Prior antigen exposure enhances the T cell response to Bispecific T cell Engager therapy

Mark Batistick[1], Mathias Oelke[2], Mital Gandhi[1,3], Sojung Kim[3], Ruipeng Wang[2], Adam Parks[2], Elena Cassella[1], Shaina Anuncio[1], Manpreet Bariana[1], Jack A. Ragheb[2], Jerome Zeldis[2], Johannes Zakrzewski[1,234]

1: Center for Discovery and Innovation, Hackensack Meridian Health
2: NexImmune Inc.
3: Department of Pediatrics, Hackensack University Medical Center
4: Department of Oncology, Georgetown University

INTRODUCTION

NexImmune's Artificial Immune Modulation for Adoptive Cell Therapy (AIM ACT) platform uses nanoparticles (core particle coupled with MHC peptide complex A*02:01 and aCD28) acting as synthetic dendritic cells to stimulate and expand antigen-specific T cells ex vivo without genetic manipulation. With this methodology, therapeutic concentrations of T cells specific for HLA-A2 restricted Multiple Myeloma (MM) epitopes (derived from WT1, CD138, CS1 and NY-ESO-1) or HLA-A2 restricted Acute Myeloid Leukemia (AML) epitopes (derived from WT1, PRAME and Cyclin A1) can be generated from patient or donor-derived peripheral blood mononuclear cells (PBMC).

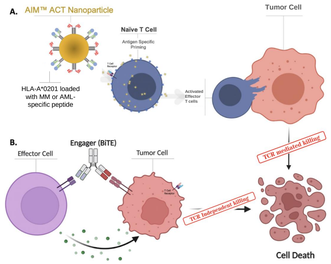


Figure 1: Multimodal tumor cell targeting through cytotoxic T Cell engagement. A. The AIM ACT platform activates naive T Cells into activated antigen-specific effector CD8+ T lymphocytes. **T Cell Receptor (TCR)-mediated** cytotoxicity occurs through HLA A*02:01 restricted tumor-specific epitope targeting. **B.** Bispecific T Cell Engager (BiTE) therapy engages **TCR-independent** cytotoxicity through T lymphocyte and tumor cell targets.

Additionally, Bispecific T cell Engager (BiTE) antibody therapy recruits T Cells to engage T cell receptor-independent cytotoxicity mediated by immune synapse formation between effector and target cells. Current BiTE therapy allows off-the-shelf treatment of MM through B Cell Maturation Antigen (BCMA), with ongoing trials exploring potential AML cell targets including CD33, CD123, and Flt3. **We hypothesize that the AIM ACT platform and BiTE therapy will act synergistically when used in combination rendering a distinct advantage over endogenous CD8 T cells + BiTE.**

AIMS

- Generate in vitro and in vivo evidence supporting synergistic effects of AIM ACT /BiTE combination therapy.
- Investigate whether prior antigen exposure enhances the BiTE effector function of T cells.

METHODS

- **In Vitro:** Firefly luciferase-transduced U266 MM cells or Molm-13 AML cells were cultured with effector T cells at various effector:target ratios. T cells investigated include PBMC derived bulk CD4, bulk CD8, naïve CD8, MM-specific CD8 (AIM ACT), AML-specific CD8 (AIM ACT), and bone marrow derived bulk CD8 T cells. The MM cells were also cultured +/- BCMA x CD3 BiTE and the AML cells were cultured +/- CD123 x CD3 or Flt3 x CD3 BITE. Cell survival was measured via luminescence at 24, 48, and 72 hours.
- **In Vivo:** Firefly luciferase-transduced U266 MM cells were injected into NSG mice followed by Neximmune MM-specific AIM ACT T Cells, bulk CD8 T cells, or PBS. Bioluminescence was measured weekly. Mice were also treated with either no BiTE or BCMA x CD3 BiTE at 2 ug/kg (low dose) or 20 ug/kg (standard dose) bi-weekly.

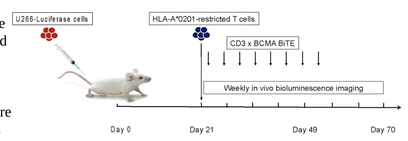


Figure 2. In vivo xenograft mouse model assay experimental layout.

RESULTS

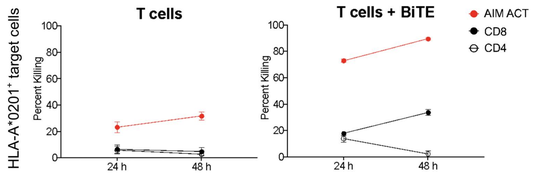


Figure 3. Tumor antigen-specific CD8 T cells are strong effectors of BiTE mediated killing. Left panel: U266-luc cells (HLA-A*0201+) human MM cells were cultured on the presence of HLA-A*0201 restricted control T cells (CD4, CD8) or MM-specific AIM ACT at an effector:target (E:T) ratio of 1:1. Right panel: CD3 x BCMA BiTE (concentration: 0.8 pM) was added to the media. Target cell numbers were measured after 24 and 48 h by luciferase assay. Target cells cultured in the absence of T cells and BiTE were used to normalize the data and calculate killing percentages. One of two independent experiments is shown. Mean and SEM are presented.

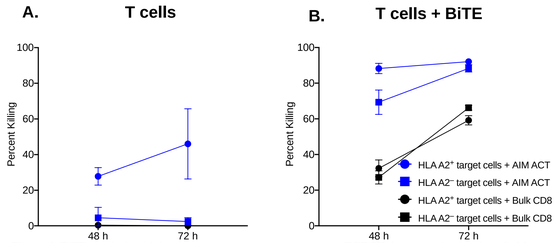


Figure 4. BiTE-mediating killing acts on target cells regardless of TCR/HLA-peptide interaction. A. Molm-13-luc human AML cells with or without HLA-A*0201 expression were cultured in the presence of AML-specific AIM ACT CD8+ T cells or bulk CD8 T cells at an E:T ratio of 0.5:1. Luciferase activity was measured at 48 and 72 hours. **B.** CD3 x CD123 BiTE 0.8 pM was added to culture. Luciferase activity was measured at 48 and 72 hours. Mean and SEM are presented.

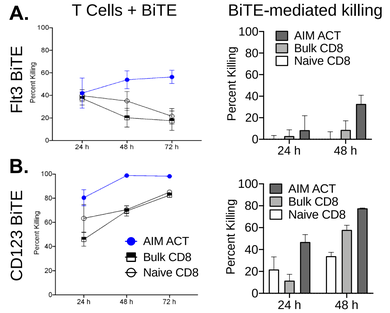


Figure 5. Antigen-experienced T cells are superior to naïve or bulk CD8 T cells as mediators of BiTE potency against AML target cells in vitro. Molm-13 (HLA-A*201+) human AML cells were cultured in the presence of HLA-A*0201 restricted AML-specific AIM ACT T cells, Bulk CD8 T cells, or naïve T cells at an effector to T cell ratio of 0.5:1. Target cells were also co-cultured with either CD3 x Flt3 (**A**) or CD3 x CD123 (**B**). BiTE-mediated killing percentage left panels) of Molm-13 cells was estimated at effector:target ratios of 0.5:1 by subtracting T-Cell only killing (data not shown) from combined killing. Mean and SEM are presented.

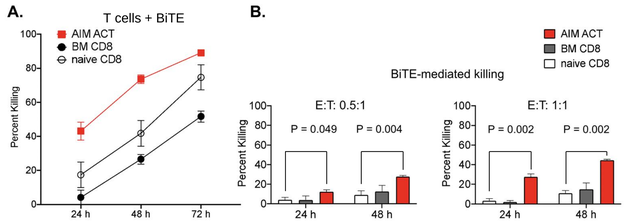
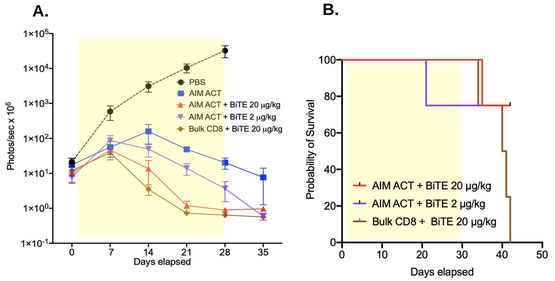


Figure 6. Antigen-experienced T cells are superior to naïve or BM-derived CD8 T cells as mediators of BiTE potency against MM target cells in vitro. A. U266-luc (HLA-A*0201+) human MM cells were cultured in the presence of BCMAxCD3 BiTE 0.8 pM and CD8+ T cells (naive, BM, or MM-specific AIM ACT) at an E:T ratio of 1:1. **B.** BiTE-mediated killing percentage of U266-luc cells was estimated at E:T ratios of 0.5:1 (left panel) and 1:1 (right panel) by subtracting T-Cell only killing (data not shown) from combined killing at respective E:T ratio (panel A and data not shown). Mean And SEM are presented. P values refer to comparisons of naïve CD8 T cells with AIM ACT CD8 T cells.



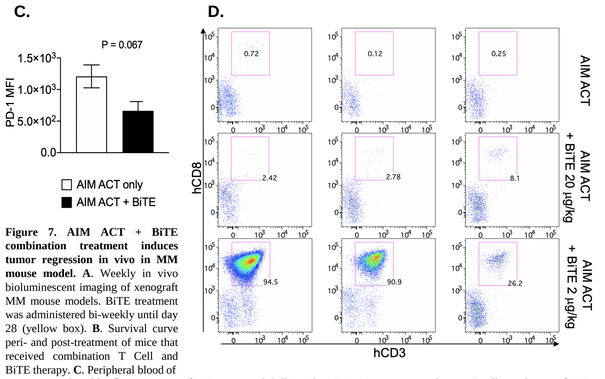


Figure 7. AIM ACT + BiTE combination treatment induces tumor regression in vivo in MM mouse model. A. Weekly in vivo bioluminescent imaging of xenograft MM mouse models. BiTE treatment was administered bi-weekly until day 28 (yellow box). **B.** Survival curve peri- and post-treatment of mice that received combination T Cell and BiTE therapy. **C.** Peripheral blood of mice was analyzed by flow cytometry for Programmed Cell Death-1 (PD-1) expression on human T cells on day 14 after T cell injection. Mean and SEM of the PD-1 median fluorescence intensity (MFI) of human CD3+ cells are presented. **D.** Peripheral blood of surviving mice on day 45 after T cell injection was analyzed for the presence of CD3+CD8+ human T cells. Pseudocolor dot plots of individual mice are presented.

CONCLUSIONS

- Antigen-specific AIM ACT T cells are superior to non-antigen specific (bulk) CD4 T cells, non-antigen specific (bulk) CD8 T cells, and non-antigen specific naïve CD8 T cells as effectors of BiTE-mediated killing in vitro.
- Combining TCR-mediated and TCR-independent (BiTE-mediated) killing enhances the overall anti-tumor efficacy, but BiTE activity does not require TCR engagement of MHC-peptide complex.
- Antigen experience of T cells (regardless of the TCR specificity) correlates with the potency of BiTE activity in vitro.
- In vivo, both AIM ACT and unmanipulated (bulk) CD8 T cells are potent effectors of BiTE activity, but only AIM ACT T cells are capable of T cell receptor-mediated tumor immunosurveillance after withdrawal of BiTE therapy.
- Therefore, BiTE withdrawal may be associated with a significant relapse risk in recipients of bulk CD8 T cells, while maintenance of remission can be achieved in recipients of AIM ACT.
- BiTE therapy induced PD-1 downregulation in human T cells and modulated persistence of human T cells in a MM xenograft model: while standard dose BiTE only slightly improved persistence, low-dose BiTE resulted in substantially improved persistence of human T cells.

REFERENCES

1. Perica, K., *et al.* Enrichment and Expansion with Nanoscale Artificial Antigen Presenting Cells for Adoptive Immunotherapy. *ACS Nano* **9**, 6861-6871 (2015).
2. Perica, K., *et al.* Nanoscale artificial antigen presenting cells for T cell immunotherapy. *Nanomedicine* **10**, 119-129 (2014).
3. Durai, M., *et al.* In vivo functional efficacy of tumor-specific T cells expanded using HLA-Ig based artificial antigen presenting cells (aAPC). *Cancer Immunol Immunother* **58**, 209-220 (2009).
4. Ichikawa, J., *et al.* Rapid Expansion of Highly Functional Antigen-Specific T Cells from Patients with Melanoma by Nanoscale Artificial Antigen-Presenting Cells. *Clin Cancer Res* **26**, 3384-3396 (2020).
5. Ugel, S., *et al.* In vivo administration of artificial antigen-presenting cells activates low-avidity T cells for treatment of cancer. *Cancer Res* **69**, 9376-9384 (2009).
6. Caraccio, C., Krishna, S., Phillips, D.J. & Schürch, C.M. Bispecific Antibodies for Multiple Myeloma: A Review of Targets, Drugs, Clinical Trials, and Future Directions. *Front Immunol* **11**, 501 (2020).
7. Hipp, S., *et al.* A novel BCMA/CD3 bispecific T-cell engager for the treatment of multiple myeloma induces selective lysis in vitro and in vivo. *Leukemia* **31**, 1743-1751 (2017).
8. Klinger, M., Benjamin, J., Kischel, R., Stienen, S. & Zugmaier, G. Harnessing T cells to fight cancer with BiTE® antibody constructs--past developments and future directions. *Immunol Rev* **270**, 193-208 (2016).
9. Kischel, R., Hausmann, S., Baeuerle, P. & Kufer, P. Effector memory T cells make a major contribution to redirected target cell lysis by T cell-engaging BiTE antibody MT110. *Cancer Res* **69**, 3252 (2009).
10. Raje, N., *et al.* Safety, Clinical Activity, Pharmacokinetics, and Pharmacodynamics from a Phase I Study of PF-06863135, a B-Cell Maturation Antigen (BCMA)-CD3 Bispecific Antibody, in Patients with Relapsed/Refractory Multiple Myeloma (RRMM). *Blood Cancer Discov* **134**, 1869 (2019).
11. DiLillo, D.J., *et al.* A BCMAxCD3 bispecific T cell-engaging antibody demonstrates robust antitumor efficacy similar to that of anti-BCMA CAR T cells. *Blood Adv* **5**, 1291-1304 (2021).
12. Brossart, P. The Role of Antigen Spreading in the Efficacy of Immunotherapies. *Clin Cancer Res* **26**, 4442-4447 (2020).

ACKNOWLEDGEMENTS

Funding for this project was provided by NexImmune Inc.

CONTACT INFORMATION

Johannes Zakrzewski, MD
Center for Discovery and Innovation
111 Ideation Way, Nutley, NJ 07110
johannes.zakrzewski@hmh-cdi.org
Phone: 201-880-3420

Jack Ragheb, PhD
NexImmune
9119 Gaither Rd, Gaithersburg 20877
jragheb@neximmune.com
Phone: 301-825-9810