NL-201 induces inflammation in a 'cold' tumor microenvironment through upregulation of MHC-I, expansion of the TCR repertoire, and potent anti-tumor activity when combined with PD-1 inhibition

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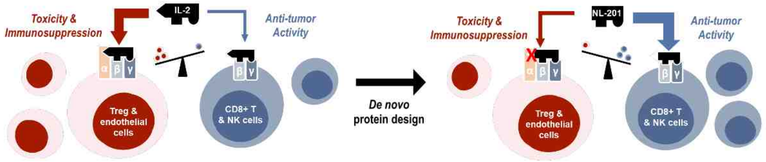
Abstract # 716

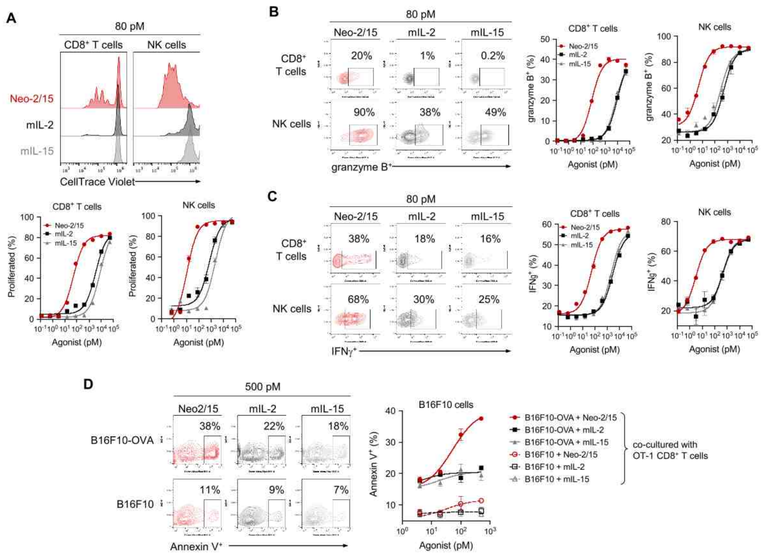
1 NL-201 was designed to overcome limitations of IL-2 immunotherapy

- Recombinant IL-2 (aldesleukin) was the first effective cancer immunotherapy, however, severe toxicity limits its widespread use.[1,2]
- The toxicity of IL-2 is mediated by high-affinity interactions with the alpha chain of the IL-2 receptor (IL-2Rα, CD25), expressed on off-target cells, including Treg and endothelial cells. Stimulation of Treg cells can promote an immunosuprressive TME while signaling in other non-targeted cells can result in cytokine release or capillary leak syndrome at high doses.[3,4]
- NL-201 is a potent, selective, and long-acting computationally designed alpha-independent agonist of the IL-2 and IL-15 receptors that is being developed as an immunotherapy for cancer.[5-7]
- NL-201 was developed from Neo-2/15, a *de novo* protein agonist of the IL-2 and IL-15 receptors, by introducing a cysteine residue that is subsequently conjugated to an unbranched 40 kDA PEG molecule to extend the systemic half-life.[6,8]
- NL-201 displays single-agent activity at well-tolerated doses in all syngeneic tumor models tested, including those resistant to checkpoint inhibitors.[8]
- While ICIs have become a mainstay of cancer treatment, most patients with advanced cancer do not respond or become resistant to current regimens that include ICIs; as a result, combination therapies have been proposed to treat patients with anti-PD-1-resistant disease, including combination with other immunotherapeutics, chemotherapy, and radiation.[9,10]



2 Neo-2/15 induced greater CD8⁺ T cell and NK cell effector functions compared to IL-2 or IL-15 *in vitro*

- Mouse splenocytes were cultured *in vitro* and assessed for proliferation after 3 days (**A**), and granzyme B (**B**) and IFNγ production after 1 day (**C**). OT-1 ($OVA_{257-264}$-specific) CD8⁺ T cells were co-cultured with B16F10-OVA cells for 1 day to assess antigen-dependent cytotoxicity; Annexin V was used to identify dead/dying tumor cells (**D**).
- Relative to IL-2 and IL-15, Neo-2/15 was superior in induction of immune effector functions, including direct antigen-dependent killing of tumor cells.

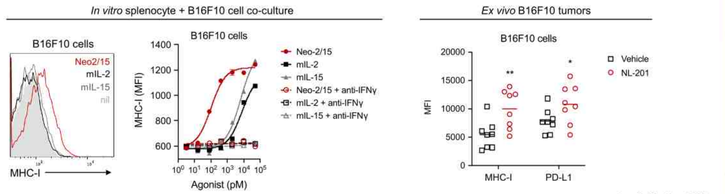


3 In the B16F10 syngeneic tumor model, a single dose of NL-201 was associated with an inflammatory gene signature in the tumor

- B16F10 tumor-bearing mice were given a single dose of NL-201 (5.5 μg) and harvested after 7 days for gene expression analysis using the Nanostring PanCancer Immune Profiling panel.
- NL-201 treatment was associated with an increase in immune activation signature scores, demonstrating the concerted anti-tumor immune response driven by NL-201 monotherapy in traditionally poorly immunogenic tumors.

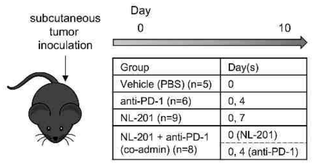
4 NL-201 increases B16F10 tumor cell MHC-I and PD-L1 expression

- In B16F10 tumor cells co-cultured *in vitro* with syngeneic mouse splenocytes for 1 day, Neo-2/15 induced greater IFNγ-dependent MHC-I expression on tumor cells than IL-2 and IL-15.
- B16F10 tumor cells harvested 7 days after NL-201 treatment (single 5.5 μg dose) had significantly increased MHC-I, potentiating conversion to an immunologically 'hot' tumor.
- B16F10 tumor cells treated with NL-201 had increased PD-L1 expression relative to tumors from vehicle (PBS)-treated mice.
  - PD-L1 immunosuppression may be subverted by the inclusion of immune checkpoint inhibitors in the treatment regimen (i.e., anti-PD-1).

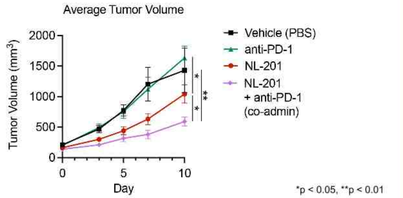


5 NL-201 monotherapy reduced B16F10 tumor growth and synergized with anti-PD-1 to significantly improve anti-tumor activity

- B16F10 tumor-bearing mice were dosed with NL-201 (5.5 μg), anti-PD-1 (200 μg), and NL-201 with anti-PD-1 (5.5 μg and 200 μg, respectively) as outlined in the table below (left) to evaluate the anti-tumor efficacies of NL-201 monotherapy and NL-201 plus anti-PD-1 combination therapy (right graph).



Group	Day(s)
Vehicle (PBS) (n=5)	0
anti-PD-1 (n=6)	0, 4
NL-201 (n=9)	0, 7
NL-201 + anti-PD-1 (co-admin) (n=8)	0 (NL-201) 0, 4 (anti-PD-1)



6 NL-201 monotherapy, or in combination with anti-PD-1, drives an expansion of TCR repertoire diversity in B16F10 tumors

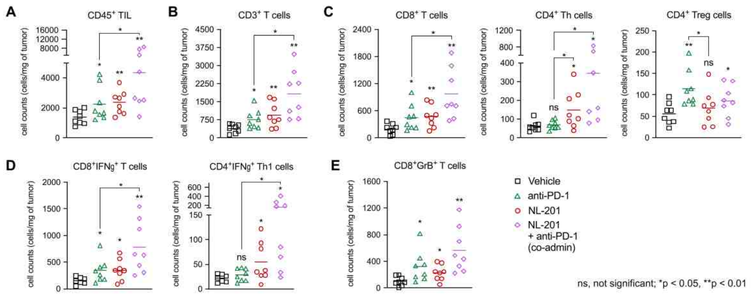
- B16F10 tumor-bearing mice were given a single dose of NL-201 (5.5 μg), anti-PD-1 (200 μg), and NL-201 with anti-PD-1 (5.5 μg and 200 μg, respectively). Tumors were harvested after 7 days and processed for TCRβ sequencing analysis using the Adaptive Biotechnologies immuno-SEQ platform. Results below are the mean and range of number of TCRβ sequence reads in total amount of tumor gDNA (~500 ng per sample).
- An increase in the number of intratumoral total T cells was observed with all treatments relative to vehicle control, however the number of unique T cells was highest with NL-201 alone or combined with anti-PD-1.
- NL-201 expansion of unique T cell clones at the tumor potentiates new antigen recognition and reversal of a 'cold' tumor.

TCRβ Sequencing Summary

Mean (range)	Total T cells	Unique T cells	Simpson Clonality
Vehicle (n=5)	1,406 (358-2,708)	445 (196-807)	0.194 (0.106-0.411)
anti-PD-1 (n=5)	2,456 (987-4,713)	464 (314-775)	0.34 (0.138-0.57)
NL-201 (n=5)	2,664 (1,578-3,816)	869 (611-1,064)	0.206 (0.11-0.292)
NL-201 plus anti-PD-1 (co-admin) (n=5)	2,865 (1,504-3,456)	1,042 (536-1,486)	0.128 (0.073-0.165)

7 NL-201 cooperates with anti-PD-1 to increase the presence of pro-inflammatory effector T cells in B16F10 tumors

- B16F10 tumor bearing mice were given a single dose of NL-201 (5.5 μg), anti-PD-1 (200 μg), and NL-201 with anti-PD-1 (5.5 μg and 200 μg, respectively). Tumors were harvested after 7 days and processed for TIL analysis by flow cytometry.
- Relative to vehicle, dosing of all treatment regimens resulted in significantly greater cell counts of CD45⁺ TILs (**A**) and CD3⁺ T cells (**B**) in the tumors with the NL-201 and anti-PD-1 combination resulting in the greatest increase.
- Similarly, the greatest increase in cell counts of CD8⁺ T cells and CD4⁺ Th cells occurred with the NL-201 plus anti-PD-1 combination treatment (**C**).
- Anti-PD-1 monotherapy showed a significant increase in CD4⁺ Treg cell numbers, but NL-201 monotherapy did not result in increased Treg numbers (**C**).
- NL-201 plus anti-PD-1 co-therapy resulted in the greatest number of pro-inflammatory IFNγ producing CD8+ T cells and CD4⁺ Th1 cells (**D**) and granzyme B producing CD8⁺ T cells (**E**).



8 Summary and Future Directions

- Neo-2/15 is superior to IL-2 and IL-15 at driving pro-inflammatory immune effector functions, such as CD8⁺ T cell and NK cell proliferation, granzyme B and IFNγ production, and direct tumor cell killing.
- NL-201 turns 'cold' tumors 'hot' by increasing both pro-inflammatory T cells and an immune signature in the TME and upregulating MHC-I in tumors.
- NL-201 stimulates pro-inflammatory tumor reprogramming without the coincident Treg expansion observed with PD-1 antibodies and other immuno-oncology agents.
- NL-201 drives anti-tumor efficacy in a manner that is cooperative with PD-1 inhibition and includes increasing TCRβ repertoire diversity.
- A Phase 1 study of NL-201 in patients with advanced solid tumors is currently underway (NCT04659629, SITC 2021 poster # 509).

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Abbreviations

gDNA, genomic DNA; GrB, granzyme B; ICI, immune checkpoint inhibitors; IFNγ, interferon gamma; IL, interleukin; MFI, mean fluorescence intensity; MHC, major histocompatibility complex; NK, natural killer; ns, non-significant; PBS, phosphate buffered saline; PD-1, programmed cell death protein-1; PEG, polyethylene glycol; TCR, T cell receptor; Th, T helper; TIL, tumor-infiltrating lymphocyte; TLR, toll-like receptor; TME, tumor microenvironment; TNF, tumor necrosis factor; T_{reg}, regulatory T cell

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Disclosures

CM, BD, JC, AC, JH, LB, CW, RS: Employees of Neoleukin Therapeutics, Inc.