NL-201: A *de novo* CD25-independent combined IL-2 and IL-15 receptor agonist designed to selectively stimulate anti-tumor CD8+ effector T and NK cells

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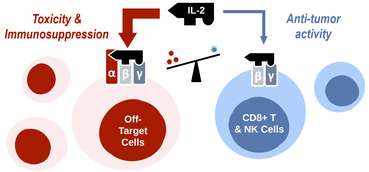
Abstract # 4518

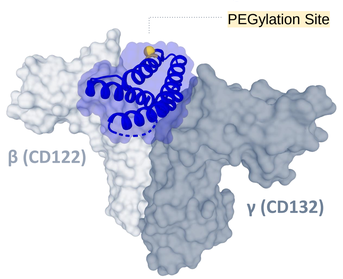
1 NL-201 was designed to overcome limitations of IL-2 immunotherapy

- Recombinant IL-2 (aldesleukin) is an approved cancer immunotherapy that leads to 5-8% durable remission; however, severe toxicity has limited its widespread use.
- IL-2's toxicity is mediated by its high-affinity interactions with the alpha chain of the IL-2 receptor (CD25), selectively expressed on off-target cells, including regulatory T cells (Tregs) and eosinophils. Moreover, Treg stimulation can contribute to immunosuppression in the tumor microenvironment.



2 NL-201 is optimized for pharmacologic activity and clinical dosing

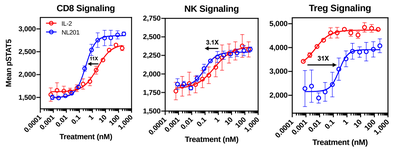
NL-201, is a *de novo* protein designed to dimerize the beta and gamma chains of the IL-2 and IL-15 receptors in the absence of CD25.



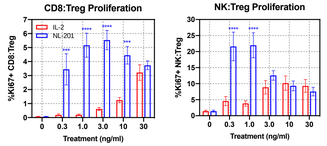
- NL-201 was developed from Neo-2/15 (Silva *et al.* **Nature,** 2019; 565, 186-191) by introducing one cysteine residue that is subsequently conjugated to a single 40kDa maleimide-modified PEG.
- PEGylation was engineered to extend blood half-life and decrease the risk of immunogenicity while having minimal impact on activity.
- NL-201 is a uniform, readily manufactured, and highly stable combined IL-2/IL-15 receptor agonist.

3 NL-201 stimulates CD8 effector T and NK cells more selectively than IL-2

- STAT5 phosphorylation in CD8+ T cells, NK cells, and Tregs was measured by flow cytometry using PBMCs from 10 healthy human donors. Proliferation was evaluated using Ki67.
- NL-201 is ~330-fold and ~90-fold more selective for CD8+ T and NK cells (vs. Tregs) than IL-2, respectively.



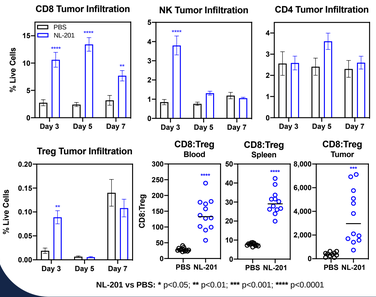
- NL-201 stimulates dose-dependent CD8:Treg and NK:Treg proliferation more selectively than IL-2.



NL-201 vs IL-2: * p<0.05; ** p<0.01; *** p<0.001; **** p<0.0001;

4 NL-201 stimulates selective CD8 and NK proliferation and tumor infiltration

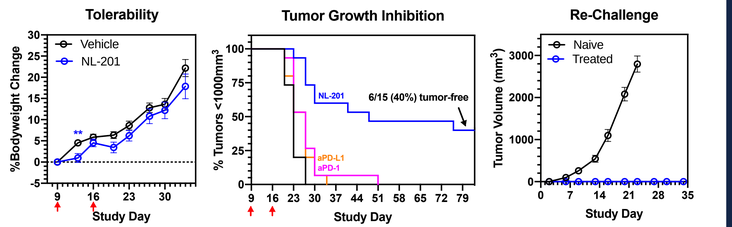
- NL-201 was administered 375µg/kg IV to mice bearing established B16F10 tumors. Lymphocyte populations in the blood, spleen, and tumor were characterized by flow cytometry 3, 5, and 7 days post-dose.
- Peak mean CD8:Treg ratios in the blood, spleen, and tumors of animals treated with NL-201 (5 days post-dose) were approximately 140, 30, and 3500, respectively: a 5X (blood), 4X (spleen), and 10X (tumor) increase vs. untreated animals.



NL-201 vs PBS: * p<0.05; ** p<0.01; *** p<0.001; **** p<0.0001

5 NL-201 is well-tolerated and promotes durable anti-tumor activity in a checkpoint inhibitor-resistant murine model

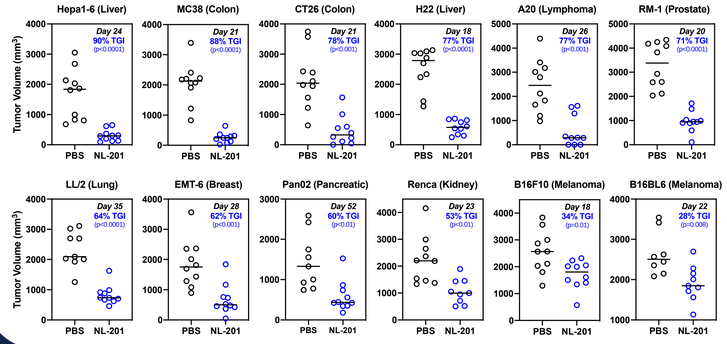
- Mice (15 per group) were implanted subcutaneously with CT26 tumor cells. Treatment with NL-201 (150µg/kg IV QWx2) began when tumors reached an average size of ~100mm^3.
- **NL-201 is well-tolerated at therapeutic doses:** Bodyweight gain is similar in NL-201-treated and untreated mice with a transient difference observed 4 days after the first dose (p<0.01).
- **NL-201 treatment exhibits single-agent activity:** Tumor growth inhibition was significantly better in NL-201 treated mice than mice treated with vehicle, anti-PD-1, or anti-PD-L1 antibodies (10mg/kg; IP; twice weekly for 3 weeks; anti-PD-1: RPM1-14; anti-PD-L1: 10F.9G2). On Day 79, 6 of 15 (40%) NL-201-treated mice were tumor-free.
- **NL-201 promotes durable anti-tumor immunity:** When tumor-free mice were re-challenged with CT26 tumor cells, 0% (0/8) of NL-201-treated mice re-grew tumors, while 100% (9/9) of naïve mice grew tumors.



NL-201 vs Vehicle: * p<0.05; ** p<0.01; *** p<0.001; **** p<0.0001

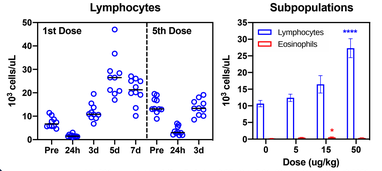
6 NL-201 inhibits tumor growth across a diverse panel of syngeneic murine tumor models, including those refractory to checkpoint inhibitors

- Immunocompetent mice (10 per group) were implanted subcutaneously with one of 12 syngeneic tumor cell lines. NL-201 was administered QWx2 when tumors reached ~100mm^3. Tumor growth inhibition (TGI) is reported in each graph vs. control.
- **NL-201 treatment inhibited tumor growth in all models:** NL-201 significantly inhibited tumor growth in models that are typically refractory to anti-PD-1 checkpoint inhibitors, including RM-1, LL/2, Pan02, Renca, B16F10, and B16BL6.



7 NL-201 stimulates sustained and dose-dependent lymphocyte expansion in NHP

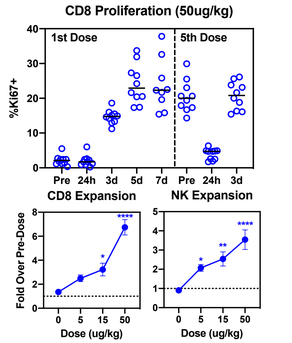
- NL-201 was administered IV to cynomolgus macaques at 5, 15, or 50µg/kg QWx5. Lymphocyte and eosinophil levels in blood were analyzed.
- All animals received scheduled doses and survived to study completion. No animal experienced detectable hypotension or showed evidence of severe vascular leak.
- Peak mean lymphocyte levels in animals receiving 50µg/kg NL-201 were approximately 2.5-fold higher than in untreated animals (p<0.0001; 5 days after first dose). Eosinophils were modestly increased at 15µg/kg (p<0.05) but not significantly increased at either 5 or 50µg/kg compared to untreated animals.



NL-201 vs. untreated (0ug/kg): * p<0.05; ** p<0.01; *** p<0.001; **** p<0.0001

8 NL-201 stimulates sustained, dose-dependent CD8 and NK cell proliferation

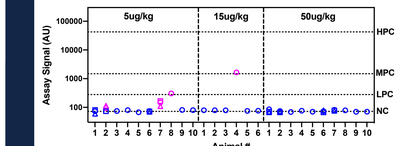
- CD8 and NK cells in the blood of NHP treated with NL-201 were evaluated by flow cytometry. Proliferation was evaluated by Ki67 expression.
- CD8 proliferation in response to NL-201 administration was similar after the 1st and 5th doses, showing that NL-201 leads to sustained CD8 proliferation when dosed weekly.
- CD8 and NK cell fractions were ~7-fold and ~3.5-fold higher than pre-dose levels, respectively, 5 days following 50µg/kg NL-201 administration.



NL-201 vs untreated (0ug/kg): * p<0.05; ** p<0.01; *** p<0.001; **** p<0.0001

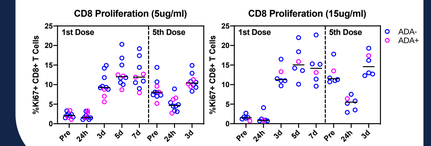
9 Infrequent, low-titer ADAs in NHPs do not impact tolerability or activity

- A highly sensitive (<100ng/ml) anti-drug antibody (ADA) assay was developed and used to detect anti-NL-201 ADAs in cynomolgus macaques receiving NL-201 QWx5 on day 29 (terminal) or days 29, 43 and 57 (recovery).
- **ADAs were not detected in any animal receiving 50µg/kg NL-201.**
- **Most ADA+ animals have low titers:** 1/6 animals receiving 15µg/kg and 3/10 animals receiving 5µg/kg had detectable ADAs; ADA levels in 3/4 animals were at or below the 100ng/ml low positive control (LPC) level.



NC: 0ng/ml; **LPC:** 100ng/ml; **MPC:** 500ng/ml; **HPC:** 5000ng/ml

- **ADAs do not impact activity or tolerability of NL-201:** Lymphocyte proliferation in ADA-positive animals was comparable to ADA-negative animals and ADAs did not significantly impact the tolerability of NL-201.



10 Summary and Future Direction

- **NL-201 stimulates CD8 T and NK cells more selectively than IL-2:** NL-201 preferentially stimulates CD8 T and NK cell proliferation at tolerated doses, giving it the potential for greater activity and less toxicity than IL-2.
- **NL-201 demonstrates potential as a monotherapy:** Robust single-agent activity in multiple tumor models, including those resistant to checkpoint inhibitors, at tolerated doses demonstrates that NL-201 may be active as a monotherapy.
- **NL-201 shows minimal immunogenicity in NHPs:** ADAs in NHPs receiving repeated doses of NL-201 are infrequent, low-titer, and do not adversely impact pharmacology or tolerability.
- **NL-201 is on track for an IND submission by the end of 2020:** GLP toxicology and GMP manufacturing are complete; a phase 1 trial in patients with advanced solid tumors is planned.