

Preclinical development and mechanism of action studies of NM21-1480, a PD-L1/4-1BB/HSA trispecific MATCH3 therapeutic clinical candidate

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AACR Poster # 2276

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Background: The combined immunomodulation of PD-L1/PD-1 blockade and 4-1BB stimulation is considered a promising strategy to increase response rates among cancer patients who are eligible to receive PD-L1/PD-1 inhibitors. Unfortunately, encouraging pre-clinical results achieved with such regimens have not yet translated into durable clinical success, due to co-administration of 4-1BB-agonistic antibodies being either intolerable at effective doses or ineffective at all evaluated doses. To eliminate this safety/efficacy tradeoff, we engineered a novel, PD-L1/4-1BB/HSA trispecific scMATCH3 immunomodulatory therapeutic candidate (NM21-1480) that agonizes 4-1BB conditionally upon PD-L1-binding and blockade. Here, we show preclinical proof of concept data of NM21-1480 *in vitro* and *in vivo*. We also describe the toxicology profile determined in a GLP study in cynomolgus monkeys. Clinical testing of NM21-1480 is due to start in Q3 2020.

Concept: Tumor-localized activation of 4-1BB combined with PD-(L)1 blockade

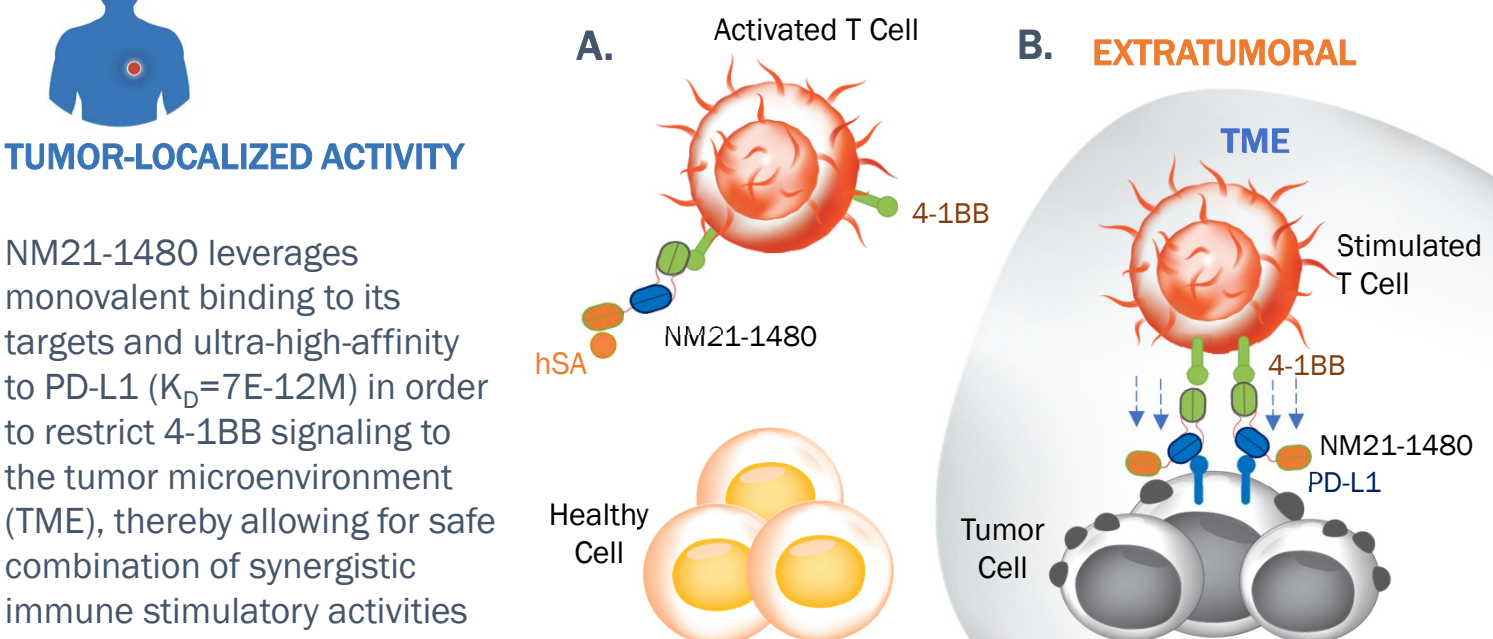


Figure 1. A. NM21-1480 cannot intrinsically trigger 4-1BB clustering and signaling upon binding to 4-1BB alone. B. Clustering of 4-1BB occurs following simultaneous binding of NM21-1480 to 4-1BB+ and PD-L1+ cells, resulting in 4-1BB signaling and concomitant blocking of the PD-1 / PD-L1 pathway.

NM21-1480 consists of three λ capTM-stabilized antibody Fvs fused in a single chain



Figure 2. A. Schematic representation of NM21-1480: a trispecific scMATCH3 molecule with α PD-L1 (VL1/VH1 in blue), α 4-1BB (VL2/VH2 in green) and α HSA (VL3/VH3 in red) domains connected by Gly-Ser peptide linkers, as indicated by red lines.

Selection of optimal epitope on 4-1BB increases T cell co-stimulation

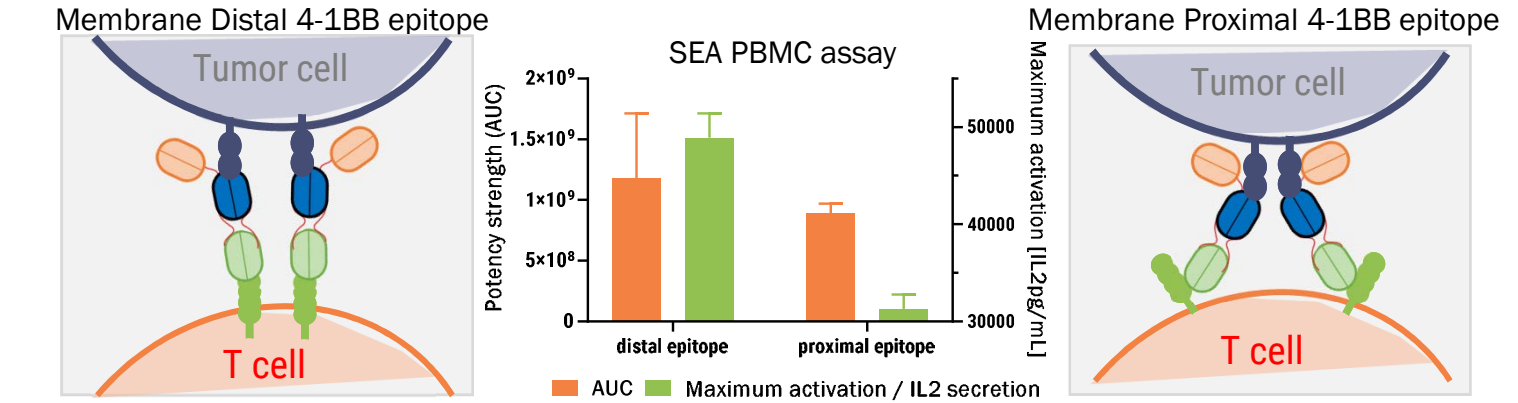


Figure 4. Human PBMCs were stimulated with Staphylococcal Enterotoxin A (SEA) to induce T cell-activation and PD-L1/4-1BB expression. Subsequently, NM21-1480 binding a membrane-distal epitope on 4-1BB or a scMATCH3 molecule binding a membrane-proximal epitope on 4-1BB was added to the culture. IL-2 secretion was assessed by ELISA. Clustering via binding to the distal tip of 4-1BB more potently co-stimulates 4-1BB-positive cells than clustering via binding to a proximal epitope on 4-1BB.

NM21-1480 potently blocks PD-L1 interaction with B7.1 and PD-1

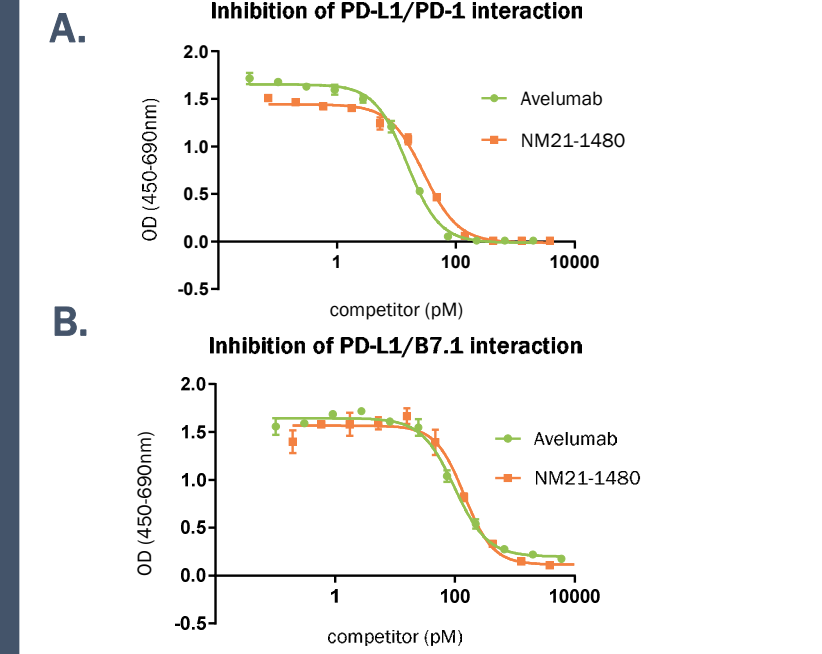


Figure 5. Representative curves illustrating the inhibition of binding of PD-L1 to A. PD-1 or B. B7.1 by NM21-1480 in an ELISA compared to avelumab.

Monovalent binding assures predictable effective dose range, independent of PD-L1 expression level

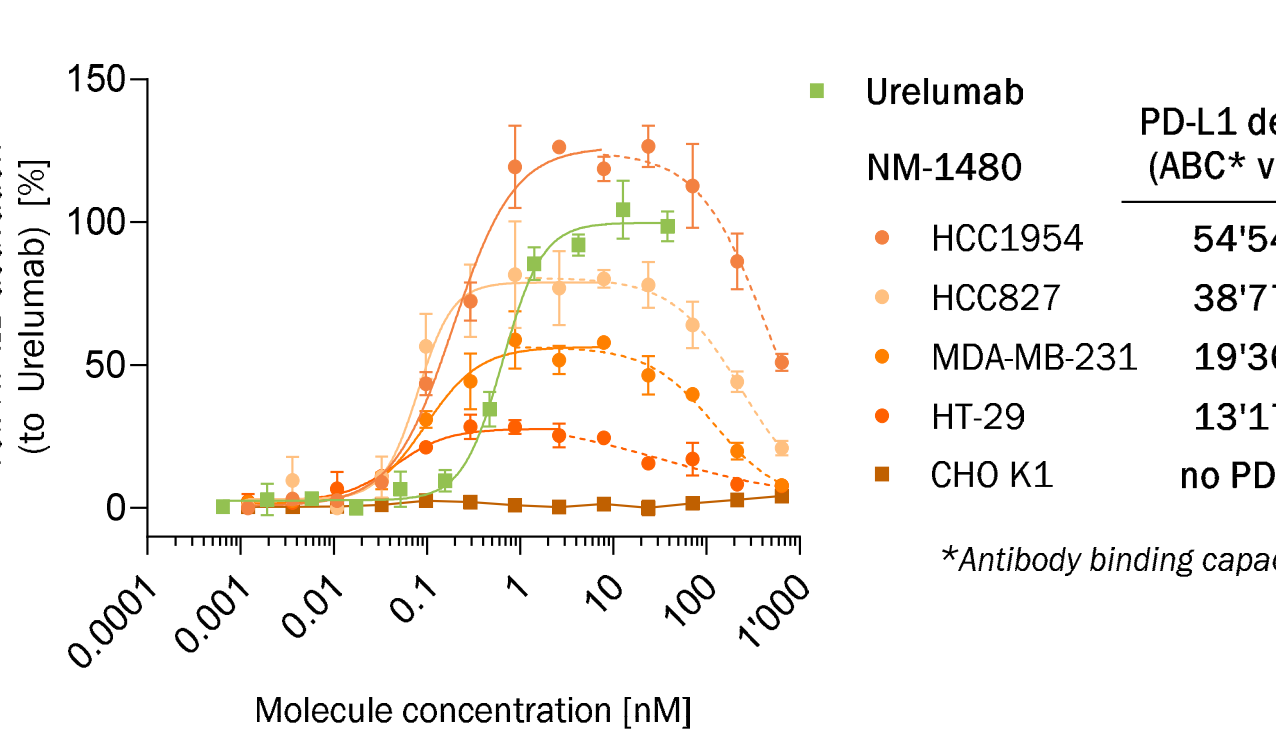


Figure 6. NM21-1480 concentration-dependent activation of 4-1BB-driven NF-kB signaling in a Jurkat reporter cell line. The assay is done in the presence of different cancer cell lines stimulated with IFN-gamma and expressing PD-L1 at different densities. PD-L1 density on co-cultivated cancer cells was quantified by flow cytometry. 4-1BB activation is represented as relative NF-kB activation, normalized to reference antibody avelumab. NM21-1480 only activates 4-1BB in the presence of PD-L1 positive cells (CHO K1). Potency of 4-1BB stimulation is independent of PD-L1 density. Maximal 4-1BB stimulation is correlated with PD-L1 density.

Affinity-optimized domains enable overlapping doses for maximal functional activity and maximal window of 4-1BB stimulation

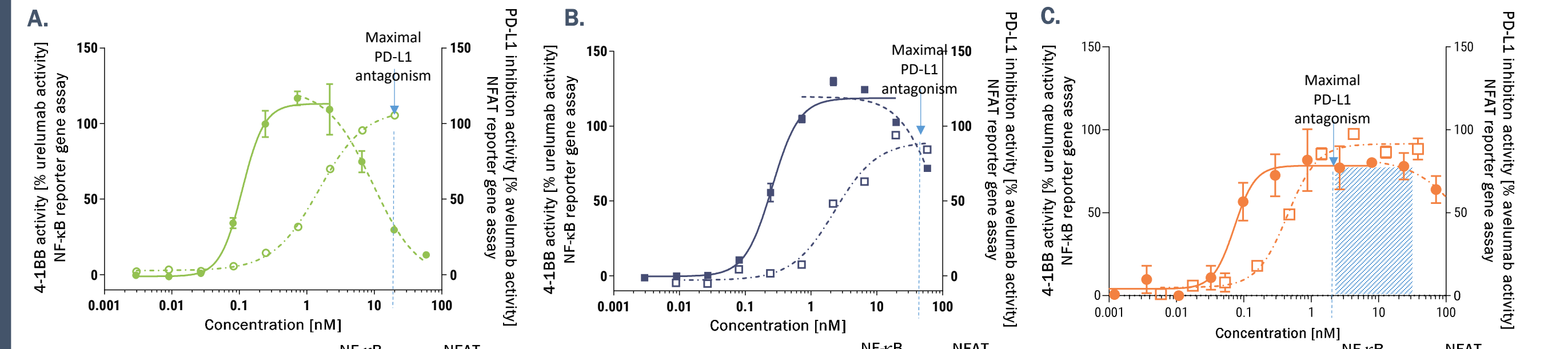


Figure 7. Concentration-dependent activation of NF-kB signaling in Jurkat reporter cells (filled symbols) and inhibition of PD-L1 activity in NFAT reporter cells (open symbols) of A. PRO1124 with PD-L1 binding affinity (K_D) greater/worse than that of 4-1BB K_D . B. PRO885 with PD-L1 K_D less than (better) than that of 4-1BB K_D . C. NM21-1480 with PD-L1 K_D considerably less (better) than that of 4-1BB K_D . The blue arrow and blue dotted line indicate the concentration at which maximal PD-L1 antagonism is reached. The corresponding level of 4-1BB agonism at that concentration and the breadth of concentrations where maximal activity of both PD-L1 blockade and 4-1BB stimulation are important for optimal dose finding, as indicated by the shaded blue box in C. By altering the K_D to PD-L1 to levels significantly improved than the K_D to 4-1BB, the EC50 and IC50 values for the respective functional activities become more closely matched and the plateau of 4-1BB stimulation becomes broader. NM21-1480 has exquisitely optimized PD-L1 and 4-1BB binding domains to allow activity of both PD-L1 blockade and 4-1BB activation at the same optimal dose and to extend the concentration range of maximal 4-1BB signalling.

Mixed Leucocyte Reaction: Activation of T cells and monocyte-derived dendritic cells to boost immune response within TME

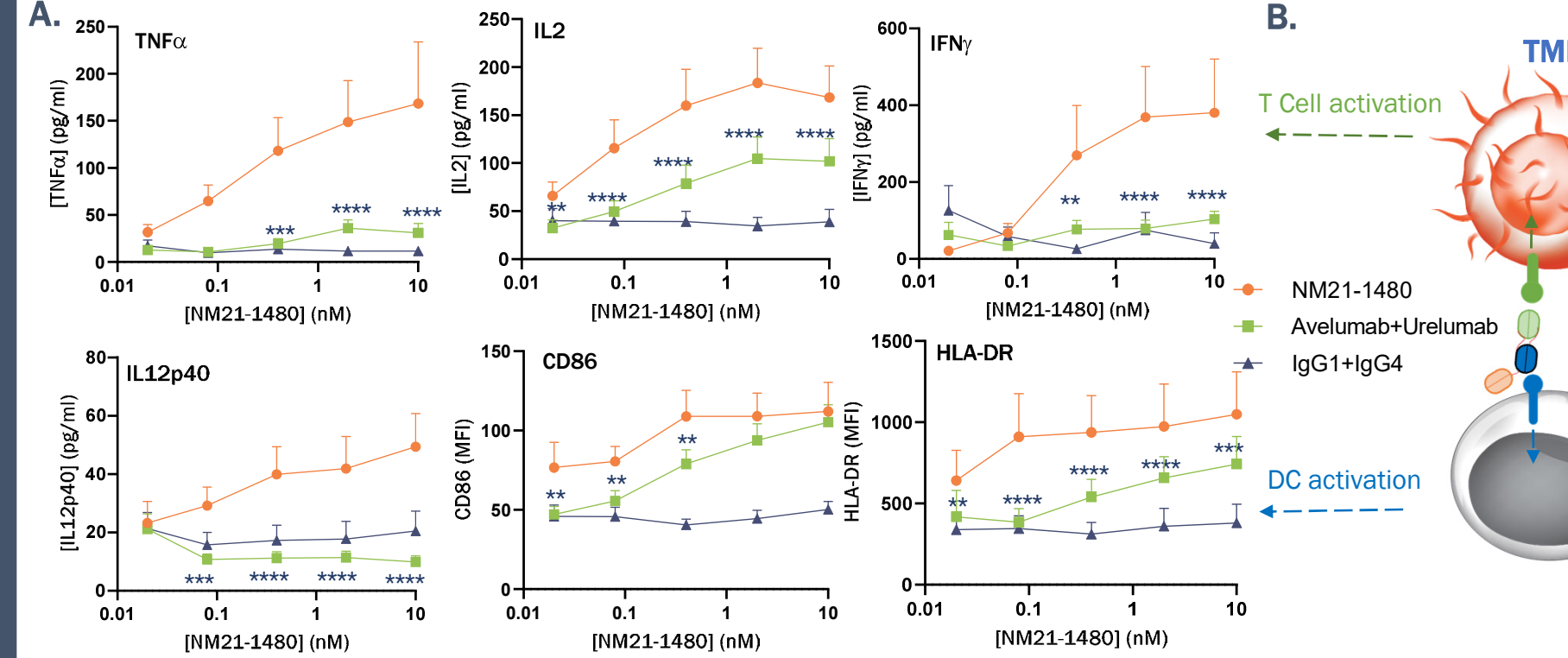


Figure 8. Monocyte derived dendritic cells (MoDCs) were prepared from CD14+ cells cultured for 7 days. MoDCs were then cultured together with T cells from a separate donor for 5 days in the presence of NM21-1480, avelumab and urelumab, IgG1 and IgG4 (0.016, 0.08, 0.4, 2, 10 nM). A. Supernatants and cells were collected at the end of culture (or 48hrs for IL2) and cytokine production was measured by ELISA or cell surface markers measured by flow cytometry. Data are presented as mean + SEM (n = 6 biological replicates). **p<0.01, ****p<0.0001, ****p<0.0001 comparing to NM21-1480, as determined using a repeated measures two-way ANOVA with Dunnett's multiple comparisons test. B. Schematic representation of potential mechanism of cellular activation.

Positive feedback loop for sustained activity of NM21-1480

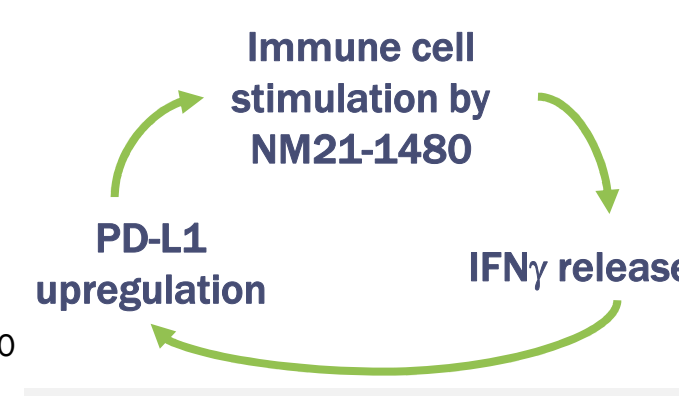


Figure 9. The activity of NM21-1480 has the potential to elicit a positive feedback loop, augmenting its effects over time. We have demonstrated the ability of NM21-1480 to potentially elicit the release of IFN-gamma from T cells following NM21-1480 engagement. We have also demonstrated the upregulation of PD-L1 on tumor cells by the presence of IFN-gamma (data not shown). In addition, the maximal activity of T cell stimulation by NM21-1480 is correlated with PD-L1 density. Therefore a positive feedback loop is created which has the potential to perpetuate the pro-inflammatory activity of NM21-1480 in the tumor microenvironment.

Stronger CD8+ T cell expansion, and higher response rates than IgG combination therapy

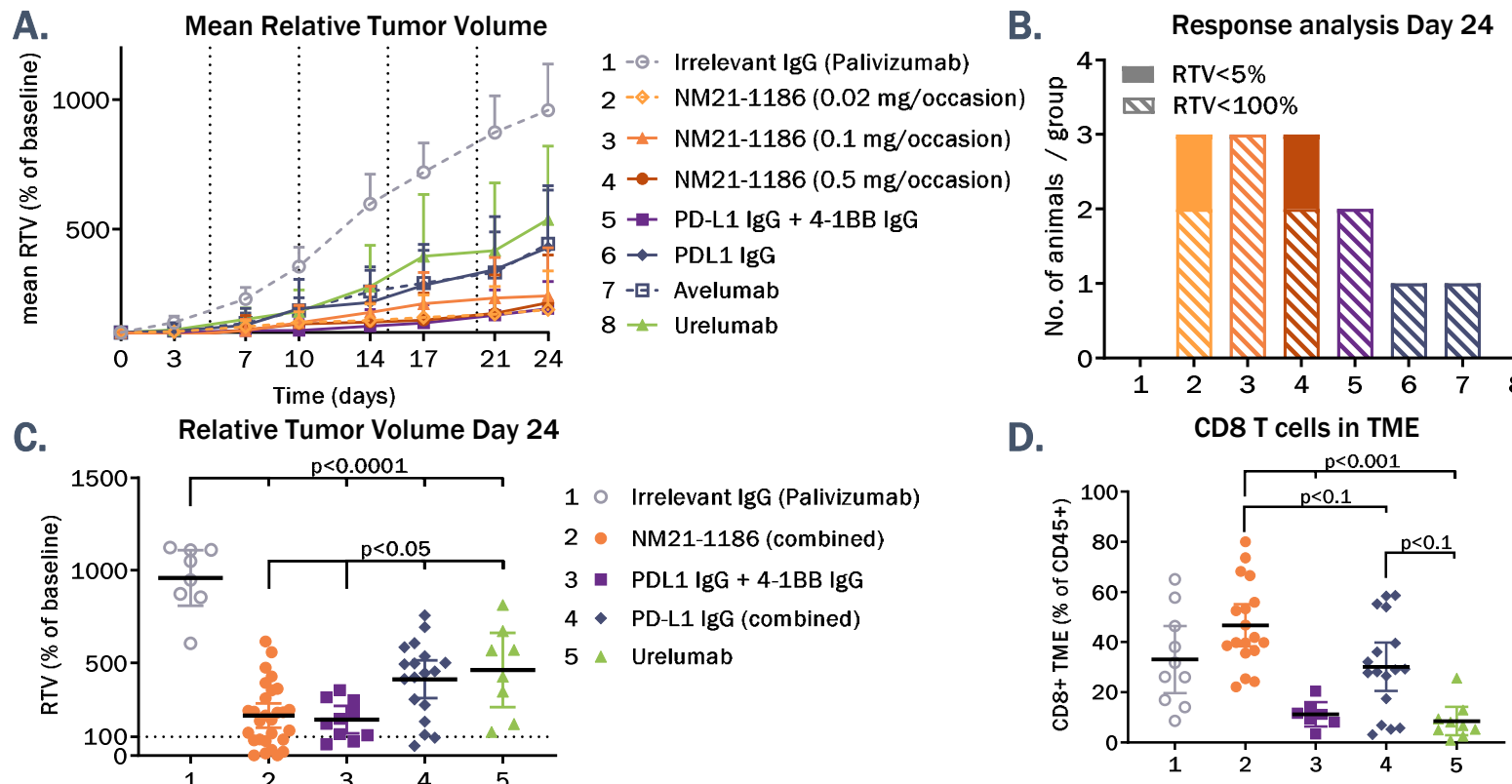


Figure 10. CD34+ stem cell-substituted NOG mice were engrafted with HCC827 NSCLC (n=10 each). A, B, C. Mice were treated on day 0, 5, 10, 15 and 20 (dotted vertical lines). Tumor growth and body weight was recorded twice weekly. Tumors were harvested at the end of the study and D. assessed for infiltration of human T cells by flow cytometry (mean and 95% CI). NM21-1186 was used for the study as a mouse surrogate molecule. Therapy with NM21-1186 elicited greater antitumor efficacy than PD-L1 mAb or 4-1BB mAb monotherapy. NM21-1186 therapy led to higher response rates (30% vs 20%) than α PD-L1 mAb + α 4-1BB mAb combination therapy.

4-week GLP Toxicology Study: NM21-1480 is well-tolerated in non-human primates. No treatment related toxicity observed up to 140mg/kg

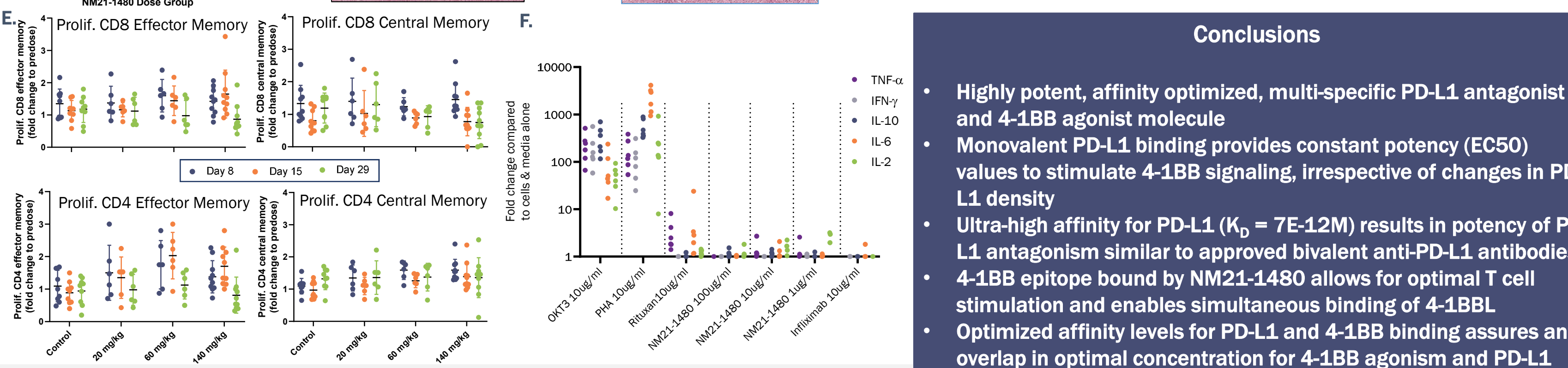
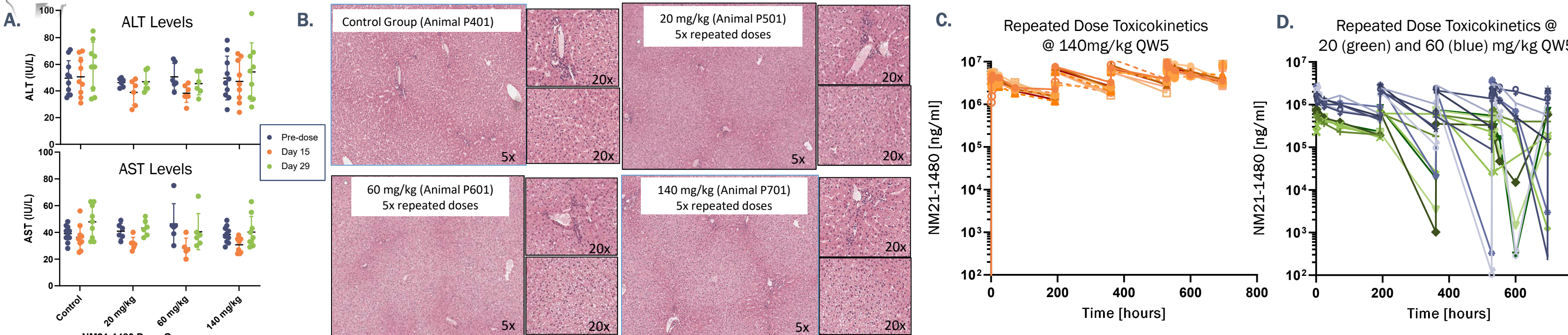


Figure 12. A GLP toxicology study in cynomolgus monkeys was conducted with 5 doses administered weekly at 0, 20, 60 and 140 mg/kg of NM21-1480. A. Liver enzymes ALT and AST were measured pre-dose, day 15 and day 29 from all animals. No changes observed compared to predose. B. Representative histology images from the livers of monkeys at the end of the study are shown. No histopathological changes observed with NM21-1480 treatment and no signs of liver inflammation. Toxicokinetic measurements were taken throughout the study. C. Data from all animals at 140 mg/kg are shown. All 10 animals maintained exposure throughout the study. D. Data from all animals at 20 and 60 mg/kg are shown. Exposure was maintained throughout the study for 1/6 animals in 20 mg/kg group and 3/6 animals in 60 mg/kg group. E. Memory cell populations were measured by flow cytometry from the blood of all study animals at day 8, 15 and 29. No changes in cellular populations were observed. F. Cytokine release assay was performed with human PBMCs from 6 donors at 1, 10, 100 micrograms/ml in comparison to positive (OKT3, PHA, Rituxan) and negative (Infliximab) controls. Data is shown as fold change compared to signal from cells and media alone. No significant increase in cytokines were observed with NM21-1480.

Biodistribution shows accumulation and retention of NM21-1480 in PD-L1-positive tumor

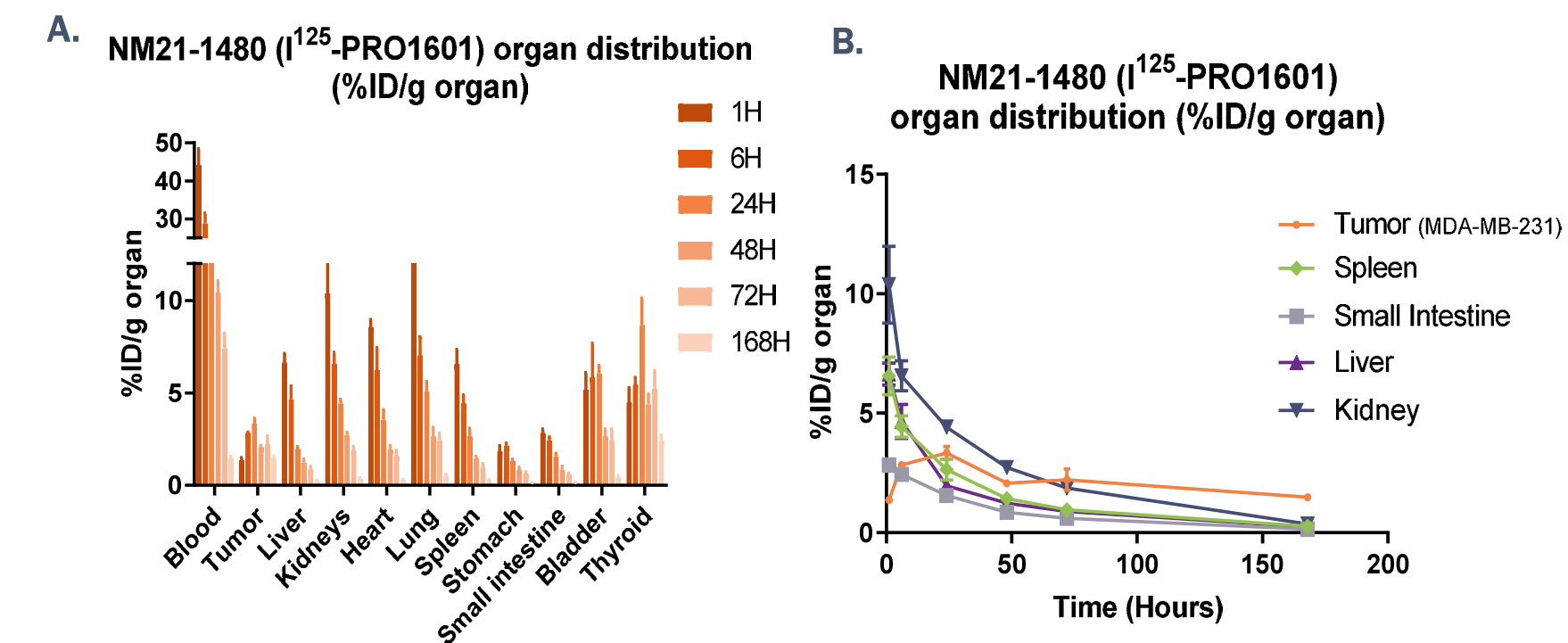


Figure 11. The distribution of NM21-1480 to normal organs and to PD-L1-positive tumor tissue was assessed and quantified using a mouse model bearing a PD-L1-positive tumor, MDA-MB-231. Detection in specific tissues was measured through radiolabelling with iodine-125 (I^{125}). A. Quantification of NM21-1480 (I^{125} -PRO1601) in organs of the mouse was conducted over time by scintillation counting and values calculated as % injection dose of I^{125} per gram of organ (%ID/g). B. %ID/g organ plotted from specific organs. The highest concentration of radioactivity in the tumor was observed at 24 hours, in contrast to other organs where highest concentrations were seen at 1 hour post-dose. Elimination from the tumor was slower than from other organs, showing retention. Clearance of NM21-1480 occurred predominantly via excretion through the kidney.

Conclusions

- Highly potent, affinity optimized, multi-specific PD-L1 antagonist and 4-1BB agonist molecule
- Monovalent PD-L1 binding provides constant potency (EC50) values to stimulate 4-1BB signaling, irrespective of changes in PD-L1 density
- Ultra-high affinity for PD-L1 ($K_D = 7E-12M$) results in potency of PD-L1 antagonism similar to approved bivalent anti-PD-L1 antibodies
- 4-1BB epitope bound by NM21-1480 allows for optimal T cell stimulation and enables simultaneous binding of 4-1BBL
- Optimized affinity levels for PD-L1 and 4-1BB binding assures an overlap in optimal concentration for 4-1BB agonism and PD-L1 antagonism and extends the window for maximal dual activity
- NM21-1480 is well tolerated in GLP toxicology assessment with NOAEL at the highest tested dose of 140 mg/kg
- Pharmacokinetic profile predicts a 2-week half-life in humans
- Clinical assessment of NM21-1480 due to commence in Q3 2020