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POSTER ABSTRACTS

703.CELLULAR IMMUNOTHERAPIES: BASIC AND TRANSLATIONAL

Fine Tuning Bispecific Activity in CLL: Harmonizing a CD19/20-T Cell Bispecific with a CD28 or 4-1BBL Costimulatory Bispecific

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Introduction:
T cell bispecific antibodies (TCBs), such as blinatumomab, mosunetuzumab or glofitamab, redirect T cells toward cancer cells and rely on endogenous T cell activation and proliferation (Boissel et al., Nature 2023). Physiological T cell activation not only requires signal 1 provided by TCR engagement but also a signal 2 delivered by positive costimulatory molecules. Like a first-generation CAR-T, TCBs only provide signal 1 through CD3 engagement. Hence, TCB-mediated T-cell activation depends on the expression profile of inhibitory and costimulatory checkpoint molecules expressed by tumor cells (Marcinek et al., Cancer Immunology 2023). As tumor cells often lack expression of positive co-stimulatory ligands like CD80, CD86 and 4-1BBL, we hypothesized that the combination of target-antigen specific TCBs with a co-stimulatory agonistic bispecific can increase TCB efficacy by promoting T cell activity and persistence.

Methods:
We first evaluated the expression level of CD80, CD86 and 4-1BBL on CD19 positive, primary CLL (pCLL) cells by multi parameter flow cytometry (MPFC, n = 6). Next, we co-cultured healthy donor T cells with pCLL cells at a ratio of 1:1 and 1:5 for 6 days in the presence of a CD19- or CD20-TCB in conjunction with a co-stimulatory CD19 directed bispecific providing either CD28 or 4-1BB co-stimulation. T-cell proliferation and cytotoxicity against pCLL cells were analysed by MPFC at day 3 and day 6. Cytokine secretion (IFNγ, IL-2, TNFα) was assessed by cytokine bead array. To further dissect the impact of positive co-stimulation in counteracting evolving TCB-mediated T cell exhaustion, an *in vitro* model system was used. For this, healthy donor T cells were co-cultured with the lymphoma cell line OCI-Ly1 and continuously stimulated with a CD20 directed TCB over 28 days. The exhausted T cells from different time points were co-cultured with pCLL cells and stimulated with CD19 directed costimulatory bispecifics (CD19-CD28 or CD19-4-1BBL) for the assessment of T-cell fitness.

Results:
The phenotype profile of pCLL cells showed only dim expression of various positive costimulatory molecules (CD80: 0.5 %, CD86: 14.4 %, 4-1BBL: 9.7 %). Looking at the impact of positive costimulatory bispecifics in short term cultures with healthy donor T cells and pCLL cells, a significant increase in CD19 and CD20-TCB-mediated cytotoxicity was observed through the addition of CD19-CD28 and CD19-4-1BBL bispecific (CD19-TCB vs CD19-TCB + CD19-CD28: 29.9 % vs 56.1 %, p = 0.0014, CD20-TCB vs CD20-TCB + CD19-CD28: 30.2 % vs 64.8 %, p = 0.0112, CD20-TCB vs CD20-TCB + CD19-4-1BBL: 30.2 % to 50.4 %). In line with these findings, T-cell proliferation increased significantly through the addition of the costimulatory bispecifics (CD19-TCB vs CD19-TCB + CD19-CD28: 12.1 % vs 50.0 %, p = 0.0009; CD20-TCB vs CD20-TCB + CD19-CD28: 24.7 % vs 51.8 %, day 3, E:T = 1:1; CD19-TCB vs CD19-TCB + 4-1BBL: 30.5 % vs 63.2 %, p = 0.0048, day 6, E:T = 1:5). Interestingly, the secretion of IFNy, IL-2 and TNFα was mainly increased through the addition of the CD19-CD28 bispecific (IFNy: 141.6 vs 3151.9 pg/mL, IL-2: 21.5 vs 2311.1 pg/mL, TNFα: 60.8 vs 1945.0 pg/mL, n = 4). Importantly, exhausted T cells exposed to CD20-TCB for 28 days in our long-term culture were characterized as dysfunctional showing i.e. an increased expression of exhaustion markers (LAG3 $^{+}$PD1 $^{+}$Tim3 $^{+}$ 37.4 % to 70.4 %, day 3 vs day 28) and a reduced cytokine secretion ability (IFNγ: 7949.9 to 693.5 pg/mL, day 3 vs day 24). T cell fitness could be reinvigorated in follow up short term assays through the addition of both CD19 directed costimulatory bispecifics. Specifically, exhausted T cells stimulated with CD20-TCB and CD19-CD28 or CD19-4-1BBL

showed a significant increase in TCB-mediated cytotoxicity against primary pCLL cells (CD20-TCB vs CD19-CD28: 15.5 % to 37.7 %, p = 0.0109, CD20-TCB vs CD19-4-1BBL: 15.5 % to 32.9 %, n = 3).

Conclusion:

In conclusion, our data demonstrates the impact of positive co-stimulation on TCB-mediated T cell function. Costimulatory bispecifics provide co-stimulation to T cells in cases of missing expression of costimulatory molecules on tumor cells as well as improving function of exhausted T cells. Clinical trials will be needed to assess the impact of combinatorial strategies to improve T-cell efficacy and to overcome resistance.

Disclosures Korfi: *Roche:* Current Employment. **Herter:** *ROCHE:* Current Employment, Current equity holder in publicly-traded company. **Sam:** *Roche:* Current Employment. **Schuler:** *Roche:* Current Employment. **Klein:** *Roche/Genentech:* Current Employment, Current equity holder in publicly-traded company, Other: Stock ownership, Patents & Royalties. **Bacac:** *Roche:* Current Employment, Current equity holder in publicly-traded company, Patents & Royalties. **Umana:** *Roche/Genentech:* Current Employment, Current equity holder in publicly-traded company, Patents & Royalties. **Subklewe:** *Miltenyi Biotec:* Consultancy, Honoraria, Research Funding; *Janssen:* Consultancy, Honoraria, Research Funding, Speakers Bureau; *Seagen:* Research Funding; *Gilead/Kite:* Consultancy, Honoraria, Other: Travel Support, Research Funding, Speakers Bureau; *Pfizer:* Consultancy, Honoraria, Other: Travel Support, Speakers Bureau; *Takeda:* Consultancy, Honoraria, Research Funding; *AstraZeneca:* Speakers Bureau; *Novartis:* Consultancy, Honoraria, Research Funding, Speakers Bureau; *BMS/Celgene:* Consultancy, Honoraria, Research Funding, Speakers Bureau; *Amgen:* Consultancy, Honoraria, Research Funding; *Roche:* Consultancy, Honoraria, Other: Travel Support, Research Funding, Speakers Bureau; *Ichnos Sciences:* Consultancy, Honoraria; *AvenCell:* Consultancy, Honoraria; *Incyte Biosciences:* Consultancy, Honoraria; *Molecular Partners:* Consultancy, Honoraria, Research Funding; *GSK:* Speakers Bureau; *LAWG:* Speakers Bureau; *Springer Healthcare:* Speakers Bureau; *AbbVie:* Consultancy, Honoraria; *Autolus:* Consultancy, Honoraria; *advesya (CanCell Therapeutics):* Consultancy, Honoraria; *Genmab US:* Consultancy, Honoraria; *Interius BioTherapeutics:* Consultancy, Honoraria; *Nektar Therapeutics:* Consultancy, Honoraria; *Orbital Therapeutics:* Consultancy, Honoraria; *Sanofi:* Consultancy, Honoraria; *Scare:* Consultancy, Honoraria.

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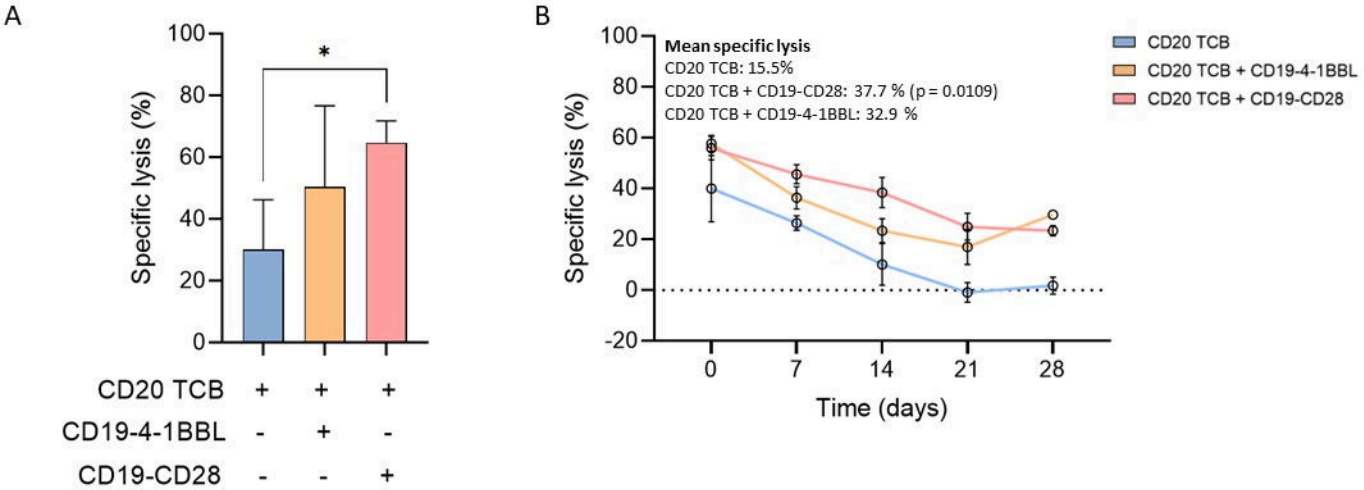

Figure 1: **(A) Co-stimulatory bispecifics increase TCB-mediated T-cell cytotoxicity against primary CLL cells**. T cells were co-culture with pCLL for 6 days and stimulated with CD20 TCB only or in combination with CD28 or 4-1BB co-stimulatory bispecifics. **(B) Reinvigoration of exhausted T cells mediated by co-stimulatory bispecifics**. Healthy donor T cells were co-cultured with the lymphoma cell line OCI-Ly1 and continuously stimulated with a CD20 directed TCB over 28 days. These precultured T cells were put in co-culture with pCLL and stimulated with CD20 TCB only or in combination with CD28 or 4-1BBL co-stimulatory bispecifics. Data represent mean +/- SEM. The statistical analysis was performed using a two-way ANOVA with Bonferroni correction.

Figure 1