

Lipid Nanoparticle mRNA Delivery of a Dual Target TACI-BCMA NK Engager for Potent and Scalable Multiple Myeloma Treatment

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LBA-31

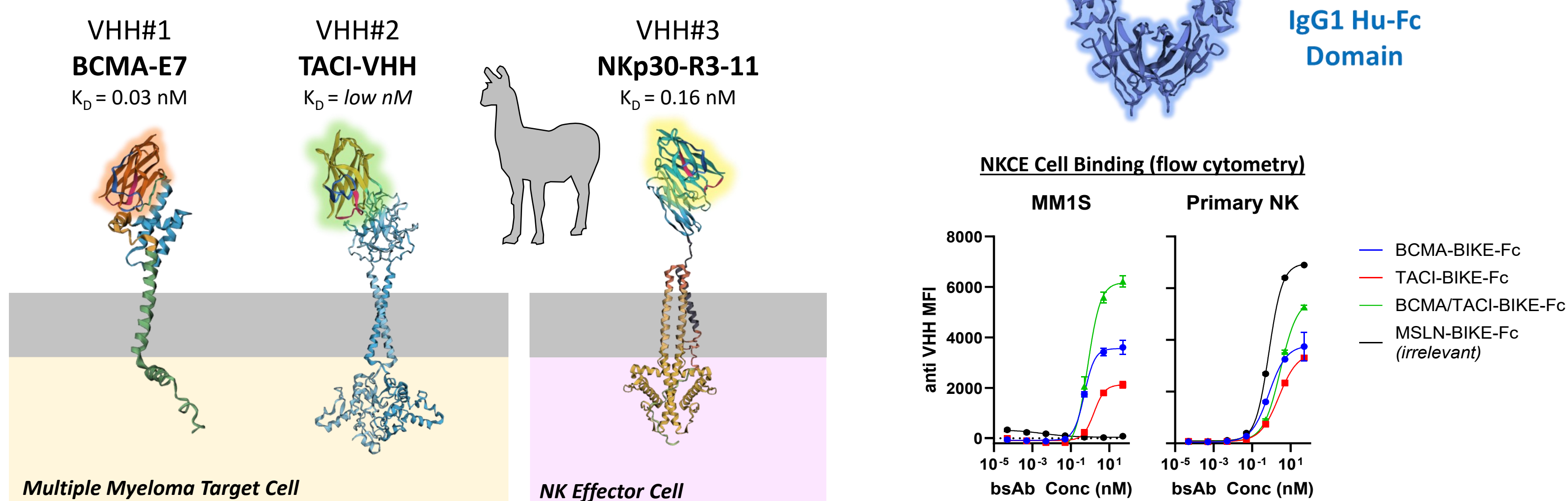
INTRODUCTION

Developing innovative therapies for multiple myeloma (MM) is both timely and crucial, given the persistent challenge of relapses in this incurable malignancy originating from plasma cells. Targeting B-cell maturation antigen (BCMA) with antibody-based therapies is a well-established strategy due to its selective and elevated expression on MM cells, but can result in mutational escape. Here we report development of multi-specific immune engagers that simultaneously target both BCMA and the related antigen, transmembrane activator and CAML interactor (TACI) using tandem variable heavy domains of camelid heavy-chain antibodies (VHHs). We have further functionalized this engager with an NKp30-activating VHH and a human IgG-Fc domain for multi-functional engagement of NK, macrophages, and other immune cells.

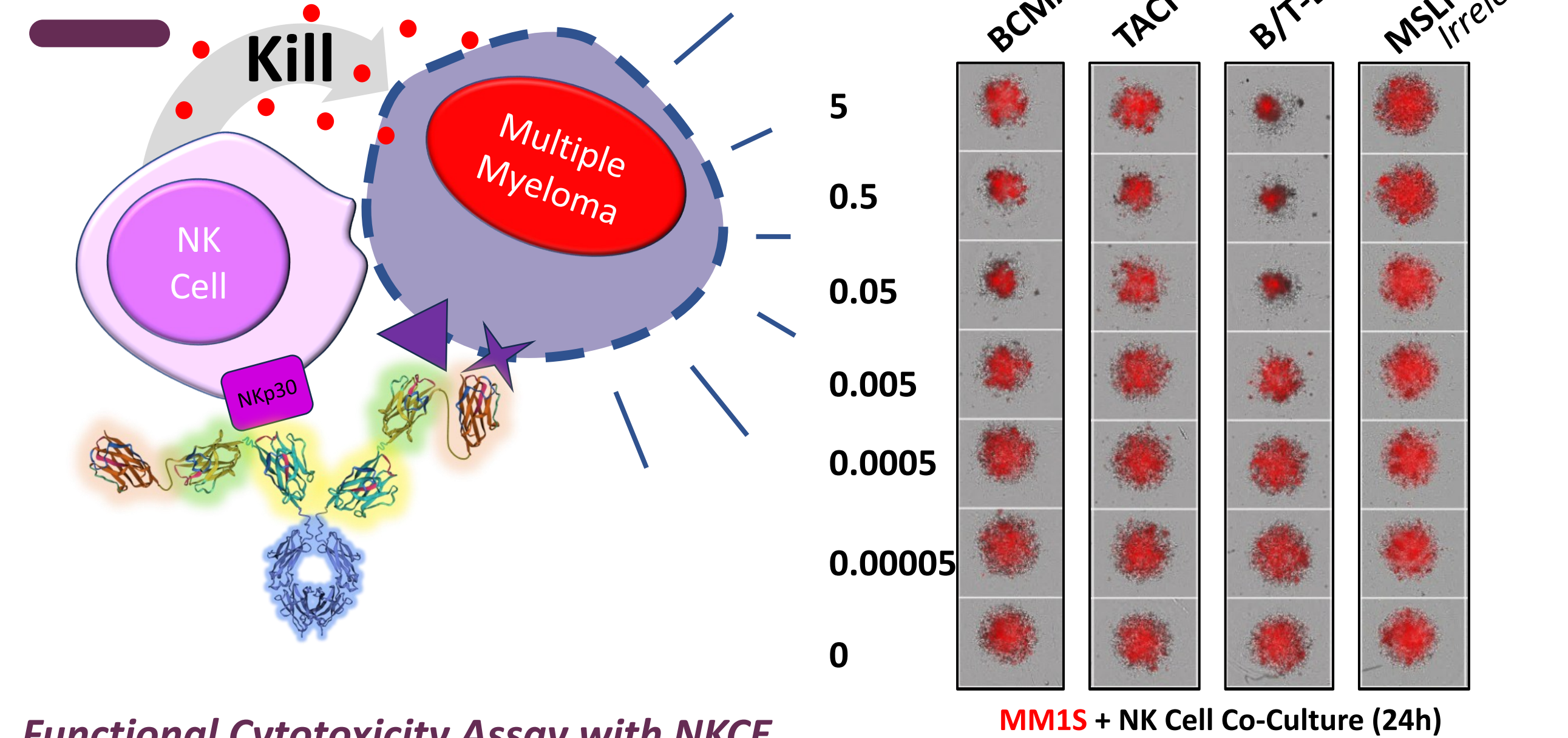
METHOD

BCMA-TACI-NKp30-hFc (B/T-BIKE-Fc) proteins and corresponding single-antigen targeting NK-engagers were synthesized and produced using NRC CHO-cell line recombinant protein production and protein A purification. Constructs were then encoded in mRNA format and encapsulated in lipid nanoparticles (LNP) of varying composition. Single- and dual-target engagers expressed as proteins or LNP-mRNA, were assessed for binding to MM and immune cells by flow cytometry. Functional engagement of NK and macrophage cytotoxicity were confirmed in vitro by fluorescence microscopy and flow-based assays, and in vivo via xenograft mouse studies for efficacy and pharmacokinetics.

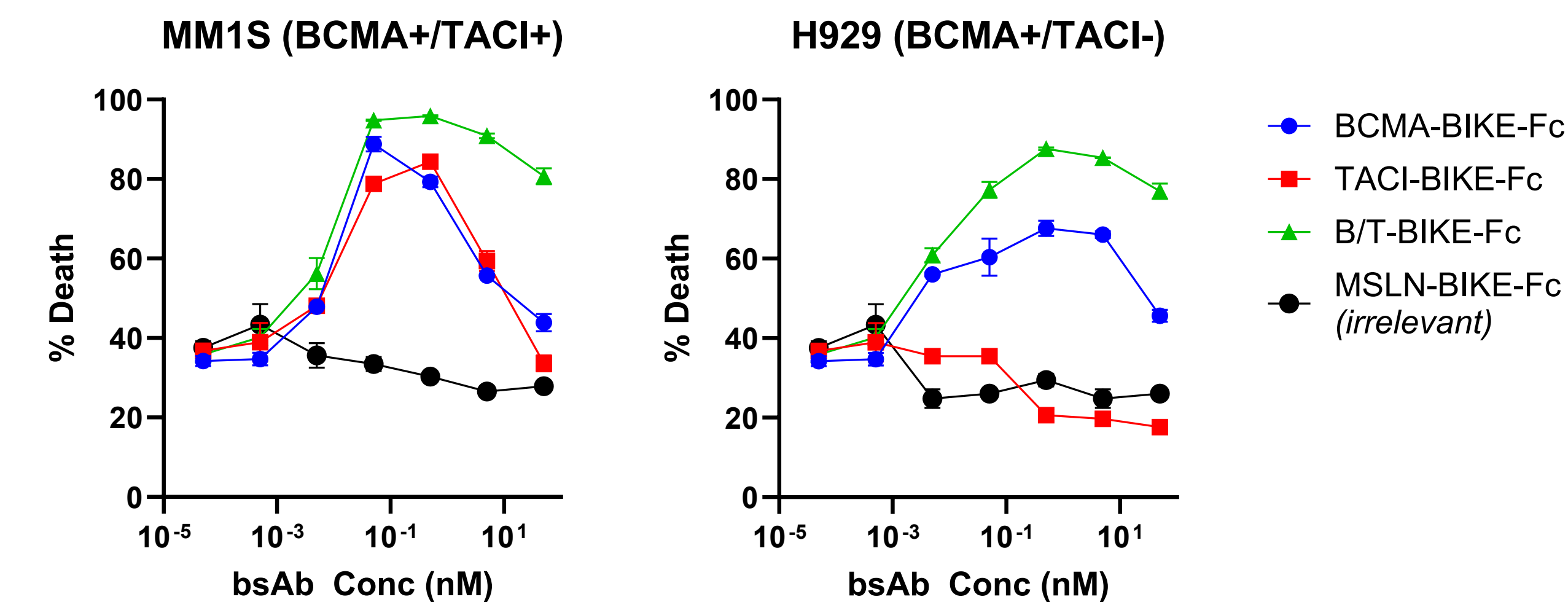
DUAL TARGET BCMA/TACI NK-Engager (B/T-BIKE-Fc)



RESULTS



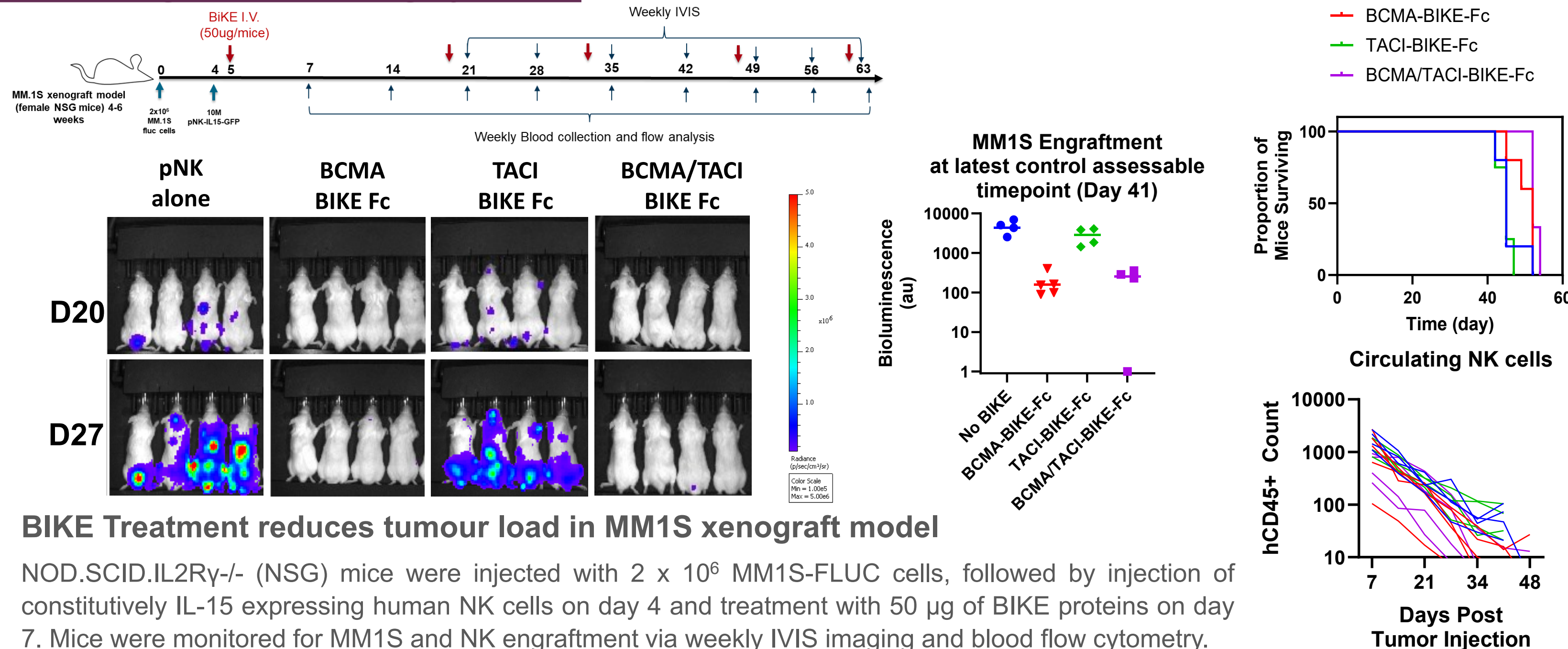
Functional Cytotoxicity Assay with NKCE



Dual BCMA and TACI Target Engagement Enhances NK-Mediated MM Killing

BCMA-TACI-NKp30-hFc proteins were produced using standard CHO-cell recombinant protein production and purified by protein A affinity chromatography. BCMA-, TACI-, or B/T-BIKE-Fc proteins were tested for in vitro cytotoxicity by co-culture of primary NK cells and target cells.

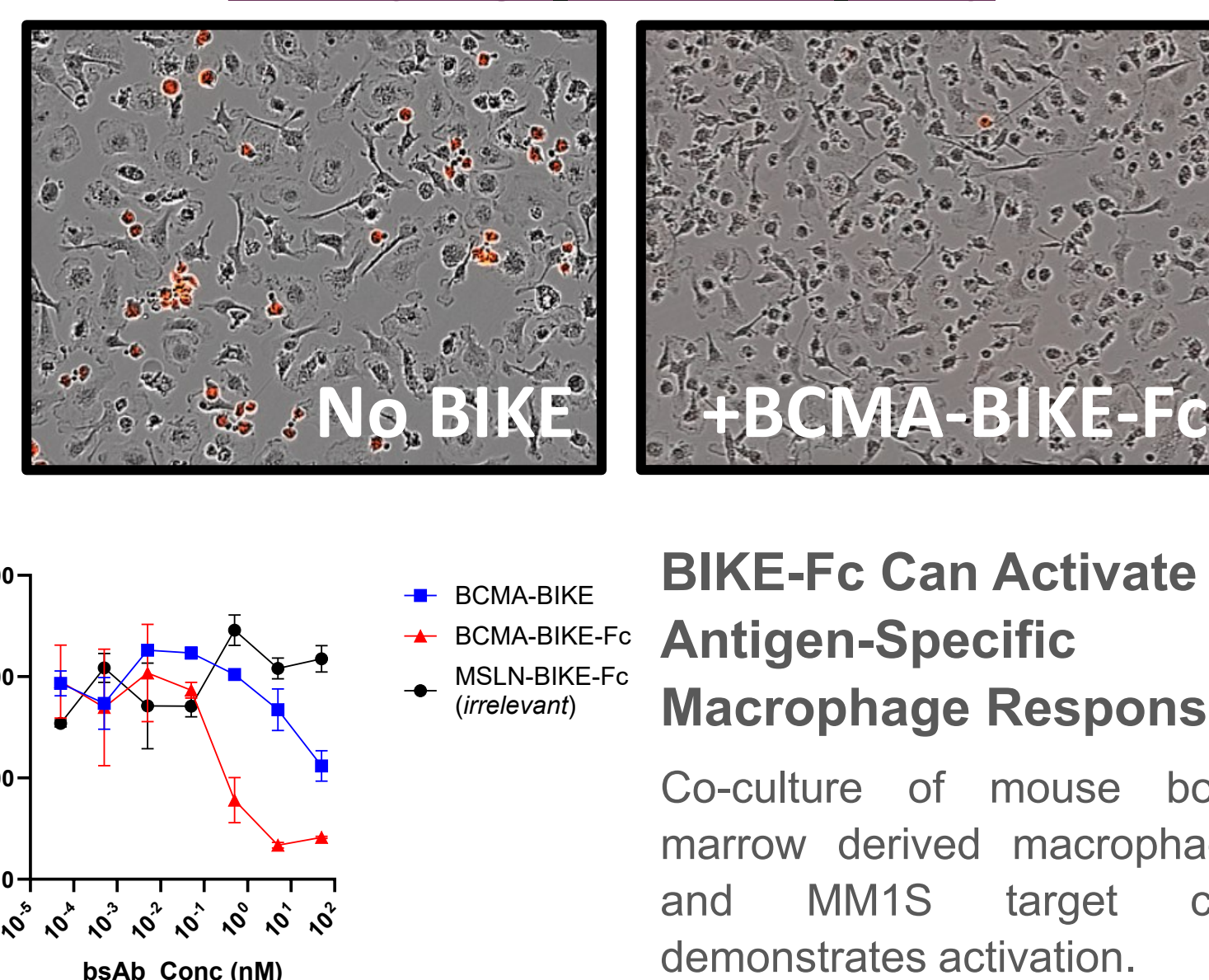
In vivo testing in MM1S Xenograft Model



BIKE Treatment reduces tumour load in MM1S xenograft model

NOD.SCID.IL2Rγ^{-/-} (NSG) mice were injected with 2×10^6 MM1S-FLUC cells, followed by injection of constitutively IL-15 expressing human NK cells on day 4 and treatment with 50 μg of BIKE proteins on day 7. Mice were monitored for MM1S and NK engraftment via weekly IVIS imaging and blood flow cytometry.

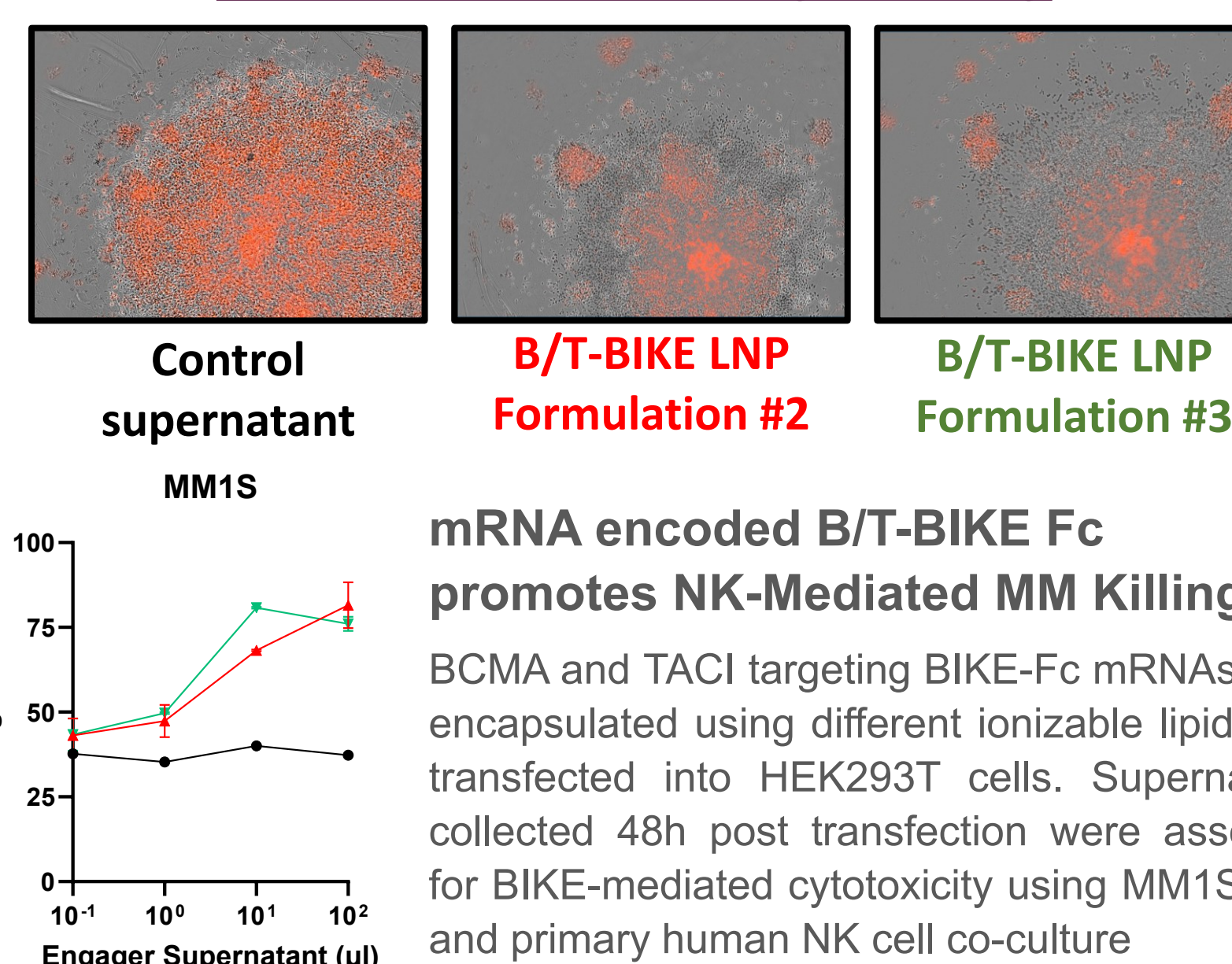
Macrophage Functional Assay



BIKE-Fc Can Activate Antigen-Specific Macrophage Responses

Co-culture of mouse bone-marrow derived macrophages and MM1S target cells demonstrates activation.

LNP-mRNA Functional Cytotoxicity

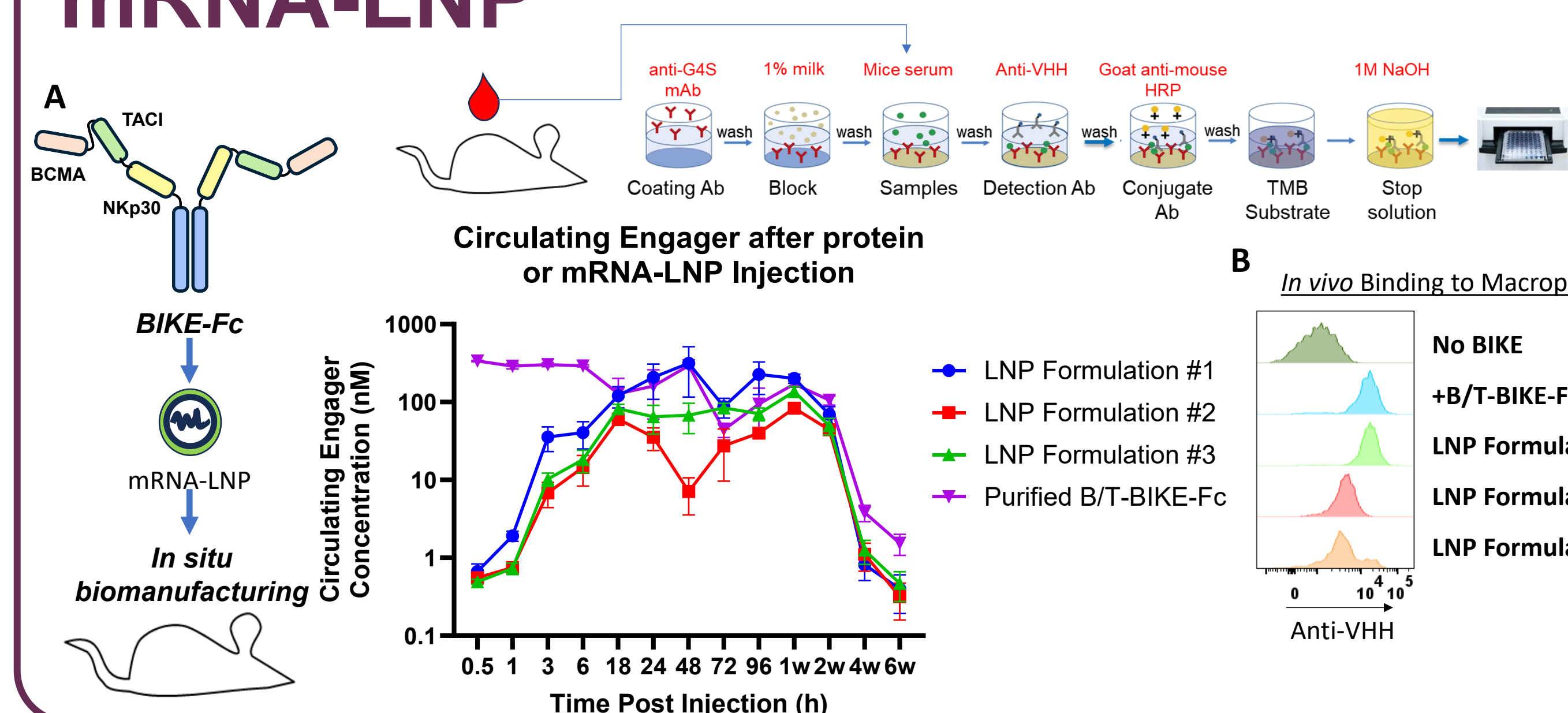


mRNA encoded B/T-BIKE Fc promotes NK-Mediated MM Killing

BCMA and TACI targeting BIKE-Fc mRNAs were encapsulated using different ionizable lipids and transfected into HEK293T cells. Supernatants collected 48h post transfection were assessed for BIKE-mediated cytotoxicity using MM1S cells and primary human NK cell co-culture.

mRNA-LNP

In vivo Pharmacokinetics and Cell Binding



mRNA encoding B/T-BIKE-Fc can be effectively delivered via intravenous LNP injection

B/T-BIKE-Fc mRNA was synthesized via in vitro transcription and encapsulated in LNP formulations with varying ionizable lipids. (A) BL6 mice were injected intravenously with a single dose of B/T-BIKE-Fc LNP-mRNA (0.3 mg/kg mRNA dose) or protein (3 mg/kg dose). Results show a progressive increase in circulating protein over 24 hours, with a similar long half-life for both LNP-mRNA and protein bolus injection. (B) Engager binding to circulating mouse macrophages was also confirmed via flow cytometry.

SUMMARY

- BIKE engagers show strong binding to both MM and NK cells
- Dual BCMA/TACI-engagement resulted in higher density staining and higher NK mediated cytotoxicity.
- BIKE engagers activate cytotoxicity with either BCMA alone or BCMA and TACI expression
- Functional Fc domain engages both NK and macrophages, and likely other immune processes
- Encoding in mRNA-LNP format resulted in strong expression of engagers and favourable in vivo pharmacokinetics.

ACKNOWLEDGEMENT

Current and previous funding for this work was provided by the National Research Council Canada (NRC), Myeloma Canada, Ontario Institute of Cancer Research, and Myellama Therapeutics.

CONTACT INFORMATION

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CONCLUSION

This work provides strong proof of concept that the small modular nature of VHH domains makes them highly conducive to engineering multi-antigen multi-functional immune engager therapies targeting multiple cancer associated antigens. Furthermore, such multi-VHH Fc engagers can be effectively encoded in mRNA format for more rapid preclinical development and therapeutic scalability.