NM28-2746, a reduced affinity bivalent mesothelin-binding MATCH™4 T cell engager with half-life extension increases selectivity for killing of mesothelin-overexpressing cells

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NUMAB Drug Innovators

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Background: The effective treatment of solid tumors remains an unmet medical need. Several concepts exist to treat malignancies, including antibody-drug or -immunotoxin conjugates, immune checkpoint inhibition, CAR- T cells, as well as bispecific T cell engagers. CD3-based T cell engagers are highly potent therapeutic molecules with T cell cytotoxicity activities in the picomolar range. Alongside this highly potent anti-tumor activity is the risk of on-target off-tumor effects due to low levels of expression of the target antigen in normal tissue, as has been observed for the tumor-associated antigen mesothelin (MSLN).

Concept: Selective T cell-mediated depletion of tumor cells

Improved Therapeutic Window

Numab's bivalent biMSLN.CD3.hSA MATCH™4 NM28-2746 efficiently depletes mesothelioma cells expressing high levels of MSLN and spares normal epithelial cells. The bivalent binding provides further resistance to soluble MSLN shed from tumor cells.

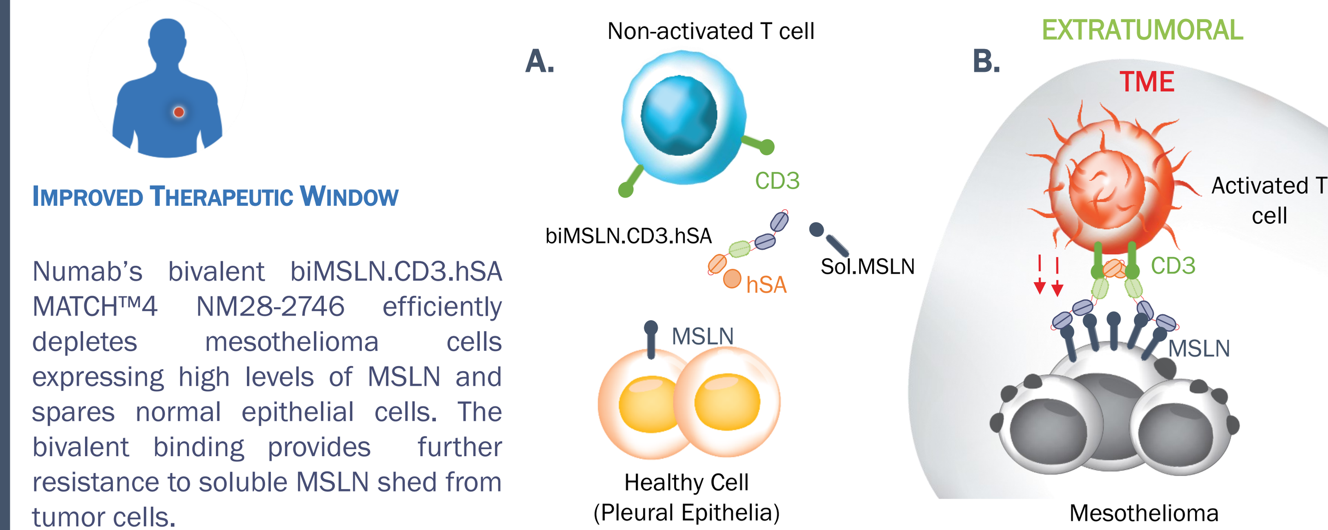


Figure 1. A. BiMSLN.CD3.hSA does not deplete healthy cells due to its low affinity binding domains and is minimally impacted by shed soluble MSLN in circulation. **B.** Avidity-based binding of high MSLN expressing cells like mesothelioma, concomitant with CD3 engagement on T cells, causes the depletion of tumor cells.

Tumor Type	% of patients MSLN+
Mesothelioma	82 - 95
Pancreatic cancer	85
Ovarian cancer	70
NSCLC	57 - 64
Biliary cancer	95
TNBC	66

Table 1. Mesothelin is expressed on many types of malignancies. Frequency of patients with MSLN-positive malignancies, adapted from Hassan et al. J.Clin. Onc. 2016. MSLN expression is associated with a poor prognosis for several of these cancers, including pancreatic adenocarcinoma, biliary cancer, and breast cancer. Targeting MSLN in these patient populations has the potential for improvement in patient outcome and to address unmet medical need for these individuals.

MATCH™4: combinations for optimized potency

Advantages of the MATCH™ technology:

- ✓ Convenient permutation of binding domains by simple plasmid exchange
- ✓ Multispecific format without the risk of light chain mispairing
- ✓ No Fc region required to drive heterodimerization

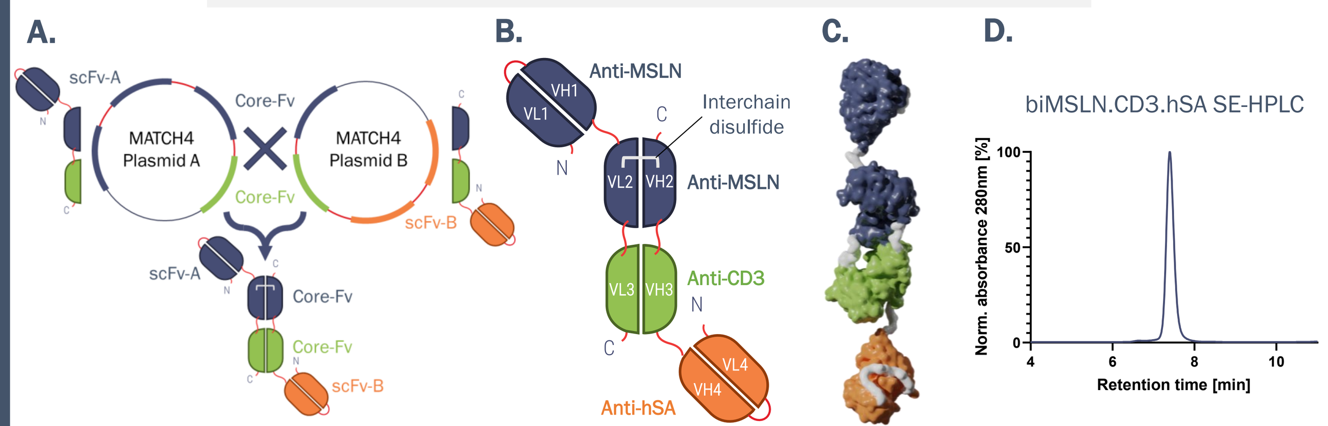


Figure 2. A. MATCH™ (Multispecific Antibody-based Therapeutics by Cognate Heterodimerization) molecules are modular and can be combined in numerous ways and formats. **B.** Schematic representation of biMSLN.CD3.hSA, NM28-2746 MATCH™4 molecule. **C.** Structural model of NM28-2746 MATCH™4 molecule (prepared in BIOVIA Discovery Studio software). **D.** Representative SE-HPLC chromatogram of NM28-2746.

NM28-2746, the bivalent anti-MSLN T cell engager shows superior anti-tumor activity compared to a monovalent binder

The bivalent T cell engager NM28-2746 kills cells in a MSLN-density-dependent manner and decreases the risk for on-target off-tumor adverse effects

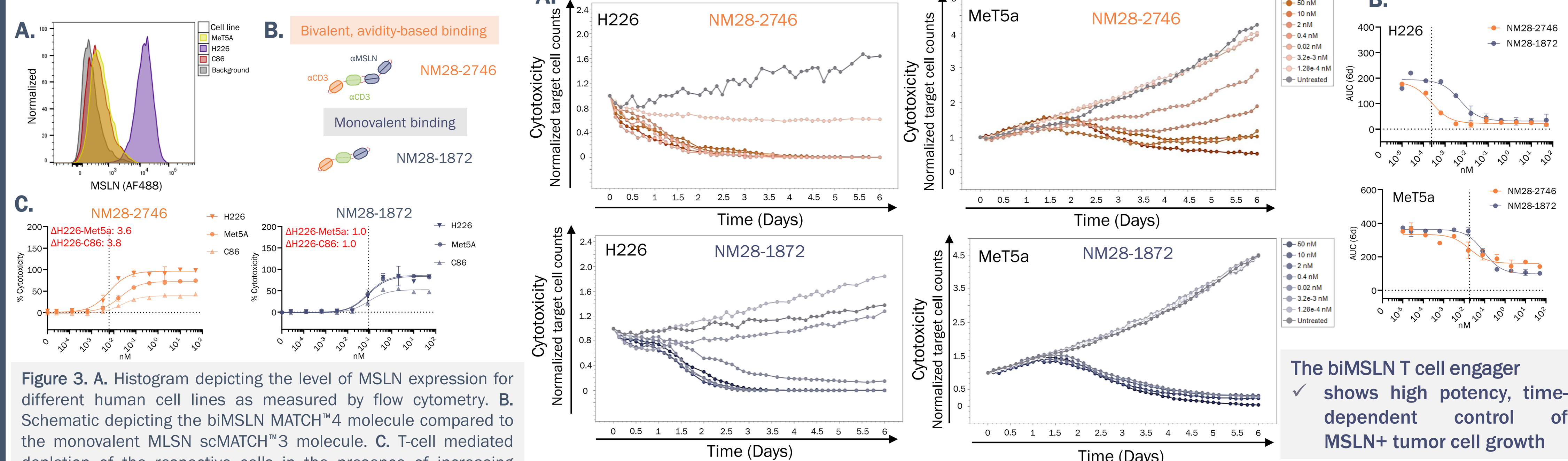


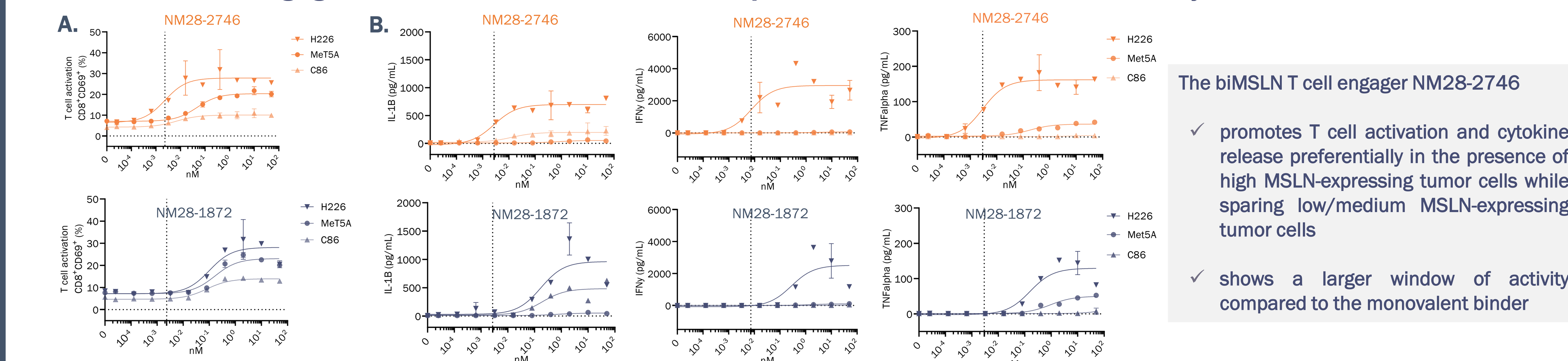
Figure 3. A. Histogram depicting the level of MSLN expression for different human cell lines as measured by flow cytometry. **B.** Schematic depicting the biMSLN MATCH™4 molecule compared to the monovalent MLSN scMATCH™3 molecule. **C.** T-cell mediated depletion of the respective cells in the presence of increasing amounts of NM28-2746 (orange) or NM28-1872 (blue). Cells were cultured together with PBMCs (E:T=30:1) for 40 hours and cytotoxic activity was assessed by LDH release. H226: lung cancer; Met5a: mesothelial cells from pleura; C86: Mesothelial cells from ascites (AG07086 or LP-9, obtained from the Coriell Institute).

The biMSLN T cell engager

- ✓ shows high potency, time-dependent control of MSLN+ tumor cell growth

Figure 4. A. H226- or MeT5A-Nuclight Red labeled tumor cells were cocultured with total T cells for up to 6 days at an E:T ratio of 5:1 in the presence of increasing amounts of NM28-2746 or NM28-1872 (darker colors indicate higher concentration), and imaged at regular intervals using the Incucyte, a real time live cell imaging platform. The y-axis depicts the numbers of target cells normalized to time 0. The "untreated" control demonstrated tumor cell growth in the absence of treatment. **B.** The integrated area under the curve data from A is depicted. Vertical line illustrates the EC50 value for NM28-2746

The bivalent T cell engager NM28-2746 mediates MSLN-dependent CD8 T cell activation and cytokine release

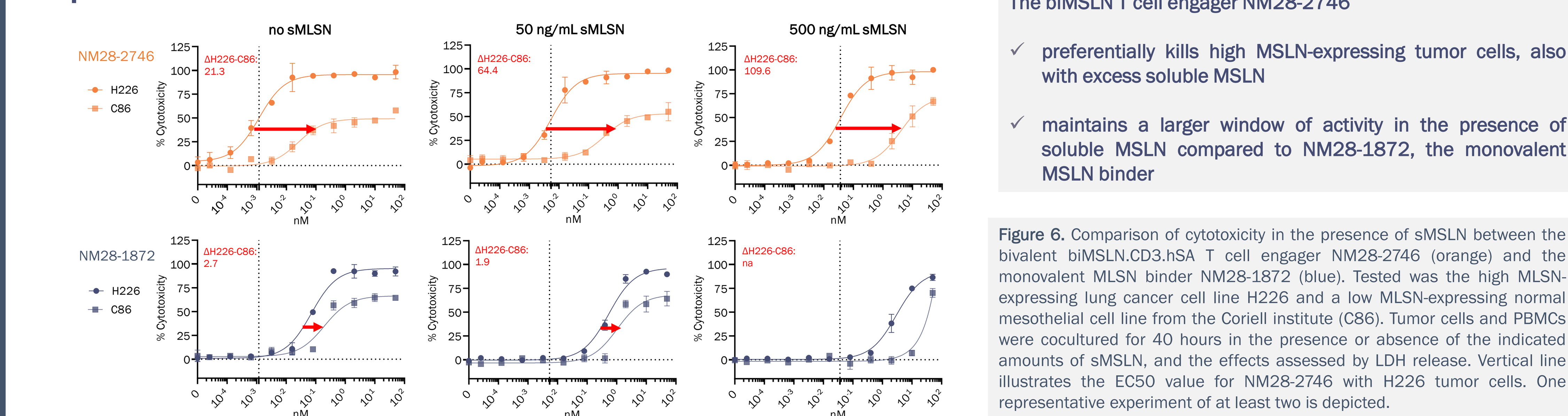


The biMSLN T cell engager NM28-2746

- ✓ promotes T cell activation and cytokine release preferentially in the presence of high MSLN-expressing tumor cells while sparing low/medium MSLN-expressing tumor cells
- ✓ shows a larger window of activity compared to the monovalent binder

Figure 5. Human cell lines were cocultured together with human PBMCs (E:T=30:1) for 40 h. **A.** CD8 T cell activation was determined by examining the frequency of CD69-expressing CD8 cells within the coculture using flow cytometry. **B.** Cytokine release was assessed from the coculture supernatant using a cytometric bead array-based multiplexing system. One representative experiment of at least two is depicted. Vertical line illustrates the EC50 value for NM28-2746 with H226 tumor cells.

The bivalent T cell engager NM28-2746 has a larger therapeutic window, also in the presence of soluble MSLN (sMSLN) as compared to the monovalent MSLN binder



The biMSLN T cell engager NM28-2746

- ✓ preferentially kills high MSLN-expressing tumor cells, also with excess soluble MSLN
- ✓ maintains a larger window of activity in the presence of soluble MSLN compared to NM28-1872, the monovalent MSLN binder

Figure 6. Comparison of cytotoxicity in the presence of sMSLN between the bivalent biMSLN.CD3.hSA T cell engager NM28-2746 (orange) and the monovalent MLSN binder NM28-1872 (blue). Tested was the high MLSN-expressing lung cancer cell line H226 and a low MLSN-expressing normal mesothelial cell line from the Coriell institute (C86). Tumor cells and PBMCs were cocultured for 40 hours in the presence or absence of the indicated amounts of sMSLN, and the effects assessed by LDH release. Vertical line illustrates the EC50 value for NM28-2746 with H226 tumor cells. One representative experiment of at least two is depicted.

Targeting MSLN and CD3 synergizes with Numab's next generation checkpoint modulator NM21-1480, resulting in enhanced tumor eradication

Concept: Tumor-localized activation of 4-1BB combined with PD-L1 blockade

TUMOR-LOCALIZED ACTIVITY

NM21-1480 leverages monovalent binding to its targets and ultra-high-affinity to PD-L1 (K_D=7E-12M) in order to restrict 4-1BB signaling to the tumor microenvironment (TME), thereby allowing for safe combination of synergistic immune stimulatory activities.

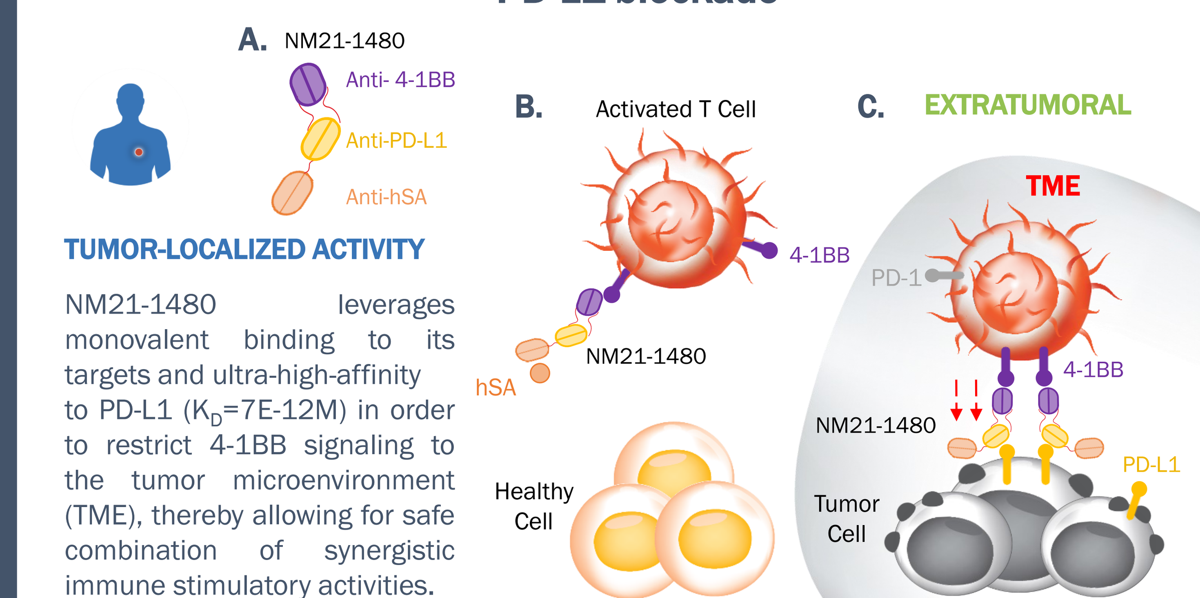


Figure 7. A. NM21-1480 is a trispecific scMATCH™3 molecule that binds to 4-1BB, PD-L1, and hSA. **B.** NM21-1480 cannot intrinsically trigger 4-1BB clustering and signaling upon binding to 4-1BB alone. **C.** Clustering of 4-1BB occurs following simultaneous binding of NM21-1480 to 4-1BB+ and PD-L1+ cells, resulting in 4-1BB signaling and concomitant blocking of the PD-1 / PD-L1 pathway.

Combination immunotherapy through mix and MATCH™ targeting

FORMATION OF A SUPER-AGONISTIC IMMUNE SYNAPSE

Targeted modulation of the tumor microenvironment by combining T cell recruiting T cell engager NM28-2746 with the next generation checkpoint inhibitor NM21-1480 to form a super-agonistic immunological synapse.

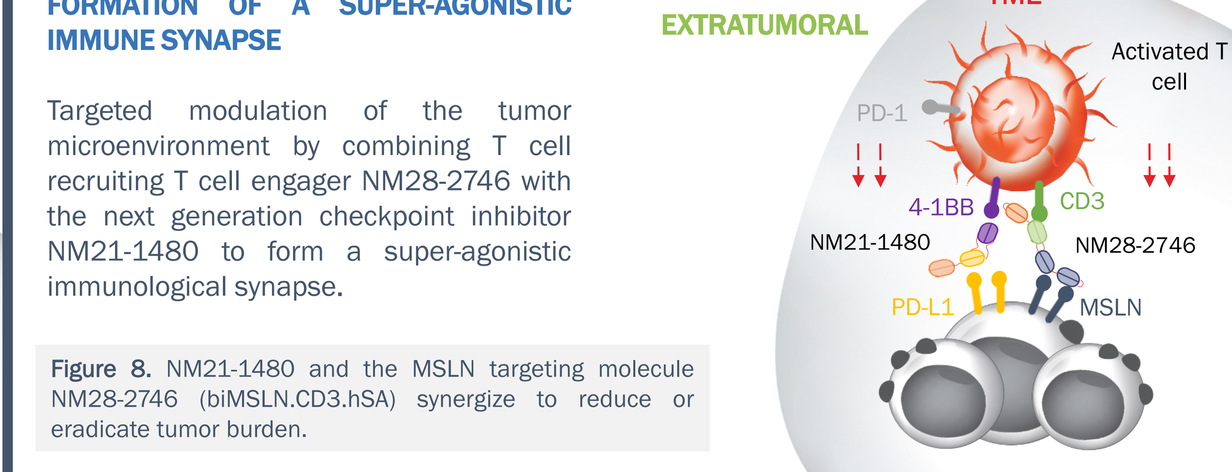
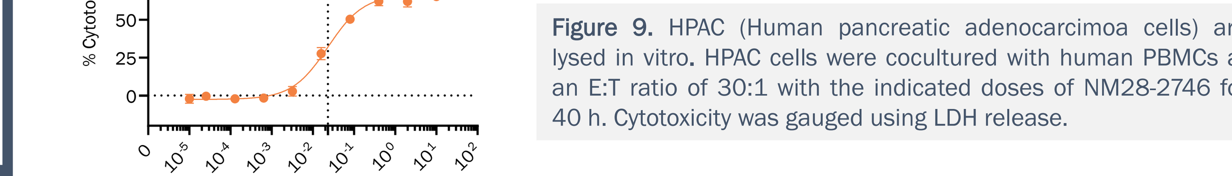


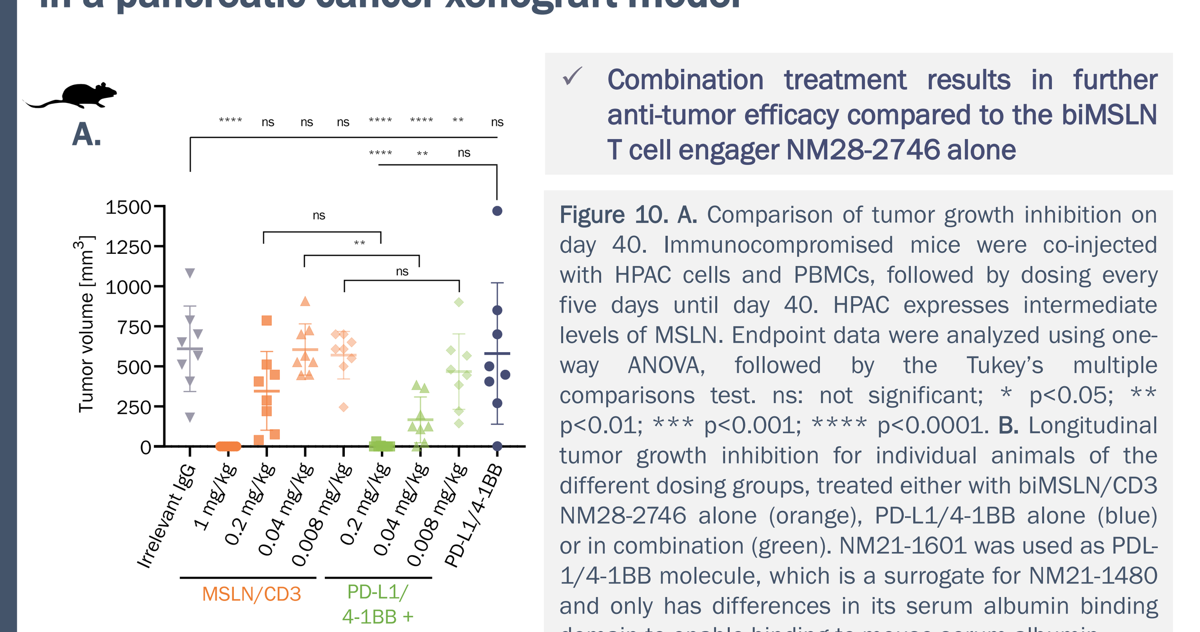
Figure 8. NM21-1480 and the MSLN targeting molecule NM28-2746 (biMSLN.CD3.hSA) synergize to reduce or eradicate tumor burden.



- ✓ Human pancreatic adenocarcinoma cells are lysed in vitro

Figure 9. HPAC (Human pancreatic adenocarcimoa cells) are lysed in vitro. HPAC cells were cocultured with human PBMCs at an E:T ratio of 30:1 with the indicated doses of NM28-2746 for 40 h. Cytotoxicity was gauged using LDH release.

PDL-1/4-1BB and NM28-2746 synergize to inhibit tumor growth in a pancreatic cancer xenograft model



- ✓ Combination treatment results in further anti-tumor efficacy compared to the biMSLN T cell engager NM28-2746 alone

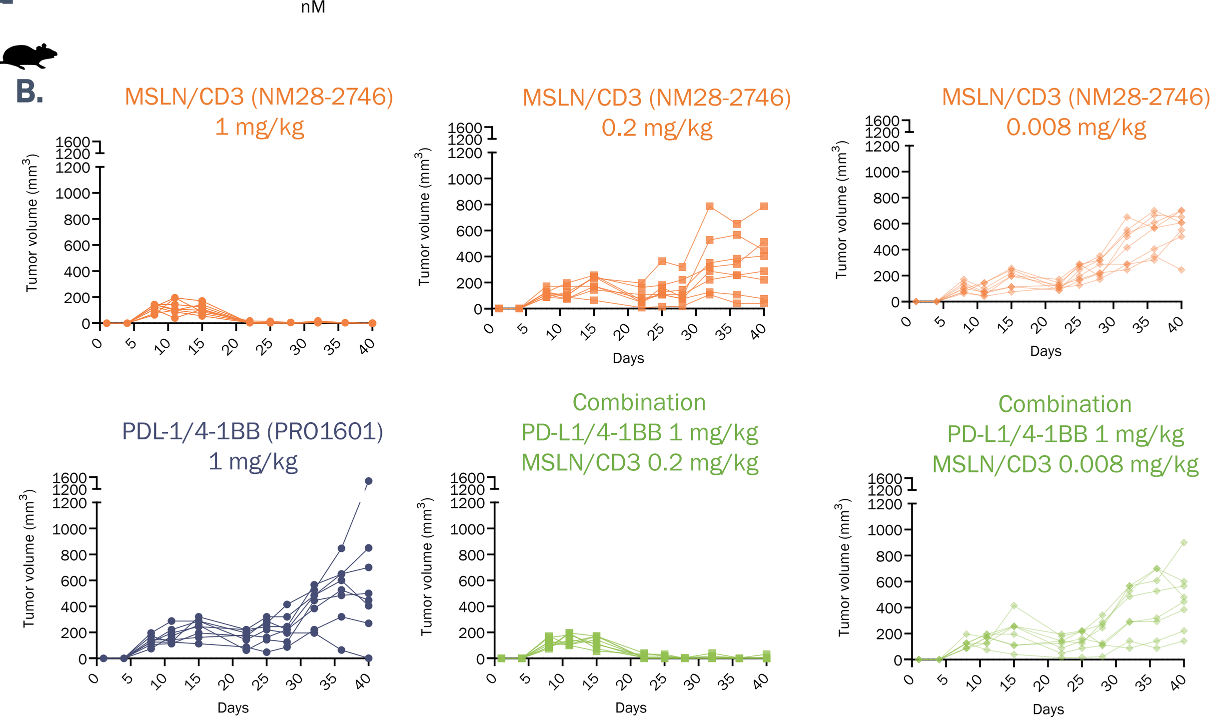


Figure 10. A. Comparison of tumor growth inhibition on day 40. Immunocompromised mice were co-injected with HPAC cells and PBMCs, followed by dosing every five days until day 40. HPAC expresses intermediate levels of MSLN. Endpoint data were analyzed using one-way ANOVA, followed by the Tukey's multiple comparisons test. ns: not significant; * p<0.05; ** p<0.01; *** p<0.001; **** p<0.0001. **B.** Longitudinal tumor growth inhibition for individual animals of the different dosing groups, treated either with biMSLN/CD3 NM28-2746 alone (orange), PD-L1/4-1BB alone (blue) or in combination (green). NM21-1601 was used as PDL-1/4-1BB molecule, which is a surrogate for NM21-1480 and only has differences in its serum albumin binding domain to enable binding to mouse serum albumin.

- ✓ Remodeling of the tumor microenvironment in vivo upon combination treatment, to create a favorable environment for anti-tumor efficacy

A. CD8+ T cells in tumor

B. CD4+ T cells in tumor

Irrelevant IgG (Palivizumab); MSLN/CD3 0.04 mg/kg; PD-L1/4-1BB + MSLN/CD3 0.04 mg/kg; MSLN/CD3 0.008 mg/kg; PD-L1/4-1BB + MSLN/CD3 0.008 mg/kg; PD-L1/4-1BB

Figure 11. A. Comparison of CD8+ T cell infiltration into the tumor. **B.** Comparison of CD4+ T cell infiltration into the tumor. Data were analyzed using one-way ANOVA, followed by the Tukey's multiple comparisons post-hoc test. ns: not significant; * p<0.05; ** p<0.01; *** p<0.001; **** p<0.0001. NM21-1601 was used as PDL-1/4-1BB molecule, which is a surrogate for NM21-1480 and only has differences in its serum albumin binding domain to enable binding to mouse serum albumin.

Conclusions

Bivalent MSLN binding	• Low-affinity, bivalent αMSLN domain preferentially engages MSLN-overexpressing malignant cells while sparing healthy, MSLN+ cells
Tumor-directed T cell stimulation	• Tumor-restricted T cell activation and tumor killing in presence of high mesothelin expressing cells
Minimal impact of soluble MSLN	• High avidity, low affinity binding to MSLN renders NM28-2746 resistant to high concentrations of soluble MSLN present in patient blood
Fc-less	• Avoids Fc-mediated adverse effects • Avoids internalization and degradation by macrophages
Extended half-life	• Half-life comparable to conventional IgG due to serum albumin binding domain