

Manchester

2nd September 2014

**High Throughput/High Content Screen to
Identify Modulators of Neutrophil
Extracellular Trap (NET) Formation**

Agenda

- Neutrophil biology and pathogenicity caused by neutrophil actions
- Screening set-up and first results
- Future directions

- Project to identify modulators of NET formation
is a joint collaboration between

A. Zychlinsky/MPI Berlin,

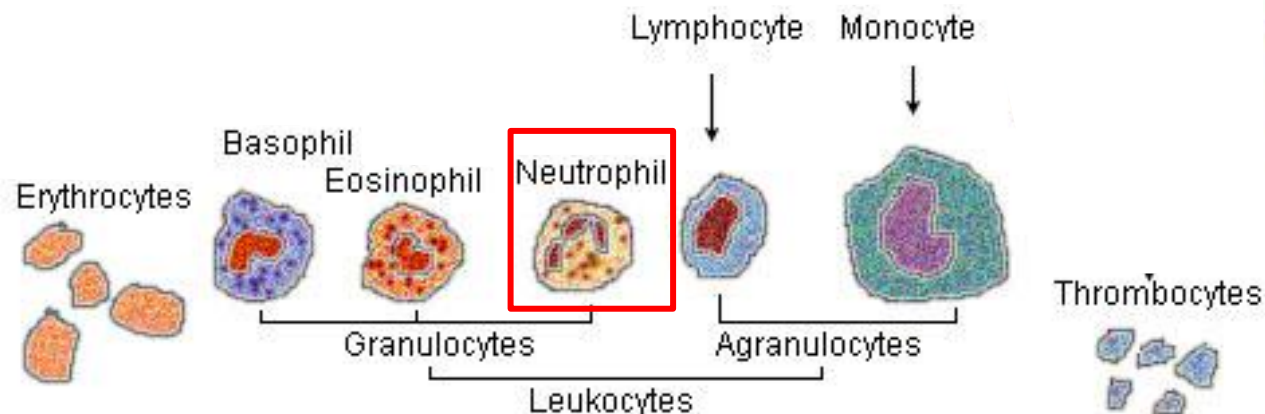
H. Waldmann/MPI Dortmund &

Lead Discovery Center

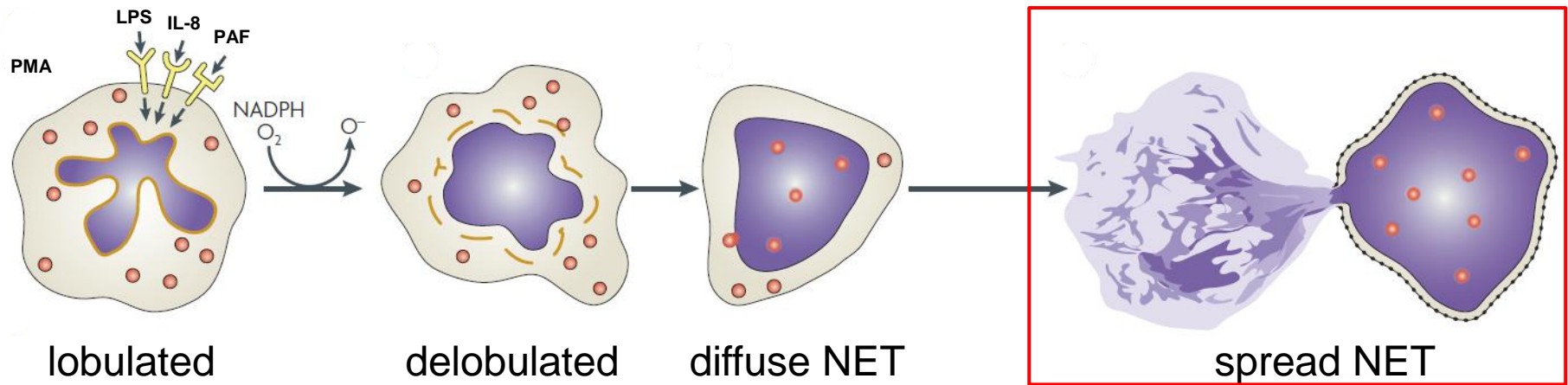


Neutrophils/Neutrophilic Granulocytes

- Neutrophils (alias polymorphonuclear leukocytes (PMN)) are specialized blood cells.
- 10^{11} neutrophils are generated daily in humans -> $\sim 4 \times 10^6$ neutrophils in 1ml blood. Their average lifetime is 5,4 days.
- Functions:
 - Neutrophils are seen as the 'infantry of host defense' (Yipp & Kubes, 2013).
 - Neutrophils generate NETs in a special form of cell death (i.e. NETosis), which is different from apoptosis (Brinckmann & Zychlinsky, 2004)

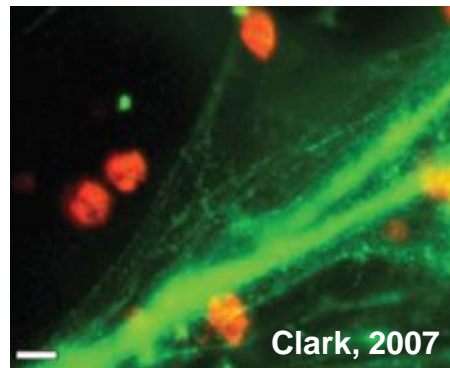


NETs (Neutrophil Extracellular Traps)

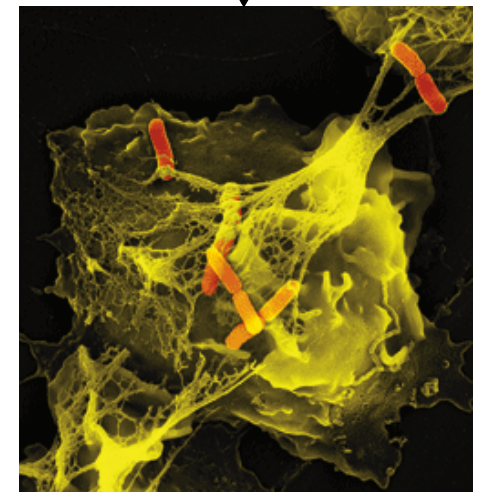


NETs are produced by neutrophils in a process called NETosis. NETs are DNA/protein complexes with antimicrobial functions (e.g. histones).

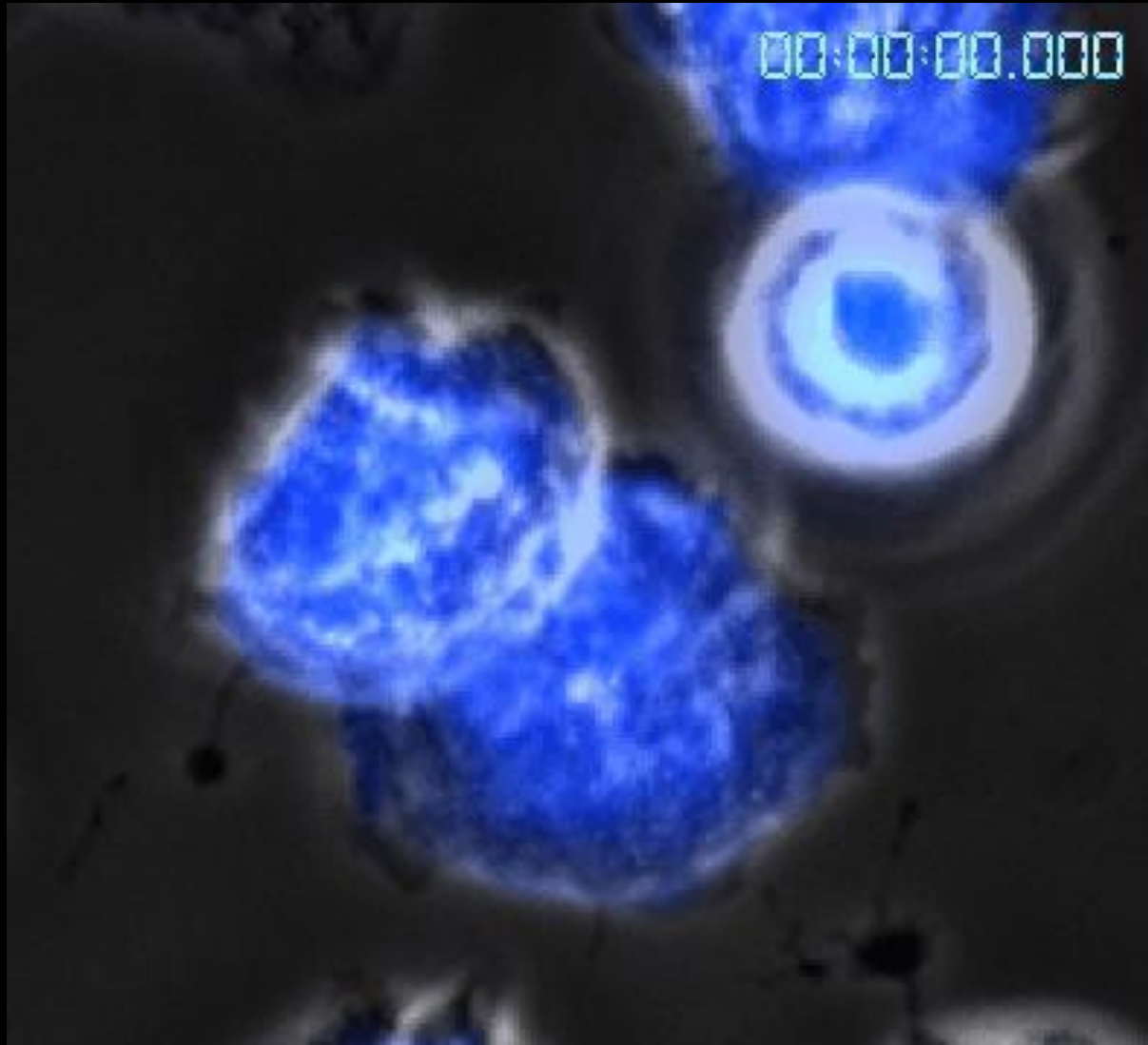
Brinckmann & Zychlinsky, 2007



Clark, 2007



Live Imaging of NET Formation

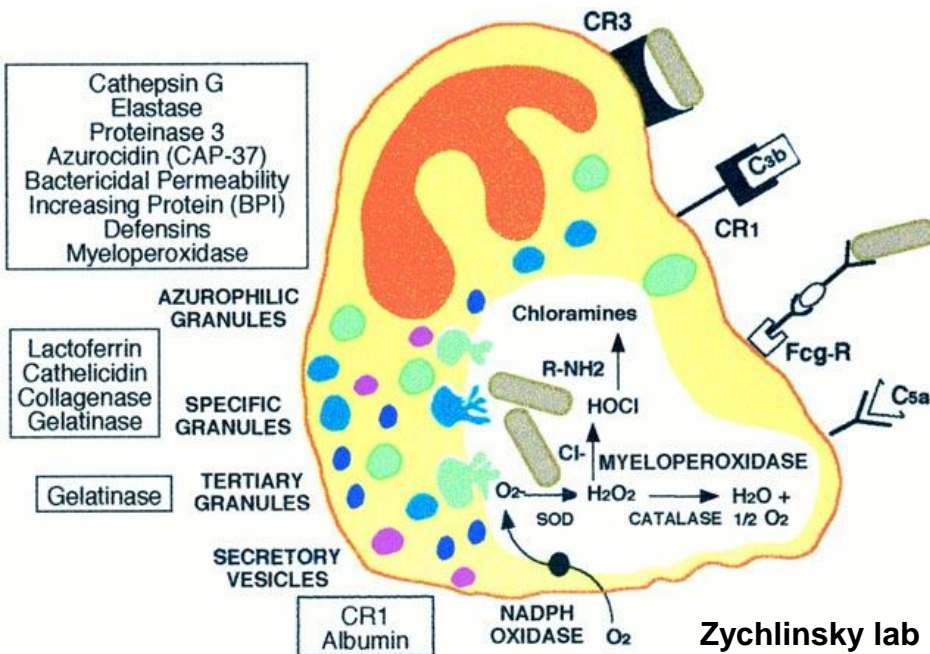


blue neutrophil

red extracellular DNA

Zychlinsky lab

Signalling Molecules Involved in NET Formation



- Limited set of enzymes/proteins involved in the regulation of NET formation:
- NADPH oxidase (NOX2; Fuchs, 2007)
 - neutrophil elastase (NE; Papayannopoulos, 2010)
 - myeloperoxidase (MPO; Metzler, 2011)
 - PKC/MEK/Erk pathway (Hakkim, 2012)
 - PAD4 (Wang, 2009)

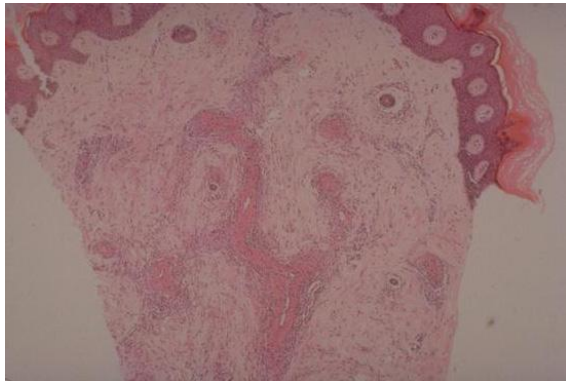
Rather effectors, than controllers have been identified

Reasons/Challenges:

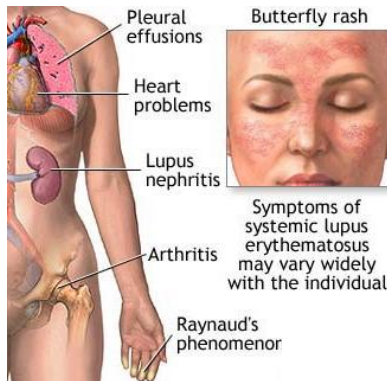
- Genetic manipulation is difficult, if no impossible
- Neutrophils are primary cells, there is no neutrophil cell line available
- Handling is not trivial (purification, sensibility, culture conditions)

-> Identification of modulating compounds may gain knowledge on NET formation and reveal new therapeutic intervention points

NET Formation and Disease



UW Washington, pathology



healthcentral.com

- **Autoimmune diseases:**
 - Small vessel vascularitis (Kessenbrock, 2009)
 - Systemic Lupus Erythematoses (SLE; Villanova, 2011; Knight, 2013)
 - Rheumatoid arthritis (Li, 2010)
- **Transfusion-related acute lung injury** (TRALI; Thomas, 2012)
- **Cancer metastasis** (Berger-Achituv, 2013, Cools-Lartigue, 2013)
- **Thrombosis** (Fuchs; von Brühl; both 2012)
- **Preeclampsia** (Gupta, 2007)
- **Gout** (Schauer et al.; 2014)
- **COPD** (Obermayer et al., 2014)

-> Excessive NET formation is a pathogenic principle
(the chicken or the egg)

Screening Set-up

Activation of the Raf-MEK-ERK pathway is required for neutrophil extracellular trap formation

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Arturo Zychlinsky^{1*} & Herbert Waldmann^{2,3*}

NATURE CHEMICAL BIOLOGY | VOL 7 | FEBRUARY 2011 | www.nature.com/naturechemicalbiology

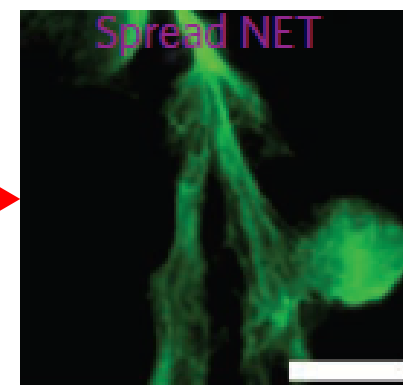
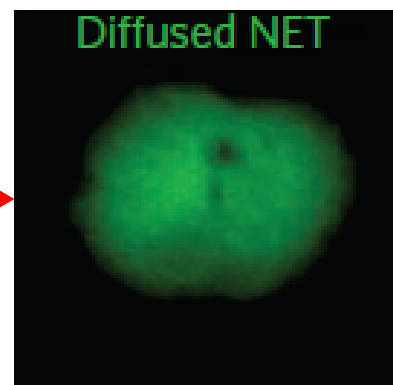
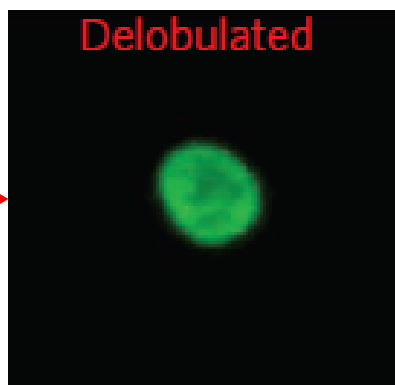
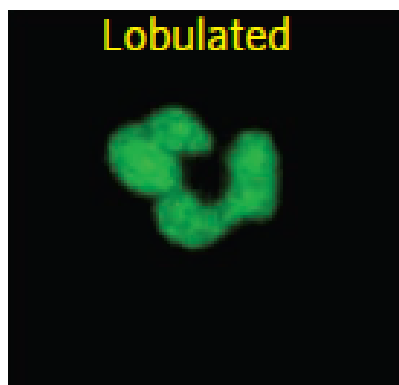
75

10

120

240

time after PMA stimulation [min]



Neutrophil support

-> 1536-well format

Read-out quantification

-> simplification to NET formation inhibition

Selection of stimulus

-> selection of PMA/validation in later stages on additional stimuli

Donor variation

-> re-test/validation using different donors

Conclusion and Next Steps

- High content screening of small molecules on NET formation (primary cells) in a 1536-well format resulted in the identification of several potent hit inhibitor classes
- Initial SAR was identified, currently working on hit optimization of several compound classes
- Selected compound classes affect neither MPO nor NE nor proliferation and therefore affect probably unknown NET formation regulators
- Identification of the mode of action as well as target deconvolution of some compound classes is ongoing
- *in vivo* proof of concept model will be performed



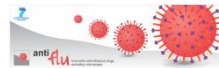
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