A novel, monovalent tri-specific antibody-based molecule that simultaneously modulates PD-L1 and 4-1BB exhibits potent anti-tumoral activity *in vivo*

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Background

The combined immunomodulation of PD-L1/PD-1 and 4-1BB 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 scMATCH™3 immunomodulatory drug candidate (NM21-1480) that agonizes 4-1BB conditionally upon PD-L1-binding/-blockade. Here, we show that a NM21-1480 surrogate (NM21-1186) was equally effective as a αPD-L1 mAb + α4-1BB mAb combination at slowing tumor progression in vivo, while being better tolerated and eliciting greater proliferation of CD8+ T cells in the TME. In PK/PD studies in healthy cynomolgus monkeys, we confirmed the tolerability and extended serum half-life of NM21-1480. In conclusion, our data confirm the successful development of a novel therapeutic designed to unlock the full potential of combined immunomodulation and overcome the limitations of conventional strategies.

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

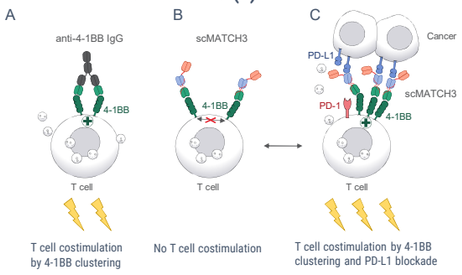


Figure 1. A) Cross-linking of 4-1BB with a bivalent mAb triggers costimulation of T cells that may lead to toxicity-inducing cytokine release. **B)** scMATCH3 molecules cannot intrinsically trigger 4-1BB clustering and signaling, unless **C)** clustering of the α4-1BB domain occurs due to the scMATCH3 binding to PD-L1+ cells.

NM21-1480 consists of three λcap™-stabilized antibody Fvs specific for PD-L1, HSA and 4-1BB, respectively, fused in a single chain

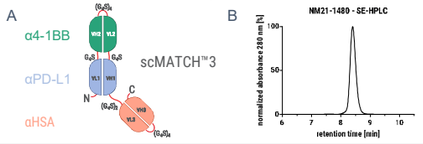


Figure 2. A) Schematic representation of NM21-1480: a trispecific scMATCH3 molecule with αPD-L1 (VL1/VH1 in blue), α4-1BB (VL2/VH2 in green) and αHSA (VL3/VH3 in red) domains connected by Gly-Ser peptide linkers, as indicated by red lines. **B)** Representative SE-HPLC chromatogram of NM21-1480.

Affinity-optimized domains for high, extended activity

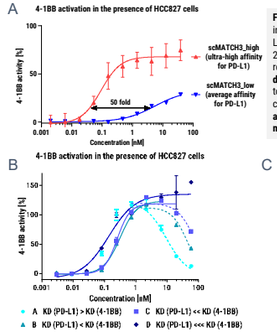


Figure 3. PD-L1+ HCC827 NSCLC cancer cells were incubated with reporter Jurkat T cells. **A)** Effects of PD-L1-affinity on potency. KD(PD-L1) is $1x10^{-10}$ M and $2x10^{-12}$ M for scMATCH3_low and scMACTH3_high, respectively **Ultra-high-affinity, monovalent αPD-L1 domain maximizes potency. B)** and **C)** Effects of affinity to targets on potency and concentration-response curves. **By carefully balancing the αPD-L1/α4-1BB affinity-ratio, the effective dose-range of PD-L1x4-1BB molecules is considerably extended.**

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

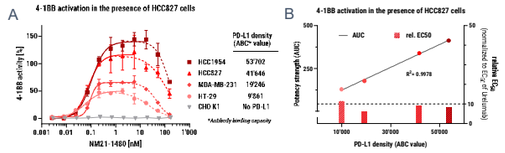


Figure 4. Effects of PD-L1 expression-levels on tumor cell-lines on potency and concentration-response curves. **A)** PD-L1+ cancer cells were incubated with reporter Jurkat T cells. **B)** PD-L1 expression-levels correlate with T cell-costimulation potency. **NM21-1480 elicits overlapping dose-response curves across tumor cell-lines with varying PD-L1 expression-levels. Thus, the optimal biologic dose observed in clinical trials should be applicable to all patients above a certain threshold of PD-L1 expression.**

Fc-less format spares APCs from depletion and prevents extratumoral 4-1BB agonism

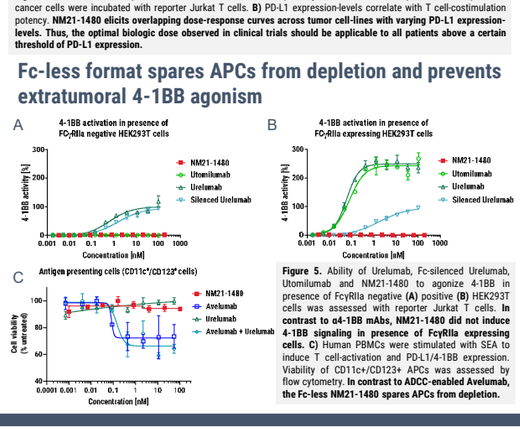


Figure 5. Ability of Urelumab, Fc-silenced Urelumab, Utomilumab and NM21-1480 to agonize 4-1BB in presence of FcγRIIa negative **(A)** positive **(B)** HEK293T cells was assessed with reporter Jurkat T cells. **In contrast to α4-1BB mAbs, NM21-1480 did not induce 4-1BB signaling in presence of FcγRIIa expressing cells. C)** Human PBMCs were stimulated with SEA to induce T cell-activation and PD-L1/4-1BB expression. Viability of CD11c+/CD123+ APCs was assessed by flow cytometry. **In contrast to ADCC-enabled Avelumab, the Fc-less NM21-1480 spares APCs from depletion.**

Binding to optimal epitope on 4-1BB increases T cell-costimulation capacity

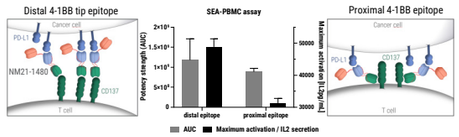


Figure 6. Human PBMCs were stimulated with SEA to induce T cell-activation and PD-L1/4-1BB expression. Subsequently, NM21-1480 binding a membrane-distal epitope on 4-1BB or a scMATCH3 molecule binding a membrane-proximal epitope on 4-1BB was added to the culture. IL-2 secretion was assessed by ELISA. **Clustering via binding to the distal tip of 4-1BB more potently costimulates 4-1BB+ cells than clustering via binding to a proximal epitope on 4-1BB.**

Rational design of the monovalent αPD-L1/4-1BB/HSA NM21-1480 results in:

- Superior activity, when compared to combinations of αPD-(L)1 and α4-1BB mAbs
- Very high potency
- Wide range of active concentrations (broad bell-shape)
- Constant EC50, largely independent of PD-L1 expression level
- Tumor restricted 4-1BB signaling
- Lack of Fc-mediated adverse effects

Stronger T cell costimulatory capacity than clinical-stage αPD-(L)1 + α4-1BB mAb combinations

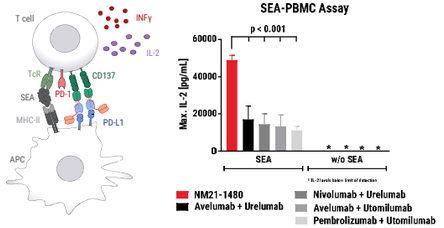
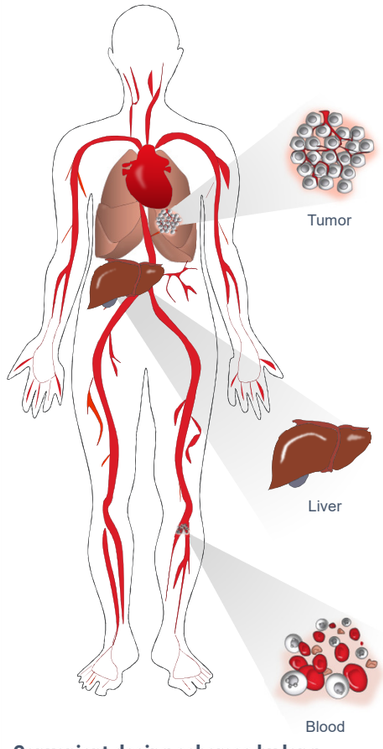


Figure 7. Human PBMCs were stimulated with Staphylococcal enterotoxin A (SEA) to induce T cell-activation and PD-L1/4-1BB expression. Subsequently, NM21-1480 or αPD-L1/PD-1 mAb + α4-1BB mAb combinations were added to the culture. IL-2 secretion was assessed by ELISA. **NM21-1480 more potently costimulates 4-1BB+ cells than the combination of clinical-stage αPD-L1/PD-1 mAb + α4-1BB mAb combinations.**



Convenient dosing schemes by long serum half-life

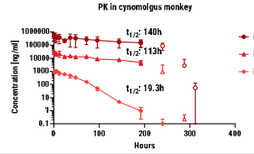


Figure 11. Pharmacokinetic analysis to quantify NM21-1480 serum samples from cynomolgus monkeys. **Serum half-life of ~6 days was observed in cynomolgus monkeys, suggesting a drug candidate compatible with dosing every 3-4 weeks in human subjects.**

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

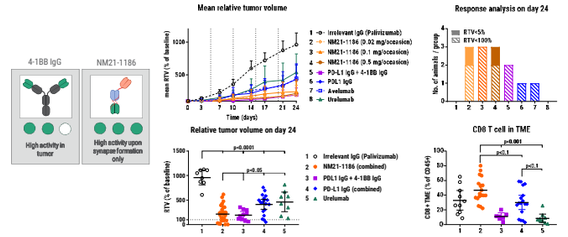


Figure 8. CD34+ stem cell-substituted NOG mice were engrafted with HCC827 NSCLC (n=10 each). Mice were treated on day 0, 5, 10, 15 and 20 (dotted vertical lines). Tumor growth and body weight was recorded twice weekly. Tumors were harvested at the end of the study and assessed for infiltration of human T cells by flow cytometry (mean and 95% CI). **NM21-1186 therapy elicited greater antitumoral efficacy than PD-L1 mAb or 4-1BB mAb monotherapy. NM21-1186 therapy led to higher response rates (30% vs 20%) than αPD-L1 mAb + α4-1BB mAb combination therapy. This correlated with higher frequency of CD8+ T cells and CD8+GrB+ double-positive T cells in the TME (not shown).**

No signs of hepatoxicity in non-human primates

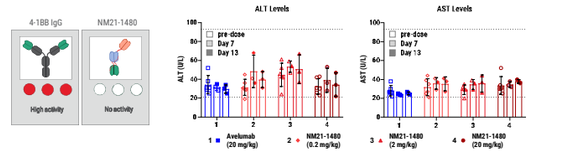


Figure 9. NM21-1480 or Avelumab was administered intravenously as single dose to cynomolgus monkeys (n=3 per group). **No elevations of aminotransferases were detected in any treatment group. All post-dose ALT/AST levels were within the normal healthy range for juvenile female cynos (hashed lines in graphs).**

Lack of systemic T cell activation in non-human primates

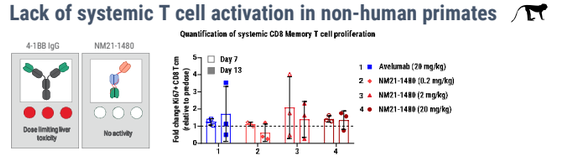


Figure 10. NM21-1480 or Avelumab was administered intravenously as single dose to cynomolgus monkeys (n=3 per group). Ki-67+ CD8+ central memory T cells (Tcm, CD95+/CD28+) were measured 7 and 13 days following administration. **NM21-1480 did not show any significant expansion of memory T cells.**

The αPD-L1/4-1BB/HSA NM21-1480 shows:

- Superior in-vivo efficacy, when compared to combinations of αPD-(L)1 and α4-1BB mAbs
- No signs for hepatotoxicity in NHPs
- No signs for systemic T cell activation in NHPs
- Prolonged half-life supporting convenient dosing schemes