



A Tumor-Agnostic TCR-mimic CAR T cell Specific for NDC80 Targets Multiple Hematological Malignancies

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Abstract

Target identification for CAR T cell therapies remains challenging due to the limited repertoire of tumor-specific surface proteins. Intracellular proteins presented in the context of cell surface HLA provide a wide pool of potential antigens targetable through TCR mimic antibodies. Mass spectrometry (MS) of HLA ligands from eight hematological and non-hematological cancer cell lines identified a shared, non-immunogenic, HLA-A*02 restricted ligand (ALNEQIARL) derived from the kinetochore-associated *NDC80* gene. CAR T cells directed against the ALNEQIARL:HLA-A*02 complex demonstrated high sensitivity and specificity for recognition and killing of multiple cancer types, especially those of hematological origin and were efficacious in mouse models against a human leukemia and a solid tumor. In contrast, no toxicities towards resting or activated healthy leukocytes as well as hematopoietic stem cells were observed. This demonstrates how MS can inform the design of broadly reactive therapeutic TCR mimic CAR T cell therapies that can target multiple cancer types currently not druggable by small molecules, conventional CAR T cells, T cells or antibodies.

Key Points

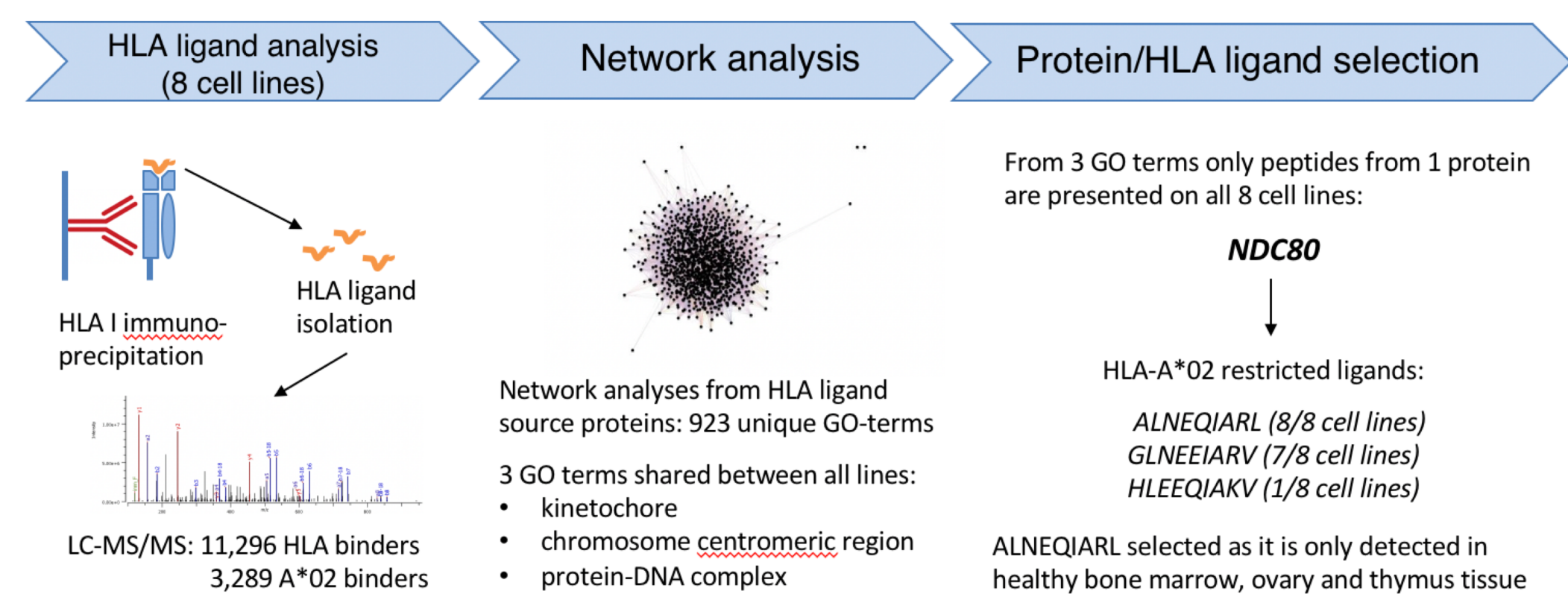
- HLA-A*02:ALNEQIARL derived from NDC80 is a broadly presented cancer immunotherapy target with high abundance in hematological malignancies
- CAR T Cells designed for this target demonstrate high sensitivity and specificity with no toxicity towards healthy leukocytes or HSCs

Objective

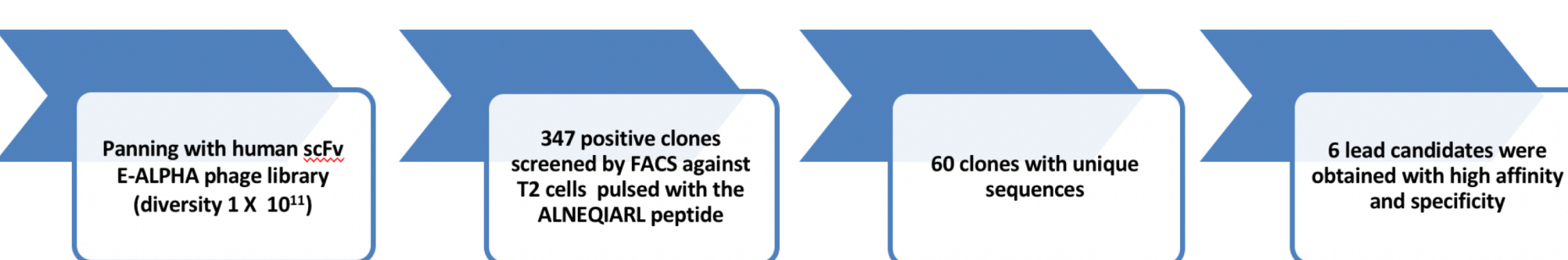
We hypothesized that mass spectrometry (MS)-based analysis of the presented HLA ligands on multiple cancer cell lines can be utilized to identify a shared, tumor-associated HLA ligand, which could then be targeted by TCR-mimic CAR-T cells specific for this particular HLA ligand

Materials & Methods

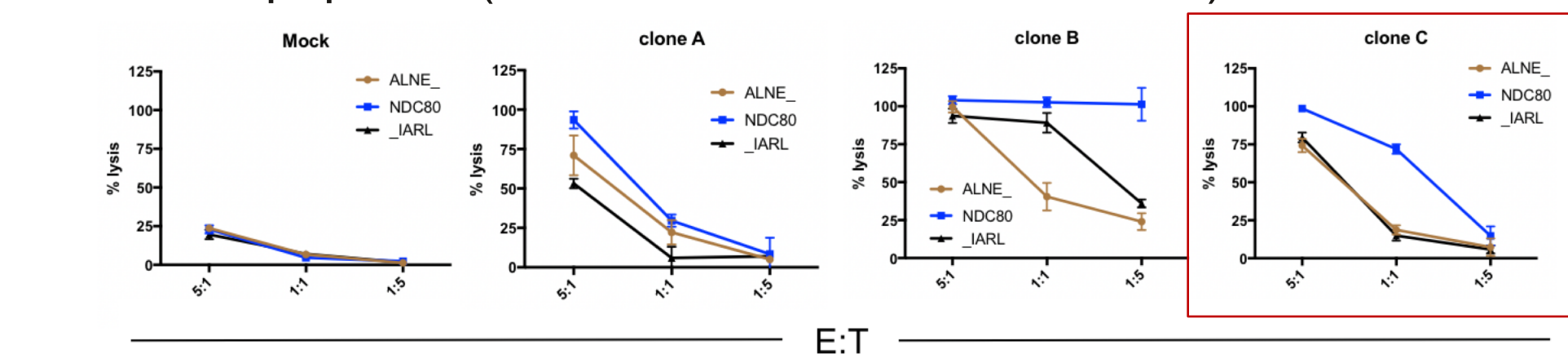
I. Target Identification: HLA ligand isolation, analysis via MS and selection



II. TCR-mimic CAR-T cell development: Phage Library Screen

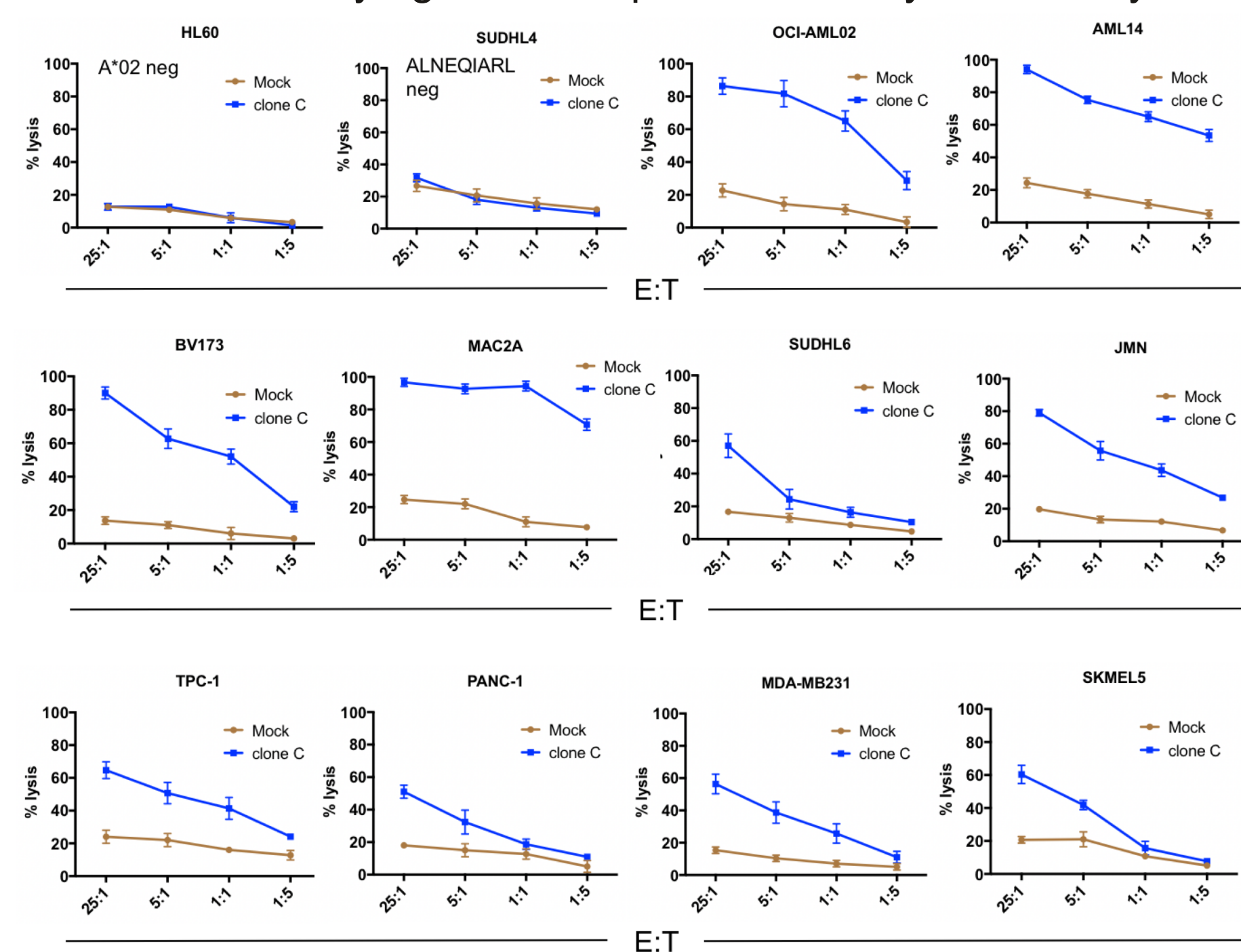


III. Selection of the final clone (representative data of 3 clones): T2 cells pulsed with target peptide (NDC80=ALNEQIARL) and control peptides (ALNEKLVNL and MLANDIARL)

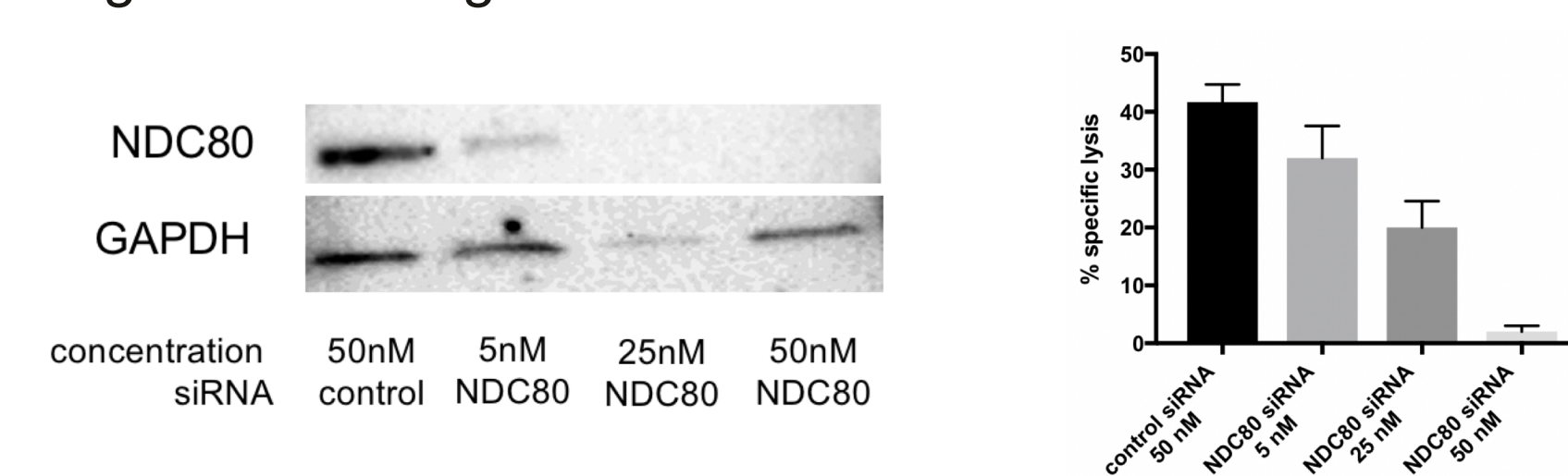


Results (I)

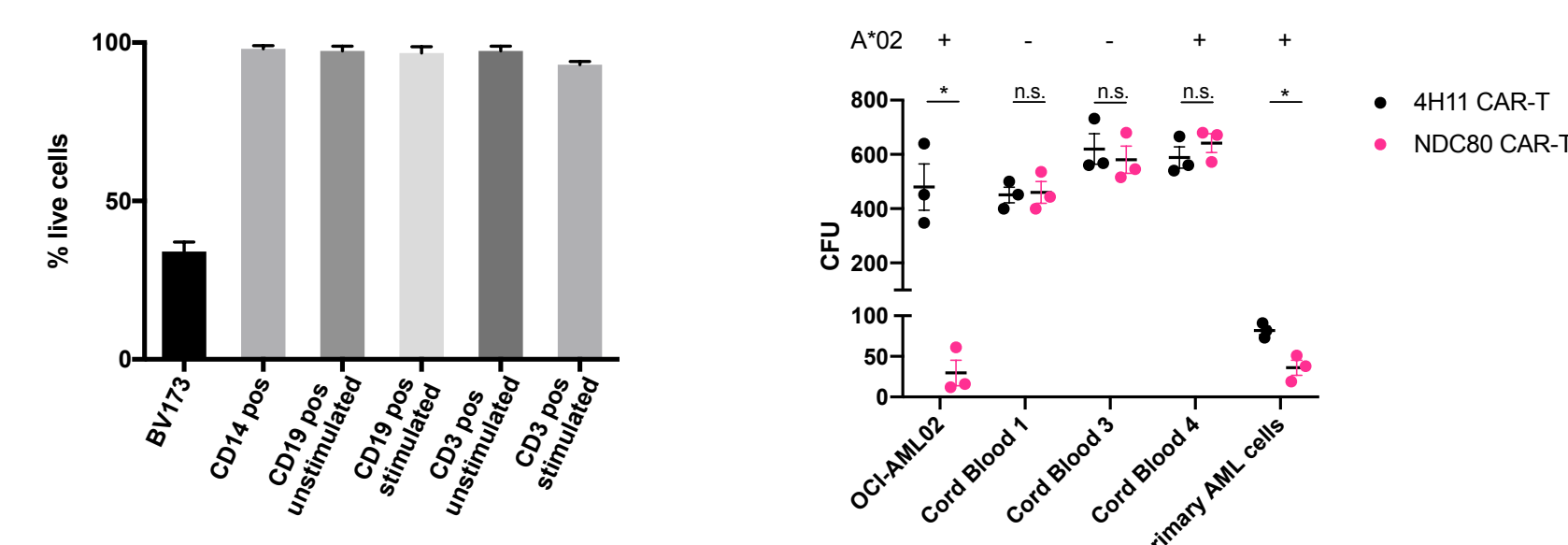
I. Broad *in vitro* activity against multiple cell lines by LDH assay



II. Abrogation of killing after NDC80 knockdown

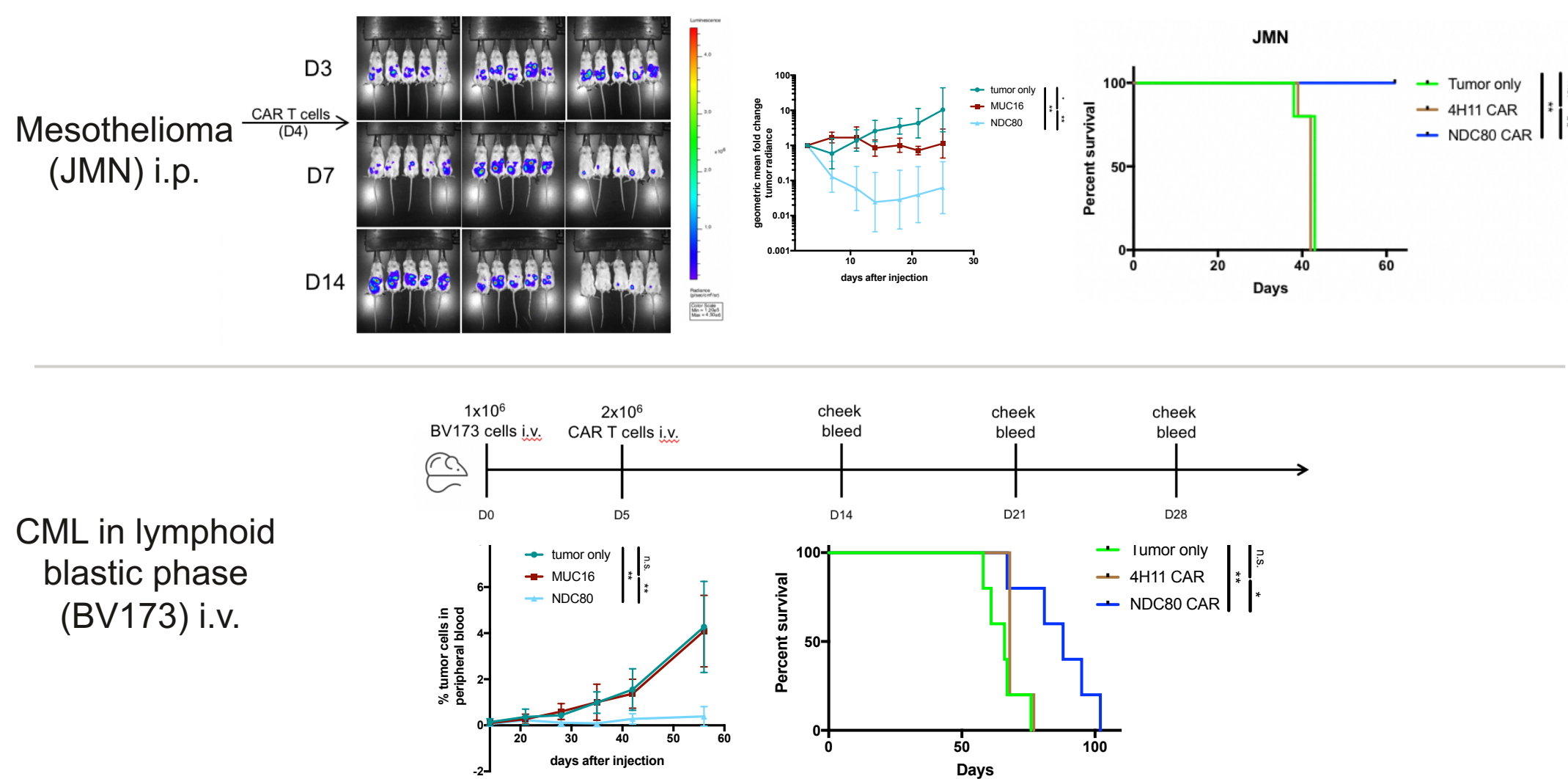


III. No toxicities against PBMCs or HSCs



Results (II)

IV. *In vivo* activity against mesothelioma and leukemia cells



Summary

Our study demonstrates how MS can inform the design of tumor-agnostic TCR-mimic therapeutic platforms that target structures that are currently not druggable by small molecules, conventional CAR-T cells, or antibodies. This strategy lays the groundwork for a potential antibody platform therapy or CAR-T cell with efficacy against highly proliferative A*02-positive cancer cells, independent of the respective cancer type.

Acknowledgements

We thank Alex Kentsis for access to the Byonic Software and the Proteomics Resource Center at The Rockefeller University for the performance of all LC/MS-MS experiments. This study was supported by the Leukemia and Lymphoma Society; NIH P30CA 008748, R01 CA55349, P01 CA23766 and R35 CA241894; The Experimental Therapeutics Center of MSK, and Tudor Funds. MGK is supported by the German Research Foundation (DFG) with an individual Research Grant KL3118/1-1.