Nanometer-Scale Assembly and High-Throughput Screening of Bispecific T Cell Engaging Cytokine (BiTEokine) Immunotherapies

Priscilla Do,[1] Lacey A Perdue,[1] Andrew Chyong,[1] Rae Hunter,[2,3,4] Jodi Dougan,[2,3,4] Curtis J Henry,[2,3,4] Christopher C Porter,[2,3,4,*] **Erik C Dreaden**[1,3,4,5*] (**presenting author**)

[1] Coulter Department of Biomedical Engineering, Georgia Institute of Technology and Emory University
[2] Department of Hematology and Medical Oncology, Winship Cancer Institute of Emory University
[3] Department of Pediatrics, Emory School of Medicine
[4] Aflac Cancer and Blood Disorders Center, Children's Healthcare of Atlanta and Emory School of Medicine
[5] Petit Institute for Bioengineering and Bioscience, Georgia Institute of Technology

Statement of Purpose: Therapies that bind with immune cells and redirect their cytotoxic activity towards diseased cells represent a promising and versatile approach to immunotherapy with applications in cancer, HIV, lupus, and other diseases; currently however, only one such drug is approved for clinical use in the US: the bispecific antibody, blinatumomab, which redirects T cell killing towards leukemic B cells. Recently, we identified deficiency of the cytokine, IL-12, as a key mediator of immune evasion in mouse models of leukemia and found that recombinant IL-12 therapy alone could improve T cell activation, immunologic memory, and overall survival in mouse models of the disease. Based on these findings, we hypothesized that the activity of immune cell-redirecting therapies targeting T cells and leukemic B cells may by improved by concurrent delivery of IL-12, particularly if the two agents were tethered to one another in order to improve the typically poor circulation of IL-12 that limits its therapeutic potential.

Methods: Nanometer-scale test compound libraries were synthesized via sequential addition of (i) antibodies directed against human CD19, CD3ε, isotype control, or (non-neutralizing) IL-12, (ii) protein G-conjugated 50 nm iron oxide nanoparticles, and (iii) single-chain human IL-12 with intermediate purification and/or sterile filtration steps. Phenotypic screening of drug-induced activity was performed via co-culture of test compounds with primary human $CD8^+$ T cells with $CD19^+$ NALM-6 leukemia cells, followed by flow cytometric analysis of leukemia cell lysis and T cell division. Test compound composition, size, and morphology was characterized via spectrofluorimetry, dynamic light scattering, and TEM, respectively. Cell specificity and drug-induced cell-cell contact was examined by flow cytometry and imaging flow cytometry, respectively.

Results: Using this approach, we assembled 47 unique bi-specific T cell engaging cytokine (BiTEokine) drug candidates (ca 108 nm dia) which varied widely in antibody composition (αCD19: 1.5-42; αCD3: 2.5-59; αIL12: 0.12-15 per particle). We then performed high-throughput phenotypic screening on these BiTEokines test compounds via co-culture of primary human $CD8^+$ T cells and leukemic B cells, followed and multicolor flow cytometry in order to characterize drug-induced lytic activity. Next, we rank-ordered top screening hits and correlated BiTEokine composition and T cell proliferation with leukemia cell lysis via regression modeling. Top screening hits were strongly non-obvious in their composition and exhibited lytic activity closely comparable to that of the FDA-approved leukemia immunotherapy, blinatumomab. We also observed high specificity for T cell CD3 and B cell CD19 via flow cytometry, as well as drug-induced B/T cell dimerization via imaging flow cytometry.

Conclusions: In summary, here we describe a novel method for the parallel assembly of compositionally diverse libraries of bi-specific T cell engaging cytokines (BiTEokines) and their high-throughput phenotypic screening, requiring just days for hit identification and the analysis of structure-function relationships. Using this approach, we identified CD19 x CD3 x IL12 compounds that exhibit *ex vivo* lytic activity comparable to current FDA-approved therapies for leukemia as well as surprising relationships between BiTEokine structure and activity. We further correlated this activity with drug-induced cell-cell contact, cytokine delivery, and leukemia cell lysis. Given the modular nature of these multivalent compounds and their rapid assembly/screening, we anticipate facile extension of this therapeutic approach to a wide range of immune cells, diseased cells, and soluble protein combinations in the future.

References: Do P, Perdue LA, Chyong A, Hunter R, Dougan J, Henry CJ, Porter CC,* Dreaden EC,* Rapid Assembly and Screening of Multivalent Immune Cell-Redirecting Therapies for Leukemia. *ACS Comb. Sci.,* 2020: 22: 533-541.