Nasdaq: CBIO

CATALYST BIOSCIENCES

ARDS summit, July 13th 2021

Complement System in ARDS

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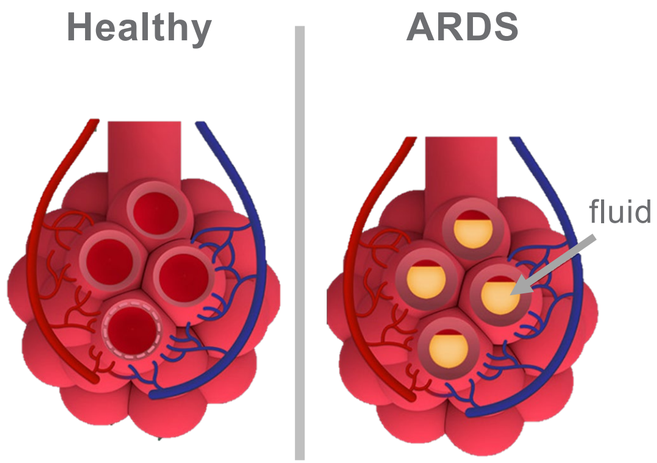
Target the complement cascade to maximize the success of your ARDS therapeutic

- ARDS: unmet need after half a century of research
- Complement involvement in ARDS
- Challenges in developing complement therapeutics for ARDS patients
- Shifting the paradigm of preclinical models
- Translational research at Catalyst Biosciences: “CFB degraders”

Acute Respiratory Distress Syndrome (ARDS)

A potentially fatal respiratory condition with serious lung injuries



+ **~200k cases** annually in the United States
+ **~40-50%** of hospital mortality, majority of deaths occurring within the first few weeks of disease onset
+ **~10%** of ARDS patients will need permanent renal replacement therapy
+ **~10%** of intensive care unit admissions worldwide
+ Significant burden on healthcare resources with an estimated cost of **$20 billion** every year in the US

Toner et al. Critical Care (2015)

Acute Respiratory Distress Syndrome (ARDS)

A potentially fatal respiratory condition with serious lung injuries

Major risk factors

- Sepsis
- Pneumonia
- Trauma injury
- COVID-19

Treatment focused on general supportive care

+ mechanical ventilation
+ supplemental oxygen
+ fluid management
+ positive expiratory pressure (PEEP) to help push the fluid out of air sacs
+ Medication to manage symptoms (antibiotics, corticosteroids, diuretics…)

Multiple pharmacotherapies evaluated but no clear benefit

Corticosteroids, Surfactants, β2-adrenergic agonists, Prostaglandin E1, Activated protein C, Antioxidants (NAC), Omega-3, Ketoconazole, Recombinant hFVIIa, GMCSF, Statins, Interferon beta, Vitamin C…

Pham et al.. American Journal of Respiratory and Critical Care Medicine (2017)

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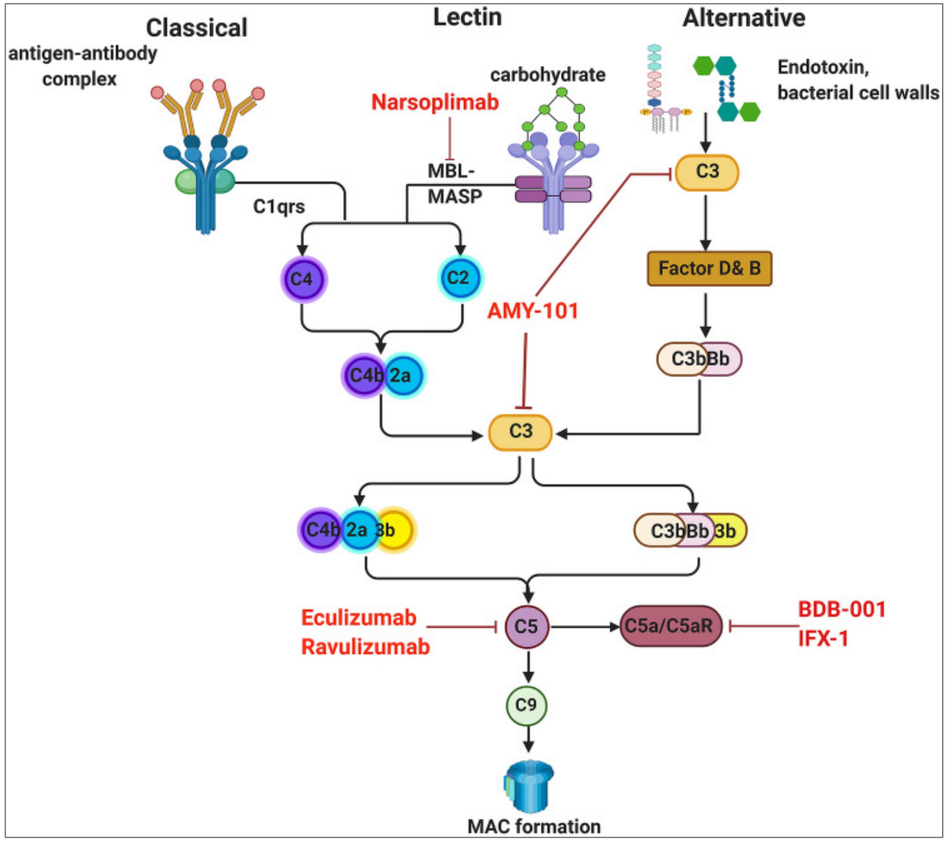
Complement system activation – a pathogenic factor in ARDS

Complement inhibitors decrease lung inflammation in COVID-19 patients with ARDS

+ **Complement inhibition in COVID-19 patients with ARDS**: decrease systemic inflammation (AMY-101, IFX-1) recovery after severe ARDS cases (Eculizumab) and improved clinical inflammation indicators and recovery (Narsoplimab)[1].
+ **Serum/plasma**: upregulation of both the classical complement pathway (C1R, C1S, and C8A), the alternative pathway CFB, the complement modulators CFI and CFH, the MAC proteins such as sC5b-9, C5, C6, and the anaphylatoxins C5a and C3a[1].
+ **Lung tissue**: upregulation of complement proteins (MBL, MASP2, C4a, C4d, C3, and MAC C5b-9) seen in post-mortem lung tissues from COVID-19 patients[2, 3].



[1] *Li et al.. Medical Microbiology and Immunology (2021)*
[2] *Magro C et al. Transl Res (2020)*
[3] *Ting Gao et al medRxiv (2020)*

Complement system activation in ARDS patients

Conflicting observations due to heterogenous population and measurement methods

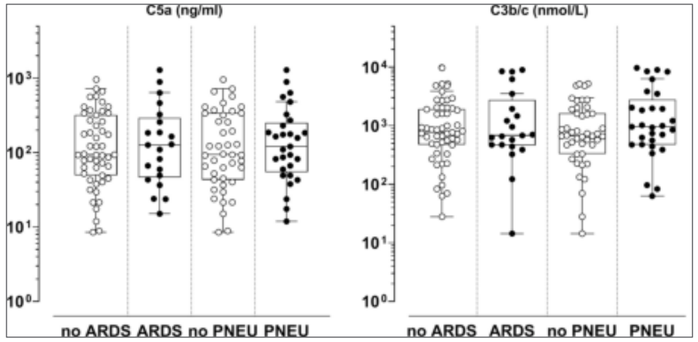
+ Lower C3 in patients with severe ARDS associated with death[1]

+ Higher C3a in trauma patients predisposed to ARDS[2]

+ No changes in C5a/C3b patients under invasive ventilation[3]

	Total	Mild	Moderate	Severe
# Patients	201	31	61	109
C3	**5.62** ± 1.78	**12.27** ± 1.83	**7.13** ± 3.14	**0.84** ± 0.33
C4	**1.33** ± 0.58	**0.25** ± 0.13	**0.24** ± 0.11	**2.25** ± 0.95

Time after admission	+ ARDS Median	+ ARDS Range	− ARDS Median	− ARDS Range	*P*
0 h	10·4	0–54·7	7·9	2·1–74·1	0·835
6 h	15·0	4·9–36·2	8·0	2·0–20·0	0·007*
12 h	10·8	3·4–23·2	6·3	2·8–14·3	0·047*
24 h	6·6	2·5–26·7	4·9	2·7–30·0	0·404
48 h	8·0	4·2–31·6	6·9	3·0–20·8	0·482
5 d	12·1	6·1–44·4	6·1	2·3–14·7	0·004*
7 d	19·5	5·1–44·4	6·9	4·8–17·6	0·003*
9 d	15·5	8·3–48·8	9·9	5·4–24·4	0·029*
11 d	17·1	7·7–39·2	7·1	3·8–26·7	0·008*
13 d	20·6	9·2–34·5	6·8	4·8–30·8	0·020*



Clarify complement role with the identification of patient subpopulations based on:

- ARDS severity
- Inflammatory markers such as anaphylatoxins
- Supportive care choice
- Standardized methods to measure complement components

[1] *Song et al. BMC Pulmonary Medicine (2020)*
[2] *Zilow et al. Clin. exp. Immunol. (1990)*
[3] *De Beer et al. Intensive Care Medicine Experimental (2020)*

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ARDS treatments – a double edge sword

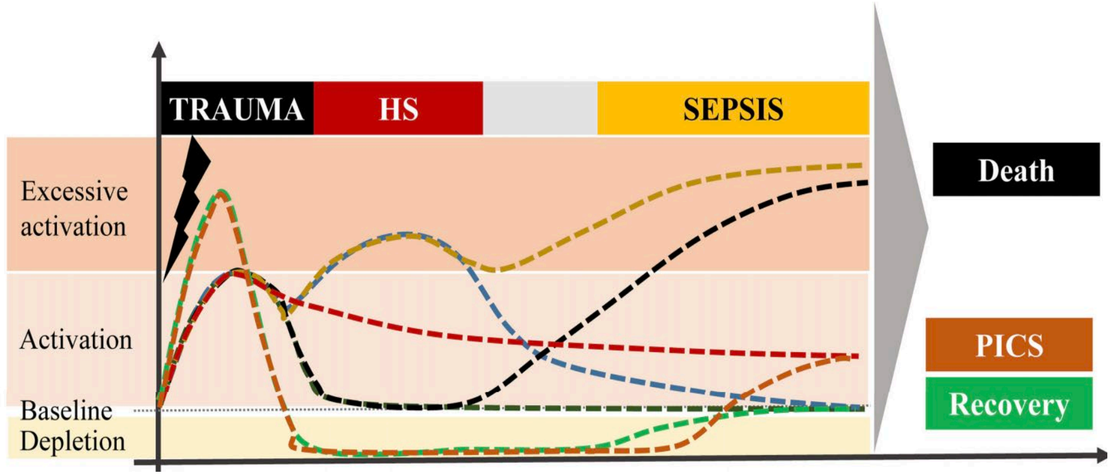
Therapeutic interventions to stabilize patients lead to dysregulated complement

Early therapeutic approaches driving complement dysregulation[1]

TRAUMA

Scene	ER	OR	ICU	Pathophysiology	Complement
Intubation/ventilation				ROS	Activation
Volume resuscitation				Hyperdilution	Depletion
Transfusion of blood products				Feed in of complement	Anaphylatoxins
Instrumentation/monitoring				Artificial surfaces	Activation
Tranexamic acid				Plasmin inhibition	Activation, C5a↓↑
	Surgical damage control			Additional tissue injury	Activation, depletion
	Osteosynthesis			Artificial surfaces	Activation
	Haemodialysis/ECMO			Artificial surfaces	Activation, depletion

Potential complement responses after trauma[2]



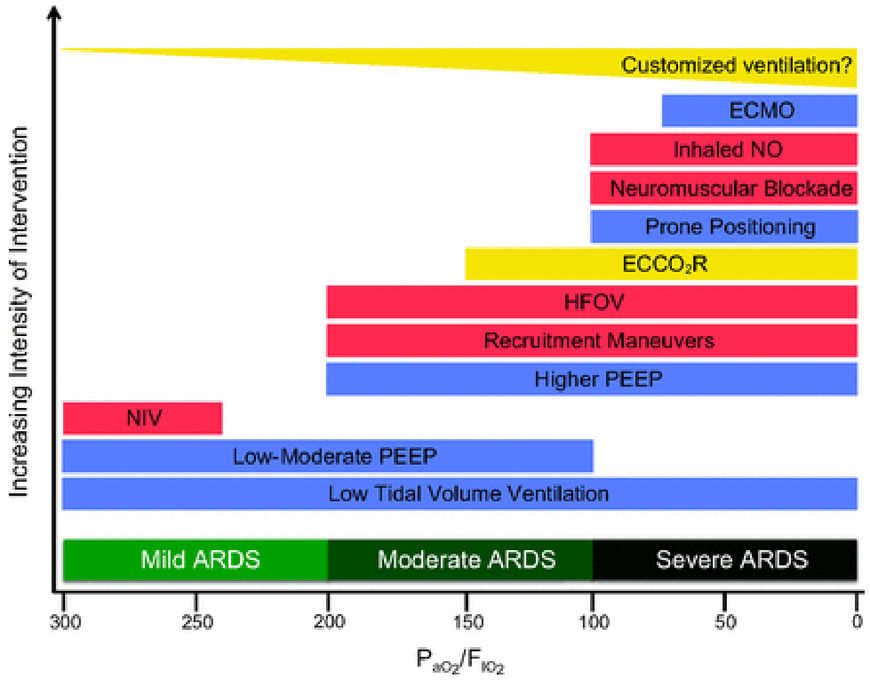
- Which complement target?
- When to start treatment?
- How long to keep treating?
- Which patients?
- Which biomarkers to use?

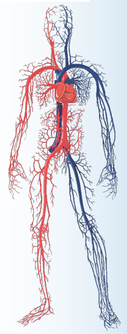
[1] *Huber-Lang et al. Br J Pharmacol. (2021)*
[2] *Karasu et al. Front Immunol (2019)*

Challenges in ARDS therapeutics targeting complement

Early recognition of severe ARDS is key for optimal treatment strategy

Potential interventions for management of ARDS





Improve clinical understanding of ARDS

+ Identify “treatment responders”
+ Develop specific tools to diagnose severe ARDS
+ Dedicate specialized teams/protocols for patients with severe ARDS
+ Identify failure of the initial treatment strategy in early management
+ Identify surrogate endpoints in addition to mortality

Develop “new” animal models

Ratano & Fan. Respiratory Care (2020)

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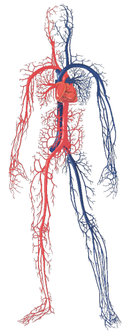
Develop animal models “as clinically relevant as possible”

Not such a basic concept in ARDS

+ **Interspecies differences:**
  - Lung anatomy
  - Innate and adaptive immune responses
  - Histopathological hallmarks of lung damage
+ **Paradigm of ARDS animal models:**
  - Lack of co-interventions and organ support
  - Lack of pre-existing lung injury
  - Pretreatment or peri-injury therapeutic approaches
  - Lack of translatable endpoints

Translational challenges



Clinical situation

+ Heterogenous clinical population
+ Difficult patient stratification
+ No ARDS specific biomarker for diagnosis
+ Unclear timing of treatment vs clinical evolution of ARDS
+ Challenges in trial designs (sample size, blinded, endpoints…)

Toward a paradigm shift in ARDS preclinical models

Similarities between stroke and ARDS translational research

Preclinical recommendations in Stroke (STAIR)

- Multiple models and species including large ones
- Comparison with SoC
- Aged animals with co-morbid conditions
- Delayed therapeutic treatment & late endpoints
- Randomized & blinded

Preclinical progress in ARDS models

+ Multiple models: ‘high throughput’ model (LPS) & by ‘clinically relevant’ models (two-hits)
+ Large animal models with imaging, long-term endpoints, co-interventions and organ support
+ Comparison with failed therapeutics to benchmark new drug
+ Avoid pretreatment or peri-injury approaches to prove benefit

Genetic depletion of complement components in animal models

Not all pathologies will benefit equally from complement depletion

Genotype	Mouse model	Conclusions [1, 2, 3, 4]	Benefit
WT	SARS-CoV infection	Elevated C3 activation products	-
C3 $^{-/-}$	SARS-CoV infection	Partial reduction of respiratory dysfunction, immune cell infiltrations and cytokine response	+
CFB $^{-/-}$	SARS-CoV infection	Lower weight loss	+
C4 $^{-/-}$	SARS-CoV infection	Lower weight loss	+
MASP2 $^{-/-}$	S. pneumoniae infection	Increase mortality rate	-
C1q $^{-/-}$	S. pneumoniae infection	Increase mortality rate	-
C4 $^{-/-}$	S. pneumoniae infection	Increase mortality rate	-
CFB $^{-/-}$	S. pneumoniae infection	Increase mortality rate	-
C3 $^{-/-}$	S. pneumoniae infection	Increase mortality rate	-
C3 $^{-/-}$	LPS infusion	No effect	n/a
C5 $^{-/-}$	LPS infusion	No effect	n/a

+ Dissect the role of complement activation products (e.g., C3a, C3b, iC3b, C3dg, C4a, C4b, iC4b) to identify therapeutic targets
+ Characterize the role of cofactors (FH, FI, DAF) and receptors (C3aR, C5aR, CR1) in specific cell types and organs to guide disease-tailored complement intervention

[1] *Gralinski et al. mBio (2018)*
[2] *Ali et al. PLOS Pathogens (2012)*
[3] *Brown et al. PNAS (2002)*
[4] *Rittirsch et al. J. Immunol (2008)*

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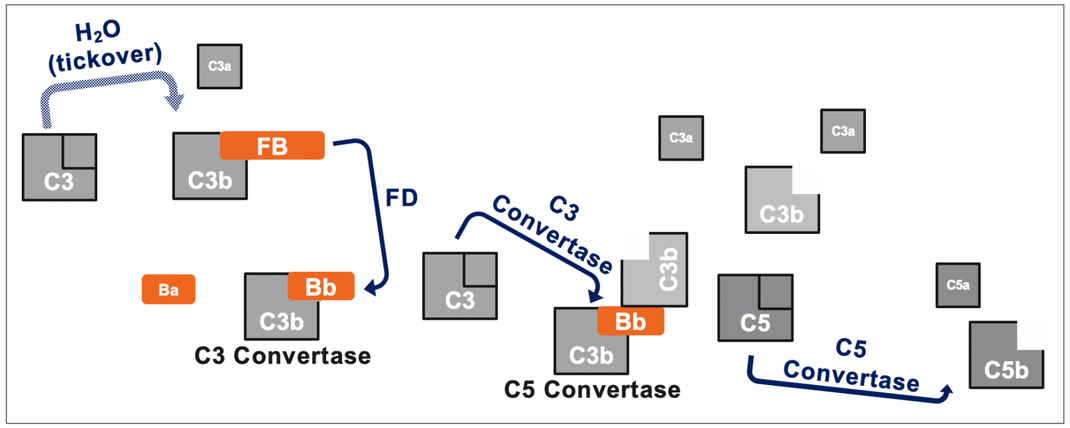
Gold standard assay to evaluate complement therapeutics

Hemolysis assay principle

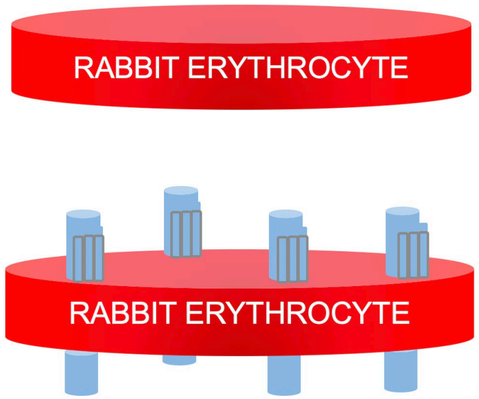
1 Serum pre-incubation

C3 and C5 convertase formation drives C5b accumulation



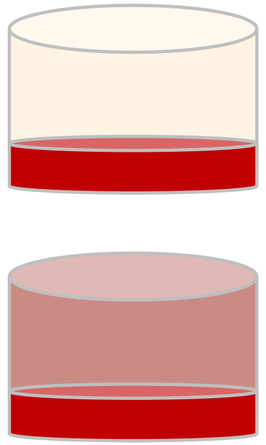
2 Add serum to RBC

Accumulated components drive C5b-C9 membrane attack complex (MAC) formation



3 RBC lysis

Measure released hemoglobin



No lysis

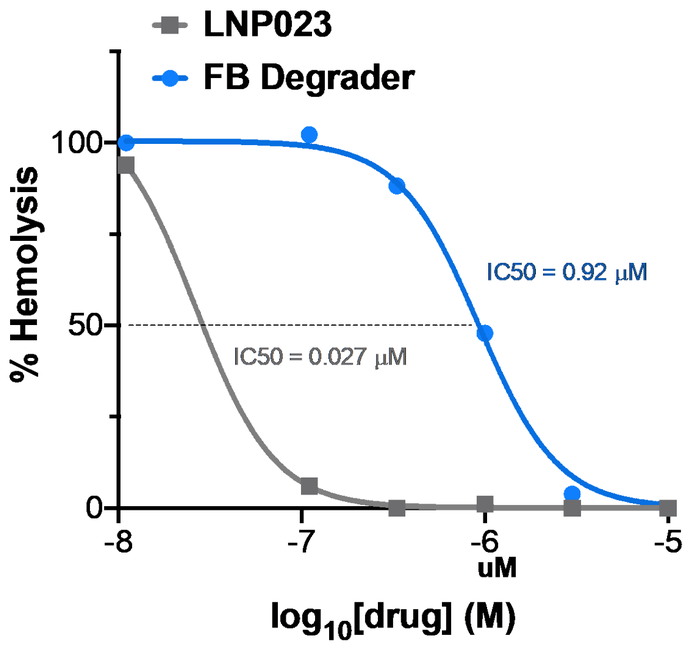
Rabbit red blood cells express rabbit complement cofactors and receptors

Blom et al. Clinical and Experimental Immunology (2014)

Standard and modified alternate pathway hemolysis assays

Comparison of CFB degrader vs LNP023 in two hemolysis assays

Standard AP Hemolysis Assay (10 min)



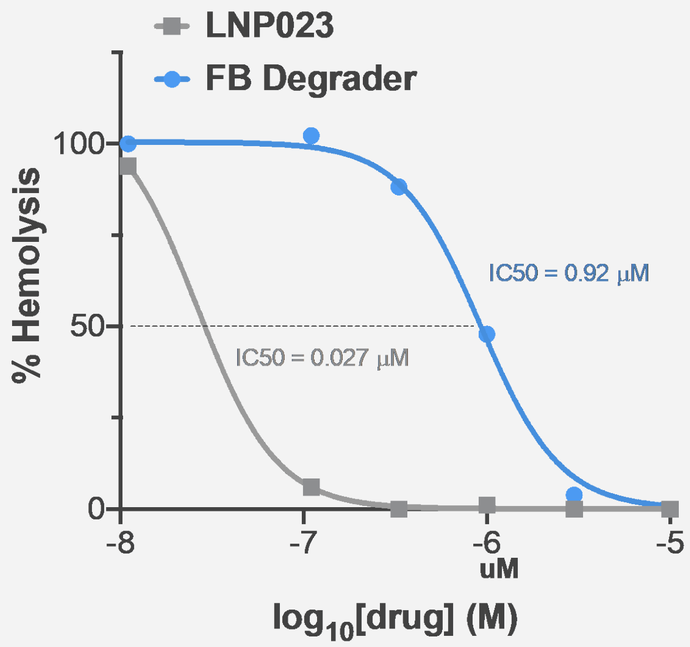
- **In an acute setting, LNP023 potency in hemolysis assay is comparable to literature findings[1]**

[1] *Schubart et al. PNAS (2019)*

Standard and modified alternate pathway hemolysis assays

Comparison of CFB degrader vs LNP023 in two hemolysis assays

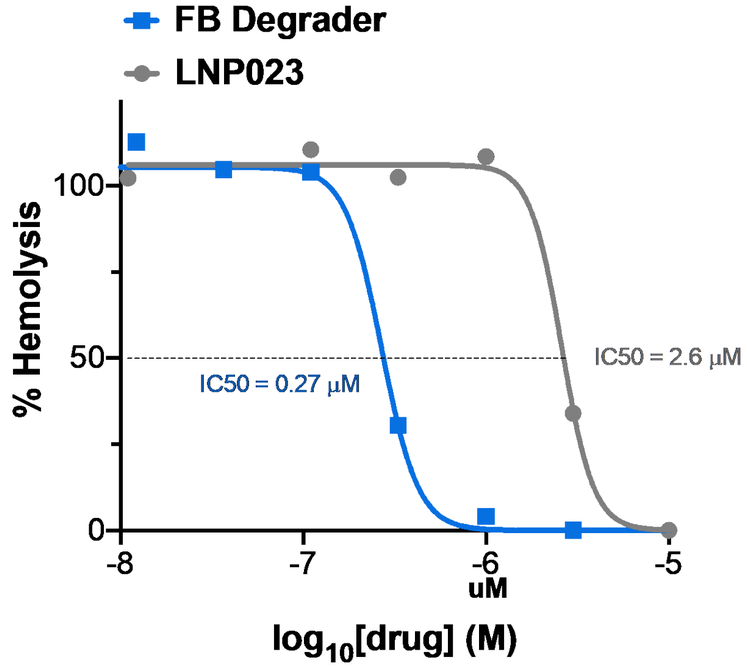
Standard AP Hemolysis Assay (10 min)



~16-fold increase in **CFB**

+ increase incubation time

"Modified" AP Hemolysis Assay (180 min)



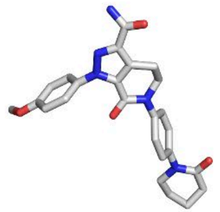
Drug : CFB ratio → **C5b9 accumulation**

- **LNP023 potency is markedly reduced from 10 to 180 minutes due to 16-fold increase in target (Bb)**
- **FB degrader potency is independent of target concentration and incubation duration**

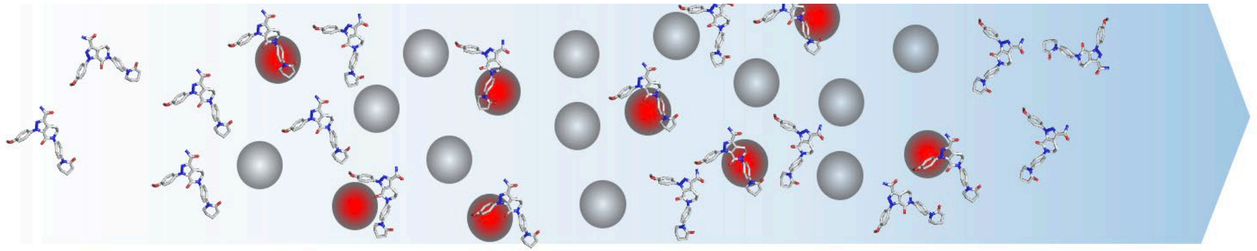
Proteases are ideal for high abundancy targets & cascades

A better way to regulate biological processes compared with small molecules

Small molecules



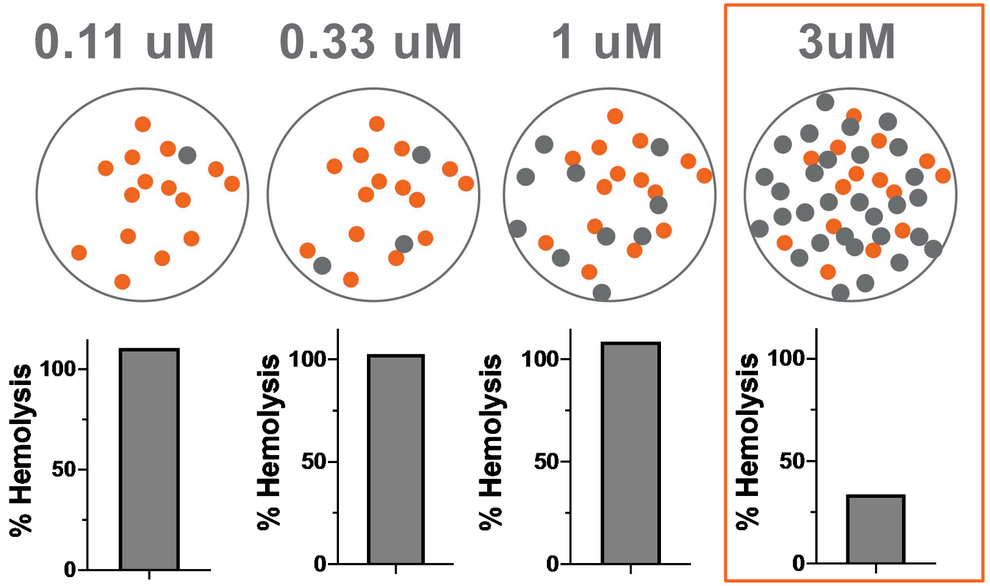
Therapeutic target neutralization



Requires high concentrations & frequent dosing

1.5 uM CFB target (180 min)

LNP023:

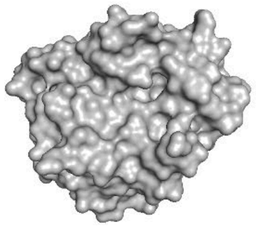


Hemolysis inhibition when LNP023 is 2-fold higher than CFB target

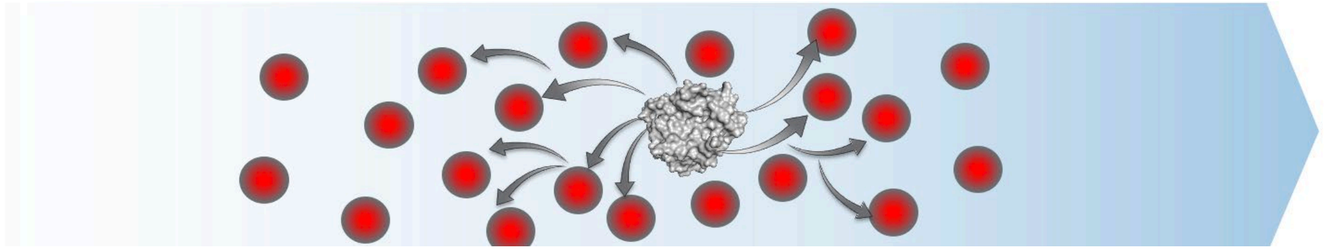
Proteases are ideal for high abundancy targets & cascades

A better way to regulate biological processes compared with small molecules

Protease therapeutics



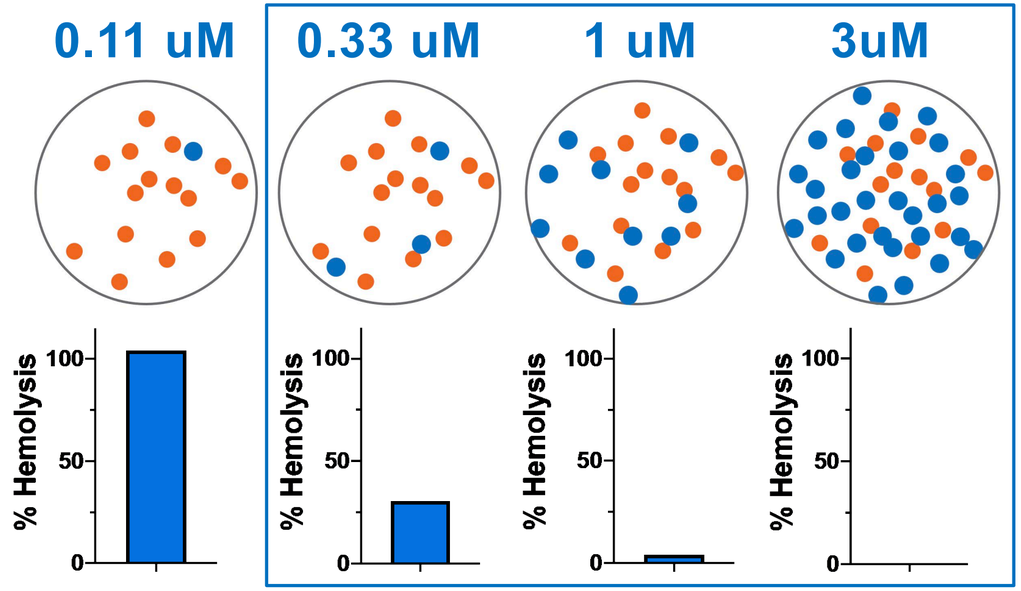
Therapeutic target neutralization



Efficient regulation at low concentrations of therapeutic protease

CFB degrader:

1.5 uM CFB target (180 min)

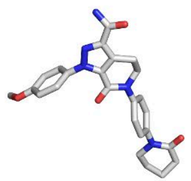


Hemolysis inhibition when CFB degrader is 3-fold lower than CFB target

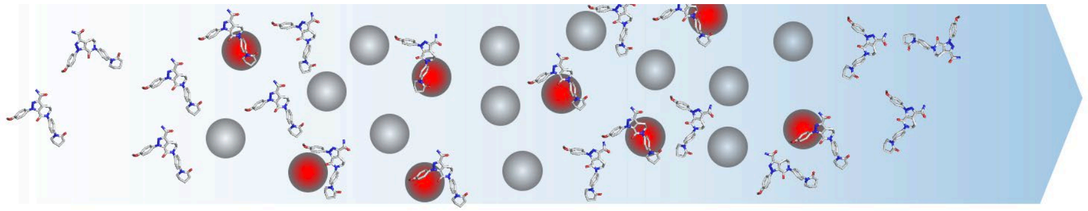
Proteases are ideal for high abundancy targets & cascades

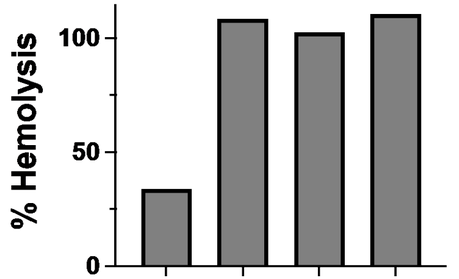
A better way to regulate biological processes compared with small molecules

Small molecules



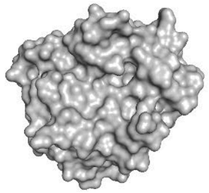
Therapeutic target neutralization



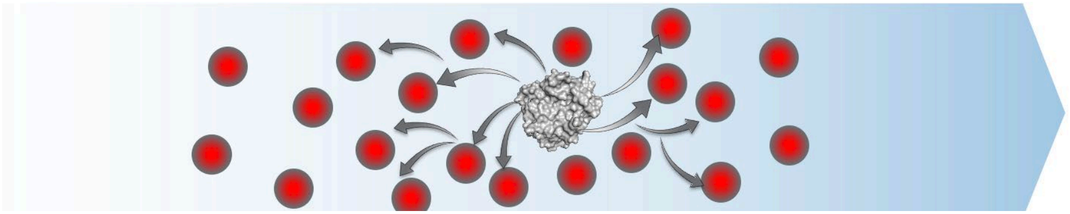


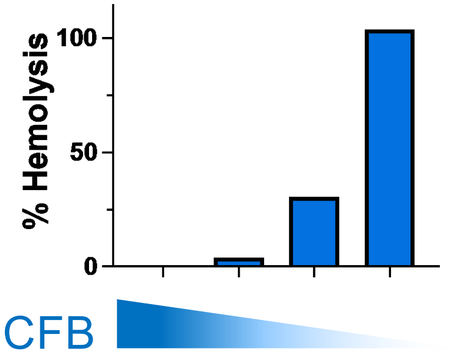
Requires high concentrations & frequent dosing

Protease therapeutics



Therapeutic target neutralization





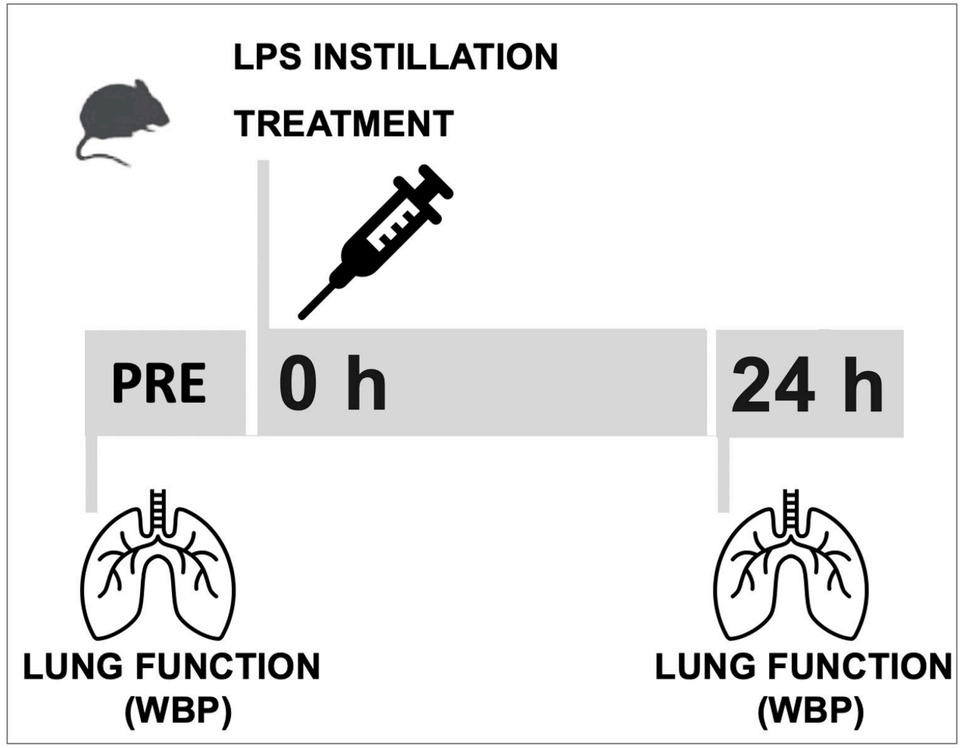
Efficient regulation at low concentrations of therapeutic protease

+ CFB degraders offer potential advantages over small molecules to efficiently sustain low levels of CFB over time to prevent complement activation in patients at risk of developing ARDS

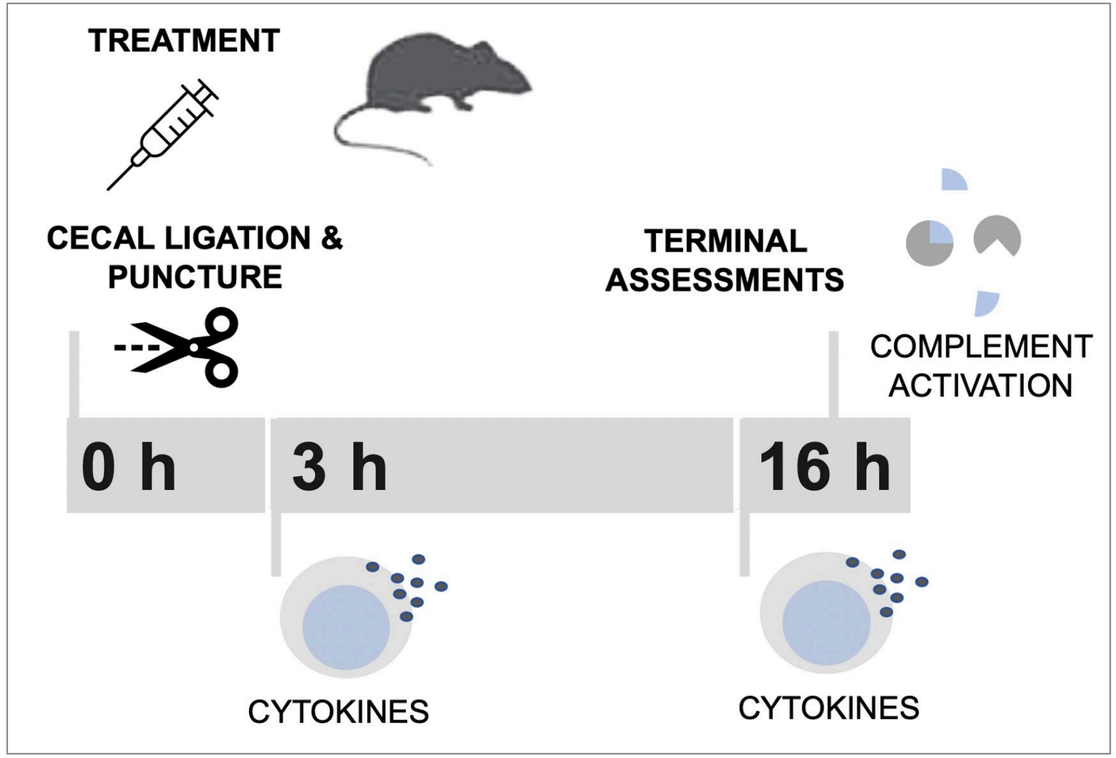
Iterative translational approach to screen for complement therapeutics

"High throughput" screening in acute models of complement activation

Mouse LPS instillation



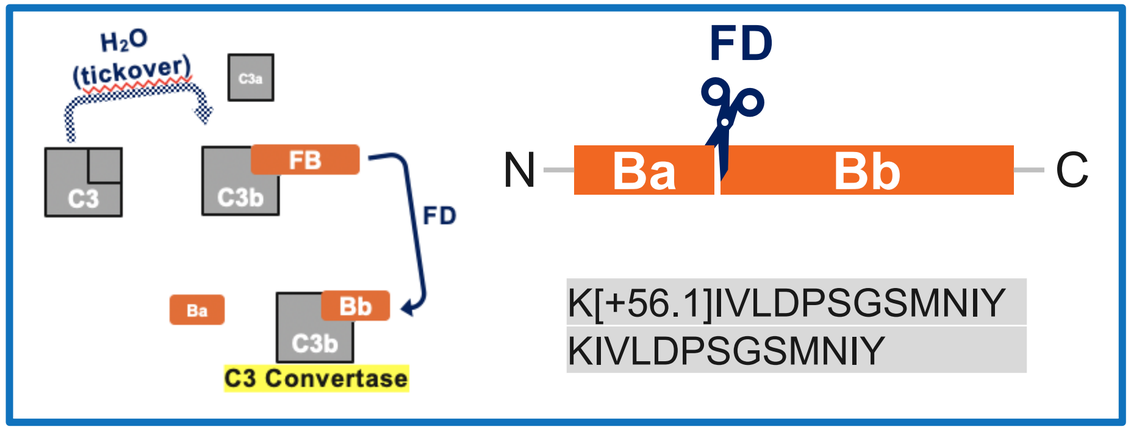
Rat microbial sepsis (cecal ligation puncture)



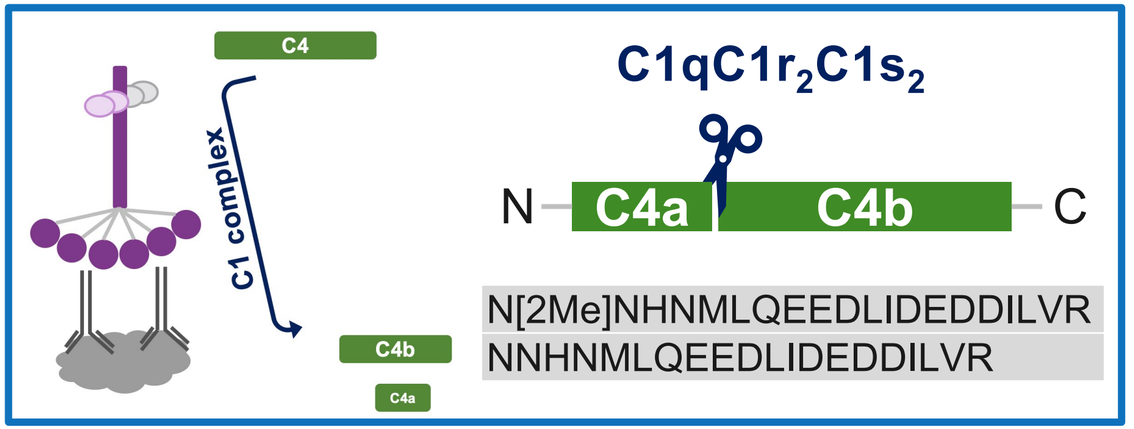
Mass spectrometry enables detection of complement fragments

Measure classical & alternate pathway activation with one method

Alternate pathway (Bb fragment)



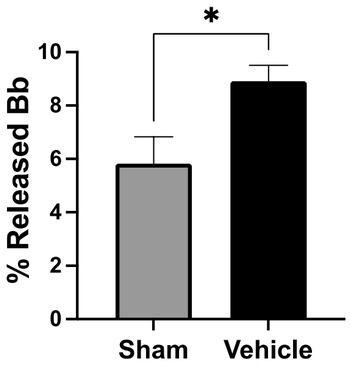
Classical and lectin pathways (C4b fragment)

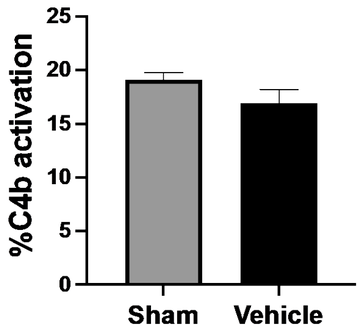


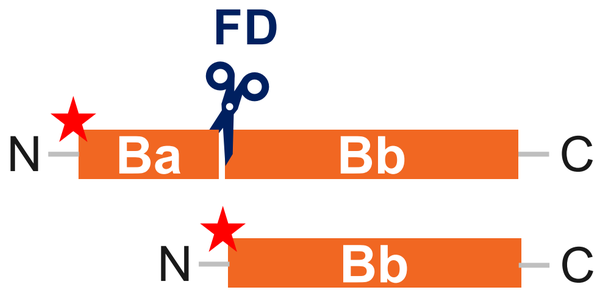
Mass spectrometry in LPS-induced ARDS & microbial sepsis

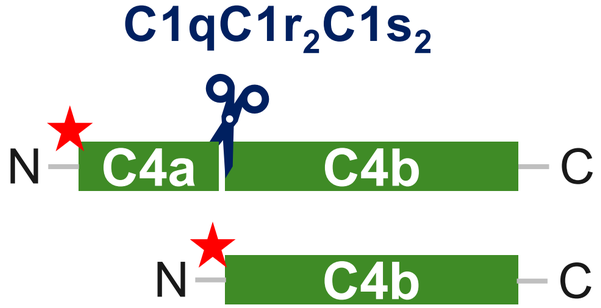
Differences in classical & alternate pathway activation in two models

LPS instillation

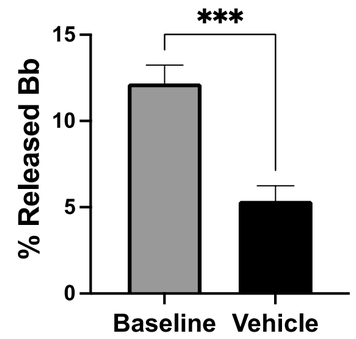


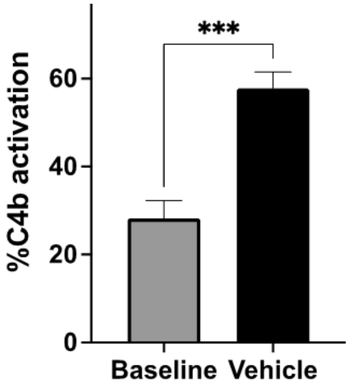






Microbial sepsis





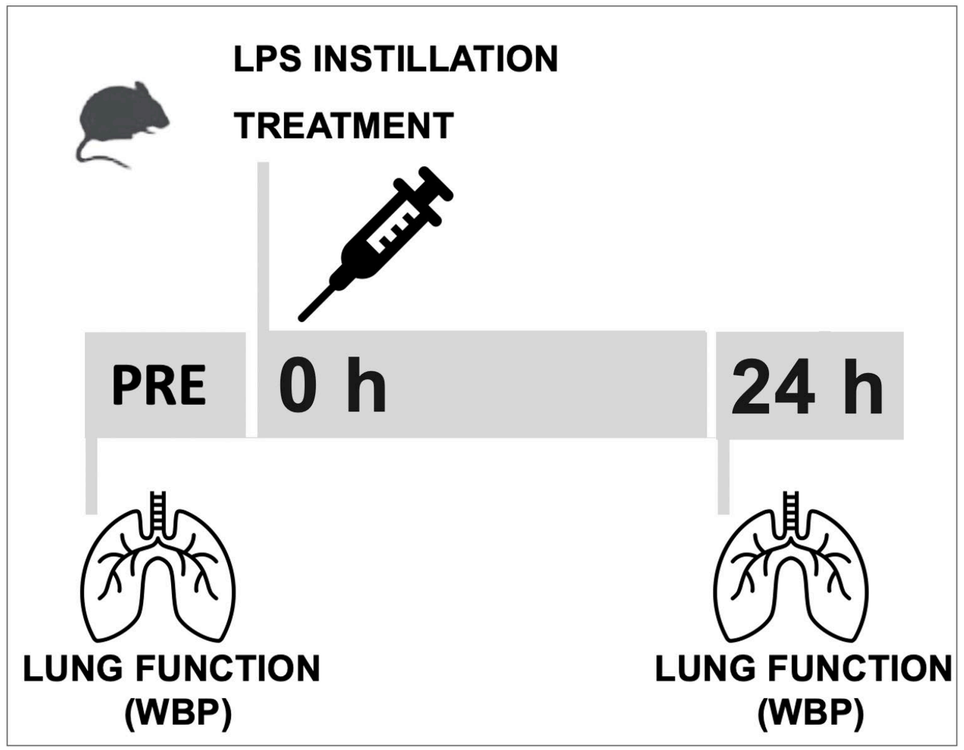
Alternate pathway activation

Classical pathway activation

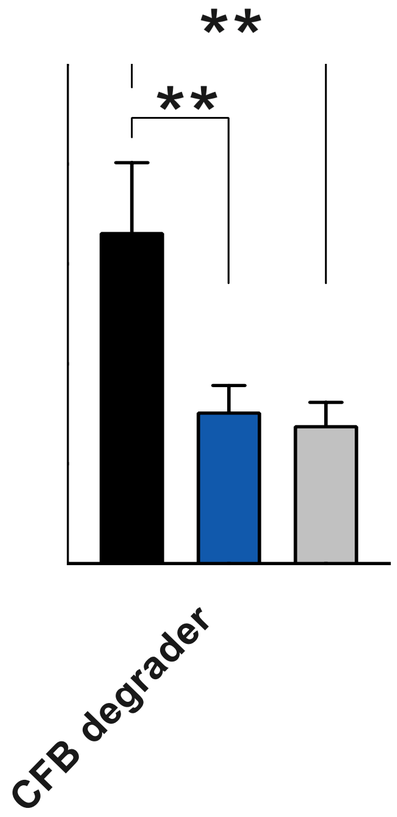
CFB degraders compare well vs LNP023

CFB degraders reduce inflammation in acute rodent model of ARDS

Study paradigm:



Improvement of respiratory functions at 24h#



Drug level at time of dosing compared with circulating levels of the endogenous target (CFB):

- **LNP023** is ~650-fold higher
- **CFB degrader** is 2-fold higher

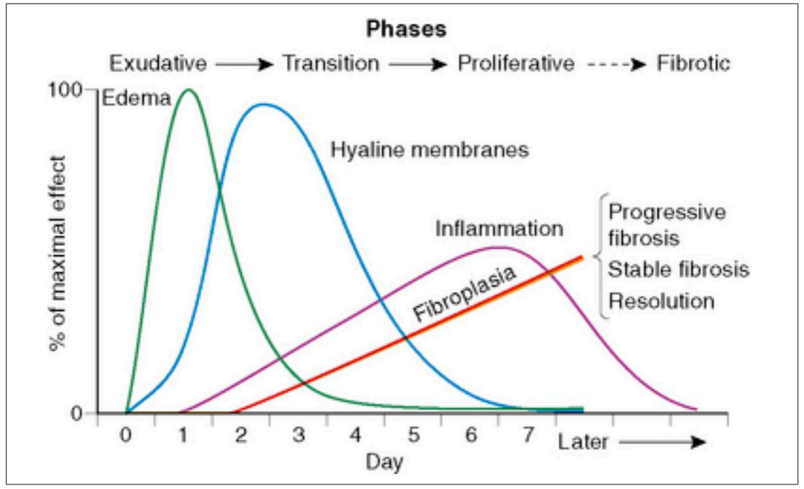
#CFB degrader 325-fold lower dose vs LNP023 (molarity)

Discussion points

- **Which complement targets should be selected in the acute, sub-acute and chronic phase of ARDS?**

- **In absence of clear subpopulation within ARDS patients, which animal models may be suitable to examine the efficacy of complement therapeutics?**

- **Which complement measurements would be meaningful in ARDS models?**



Modified from Katzenstein A: Acute lung injury patterns: diffuse alveolar damage and bronchiolitis obliterans–organizing pneumonia. In: Katzenstein A, Askin F, eds. Katzenstein and Askin's Surgical Pathology of Non-Neoplastic Lung Disease, 3rd ed. Philadelphia: Saunders; 1997.