NUMAB Drug Innovators

SITC 2021 Poster # 844

A trispecific ROR1xCD3xHSA T cell engager mediates in vitro tumor cell killing and in vivo tumor eradication

Bithi Chatterjee, Daniel Snell, Christian Hess, Matthias Brock, Fabio Spiga, Maria Johansson, Alexandre Simonin, Julia Tietz, Tea Gunde, Stefan Warmuth, Christopher Weinert, Nicole Bassler, Niels Kirk, Nina Schumacher, Dana Mahler, Yasemin Yaman, Bettina Bommer, Giorgio Gambino, Noreen Giezendanner, Benjamin Kuettner, Naomi Flueckiger, Robin Heiz, Sandro Wagen, Dania Diem, Julia Zeberer, Belinda Wickihalder, David Urech

Numab Therapeutics AG, Einsiedlerstrasse 34, 8820 Wädenswil, Switzerland

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Background: Receptor tyrosine kinase-like orphan receptor 1 (ROR1) is expressed on a variety of difficult to treat solid and hematological malignancies. Several therapeutic molecules targeting ROR1 are currently in clinical studies, including antibody-drug conjugates (ADCs), chimeric antigen receptor engineered T cells (CAR-T), as well as a bispecific T cell engager. In contrast to ADCs, T cell engagers have the capacity to induce tumor cell depletion irrespective of tumor cell mitotic activity. For the therapy of ROR1 expressing tumors, we engineered a T cell engager with half-life extension to support convenient dosing regimens: scMATCH™3-ROR1xCD3xHSA (NM32-2668).

Tumor Type	Frequency of patients with ROR1-positive disease (%)	Correlation with survival
Chronic Lymphocytic Leukemia (CLL)	90-95%	Poor OS and TFS
Mantle cell lymphoma (MCL)	~13-90%	-
Triple negative breast cancer	57%	Poor OS, MFS, DFS
Lung adenocarcinoma	40-65%	Poor OS
Ovarian cancer	52-57%	Poor OS and DFS

Table 1. Frequency of patients with ROR-positive disease, and correlation of this with patient survival. OS: Overall survival; TFS: Therapy free survival; MFS: Metastasis free survival; DFS: Disease free survival: adapted in part from Menck et al. 2021

NM32-2668 mediates T cell-dependent killing of ROR1 positive tumor cells

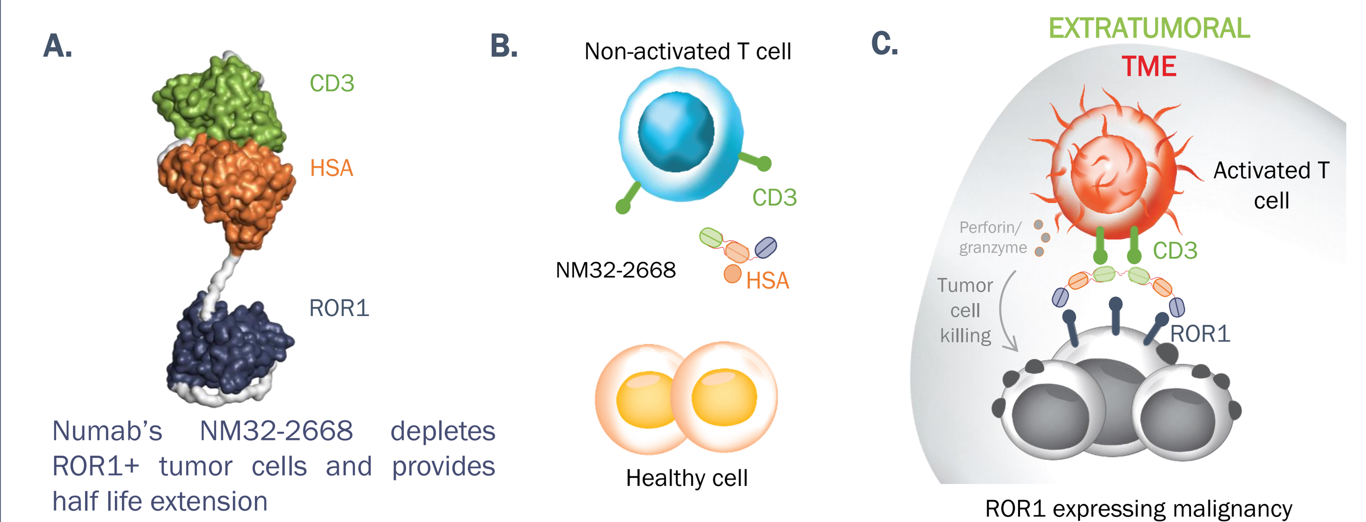


Figure 1. A. Structural model of NM32-2668 (prepared in BIOVIA Discovery Studio software). B. NM32-2668 does not activate T cells in the absence of target. C. In the presence of target, CD3 T cells are engaged and activated to kill ROR1+ cells.

Key properties of NM32-2668: ROR1xCD3xHSA

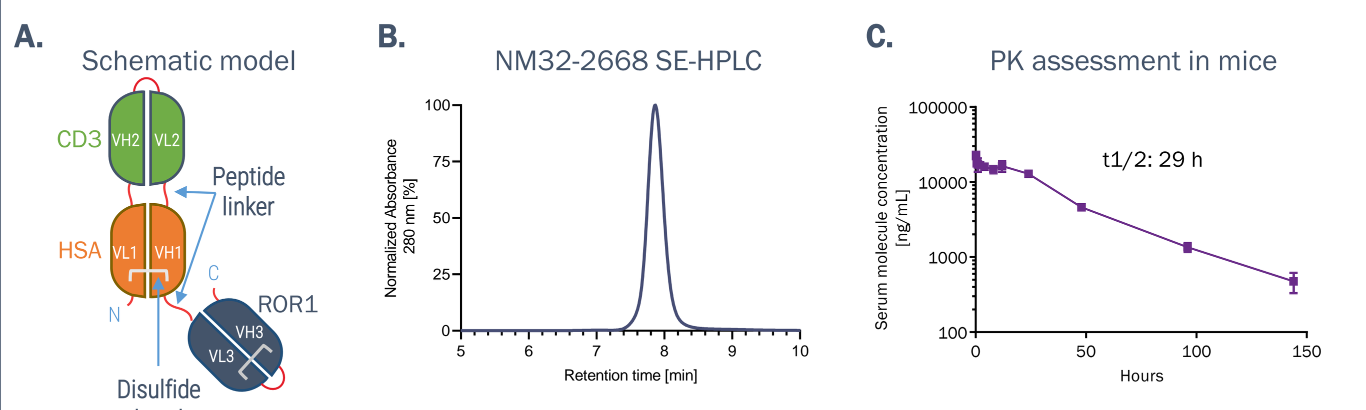
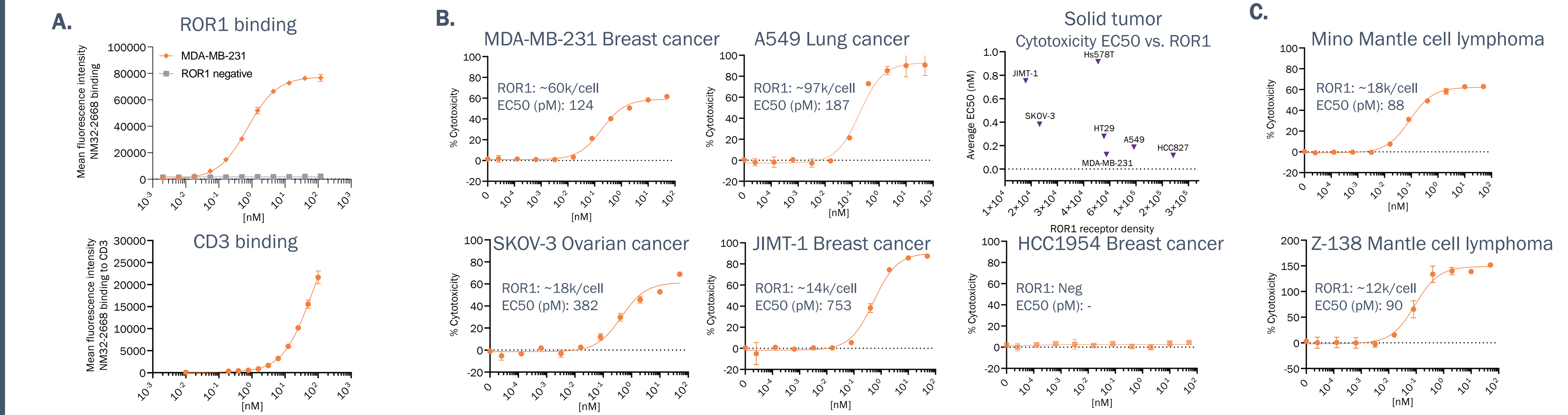


Figure 2. ScMATCH™3 (Multispecific Antibody-based Therapeutics by Cognate Heterodimerization) molecule advantages include: 1. Convenient permutation of binding domains, 2. Multispecific format on a single peptide chain without the risk of light chain mispairing, and 3. No Fc domain requirement. A. Schematic representation of an scMATCH™3 molecule. B. Representative SE-HPLC chromatogram of NM32-2668. C. Half-life assessment of TAAxCD3xHSA MATCH™ molecule in non-tumor bearing CD1 mice. The anti-human serum albumin (HSA) domain is cross reactive to mouse and cynomolgus serum albumin.

NM32-2668 induces specific T cell-mediated lysis of ROR1+ solid and hematological cancer cell lines



✓ Treatment with NM32-2668 results in ROR1-dependent cytotoxic potency on solid tumor and hematological tumor cell lines

Figure 3. A. Binding of NM32-2668 to a human tumor cell line expressing ROR1 (MDA-MB-231, top) and to human CD3+ T cells in PBMCs (bottom). A plateau was not reached for CD3 binding due to the lower affinity of this domain to CD3. B. T cell-mediated depletion of solid tumor cell lines and C. hematological tumor cell lines. Tumor cell lines were cocultured together with T cells for 40 h, and cytotoxicity was assessed using lactate dehydrogenase release relative to controls. The average number of ROR1 receptors on the cell surface, as well as average EC50 values for cytotoxicity are shown in the graph insets.

NM32-2668 time-dependent control of ROR1+ tumor cell growth via cytotoxicity

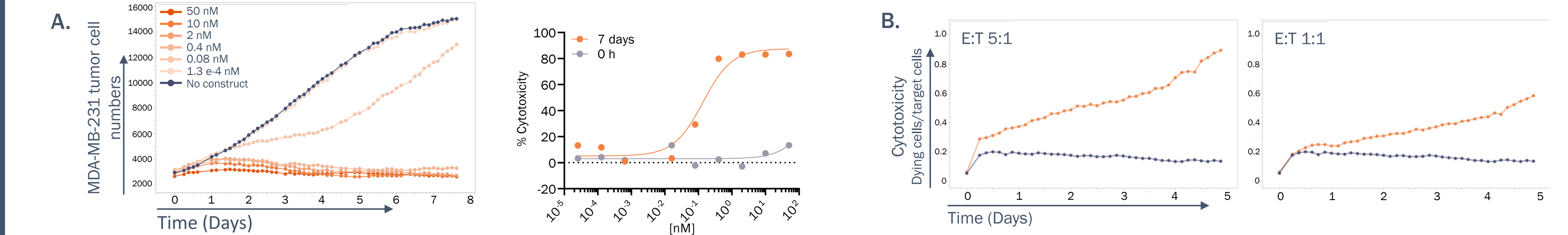


Figure 4. A. (left) MDA-MB-231 cells were cocultured with T cells for up to 7.5 days at an E:T ratio of 5:1 in the presence of increasing amounts of NM32-2668 (darker orange indicates higher concentration), and imaged at regular intervals using the IncuCyte, a real time live cell imaging platform. The y-axis depicts the numbers of target cells. No construct (blue) demonstrated tumor cell growth in the absence of treatment. (right) The extent of specific killing relative to the no construct control from this data is depicted. B. NM32-2668 mediates sustained specific lysis using multiple E:T ratios as observed on the IncuCyte. MDA-MB-231 cells were used as targets. The y-axis plots the number of dying cells divided by the number of target cells.

NM32-2668 mediates ROR1-dependent CD8 T cell activation and proliferation

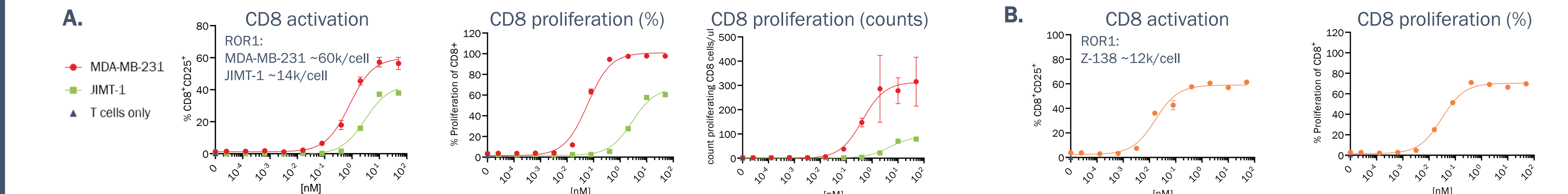
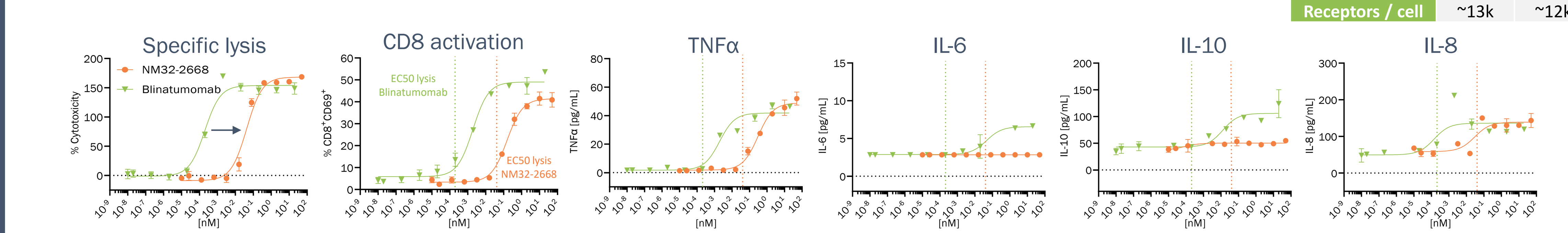


Figure 5. A. CD8 activation and proliferation at day 6 mediated by NM32-2668 using MDA-MB-231 or JIMT-1 as solid tumor target cells. B. CD8 activation and proliferation at day 3 mediated by Z-138 as hematological tumor target cells. Targets and CellTrace violet-labeled healthy T cells were cocultured at an E:T ratio of 5:1. The frequency of proliferating cells was determined by CellTrace violet dilution relative to controls. Activated cells were identified by CD25 labeling. One representative experiment of two is depicted.

NM32-2668 mediates reduced release of IL-6 and IL-10 compared to blinatumomab

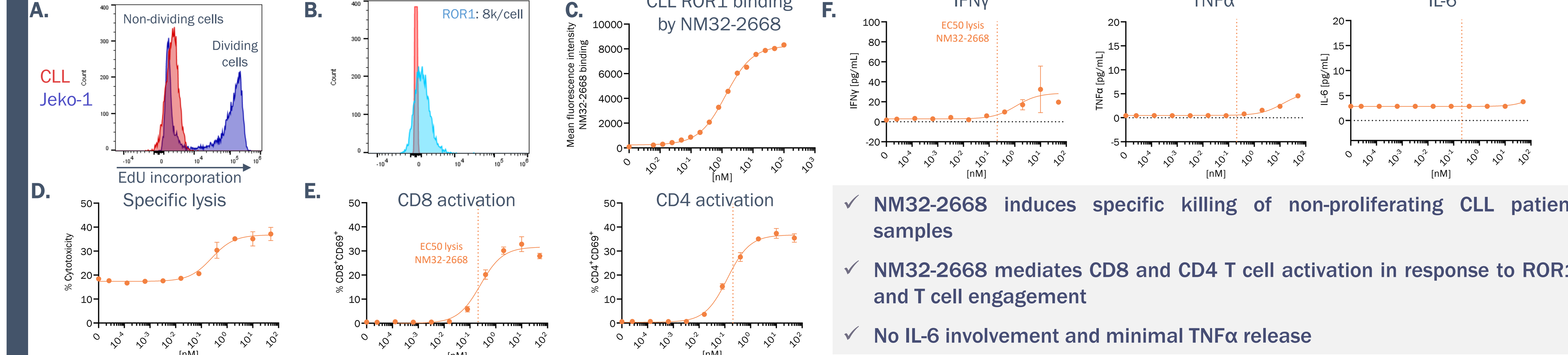
	CD19	ROR1
Receptors / cell	~13k	~12k



✓ Treatment with NM32-2668 results in similar maximal killing levels as blinatumomab, but with no IL-6 or IL-10 release

Figure 6. Human mantle cell lymphoma line Z-138 was cocultured together with human T cells for 40 h in the presence of either blinatumomab or NM32-2668. The relative surface levels of CD19 and ROR1 are similar on this tumor cell line. Cytotoxicity was assessed using lactate dehydrogenase release relative to controls; CD8 T cell activation was determined by examining the frequency of CD69 expressing CD8 cells within the coculture; cytokine release was assessed from the coculture supernatant using a cytometric bead array-based multiplexing system. One representative experiment of three is depicted.

NM32-2668 treatment results in specific lysis of non-mitotic, low ROR1-expressing primary human CLL patient samples



✓ NM32-2668 induces specific killing of non-proliferating CLL patient samples

✓ NM32-2668 mediates CD8 and CD4 T cell activation in response to ROR1 and T cell engagement

✓ No IL-6 involvement and minimal TNFα release

Figure 7. A.. CLL samples do not proliferate. EdU incorporation was assessed on an actively dividing Jeko-1 tumor cell line (blue histogram) compared to CLL patient PBMCs (red histogram). The incorporation of EdU indicates cell division. B. The ROR1 surface expression and quantification is shown (blue histogram, relative to negative control in red). C. Binding of NM32-2668 gated on CLL patient tumor cells. D. PBMCs from a CLL patient were cocultured with allogeneic healthy T cells at an E:T ratio of 5:1 for 40 h. Specific killing was determined by the proportion of Annexin V and live/dead dye positive CLL cells upon treatment. E. T cells from D. were stained for the activation marker CD69. F. Supernatants from D. were assessed for cytokine release using a cytometric bead array-based multiplexing system. Data depicted are one of four independent donors/experiments.

Treatment with NM32-2668 results in tumor eradication in a mantle cell lymphoma model

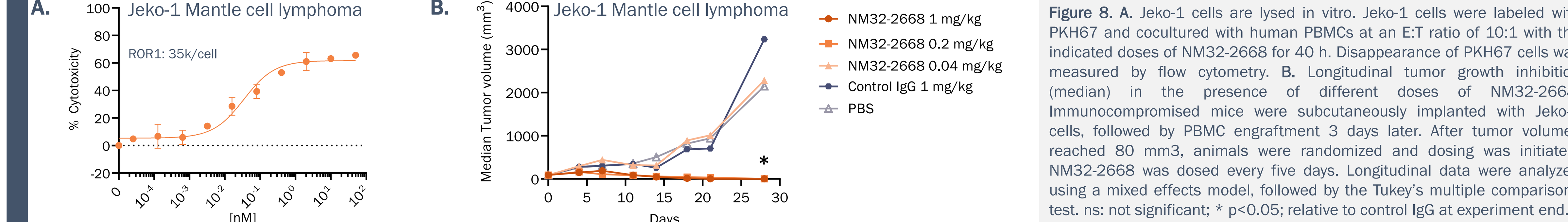


Figure 8. A. Jeko-1 cells are lysed in vitro. Jeko-1 cells were labeled with PKH67 and cocultured with human PBMCs at an E:T ratio of 10:1 with the indicated doses of NM32-2668 for 40 h. Disappearance of PKH67 cells was measured by flow cytometry. B. Longitudinal tumor growth inhibition (median) in the presence of different doses of NM32-2668. Immunocompromised mice were subcutaneously implanted with Jeko-1 cells, followed by PBMC engraftment 3 days later. After tumor volumes reached 80 mm3, animals were randomized and dosing was initiated. NM32-2668 was dosed every five days. Longitudinal data were analyzed using a mixed effects model, followed by the Tukey's multiple comparisons test. ns: not significant; * p<0.05; relative to control IgG at experiment end.

✓ NM32-2668 treatment results in tumor eradication at doses of 1 mg/kg and 0.2 mg/kg

NM21-1480, Numab's next generation checkpoint modulator, synergizes with Numab's MATCH™ CD3 T cell engagers to inhibit tumor growth

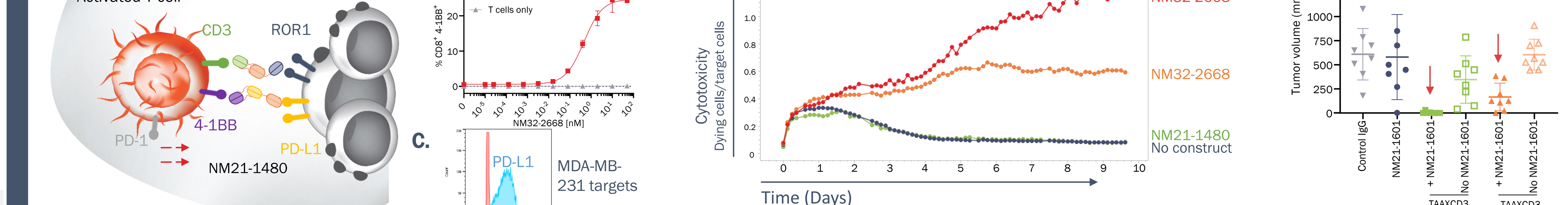


Figure 9. A. Schematic for coengagement of NM32-2668 and NM21-1480. B. Coculture of MDA-MB-231 targets and T cells with NM32-2668 results in dose dependent upregulation of 4-1BB after 40 h. C. Target cells express PD-L1. D. MDA-MB-231 cells were cocultured with T cells for up to 9.5 days at an E:T ratio of 5:1 in the presence of 0.4 nM NM32-2668 (orange), 0.4 nM NM21-1480 (green), or the combination of these two molecules (red) and imaged at regular intervals using the IncuCyte. The y-axis plots the number of dying cells divided by the number of target cells. E. The combination of NM21-1601 (a surrogate for NM21-1480 with a mouse cross-reactive serum albumin domain) with TAAxCD3 results in tumor regression compared to either alone. Data depicted are from the end of the experiment at Day 40.

Conclusions and potential benefits

Benefit	
ROR1-specific activity	• Anti-ROR1 domain ensures consistent anti-tumor potency across varying degrees of ROR1 expression in vitro, and tumor eradication in vivo
Tumor-directed T cell stimulation	• Tumor-restricted T cell activities on dividing and non-dividing cells, unlike ADCs, which require cell division and can release cytolytic agents into circulation causing DLTs
Safety	• No release of IL-6 observed when a low ROR1-expressing cell line and CLL patient PBMCs are used as targets for NM32-2668-mediated specific lysis by T cells
Fc-less	• Designed to avoid Fc-mediated adverse effects and to avoid internalization and degradation by macrophages
Extended half-life	• Half-life comparable to conventional IgG due to serum albumin binding domain to allow for convenient dosing