A low affinity bivalent mesothelin-binding MATCH4 multispecific T cell engager increases cytotoxic selectivity for high mesothelin expressing cells

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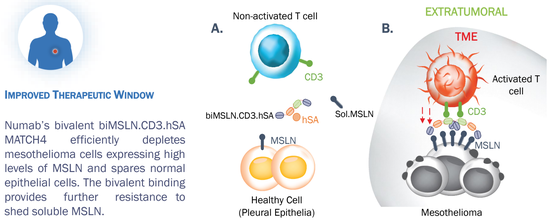
Background: The effective treatment of solid tumors remains an unmet medical need. Several concepts exist to treat malignancies, including antibody-drug or -immunotoxin conjugates, immune checkpoint inhibition, CAR- T cells, as well as bispecific T cell engagers. CD3-based T cell engagers are highly 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



Numab's bivalent biMSLN.CD3.hSA MATCH4 efficiently depletes mesothelioma cells expressing high levels of MSLN and spares normal epithelial cells. The bivalent binding provides further resistance to shed soluble MSLN.

Figure 1. **A.** BiMSLN.CD3.hSA does not deplete healthy cells due to its low affinity binding domains and does not bind to 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	No. of patients with MSLN-positive disease (%)
Mesothelioma	290 of 352 (82)
Epithelioid	248 of 261 (95)
Sarcomatous	0 of 23 (0)
Pancreatic adenocarcinoma	303 of 357 (85)
Epithelial ovarian cancer	346 of 494 (70)
High grade serous	248 of 332 (75)
Endometroid	36 of 52 (69)
Mucinous	2 of 19 (11)
Clear cell	11 of 21 (52)
NSCLC	1,157 of 2,036 (57)
Adenocarcinoma	1,082 of 1,686 (64)
Squamous	40 of 188 (21)
Biliary cancer, extrahepatic	93 of 98 (95)
Triple negative breast cancer	33 of 50 (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.

MATCH4™: combinations for optimized potency

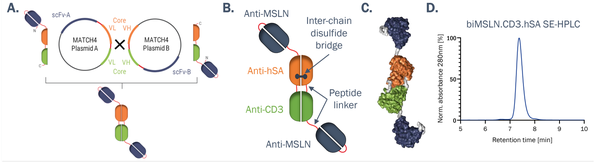


Figure 2. **A.** MATCH™ (Multispecific Antibody-based Therapeutics by Cognate Heterodimerization) molecules are modular and can be combined in numerous ways and formats. **The advantages include: 1. Convenient permutation of binding domains by simple plasmid exchange, 2. Multispecific format without the risk of light chain mispairing, and 3. No Fc domain requirement. B.** Schematic representation of a MATCH4 molecule. **C.** Structural model of a MATCH4 molecule (prepared in BIOVIA Discovery Studio software). **D.** Representative SE-HPLC chromatogram of biMSLN.CD3.hSA.

Bivalent biMSLN.CD3.hSA T cell engager actively kills tumor cells in a MSLN-dependent manner

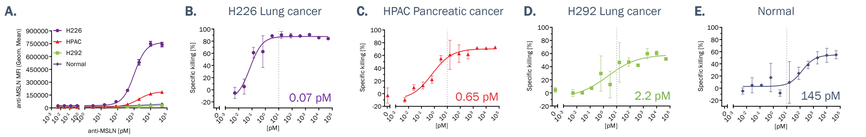
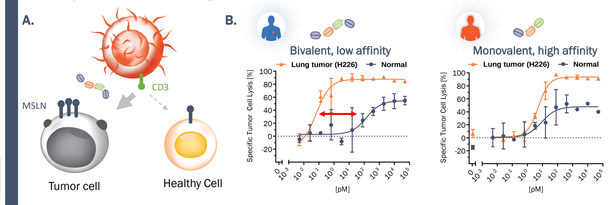


Figure 3. **A.** Surface binding of anti-MSLN antibody to various cell types with different MSLN expression levels. **B.-E.** Tumor cell killing and concomitant CD8 T cell activation in the presence of biMSLN.CD3.hSA engager. H226 (**B**), HPAC (**C**), H292 (**D**), or normal pleural cells (**E**) were cocultured together with PBMCs for 40 hours and cytotoxic activity was assessed by flow cytometry.

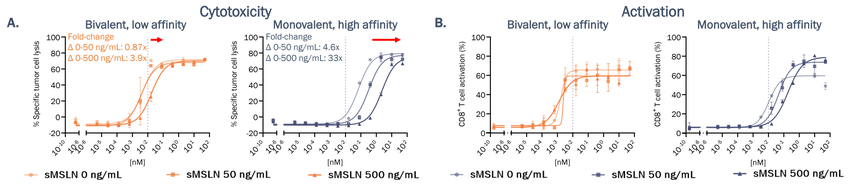
Larger therapeutic window for bivalent biMSLN.CD3.hSA T cell engager due to preferential avidity-based binding to tumor cells



- ✓ Bivalent biMSLN.CD3.hSA engager spares normal MSLN-expressing cells at doses where tumor cell killing is observed.

Figure 4. **A.** Schematic depicting preferential avidity-based binding of biMSLN.CD3.hSA. **B.** Comparison of cell killing on H226 tumor cells (orange line) vs. normal cells (blue line). The bivalent, low affinity biMSLN.CD3.hSA engager is depicted on the left, while the monovalent, higher affinity MSLN.CD3.hSA molecule is depicted on the right. The window of activity is shown in red (double-headed arrow). Tumor cells or normal pleural cells and PBMCs were cocultured for 40 hours and cytotoxic activity was assessed by flow cytometry.

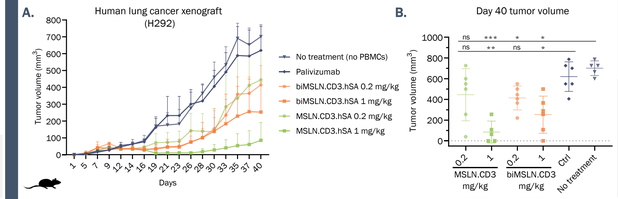
Bivalent biMSLN.CD3.hSA T cell engager triggers potent tumor cell lysis and T cell activation in the presence of excess soluble MSLN (sMSLN) as compared to monovalent MSLN.CD3.hSA



- ✓ Cytotoxic activity of biMSLN.CD3.hSA to MSLN-positive tumor cells is not impacted by the presence of high concentrations of soluble MSLN.

Figure 5. **A.** Comparison of specific killing of H226 tumor cells in the presence of soluble MSLN. The bivalent, low affinity biMSLN.CD3.hSA T cell engager is depicted in orange, while the monovalent, higher affinity molecule is depicted in blue. **B.** Comparison of CD8 T cell activation in the presence of soluble MSLN. Tumor cells or normal cells and PBMCs were cocultured for 40 hours and the effects assessed by flow cytometry.

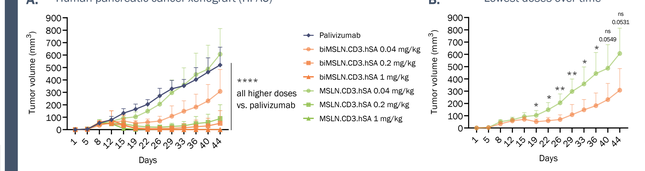
Human lung cancer growth is inhibited upon MSLN targeting



- ✓ Treatment with bivalent biMSLN.CD3.hSA significantly reduces the growth of MSLN+ human H292 lung cancer in humanized mice
- ✓ Similar potency to monovalent MSLN.CD3.hSA

Figure 6. **A.** Longitudinal tumor growth inhibition (mean + SD) in the presence of either biMSLN.CD3.hSA (orange) or MSLN.CD3.hSA (green). Immunocompromised mice were co-injected with H292 lung cancer cells and PBMCs, followed by dosing every five days. H292 expresses low levels of MSLN. **B.** Comparison of tumor growth inhibition on day 40. Longitudinal data were analyzed using two-way ANOVA, followed by the Tukey's multiple comparisons test. ns: not significant; * p<0.05; ** p<0.01; *** p<0.001

Human pancreatic cancer growth is inhibited by biMSLN.CD3.hSA

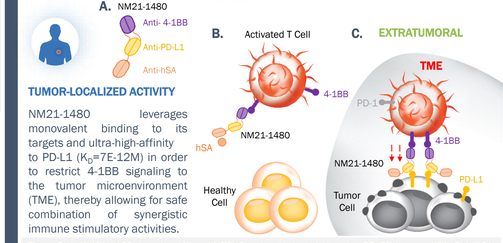


- ✓ Bivalent biMSLN.CD3.hSA shows significant inhibition of MSLN+ human HPAC pancreatic adenocarcinoma growth in humanized mice (co-injection of cancer cells and human PBMCs)
- ✓ Improved potency of bivalent biMSLN.CD3.hSA compared to monovalent MSLN.CD3.hSA with HPAC pancreatic xenograft expressing higher levels of MSLN

Figure 7. **A.** Longitudinal tumor growth inhibition (mean + SD) in the presence of different doses of either biMSLN.CD3.hSA (orange) or MSLN.CD3.hSA (green). Immunocompromised mice were co-injected with HPAC pancreatic cancer cells and PBMCs, followed by dosing every five days. **B.** Comparison of tumor growth inhibition with the lowest doses over time. Longitudinal data were analyzed using two-way ANOVA, followed by the Tukey's multiple comparisons test. ns: not significant; * p<0.05; ** p<0.01; *** p<0.001

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

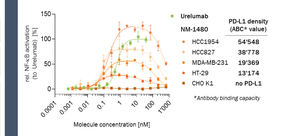
Concept: Tumor-localized activation of 4-1BB combined with PD-L1 blockade



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 8. **A.** NM21-1480 is a trispecific scMATCH3 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.

4-1BB is only activated in the presence of PD-L1 expression



- ✓ Potency of 4-1BB stimulation is independent of PD-L1 density. Maximal 4-1BB stimulation is correlated with PD-L1 density.

Figure 9. NM21-1480 concentration-dependent activation of 4-1BB-driven NF-κB signaling in a Jurkat reporter cell line. The assay is done in the presence of different cancer cell lines stimulated with IFNγ 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-κB activation, normalized to reference antibody Urelumab.

Combination immunotherapy through mix and MATCH™ targeting

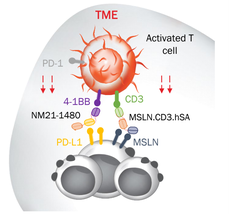
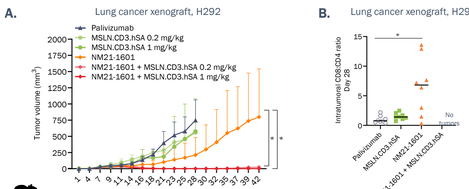


Figure 10. NM21-1480 and MSLN targeting molecules such as MSLN.CD3.hSA synergize to reduce or eradicate tumor burden.

NM21-1480 surrogate (NM21-1601) synergizes with MSLN.CD3.hSA to promote human lung cancer growth inhibition in vivo



- ✓ Remodeling of the tumor microenvironment is observed upon NM21-1601 treatment, to create a favorable environment for anti-tumor efficacy observed in combination,

Figure 11. **A.** Longitudinal tumor growth inhibition (mean + SD) in the presence of NM21-1601 (orange), MSLN.CD3.hSA (green), or a combination of the two (red). Immunocompromised mice were co-injected with H292 lung cancer cells and PBMCs, followed by dosing every five days. H292 express low levels of MSLN and moderate levels of PD-L1. **B.** Comparison of CD8 infiltration between conditions. Data were analyzed using either two-way ANOVA (B) or one-way ANOVA (C), followed by the Tukey's multiple comparisons post-hoc test. ns: not significant; * p<0.05; ** p<0.01; *** p<0.001. **NM21-1601 is a surrogate for NM21-1480 and only has differences in its serum albumin binding domain to enable binding to mouse serum albumin.**

Conclusions and potential benefits

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 activities, unlike ADCs, which can release cytolytic agents into circulation causing dose-limiting toxicities
Minimal impact of soluble MSLN	• High avidity, low affinity binding to MSLN render biMSLN.CD3.hSA 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