NL-201 INDUCES INFLAMMATION IN A 'COLD' TUMOR MICROENVIRONMENT THROUGH UPREGULATION OF MHC-I, EXPANSION OF THE TCR REPERTOIRE, AND POTENT ANTITUMOR ACTIVITY WHEN COMBINED WITH PD-1 INHIBITION

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Background 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. Downregulation of MHC class I (MHC-I) expression by tumors is a well-known mechanism of immune escape, and IFN γ is known to upregulate MHC-I. Here, we investigated whether NL-201 monotherapy can convert a 'cold' tumor microenvironment (TME) to an immunologically 'hot' TME through IFN γ -mediated MHC-I expression. This effect could expand the TCR repertoire for increased antitumor response and improve anti-PD-1 combination therapy.

Methods For *in vitro* assays, mouse splenocytes were cultured with Neo-2/15 to assess effector cell function, as well as co-cultured with B16F10 cells to assess IFN γ -induced MHC-I and PD-L1 expression. B16F10 tumors were established in C57BL/6 mice and dosed with NL-201, anti-PD-1, or both to assess *in vivo* efficacy. B16F10 tumors were excised and dissociated for phenotyping of tumor-infiltrating lymphocytes (TILs) using flow cytometry. For gene expression analysis, RNA and genomic DNA were extracted from tumors and submitted for NanoString Pancancer Immune Profiling and Adaptive ImmunoSEQ analysis, respectively.

Results *In vitro*, Neo-2/15 induced greater CD8+ T cell and NK cell proliferation, as well as granzyme B production and IFN γ -dependent MHC-I upregulation on B16F10 tumor cells, compared to IL-2 or IL-15. In 'cold' B16F10 syngeneic tumors, NL-201 monotherapy reduced tumor growth and induced MHC-I, IFN γ , and granzyme B upregulation. Gene expression analysis of NL-201-treated tumors demonstrated increased TCR repertoire diversity and inflammatory signature at the tumor. In addition, PD-L1 was significantly upregulated on B16F10 cells. While the B16F10 tumors exhibited resistance to anti-PD-1 monotherapy, combination treatment with NL-201 significantly improved anti-PD-1 activity. This may explain the potent anti-tumor activity of NL-201 with anti-PD-1 combination therapy.

Conclusions NL-201 induces potent inflammatory effects on effector cells and is able to turn 'cold' TMEs 'hot'. We demonstrate that NL-201 strongly upregulated MHC-I expression *in vitro* and *in vivo* via an IFN γ -dependent pathway. Increased antigen presentation drives TCR diversity while augmenting the inflammatory signature at the tumor. This adaptive response also upregulates PD-L1 expression and results in impressive antitumor activity when NL-201 and PD-1 inhibitors are co-administered. The demonstration that NL-201 can convert 'cold' tumors to immunologically 'hot' tumors may provide a novel therapeutic option for patients unresponsive to current standard of care checkpoint inhibitors. A Phase 1 study of NL-201 in patients with advanced solid tumors is currently underway (NCT04659629).

Ethics Approval All experiments were approved by the Institutional Animal Care and Use Committee of Bloodworks Northwest and performed under protocol 5360-03.

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