

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

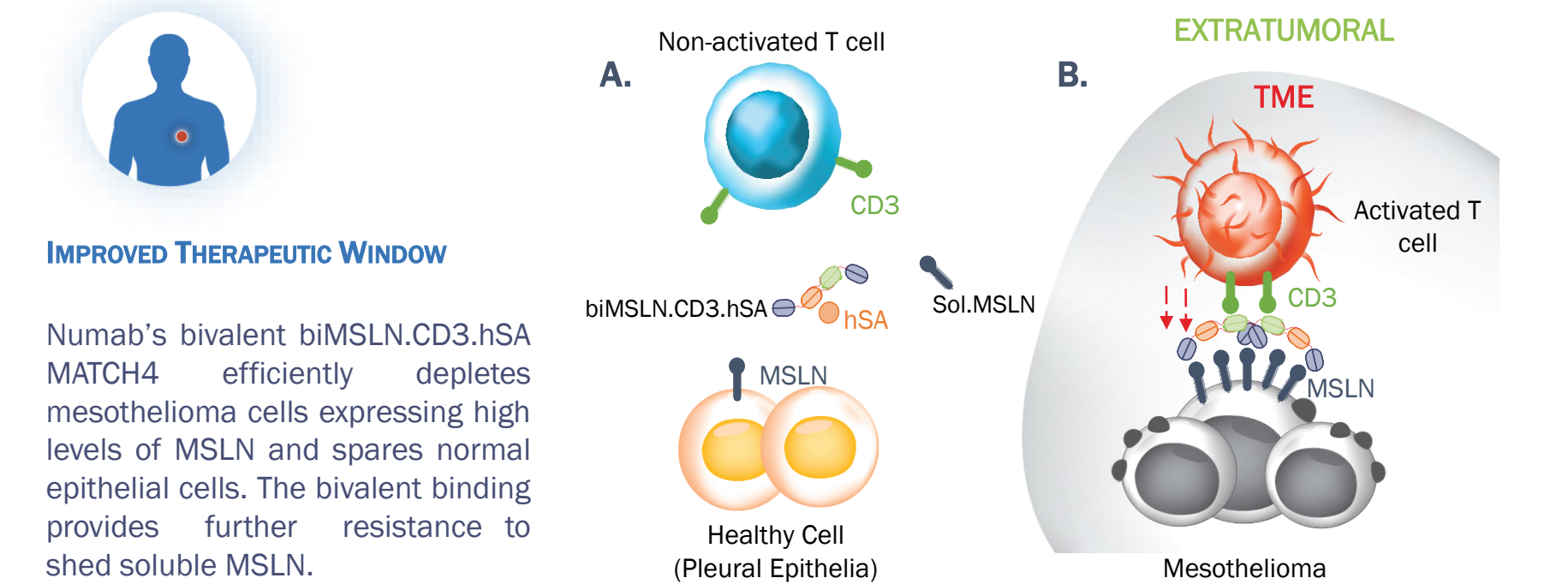
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**Background:** The effective treatment of solid tumors remains an unmet medical need. Several concepts exist to treat malignancies, including antibody-drug or -immunotoxin conjugates, immune checkpoint inhibition, CAR- T cells, as well as bispecific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

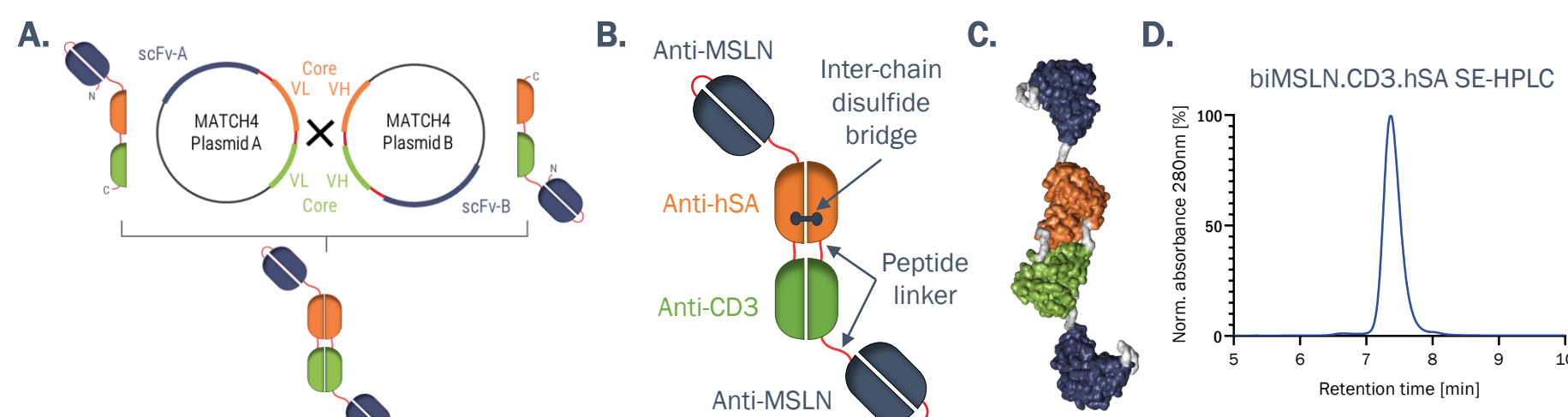


**Figure 1. A.** BiMSLN.CD3.hSA does not deplete healthy cells due to its low affinity binding domains and does not bind to shed soluble MSLN in circulation. **B.** Avidity-based binding of high MSLN expressing cells like mesothelioma, concomitant with CD3 engagement on T cells, causes the depletion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

| Tumor Type                    | No. of patients with MSLN-positive disease (%)                                       |
|-------------------------------|--------------------------------------------------------------------------------------|
| Mesothelioma                  | 290 of 352 (82)<br>248 of 261 (95)<br>0 of 23 (0)                                    |
| Pancreatic adenocarcinoma     | 303 of 357 (85)                                                                      |
| Epithelial ovarian cancer     | 346 of 494 (70)<br>248 of 332 (75)<br>36 of 52 (69)<br>2 of 19 (11)<br>11 of 21 (52) |
| NSCLC                         | 1,157 of 2,036 (57)<br>1,082 of 1,686 (64)<br>40 of 188 (21)                         |
| Biliary cancer, extrahepatic  | 93 of 98 (95)                                                                        |
| Triple negative breast cancer | 33 of 50 (66)                                                                        |

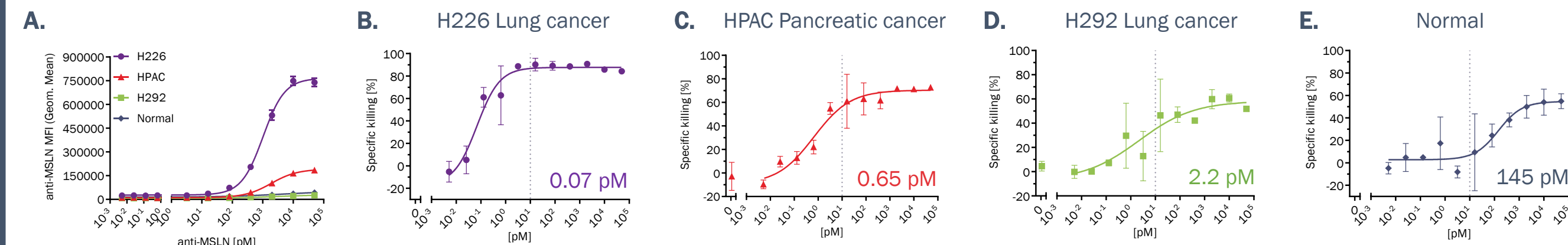
**Table 1. Mesothelin is expressed on many types of malignancies.** Frequency of patients with MSLN-positive malignancies, adapted from Hassan et al. J.Clin. Onc. 2016. MSLN expression is associated with a poor prognosis for several of these cancers, including pancreatic adenocarcinoma, biliary cancer, and breast cancer.

## MATCH4™: combinations for optimized potency



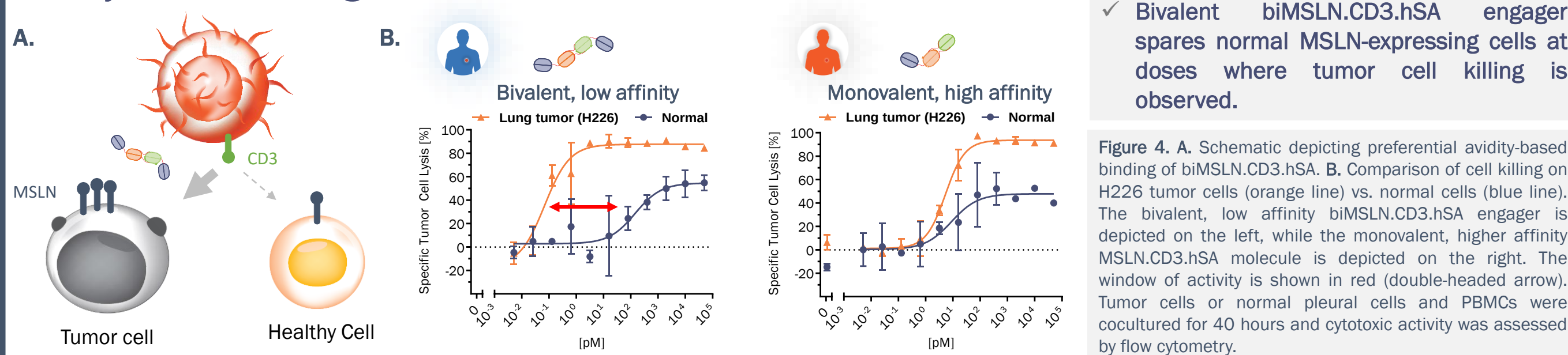
**Figure 2. A.** MATCH4™ (Multispecific Antibody-based Therapeutics by Cognate Heterodimerization) molecules are modular and can be combined in numerous ways and formats. The advantages include: 1. Convenient permutation of binding domains by simple plasmid exchange, 2. Multispecific format without the 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

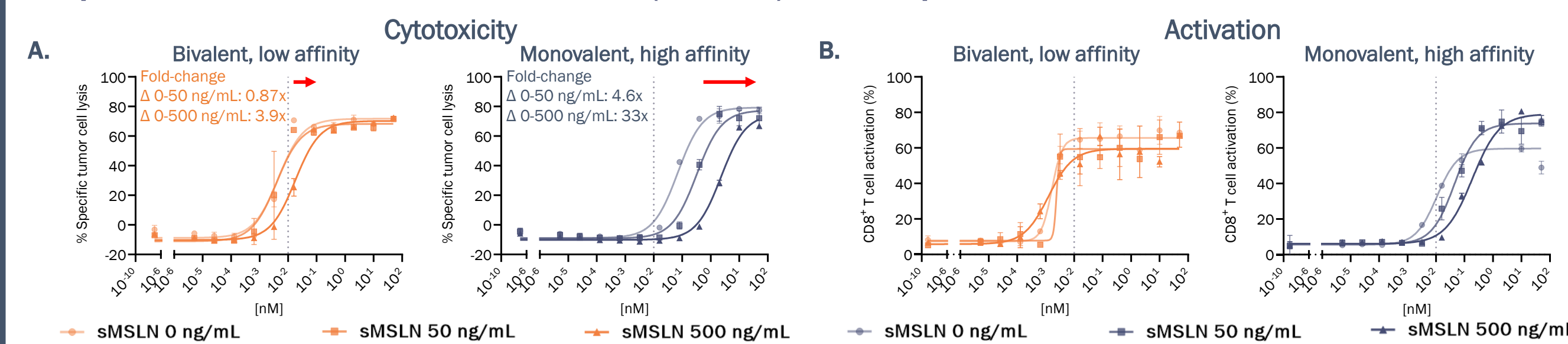


**Figure 3. A.** Surface binding of anti-MSLN antibody to various cell types with different MSLN expression levels. **B.-E.** Tumor cell killing and concomitant CD8 T cell activation in the presence of biMSLN.CD3.hSA engager. H226 (B), HPAC (C), H292 (D), or normal pleural cells (E) were cocultured together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

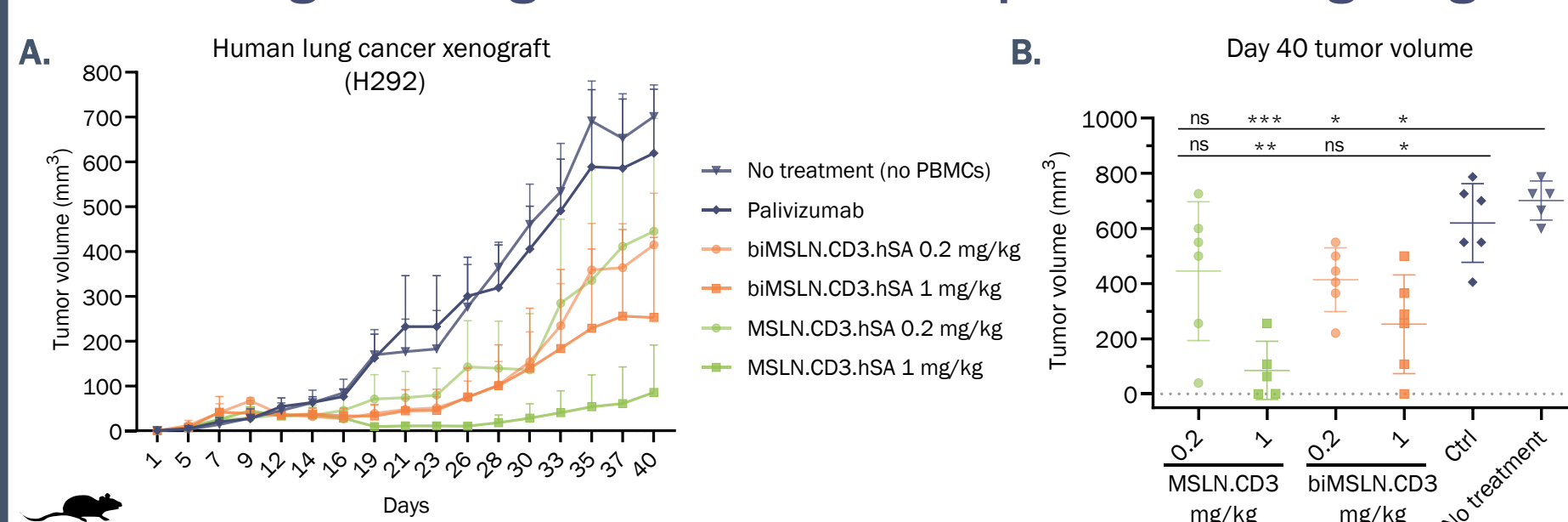
## Larger therapeutic window for bivalent biMSLN.CD3.hSA T cell engager due to preferential avidity-based binding to tumor cells



## Bivalent biMSLN.CD3.hSA 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



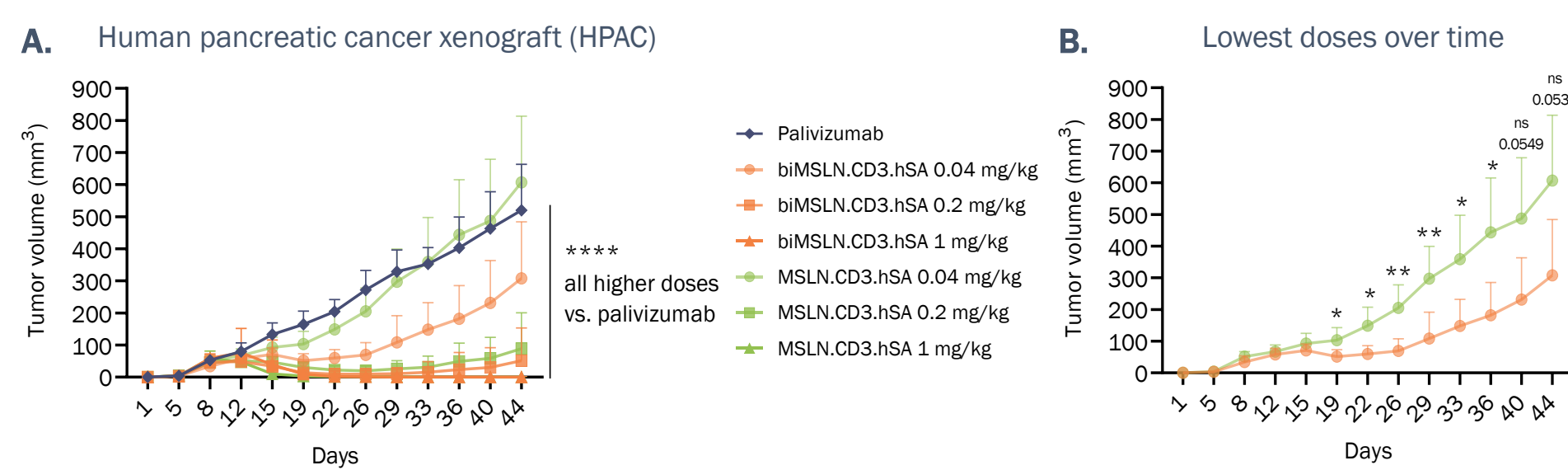
## Human lung cancer growth is inhibited upon MSLN targeting



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

**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

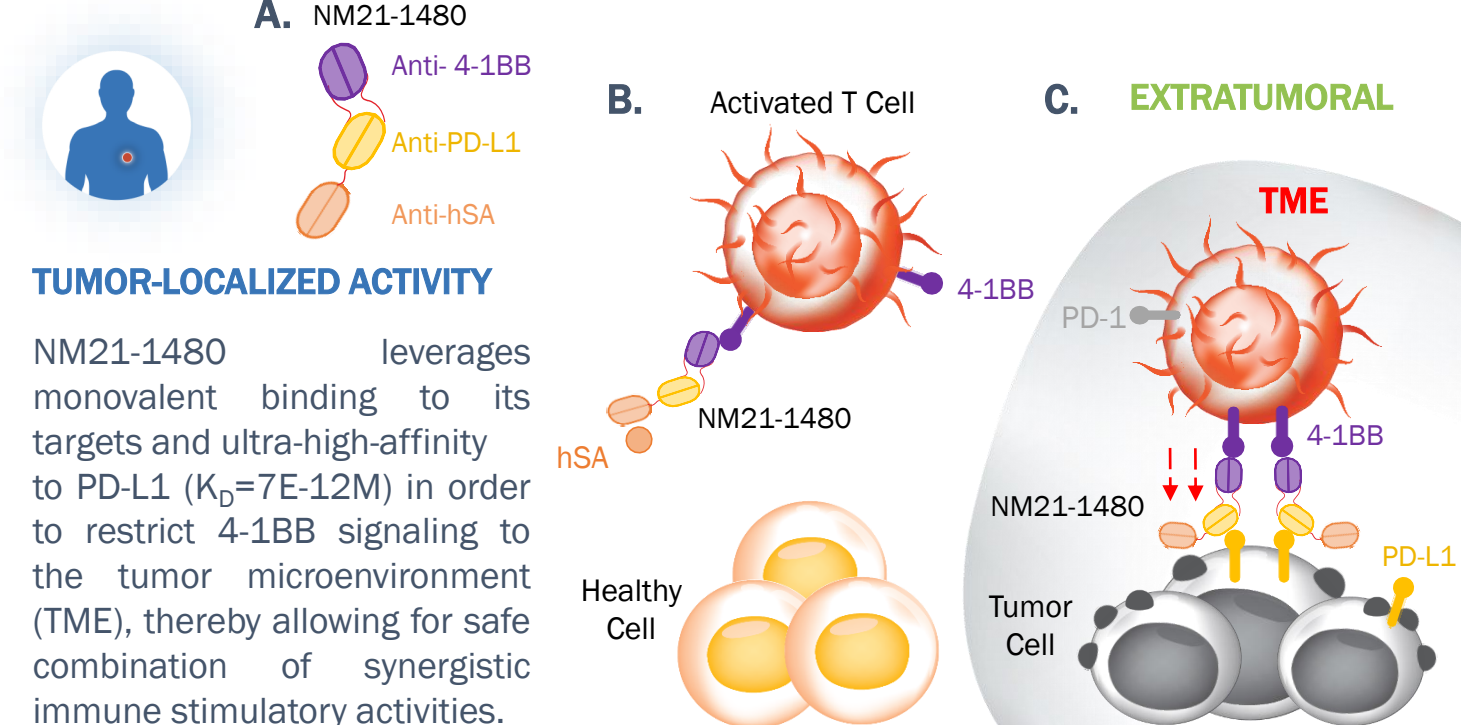
## Human pancreatic cancer growth is inhibited by biMSLN.CD3.hSA



**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

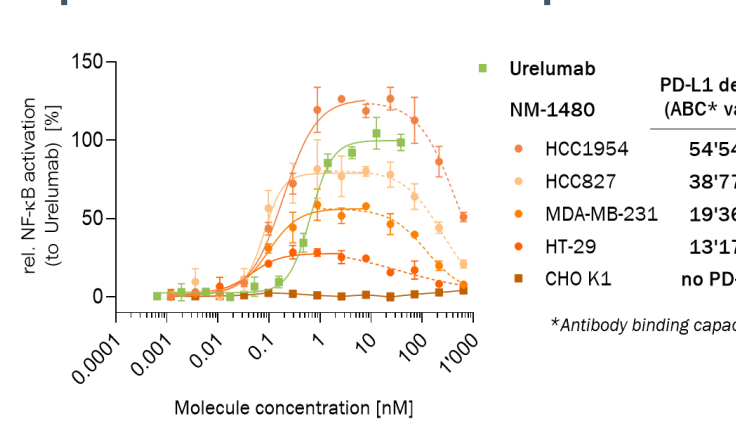
## Targeting MSLN and CD3 synergizes with Numab's next generation checkpoint modulator NM21-1480, resulting in tumor eradication

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



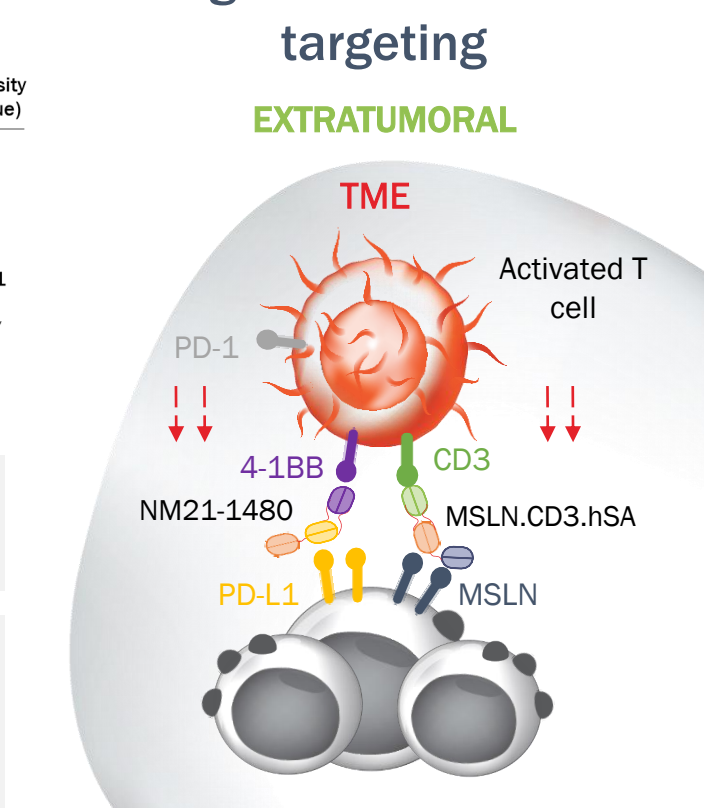
**Figure 8. A.** NM21-1480 is a trispecific scMATCH3 molecule that binds to 4-1BB, PD-L1, and hSA. **B.** NM21-1480 cannot intrinsically trigger 4-1BB clustering and signaling upon binding to 4-1BB alone. **C.** Clustering of 4-1BB occurs following simultaneous binding of NM21-1480 to 4-1BB+ and PD-L1+ 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



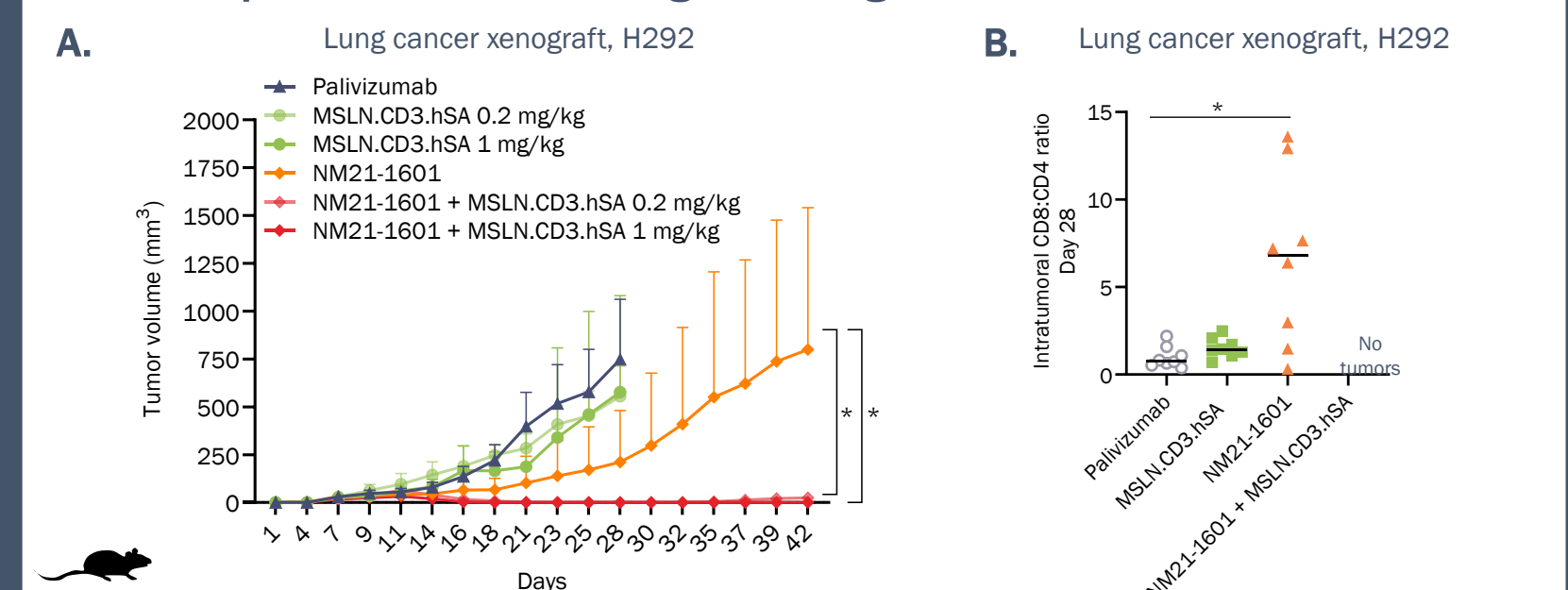
**Figure 9.** NM21-1480 concentration-dependent activation of 4-1BB-driven NF-κB signaling in a Jurkat reporter cell line. The assay is done in the presence of different cancer cell lines stimulated with IFNγ and expressing PD-L1 at different densities. PD-L1 density on co-cultured cancer cells was quantified 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



**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 of 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
- ✓ Extended half-life**
  - Avoids internalization and degradation by macrophages
  - Half-life comparable to conventional IgG due to serum albumin binding domain