Methods In order to manipulate SAMDH1 levels using Vpx, different Vpx delivery systems were developed. These were virus-like particles (VLPs) packaged with different homologs of Vpx from SIV and HIV-2, and cell-penetrating peptides (CPPs) bound to either a 67 amino acid truncated SIVmac Vpx (67aaVpx) or to the WT full-length form. Two different CPPs were used in the synthesis: TAT and CPP44, the latter is based on a study by Kondo et al..[3]

Results Upon treating different AML cell lines with the VLPs, we observed different SAMHD1-degradation capacities of the different Vpx homologs. Vpx from SIV isolated from macaques (mac239 and mac251) performed the best, compared to Vpx from other lineages. They also increased the ara-C sensitivity of THP-1 cells, which is an AML cell line with high SAMHD1 expression levels, up to 45-fold. Vpx from HIV-2 7312a only partially increased ara-C sensitivity, while HIV-2 Rod9 Vpx did not show any SAMHD1 degradation or improvement in ara-C sensitivity despite its high packaging efficiency in the VLPs.

As for the CPPs, CPP44 bound to 67aaVpx showed better uptake and SAMHD1 degradation compared to the TAT bound 67aaVpx in THP-1 cells. Upon co-treatment with ara-C, up to a 5-fold reduction in IC50 was observed when treated with CPP44-bound 67aaVpx. In an attempt to increase efficiency, full-length Vpx-bound CPPs will be prepared, and trials using these CPPs are currently underway.

Conclusion We demonstrate that inducing SAMHD1 degradation by Vpx delivered via VLPs or CPPs efficiently improved ara-C sensitivity in AML cell lines. Since the VLPs presented a better efficiency compared to the CPPs, we are currently testing their efficiency in primary AML blasts, *ex vivo*. Ultimately, combining a Vpx delivery system with treatments containing ara-C could improve treatment outcomes in high-SAMHD1 patients who fail to respond effectively to ara-C treatment.

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P08.06 USE OF LUCIFERASE-LABELED TARGET CELLS TO EXPLORE IMMUNE CELL KILLING IN HIGH THROUGHPUT FORMAT

C Castro*, Daniel Feger, Sarah Ulrich, Oliver Siedentopf, JanErik Ehlert. *Reaction Biology GmH Europe, Freiburg, Germany*

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With the development and approval of cutting-edge immune-based approaches for cancer treatment in the latest years, the global immune-oncological (IO) pipelines have dramatically increased. To overcome the challenges of growing treatment resistance many new strategies are being developed pointing towards enhancing and sustaining T cell-mediated cytotoxicity, exploiting NK, monocytes and γδT cell killing capacity, and combinatorial therapies, among others.

Examples of such strategies are the bispecific antibodies, which are mainly engagers of T-cells (BiTE´s), but also NK-cell engagers, several of which have entered clinics, such as Blinatumomab (anti-CD3/anti-CD19). Also, apart from the classical anti-PD1/PDL1 and anti-CTLA4, novel immune checkpoint inhibitors are being discovered, studied, and used in combination. Monoclonal antibodies against tumor antigens are being developed to enhance the cytotoxic response of NK and macrophages/monocytes through antibody-induced cell cytotoxicity (ADCC).

To facilitate the efficacy analysis of BiTE´s, and other biologicals in high throughput, we developed a 384-well format assay system based on Luciferase-labeled tumor cells. This detection technology has many advantages. As only the tumor cells are labeled, the specific detection of luciferase exclusively correlates to the number of viable tumor cells, allowing for co-cultures with high excess of effector cells without them interfering in the detection. Due to high sensitivity, only few tumor cells are required for a decent signal/noise ratio, keeping the overall need for primary effector cells to the lowest, even at high effector/target cell ratios. That can be of great advantage when working with rare cytotoxic subpopulations (e.g. NK cells), precious samples from patients, or simply saves resources when performing large exploratory studies. In addition, combination of low 384-well format volumes with nanodrop agent dispensing technology minimizes amounts of expensive antibodies and other agents.

Applying this technology, we evaluated the combinatorial effects of Blinatumomab with immune modulators (e.g. Lenalidomide), kinase inhibitors (e.g. MEK-inhibitor, Selumetinib) and other clinically relevant agents (e.g. MCL1-inhibitor, S63845) on the Luciferase-labeled GCB-like DLBCL cell line OCI-LY1. Depending on compound combination, we observe synergistic but also antagonistic effects. We developed as well, CD3-stimulated T cell killer assays, specially thought to study checkpoint inhibitors or other T cell activation modulators. And finally, we show an ADCC example using NK as cytotoxic cells. Our data supports the outstanding usefulness of this methodological approach for the exploration of bispecific cytotoxic immune cell engager agents.

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