Enhancement of bispecific T cell engagers (bispecific TCE) killing potency with the NexImmune Artificial Immune Modulation (AIM™) adoptive cell therapy (ACT) product NEXI-001

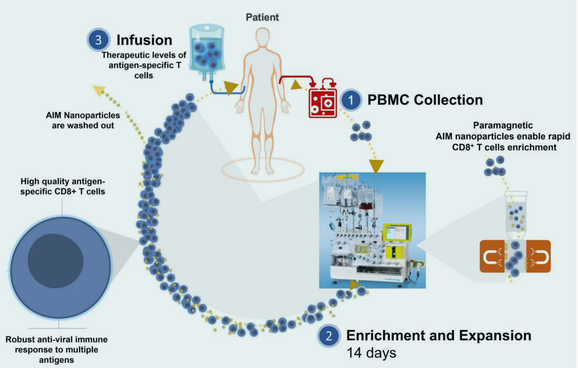
Ruipeng Wang, Mathias Oelke, Shweta Jain, Sojung Kim, Jack Ragheb, Adam Parks, Rui Wang, Charles Reed, Brian Alvarez, Jerome Zeldis, Kristi Jones, Daniel Bednarik
NexImmune Inc., Gaithersburg, MD

INTRODUCTION

Bispecific T cell engagers (TCEs) are a new class of immunotherapeutic molecules for the treatment of cancer. TCE molecules enhance the patient's immune response to tumors by redirecting T cells to tumor cells. However, TCEs frequently show rapid clearance with shorter serum half-life, requiring higher and more frequent doses. Increasing TCE exposure (e.g., dose, frequency) has been associated with T cell exhaustion[1]. Importantly, the phenotype of existing T cell landscape has been associated with clinical response[2]. Here we demonstrated the combination of TCE's with NexImmune's multi-antigen specific AIM ACT T cells (AIM ACT) results in superior anti-tumor potency at low doses due to increasing tumor targets and synergistic killing mechanisms. The combination offers the potential for superior durability and T cell persistence due to fit phenotype (consisting of antigen specific Tscm, Tcm and Tem). For this study we have generated 5 AML specific TCEs targeting FLT3 (two different molecules), CD123, CD33 and Siglec-6 and tested them in vitro for AML-specific tumor cell killing in combination with either AML-specific AIM ACT or non-specific naïve or bulk CD8 T cells, which represent TCE monotherapy.

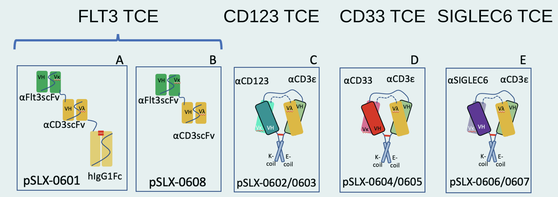
IO/IO combination as a novel treatment paradigm

Manufacturing of NEXI-001



Artificial Immune Modulation Adoptive Cell Therapy (AIM ACT). (1) Leukapheresis material is collected from HLA matched healthy donors. (2) The CD8+ T cells are enriched for using paramagnetic AIM nanoparticles that function as artificial APCs to expand CD8+ T cells specific for tumor associated antigens from AML. (3) After 14 days expansion, AIM nanoparticles are washed out and the AIM ACT is ready to be infused into HLA matched patients. Adopted from Frontiers in Immunology.

Structures of the bispecific T cell engager proteins



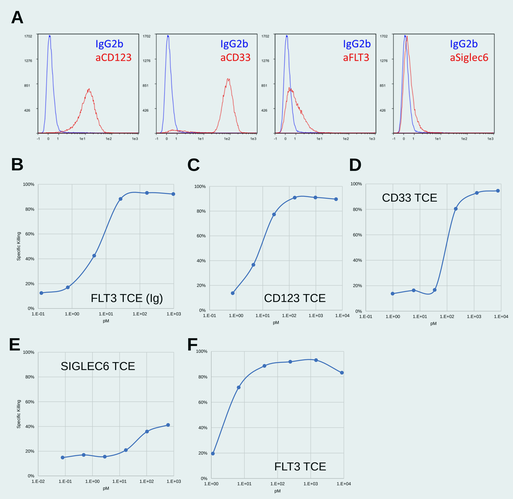
Antigen-specific killing assay with NEXI-001 cells

NEXI-001: Antigen-specific killing tumor cells

T cells and target cells (K562, U266, THP1 and autologous CD4+ T cells) were co-cultured at Effector to Target (E:T) ratios of 5:1 and 10:1 respectively. Before the target cells are added to the T cells, they are labeled with fluorescent dye so that they can be distinguished from the T cells in the assay. A caspase detection reagent is added to this co-culture and the co-culture is incubated at 37ºC for 4 hours. After incubation, caspase activity in target cells is measured by FACS.

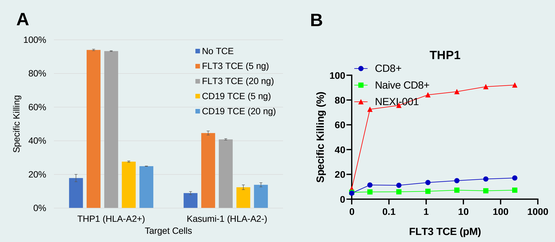
Synergy between NEXI-001 and TCEs

Increased tumor cell killing potency by combining NEXI-001 and TCEs



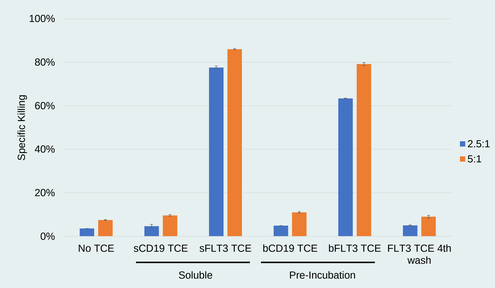
Coincubation of TCEs with NEXI-001 results in highly potent tumor cell killing dependent on AML antigen expression. THP1 cells were stained with fluorescence labeled monoclonal antibodies against human CD123, CD33, FLT3 and SIGLEC6, and the expression of these proteins were analyzed by flow cytometry (A). NEXI-001 killing assay using THP1 as target cells in the presence of various concentration of TCEs, including FLT3 TCE, Ig version (B), CD123 TCE (C), CD33 TCE (D), SIGLEC6 TCE (E) and FLT3 TCE (F). E:T ratio was 2.5:1.

AIM ACT T cells are superior effector cells for TCEs



A. Synergy between NEXI-001 and TCEs depend on both HLA subtype and tumor antigen expression on target cells. CD19 TCE was used as a negative control, and HLA-A2 positive cell line THP1 (Left) and HLA-A2 negative cell line Kasumi-1 (Right) were used as target cells at E:T ratio of 2.5:1. B. Combining TCEs with AIM ACT cells (Red) mediated superior target cell killing when compared to naïve CD8 (Green) and bulk CD8 (Blue) T cells (E:T = 2.5:1).

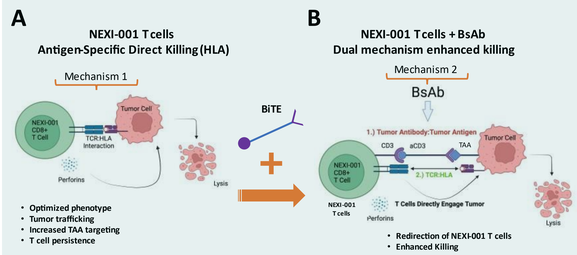
Pre-incubation of NEXI-001 with TCEs works similarly as soluble TCEs



Pre-incubation of NEXI-001 with FLT3 TCE followed by removing unbounded TCEs by repeated washing demonstrates T cells can be armed with TCEs before target exposure. E:T ratios were set to 2.5:1 and 5:1 respectively. Groups (left to right): Control (No TCE), specificity control (sCD19 TCE), positive control (sFLT3 TCE), pre-incubation with CD19 TCE (bCD19 TCE), pre-incubation with FLT3 TCE (bFLT3 TCE) and supernatant from the 4th wash. Absence of activity of 4th wash indicated all unbounded TCEs had been removed.

Dual targeting hypothesis

Directed killing by NEXI-001 T cells combined with TCEs result in superior anti-tumor potency at low doses due to synergistic killing mechanisms



The mechanism of NEXI-001 mediated tumor cell killing as illustrated in Panel A (mechanism 1) is dependent on TCR/MHC interaction. Panel B (mechanism 2) shows the synergistic anti-tumor effect of NEXI-001 T cells in combination with the bispecific T cell engager. These combined mechanisms greatly increase the tumor cell killing even at low doses. Addressing known issues of tumor escape, TCE mediated exhaustion and other concentration dependent SAEs.

CONCLUSION

We present data that if bispecific target is expressed, the combination of bispecific TCE and AIM ACT "fit" T cells offers unique synergy and the potential for superior benefit compared with bispecific TCE alone which relies on engaging with the host endogenous tumor non-specific T cell repertoire. This was confirmed by all TCEs tested, including the SIGLEC6-specific TCE as a negative control. Specifically, we interrogated the potency of different types of T cells as bispecific effectors including naïve and non-specific bulk CD8 T cells isolated from PBMC of healthy volunteers in comparison to our AIM ACT. Analysis of TCR-mediated killing (without TCEs) showed that, non-specific bulk CD8 T cells had little potency while AML-specific AIM ACT can mediate effector to target cell ratio dependent target cell killing. In summary, combining TCEs with AIM ACT cells mediated superior target cell killing than bulk CD8 T cells (representing TCE monotherapy). This enables opportunities for A) increase the spectrum of target by combining TCEs with multi-antigen specific T cells, thus reducing the risk of immune escape. B) increase durability beyond TCEs treatment due to persistence of tumor antigen specific T cells. C) reducing TCE concentration or frequency with the potential to reduce T cell exhaustion and other SAEs.

REFERENCES

1. Nora Philipp et. al. T-cell exhaustion induced by continuous bispecific molecule exposure is ameliorated by treatment-free intervals. Blood. 2022 Sep 8;140(10):1104-1118.
2. Mirco J Friedrich et. al. The pre-existing T cell landscape determines the response to bispecific T cell engagers in multiple myeloma patients. Cancer Cell. 2023 Apr 10;41(4):711-725.e6.

Abstract No. 395 | Presented at the Society for Immunotherapy of Cancer (SITC) 38th Annual Meeting, November 1–5, 2023, San Diego, CA