ACR Convergence
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Development of a computationally designed, hyperstable dual inhibitor of the IL-2 and IL-15 receptors: a novel therapeutic candidate for inflammatory conditions

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Disclosure

- All authors contributed to this work as employees of Neoleukin Therapeutics, Inc.
- All authors have equity in Neoleukin Therapeutics, Inc. (NASDAQ: NLTX)

IL-2 and IL-15 are critical cytokines in T and NK cell biology

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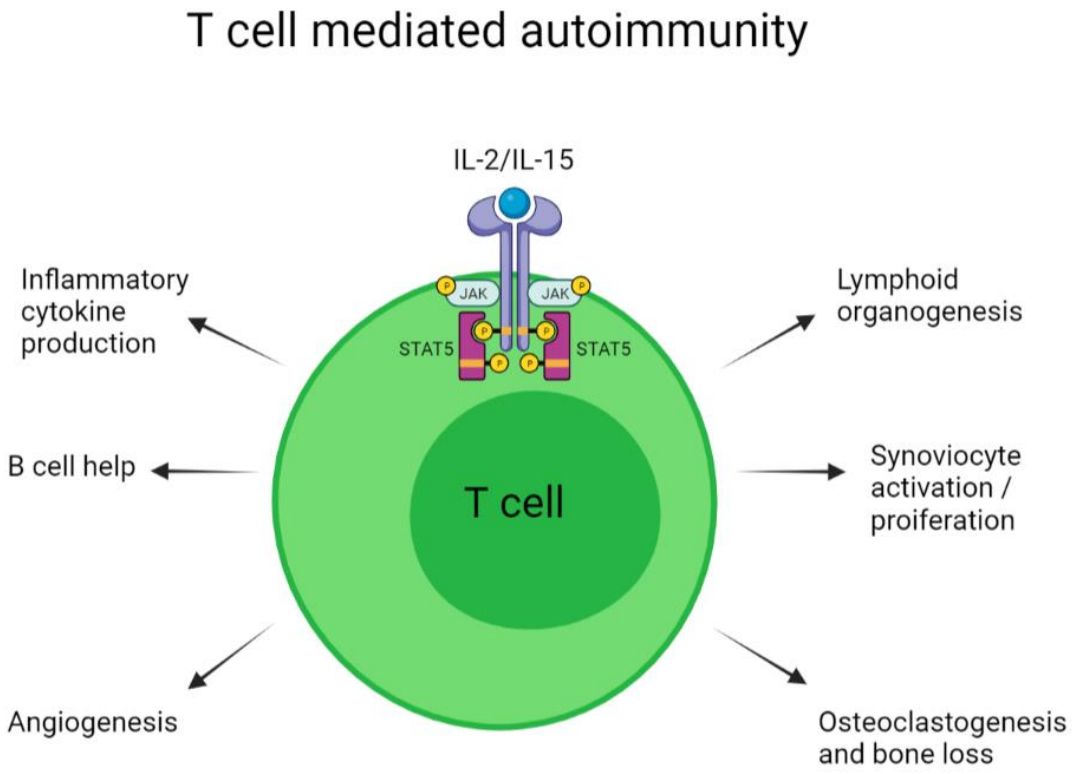
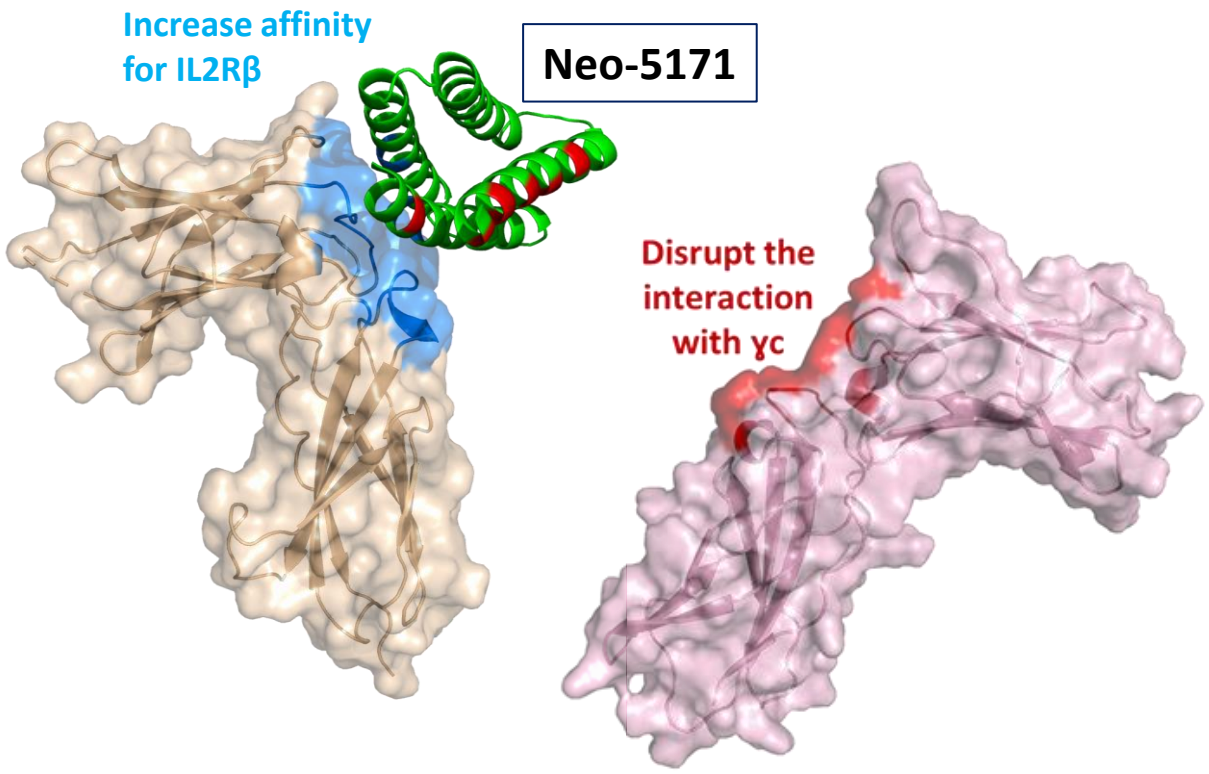
- IL-2 and IL-15 signal through the shared IL2Rβ/IL2Rγ receptor complex on both T and NK cells, activating STAT5 transcriptional activity
- Both cytokines promote T cell and NK cell survival, activation, expansion, and effector functions
- Elevated IL-2 can be found in serum from RA, SLE, and MS patients
  - Soluble IL2RA is a serum marker in many inflammatory conditions
- Anti-CD25 (IL2Rα) mAbs daclizumab/basiliximab are approved in the renal transplant setting, validating clinical benefit with IL-2 pathway antagonists



Neo-5171: A computationally designed de novo protein inhibitor of IL-2 and IL-15 signaling



- Neo-5171 was designed from a de novo IL-2 mimetic scaffold to:
  - increase affinity for IL2Rβ (CD122)
  - eliminate binding to IL2Rα (CD25)
  - eliminate binding to IL2Rγ (CD132)

- Neo-5171 prevents IL2Rβ/IL2Rγ heterodimerization and JAK/STAT phosphorylation, blocking both IL-2 and IL-15 signaling

Neo-5171 has high affinity for human IL2Rβ with no detectable binding to human IL2Rγ

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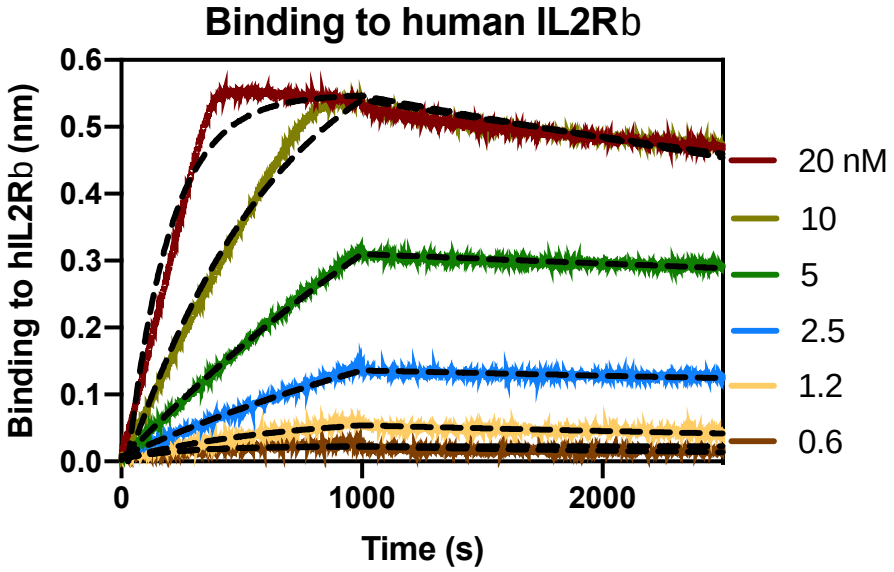
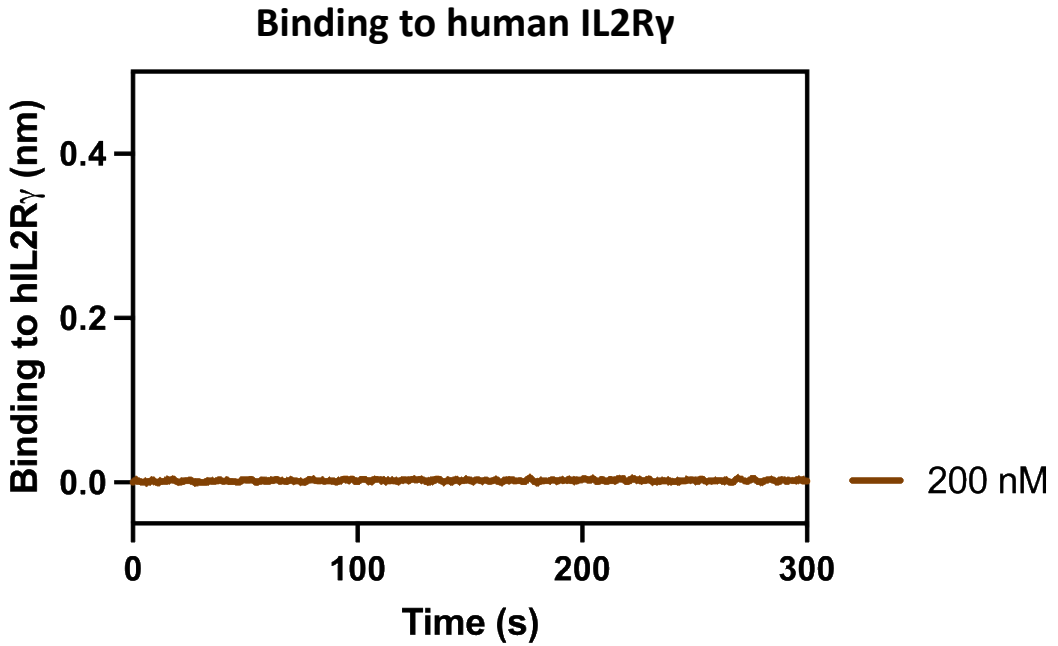
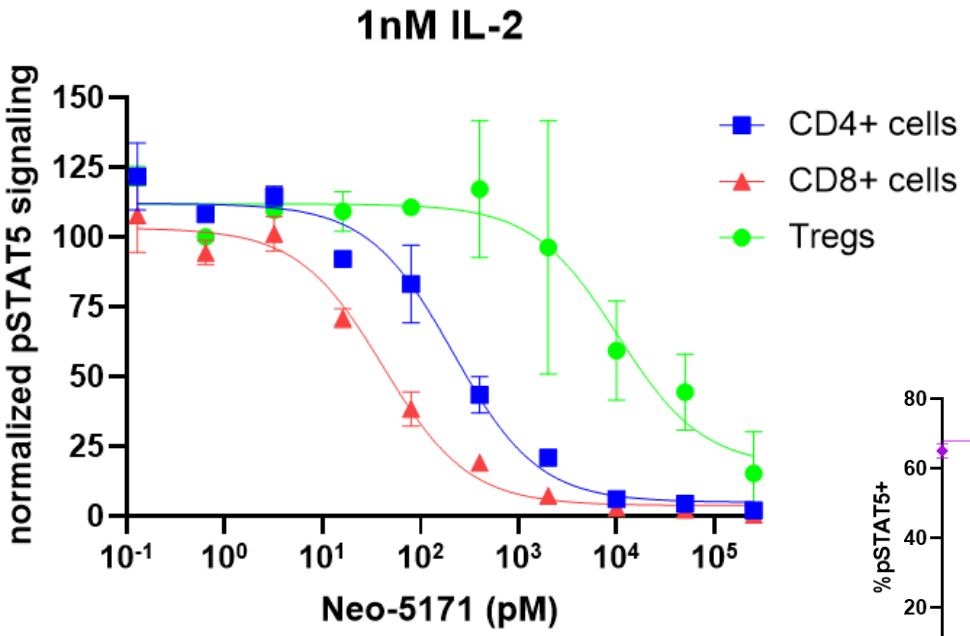




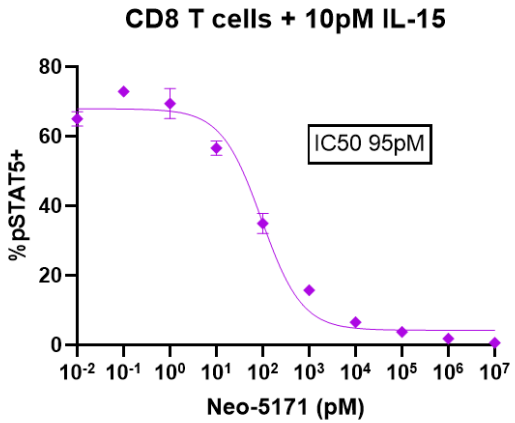
- Neo-5171 binds recombinant human IL2Rβ with high affinity via Octet biolayer interferometry
  - K_D ~ 0.3 nM for Neo-5171 and human rIL2Rβ
- 1000x higher affinity than native human IL-2
  - K_D ~ 327 nM for human rIL-2 and rIL2Rβ

- Neo-5171 mixed with rhIL2Rβ has no detectable binding to human recombinant IL2Rγ via biolayer interferometry

Neo-5171 inhibits IL-2 signaling in CD4 and CD8 T cells at low concentrations, with reduced impact on Treg signaling

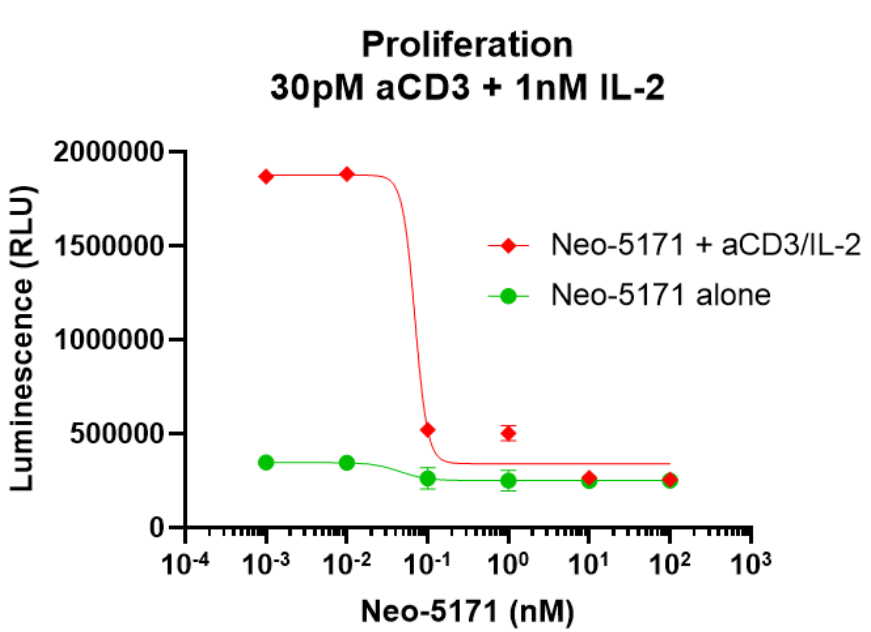


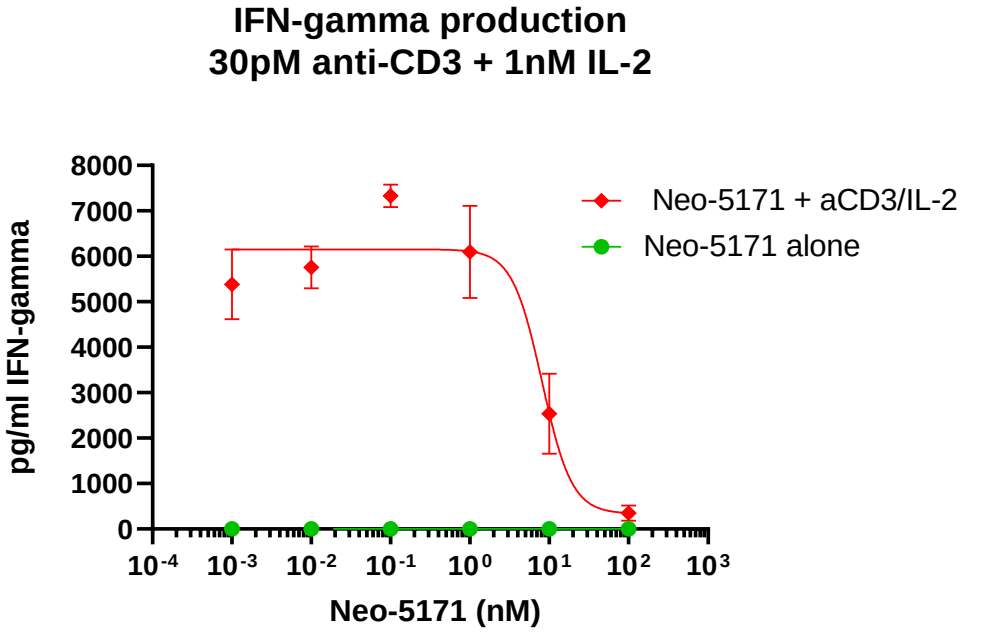
	CD4+ cells	CD8+ cells	Tregs
IC_{50} (pM)	215	42	10,265



- Purified human T cells were stimulated with 1nM IL-2 or 10pM IL-15 and pSTAT5 levels were determined via flow cytometry
- Neo-5171 potently inhibits human CD4 and CD8 IL-2-induced STAT5 signaling at sub-nM concentrations
- Human Treg IL-2 STAT5 signaling is less sensitive to Neo-5171 antagonism
- Therapeutic window for Neo-5171 Teff antagonism that does not negatively affect human Treg IL-2 usage (roughly 50X)

Neo-5171 potently inhibits IL-2 -induced T cell proliferation and IFN-gamma production by human T cells





- Human PBMCs were stimulated with anti-CD3 and IL-2 to induce T cell proliferation, measured by Cell Titer Glow on culture Day 6
- Neo-5171 potently inhibited proliferation of T cells with an IC_{50} of 70pM
- Neo-5171 cultured alone did not induce proliferation at any concentration

- Day 6 culture supernatant IFN-gamma levels were quantified by ELISA
- Neo-5171 inhibited IFN-gamma production of PBMCs stimulated with an IC_{50} of 8.1nM
- Neo-5171 cultured alone did not induce IFN-gamma production at any concentration

Neo-5171 is resistant to proteolysis and hyperstable at high temperature or low pH

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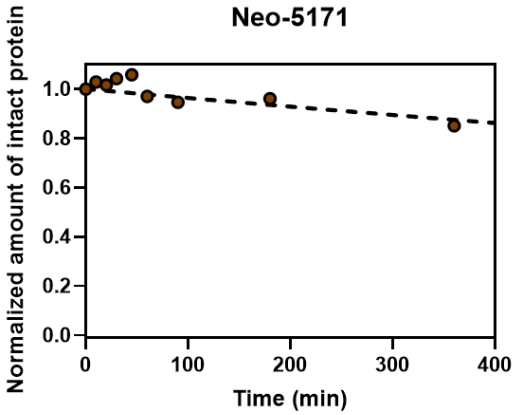
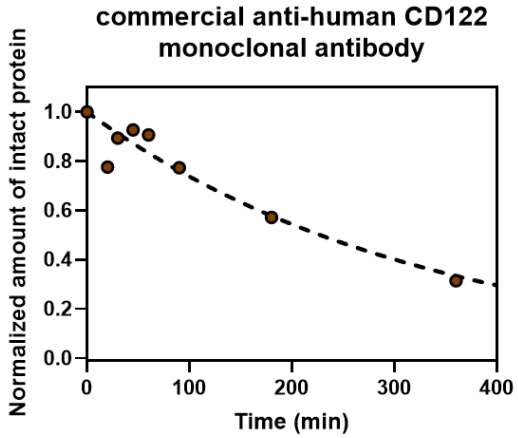
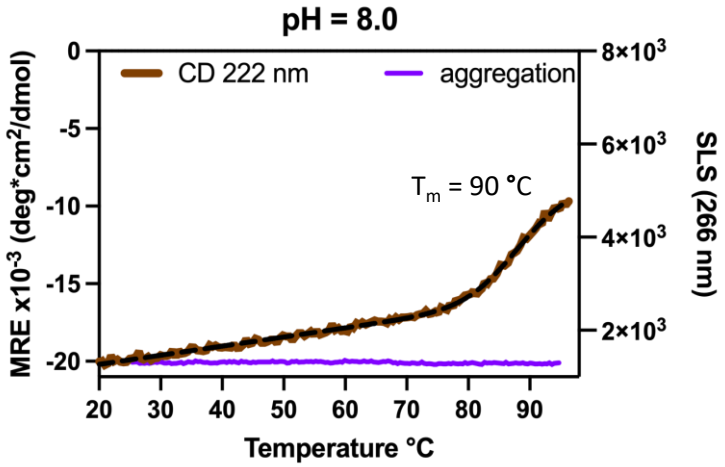
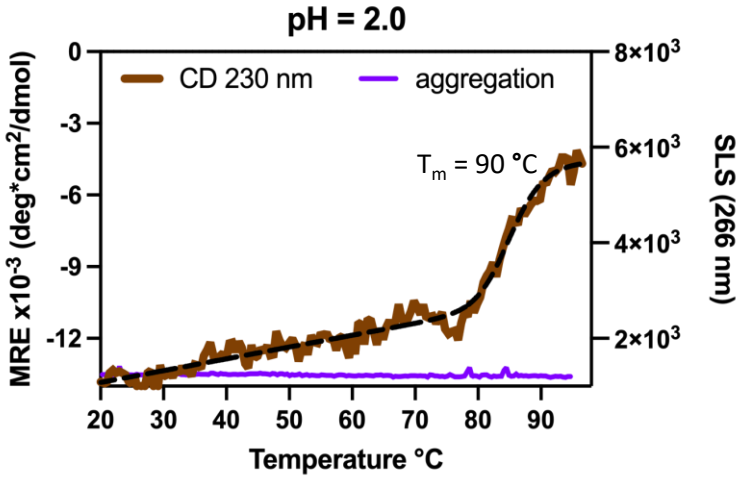




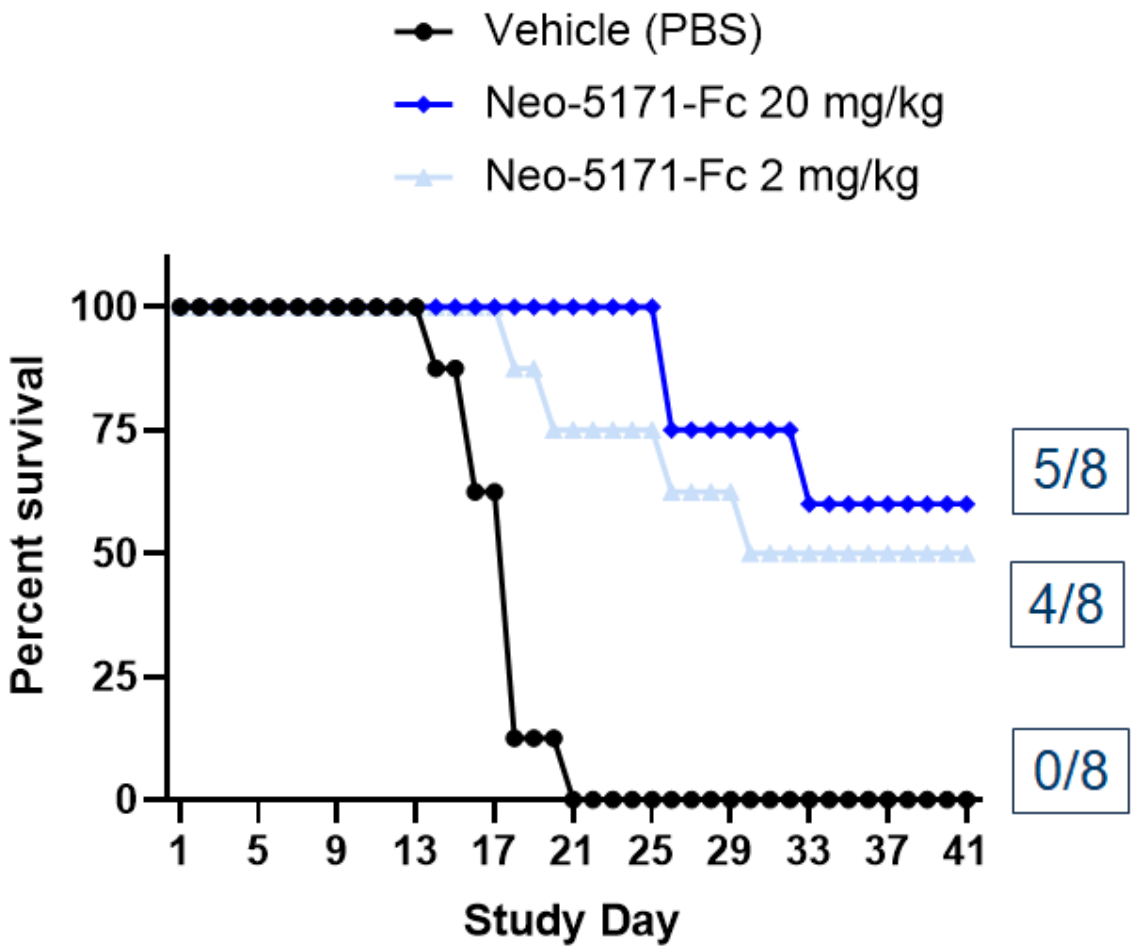
- Indicated proteins were incubated with trypsin, chymotrypsin, and carboxypeptidase (1:1 weight ratio) at 37°C for indicated time
- Neo-5171 demonstrates superior proteolytic resistance compared to an exemplary monoclonal antibody

- Thermal induced unfolding was determined by circular dichroism (brown)
- Aggregation propensity was determined by static light scattering (purple)
- At both pH 8.0 and pH 2.0, Neo-5171 displays high thermal stability and no aggregation <90°C





Neo-5171-Fc prolongs survival in a preclinical model of graft-vs-host disease (GVHD)



- Neo-5171 was fused to the C terminal end of human IgG1
- Immunodeficient NSG mice were irradiated and received 10 million human PBMC on Day -1
- Intraperitoneal dosing with Neo-5171-Fc began on Day 0 and was performed every 3 days throughout study
- Mice were euthanized when experiencing >20% body weight loss
- Neo-5171-Fc prolonged survival in a dose-dependent manner
- Boxed number is the number of surviving mice at study end (Day 42)

Summary

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- De novo protein Neo-5171 is a dual IL-2/IL-15 antagonist that binds with high affinity to IL2Rβ without binding IL2Rα and IL2Rγ
- Neo-5171 potently inhibits human IL-2 and IL-15 signaling and effector function of CD8, CD4, and NK cells with reduced potency on Treg, a unique mechanism of action for antagonists of these pathways
- Neo-5171-Fc fusion protein demonstrated *in vivo* efficacy in mouse GVHD preclinical modeling
- Neo-5171 demonstrates protease resistance and biophysical stability in extreme temperatures and pH conditions
- Neo-5171 is being evaluated as a potential clinical candidate for the treatment of inflammatory conditions