
SVAr
Answers in Life Science

Functional “potency” assay feasibility of therapeutics and new modality drug candidate targeting the complement cascade

Ready to use cells and applications in the bioanalytical laboratory

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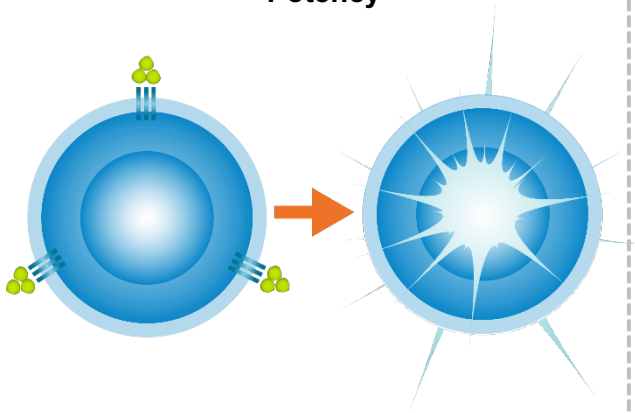
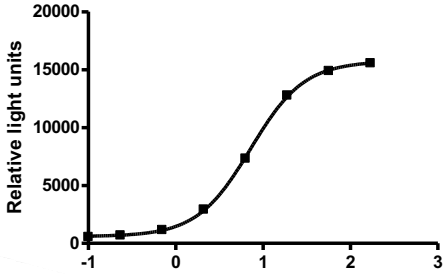
14th EBF Open Symposium,
Session 9, LBA/CBA – Technical Challenges
November 25th 2021

Cell-Based Assays in Bioanalysis

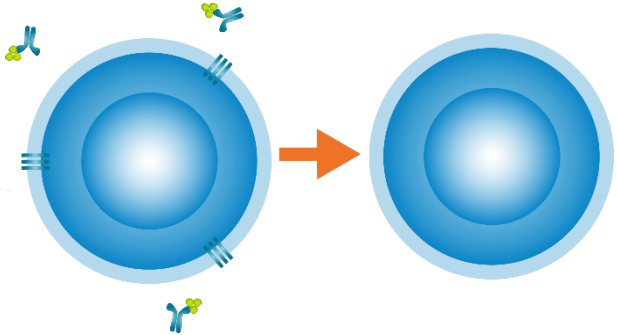
Applications

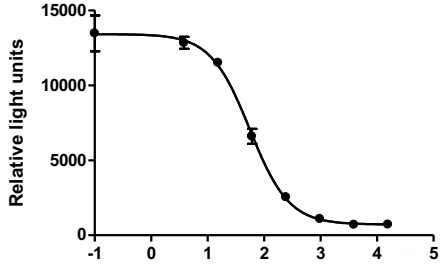
Potency



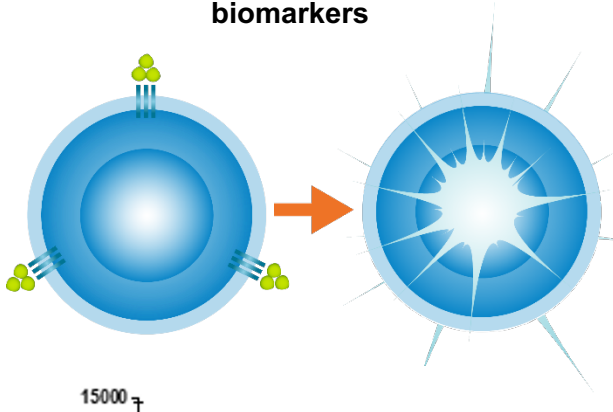


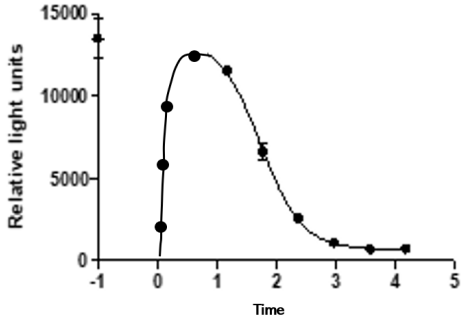
Immunogenicity/ Nab assays





Pharmacodynamics/ biomarkers





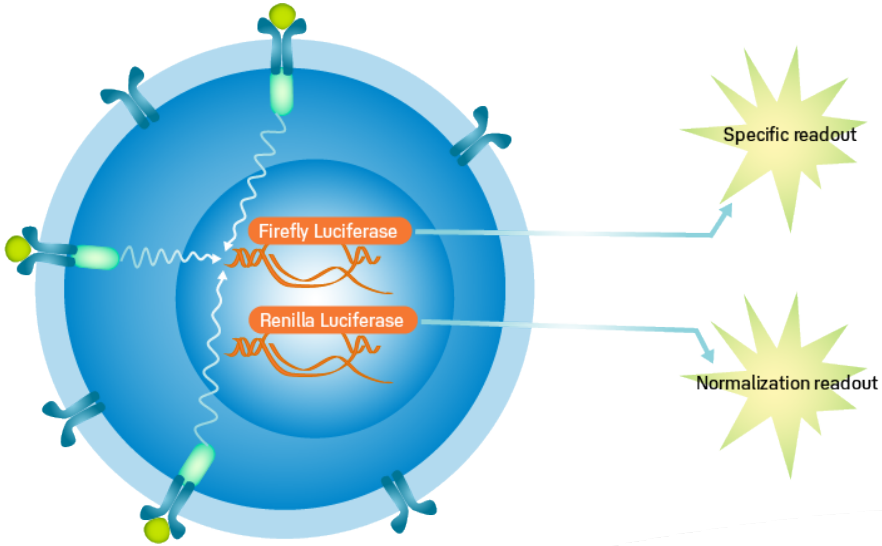
Challenges cell-based assays

- Development of robust and consistent assays for specific indications
- Optimization is often necessary after obtaining preliminary results
- Time consuming and labor-intensive workflow
- Cell Maintenance - Continuous culture and harvesting as needed.
- Specific protein expression - falling expression levels with increasing passages.
- Variability in cells and among subjects
- Multiple and complex MoA's
- Lack of readily available reference materials
- Complicated analytical methods and instruments underlie the major technical difficulties

iLite® Cell-Based Assays

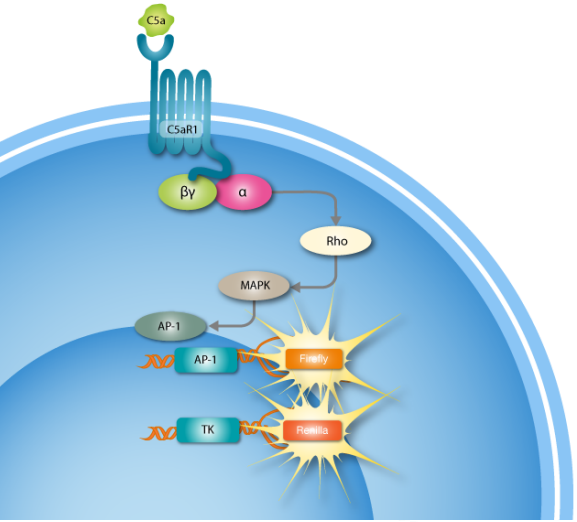
Features and Benefits

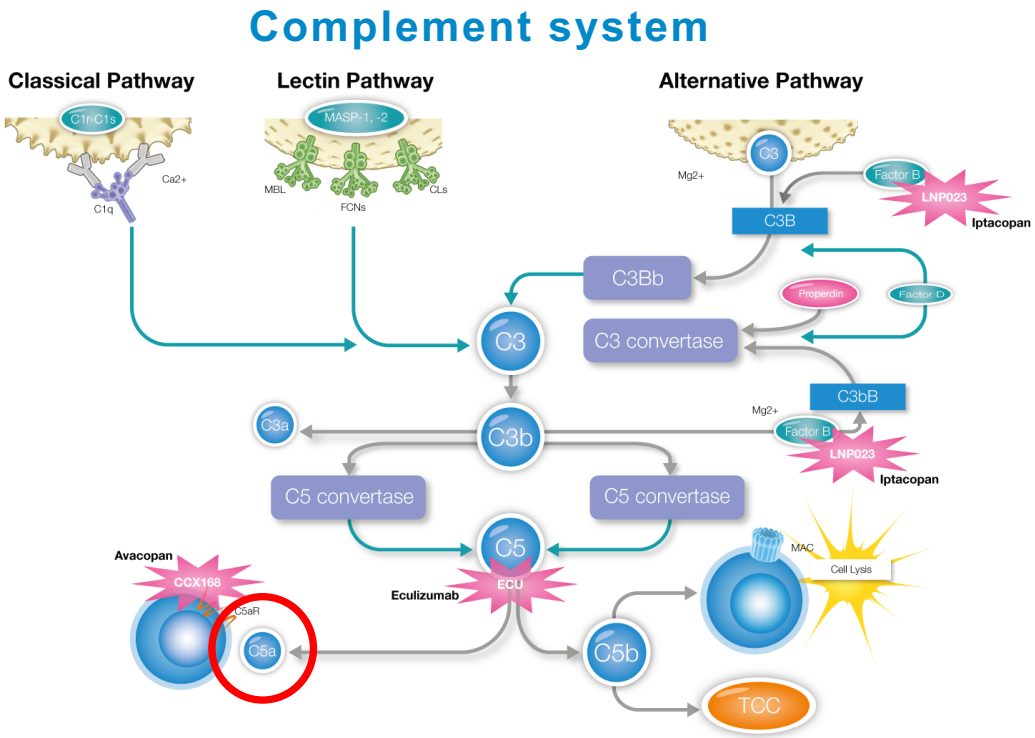
- Assay Ready Cells – ready-to-use from the freezer, without culturing of cells
  - High reproducibility
  - Assays within a workday
- Normalization gene



iLite C5a responsive reporter gene cell line

- **Ligand C5a**
- **C5a receptor 1**
- **Firefly (Specific)**
- **Renilla (Normalization)**





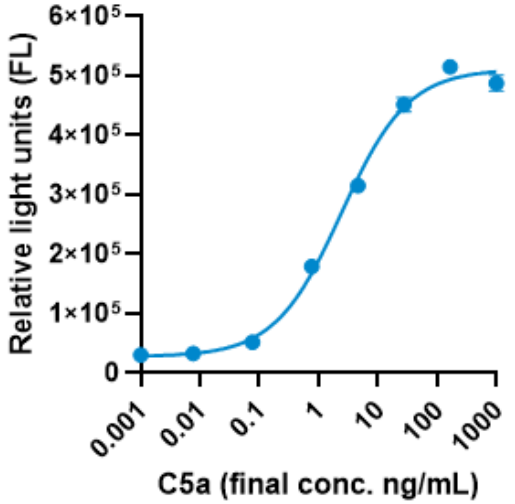
iLite C5a cell-based assay
– Protocol and Calibration

Sample dilution
- Dilute calibrators, controls and samples and add to wells.

Addition of cells
- Thaw, dilute and add cells
- Incubate (37°C, 5% CO_2) – **5 h**

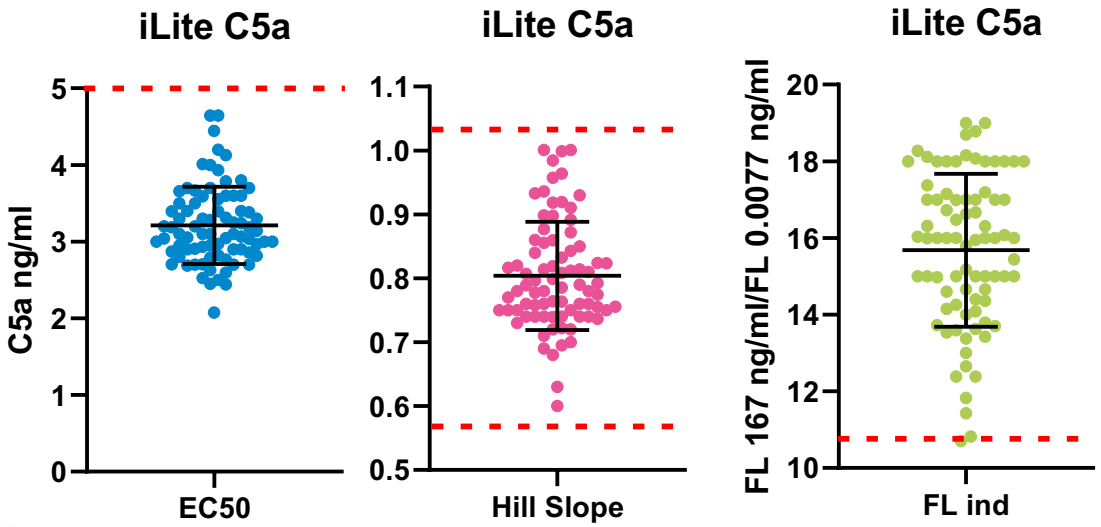
Develop light reaction
- Add substrate
- Incubate (RT) – **10 min**

Read plate
- Read plate in a luminometer



iLite C5a cell-based assay

- Robustness



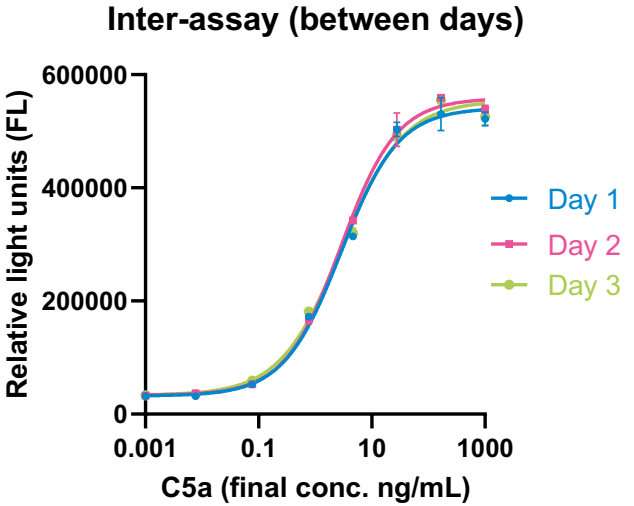
Data from pre-val & validation runs
- 80 dose resp. curves
- 19 assay occations /plates
- 6 operators
- 3 cell batches

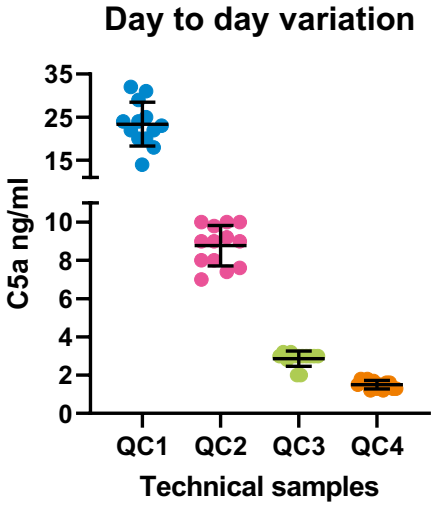
QC specs marked with red dotted line

	Mean	CV%
EC50	3.2	16
FL induction	16	13
Hill Slope	0.80	11

iLite C5a cell-based assay

– Inter-assay precision



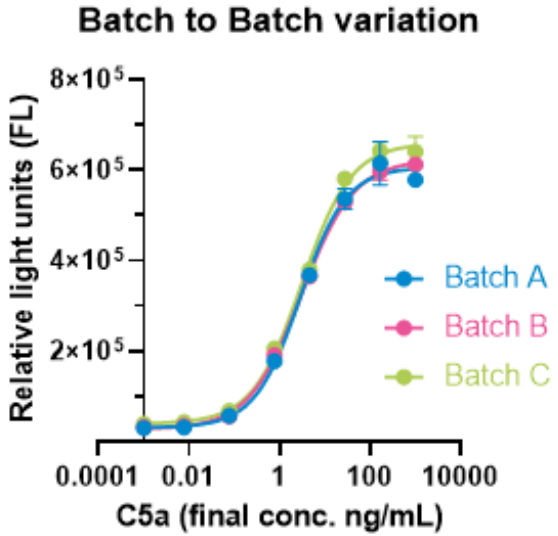


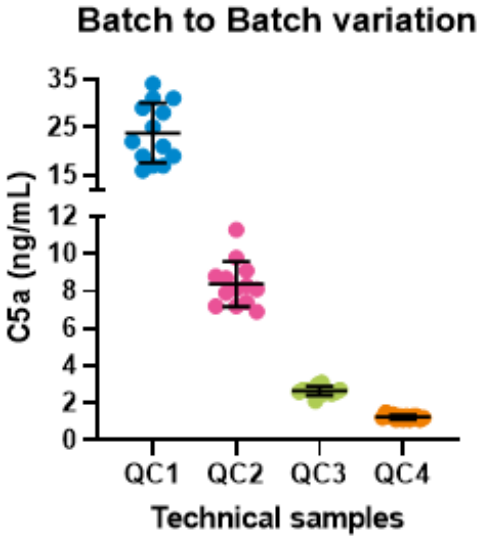
	Mean	CV%
EC50	2.9	2%
FL induction	16	5%
Hill Slope	0.84	5%

Sample	Mean	CV%
1	24	16
2	8.7	3
3	2.8	11
4	1.5	10

iLite C5a cell-based assay

-Batch to Batch variability





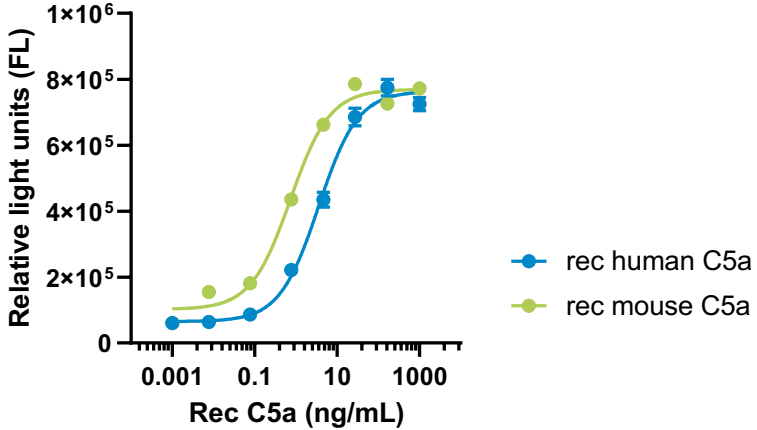
	Mean	CV%
EC50	3.0	4%
FL induction	17	14%
Hill Slope	0.80	7.1%

Sample	Mean	CV%
1	23	16
2	8.3	8
3	2.7	2
4	1,2	3

iLite C5a cell based-assay

– Pre-Clinical use

Comparison recombinant human C5a and recombinant mouse C5a using iLite C5a assay ready cells



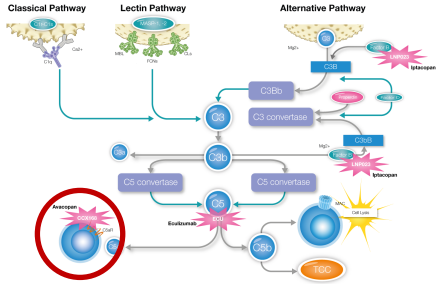
Modalities tested

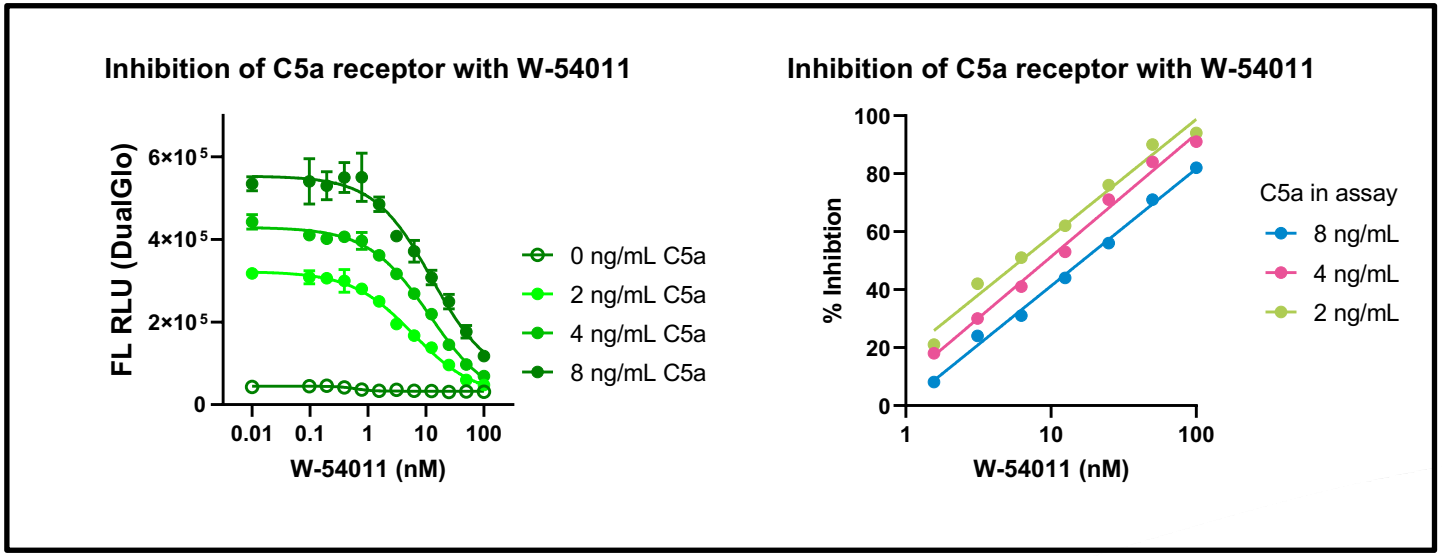
– Small Molecule, Antibodies and RNA Aptamer

- Inhibition by Small Molecule C5aR1 receptor antagonist, W-54011
- Inhibition by Large Molecule C5aR1 receptor binding antibody C5aR-mab, MAB3648
- Inhibition by Large Molecule C5a binding antibody C5a-mab, R&D MAB2037
- Inhibition by New Modality C5a binding L-RNA-Aptamer

iLite C5a cell-based assay

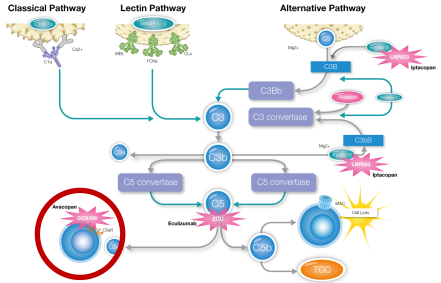
– inhibition with C5aR1 receptor antagonist W-5401



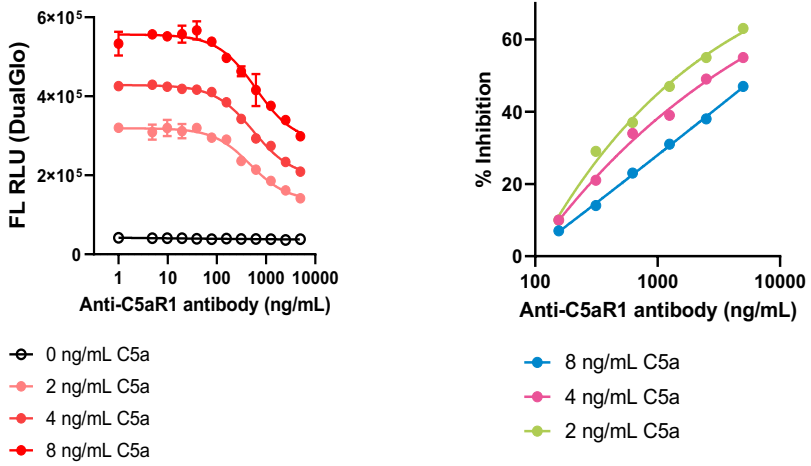


iLite C5a cell-based assay

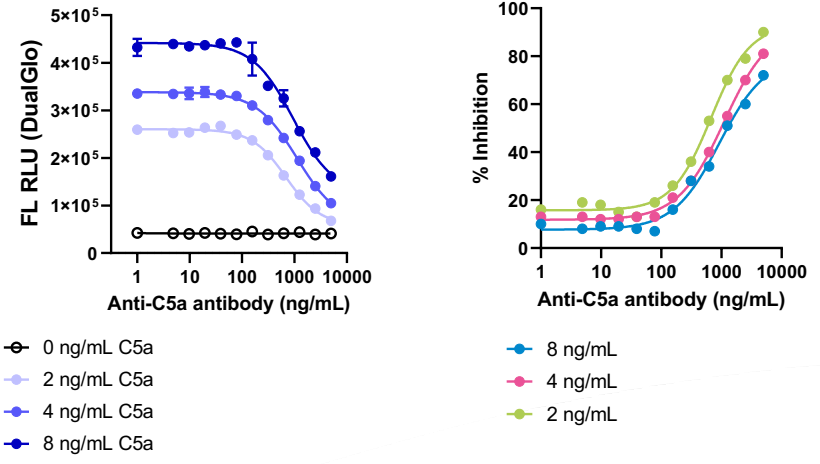
- inhibition with C5aR1 and C5a antibodies



Inhibition of C5a receptor with C5aR1 antibody



Inhibition of C5a activity with C5a antibody



MTOpen

original article

A Novel C5a-neutralizing Mirror-image (L-)Aptamer Prevents Organ Failure and Improves Survival in Experimental Sepsis

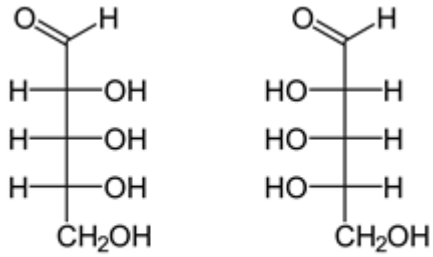
Kai Hoehlig[1], Christian Maasch[1], Nelli Shushakova[2], Klaus Buchner[1], Markus Huber-Lang[3], Werner G Purschke[1], Axel Vater[1] and Sven Klussmann[1]

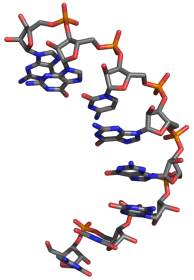
[1]NOXXON Pharma AG, Berlin, Germany; [2]Phenos GmbH, Hannover, Germany; [3]Department of Traumatology, Hand, Plastic, and Reconstructive Surgery, Center of Surgery, University of Ulm, Ulm, Germany

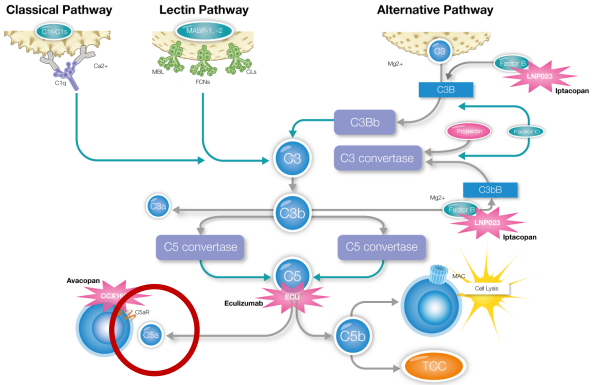
iLite C5a cell-based PD assay for Clinical Trial

- inhibition by C5a specific L-RNA Aptamer drug candidate

- **L-RNA Aptamer built from L-ribose units**
- **Natural oligonucleotides are build from D-ribose units.**
- **Artificial and mirror image of natural oligonucleotides.**
- **The L-nucleotides increase resistance to nuclease degradation**
- **Can bind to proteins, peptides and low molecular weight molecules**
- **Drug candidates**



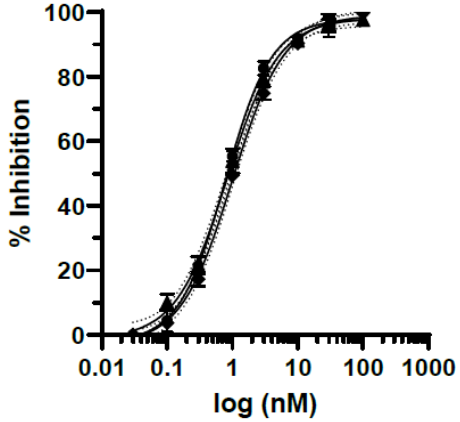




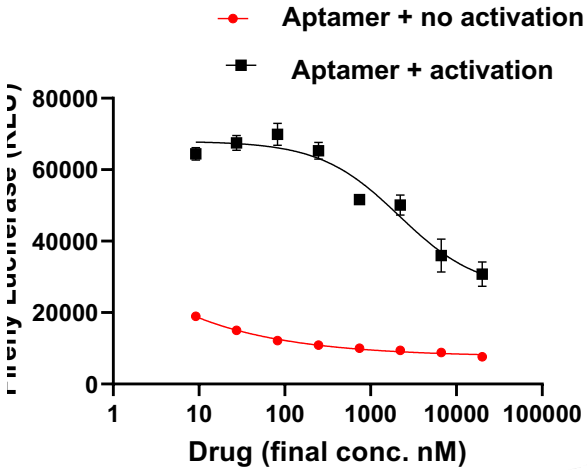
iLite C5a cell-based PD assay for Clinical Trial

- C5a specific L-RNA Aptamer dose response and inhibition of endogeneous C5a in activated matrix

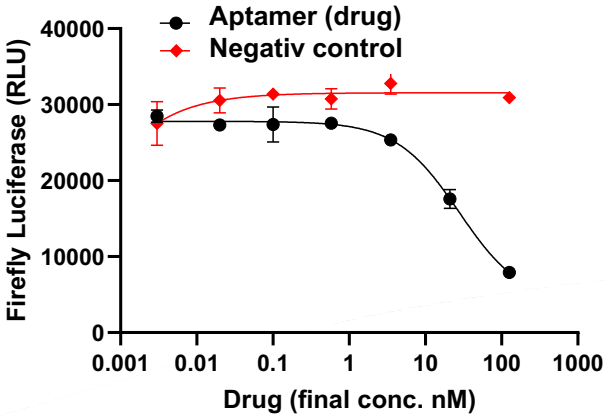
Drug titration



Activation of matrix

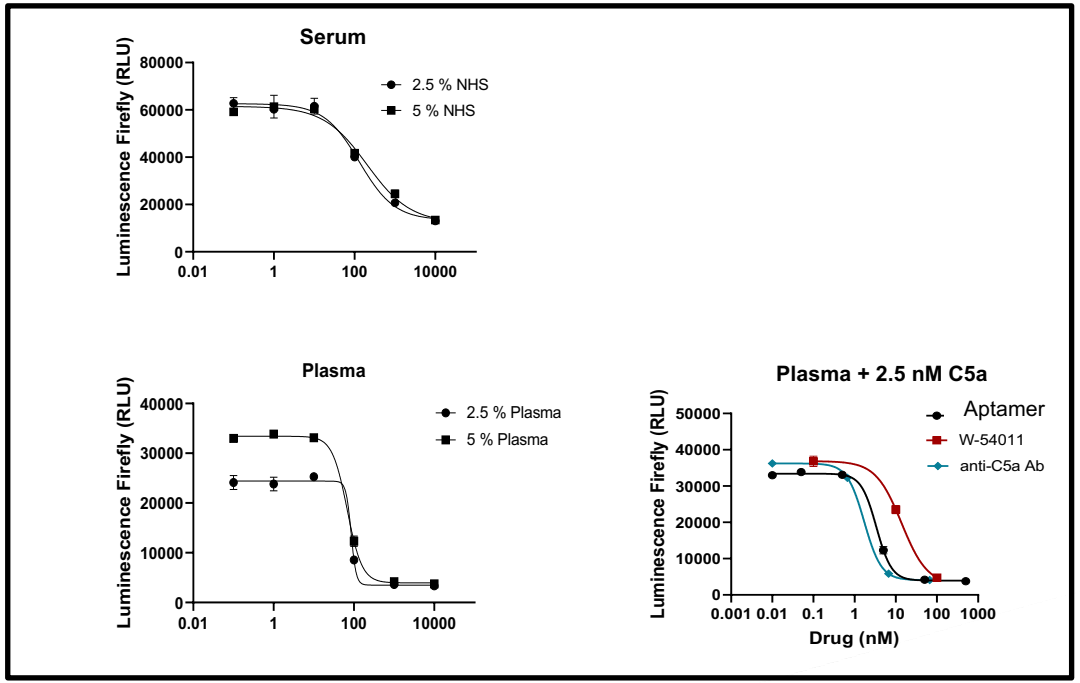


No inhibition by control molecule



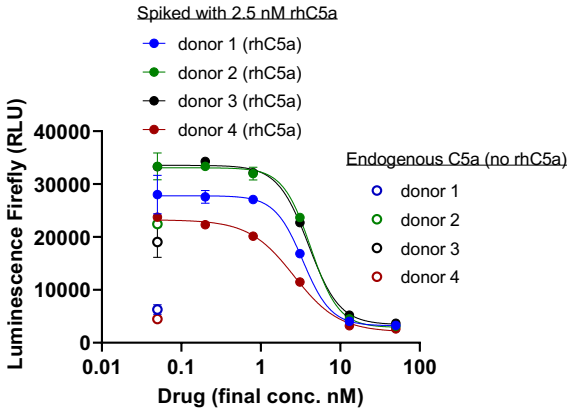
iLite C5a cell-based PD assay for Clinical Trial

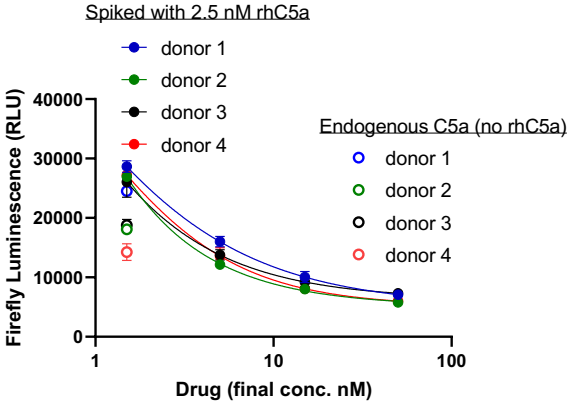
- L-RNA Aptamer and matrix optimization

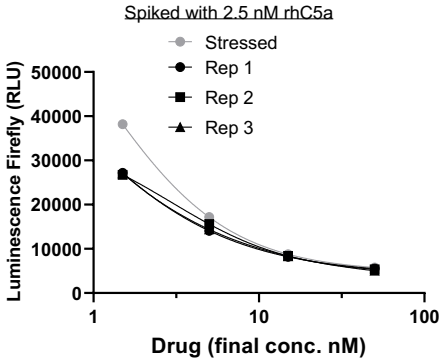


iLite C5a cell-based PD assay for Clinical Trial

- L-RNA Aptamer and matrix pre-processing







Plasma:

- **Stored for > one year**
- **<-20 C**

Plasma:

- **<-80 C within 60 min**
- **Stressed = Blood at RT for 4 h**

In Summary…

iLite® cell-based assays can be used for different drug modalities such as small molecules, Antibodies and RNA Aptamers.

Sample material and handling highly important

Feasible for PD assay


THANK YOU!