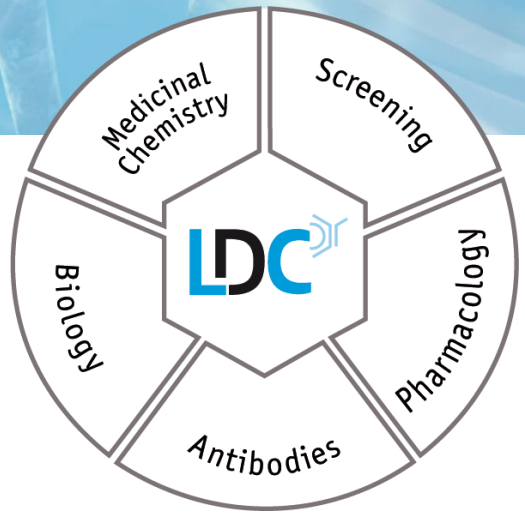
Lead Discovery Center LDC

NETosis Inhibitors

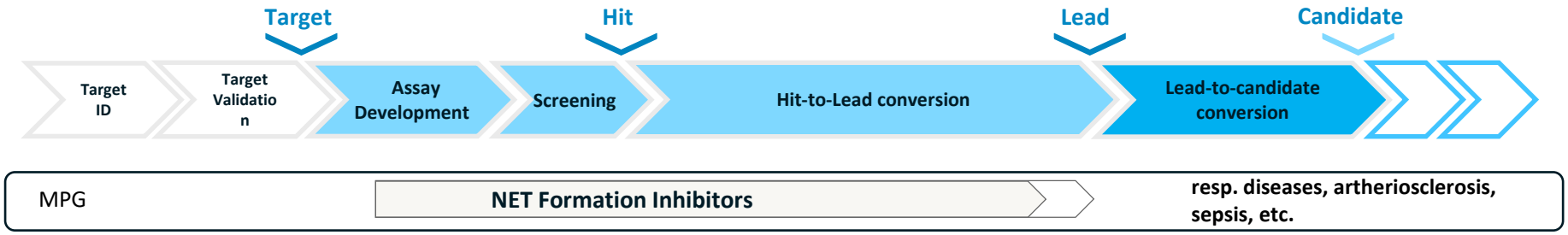


NET Formation

Neutrophil **E**xtracellular **T**raps

Academic Partners:
Prof. Arturo Zychlinsky, Max Planck Institute Infection Biology, Berlin
(Prof. Herbert Waldmann, MPI Molecular Physiology, Dortmund)

Executive Summary



Target rationale:

- Excessive NET formation is linked to several diseases incl. SLE, thrombosis, fibrosis, etc.
- Addressing a completely novel therapeutic principle with first-in-class potential

Key achievements:

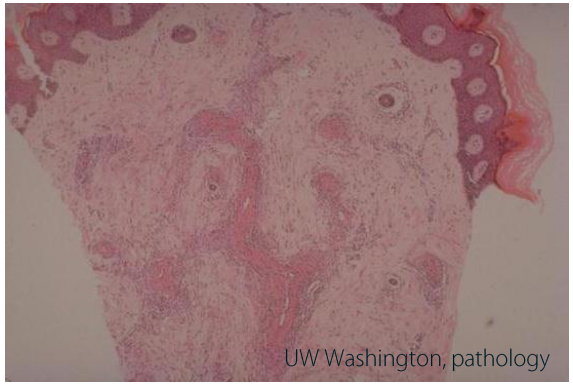
- Imaging-based phenotypic HTS of human primary neutrophils (190k compound library) identified multiple sub-µM hits series for different phenotypes
- Triaging and validation of hit compounds through assay cascade and additional functional PBMC assays
- Several unexplored hits available
- Molecular NET formation target class II identified

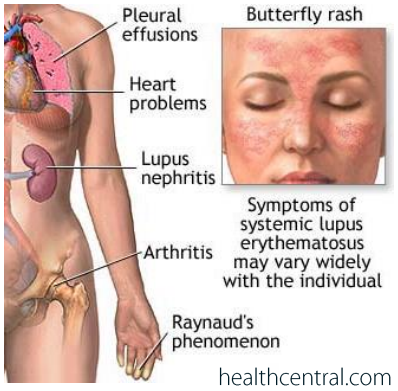
Next steps:

- Investigating frontrunner compounds under disease-specific stimuli
- Selection of in vivo efficacy model
- To establish binding assays for target class II

NETosis and Diseases

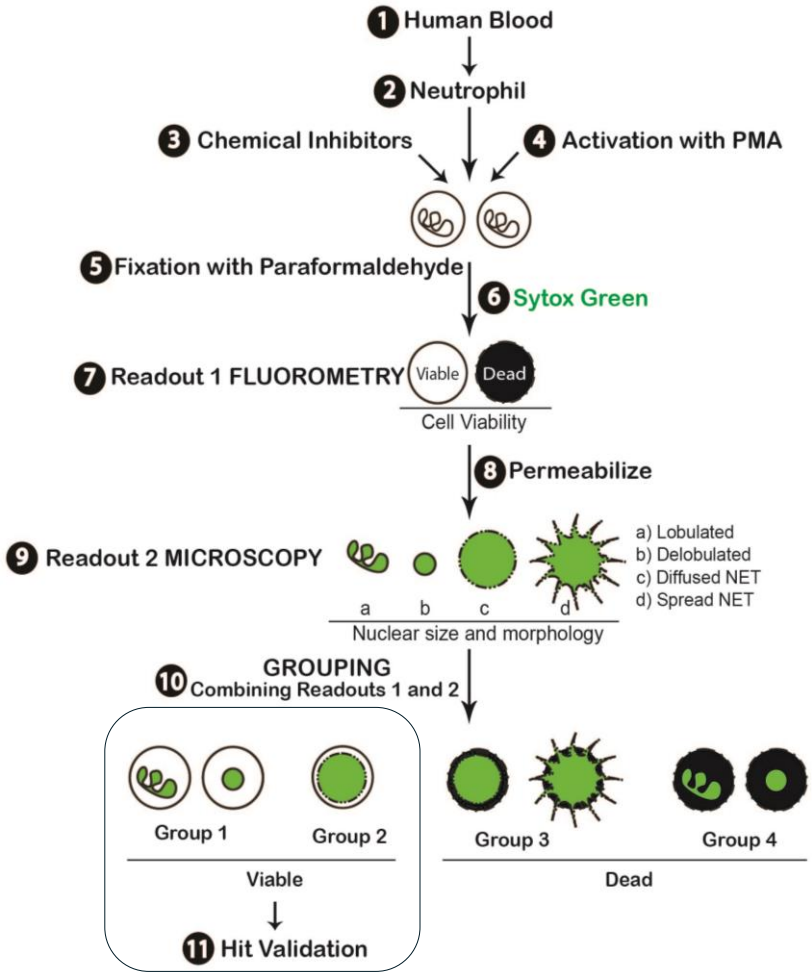
- *Systemic Lupus Erythematosus* (Villanova, 2011; Knight, 2013)
- Rheumatoid arthritis (Li, 2010)
- Atherosclerosis (Warnatsch, 2015)
- Small vessel vascularitis (Kessenbrock, 2009)
- Thrombosis (Fuchs, von Brühl; both 2012)
- Sepsis (Xu, 2009)
- ALI, TRALI (Narasaraju, 2011; Thomas, 2012)
- COPD (Obermayer, 2014)
- Cystic fibrosis (Papayannanopoulos, 2011)
- Cancer metastasis (Berger-Achituv, 2013; Cools-Lartigue, 2013; Podaza, 2016; Park, 2016)
- Preeclampsia (Gupta, 2007)
- Cardiac (or brain) reperfusion (Ge, 2015)
- Asthma (Dworski, 2011)
- *Diabetes*, diabetic wound healing (Wong, 2015)
- Periodontitis (White , 2016)
- Psoriasis (Chu-Sung Hu, 2016)
- Dermatomysitis, Polymyositis (Zhang, 2014)

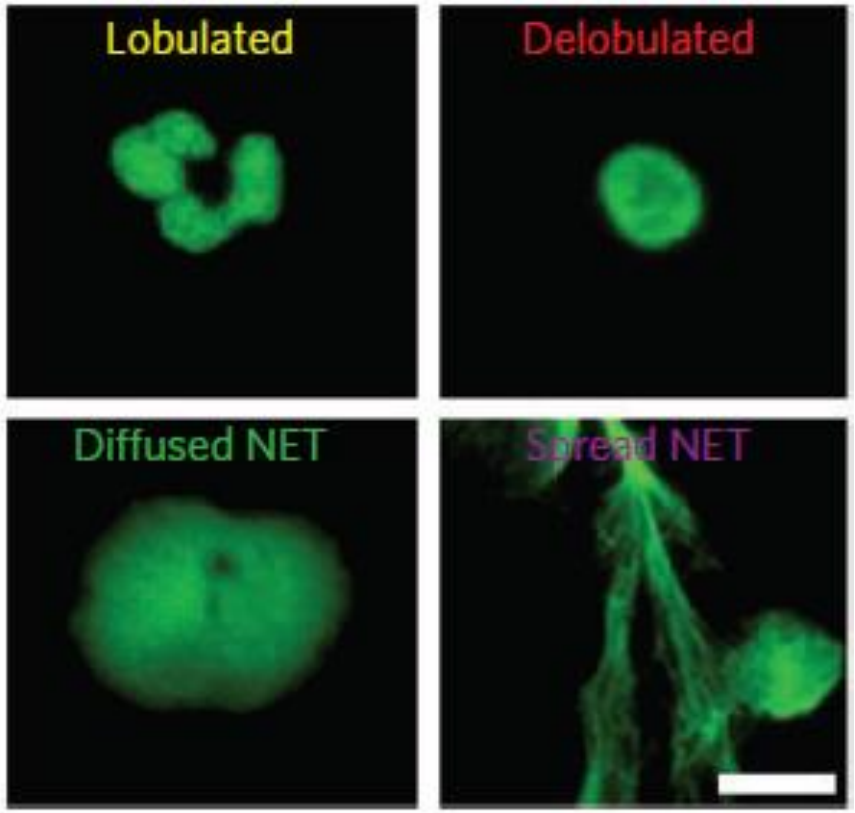




➢ Excessive NET formation is a pathogenic principle (cause or consequence?) associated with many inflammatory pathologies

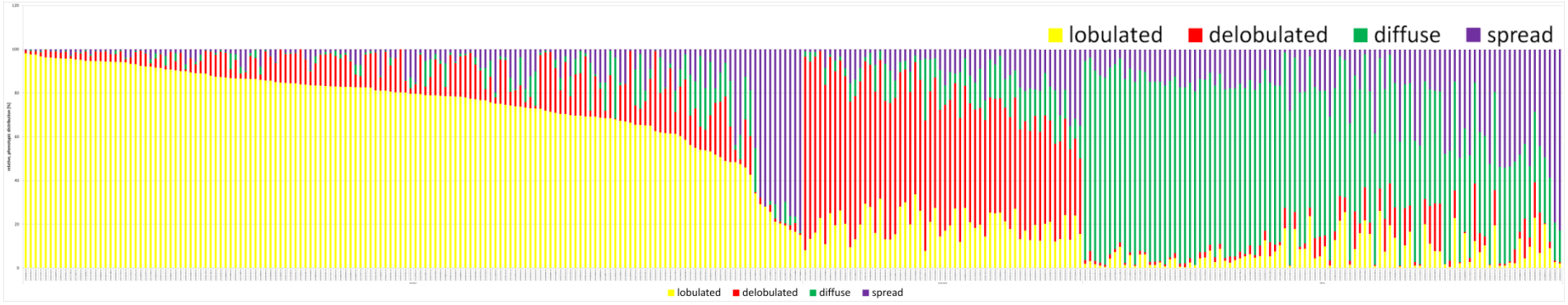
Screening Set-up (NETosis Inhibitors)



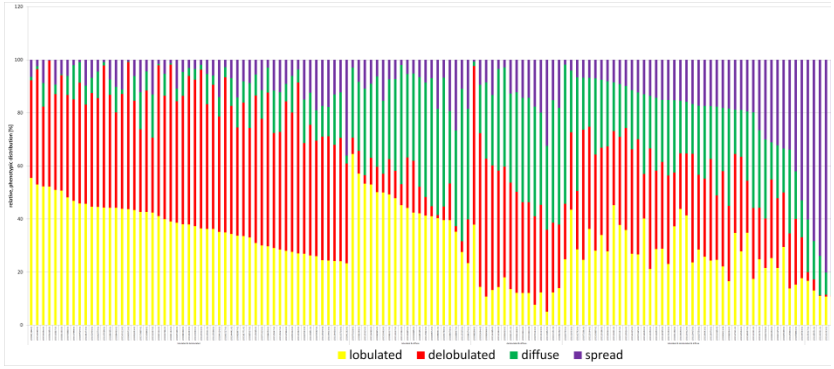


- Novel phenotypic screening set-up developed by our academic partners suitable for high-throughput HCS

Screening Results



lobulated | **delobulated** | **diffuse**

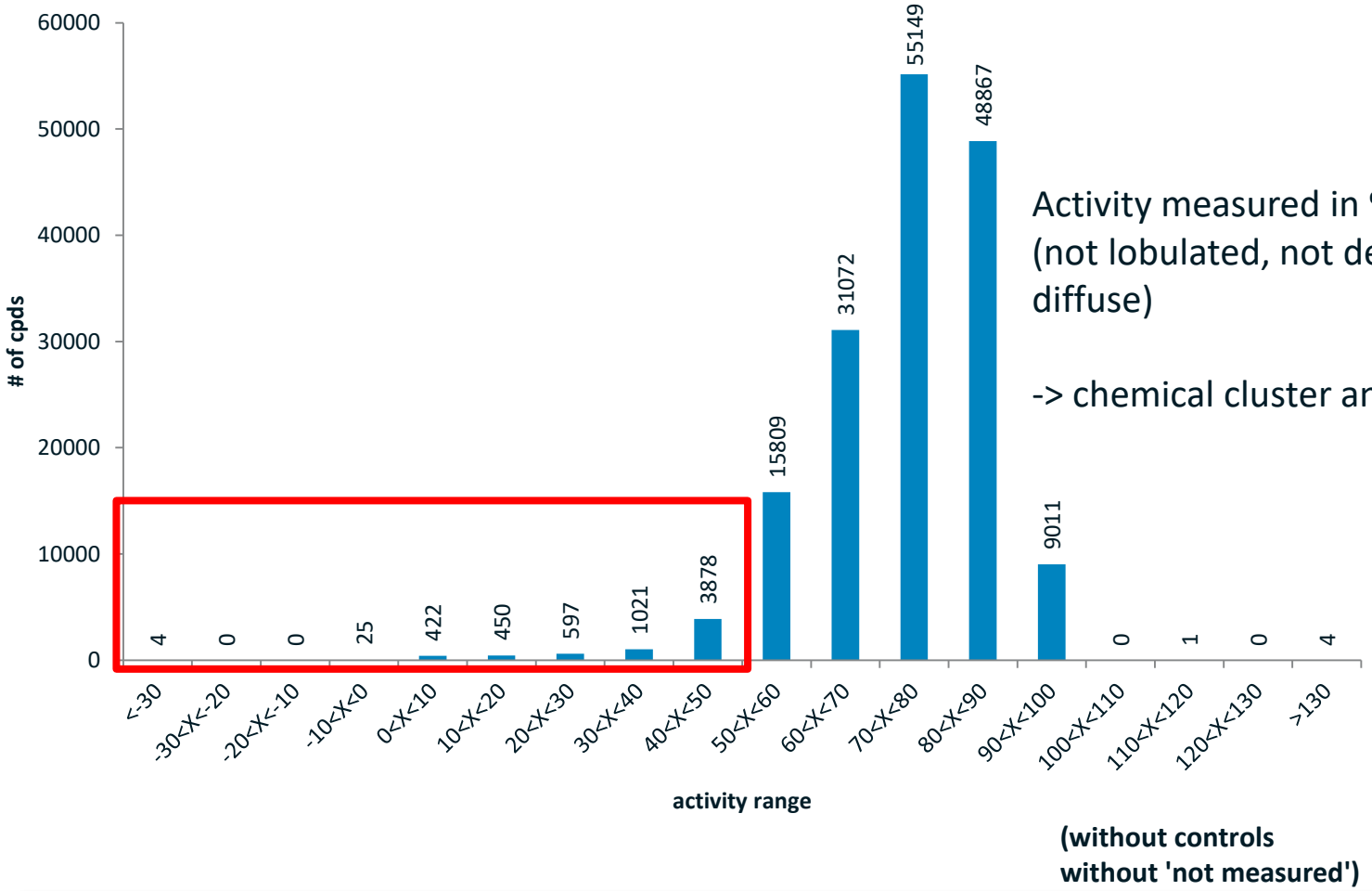


lobulated & delobulated | **delobulated & diffuse** | **lobulated & diffuse** | **lobulated & delobulated & diffuse**

Phenotype(s)	Number of hits
Lobulated	163
Delobulated	65
Diffuse	83
Lobulated & Delobulated	52
Lobulated & Diffuse	20
Delobulated & Diffuse	15
Lobulated & Delobulated & Diffuse	41

- Multiple hits series could be identified for different phenotypes
- „Known“ NET inhibitors (NE, MPO) have been identified further validating the assay performance

Screening Results – Source of Compounds



Activity measured in % NET formation (not lobulated, not delobulated & not diffuse)

-> chemical cluster analysis

(without controls without 'not measured')

- Multiple hits series could be identified for different phenotypes
- Valuable source of compounds acting on NETosis via novel MoAs

Frontrunner Profile

Class II		
Activity		Best compounds: <10nM
hPBMC toxicity		no
SAR		clear, broad, activity improved
in vitro SPR	Solubility	low
	Permeability	medium
	MLM stability	good
	MLM Phase 2	compound-dependent
	Plasma stability (mouse)	compound-dependent
Metabolites analysis		*preliminary:* soft are addressable
Tool compound for target fishing		available
in vivo PK	$t_{1/2}$	medium
	V_d	low (except for LDC 202565)
	CL	low
	F%	good
	$\uparrow AUC_{0\text{-inf}}$	linear
IP position		requires clear definition of scope

Conclusions & Next Steps

- Completely novel therapeutic principle & novel mechanism identified for NET formation *per se*
  - ➢Potential for application in several possible indications associated with NETs
- Proteomics –based target ID – potential & novel candidate targets identified
- H2L MedChem advancing 2 frontrunner classes; *in vivo* PK profiles known
  - ➢eADME as well as PK/tolerance results favorable for further *in vivo* testing

Next steps:

- **Profiling frontrunner compounds in murine ConA-induced NETosis assays**
- Extended PK/mechanistic PD model (ConA-induced NETosis and/or thrombosis)
- Selection of therapeutic relevant *in vivo* efficacy model (Sepsis, COPD, TRALI, lung diseases, etc.)
- Further validation of MoA for frontrunner series