
- **Overview of Current Culture method**
- **Today Challenges in the Tissue Laboratory**
- **Ruskinn Hypoxia workstation**
- Summary: what's advantages in Ruskinn workstations

Ruskinn- Key Milestones

Founded by Andrew Skinn

1993

1994 Anaerobic Workstations "Bugbox"

1999 Hypoxia Workstations "Invivo2" O2 and CO2 control

1000 workstations

2008 New Gas Mixer

2011 Acquired by The Baker Company

2010 Stem Cell Workstations "SCI-tive"

1800 workstations

2013

2013 Media Pre-Conditioning " HypoxyCOOL"

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Hypoxia definition

Q: What is Hypoxia?

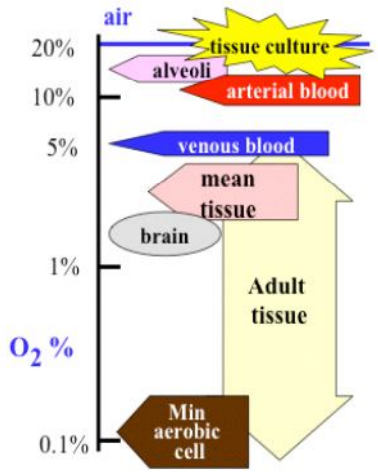
A: When O_2 in the cell or organ drops below physiologically normal levels

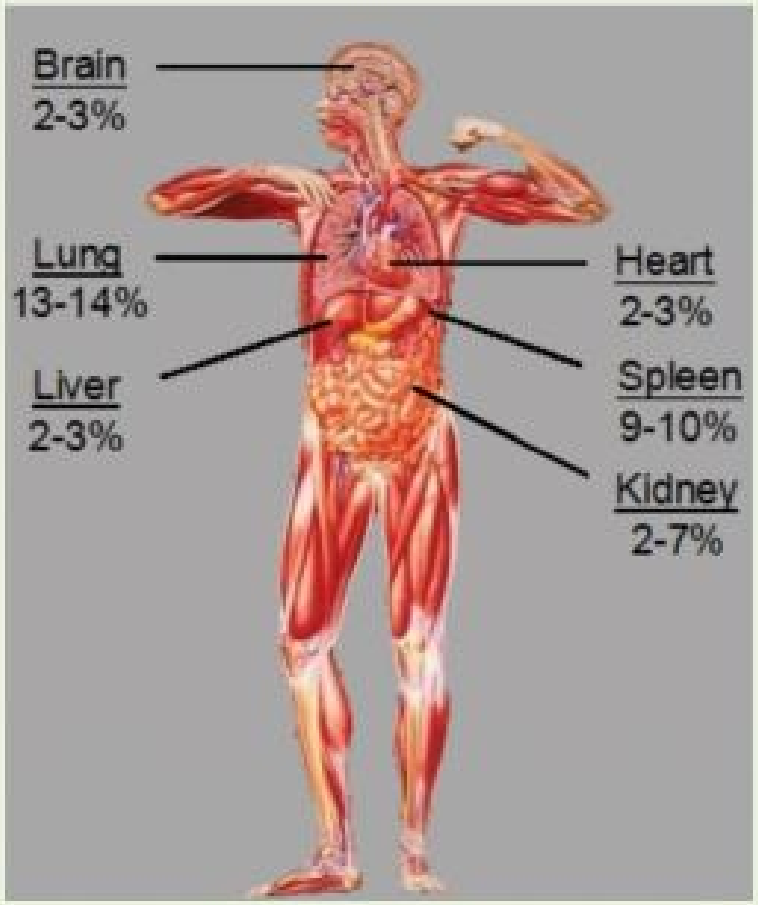
Q: What is the physiologically normal O_2 level?

A: It depends on cell or organ but is <20.9%

Why is Low Oxygen Important in Tissue Culture?

- Most Cells live within 2-8 % Oxygen (except lung)
- *in vivo* cell interaction does not occurs at ambient oxygen





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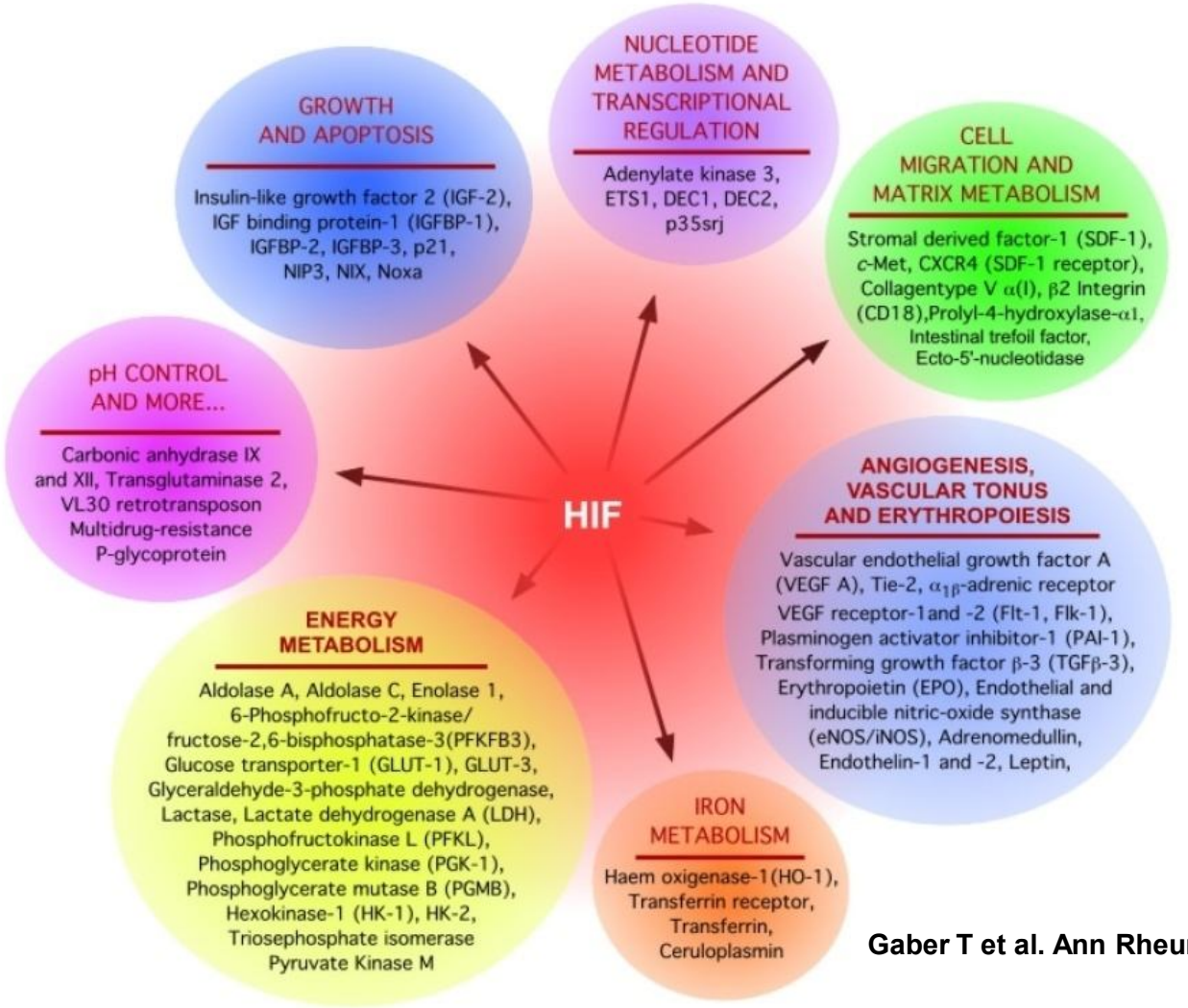
Low Oxygen Tissue or Cell Culture and Research

1. Cancer
2. Diabetes
3. Aging
4. Stem Cells

Mainly HIF (Hypoxia Inducible Factor) dependent signalling pathways

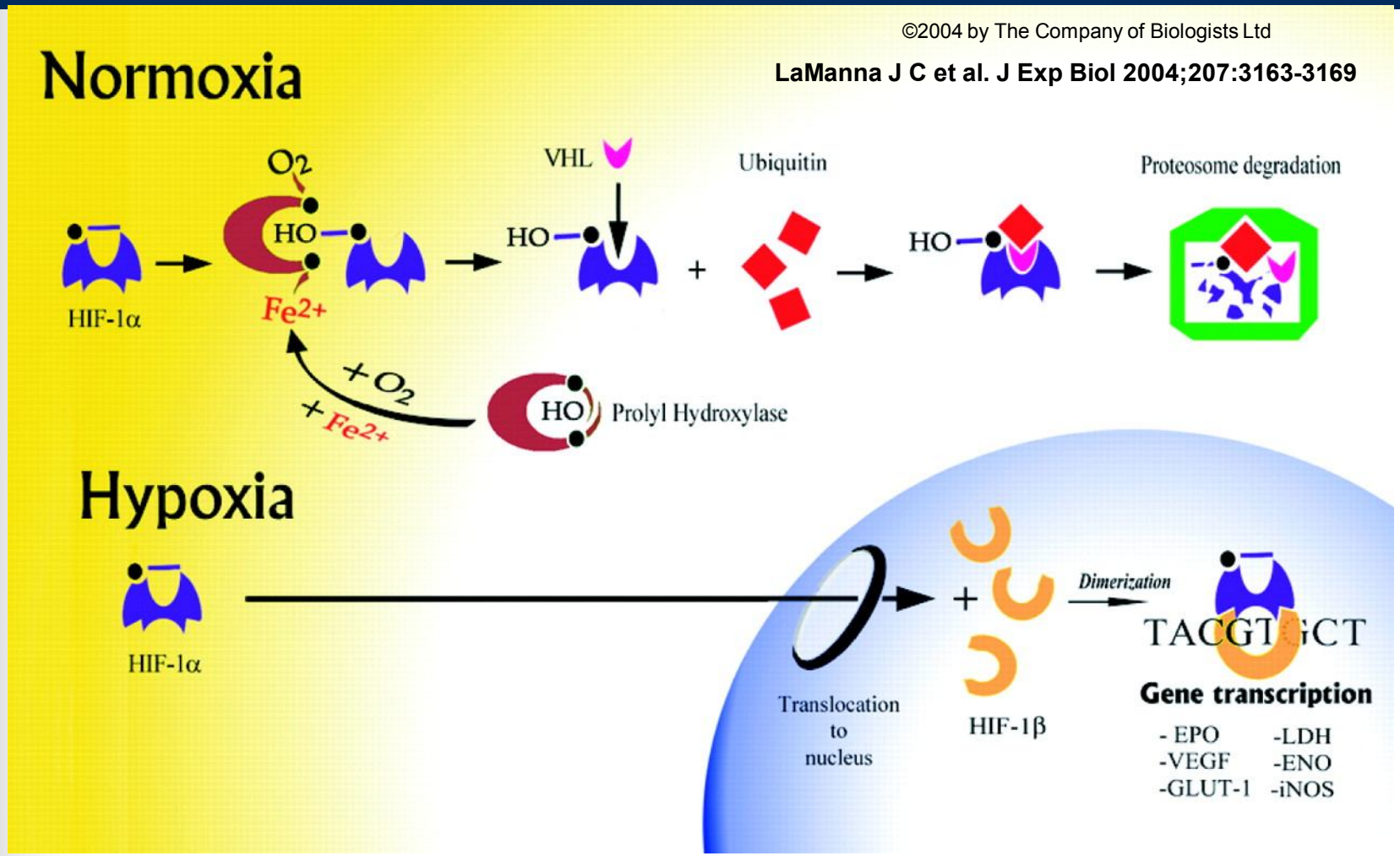
HIF, master regulator of hypoxia-induced gene expression



Gaber T et al. Ann Rheum Dis 2005;64:971-980

This scheme represents the current concept for hypoxia-inducible factor-1 (HIF-1) pathways

LaManna J C et al. J Exp Biol 2004;207:3163-3169



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Important of Low Oxygen in Cancer



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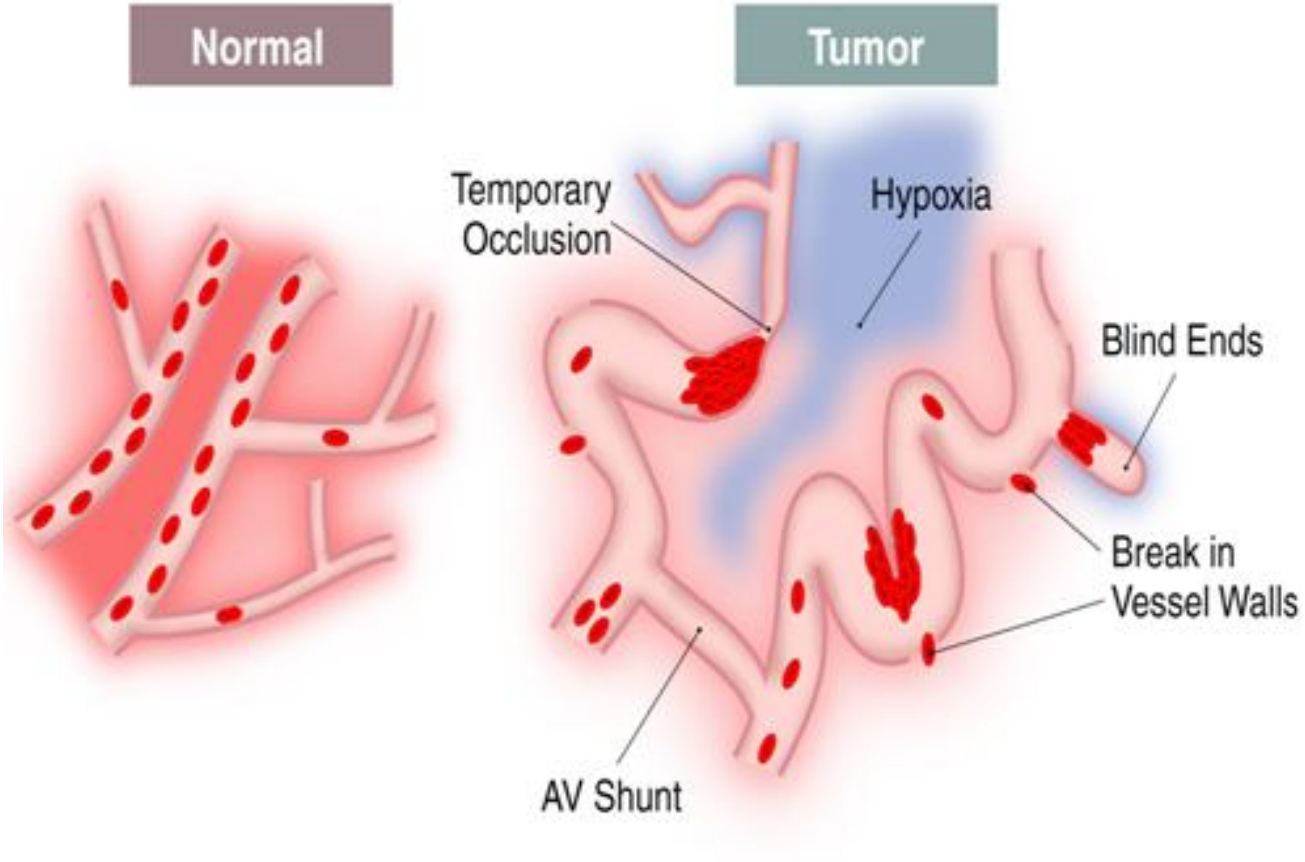
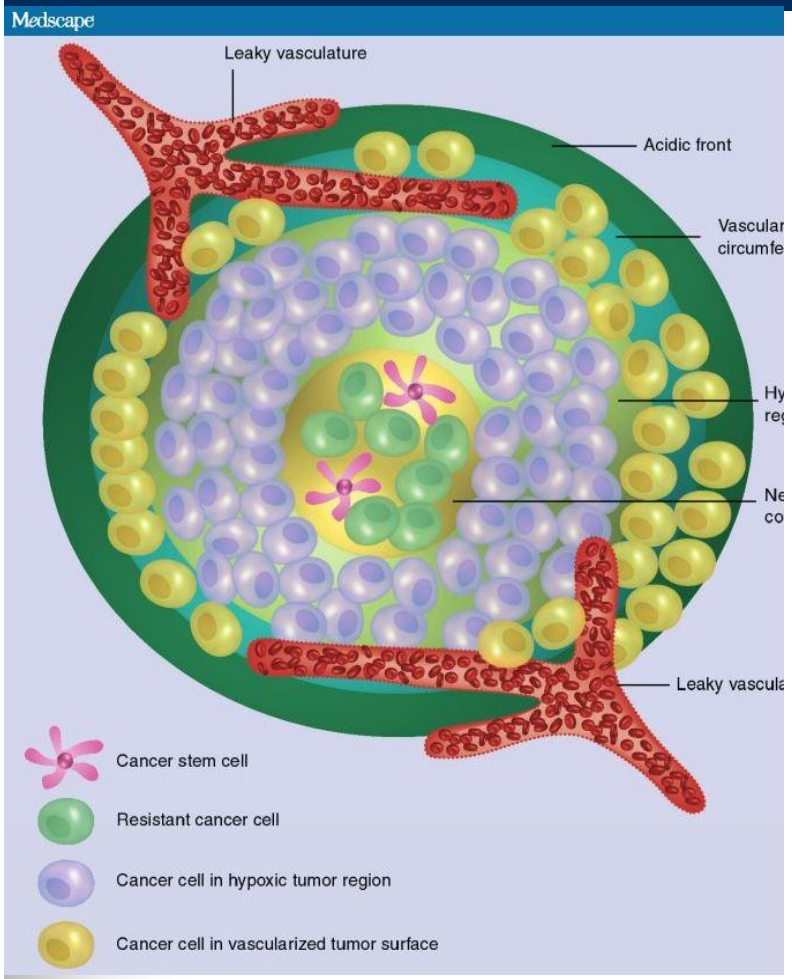
Hypoxic Tumor Cells

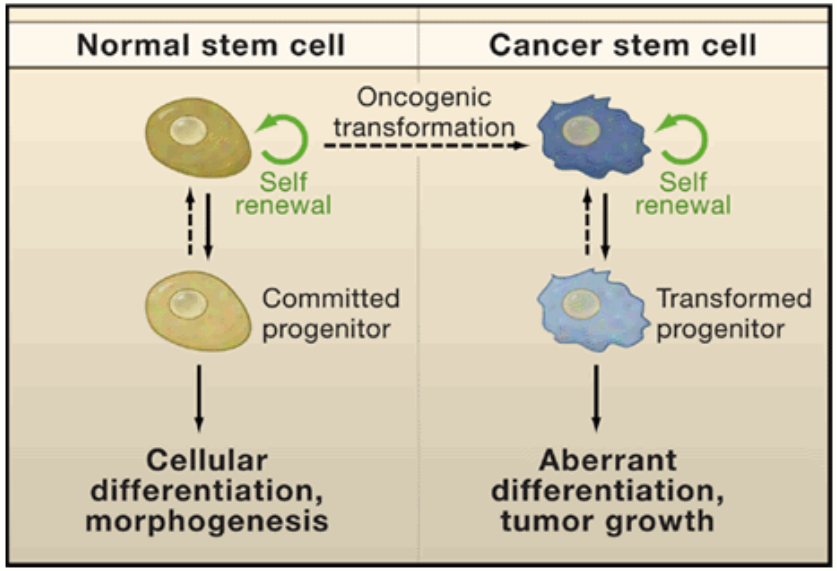


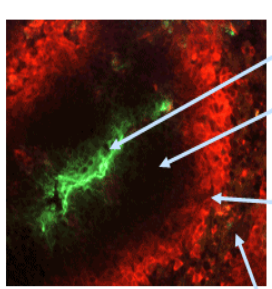
From Brown and Giaccia, Cancer Res., 58: 1408-16 (1998)

- Many solid cancers receive less blood, causing hypoxic environment
- Hypoxic tumor often more resistant to cancer treatments and are more likely to become invasive and spread to other parts of the body

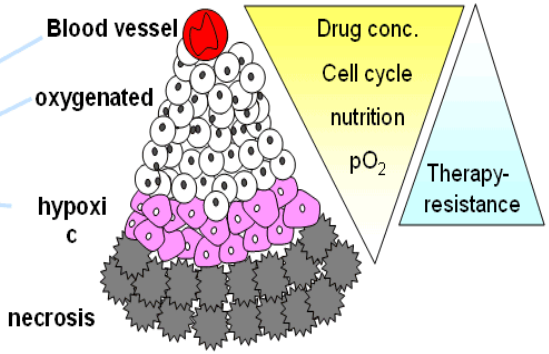
Cancer Research Tumour microenvironment







Green: CD31
Red: anti-Pimonidazole



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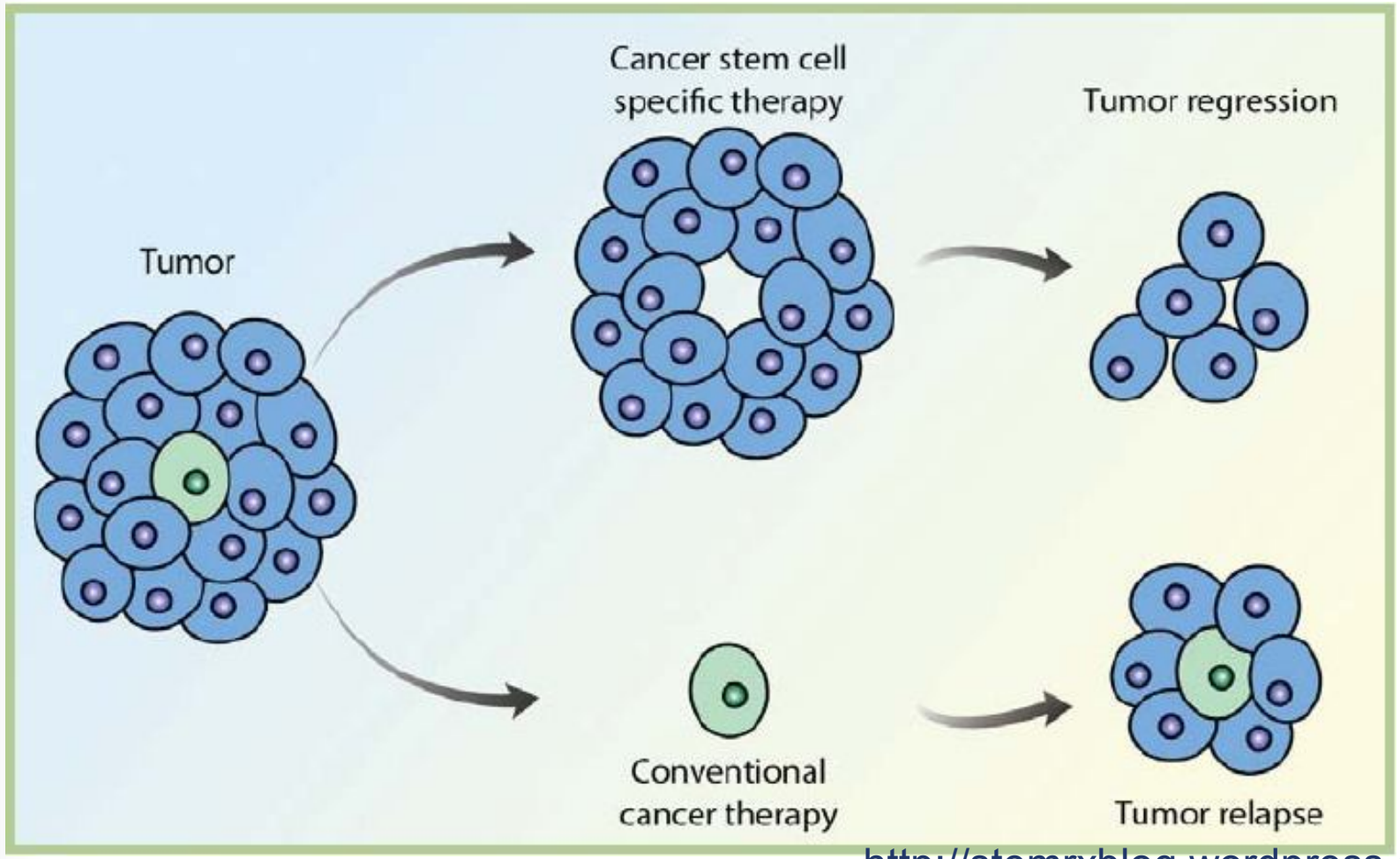
Post Chemotherapy Stem Cells Treatment For Cancer



http://stemrxblog.wordpress.com/

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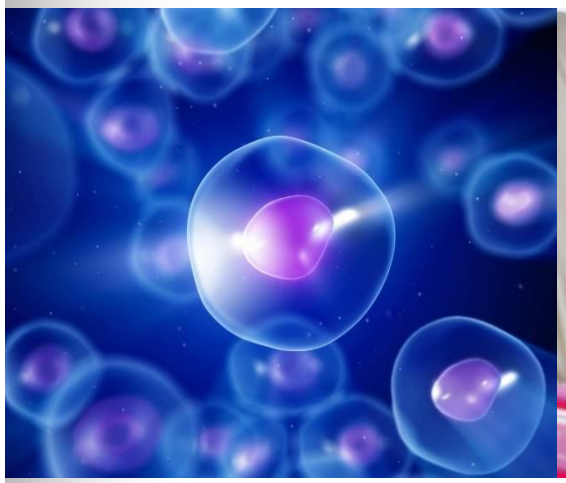
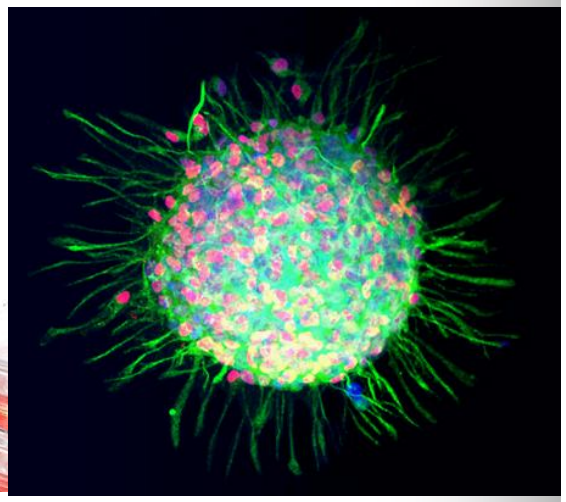
Important of Low Oxygen in Stem Cell





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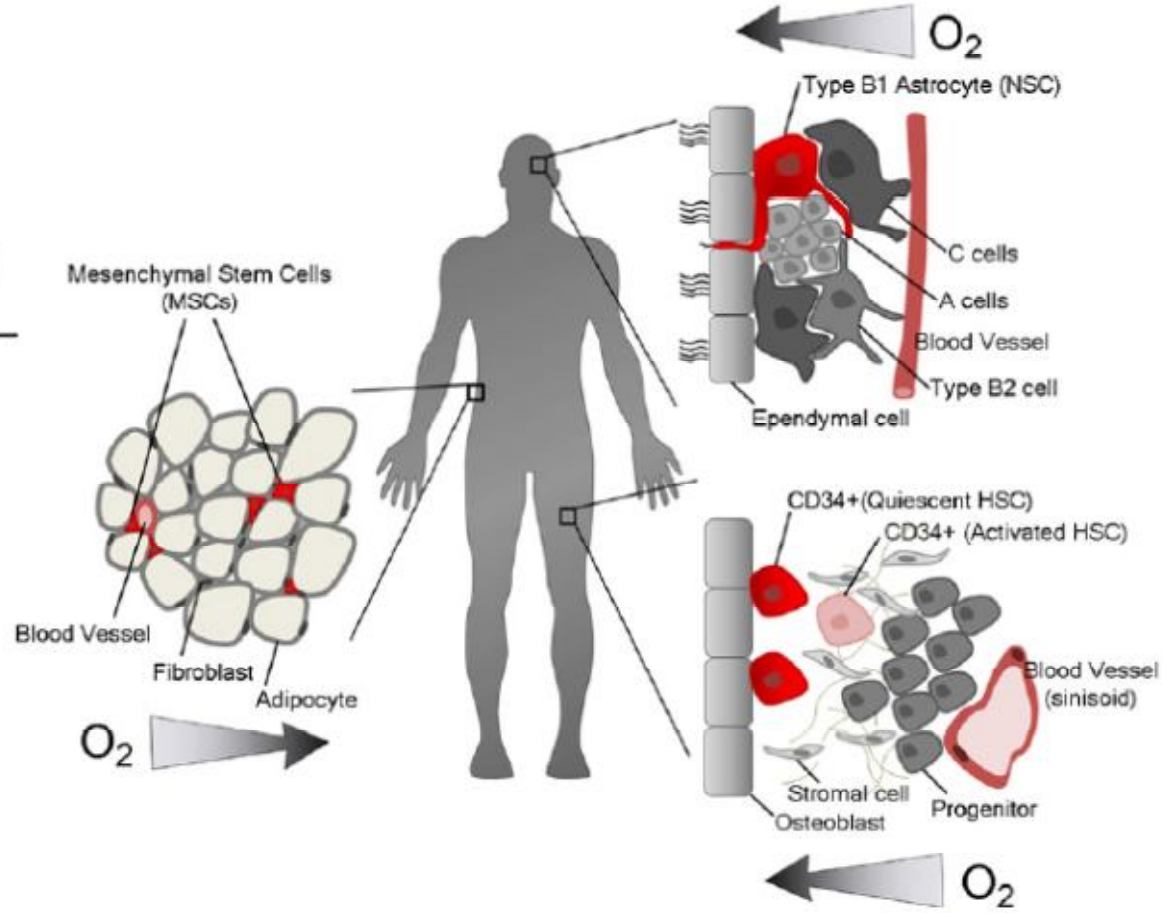
In Stem Cell Research

The Mesenchymal Stem Cell Niche

2-8% O_2

Kofoed et. al., 1985
Harrison et. al., 2002
Matsumoto et. al., 2005
Pasarics et. al., 2009



The Neural Stem Cell Niche

<1-8% O_2

Dings et. al., 1998
Erecinska and Silver, 2001
Panshision, 2009

The Hematopoietic Stem Cell Niche

1-6% O_2

Grant and Root, 1947
Cipolleschi et. al., 1993
Chow et al., 2010
Eliasson and Jonsson, 2010

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Summary

1. More representative of *in vivo* physiology
2. Faster growth rate of cell cultures
3. More representative of tumur microenvironment
4. Maintenance of pluripotency when expanding Stem Cells

Today Challenges in the Tissue Laboratory

However, there are changes in….

1. Temperature
2. Oxidative Stress
3. Humidity
4. pH

➡ **Leading to cell stress**

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- To mimic the physiological *in vivo* cellular process on an *in vitro* level …

Ruskinn Hypoxia Workstations:
SCI-tive Workstations

Stem Cell Investigations Total In Vitro Environment

1. O_2: 0.0% to 23.0%, in 0.1% increments
2. CO_2 : 0.0% to 30.0%, in 0.1% increments
3. Temperature Control: from ambient +5C to 45 C
4. Humidity Control: ambient to 85% RH
5. Pre-programmable cycling
6. Auto Calibration of O_2 sensor
7. Hepa-filtered Air: Class 5/Class 100
8. Larger Workstation (1.1m internal width) and Interlock
9. Ability to accept different stereo and inverted microscopes

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Ruskinn Hypoxia Workstations:
Sci-tive Workstations: 2 Models

Sci-tive

1650mm

Dual Sci-tive

2610mm

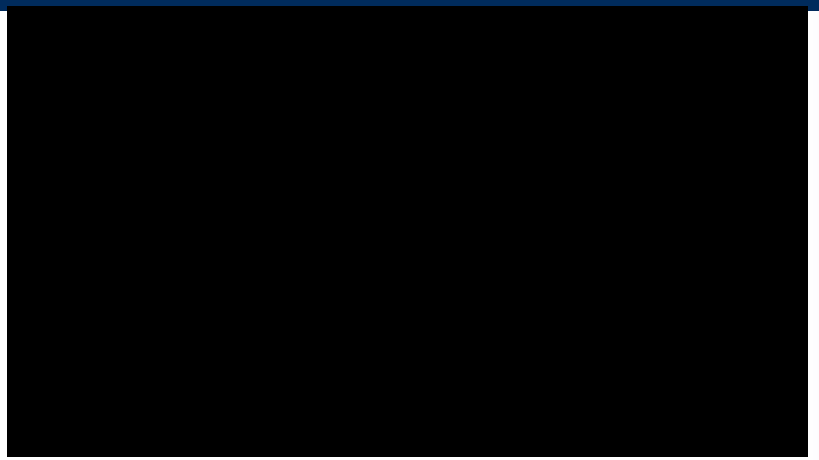
Cell/Tissue Culture Challenge: SCI-tive

1. Quick direct-hand access into Workstation: Ezee Plug (not Gas/Vac)

2. Main Chamber atmosphere is not compromised

Cell/Tissue Culture Challenge: SCI-tive

1. Very fast transfer of samples into and out of the workstation via an Interlock
2. Main Chamber atmosphere is not compromised

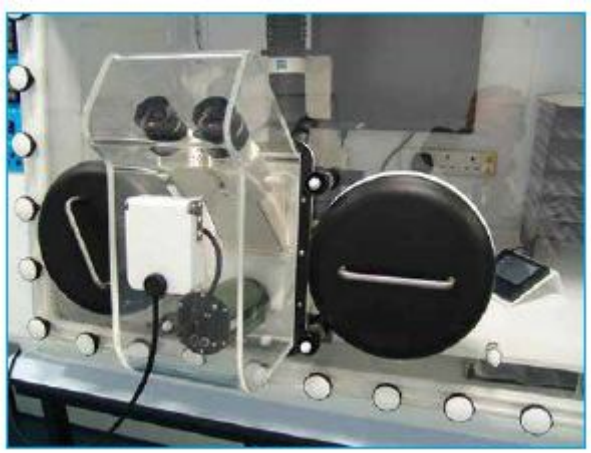


Ruskinn Hypoxia Workstations: SCI-tive Workstations

Option of HEPA-filtration for enhanced internal atmosphere quality

Sci-tive- Brings more benefits

Easily accommodates analytical tools



Microscope with oculars protruding through front cover

Quick access

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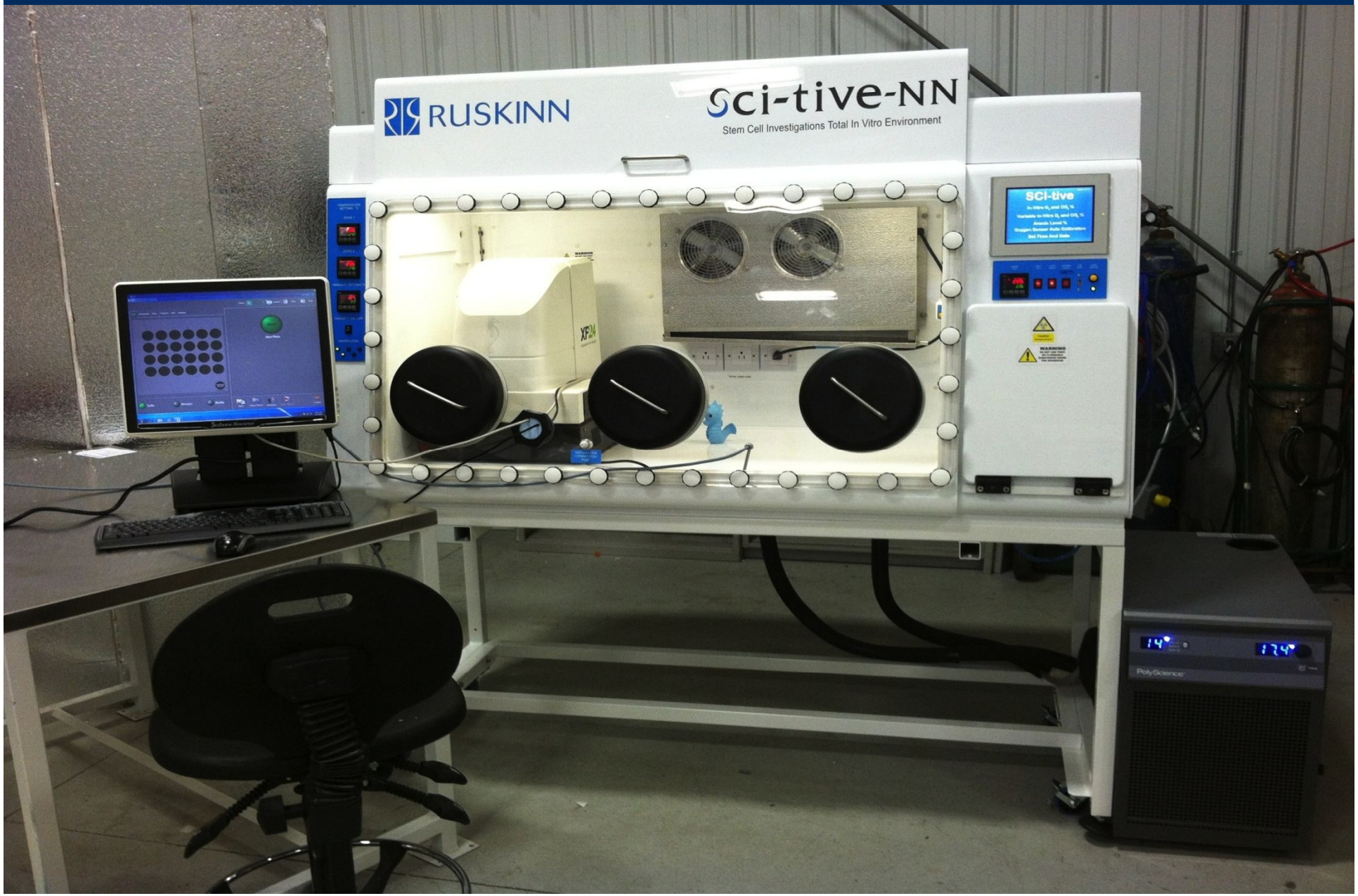
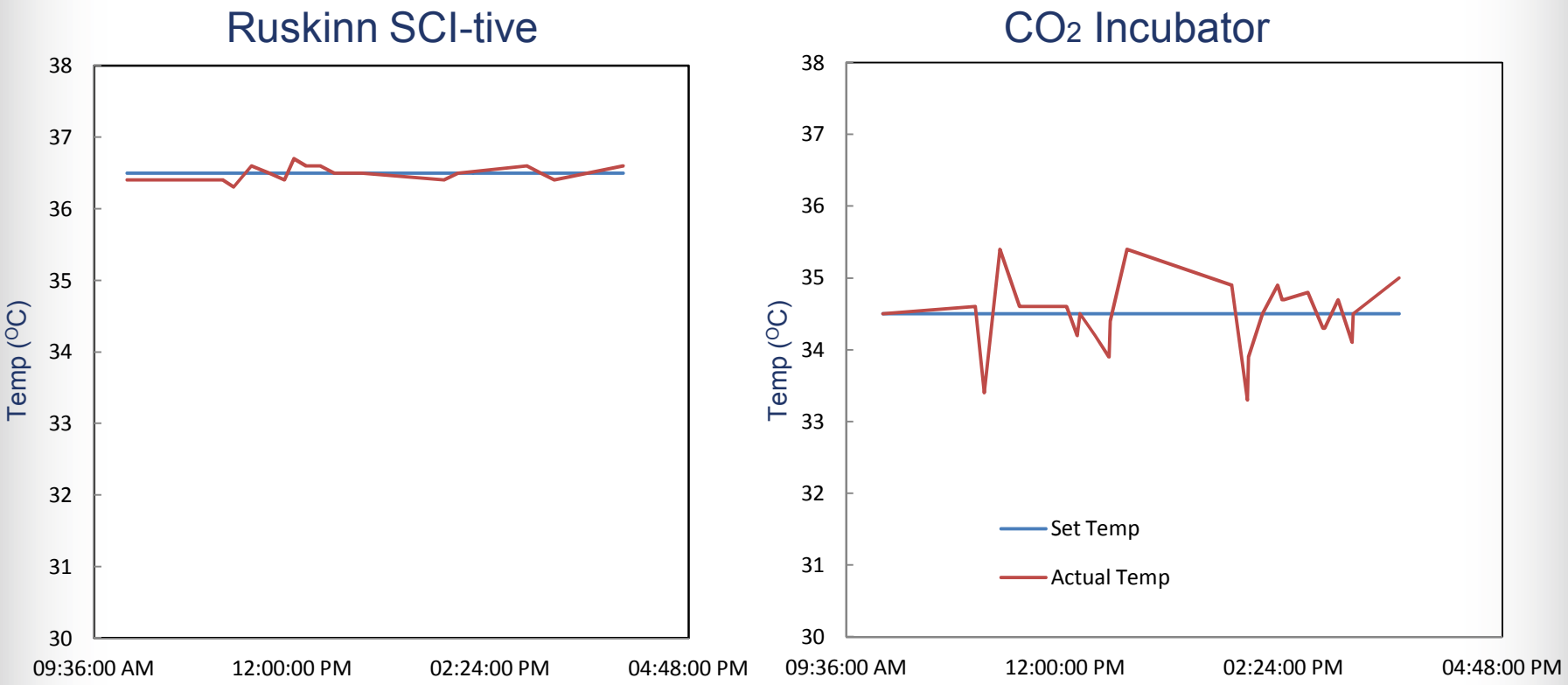
Ruskinn Hypoxia Workstations:
SCI-tive Workstations



Environmental Control Comparison: Temperature

CO2 incubator vs Closed Cell Culture System



Environmental fluctuations during access to experiments e.g. MEA plate set ups and administration of drugs

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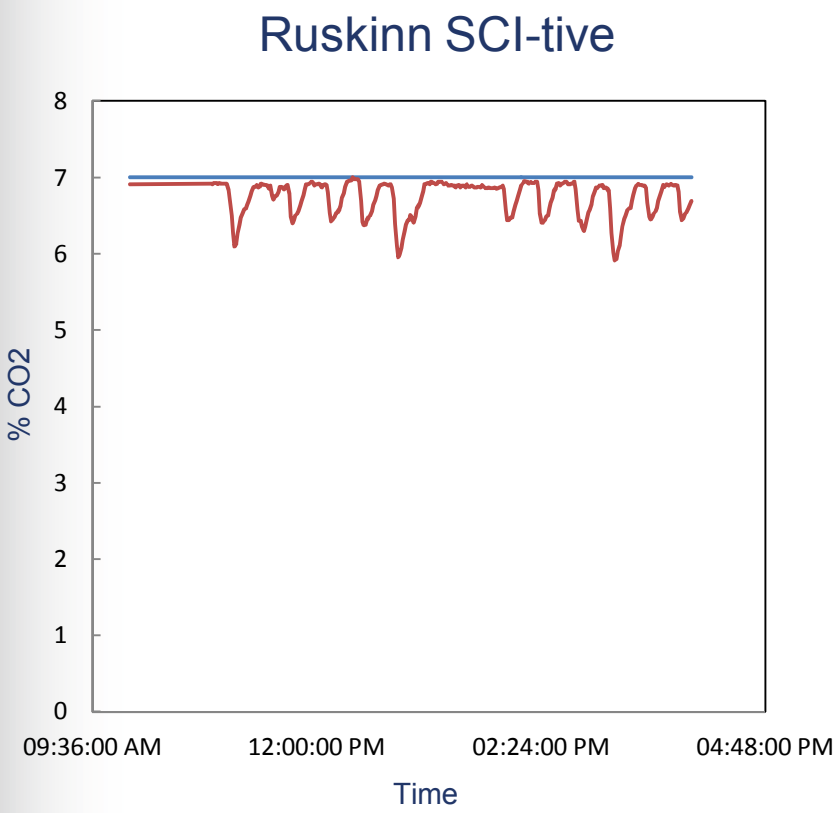
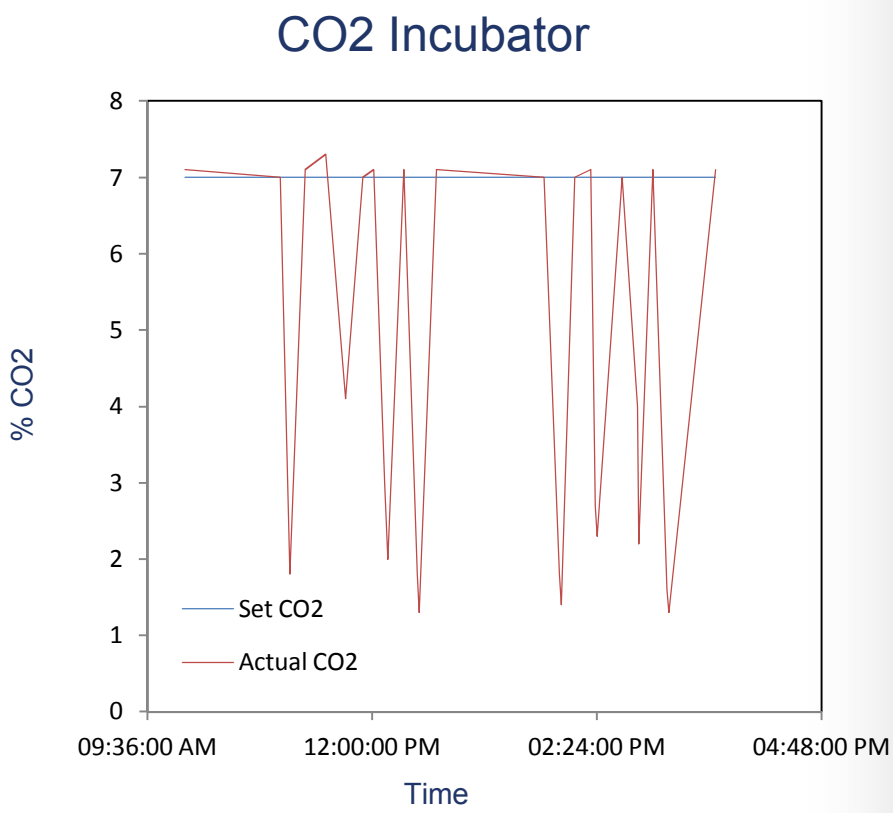
Environmental Control Comparison: CO_2 levels (CO_2 incubator vs Closed Cell Culture System)





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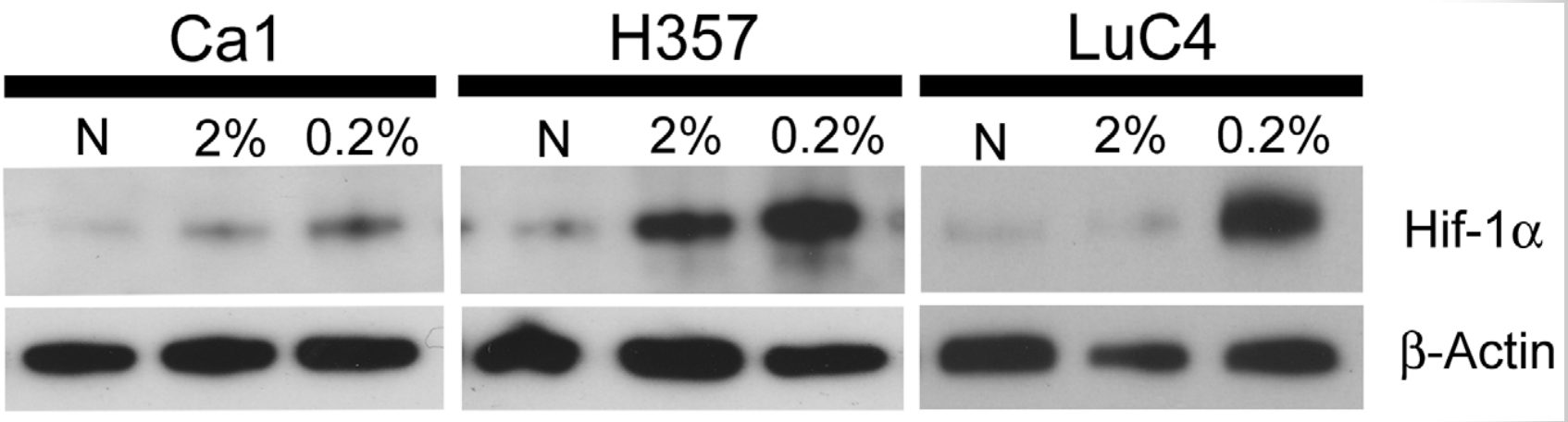
Experimental Results: HIF

HNSCC cell lines express increased HIF-1a at lower oxygen concentrations.



Western blot of protein lysates of Ca1, H357 and LuC4 cell lines after culture under normoxia (N), 2% and 0.2% oxygen.

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Bergen, Haukeland University Hospital, Bergen, Norway, 3 School of Engineering and Materials Science, Queen Mary University of London, London, United Kingdom

Thanks for your listening

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