ABSTRACT

Background: The PharmaFlow automated flow platform has achieved 85% clinical concordance with AML samples with in vitro immunofluorescence assay. Recently, novel Bispecific Antibodies (BisAbs) or analogous constructs acting through the formation of an immunologic synapse between T-cells (CD25+) and a tumor-associated surface antigen (TAA) have been used in immunotherapy leading to T-cell activation and serial lysis of cells.

Aims: The aim of the present study is to develop and in vitro assay with multiple parameters to better quantify the activity of Bispecific Antibodies and reflect the interpatient variability.

Material & Methods: For this purpose, different fresh whole Bone Marrow (BM) or Peripheral Blood (PB) were tested with their corresponding BisAbs at 4 different concentrations in different time points (24h-48h). In this sense, we tested 31 AML AM samples (5 paired BM and PB) with the CD123xCD5 (Bisako Biolabs) and 7 T-CLL and 2 B-ALL samples with Blnatumab (Angene). When appropriate, basal quantification of TAA was performed by flow cytometry (FCM). The PharmaFlow platform effective dose (E) was calculated by FCM, how many tumor cells are killed by each activated T-cells, hence called effective E:T-ratios. For each sample, 8-color FCM staining was performed to simultaneously analyse the leukemic population, activated CD4+ and CD8+ T-cells and the residual normal cells. EC50 or Emax was evaluated to calculate potential efficiency or efficacy. Kinetics of activity was analyzed repeating the dose-response curves in 3 different days.

Results: Most of the samples present both T-cell activation (CD25+) and an effective lysis of tumor cells after BisAbs-exposure in a 24h-48h dose dependent manner, even starting with low basal E:T ratios (1:100). AML, basal quantification of CD25 by FCM staining was performed to simultaneously analyse the leukemic population, activated CD4+ and CD8+ T-cells and the residual normal cells. EC50 or Emax was evaluated to calculate potential efficiency or efficacy. Results show that the T-cell activation and killing of leukemic cells are dose-dependent. Basal E:T ratios and T-cell kinetics allow us to generate an in vitro response model and select those samples with higher T-cell cytotoxicity after the BisAbs exposure. Interestingly, many of the samples for all the BisAbs have a significant proportion of live cells, even at the highest BisAbs concentrations or with a remarkable expansion of activated T-cells that suggest the use of Immunomodulators to enhance the immunoresistant status.

Conclusions: We have developed an automated flow cytometry assay for Bispecific Antibodies screening that keep intact both the antigen affinity to target (E:T) ratios and Native environment using whole blood or bone marrow samples. In this context, the PharmaFlow platform selects different in vitro T-cytotoxicity effects across patient populations in a cost-effective and fast screening assay for adoptive antitumor immunotherapy with BisAbs. The integration of Effective E:T ratios and pharmacological parameters better predict the in vitro response of BisAbs. Because of the high capacity of the PharmaFlow platform, additional antibodies constructed alone or in combinations with immunomodulatory agents could be tested to identify the better agents or immunomodulatory combinations in hematological diseases.

RESULTS

Simple Version Immunoreactive Tumor

Table 1: Immunoreactive Tumor

Bispecific Antibody

Table 2: Bispecific Antibody

Activated T cells (CD4+/CD8+) T Cells Lead to Tumor Depletion??

Table 3: Tumor Depletion

Activated T cells are the real drug: Effective E:T Ratios

Table 4: Effective E:T Ratios

Figure 1. Screening set-up and Workflow

Figure 2. Dose response curves to assess the CD25+CD4//CD8+ immune effectivity of new BisAbs in AML samples. Upper panel displays leukemic cell killing. The survival index (SI) ranges from 100% to 0% displaying the leukemic cell count after responses to dose response CD25xCD123 Bispecific antibody concentrations (x-axis). A lower panel shows the simultaneous T-cell activation and proliferation taking different time intervals. Absolute cell count of activated T-cells (y-axis) after CD25xCD123 Bispecific antibody dose response concentrations.

Figure 3. Representative example of the Effective E:T (x-axis) ratio from 8 AML samples (x-axis). This method clearly straitly patients with high (left side) vs low T-cell killing activity (right side).

Figure 4. The integration of all these parameters quantifies the BisAb activity selecting cases with higher possibility to Bllatunomab which is the real drug.

Figure 5. Effective E:T ratios: The real drug (CD25+) T cells lead to tumor depletion?

Figure 6. Effective E:T ratios on CLL samples (5): after the CD25xCD123 exposure (24h). Red Line corresponds to the leukemic cells and green line to the activated T-cells (CD4+ or CD8+). Effective E:T ratios, AUC values, EC50, and Emax reflect both T-cell efficacy and leukemic immunomodulation identifying patients likely to receive Bllatunomab therapy.

Figure 7. Effective E:T ratios on AML samples (x-axis) after the CD25xCD123 exposure (24h). Upper panel correspond to the BM and bottom panel to the PB complement. Red Line corresponds to the T-cell population and green line to the activated T-cells (CD4+ or CD8+). BM T-cells are better killers in 3 samples (1, #4, and #5) containing BM immunomodulators: Tumor Infiltrated Lymphocytes (TILs).

Figure 8. Effective E:T ratios on CLL samples (5): after the CD25xCD123 exposure (24h). Red Line correspond to the leukemic cells and green line to the activated T-cells (CD4+ or CD8+). Effective E:T ratios, AUC values, EC50, and Emax reflect both T-cell efficacy and leukemic immunomodulation identifying patients likely to receive Bllatunomab therapy.

Figure 9. Effective E:T ratios on ALL samples (5): after the CD25xCD123 exposure (24h). Red Line correspond to the leukemic cells and green line to the activated T-cells (CD4+ or CD8+). Effective E:T ratios, AUC values, EC50, and Emax reflect both T-cell efficacy and leukemic immunomodulation identifying patients likely to receive Bllatunomab therapy.

Figure 10. Absolute cell counts in a CLL sample for activated T-cells (left panel) and Live tumor cells (right panel) incubated with Bislatumab (presence green (x-axis)) or absence (blue axis) of the immunomodulatory inhibitor checkpoint PD1. Results show increased numbers of both activated T-cells (CD4 and CD8) in presence of PD1, enhancing the overall tumor killing.

CONCLUSION

We report a novel proprietary ex vivo automated flow cytometry assay for I/O drugs keeping intact in a cost-effective target (E:T) ratios and native environment using whole BM or PB.

The PharmaFlow platform selects different in vitro T-cytotoxicity effects across patients identifying best patient candidates for adoptive antitumor immunotherapy with BisAbs in the integration of Effective E:T ratios and pharmacological parameters (EC50, Emax): quantitative pharmacology of BisAbs in patient samples.

Our findings are consistent with a model in which BisAbs can enrich highly cytotoxic clonal T-subsets with Tumor-Specific Antigen in some patients. This assay enable evaluate multiple combinations with immunomodulators (PDT, CTLA-4, TIM-3, LAG-3) or BisAbs candidates for hematological diseases.

Clinical trials should not exclude patient for low expression of TargetCD3.

New design of multiple anti-CD antibodies from our new MOA are empowered by our screening of hundreds constructs ex vivo.

CD8+ T cells may increase substantially the clinical outcomes (IBT).

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