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# ORAL ABSTRACTS

## 704.CELLULAR IMMUNOTHERAPIES: EARLY PHASE AND INVESTIGATIONAL THERAPIES

### Cytokine Release Syndrome Results in Reduced AML Killing By CD123 CAR T Cells

*Anand S Bhagwat, MDPhD[1,2], Leonel Torres[3], Olga Shestova, PhD[4], Maksim Shestov[5], Noelle V. Frey, MD MS[5], Elizabeth O Hexner, MD MSTR[5], Selina M Luger, MDFRCPC[6], Alison W Loren, MD[7], Mary Ellen Martin, MD[5], Shannon R McCurdy, MD[5], Alexander Perl[5], Edward A. Stadtmauer, MD[8], James K. Mangan, MD[9], Jennifer Brogdon, PhD[10], Joseph A Fraietta, PhD[11], Wei-Ting Hwang, PhD[12], Don L. Siegel, MDPhD[2], Saad S. Kenderian, MD[13], David L. Porter, MD[14], Carl June, MD[3], Saar Gill, MD PhD[5]*

[1] Children's Hospital of Philadelphia, Philadelphia, PA
[2] University of Pennsylvania, Philadelphia, PA
[3] Center for Cellular Immunotherapies, University of Pennsylvania, Philadelphia, PA
[4] Center for Cellular Immunotherapies, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA
[5] Division of Hematology/Oncology, Department of Medicine, University of Pennsylvania, Philadelphia, PA
[6] Perelman School of Medicine, Hospital of the University of Pennsylvania, Philadelphia, PA
[7] Cellular Therapy and Transplantation, Perelman Center for Advanced Medicine, Philadelphia, PA
[8] Abramson Cancer Center, University of Pennsylvania, Philadelphia, PA
[9] UCSD Moores Cancer Center, La Jolla, CA
[10] Novartis Institutes For Biomedical Research, Cambridge, MA
[11] Center for Cellular Immunotherapies, University of Pennsylvania, Perelman School of Medicine, Philadelphia, PA
[12] Department of Biostatistics, Epidemiology, and Informatics, The University of Pennsylvania Perelman School of Medicine, Philadelphia, PA
[13] Division of Hematology, Mayo Clinic, Rochester, MN
[14] Abramson Cancer Center, University of Pennsylvania, Perelman School of Medicine, Philadelphia, PA

**Background**: Chimeric antigen receptor (CAR) T cells have repeatedly demonstrated capacity to induce high rates of response and durable remissions for B cell cancers. In contrast, CAR T cells for the treatment of acute myeloid leukemia (AML) have not yet achieved this impact. We previously demonstrated that CD123 is expressed on AML blasts at high frequency, thus making it an attractive target antigen for AML-directed CAR T cells.

**Methods and Results**: We conducted a pilot study of CD123-directed CAR T cells (CART-123) in adults with relapsed or refractory AML. The primary objective was safety with a secondary objective of anti-leukemia efficacy. Twenty-two subjects were screened, and 20 were eligible for the trial. Fludarabine and cyclophosphamide were used for lymphodepletion (LD). Twelve subjects were infused with CART-123. There were two treatment-related deaths due to cytokine release syndrome (CRS) and infection. Four subjects (33%) achieved complete response with incomplete count recovery (CRi), three subjects had progressive disease, and five had stable disease. Of the four who achieved CRi, one subject remains alive in remission after a relapse and subsequent consolidative hematopoietic stem cell transplant (HSCT). One other subject is alive after HSCT following CART-123 failure. There was evidence of CAR T cell expansion and ten subjects (83%) experienced clinical CRS ranging from Grade I to Grade V. There was no neurotoxicity.

To address the apparent paradox of high rates of CART-123 bioactivity (clinical CRS and in vivo expansion) co-occurring with low rates of response, we hypothesized that cytokines produced during CRS may undermine the anti-AML effect of CART cells. We obtained serum drawn from trial subjects at baseline (prior to LD chemotherapy) or during CRS. Serum drawn during CRS promotes primary AML survival in cell culture, while matched baseline serum from the same subjects does not. Individual cytokines secreted during CRS that signal through receptors expressed on myeloid progenitors (such as GM-CSF and Flt3 ligand) reproduced this survival advantage in primary AML blasts when tested at physiologically relevant levels. In contrast, these cytokines did not produce a pro-survival effect in primary patient B-ALL blasts. Crucially, candidate myeloid-acting cytokines, as well as serum drawn during the peak of clinical CRS, promoted resistance of AML to killing by CART-123 *in vitro*. Using scRNAseq of bone marrow aspirates from subjects on this clinical trial, we found that GM-CSF and FLT3L were most frequently expressed by CD4+ T cells. Exposure of primary AML samples to CRS cytokines led to upregulation of signaling (e.g.

JAK/STAT5) and pro-survival pathways. In previous work in B-ALL, we showed that relative resistance to killing can translate to a functional deficit in CART-19 due to development of exhaustion in the CART cells. We similarly find that that exposure to GM-CSF reduces AML blast sensitivity to CART-123, and that the persistence of antigen leads to exhaustion in CART cells (Figure 1). Specifically, co-incubation of CART-123 cells and cytokine-exposed primary AML blasts leads to increased populations of PD-1+/CD39+/CTLA4+/LAG3+ CAR T cells, consistent with an exhaustion phenotype. Finally, we found that anti-apoptotic effects of cytokines can be prevented, and CART-123 killing of AML can be restored, via ruxolitinib blockade of JAK/STAT signaling in both *in vitro* and *in vivo* settings.

**Significance:** Our work reveals CRS as active in undermining CART-123 lethality first by promoting survival of AML, and subsequently by exhausting CAR T cells. This is in stark contrast to its role in lymphoid malignancies and underscores biological differences in myeloid versus lymphoid neoplasms. These results highlight a need to tailor therapy to limit cytokine effects on AML blasts to improve outcomes with AML-directed CAR T cell therapy.

**Disclosures Bhagwat:** *Bristol Meyers Squibb:* Current equity holder in publicly-traded company. **Frey:** *Kite Pharma:* Consultancy; *Sana Biotechnology:* Consultancy. **Luger:** *Onconova:* Research Funding; *AbbVie:* Membership on an entity's Board of Directors or advisory committees; *Marker Therapeutics:* Membership on an entity's Board of Directors or advisory committees; *Amgen:* Honoraria, Membership on an entity's Board of Directors or advisory committees; *Novartis:* Consultancy; *Bristol-Myers Squibb:* Honoraria; *Astellas:* Honoraria. **Perl:** *Syndax:* Research Funding; *Bayer:* Research Funding; *BMS:* Honoraria; *Foghorn:* Consultancy; *Genentech:* Honoraria; *Abbvie:* Consultancy, Honoraria, Research Funding; *Immunogen:* Honoraria; *Beat AML:* Other: Participation on a Data Safety Monitoring Board or Advisory Board; *BerGen Bio:* Honoraria; *FujiFilm:* Research Funding; *Forma:* Consultancy; *Astellas:* Consultancy, Honoraria, Membership on an entity's Board of Directors or advisory committees, Research Funding; 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*Mettaforge:* Patents & Royalties; *Lentigen:* Research Funding; *Tolero/Sumtomo:* Research Funding; *Morphosys:* Research Funding; *LEAHLabs:* Consultancy, Current equity holder in private company, Research Funding; *CapstanBio:* Consultancy, Other: Scientific advisory board. **Porter:** *Tmunity:* Patents & Royalties; *Novartis:* Membership on an entity's Board of Directors or advisory committees, Patents & Royalties, Research Funding; *National Marrow Donor Program:* Membership on an entity's Board of Directors or advisory committees; *Sana Therapeutics:* Consultancy, Current equity holder in publicly-traded company; *Mirror Biologics:* Membership on an entity's Board of Directors or advisory committees; *Kite/Gilead:* Membership on an entity's Board of Directors or advisory committees; *Janssen:* Membership on an entity's Board of Directors or advisory committees; *Genentech:* Current equity holder in publicly-traded company; *DeCart:* Membership on an entity's Board of Directors or advisory committees; 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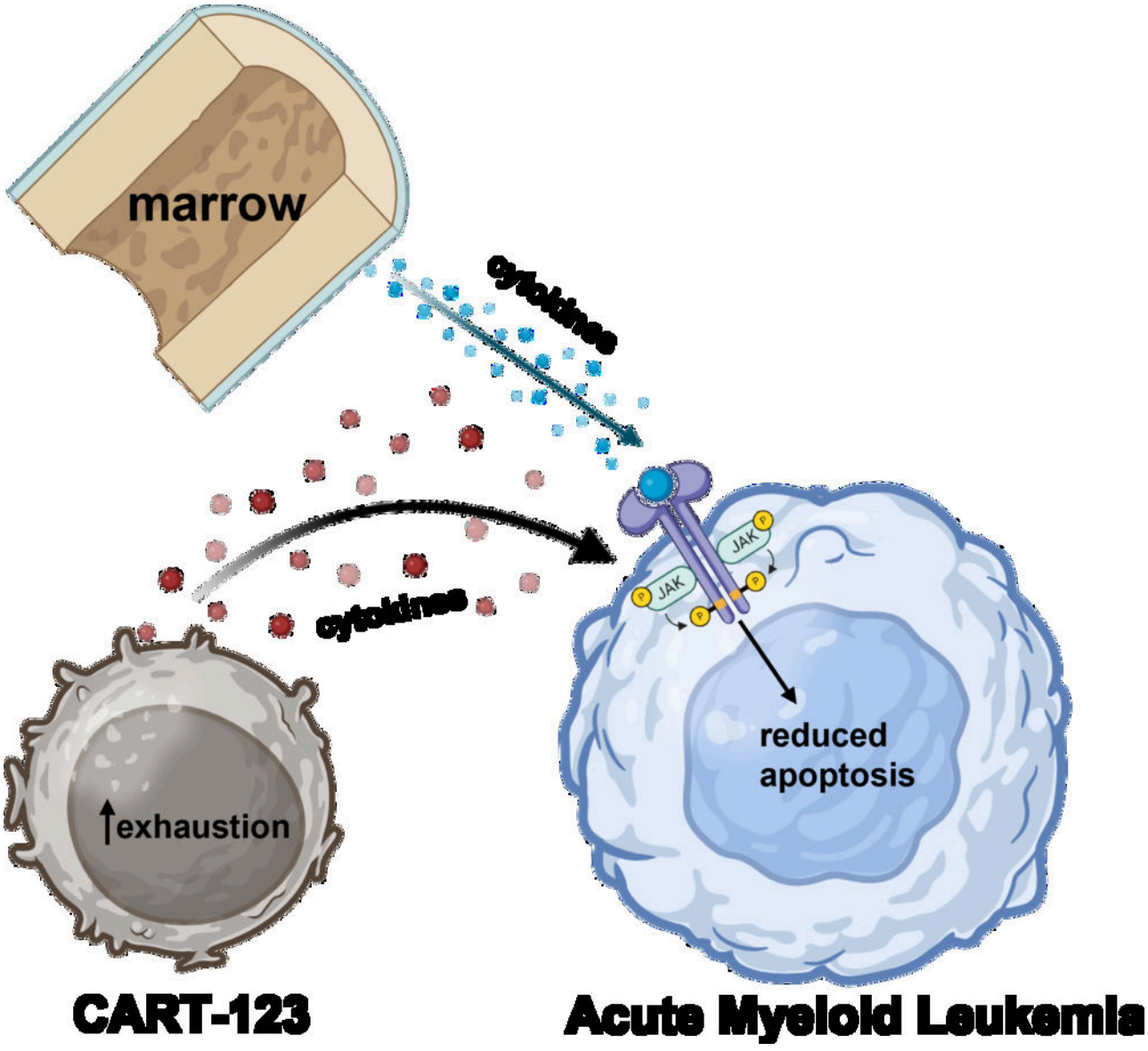


**Figure 1**

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