Detection of Inflammation and Parenchymal Damage Using Precision-cut Lung Slices

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Presentation Outline

- Disclaimer
- History and background
- Precision cut lung slices (PCLS):
  - Considerations & Methodology
  - Longevity in culture: Histology & Biochem
  - Macrophages & Collagen
  - Differential Toxicity of Analogs
  - Parenchymal damage: Aminoflavone & Phortress
  - Cytokines: important considerations and multiplexing
  - No effect level of Phortress exposure
- PCLS: summary of biomarkers and utility for COPD etiology events
- Paths forward for PCLS model use

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Disclaimer

Data to be presented on precision cut lung slices was generated at several companies working as grantees of, or as the Operations and Technical Support (OTS) contractor for, the National Cancer Institute.

all data has previously been publicized

Disclaimer:

The data to be presented was generated at SRI International (SRII) via the funding of the National Cancer Institute (NCI), supported by NIH grant CA097438 and at SAIC-Frederick (OTS contractor to the NCI). Funded by NCI Contract No. HHSN261200800001E. None of the conclusions, interpretations, or comments made represent the opinions or views of SRII, Leidos Biomedical Research, Inc. (formerly SAIC-Frederick), or the NCI.

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PCLS History and Recent Application

Brief History of Slices:

- Organ slice culture has been described since **1923** when Otto Warburg placed small pieces of tissue into physiological buffer
- The preparation of slices as "**precision-cut**" occurred in 1985 following the invention of a mechanical slicer by Carlos Krumdieck in 1980
- Brendel, K. et al. then describe the utility of slices for **toxicology** and **pharmacology** and the ability to **culture slices for days**

Background of Organ Slice Model Development:

- Application of 3D model for acute and delayed toxicities using short and long term culture; retention of endogenous cell types was expected to yield more relevant results
- Evaluate chemotherapeutics individually in an investigational setting or multiple molecules comparatively to allow "analoging" and modification of SAR
- Utilization of precision cut slices was conducted for numerous antineoplastic molecules using exposures lasting from 1 day to 4 weeks.

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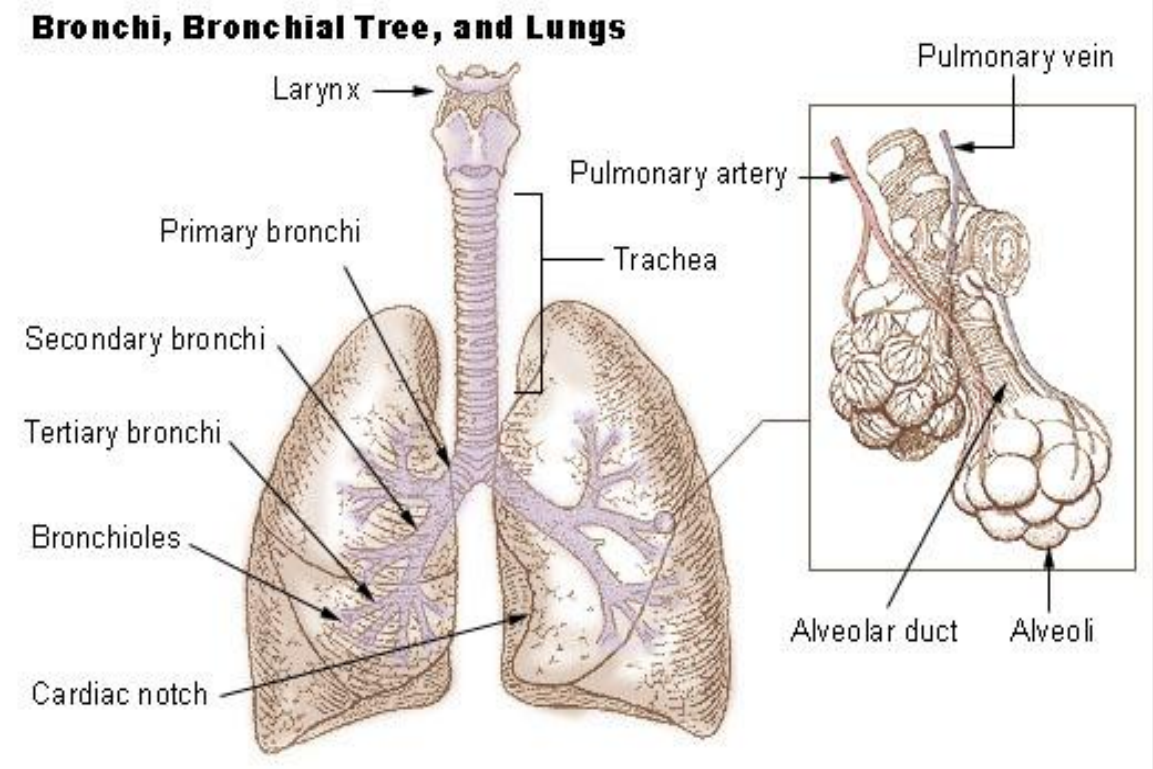
PCLS: Anatomy & Considerations for Use

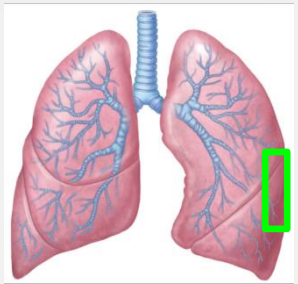
- A whole lung is required for proper inflation
- Acceptance criterion of human lungs important
- Choice of region for removal, coring, & slicing is necessary for
  - Avoidance of diseased portion
  - Targeting of small airways and alveoli



http://en.wikipedia.org/wiki/Lung#mediaviewer/File:Illu_bronchi_lungs.jpg

PCLS: Isolation and Culture

Method of creating slices is typically similar



1. Inflate lung tissue and create tissue cores

- Aseptic lung removal and storage in organ preservation solution. Inflation with 0.8% agarose, lobe dissociation, and tissue coring (8 mm).

2. Slice cores with Krumdieck slicer

- In thermostatically controlled cold UW, cores are sliced to 500 micron thickness

Method of culture can vary:

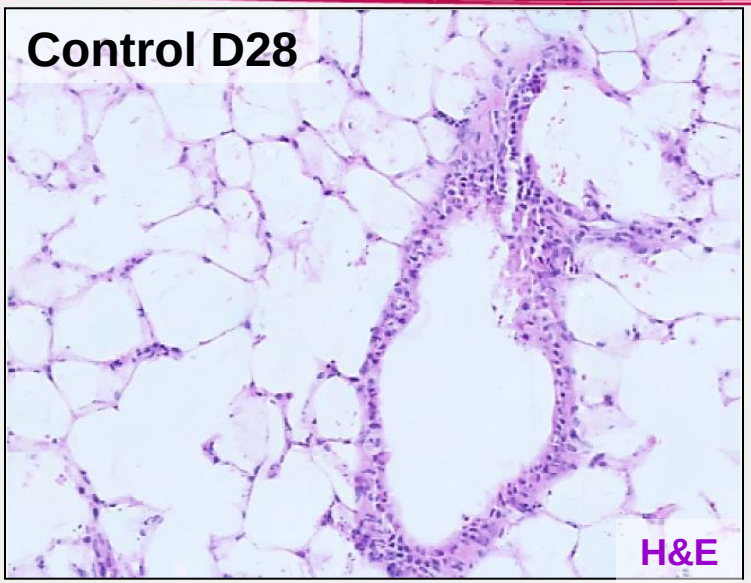
- Shaking flask
- Stirred well
- Rocker platform
- Well insert (ALI)
- **Roller system**

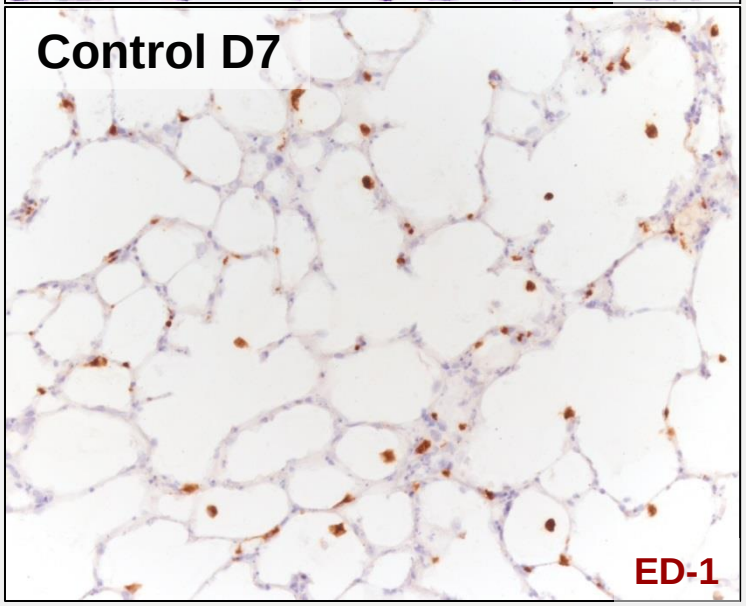
Olinga et al, J Pharmacol Toxicol Methods. 1997 Oct;38(2):59-69.

3. Slices are mounted onto HATF paper within titanium inserts and placed in vials and cultured in 1.7 mL serum-free, M199 medium

4. Vials are rotated at ~3-7 rpm in roller drum within humidified incubator set to 5% CO_2/95% air at 37°C

PCLS Long Term Culture: Histology

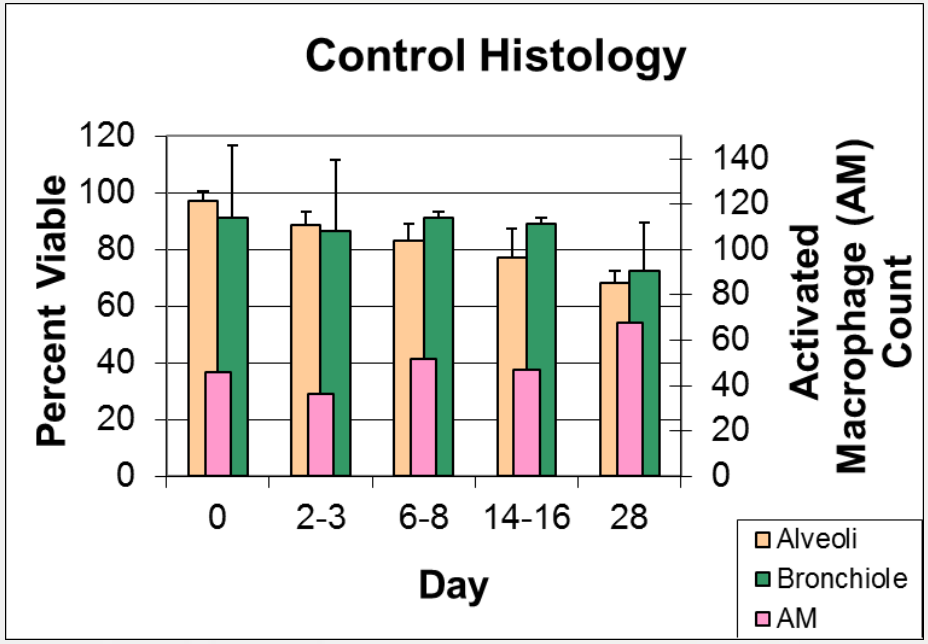


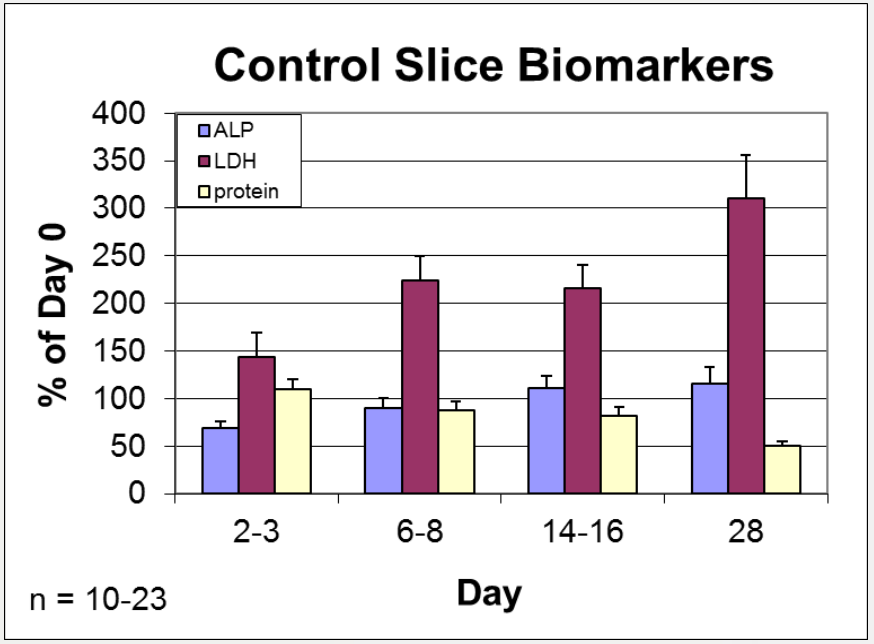


<u>Viability and macrophages</u>

- High degree of alveolar and bronchiolar viability retained over 28D
- Some loss of cellularity
- Control slices exhibit baseline numbers of activated macrophages (AM)

PCLS Long Term Culture: Viability & Biomarkers





- Retention of tissue markers over 28 days in serum-free M-199 medium
- Some loss of protein over time (coincides with minor loss of cellularity)
- ALP, a marker of Type II alveolar cells remains stable (normalized to tissue protein content) over the entire 28 day culture period

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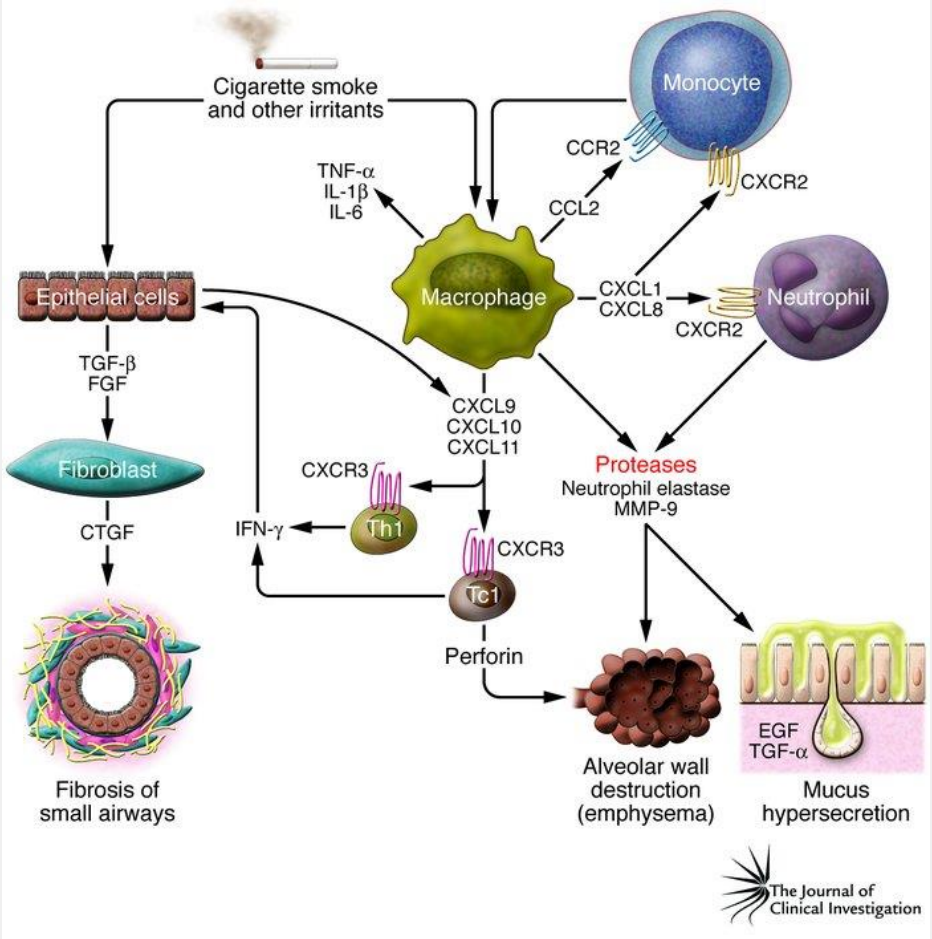
Benefit of Models Containing Relevant Cell Types

E.g. Macrophage and Cytokine Involvement in COPD



Inhaled irritants, such as **cigarette smoke**, activate epithelial cells and **macrophages** to release multiple cytokines…. resulting in **fibrosis** in the small airways.

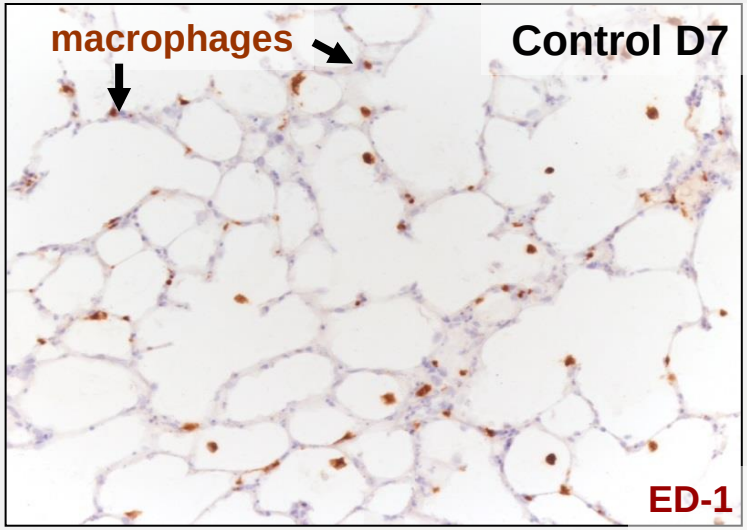
These cells also secrete the proinflammatory **cytokines** TNF-α, IL-1β, and IL-6, all of which amplify **inflammation**, and several chemokines that attract circulating cells …

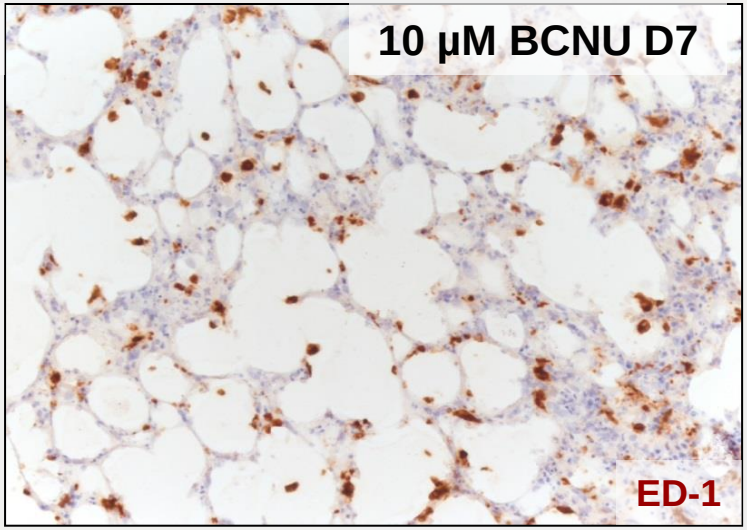
"The cytokine network in asthma and chronic obstructive pulmonary disease"
Peter J. Barnes
J Clin Invest. 2008; 118(11):3546–3556 doi:10.1172/JCI36130

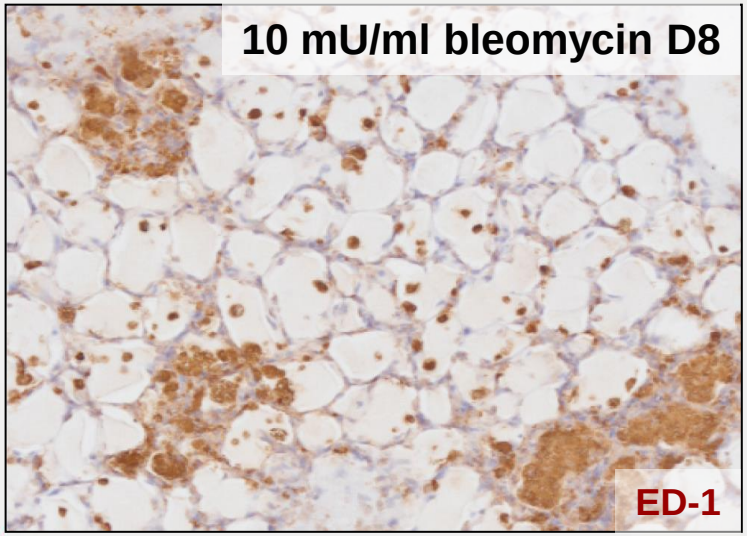
PCLS: Compound-induced Macrophage Activation

Rat PCLS:

ED-1 Stain







BCNU (carmustine) exposure shows numerous macrophages, many of which have infiltrated alveolar walls mimicking interstitial pneumonitis

Bleomycin treatment results in patches of activated macrophages filling alveolar spaces; many solitary macrophages also seen

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Lung Slices: Collagen Deposition

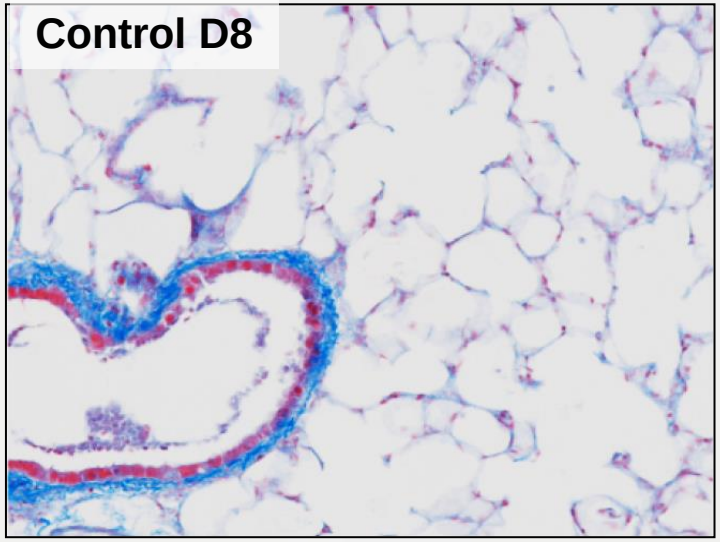
Bleomycin:

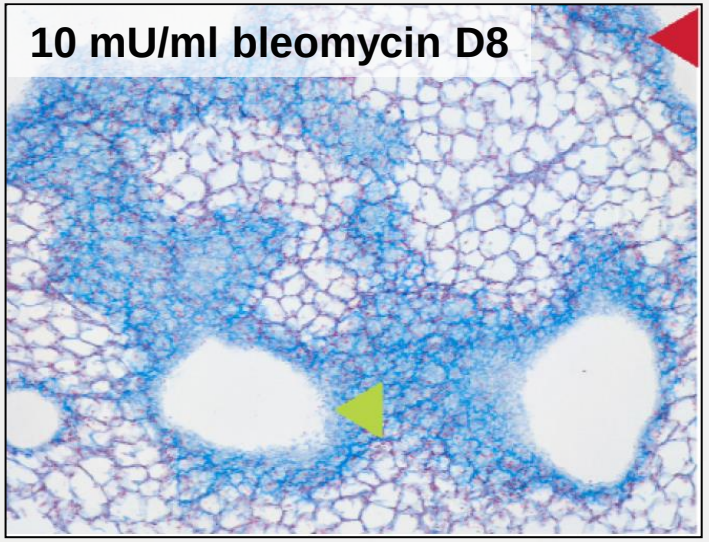
Extensive collagen deposition present in the interior of the PCLS (green arrow)

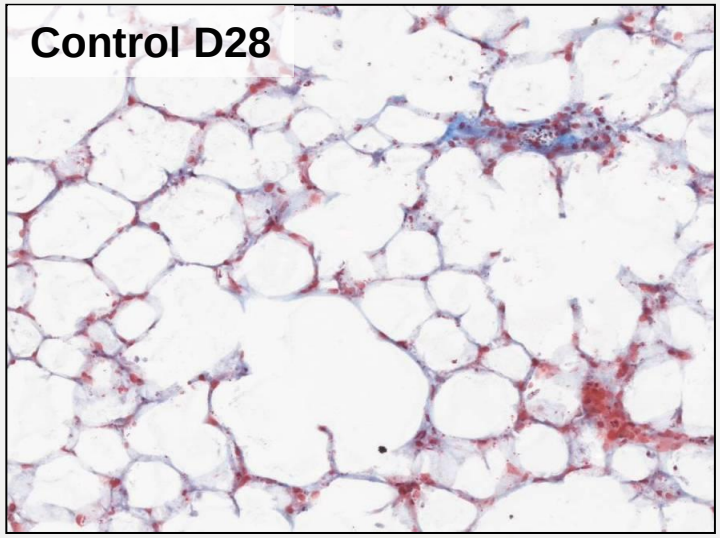
Slice margins also show deposition (red arrow)

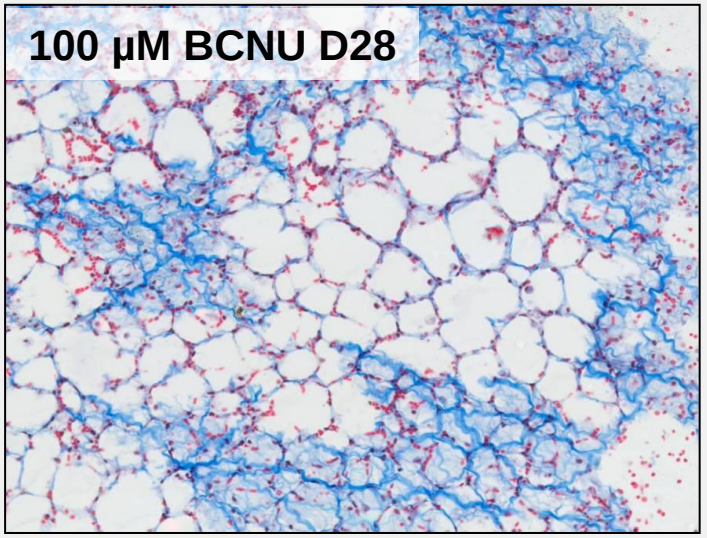
BCNU:

Large areas of parenchyma exhibit extensive deposition of collagen fibers, with intervening normal alveoli









Masson's Trichrome (MT) Stain

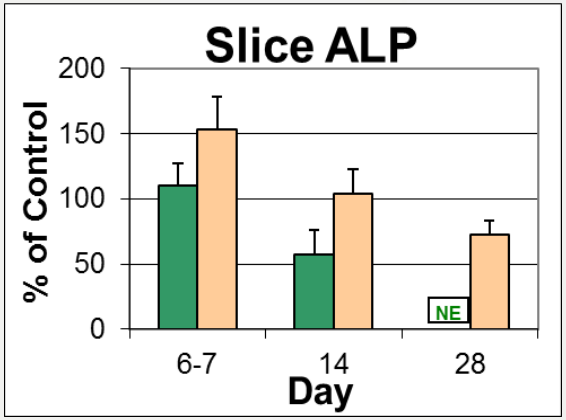
Differential (SarCNU and BCNU) Toxicity

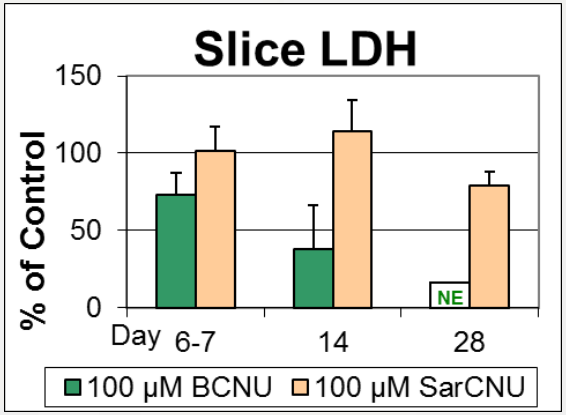
Toxicity: **BCNU > SarCNU**

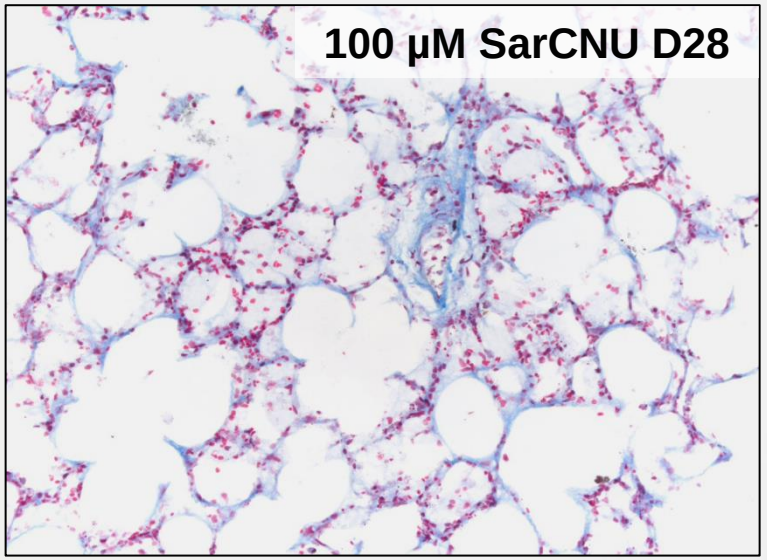
- biomarker content
- numbers of AM
- collagen deposition

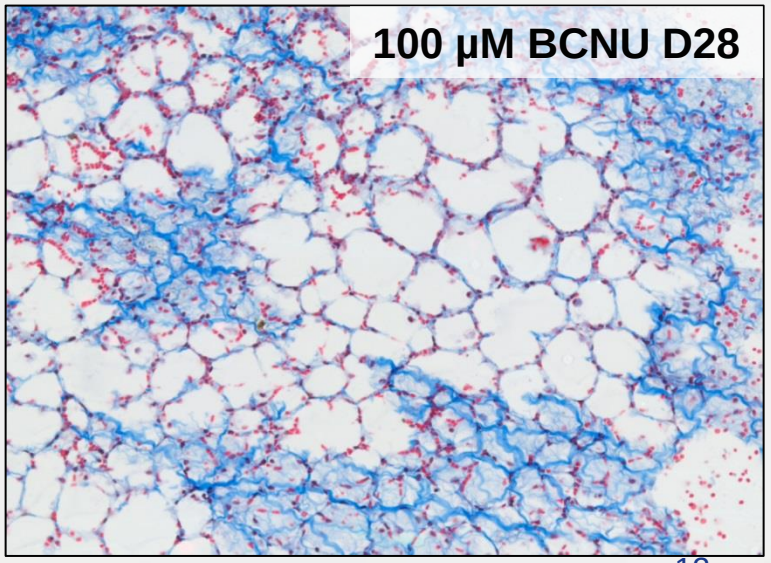
Activated Macrophages in Lung Slices				
		Counts [a]		
Compound	µM	Day 6-7	Day 14	Day 28
Vehicle	0	52	48	59
BCNU	1	60	94**	-
	10	67*	71*	-
	100	66	102**	81*
SarCNU	1	56	52	-
	10	61	57	-
	100	69*	76*	71

[a] Means of 3 measures on 3-4 replicates
* p<0.05 ** p<0.01

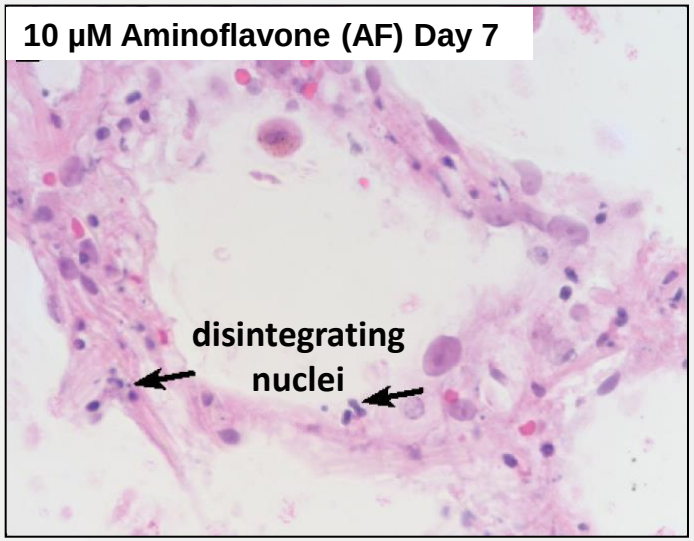


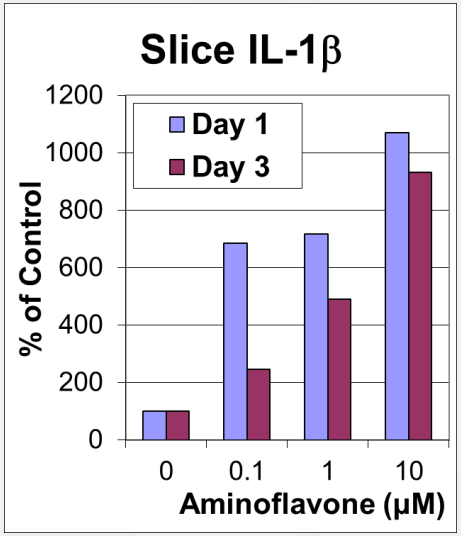






Human Lung Slices: Parenchymal Damage





Concentration-depend. IL-1β increases

Aminoflavone Exposure

- Control tissue shows alveoli lined by mostly viable cells
- Exposure of human PCLS to 10 µM AF causes cytokine increases in < 24 hr
- Days later, severe tissue damage was noted: AF-induced, decreased cellularity and nuclear changes reflecting toxicity

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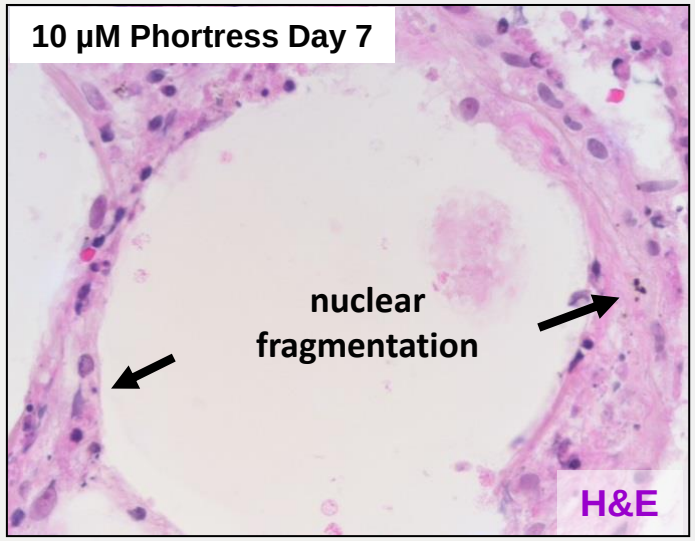
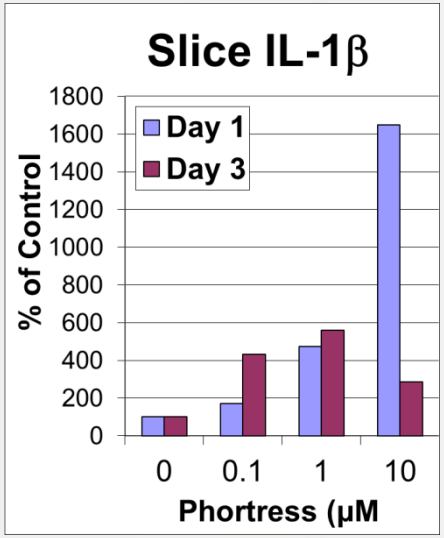
Human Lung Slices: Parenchymal Damage





Concentration-depend. IL-1β increases

Phortress Exposure

- Control tissue shows alveoli lined by mostly viable cells
- Cytokine (IL-1β) increase at Day 1 precedes traditional LDH content changes (not shown) and histology results showing damage at Day 7
- Severe injury to the lining pneumocytes and possibly other cells as indicated by nuclear fragmentation and marked decreased alveolar wall cellularity

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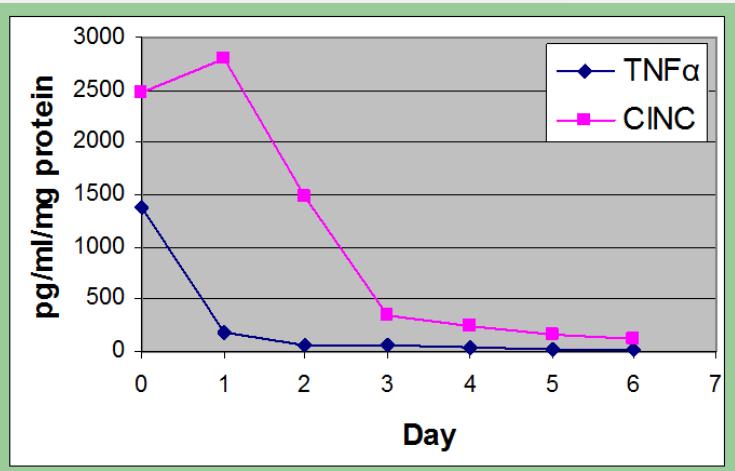
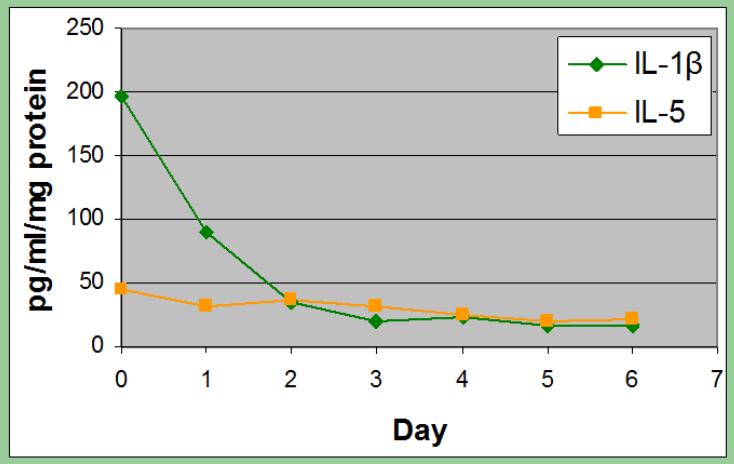
PCLS Method Refinement for Cytokine Assay

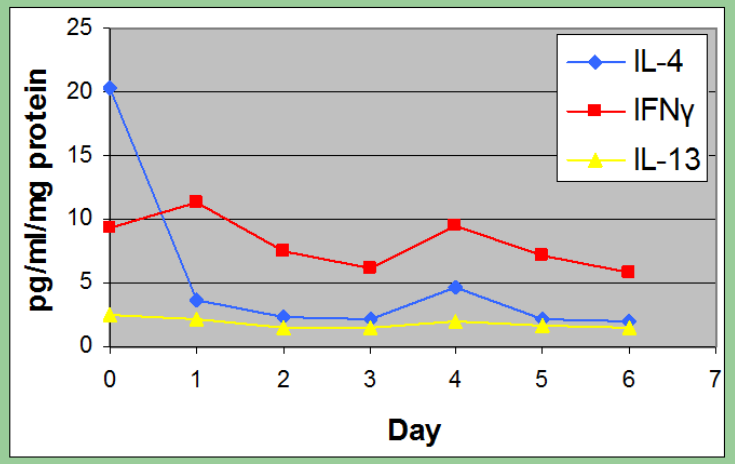
- NOTE: recent studies indicate the process of creating slices (mechanical disruption of lung tissues (coring, slicing, etc.) results in cytokine induction

CINC/GRO > TNF-α > IL-1β > IL-5

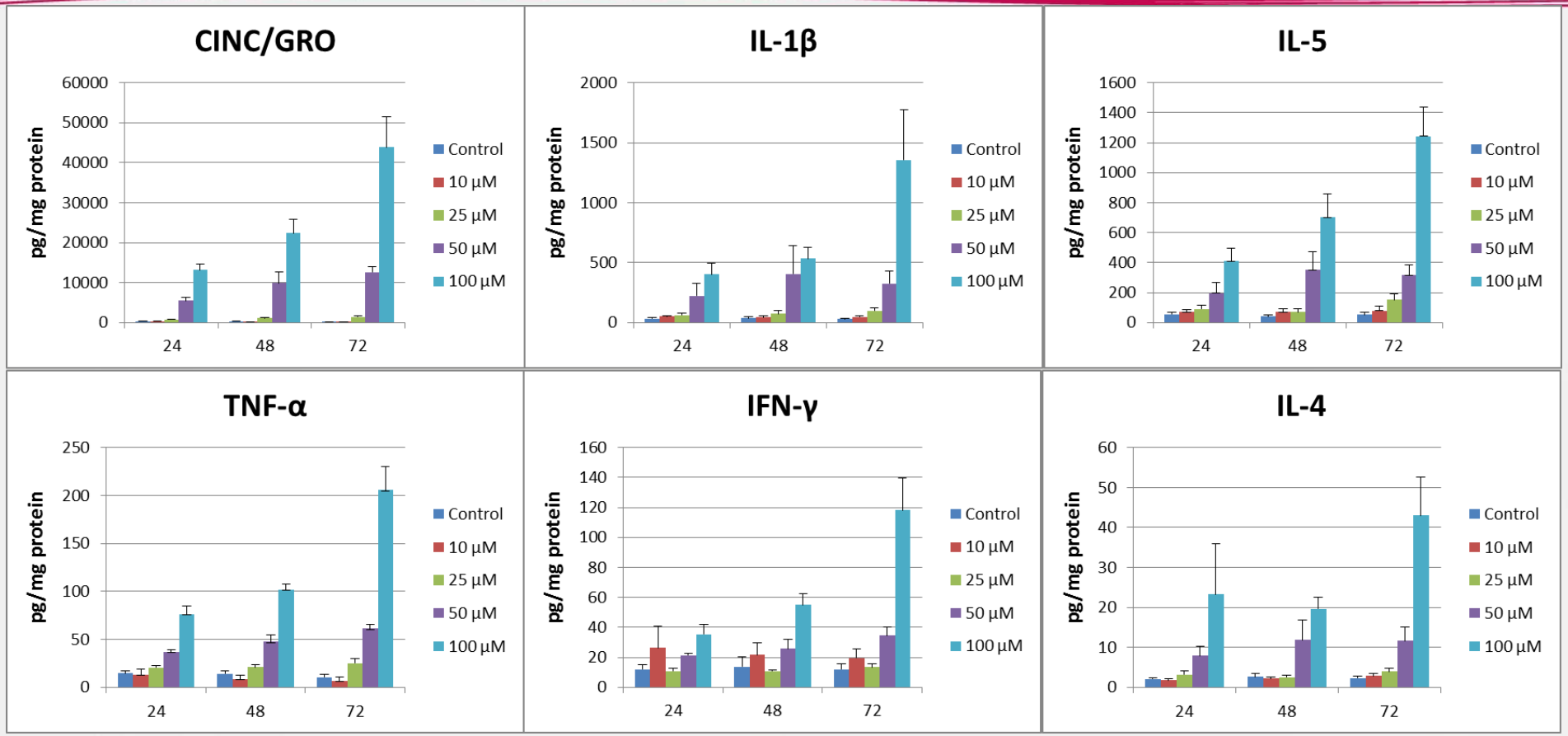
- Despite inclusion of 0.1 µg/ml hydrocortisone, slice production generates cytokine response
  - Most notable in terms of change are TNFα, CINC,IL-1β, & IL-4
- By day 2-3, we observe a substantial decline in cytokine levels
- Cytokine elevations in medium coincide with tissue responses







Phortress Exposure: Cytokine Induction



- 24hr, 48hr, and 72hr exposure to Phortress results in large increases in tissue cytokines and the chemokine KC/GRO. (IL-13 not pictured)
- ELISA based changes in IL-6 (~10x) and TGF-β (10x) also measured (not pictured)

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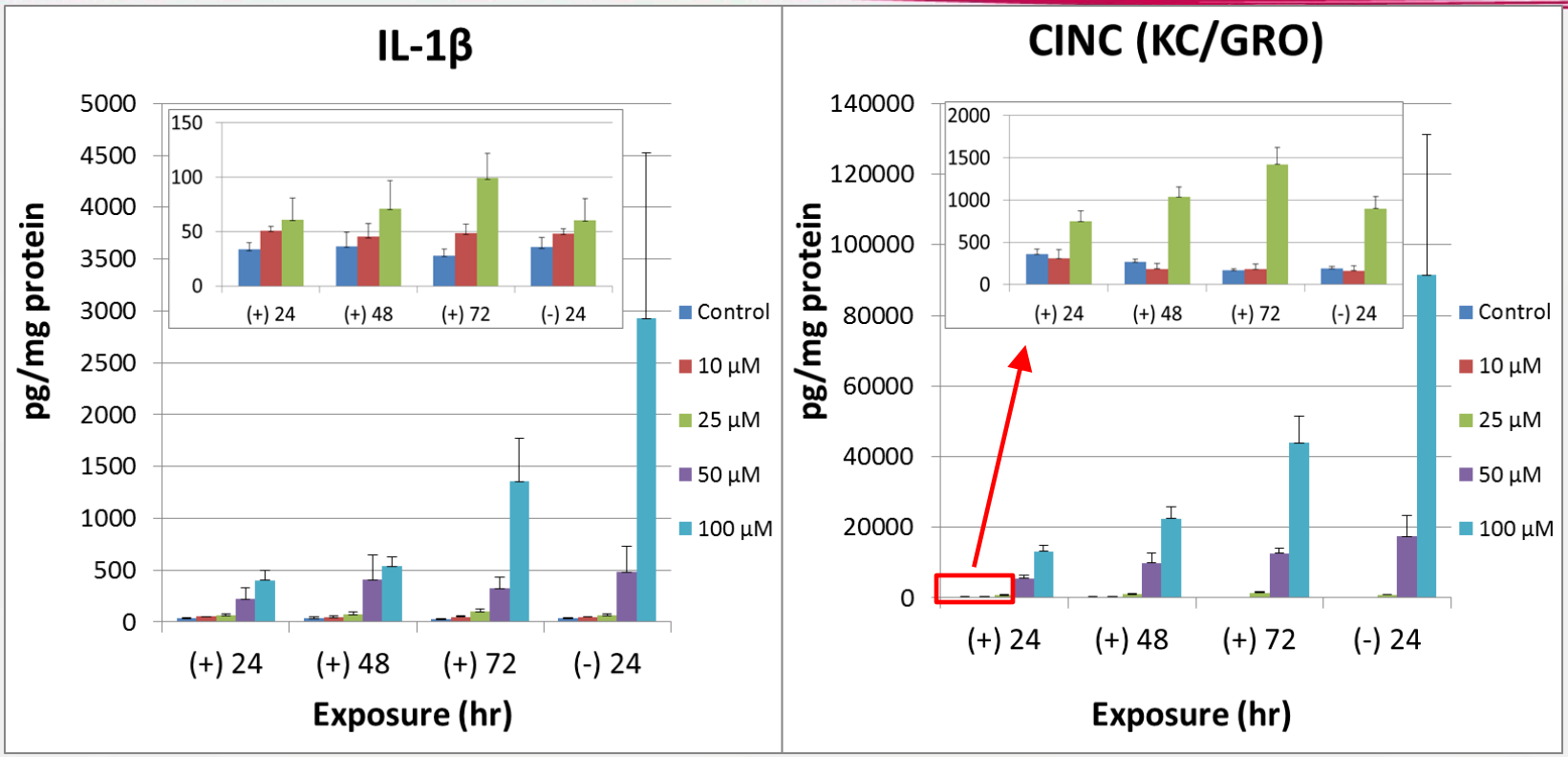
Reversibility of Cytokine Induction



- Initial cytokine/chemokine increases of PCLS treated with 25 µM subside after Phortress removal
- Despite removal of drug, C/C levels continue to increase in PCLS treated with 50 and 100 µM Phortress

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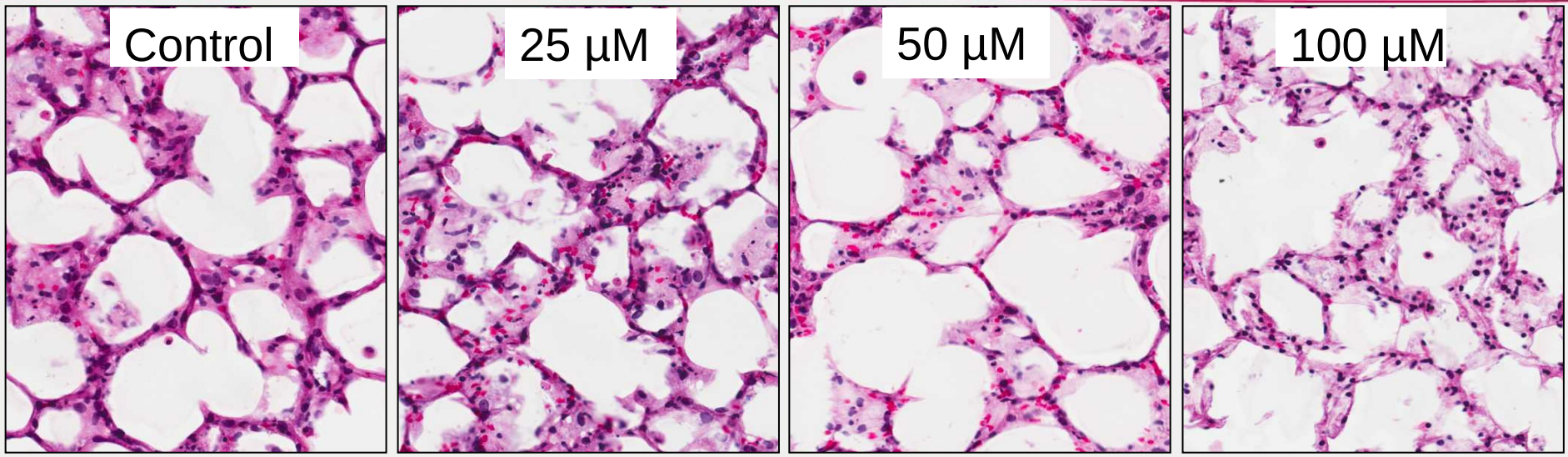
Establishment of No Effect Level: Phortress



		Protein
	Time point	mg/ml
Control	72hr Exposure	1.17
	24hr Recovery	1.01
10 µM	72hr Exposure	1.02
	24hr Recovery	1.01
25 µM	72hr Exposure	0.97
	24hr Recovery	0.97
50 µM	72hr Exposure	0.68
	24hr Recovery	0.55
100 µM	72hr Exposure	0.28
	24hr Recovery	0.25

- Phortress at 10 µM (not shown) has no effect while 25 µM shows minimal evidence of toxicity histologically.
- With 50 and 100 µM Phortress, PCLS show decreased cellularity; cells lining the alveoli display pyknotic nuclei

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PCLS as a Model for COPD Etiology Events

PCLS as a Model for COPD Etiology Events

Initiating event:

Tobacco exposure or other toxic insult to lung epithelium

1. Ligand-receptor interactions

2. Intracellular response

2. Oxidative stress

3. Initiation of autocrine, paracrine, and endocrine signaling

4. Cellular damage

Tissue Response:

1. Cytokines/chemokines

2. Increased integrin and adhesion molecule expression

3. Monocyte recruitment (persistent influx of neutrophils)

4. Protease/antiprotease imbalance

5. Adverse cellular ion homeostasis-dehydration

6. Oxidative stress

7. Inflammation

Tissue Effects:

1. Ciliary dysfunction

2. Increased mucous secretion

3. Fibroblast activation

4. Goblet cell hyperplasia

5. Bronchial epithelial squamous metaplasia

6. Narrowing of airways

7. Collagen deposition

8. Parenchyma/tissue destruction

10. Injury/repair cycling

Pulmonary Effects:

1. Reduced lung elasticity

2. Reduced airflow

3. Airspace enlargement

4. Small airway remodeling

5. Vascular remodeling

5. Hyperinflation

6. Chronic inflammation

7. Fibrosis

Clinical manifestations:

1. Chronic bronchitis

2. Emphysema

3. Small Airways Disease

4. Increased susceptibility to infection and air pollutants

COPD:

Progressive (usually) airflow limitation in airways/lungs due to noxious particles or gases and associated with inflammatory response

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Summary of PCLS as Model for COPD

- The native architecture, amenability for long term culture, and heterogeneity of cell types (including those centrally involved in inflammatory events) make PCLS attractive for examining complex pulmonary changes
- The ability to obtain human donor lungs for PCLS studies will avoid use of animals and obverts cross-species extrapolation
- Biomarker endpoints evaluated in PCLS are also involved in COPD etiology events
  - These have been used to determine or evaluate 1) no effect level, 2) detailed histopathological changes, 3) induction of proinflammatory biomarkers 4) reversal of inflammatory signals after removal of insult, and 5) retention of standard biochemical markers of toxicity
- The stage is set to evaluate PCLS as a model to detect and quantify tobacco smoke exposures and other markers of COPD

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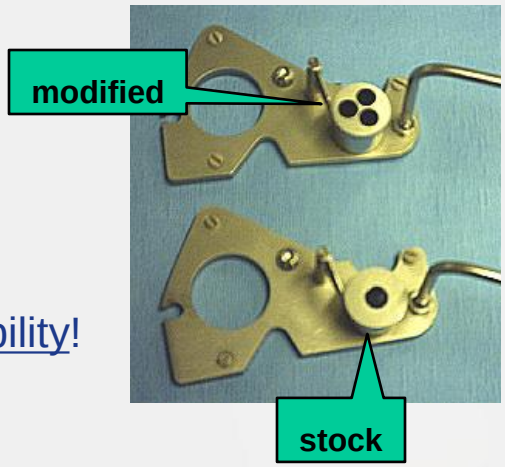
Position PCLS into Mainstream Research

- ~50 years have elapsed between Warburg's first use of slices and the invention of the precision cut slicer
- The Krumdieck slicer was first introduced ~30 years ago with no significant changes made since

- **Areas for improvement:**
  - Hardware/engineering changes can (or already have) benefit several key areas:
    - Rate of slice production
    - Experimental capacity
    - Exposure of PCLS to gases (whole smoke)
  - Tissue utilization and storage
    - Utilize more tissue from donor source
    - A key setback for PCLS is the lack of cryo-storage capability!
  - Tissue Source
    - Better quality human tissue
    - Increase donor pool/availability (to increase frequency of usable tissue)

Krumdieck Slicer



Acknowledgments and References

Thank You!

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Selected References:

- Precision-cut Lung Slices (PCLS), Christian Martin and Stefan Uhlig, Chapter 6., *Replacing Animal Models: A Practical Guide to Creating and Using Culture-based Biomimetic Alternatives* ,edited by Jamie Davies John Wiley & Sons – Publisher (2012)
- Behrsing, H. P., et al. In vitro exposure of precision-cut lung slices to 2-(4-amino-3-methylphenyl)-5-fluorobenzothiazole lysylamide dihydrochloride (NSC 710305, Phortress) increases inflammatory cytokine content and tissue damage. *Toxicol Sci* 131, 470-9. (2013)

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