Molecular glue degraders of NEK7 for treatment of inflammatory diseases

Artur Anisiewicz, Klaudia Bielak, Michał Biśta, Karolina Brodzik, Kinga Chrzanowska, Sylvain Cottens, Weronika Czachor, Paweł Dobrzański, Daniel Drzewiecki, Daria Gajewska, Przemysław Glaza, Karolina Kamińska, Jan Klajn, Marta Klejnot, Krzysztof Kołodziejczyk, Aleksandra Matuszkiewicz, Daria Nitarska, Krzysztofa Odrzywół, Paweł Pasikowski, Roman Pluta, Marta Poźniak, Aniela Przytulska, Anna Sawicka, Agata Śnieżewska, Grzegorz Statkiewicz, Anna Szlachcic, Michał J. Walczak, Karolina Wójcik, Arkadiusz Zając

Captor Therapeutics S.A.

Introduction

Targeted protein degradation (TPD) has become a widely employed strategy for discovering novel drugs and focuses on the removal of pathogenic proteins from the cell, including those considered 'undruggable'. Among them, CRBN based molecular glues have several advantages and the recent progress in structural biology allowed their rational design.

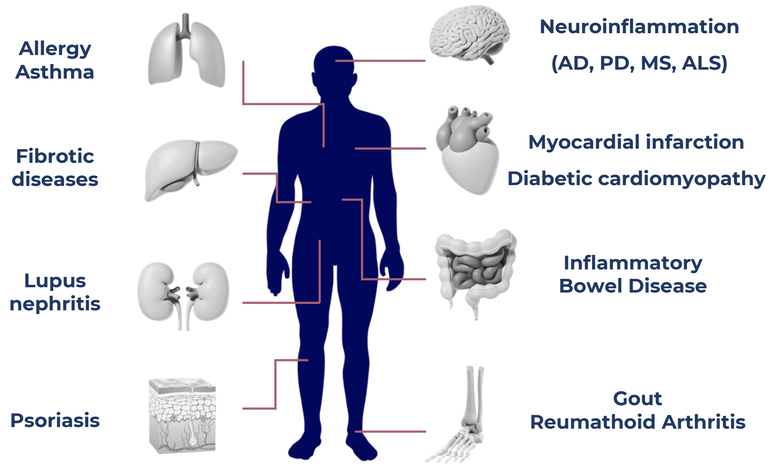
Herein, we present small-molecule glue (MW < 350 Da) NEK7 degraders with high activity in human in vitro models and with promising ADMET data.

Importantly, two separate chemical series gave lead compounds with systemic or CNS-penetrating properties, allowing targeting diverge therapeutic areas.

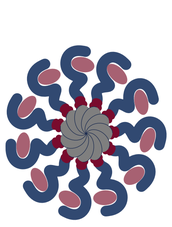
These degraders could overcome the limitations and avoid the adverse effects of NLRP3 inhibitors by weakening the activation of the NEK7-mediated NLRP3 activation pathway without completely inactivating it.

Immune diseases

- Inappropriate release of proinflammatory cytokines by NLRP3 inflammasome in various inflammatory diseases and cancers
- NLRP3 inflammasome activation mediated by NEK7 strongly indicates promising roles for targeting NEK7 in treating inflammation-related diseases
- Vast potential market for inflammasome modulators



Inflammasome activation



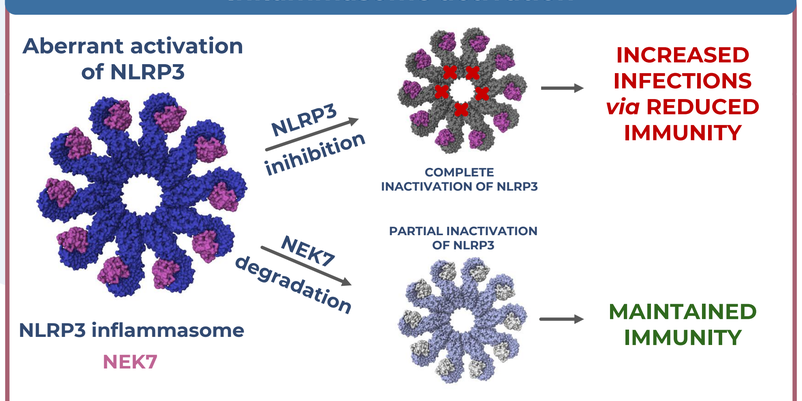
Inflammasome:NEK7 complex

→ Caspase 1 → Gasdermin D

→ Caspase 1 → IL-1β IL-18

- NLRP3 inflammasome – central role in innate immunity
  - Proteolytic activation of Caspase-1 by activation of NLRP3 inflammasome
  - Cleavage of pro-IL-1β, pro-IL-18 and Gasdermin D
  - Secretion of proinflammatory cytokines due to cell membrane disruption by GSDM D
  - Induction of pyroptosis

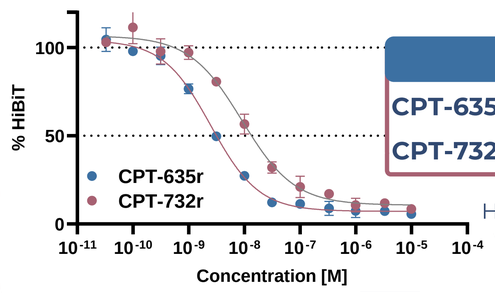
Inflammasome activation



- NEK7 degraders – a potential to overcome the limitations of NLRP3 inhibitors related to increased susceptibility to infection
- Selective degradation of NEK7 – removal the NEK7 scaffolding function leading to potent inflammatory inhibition

NEK7 degraders screening

- Biophysical assays: FP, AlphaLISA and HTRF used in a primary activity and selectivity screenings of compound libraries on recombinant proteins
- SAR model built both on biophysical and structural biology data
- NEK7-HiBiT Lytic Assay with CTG readout in HEK293 cell line used as a screening tool *in vitro*

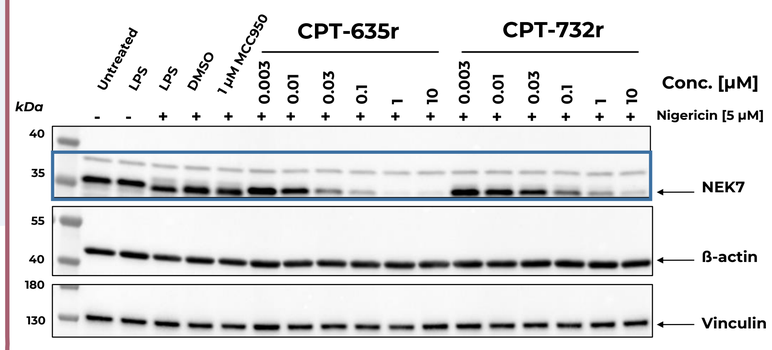


	DC_{50}	D_{max}
CPT-635r	4.03 nM ± 1.36	94%
CPT-732r	13.1 nM ± 1.06	91%

HEK293 cell line; treatment with compounds for 24h

NEK7 Degradation and selectivity in human PBMC-dMφ

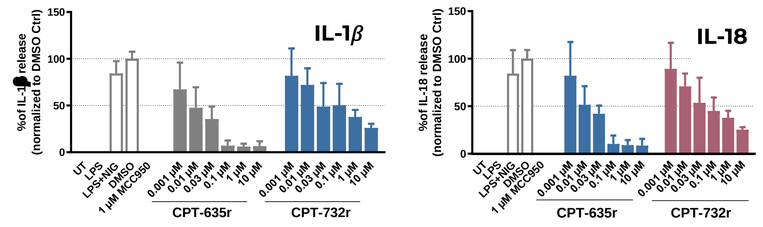
- Dose-dependent degradation of NEK7 in human PBMC-derived macrophages with LPS+Nig activated inflammasome
- No cytotoxicity in human PBMCs
- No or very weak downregulation of: IKZF1, IKZF2, CK1α, GSPT1 and ZFP91
- No clear off-targets in TMT proteomics



Human PBMC differentiated into macrophages with M-CSF; treatment with compounds – 24h; inflammasome activation: LPS – 3h, Nig. – 1h

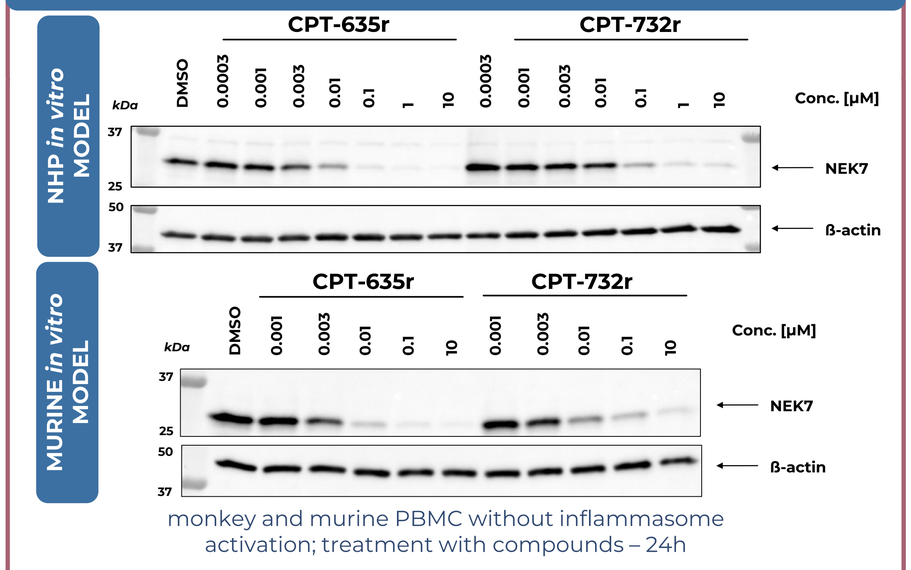
ELISA analysis of cytokines produced by human PBMC-dMφ

- Dose-dependent decrease in the production of pro-inflammatory cytokines IL-1β and IL-18



Human PBMC differentiated into macrophages with M-CSF; treatment with compounds – 24h; inflammasome activation: LPS – 3h, Nigericin – 1h

Cross-species comparison



monkey and murine PBMC without inflammasome activation; treatment with compounds – 24h

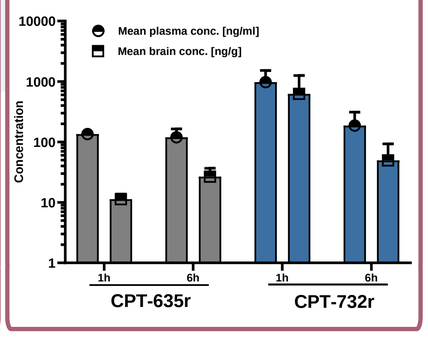
- Potent degradation NEK7 in NHP and murine unactivated PBMCs in vitro models for both compounds
- No cytotoxicity in NHP and murine PBMCs

DMPK and safety

- Favourable *in vitro* ADMET profile
- Good stability (plasma, liver microsomes)
- High unbound fraction (plasma, brain)
- Good oral absorption for both compounds
  - CPT-635r – systemic exposure
  - CPT-732r – high potential to cross the blood-brain barrier

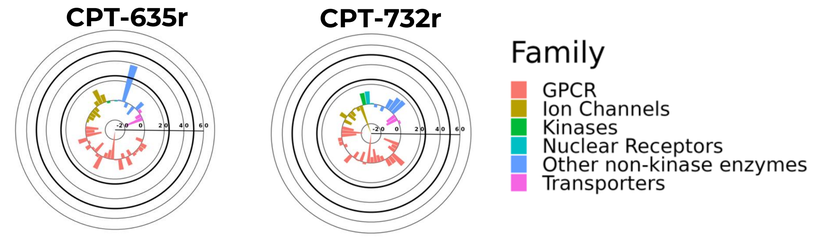
PK parameters		CPT-635r	CPT-732r
$F_{u,\ plasma}$		0.88	0.44
$F_{u,\ brain}$		0.53	0.26
IV	Dose	1	1
	AUC_{inf}	582.5	541.8
	$T_{1/2}$	3.1	0.87
	Cl	31	30.9
	V_d	8.1	2.3
PO	Dose	10	10
	C_{max}	253.1	2238
	T_{max}	1	0.4
	$T_{1/2}$	4.1	4
	AUC_{inf}	1883	5637
	F	32%	>95%

Blood–brain barrier permeation



PK studies in male CD1 mice. The data were adjusted to a noncompartmental model. Fraction unbound in plasma $F_{u,plasma}$; Fraction unbound in brain homogenate $F_{u,brain}$; Dose (mg/kg); area under the curve AUC_{inf} (ng·h/mL); half-life $T_{1/2}$ (h); clearance Cl (mL/h/kg); volume of distribution V_d (L/kg); maximum concentration C_{max} (ng/mL); time of maximum concentration T_{max} (h), bioavailability F. Administration route: intravenous IV; per os PO; Formulation vehicle: 10% DMA/5% Solutol HS-15/25% PEG-300/ 60% Normal Saline.

Off-target interactions



- Clean profile in SafetyScreen44 panel™
- No off-target above 50% threshold @ 10 μM

Conclusions

- Development of potent, low molecular weight molecular glue NEK7 degraders for treatment of inflammatory diseases
  - Chemical series with systemic and CNS exposure, with good oral bioavailability
  - Good selectivity vs. other CRBN neosubstrates (IKZF1, IKZF2, CK1α, ZFP91, GSPT1)
  - High activity of the compounds in NHP and murine *in vitro* models

The project „Discovery and development of non-toxic ligase ligands and their application in the treatment of autoimmunological diseases" is co-financed by the European Regional Development Fund. The authors would like to acknowledge all members of the CT Team that contributed to this work.

Captor Therapeutics S.A.
Duńska 11, 54-427 Wrocław, Poland
Hegenheimermattweg 167A, 4123 Allschwil, Switzerland
web: www.captortherapeutics.com
e-mail: r.pluta@captortherapeutics.com

European Funds Smart Growth
Republic of Poland
NCBR National Centre for Research and Development
European Union European Regional Development Fund