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POSTER ABSTRACTS

703.CELLULAR IMMUNOTHERAPIES: BASIC AND TRANSLATIONAL

Affinity Matured CD72 CAR-T Improves Efficacy Versus Low Antigen Density B-Cell Non-Hodgkin Lymphoma Models

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Background:
Even in the CD19 CAR-T era, many patients with relapsed/refractory B cell non-Hodgkin lymphoma (B-NHL) need additional therapeutic options. Our group has identified CD72 as a therapeutic target for high-risk B-cell malignancies and validated anti-CD72 CAR-Ts ("nanoCAR"s) for these indications in relevant preclinical models (Nix et al., *Cancer Discovery* 2021, Temple et al., *ASH* 2022). However, patient B-NHL tumors may express a wide distribution of surface CD72, and our current CAR-T designs may be less efficacious for tumors with low antigen density.
We previously described the development of affinity-matured binders extending from our initial published anti-CD72 nanobody clone (NbD4), with ~20-60x higher target affinity (Temple et al.). Here, we tested the hypothesis that these higher affinity binders would better eliminate low antigen density B-NHL models, both *in vitro* and *in vivo*, and impact other properties including avidity, and antigen binding epitopes.
Methods:
Anti-CD72 affinity-matured binders were developed by random mutagenesis of CDR regions of the nanobody clone NbD4 (described in Temple et al.). CAR-T cells were generated by lentiviral transduction. CAR-T cell efficacy was tested by *in vitro* cytotoxicity assays and Incucyte live-cell imaging against lymphoma cell lines. For *in vivo* studies, JeKo-1 tumor cells were implanted at 1e6 per mouse and then mice were treated with 3.5e6 CAR+ T-cells at day 7. Tumors from mice bearing JeKo-1 that relapsed on CAR-T treatment were isolated and used in Incucyte assays.
Results:
We analyzed publicly available datasets of patient cohorts and found that CD72 is widely expressed, though with a broad distribution, across diffuse large B cell lymphoma (DLBCL), follicular lymphoma, and Burkitt lymphoma patient samples. In both DLBCL and chronic lymphocytic leukemia (CLL), high expression of CD72 correlated with significantly shorter overall survival (DLBCL: GSE10846, n=233, $p = 0.0382$; CLL: GSE22762, n=107, $p = 0.00124$, log-rank test). We confirmed expression of CD72 on 8 lymphoma cell lines by flow cytometry. *In vitro* against a subset of these lines, affinity-matured clone NbD4.13 showed improved cytotoxicity compared to parental NbD4 and similar cytotoxicity to a humanized variant (H24 clone).
We developed a JeKo-1 model of low CD72 antigen density (4.7x MFI reduction) by CRISPR/Cas9 knockout of endogenous CD72 and re-expression of a recombinant construct. In this "CD72 low" model, affinity matured clone NbD4.13 led to improved cytotoxicity vs. H24 ($p = 5.1e-4$ by *t*-test). We previously showed that tumor isolated from JeKo-1 mice treated with

H24 CAR appear to have reduced antigen expression at relapse (Temple et al.). We cocultured these post-relapse JeKo-1 tumors with various CD72 nanoCARs and found that NbD4.13 eliminates tumor significantly faster at 24 hours than H24 by live-cell imaging ($p = 0.0054$ by t-test). *In vivo* in a JeKo-1 model, NbD4.13 also out-performed the H24 clone with respect to survival benefit ($p = 0.011$ by log-rank for survival) (**Fig. 1**).

To assess if additional differences beyond affinity may determine the efficacy of NbD4.13, we performed structural modeling using AlphaFold and HADDOCK. However, this analysis predicts NbD4, NbD4.13 and H24 bind a similar non-linear epitope on the CD72 monomer. Furthermore, acoustic force microscopy did not reveal a significant difference in binding avidity for any of the compared CD72 nanoCAR constructs.

Finally, we evaluated whether it could be possible to pharmacologically increase CD72, as co-treatment strategy to enhance efficacy of CD72 nanoCARs. We found that the Protein Kinase C inhibitor Bryostatin-1, previously demonstrated to increase CD22 antigen density (Ramakrishna et al., *Clin Cancer Res* 2019), could also significantly increase surface CD72 in vitro in both B-ALL and B-NHL models (**Fig. 2**).

Conclusion:

NbD4.13-based anti-CD72 CAR is a promising candidate for further preclinical development for treatment of relapsed/refractory B-cell non-Hodgkin lymphoma. Given anticipated heterogeneity in CD72 expression in various lymphomas, pharmacologic co-treatment strategies may be considered to modulate tumor antigen density.

Disclosures Nix: *Cartography Biosciences:* Current Employment. **Larson:** *MGH:* Patents & Royalties: author on patents related to cellular therapy. **Maus:** *Novartis:* Patents & Royalties; *TCR2:* Current equity holder in private company; *Oncternal:* Current equity holder in private company; *Neximmune:* Current equity holder in private company; *Genocea:* Current equity holder in private company; *Century Therapeutics:* Current equity holder in private company; *2SeventyBio:* Consultancy; *Promab:* Patents & Royalties; *Massachusetts General Hospital:* Patents & Royalties. **Wiita:** *Genentech/Roche:* Research Funding; *Sanofi:* Honoraria.

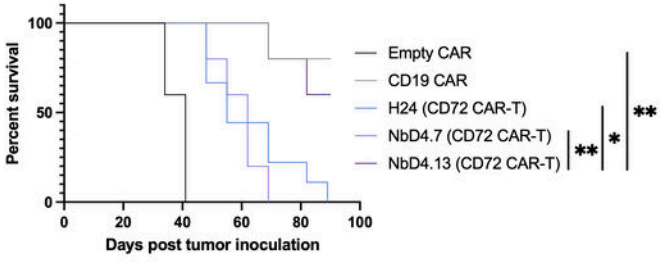

Figure 1. Affinity matured CD72 CAR NbD4.13 has superior efficacy than H24 against lymphoma in vivo. Survival curve of JeKo-1 bearing mice (n=5 per group) treated with indicated CAR-Ts. p-values are for NbD4.13 CAR compared to H24, NbD4.7 and Empty CAR groups, *p < 0.05, **p < 0.01 by log rank (Mantel-Cox) test.

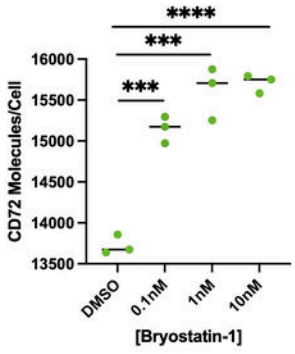

Figure 2. Potential resistance to CD72 CAR therapy can be ameliorated by antigen modulation with Protein Kinase C inhibitor Bryostatin-1. CD72 antigen per cell estimation 24 hours after JeKo-1 cell line treatment with DMSO or 3 indicated doses of Bryostatin-1. Bars represent the mean ***p < 0.001, ****p < 0.0001 by unpaired t test.

Figure 1

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