Targeting ROR1: Evaluating expression in cancer tissue and development of a therapeutic T cell engager

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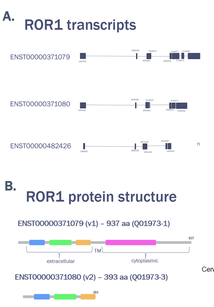
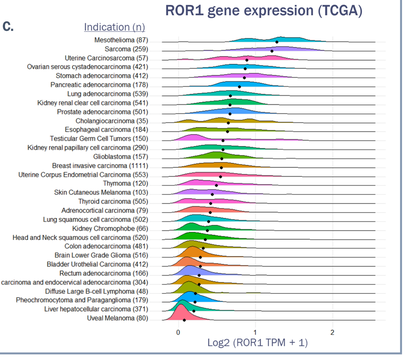
Background

Receptor tyrosine kinase-like orphan receptor 1 (ROR1) is expressed on a variety of difficult to treat solid and haematological 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 have engineered a T cell engager with half-life extension to support convenient dosing regimens: scMATCH™3-ROR1xCD3xHSA (NM32-2668).

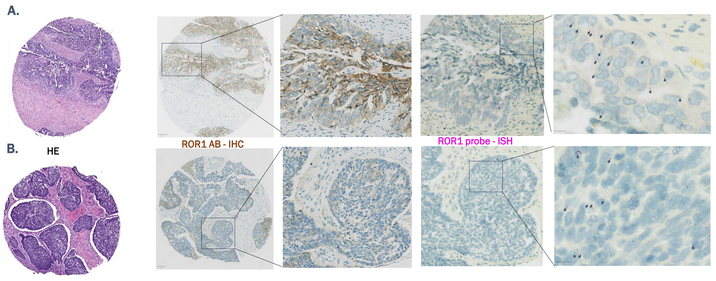
ROR1 structure and expression pattern in tumor tissue

Figure 1. **A.** Exon overview of the ROR1 transcripts according to the ensembl GRCh38.p14 annotation (numbers indicate the Exon ID) **B.** ROR1 transcripts are associated to two protein-coding phenotypes (adapted from GEPIA 2, Tang, Z. et al.[1]) **C.** TCGA RNA Seq data sets were downloaded from the GDC data portal. GDC mRNA quantification analysis pipeline measures gene level expression with STAR as raw read counts, which are augmented with transcripts per Million (TPM) transformation and aligned reads to the GRCh38 reference genome. Black dot in the density plot indicates the mean ROR1 expression with indications ranked from highest ROR1 expression to lowest.





Development of a ROR1 detection assay



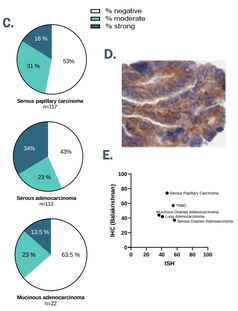
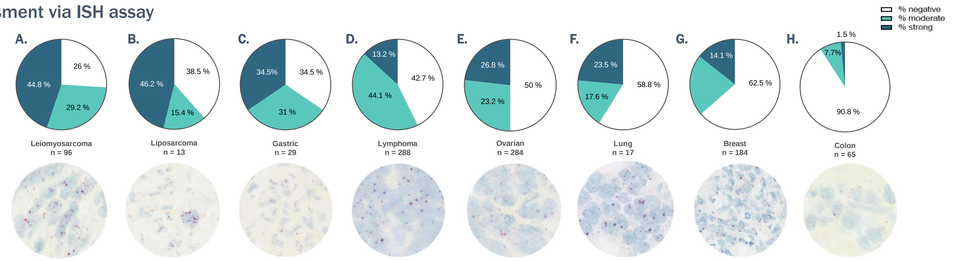


Figure 2. **ROR1 (PRO3305) IHC and ROR1 probe ISH assay comparison in ovarian cancer TMA.** Anti-ROR1 antibody (PRO3305) was used at 2µg/ml with CC1 unmasking condition on Benchmark XT and ROR1 RNAscope® ISH workflow on Leica Bond RX. **A.** Low grade serous papillary carcinoma and **B.** High grade serous papillary carcinoma show specific ROR1 cyto-membrane staining and good correlation between PRO3305 and ISH probes. **C.** Percentage of ROR1 expression in ovarian cancer subtypes assessed via ISH (negative; low and moderate % of positive cells (1-50%); high % of positive tumor cells (>50%) **D.** ROR1 antibody (6D4) staining on serous papillary carcinoma (Balakrishnan et al.[2]) **E.** Correlation of the percentage of ROR1+ tumors between IHC stained with 6D4 and ISH assay in different cancer indications.
Assays were developed at Veracyte, France.

ROR1 prevalence assessment via ISH assay

Figure 3. **Percentage of ROR1 positive tumor cells per indication in 3 different categories:** negative; low and moderate % of positive cells (1-50%); high % of positive tumor cells (>50%) with representative TMA cores with strong ROR1 expression below.
A. Leiomyosarcoma,
B. Liposarcoma,
C. Gastric adenocarcinoma,
D. B-cell lymphoma,
E. Serous ovarian adenocarcinoma,
F. Lung adenocarcinoma,
G. Breast invasive adenocarcinoma and
H. Gastric adenocarcinoma



Key properties of NM32-2668: ROR1xCD3xHSA

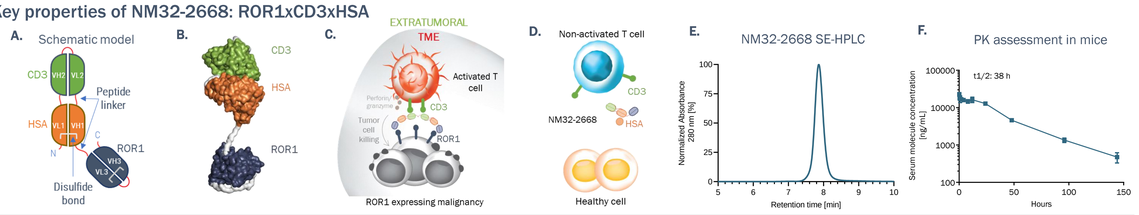
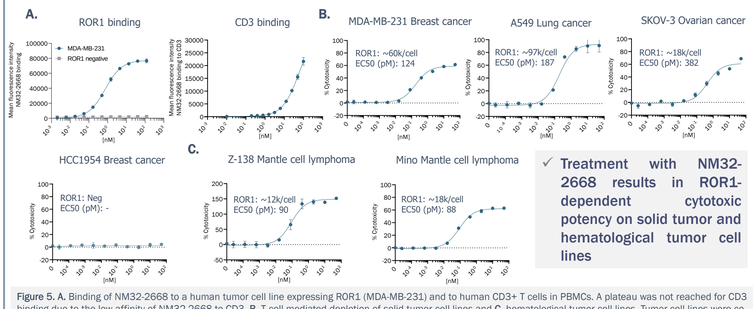


Figure 4. **Numab's NM32-2668 depletes ROR1+ tumor cells and provides half-life extension via binding to serum albumin** **A.** Schematic representation of NM32-2668 scMATCH™3 (Multispecific Antibody-based Therapeutics by Cognate Heterodimerization) molecule. **B.** Structural model of NM32-2668 (prepared in BIOVIA Discovery Studio software). **C.** In the presence of target, CD3 T cells are engaged and activated to kill ROR1+ cells. **D.** NM32-2668 does not activate T cells in the absence of **E.** Representative SE-HPLC chromatogram of NM32-2668. **F.** 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.

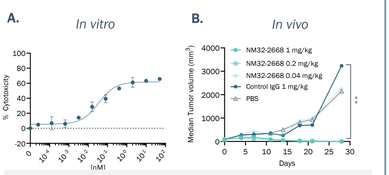
NM32-2668 induces specific T cell-mediated lysis of ROR1+ cancer cell lines



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

Figure 5. **A.** Binding of NM32-2668 to a human tumor cell line expressing ROR1 (MDA-MB-231) and to human CD3+ T cells in PBMCs. A plateau was not reached for CD3 binding due to the low affinity of NM32-2668 to CD3. **B.** T cell-mediated depletion of solid tumor cell lines and **C.** hematological tumor cell lines. Tumor cell lines were co-cultured 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.

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



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

Figure 6. **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. Loss 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 one-way ANOVA with Tukey's post hoc test. ** p<0.01; relative to control IgG at experiment end (d28).

NM32-2668 is cross-reactive with cynomolgus monkey and is well tolerated in repeat dose studies

A.

	Activity (pM)	Human T cells	Cyno T cells
CHO-ROR1 Medium expression	Cytotoxicity	20.25	12.2
	CD8 T cell activation	3.11	6.99
	CD4 T cell activation	2.66	17.5
CHO-ROR1 Low expression	Cytotoxicity	90.39	35.8
	CD8 T cell activation	65.06	26.4
	CD4 T cell activation	30.88	69.3

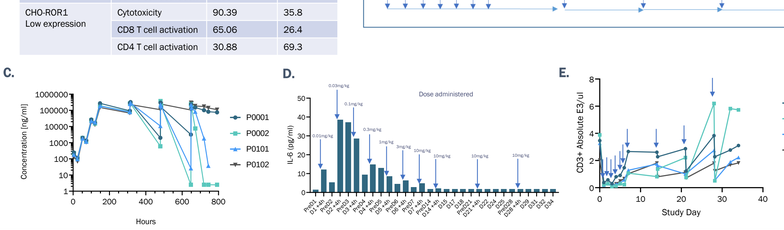


Figure 7. **NM32-2668 is cross-reactive to cynomolgus monkey and is well tolerated in repeat dose studies, with a repeat dose MTD of 10 mg/kg. A.** Cytotoxicity and T cell activation of NM32-2668 in the co-cultures of T cells and ROR1-expressing CHO cells. T cells were isolated from either human or cynomolgus monkey peripheral blood. ROR1 is 100% identical between human and cynomolgus monkey. **B.** A repeat dose study was conducted in cynomolgus monkeys using a step-dose regimen. NM32-2668 was administered intravenously via infusion for 0.5hr. Three-fold increases in dose were administered daily for 7 days from 0.01 mg/kg to 10 mg/kg. Subsequently, NM32-2668 was administered weekly at 10 mg/kg for a further 3 doses. **C.** NM32-2668 concentrations were measured from serum. Increases in serum concentration were recorded with increases in dose. Increased clearance was observed in 3 of 4 animals, associated with an anti-drug antibody response in the monkeys. **D.** IL-6 concentrations in serum were measured at pre-dose and 4 hours post-dose. Increases in IL-6 levels were observed with the initial dosing events, with peak levels at day 2 and 3. Upon maximum dose levels (10mg/kg) only low levels of IL6 release were observed reflective of an immune system adaptation to treatment as observed with other T cell engagers *in vivo*. **E.** Circulating CD3+ cells were measured from blood by flow cytometry during the study. An initial decrease of CD3+ cells is observed followed by a recovery of CD3+ cells by day 7, at maximum drug levels again illustrative of a redistribution of T cells rather than depletion.

Conclusions and potential benefits

ROR1 expression	• ROR1 is a highly selective tumor associated antigen which is upregulated across many solid tumor indications with high unmet medical need
ROR1 specific tumor killing	• Tumor-restricted T cell activity and tumor cell killing in vitro and in vivo
Safety	• Repeat dose studies in monkeys were well tolerated with a maximum tolerated dose of 10 mg/kg (maximum tested)
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

References:
1. Tang Z. et al., "GEPIA2: an enhanced web server for large-scale expression profiling and interactive analysis", Nucleic Acids Research, Volume 47, Issue W1, 02 July 2019, Pages W556–W560
2. Balakrishnan A. et al., "Analysis of ROR1 Protein Expression in Human Cancer and Normal Tissues", Clin Cancer Res. 2017 Jun 15;23(12):3061-3071.