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A Novel Therapeutically Active CSF1R Agonist Promotes Tissue Macrophage Inflammation Resolution And Induces Tissue Repair Pathways

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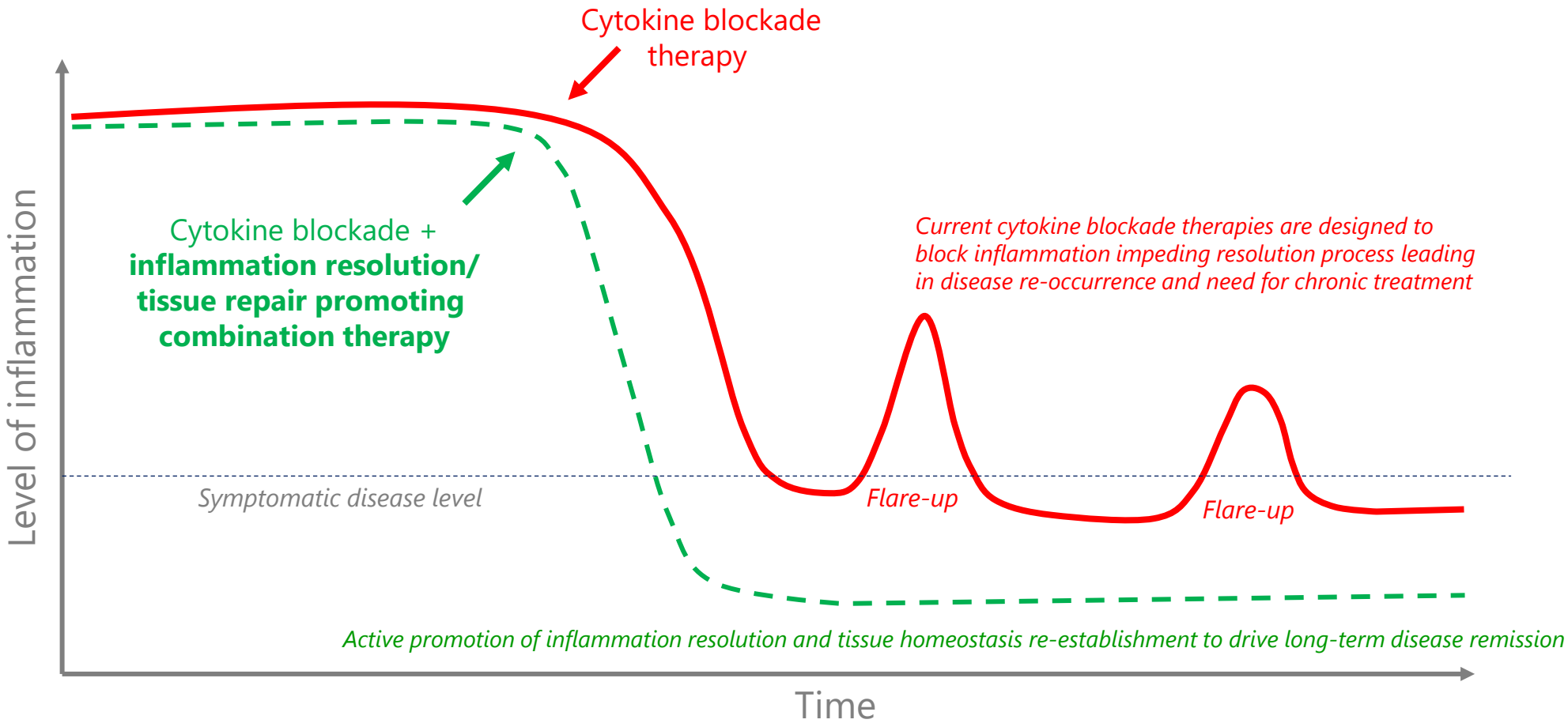
Rationale for combining novel inflammation resolution-promoting agents with existing cytokine blockade therapies for enhanced efficacy and sustained remissions in inflammatory diseases



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Reference: Nektar Therapeutics, 2024

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Supporting evidence for tissue macrophages as drug targets for inflammation resolution and tissue repair in injury and chronic inflammation conditions

- Disease remission and re-establishment of homeostasis in most disease conditions is universally characterized by a requisite phenotypic transition of pro-inflammatory monocytes and macrophages to induction of inflammation resolution and tissue repair functions

- In human rheumatoid arthritis patients, synovial tissue macrophages with inflammation resolution phenotypes drive disease remission in contrast to disease progressing inflammatory monocyte-derived macrophages

- Exogenous CSF1 or CSF1-Fc fusion cytokine administration and activation of tissue macrophages has been shown to improve tissue damage recovery in animal injury models (e.g. liver fibrosis, CNS demyelination, renal tubular injury) however they also induce systemic monocytosis and inflammatory monocytes tissue infiltration

References: Alikhan et al., 2011, Am. J. Pathol.; Laflamme et al., 2018, Front. Cell. Neurosci.; Keshvari et al., 2022, Dis. Model. Mech.; Sauter et al., 2016, Am. J. Physiol. Gastrointest. Liver Physiol.

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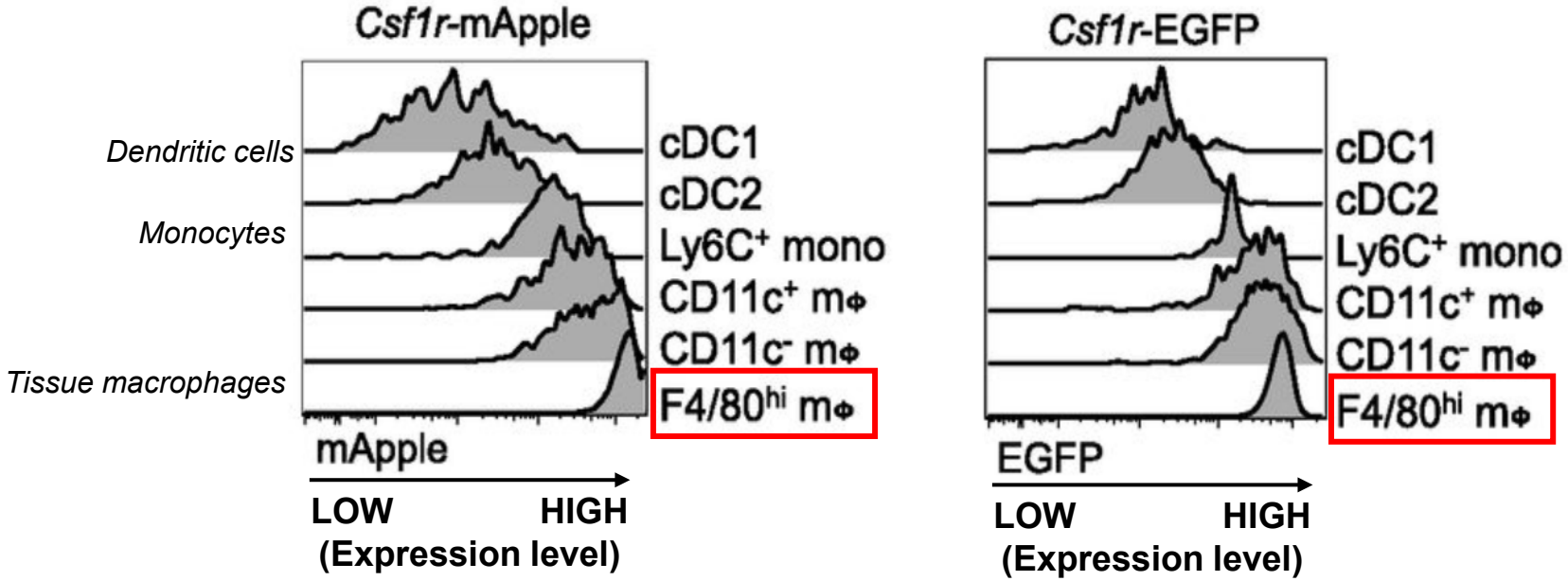
Rationale for attenuated CSF1 receptor agonists to generate selectivity in functional activation across CSF1 receptor expressing cell types

- Tissue resident macrophages show the highest levels of CSF1 receptor expression compared to monocytes and dendritic cells



- <u>Design concept:</u> Attenuated CSF1R agonist to optimize target engagement and duration of receptor occupancy on the cell types of interest and minimize effects on off-target cells

Reference: Hawley et al., 2018; DOI: 10.4049/jimmunol.1701488

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Receptor binding screen for attenuated polymer conjugated CSF1 variants

Conjugate	k_{on} ($M^{-1}s^{-1}$)	k_{off} (s^{-1})
rhCSF1		
NKTR-422-H		
NKTR-422-G		
NKTR-422-F		
NKTR-422-E		
NKTR-422-D		
NKTR-422-C		
NKTR-422-B		
NKTR-422-A		

Parameters used to generate diversity in PEG-CSF1 conjugate library:

- PEG polymer properties: polymer size, shape
- PEG polymer conjugation chemistry: single site-specific vs multiple surface residues
- CSF1 protein sequence: wildtype sequence vs mutations in receptor binding interface

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Affinity measured by BIAcore, using CSF1 receptor:Fc captured by immobilized anti-Fc antibody.

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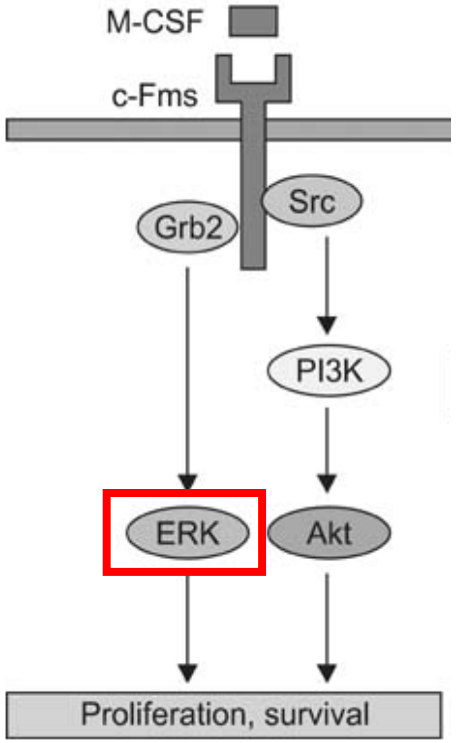
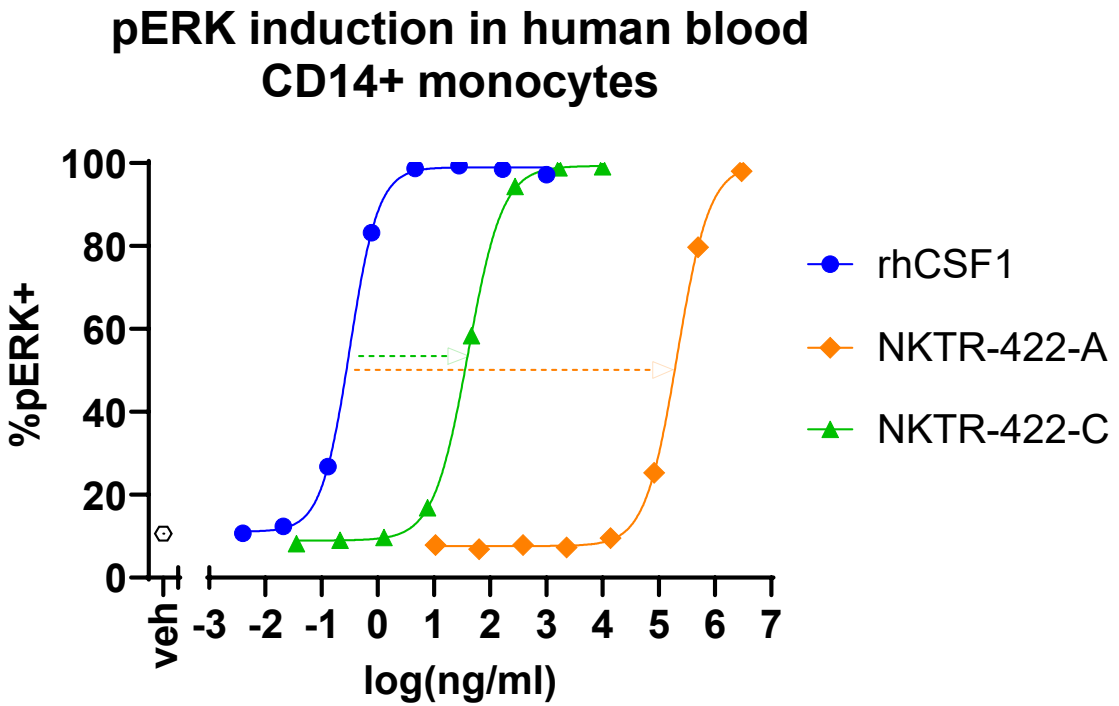
NKTR-422 conjugates with attenuated receptor binding properties are full agonists of CSF1 receptor with significantly reduced potency





Human whole blood was incubated in vitro for 10 min at 37ºC with recombinant human CSF1 (rhCSF1) or NKTR-422 conjugates shown at indicated concentrations. Intracellular ERK phosphorylation at T202/Y204 in CD14+ monocytes was measured by flow cytometry.

Reference: Kim and Kim. 2016; DOI: 10.4068/cmj.2016.52.1.12

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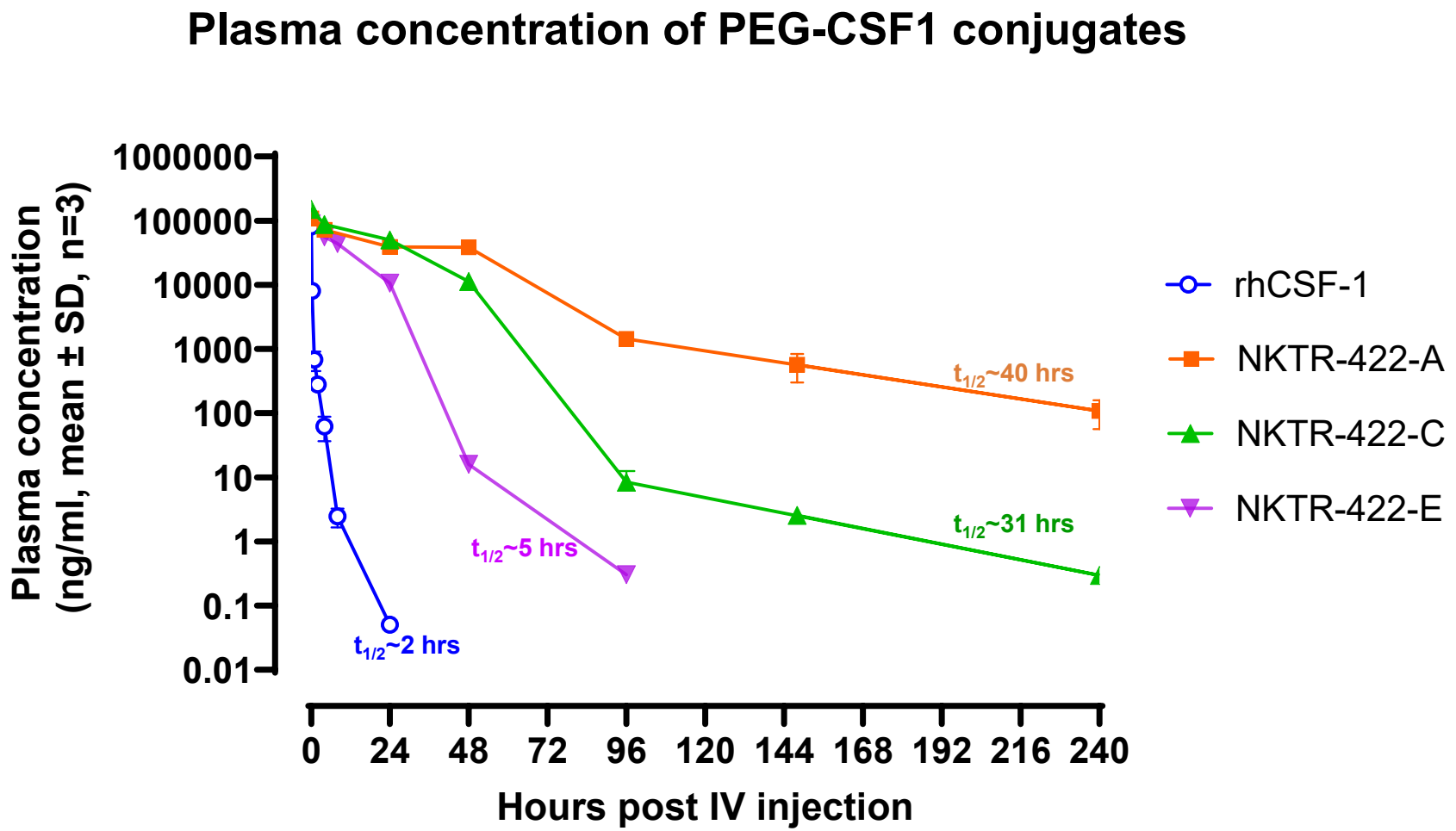
Attenuated receptor binding of NKTR-422 conjugates correlates with plasma exposure in mice



c57Bl6 and Balb/c mice (n=3 animals/timepoint) were intravenously injected with indicated NKTR-422 conjugates at shown dose levels. Plasma was collected at indicated timepoints and conjugate concentrations were measured by a bioanalytical assay designed specifically for hCSF1 PEG-polymer conjugates detection utilizing MSD assay with PEG capture and hCSF1 detection. rhCSF1 levels were measured by analogous bioanalytical assay utilizing hCSF1 capture and detection antibodies. SD - standard deviation.

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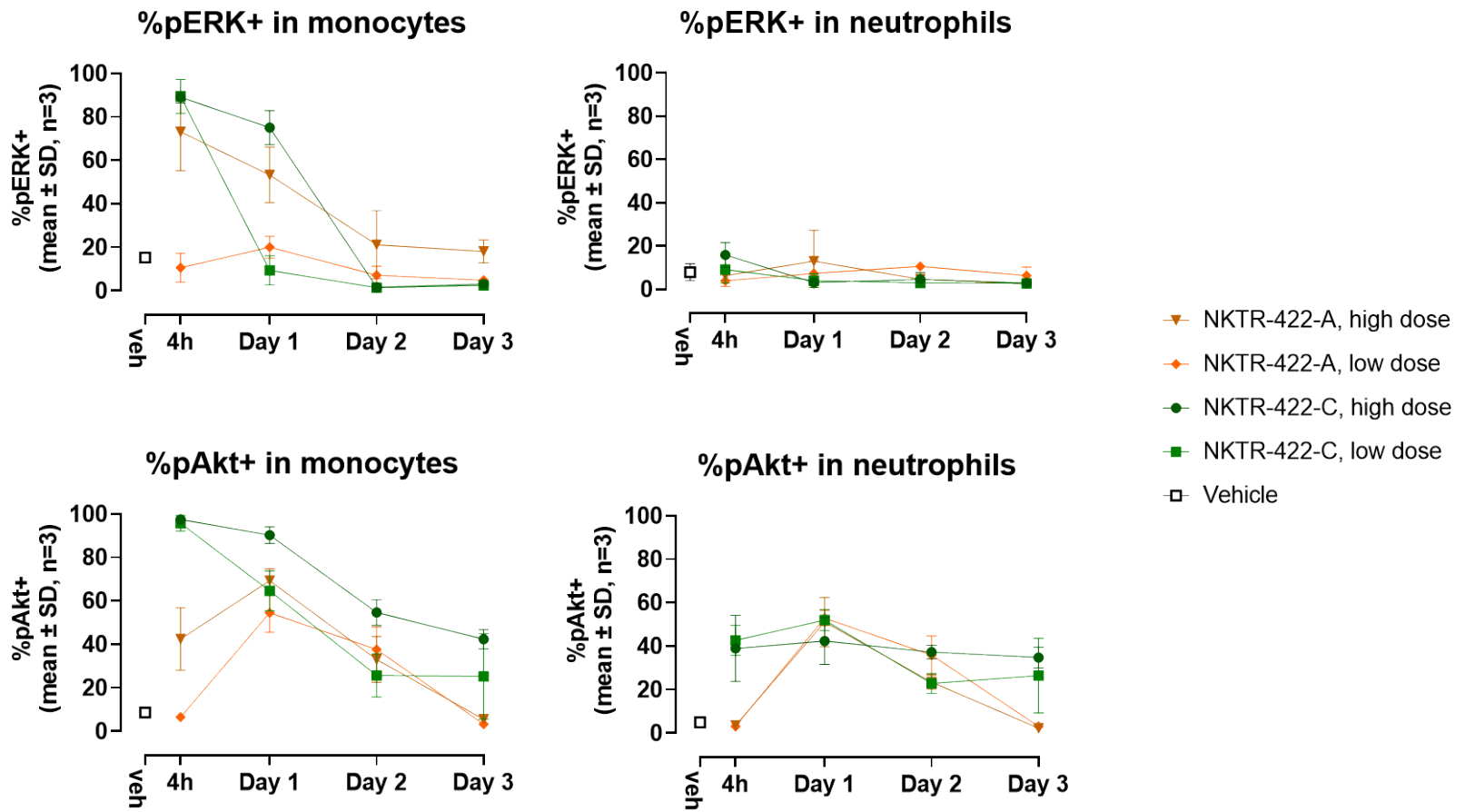
NKTR-422 conjugates induce sustained activation of ERK and PI3K pathways in vivo in mouse blood monocytes and neutrophils from a single dose



c57Bl6 mice were intravenously injected (n=3 animals/timepoint/treatment group) with indicated PEG-CSF1 conjugates at shown dose levels. Phosphorylation of intracellular phospho-ERK (T202/Y204) or phospho-Akt (S473) were measured by flow cytometry in peripheral blood monocytes and neutrophils at indicated timepoints.

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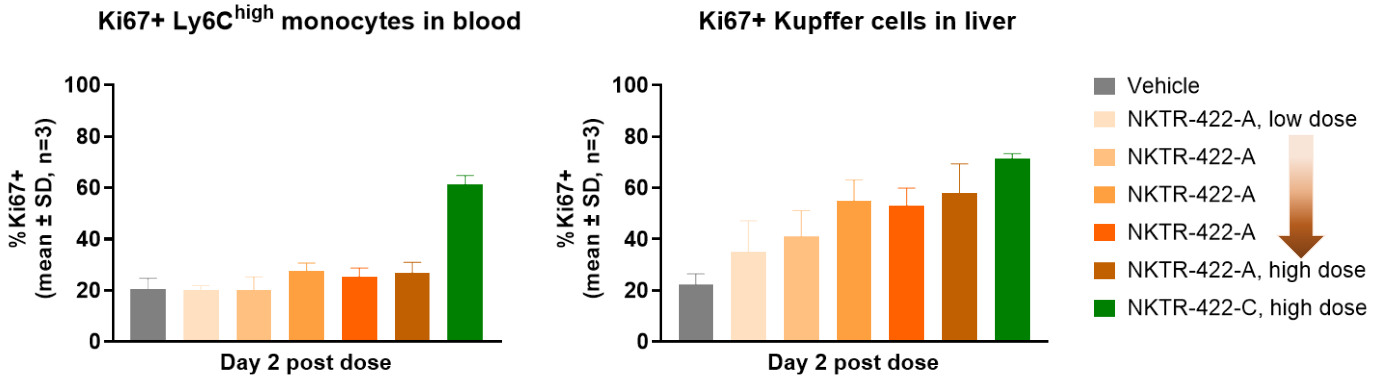
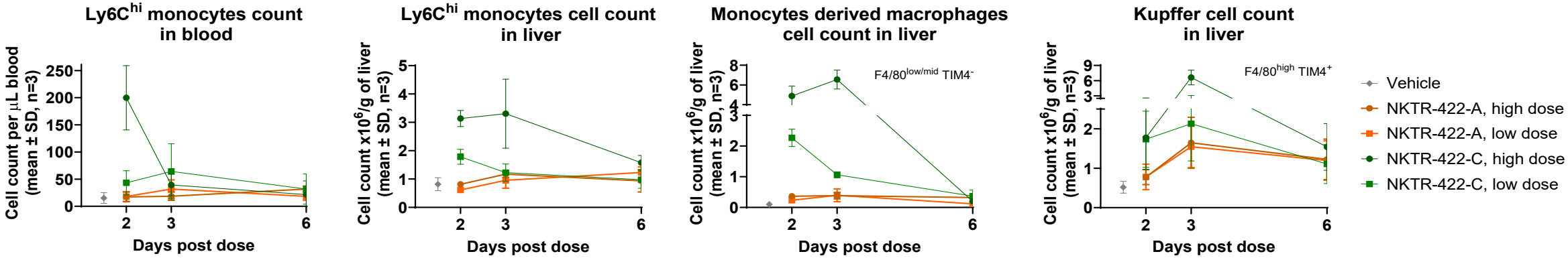
NKTR-422-A selectively proliferates and expands tissue resident macrophages without proliferation and tissue infiltration of pro-inflammatory Ly6C^{high} blood monocytes





Attenuated CSF1R agonist NKTR-422-A achieved design goal of differential tissue macrophage activation

c57Bl6 mice were intravenously injected with indicated NKTR-422 conjugates at shown dose levels. Ki67 positivity and monocyte or macrophage subsets were identified in blood and liver tissue by flow cytometry based on lineage markers. Monocyte derived vs tissue resident macrophages were identified based on cell surface F4/80 expression level and TIM4 expression.

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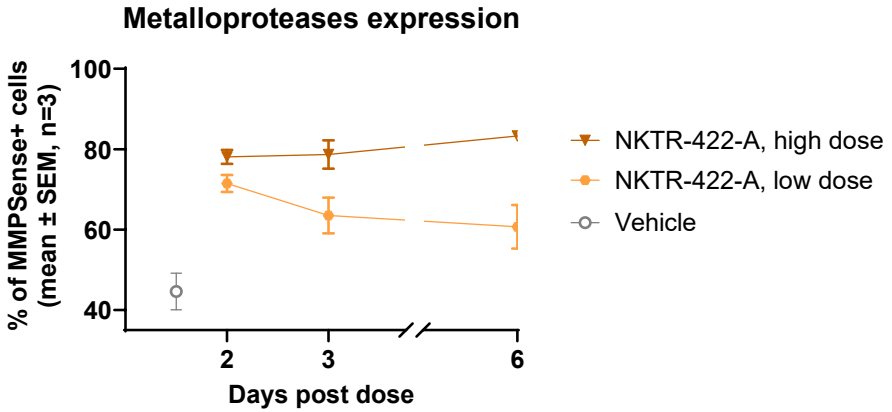
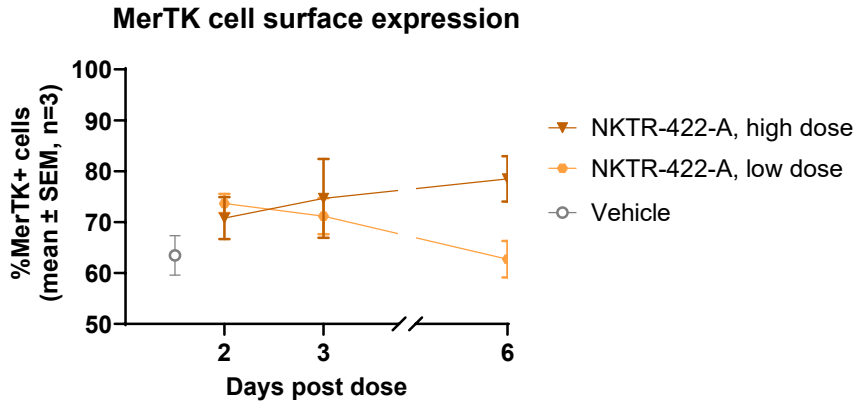
NKTR-422-A induces sustained expression of inflammation resolution and tissue repair functional markers in Kupffer cells

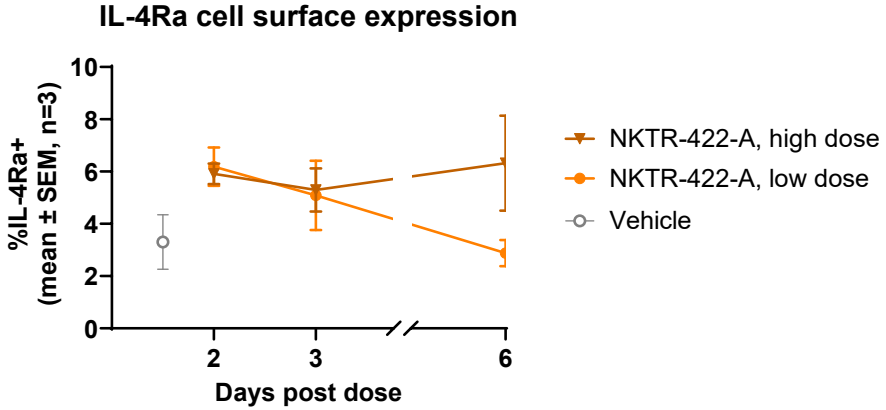
Tissue matrix remodeling

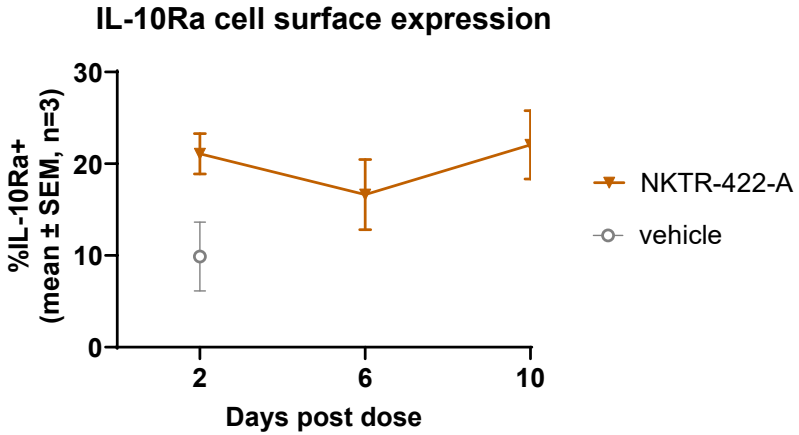


Efferocytosis enhancement



Increased sensitivity to anti-inflammatory cytokines





c57Bl6 mice were intravenously injected with indicated PEG-CSF1 conjugates at shown dose levels. MerTK, IL-4Ra and IL-10Ra cell surface expression in liver tissue macrophages Kupffer cells, defined by F4/80highTIM4+ phenotype, was measured by flow cytometry. Metalloprotease levels were measured by flow cytometric detection of MMPSense680 biosensor intravenously injected 24h prior to tissue collection.

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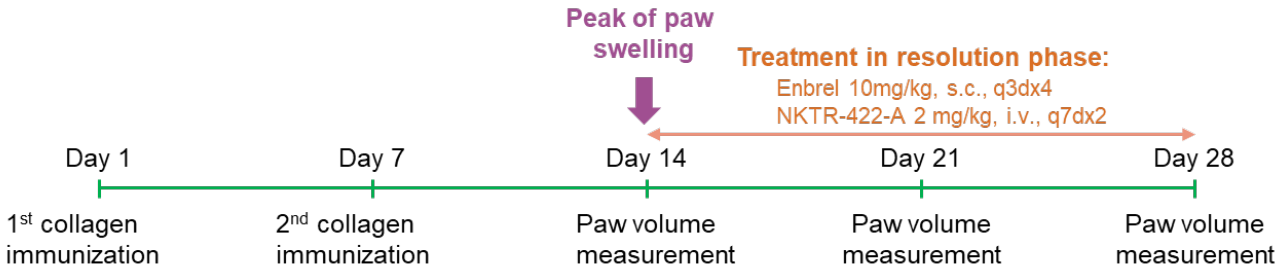
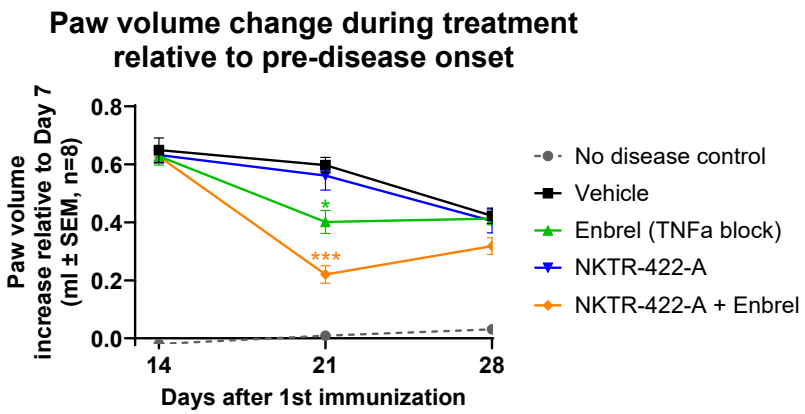
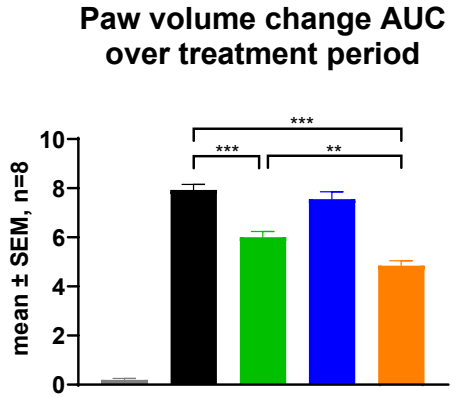
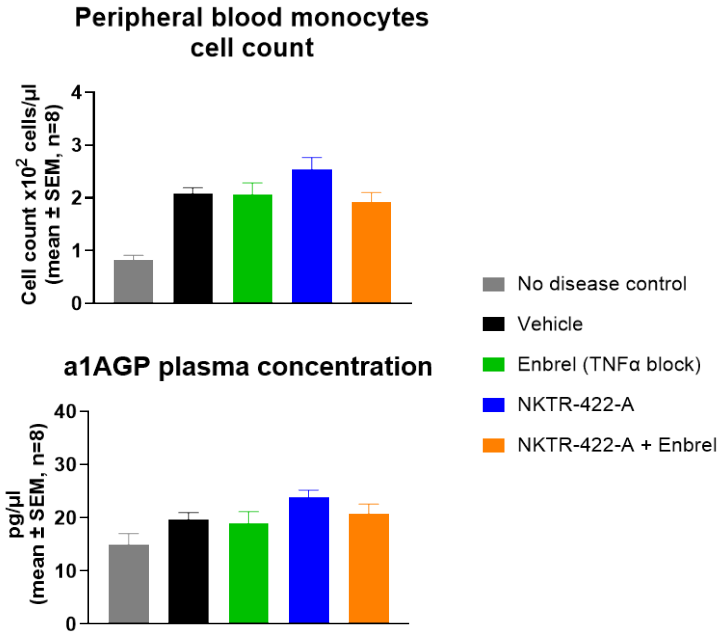
NKTR-422-A enhances TNFa blockade (Enbrel) efficacy in rat collagen induced arthritis model without increasing systemic inflammatory markers









Female Lewis rats (n=8/group) were immunized twice with bovine collagen emulsion. Hind paw volumes were measured by plethysmometer and averaged. Animals were randomized to indicated treatment groups on Day 7. Enbrel was subcutaneously (s.c.) administered twice a week and NKTR-422-A was intravenously (i.v.) administered once a week starting on Day 14 at peak inflammation. Paw volumes were measured on Days 14, 21, 28. On Day 21, Enbrel + NKTR-422-A and Enbrel vs vehicle: **** p<0.0001, * p=0.01, respectively; Enbrel vs Enbrel + NKTR-422-A: p=0.001, 2-way ANOVA, Dunnet's multiple comparison test). Total resolution phase inflammation reduction, Enbrel + NKTR-422-A vs Enbrel and vehicle (*** p<0.0001, ** p=0.0024, 1-way ANOVA, Dunnet's multiple comparison test. No significant difference between treatments and vehicle in monocyte count and alpha 1 acid glycoprotein (a1AGP) plasma concentration measured by ELISA.

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Summary

- Rational design of polymer conjugated CSF1 cytokine variants led to identification of novel attenuated CSF-1R agonists
- NKTR-422 receptor affinity modulation improves PK profile consistent with receptor mediated CSF1 clearance mechanism
- Reduced clearance enables sustained PD activity from a single dose unlike historical multiple dose per day necessary regimens of rhCSF-1 administration
- NKTR-422 shows selective activation of functional phenotypes in monocytes and tissue macrophages
  - Tissue macrophages expansion and induction of inflammation resolution/tissue repair functional markers at dose levels that do not mobilize peripheral blood pro-inflammatory monocytes
- NKTR-422 combination treatment enhances efficacy of standard of care TNFa blockade in a rat model of collagen induced arthritis

- **NKTR-422 has potential to accelerate treatment efficacy and may improve disease remission in combination treatments with standard of care inflammatory cytokine blockade drugs**

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