Bispecific T-cell engager antibodies may contribute to reactivate pre-existing Tumor specific T-Cells

Daniel Primo1, Jaime Pérez Oteyza2, Juan Miguel Bergua3, José Ángel Hernández-Rivas4, Elena Ruiz5, Aurelio López6, Carlos Javier Cerveró6, Milan Jaruninoski5, Roman Hájek12, Atanas Radinoff12, Ilina Micheva12, Rebeca González Rolle12, María Calbacho12, Julian Gorrochategui12, Antonio Valeri12, Joaquín Martínez López12, Joan Ballesteros1

1Vivía Biotech, Madrid; 2Hospital Universitario Sanchinarro, Madrid, Spain; 3Hospital San Pedro de Alcántara, Cáceres, Spain; 4Hospital Universitario Infanta Leonor, Universidad Complutense de Madrid, Madrid, Spain; 5Hospital de Tajo, Madrid, Spain; 6Hospital Universitario Germans Trias i Pujol, Badalona, Spain; 7Hospital Arnau de Vilanova, Valencia, Spain; 8Hospital Virgen de la Luz, Cuenca, Spain; 9Specialized Hospital for Active Treatment of Hematological Diseases, Sofia, Bulgaria; 10University Hospital Ostrava, Ostrava, Czech Republic; 11University Multiprofile Hospital for Active Treatment “Sveti Ivan Rilski” EAD, Sofia, Bulgaria; 12University Multiprofile Hospital for Active Treatment “Sveta Marina” EAD, Varna, Bulgaria; 13Fundación para la investigación, Hospital 12 de Octubre i12-CNIO-CIBERONC, Madrid, Spain.

INTRODUCTION

Cancer immunotherapy aim to harness the power and durability of immune responses. Bispecific T-Cell engager antibodies (BsAbs) redirect T-cells to kill tumor cells by proximity, independent of the intrinsic antigen-specific TCR recognition. However, we have previously demonstrated that following incubation of Acute Myeloid Leukemia (AML) samples with a CD3×CD123 BsAbs, isolating BsAb-activated T-cells by fluorescence-activated cell sorting (FACS), removing BsAbs by wash steps, and adding new autologous blasts, these BsAb-activated T-cells in the absence of the BsAb was able to kill blasts. We hypothesized that the recognition and killing by these isolated activated T-cells may be through classical TCR-HLA antigen-specific recognition. Interestingly, clinically a subset of patients develops a long-term response after BsAb therapy that could point to an expansion of tumor-specific cytotoxic T-lymphocytes against AML.

OBJECTIVE

We aim to identify patients with a TCR-dependent blast-T-cell interaction after in vitro incubation with a CD3×CD123 BsAb on Bone Marrow (BM) from AML patients to potentially select patients with a more durable response after BsAb therapy.

MATERIAL & METHODS

Whole BM were tested in the PharmaFlow platform, an innovative proprietary method that uses flow cytometry (FCM) to efficiently count the number of tumor cells killed by activated T-cells with a CD3×CD123 BsAbs (Creative Bioscience) at 120h at different doses. If the killing capacity of the BsAbs could be measured by the PharmaFlow platform, then the activated T-cells were isolated, washed several times to remove BsAbs, and co-incubated with new autologous isolated blast cells from a cryopreserved vial (Figure 3). For blocking experiments, tumor cells were stained with PKH67 and co-incubated at 0.5:1 ratio at 37°C for two different time points (4h & 24h). The strategy to define and block the TCR interaction was analyze the T-cells expressing PKH67 in the doublet zone by FSC-ASFCH. The Doubles (CD5−CD25+) consist of activated T-cells bound to autologous PKH67 blasts. For blocking experiments, HLA A,B,C (blast cells), oT3R (T-cells) or both were incubated 1h at 37°C before the co-culture (Figure 6).

RESULTS

We hypothesized A New MOA BsAbs

Bispecific TCR-HLA engagement would lead to a MOA distinct from simple cell killing by TAA recognition. We analyzed two distinct MOAs in our patient samples: new MOA, which corresponds to fully activated T-cells, and standard MOA that corresponds to partially activated T-cells due to the presence of BsAbs. We also looked at the correlation between the MOAs and the clinical response to BsAbs (Table 1).

Figure 1. Mechanism of action of BsAbs. In the standard MOA (higher panel), BsAbs bring T cells in proximity of other cells, which activates T cells that kill tumor cells independent of any Tumor-Associated Surface Antigen (TAA) specific recognition. However, in bone marrow of AML patients, some of the T Cells would be TAA T cells, which could also be activated by the BsAbs. We hypothesized a new MOA (lower panel), where the BsAbs activates tumor specific TAA T cells. The difference between these MOAs is that these TAA T cells shown be capable of continued activated killing of tumor cells without the BsAbs.

Figure 2. To test the hypothesis of the new MOA (Fig. 1), we designed the experiment illustrated above: First, we generated activated T Cells lysocytes by incubation with an AML BM sample with a CD3×CD123 BsAb. Second, isolation of the activated T Cells and washing to remove the BsAbs. Third, evaluation of its killing capacity on a second vial from the same sample.

Figure 3. Evaluation of T-Cell killing ex vivo on 8 AML samples generating activated T-Cells by a CD3×CD123 BsAb and incubating with new autologous frozen AML blasts.

CONCLUSIONS

➢ We speculate that proximate contact between T cells and blast cells through an BsAbs could, in some cases, directly reactivate pre-existing Tumor-Associated Antigen T-cells from the BM to recognize and kill AML blasts by themselves.
➢ This secondary T-cell response require cross-presentation by classical APCs or probably the blasts directly, independently of the BsAbs that can be blocked by Anti HLA or TCR antibodies.
➢ Reactivating the immune system could provide those patients with the benefits of long-term duration responses.
➢ Assays that could identify those patients may enable Precision Medicine tests for patient selection.

CONTACT INFORMATION

Joan Ballesteros, CSO Vivía Biotech
jballesteros@viviabiotech.com