# A New Modality for the Management of Inflammation: NEK7 Degradation

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# Summary

This white paper explores the importance of NEK7 & NLRP3 in the inflammasome, their roles in inflammatory diseases and the potential advantages of NEK7 degraders over NLRP3 inhibitors for the treatment of inflammatory diseases. It also explores the greater selectivity & potential safety that NEK7 degraders are expected to bring and identifies a number of major market opportunities where these degraders may provide significant therapeutic benefits.

# Biology of NEK7 & NLRP3

**Target Overview:** NIMA-related kinase 7 (NEK7) is a member of the NEK family of serine/threonine kinases which are involved in various cellular processes, including cell cycle regulation, mitosis, and microtubule organization. NEK7 in particular has garnered significant interest due to its critical role in the regulation of the NLRP3 inflammasome, a multiprotein complex involved in the innate immune response. This type of inflammasome is activated in response to a variety of stress signals and is responsible for the maturation and secretion of pro-inflammatory cytokines such as interleukin-1β (IL-1β) and interleukin-18 (IL-18). Therefore, targeting the pathological role of NEK7 in the mechanism of NLRP3 activation would suggest a promising novel opportunity to treat both systemic & neurological inflammatory disorders[i,ii].

**NLRP3 Inflammasome forms a Pivotal Role in Immune Response:** Inflammasomes are cytoplasmic, oligomeric complexes that activate Caspase 1 and other pro-inflammatory caspases to trigger the host defense in response to challenges. The most studied and characterized inflammasome is NLRP3, which serves as a detector of cellular stress and cell membrane damage. NLRP3 protein belongs to the family of NOD-like receptors (NLRs), primarily expressed by myeloid immune cells such as monocytes/macrophages, neutrophils, and dendritic cells, but also T and B cells, keratinocytes, as well as bronchial and intestinal epithelial cells. However, the pathological activation of NLRP3 has been demonstrated in many diseases, including systemic inflammatory and autoimmune diseases, metabolic disorders, and neurodegenerative conditions. The activation of NLRP3 that occurs in these disorders can lead to the development of chronic inflammation, progressive destruction of tissues, inhibition of repair processes, and consequently, significant disease progression[iii].

**NLRP3 Domains and Their Role in Activation:** NLRP3 consists of three main domains - a C-terminal leucine-rich repeat (LRR) domain, a central nucleotide-binding oligomerization domain (NACHT), and a N-terminal effector pyrin domain (PYD) (***Figure 1A***). Each domain plays a unique role in the process of NLRP3 activation. The LRR domain is responsible for protein-protein interactions and has been recognized as crucial for the formation of oligomeric ring-like structures of inactive NLRP3. The central NACHT domain, with ATPase activity, has been found to mediate the ATP-induced self-oligomerization of NLRP3. This process results in self-PYD interactions, and PYD domains recruit the ASC (Apoptosis-associated speck-like protein containing

a CARD) proteins which triggers the formation of prion-like ASC filaments. The effector PYD domain thus mediates the regulation of downstream signaling, and the resulting oligomeric NLRP3-ASC structure constitutes a platform for recruiting pro-Caspase 1, which then undergoes self-activation. Caspase 1 cleaves Gasdermin D protein, responsible for the formation of pores in the cell membrane that drive pyroptosis, as well as cleaves pro-IL-1β and pro-IL-18, which, upon maturation and release from the cell, trigger an inflammatory response[iv,v,vi].

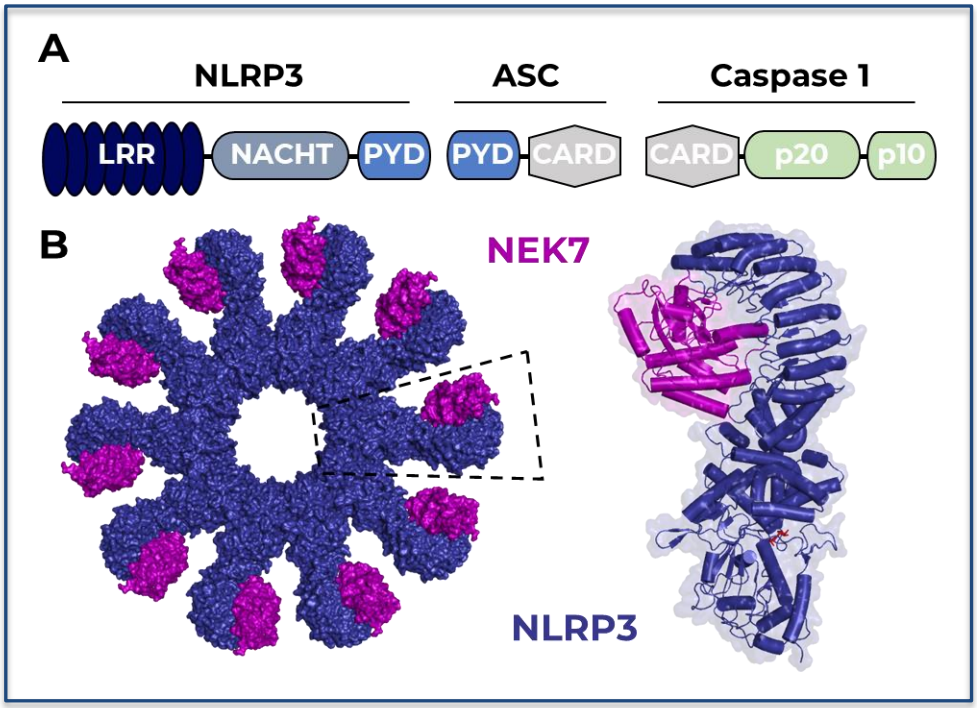


***Figure 1:*** ***NLRP3 inflammasome complex and its key elements.*** *(A) The NLRP3 protein consists of three main domains: LRR, NACHT, and PYD. The PYD domain of NLRP3 interacts with the PYD domain of the ASC protein, which in turn recruits pro-Caspase-1 due to the presence of the CARD domain. Next, Caspase 1 undergoes self-activation, which allows it to perform its effector functions. (B) The oligomeric structure of the active NLRP3 inflammasome disk. NEK7 interacts with the LRR domain of NLRP3 and cooperates in its conformational change from a closed to an open state, capable of recruiting ASC. PDB: 8EJ4.*

**The Role and Therapeutic Potential of NEK7 in Regulating the NLRP3 Inflammasome:** The NLRP3 protein is considered a general sensor of cellular perturbations rather than a direct effector of danger signals. According to the common dogma, factors that act as danger signals, referred to as pathogen- or damage-associated molecular patterns (PAMPs/DAMPs), lead to ion flux imbalance and/or mitochondrial dysfunction through various mechanisms, resulting in the disruption of the endomembrane system and trans-Golgi network. These events constitute a signal to initiate the process of the NLRP3 inflammasome assembly, supported also by the stimulation of gene expression for NLRP3, IL-1β, and IL-18 and post-translational modifications of NLRP3. It has been demonstrated that the NEK7 protein interacts with the LRR domain of NLRP3, contributing to the activation of the NLRP3 complex (***Figure 1B***). Detailed studies using cryo-EM technique have revealed that NEK7 cooperates in changing the conformation of the closed cage-like structure

of NLRP3 to form a disk-shaped inflammasome capable of recruiting ASC. Further studies have also indicated that NEK7 plays a structural role in the activation of NLRP3, while its enzymatic activity is not required in this process[vii]. The simplified scheme of NEK7-mediated NLRP3 activation is shown in ***Figure 2***.

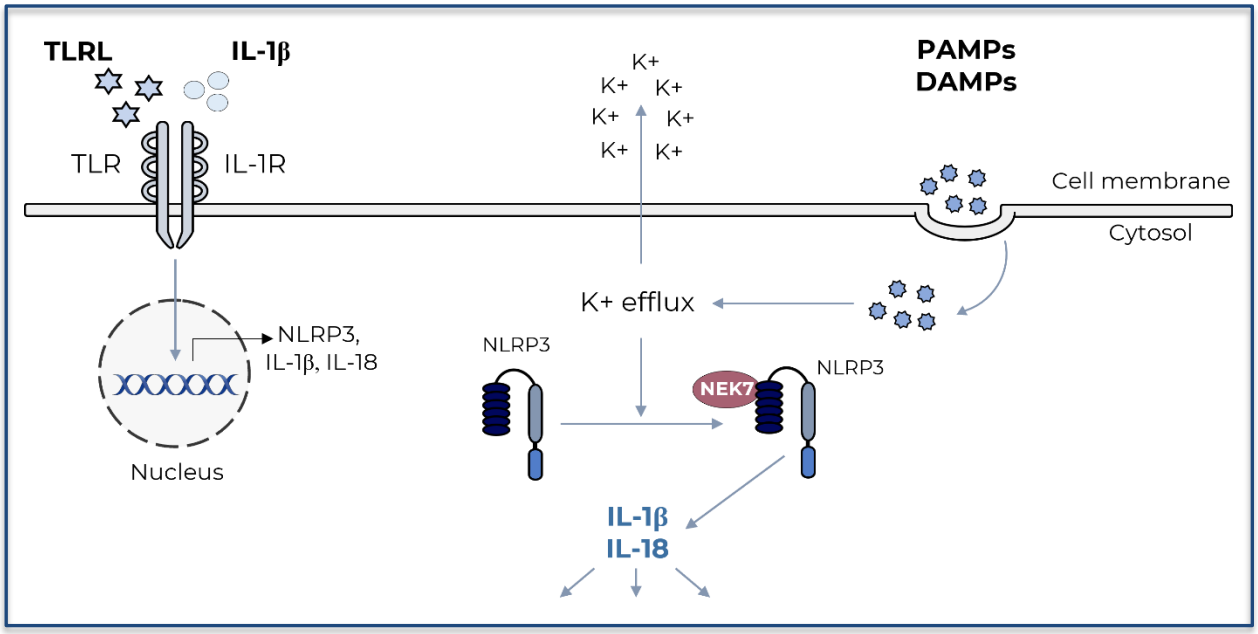


***Figure 2**: **Mechanism of NEK7-licensed NLRP3 activation.** IL-1β and TLR receptor ligands activate signaling pathways responsible for increasing the expression of genes for NLRP3, IL-1 β, and IL-18. DAMPs and PAMPs, through various mechanisms, disrupt ion flux across the cell membrane, and the efflux of K+ ions serve as a strong stimulus for the assembly of the NLRP3 inflammasome. NEK7 acts as a scaffolding protein and aids in the conformational change of the oligomeric structure of NLRP3, resulting in the recruitment of other components of the NLRP3 complex and the maturation and release of the pro-inflammatory interleukins IL-1β and IL-18.*

# NEK7 & NLRP3 as Drivers of Inflammatory Diseases

NLRP3 is increasingly recognized as central to the clinical manifestation of many inflammatory diseases and conditions with diverse etiology:

**Cryopyrin-Associated Periodic Syndromes:** The relationship between NLRP3 pathway hyperactivation and disease state has been demonstrated for a group of rare monogenic autoinflammatory diseases described as CAPS (Cryopyrin-Associated Periodic Syndromes). CAPS includes three phenotypes with varying degrees of disease severity; they are associated with gain-of-function mutations in the *NLRP3* gene and overproduction of IL-1β[viii].

**Gout:** One of the best described diseases associated with NLRP3 activation is gout, caused by the accumulation of uric acid crystals in tissues and joints. It has been

shown that monosodium urate crystals activate cells *in vitro* to produce IL-1β. Clinically, two IL-1β inhibitors, anakinra and canakinumab, have demonstrated therapeutic efficacy in patients with refractory gout indicating the significant role of the NLRP3 pathway in the pathogenesis of this condition[ix].

**Metabolic Diseases:** NLRP3-induced inflammation has also been shown to be fundamental to changes observed in both Type 1 (T1D) and Type 2 diabetes mellitus (T2D). According to Nițulescu *et al* (2023)[x] "*For T1D, NLRP3 perpetuates the autoimmune cascade, leading to the destruction of pancreatic islet cells. In T2D, its activation is associated with the presence of insulin resistance. NLRP3 activation is also instrumental for the presence of numerous complications associated with DM, microvascular and macrovascular*". Diabetic nephropathy, diagnosed in approximately 40% of patients, constitutes a major challenge in the management of diabetes and is a leading cause of death in this patient group. Patients with glomerular injury, as a manifestation of diabetic nephropathy, showed a correlation between increased glomerular NLRP3 expression and elevated levels of serum IL-1β, as well as clinical markers of nephropathy, including proteinuria and albuminuria[xi]. It has also been reported that in patients with metabolic dysfunction-associated steatohepatitis (MASH), the level of activated Caspase 1 in serum and the liver was significantly higher and correlated with disease severity[xii].

**Neuroinflammatory and neurodegenerative diseases (NDD):** Pathological activation of NLRP3 has also been demonstrated in several NDDs and CNS-related diseases such as multiple sclerosis (MS). For instance, primary progressive MS patients with an overactive NLRP3 inflammasome and increased IL-1β expression had disease symptoms that progressed significantly faster than patients with low IL-1β expression levels[xiii]. Pathogenic aggregates of α-synuclein in Parkinson's disease (PD) and p-tau or β-amyloid in Alzheimer's disease act as DAMPs resulting in NLRP3 activation in microglia, contributing to neuroinflammation and the progression of NDDs. NLRP3 inflammasome-related genes and proteins were also found to be upregulated in the brains and peripheral blood mononuclear cells (PBMCs) of patients with PD[xiv].

**Rheumatoid Arthritis:** Several single nucleotide polymorphisms (SNPs) in the genes that encode NLRP3, IL-1β, and IL-18, which result in elevated expression levels, have been linked to the onset of rheumatoid arthritis (RA) and other secondary osteoarthritis. Moreover, the expression of inflammasome-related genes in macrophages isolated from RA patients was significantly higher compared to healthy volunteers, while the plasma and synovial fluid showed markedly elevated levels of pro-inflammatory cytokines, including IL-1β[xv].

# Efficacy Considerations for NLRP3

**Uncertain Efficacy of Direct NLRP3 Inhibitors:** The long-term potential of NLRP3 as a drug target remains uncertain as clinical trials with NLRP3 inhibitors have yet to demonstrate conclusive therapeutic efficacy[xvi]. As such, NEK7 may prove to be an excellent alternative target for therapies targeting the inflammasome.

**Potential Mechanism-Based Limitations of NLRP3 Inhibitors:** Some structural models suggest that NLRP3 inhibitors might face difficulties in inhibiting a large protein-protein interaction surface once the inflammasome complex is formed[xvii]. Thus, they may only be effective in a preventive manner but unable to show efficacy as a therapeutic intervention. This might be due to conformational changes in NLRP3 that prevent an inhibitor binding after inflammasome assembly. NEK7 degraders, on the other hand, may have a more consistent effect due to the constant accessibility of the NEK7 G-loop for degradation.

**NLRP3's Pleiotropic Functions and Potential Off-Target Effects:** NLRP3 exhibits inflammasome-independent functions in various cell types, including those in the kidney and airway epithelium[xviii,xix,xx,xxi]. Direct NLRP3 inhibitors might disrupt these processes, leading to unforeseen adverse effects:

- Studies suggest NLRP3 is involved in renal epithelial cell apoptosis, mitochondrial regulation, and TGF-β signaling, independent of inflammasome assembly[xviii,xix,xx,xxi].
- Clinical trials with MCC950, a direct NLRP3 inhibitor, revealed unexpected hepatotoxicity in patients, highlighting potential off-target effects[xxii].
- Affinity-based proteomic studies suggest MCC950 might bind to unintended targets besides NLRP3, potentially contributing to off-target toxicities[xxiii].

NEK7 degraders, by targeting a protein upstream in the inflammasome pathway, could potentially avoid these complications[xviii].

**Limited Safety Data for Direct NLRP3 Inhibitors:** The long-term safety profile of NLRP3 inhibitors in patients remains unclear[xxiii] and more extensive clinical evaluations are needed to establish their safety for prolonged use.

| NLRP3 inhibitor off-target effects | NEK7 degrader selectivity |
|---|---|
| Sterile necroinflammation, fibrosis, tissue repair[xviii]<br>Apoptosis regulation in tubular cells in kidneys (mice showed renal problems upon MCC950 administration)[xix]<br>Innate immune homeostasis in the airway[xx]<br>Regulation of IL-33 production[xxi] | Degrading NEK7 does not result in total blockade of the inflammasome (complete shutdown of IL-1β can lead to fatal infections)[xxiv]<br>Preclinical data suggests the off-target effects seen with NLRP3 can be avoided by selectively degrading the upstream protein<br>Highly selective NEK7 degraders do not affect other members of the NEK family[1] |

***Table 1:* Significant off-target effects (unrelated to the inflammasome) seen with NLRP3 inhibitors in the clinic & potential benefits of NEK7 degraders.**

# Safety of NEK7 as a Target

**NEK7 in mitosis:** Several early reports implicated NEK7 kinase activity in mitosis.

[1] Studies conducted in-house by Captor

However, the data on NEK7 involvement in mitosis are inconsistent and often contradictory, with numerous reports demonstrating no impact on cell division, proliferation and viability. In particular, the more recent reports showed that the genetic or pharmacological abrogation of NEK7 expression in cellular models and *in vivo*, in mouse and non-human primates, didn't induce any toxicity associated with impaired cell proliferation and viability.

The original NEK7 knockout mouse model resulted in lethality in late embryogenesis and early post-natal stages[xxv]. Mouse embryonic fibroblasts (MEFs) derived from Nek7(-/-) embryos showed an increased tendency for chromosomal lagging, micronuclei formation and cytokinesis failure. The concept of NEK7 involvement in mitosis was developed solidified by Shi *et al*. (2016)[ii] who concluded that NLRP3 inflammasome activation and mitosis cannot occur simultaneously, and that NEK7 acts as a switch between mitosis and inflammasome activation competence, both of which require NEK7. These findings might suggest potential adverse effects on mitosis of NEK7 inhibitors and degraders.

However, the expanding knowledge of molecular mechanisms governing the NLRP3 inflammasome activation and increased understanding of the role of NEK7 suggest that these conclusions were incorrect and point to a different interpretation of these results, which explain why NEK7- NLRP3 inflammasome interactions are limited during mitosis.

**Relevance to NEK7 degradation:** The lack of any phenotype observed by Shi *et al*. (2016) in the NEK7 +/Cu mice (heterozygotes) is important because it demonstrates that an ~50% decrease in NEK7 caused no toxicity. Therefore, a partial but continual NEK7 inactivation likely to be achieved by a degrader should be safe.

A complete NEK7 knockout in Cuties had no negative impact on the proliferation and differentiation of various types of blood cells indicating that NEK7 is not involved in mitosis. This is of particular importance, because hematopoietic cells have a very high proliferative index, and any impairment of mitosis/cell proliferation would result in decreased number of hematopoietic cells.

Together, these results indicate that the pharmacological degradation of NEK7 is expected to be safe and well tolerated.

## NEK7 degradation has no impact on mitosis

**In Vitro:** Kim *et al* (2011)[xxvi] showed that NEK7 is a centrosomal kinase that is required for a proper spindle formation during mitosis and that centriole duplication was inhibited in NEK7-depleted cells. These results were generated using NEK7 inactivation by siRNA, followed by ectopic expression of various NEK7 mutants, then by a 72h exposure to hydroxyurea. The levels of transfected NEK7 were very high, >10 fold higher than the levels of endogenous NEK7. Non-physiologically high levels of

ectopic proteins are known to alter multiple cellular processes e.g. via protein squelching. Hydroxyurea used to synchronize cells is known to induce changes in multiple cellular processes including DNA strand breaks, apoptosis, ROS generation, oxidative stress, and chromatin remodeling. Therefore, the results linking NEK7 function to mitosis were generated in a highly engineered, artificial cellular systems not representative of the normal biology of NEK7. This can explain why genetic and pharmacological inactivation of NEK7 in numerous biological models failed to demonstrate any effects on mitosis, cell proliferation and cell viability. These include multiple NEK7 knockout cell lines available commercially and NEK7 KO mice discussed elsewhere in this document.

Multiple cell lines with NEK7 knockout have been generated which do not display any effects on cell proliferation, including commercially available lines (e.g.):

a. AcceGen: NEK7 stable knockout HeLa cell line # ABC-KH10015
b. Creative Biogen: NEK7 stable knockout HeLa cell line # CSC-RT0817
c. BPS BioScience: NEK7 stable knockout THP-1 cell line Catalog #82121

Therefore, in multiple cellular models, including studies conducted in-house by Captor, NEK7 elimination exhibited no negative impact on mitosis, including in rapidly proliferating cells.

***In Vivo* & Clinical:** Merck reported a novel tamoxifen-inducible NEK7 conditional KO mouse model (NEK7 cKO). NEK7 gene expression was significantly downregulated in heart, liver, kidney, and spleen of NEK7 cKOs. Protein levels were also significantly reduced in these tissues. In BMDMs, NEK7 protein levels were reduced by >90%. No adverse effects were reported further confirming that NEK7 inactivation has no impact on mitosis[xxvii].

*In vivo* studies conducted by Captor in cynomolgus monkeys did not identify any safety signals at doses which fully degraded NEK7 in monkey PBMCs.

Monte Rosa Therapeutics reported no safety signals in cynomolgus monkeys dosed with the NEK7 degrader MRT-8102 at 0.2 mg/kg, 1mg/kg and 5mg/kg for 5 days[xxviii]. The doses induced about 70% NEK7 degradation in PBMCs. The final observations were done on day 15. In another *in vivo* study of MRT-8102, cynomolgus monkeys were administered a high dose of 30mg/kg for 7 days, resulting in complete NEK7 degradation in both PBMCs & brain, with no reports of any NEK7 degradation-associated toxicity[xxix]. An IND is expected to be submitted for MRT-8102 in Q1 2025[xxx].

Halia Therapeutics completed a Phase 1 evaluation of HT-6184, the allosteric NEK7 inhibitor (NCT05447546) and reported adequate safety and tolerability in healthy volunteers dosed for 2 weeks[xxxi]. While an inhibitor is different from a degrader, the results didn't indicate any safety signal associated with interference in NEK7 activity.

Early reports suggested a role for NEK7 in mitosis, which could potentially lead to adverse effects for NEK7-targeted therapies. However, the ample evidence now available indicates that the early findings were incorrect with considerable preclinical (both *in vitro* & *in vivo*) & clinical data demonstrating that the targeting of NEK7 has

no impact on cell mitosis, proliferation or safety. **Together, multiple studies and models have also confirmed that the pharmacological degradation of NEK7 has no impact on cell proliferation/mitosis and therefore NEK7 degraders are expected to be safe and well tolerated.**

# Key Differentiators of Captor Therapeutics' NEK7 Degraders

Captor Therapeutics is developing two chemical classes of NEK7 molecular glue-type degraders. The first (CPT-635r & CPT-635) displays systemic distribution and picomolar potency, while the second (CPT-732r & CPT-732) is brain-penetrant and active in a low single-digit nanomolar regime[2]. Overall, both classes offer a unique approach to treat diseases such as: gout, inflammatory bowel disease, lupus nephritis, NASH/MASH, multiple sclerosis, amyotrophic lateral sclerosis, Alzheimer's or Parkinson's Disease. Market estimates & patient populations in NLRP3-related diseases are provided in **Table 3** below.

**Safety as a Priority for Chronic Conditions:** For chronic inflammatory diseases safety is a paramount concern due to the requirement for long-term treatment[xxxii]. Excellent selectivity and high tolerability together with innate biology of NEK7 make Captor's degraders a promising avenue for these conditions.

**No impact on proliferating cells:** In studies carried out by Captor, none of our lead NEK7 degraders affected the viability of six different actively proliferating human cancer cell lines (**Table 2**), and commercially available NEK7 KO cancer cell lines also did not show any effects on cell proliferation. Our studies conducted in NHPs have also not identified any safety signals at doses which fully degraded NEK7 in monkey PBMCs.

| Cancer Cell Line | CPT-635r | CPT-635 | CPT-732r | CPT-732 | Doxorubicin |
|---|---|---|---|---|---|
| Adenocarcinoma (SW48) | 105 ± 7.67 | 94.9 ± 5.59 | 100 ± 1.90 | 94.0 ± 10.0 | 0.33 ± 0.02 |
| Adenocarcinoma (DLD-1) | NT | 88.9 ± 4.66 | NT | 97.9 ± 0.2 | 3.42 ± 0.13 |
| CML (K562) | 117 ± 6.21 | 106 ± 2.19 | 119 ± 2.91 | 110 ± 6.09 | 2.04 ± 0.59 |
| MML (MV4-11) | 102 ± 4.40 | 99.5 ± 18.0 | 108 ± 12.8 | 97.0 ± 20.6 | 0.05 ± 0.02 |
| HCC (Hep3B) | 103 ± 0.16 | 103 ± 5.51 | 99.2 ± 8.49 | 103 ± 0.21 | 1.31 ± 0.02 |
| Lung Carcinoma (A549) | 92.7 ± 5.30 | 99.7 ± 4.44 | 91.8 ± 6.12 | 99.2 ± 3.9 | 1.46 ± 0.22 |

***Table 2:* Mean minimum viability ± SD (%) of rapidly dividing cancer cell lines to Captor NEK7 degraders and a positive control (doxorubicin). Cancer cells were incubated with the compounds for 72h at six different concentrations up to 10µM and viability was measured using the CTG assay. NT = not tested.**

**Favorable DMPK Profile:** Initial *in vitro* tests showed no inhibition of CYP isoforms at the tested concentrations (10 µM), suggesting minimal potential for drug-drug interactions mediated by CYP enzymes. No significant hERG inhibition was observed

[2] CPT-635 & CPT-732 are diastereoisomers of CPT-635r & CPT-732r respectively.

up to 10 µM for either CPT-635 or CPT-732 and no genotoxicity was observed in Ames assays with of CPT-635r or CPT-752r using TA98/T100 bacterial strains up to 1 mM. Both CPT-635 and CPT-732 were also shown to be safe in the micronucleus genotoxicity test, with no adverse effects observed in micronucleus formation, whether with or without metabolic activation (**Figure 3**). There is also no significant inhibition of any targets in CEREP44 assays for CPT-635r or CPT-732r. Captor degraders also show excellent selectivity against suspected teratogenic targets SALL4, PLZF and p63 after 24h exposure (**Figure 4**). These results demonstrate that Captor's NEK7 degraders do not show any mutagenic or teratogenic potential.

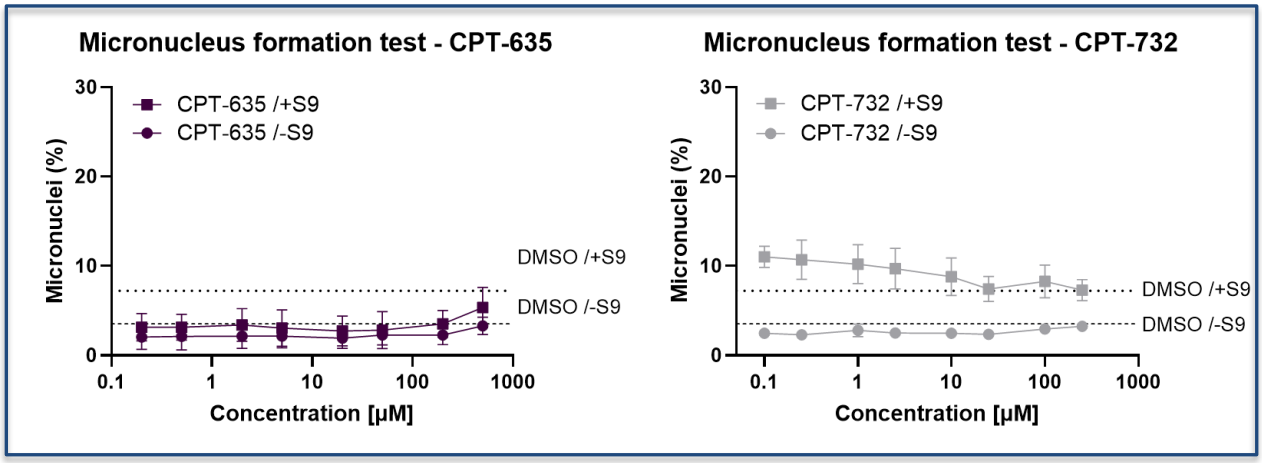


***Figure 3:** Captor NEK7 degraders CPT-635 & CPT-732 tested in micronucleus genotoxicity test using human TK6 cells with metabolic activation (+S9; 3h exposure + 21h recovery) and without metabolic activation (-S9; 24h exposure). CPT-635 & CPT-732 are non-genotoxic in the positive micronuclei induction analysis (Cyprotex).*

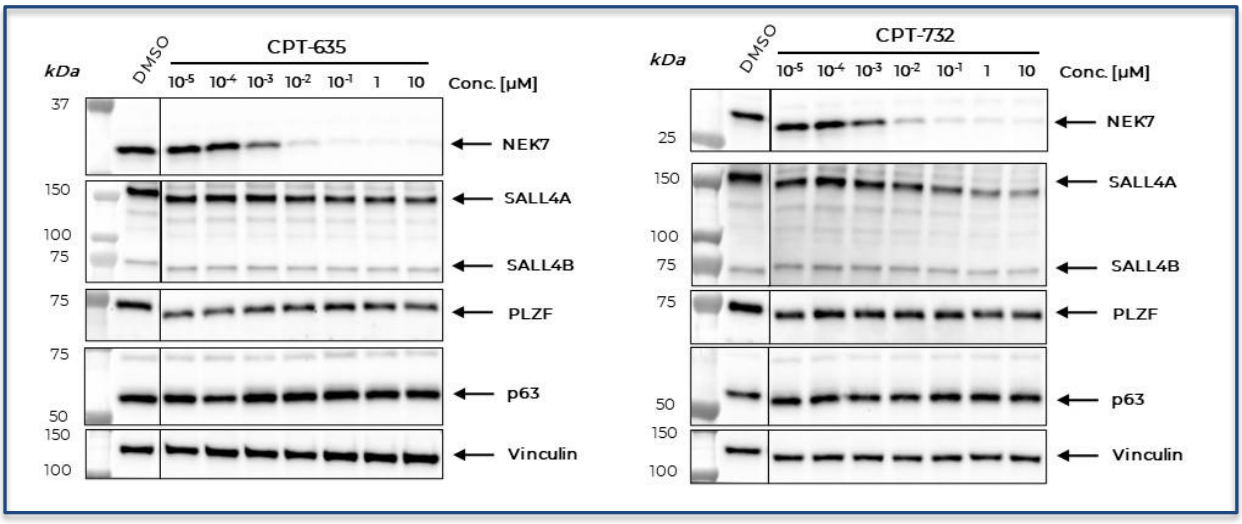


***Figure 4:** Comparison of the degradation of NEK7 and suspected teratogenic targets - SALL4, PLZF, and p63 - in Kelly neuroblastoma cells.* *The Western-blot analysis was performed after 24 hours of treating the cells with CPT-635 & CPT-732, Captor's most potent NEK7 degraders, in the concentration range of 10 pM-10 µM. Both compounds degrade NEK7 in a dose-dependent manner and exhibit excellent selectivity profiles.*

**Comparative Inhibition of IL-1β Release:** In our *in vitro* studies, with complete NEK7 degradation, we achieved around 90% inhibition of IL-1β release. MCC950, a direct NLRP3 inhibitor, achieved complete inhibition of IL-1β, which may lead to adverse, recurring infections[xxxiii].

**Cross-species Activity:** Despite significant differences in mouse and human Cereblon[xxxiv], Captor's NEK7 degraders have demonstrated therapeutic effects in *in vivo* mouse disease models, albeit at higher dose levels. Such cross-species activity has not been reported for other NEK7 degraders.

***In Vivo* Evaluation:** Our current non-human primate (NHP) studies in cynomolgus monkeys demonstrated over 95% degradation of NEK7 after 24 hours from single, oral drug administration (**Figure 5**). This is by far the most prominent evidence supporting development of once daily drugs targeting NEK7.

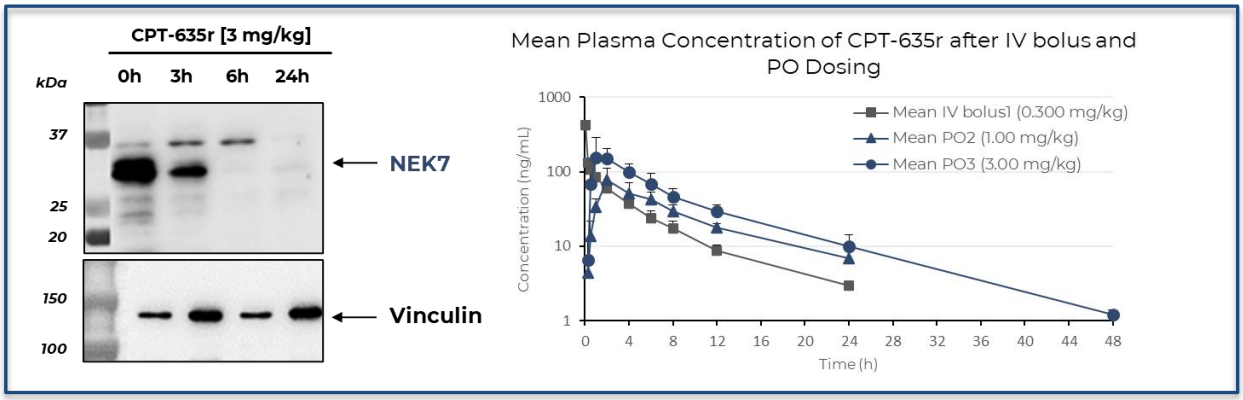


***Figure 5:* PK/PD study with CPT-635r in cynomolgus monkeys.** *CPT-635r shows excellent pharmacokinetic properties (right panel) after single intravenous (0.3 mg/kg) or oral administration (1 mg/kg or 3 mg/kg). The Western-blot analysis shows almost complete degradation of NEK7 as early as 6 hours after single oral administration, persisting up to 24 hours post-administration. Number of animals per group: N=3.*

In another *in vivo* study we showed the efficacy of NEK7 degraders in a mouse model of multiple sclerosis, Experimental Autoimmune Encephalopathy (EAE), where our approach exhibited superior activity in the interventional model compared with the NLRP3 inhibitor, MCC950 (**Figure 6**). EAE constitutes an animal model of multiple sclerosis and is mediated by autoreactive T-cells. The disease was induced by the $MOG_{35-55}$ peptide and Pertussis Toxin. Mice were observed for motor abilities and tail/limb weakness, based on which the clinical score was determined. The first symptoms of the disease appeared on day 12, at which point the treatment of animals in all groups began. The mice received oral doses of CPT-635r once daily at 10 or 30 mg/kg. Additionally, Dexamethasone was used as a control compound at an oral dose of 0.1 mg/kg once daily, and MCC950 was administered intraperitoneally at a dose of 10 mg/kg once daily. A dose-dependent therapeutic effect of CPT-635r was observed, with significant inhibition of disease progression in the final stage of the study at a dose of 30 mg/kg. MCC950 did not show a therapeutic effect in this model. No toxic effects related to the administration of the compounds were observed during the study. The body weight of mice in the groups treated with CPT-635r was higher than in the control group and the group receiving dexamethasone.

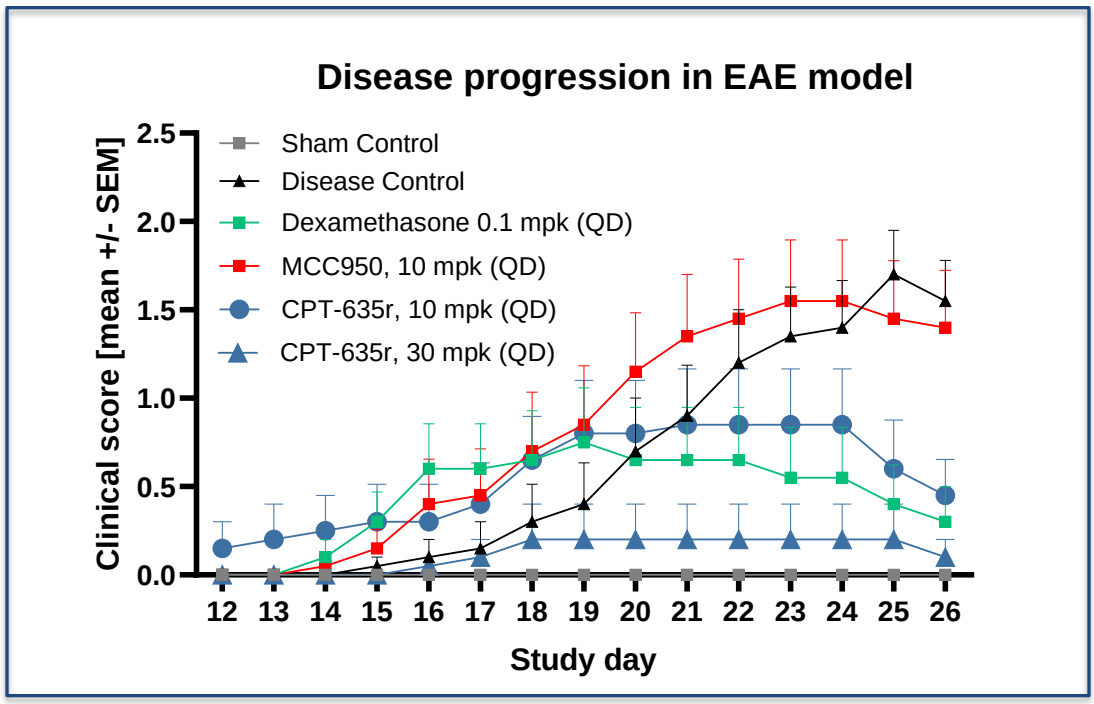


***Figure 6:*** ***Therapeutic efficacy of CPT-635r in a murine model of Experimental Autoimmune Encephalopathy (EAE).*** *The study was conducted on female C57BL/6 mice. Number of animals per group: N=10.*

This *in vivo* evaluation is complemented with a classical model of Rheumatoid Arthritis, Collagen-Induced Arthritis (CIA), where NEK7 degradation fully recapitulates its genetic knockout and strongly reduces the disease burden - on par with MCC950 (**Figure 7**). The study was conducted on male DBA1 mice. CIA is a T/B cell-driven animal model of rheumatoid arthritis. The disease was induced by collagen injection on day 0, followed by booster immunization on day 21. Clinical score was calculated based on swelling of digits/paws and erythema. The first symptoms of the disease appeared on day 18, at which point the treatment of animals in all groups began. The mice received oral doses of CPT-635r once daily at 10 or 30 mg/kg. Additionally, dexamethasone was used as a control compound at an oral dose of 0.1 mg/kg once daily, and MCC950 was administered intraperitoneally at a dose of 10 mg/kg once daily. A dose-dependent therapeutic effect of CPT-635r was observed, with a 50% reduction in clinical score at a dose of 30 mg/kg. MCC950 showed a similar effect to CPT-635r at a higher dose. No toxic effects related to the administration of the compounds were observed during the study.

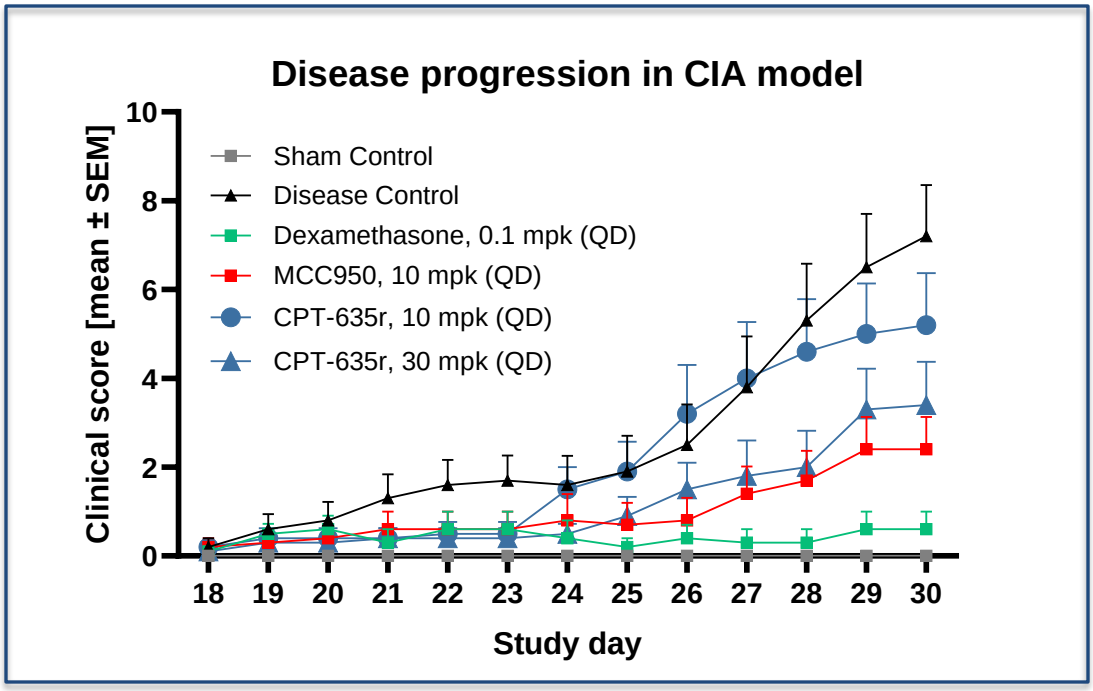


***Figure 7:*** ***The results of the study on the therapeutic efficacy of CPT-635r in a murine model of Collagen-Induced Arthritis (CIA).*** *Number of animals per group: N=10.*

# Market Opportunities

Given the vital role the inflammasome plays in many diseases, there are a number of major chronic indications, both neurodegenerative and systemic, where NEK7 degraders may bring significant therapeutic benefits (**Table 3**).

| Disease | | Global Prevalence | Market Opportunity (US$, 2024) |
|---|---|---|---|
| Rare Diseases | Amyotrophic Lateral Sclerosis (ALS) | 4.42/ 100k | $780M |
| | Cryopyrin-Associated Periodic Syndromes (CAPS) | 0.27-0.55/ 100k | $40M |
| | Huntington's | 5-10/ 100k | $720M |
| CNS | Alzheimer's | 55M | $5.5B |
| | Parkinson's | 8.5M | $5.4B |
| | Multiple Sclerosis | 2.8M | $24.5B |
| Systemic | Crohn's/ IBD | 6-8M | $18.3B |
| | Diabetic Nephropathy | 188M | $3.4B |
| | Gout | 41M | $3.8B |
| | MASH (NASH) | 264M | $4.6B |
| | Psoriasis | 4.6M | $20.5B |
| | Rheumatoid Arthritis | 18M | $40.8B |

***Table 3:*** ***Current market opportunities for indications that may be suitable for treatment***

***with NEK7 degraders.*** *Market opportunity estimates are an average of publicly available market research reports & published literature for each indication (accessed August 2024).*

# Conclusions

NLRP3 is a key component of the inflammasome and increasingly recognized as playing a significant role in the manifestation of many systemic & neurodegenerative inflammatory diseases. However, the direct targeting of NLRP3 has seen serious side-effects, in particular the associated reduction in patient immunity leading to excess infections & mortality. NEK7, which modulates the activity of NLRP3, provides a new and potentially more selective target for inflammatory diseases as targeting NEK7 has not shown the same degree of immune suppression as seen with NLRP3. Therefore, NEK7 degraders may present a promising and potentially safe therapeutic strategy for many inflammatory diseases. Their likely advantages include:

- Superior safety profile by avoiding NLRP3's pleiotropic functions;
- NEK7 may be less susceptible to inflammasome-mediated resistance due to its function;
- Previous suspected role of NEK7 in cell mitosis shown to be erroneous and multiple studies & models have since confirmed that the pharmacological degradation of NEK7 has no impact on cell proliferation/mitosis, therefore NEK7 degraders are expected to be safe and well tolerated.
- Degraders do not require constant drug coverage as, once NEK7 is degraded, levels will not recover until resynthesized. This contrasts with inhibitors which must provide constant coverage to exert their effect. The intermittent drug coverage that degradation permits allows for reduced dosing with the potential for improved side-effect profiles and reduced therapeutic costs;
- Small size of Captor's NEK7 molecular glue degraders allows for oral dosing & brain-penetrant compounds.

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