ELSEVIER

Blood 142 (2023) 768–769

The 65th ASH Annual Meeting Abstracts

ORAL ABSTRACTS

703.CELLULAR IMMUNOTHERAPIES: BASIC AND TRANSLATIONAL

Modulation of the Btla-HVEM Axis to Enhance CAR T Cell Immunotherapy Against Cancer

Puneeth Guruprasad[1], *Alberto Carturan, MD*[2], *Yunlin Zhang, MS*[2], *Kingsley Gideon Kumashie, PhD*[3], *Ivan J Cohen, PhD*[2,2], *Guido Ghilardi, MD*[2], *Ki-Hyun Kim*[4], *Jong-Seo Lee*[4], *Yoon Lee*[4], *Jong-Hoon Kim*[4], *Junho Chung, MD PhD*[4], *Maksim Shestov*[2], *Raymone Pajarillo, MS*[2], *Jaryse Harris, MD*[5], *Yong Gu Lee, PhD*[2], *Michael Wang*[2], *Hatcher Ballard, BS*[2], *Aasha Gupta, BS*[2], *Ositadimma Ugwuanyi, BS*[2], *Seok Jae Hong*[2], *Linhui Chen*[2], *Luca Paruzzo, MD*[2], *Shane Kammerman*[3], *Ruchi P. Patel, MS*[2], *Olga Shestova, PhD*[2], *Laura Vella, MD PhD*[3], *Stephen J. Schuster, MD*[6,2,7], *Jakub Svoboda, MD*[7,6], *Patrizia Porazzi, PhD*[2], *Marco Ruella, MD*[7,6,2]

[1] Center for Cellular Immunotherapies, Perelman School of Medicine, University of Pennsylvania, North Wales, PA
[2] Center for Cellular Immunotherapies, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA
[3] Children's Hospital of Philadelphia, Philadelphia, PA
[4] Biopharmaceutical Research Center, AbClon Inc., Seoul, Korea, Republic of (South)
[5] Department of Pathology, Hospital of the University of Pennsylvania, Philadelphia, PA
[6] Lymphoma Program, Abramson Cancer Center, University of Pennsylvania, Philadelphia, PA
[7] Department of Medicine, Division of Hematology/Oncology, Hospital of the University of Pennsylvania, Philadelphia, PA

Introduction: The efficacy of adoptive T cell immunotherapies against cancer, such as chimeric antigen receptor (CAR) T cells, is severely blunted by the immunosuppressive tumor microenvironment (TME). We sought to investigate the role of the TME in cancer resistance to effector T cells in order to define actionable targets to enhance CAR T cell immunotherapies. We initially used Hodgkin lymphoma (HL) as an ideal tumor model since it is characterized by a TME that is profoundly infiltrated by immunosuppressive cells, and then we expanded our findings to multiple cancer models.

We first sought to identify the dominant interactions of immunosuppressive cellular compartments and effector T cells by analyzing single-cell RNA sequencing data on a total of 26 (4 exploratory + 22 validation) HL patient tumor biopsies. Using the CellPhoneDB algorithm, we inferred that the ligand B- and T-lymphocyte attenuator (BTLA) on effector T cells and the receptor Herpesvirus entry mediator (HVEM, *TNFRSF14*) on immunosuppressive cells (e.g., regulatory T cells, monocytes) strongly interact in the TME and promote T cell dysfunction (**Fig 1a**). Akin to the canonical checkpoint PD1, BTLA recruits two potent tyrosine phosphatases, SHP-1 and SHP-2, to disable early T cell activation. Thus, we rationalized that BTLA expression on T cells might reduce their anti-tumor function. We hypothesized that deleting BTLA in CAR T cells would abolish BTLA-HVEM *trans* interactions at the immunological synapse and unleash the cytotoxic potential of CAR T cells.

Methods and Results: We first generated BTLA KO anti-CD30 CAR T (CART30) cells against HL. To test their function *in vivo*, 15x10 ^{6} HDLM-2 (CD30+HVEM+ HL) cells were subcutaneously implanted into NSG mice on day 0, and on day 62, 3x10 ^{5} CAR30+ T cells were infused intravenously. Our results demonstrated that BTLA KO significantly enhances the function of anti-CD30 CAR T cells in HVEM+ HL (**Fig 1b**), as assessed via tumor size (caliper) and CART30 expansion in the peripheral blood (flow cytometry). Additionally, we generated BTLA KO 4-1BBζ CART19 cells, which showed greater *in vivo* anti-tumor function in a subcutaneous tumor model of DLBCL (CD19+ HVEM+ OCI-Ly18). Serum collected from OCI-Ly18-bearing NSG mice infused with BTLA KO CART19 was enriched in effector cytokines (e.g., TNF, IFNγ, IL-2) as measured by Luminex. We then extended these findings into HVEM+ solid tumor models. BTLA KO improved tumor control *in vitro* in short-term killing experiments (Incucyte SX5) for both CAR and TCR T cells, respectively directed against prostate cancer (HER2+ PC-3) and melanoma (GP-100+ DM-6). Importantly, we demonstrated that BTLA KO in primary BALB/c-derived murine CAR T cells enhances tumor control in A20 murine lymphoma. In this model, BTLA KO tumor-infiltrating CAR T cells showed substantially reduced exhaustion in the TME relative to wild-type CAR T cells.

Mechanistically, we showed that targeted mutations in BTLA intracellular tyrosine motifs effectively reduce the recruitment of SHP-1/2 and preserve the docking domain of the pro-stimulatory Grb2, thus maintaining high NFAT signaling and increased persistence *in vivo*. These effects were dependent on the expression of HVEM on tumor cells. Finally, we found that high BTLA RNA expression in tisagenlecleucel infusion products correlates with poor response to treatment in patients with DLBCL and FL (NCT02030834).

Conclusion: Our results reveal a critical role of the BTLA-HVEM axis in inhibiting CAR T cell function, and demonstrate that CRISPR-Cas9 deletion of BTLA leads to enhanced anti-tumor efficacy in multiple models of cancer. The key mechanism is the reduction of SHP-1/2 recruitment and the consequent increase in CAR T cell activation. The results of this study will be translated into a first in human clinical trial of BTLA-deficient CAR T cells for relapsed or refractory cancer.

Disclosures Ghilardi: *viTToria biotherapeutics:* Consultancy. **Kim:** *Abclon:* Current Employment. **Lee:** *Abclon:* Current Employment. **Lee:** *Abclon:* Current Employment. **Kim:** *Abclon:* Current Employment. **Chung:** *Progeneer Incorporation:* Research Funding. **Patel:** *viTToria biotherapeutics:* Consultancy. **Schuster:** *Celgene/Juno Therapeutics:* Consultancy, Research Funding; *Takeda:* Honoraria; *Genentech/Roche:* Consultancy, Honoraria, Membership on an entity's Board of Directors or advisory committees, Research Funding; *Fate Therapeutics:* Membership on an entity's Board of Directors or advisory committees; *Caribou Biosciences:* Consultancy, Membership on an entity's Board of Directors or advisory committees; *viTToria biotherapeutics:* Consultancy, Membership on an entity's Board of Directors or advisory committees; *BeiGene:* Consultancy, Honoraria; *Loxo Oncology:* Consultancy; *Mustang Biotech:* Consultancy; *Noardic Nanovector:* Membership on an entity's Board of Directors or advisory committees; *Legend Biotech:* Consultancy; *Morphosys:* Consultancy; *Regeneron:* Consultancy; *Genmab:* Consultancy, Membership on an entity's Board of Directors or advisory committees; *Kite Pharmaceuticals:* Consultancy; *Novartis:* Consultancy, Honoraria, Membership on an entity's Board of Directors or advisory committees, Research Funding; *Incyte:* Consultancy, Honoraria; *Janssen:* Consultancy; *AstraZeneca:* Consultancy, Honoraria; *AlloGene:* Consultancy, Honoraria; *Abbvie:* Consultancy. **Svoboda:** *Genmab:* Consultancy; *BMS:* Consultancy, Research Funding; *Incyte:* Consultancy, Research Funding; *Merck:* Research Funding; *Atara:* Consultancy; *TG Therapeutics:* Research Funding; *ADCT:* Consultancy; *Astra Zeneca:* Consultancy, Research Funding; *Adaptive:* Consultancy, Research Funding; *Pharmacyclics:* Consultancy, Research Funding; *SEAGEN:* Consultancy, Research Funding. **Ruella:** *NanoString:* Consultancy, Research Funding; *Bristol Myers Squibb:* Consultancy; *GlaxoSmithKline:* Consultancy; *Bayer:* Consultancy; *AbClon:* Consultancy, Research Funding; *Beckman Coulter:* Research Funding; *viTToria biotherapeutics:* Consultancy, Membership on an entity's Board of Directors or advisory committees, Other: Scientific Founder, Research Funding.

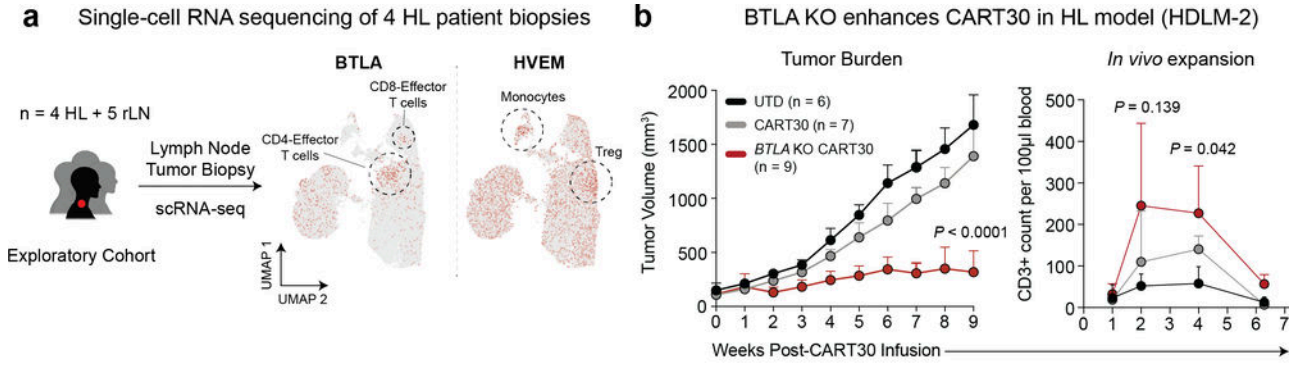

Figure 1. The BTLA-HVEM axis is enriched in HL and restricts the function of anti-CD30 CAR T cells against HL. a. UMAP of scRNA-seq (10X Genomics) peformed on 4 HL patient biopsies, showing expression of BTLA (left) and HVEM (i.e. *TNFRSF14*, right). **b.** Tumor burden of HDLM2-bearing immunodeficient (NOD scid gamma) mice following infusion with anti-CD30 CAR T cells. Briefly, 15e6 HDLM-2 cells were subcutaneously implanted in the right flank on day 0, and on day 62, 3e5 CAR30+ T cells were infused via tail vein. Tumor size was measured weekly via caliper (left), and peripheral blood was analyzed via flow cytometry for human CD45+ CD3+ cells (right). HL, Hodgkin lymphoma; rLN, reactive lymph node (healthy control); scRNA-seq, single-cell RNA sequencing; UMAP, Uniform Manifold Approximation and Projection; Treg, regulatory T cells; UTD, untransduced.

Figure 1

https://doi.org/10.1182/blood-2023-189633