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CD58 Aberrations Limit Durable Responses to CD19 CAR in Large B Cell Lymphoma Patients Treated with Axicabtagene Ciloleucel but Can be Overcome through Novel CAR Engineering

Robbie G. Majzner, MD,[1] Matthew J. Frank, MD PhD,[2] Christopher Mount, MD PhD,[*,3] Aidan Tousley,[*,3] David M. Kurtz, MDPhD,[4] Brian Sworder, MD PhD,[5] Katherine A Murphy,[*,6] Antigoni Manousopoulou,[*,7] Kennedy Kohler,[*,3] Maria Caterina Rotiroti, PhD,[*,3] Jay Y. Spiegel, MDFRCPC,[2] Yasodha Natkunam, MD PhD,[8] Sheren F. Younes, MD PhD,[*,9] Elena Sotillo, PhD,[*,1] Vandon Duong,[*,3] Charles Macaulay, MSc, BA,[*,10] Zinaida Good, PhD,[11] Peng Xu, MD,[*,3] Louai Labanieh,[*,1] Leo D Wang, MD PhD,[12] Ash A. Alizadeh, MD PhD,[13] Michelle Monje, MD PhD,[*,3] David B. Miklos, MD PhD,[2] Crystal L. Mackall, MD[11]

[1]Department of Pediatrics, Stanford University School of Medicine, Palo Alto, CA

[2]Division of Blood and Marrow Transplantation, Stanford University, Stanford, CA

[3]Stanford University School of Medicine, Palo Alto, CA

[4]Department of Medicine, Divisions of Hematology & Oncology, Stanford University, San Francisco, CA

[5]Department of Medicine, Divisions of Hematology & Oncology, Stanford University, Stanford, CA

[6]Stanford University, Palo Alto, CA

[7]City of Hope, Duarte, CA

[8]Department of Pathology, Stanford University Medical Center, Stanford, CA

[9]Department of Pathology, Stanford University School of Medicine, Stanford, CA

[10]Department of Medicine, Divisions of Hematology & Oncology, Stanford University Medical Center, Palo Alto, CA

[11]Center for Cancer Cell Therapy, Stanford University School of Medicine, Stanford, CA

[12]City of Hope National Medical Center, Beckman Research Institute, Duarte, CA

[13]Stanford University, Stanford, CA

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CD19 CAR T cells have revolutionized the treatment of relapsed and refractory (R/R) large B cell lymphomas (LBCL), mediating durable complete responses in approximately 40-50% of patients. Besides a loss or decrease in CD19 expression, no studies have identified tumor specific factors driving inherent or acquired resistance to CAR T cells in LBCL. Mutations in and loss of expression of LFA-3 (CD58) have

been described in approximately 20% of cases of LBCL. As the ligand for CD2 on T cells, CD58 provides costimulation to T cells and CD58 loss or mutation has been linked to immune resistance in LBCL.

We evaluated CD58 status in fifty-one R/R LBCL patients treated at Stanford with commercial axicabtagene ciloleucel (axi-cel) through immunohistochemistry (IHC) on tumor biopsy samples and/or deep sequencing of circulating tumor DNA by CAPP-Seq. We identified 12/51 (24%) patients with a CD58 aberration (lack of expression by IHC or mutation by CAPP-Seq). Progression-free survival (PFS) was significantly decreased in patients with a CD58 aberration (median PFS for CD58 aberration 3 months vs. not reached for CD58 intact, $p<0.0001$). In fact, only 1/12 patients with a CD58 alteration achieved a durable, complete response to axi-cel, while the remaining 11 patients progressed, most commonly after a period of initial response. Partial responses were more common among patients with CD58 aberrations (58% for CD58 aberration vs 10% for CD58 intact, $p<0.001$), and complete responses were less common (25% for CD58 aberration vs 82% for CD58 intact, $p<0.0001$).

To probe the biology of CAR T cell responses towards tumors lacking functional CD58, we generated a CD58 knockout Nalm6 model. CD19.CD28.ζ, CD19.4-1BB.ζ, and CD22.4-1BB.ζ CAR T cells demonstrated significantly reduced cytokine production and cytolytic activity in response to CD58 KO vs wildtype (WT) tumor cells. Additionally, while mice inoculated with WT Nalm6 and treated with any of the three CARs demonstrate complete responses and prolonged leukemia-free survival, mice inoculated with CD58KO Nalm6 demonstrated only partial responses, eventual tumor progression, and death from leukemia.

CD2, the T cell ligand for CD58, plays both an adhesive role and a costimulatory role in T cells. CD2 knockout resulted in significantly reduced cytokine production after CAR stimulation. Re-expression of only the CD2 extracellular domain did not rescue CAR function, indicating that CD2 signaling is essential for full CAR activation. Additionally, when we stimulated CD19 CAR T cells with anti-idiotype antibody (CAR stimulation), soluble CD58 (CD2 stimulation), or both, we observed significantly enhanced phosphorylation of both CD3ζ and ERK by western blot in CAR T cells stimulated through both the CAR and CD2. Phosphorylation analysis by mass spectrometry revealed that CD2 stimulation enhances phosphorylation of proximal signaling molecules in the TCR pathway (LCK, LAT, CD3ε among others) and also mediators of actin-cytoskeletal rearrangement in CAR T cells, consistent with effects in natural T cell responses.

To overcome CD58 loss in LBCL, we generated second- and third-generation CAR T cell constructs integrating CD2 costimulatory domains within the CAR molecule. While these *cis* constructs demonstrated increased potency against CD58KO cells *in vitro*, they were unable to ultimately overcome

CD58 loss *in vivo*. However, when CARs were co-expressed with an additional CD2 receptor *in trans*, they mediated significant anti-tumor activity *in vivo*, overcoming CD58 knockout in tumor cells.

In conclusion, we have identified that CD58 status is an important biomarker for durable response to CAR T cells in LBCL. We modeled the biologic basis for this finding and generated CAR T cells capable of overcoming CD58 loss in B cell malignancies. CD58 mutations have been reported in many cancers, including multiple myeloma and colon cancer, and are likely to play a role in immune evasion for CAR T cells as they are developed for additional histologies. These data provide rationale for investigating CD58 status for patients receiving CAR based therapeutics and devising next generation CARs capable of overcoming this newly discovered mechanism of resistance.

Disclosures

Majzner: *Xyphos Biopharma:* Consultancy; *Zai Lab:* Consultancy; *Lyell Immunopharma:* Consultancy; *GammaDelta Therapeutics:* Membership on an entity's Board of Directors or advisory committees; *Aprotum Group:* Consultancy; *Illumina Radiopharmaceuticals:* Consultancy. **Kurtz:** *Roche:* Consultancy; *Genentech:* Consultancy; *Foresight Diagnostics:* Other: Ownership. **Sotillo:** *Lyell Immunopharma:* Consultancy, Other: Consultancy. **Alizadeh:** *Janssen:* Consultancy; *Genentech:* Consultancy; *Pharmacyclics:* Consultancy; *Chugai:* Consultancy; *Celgene:* Consultancy; *Gilead:* Consultancy; *Roche:* Consultancy; *Pfizer:* Research Funding. **Miklos:** *Miltenyi Biotec:* Research Funding; *Janssen:* Consultancy, Other: Travel support; *Pharmacyclics:* Consultancy, Other: Travel support, Patents & Royalties, Research Funding; *Novartis:* Consultancy, Other: Travel support, Research Funding; *Allogene Therapeutics Inc.:* Research Funding; *Juno-Celgene-Bristol-Myers Squibb:* Consultancy, Other: Travel support, Research Funding; *Kite-Gilead:* Consultancy, Membership on an entity's Board of Directors or advisory committees, Other: Travel support, Research Funding; *Adaptive Biotech:* Consultancy, Other: Travel support, Research Funding. **Mackall:** *BMS:* Consultancy; *Allogene:* Current equity holder in publicly-traded company; *Apricity Health:* Consultancy, Current equity holder in private company; *Nektar Therapeutics:* Consultancy; *NeoImmune Tech:* Consultancy; *Lyell Immunopharma:* Consultancy, Current equity holder in private company.

Author notes

* Asterisk with author names denotes non-ASH members.