Evaluation of the Safety and Efficacy of Bi-specific Antibodies using Organoids and Colorectal Cancer Spheroids

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BACKGROUND

The tumor microenvironment (TME) involves complex interactions between cancer and immune cells, creating an immunosuppressive niche that promotes tumor growth. Developing ex vivo models that accurately mimic these dynamics is essential for advancing immunotherapy, as traditional 2D co-cultures fail to capture this complexity. Our study introduces colorectal tumoroids as a 3D model to investigate immune-cancer cell interactions under T cell bispecific (TCB) antibody treatment, providing a platform to evaluate novel therapeutic strategies with greater precision and relevance.

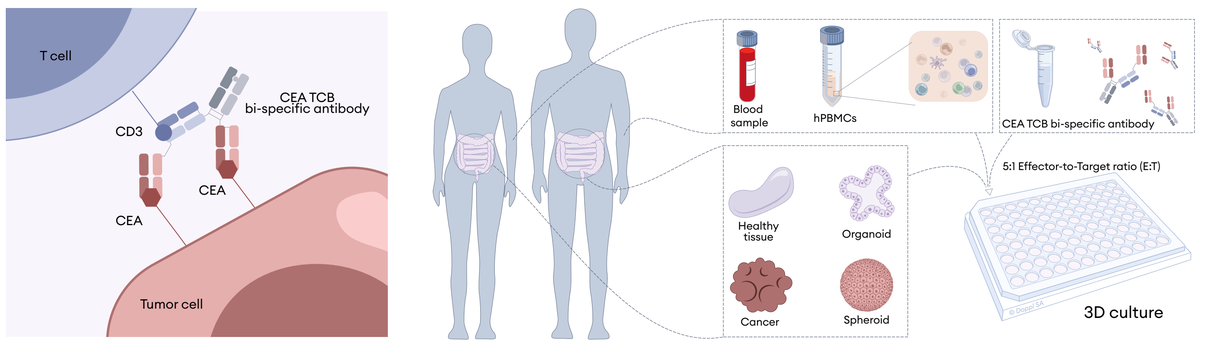

Fig. 1: Co-culture set up of human PBMCs, healthy and colorectal cancer cells and CEA TCB bi-specific antibody

METHODS

We established a comprehensive 3D redirect lysis (3D-RDL) assay using colorectal cancer spheroids and adult stem cell-derived, healthy human organoids, both co-cultured with human Peripheral Blood Mononuclear Cells (hPBMCs), to evaluate the efficacy and safety profile of *Cibisatamab* - a bispecific antibody targeting carcino-embryonic antigen (CEA) on cancer cells and CD3 on T cells. This model allows to assess the cytotoxic activity and immune responses, capturing variations in therapeutic response not observable in simpler systems.

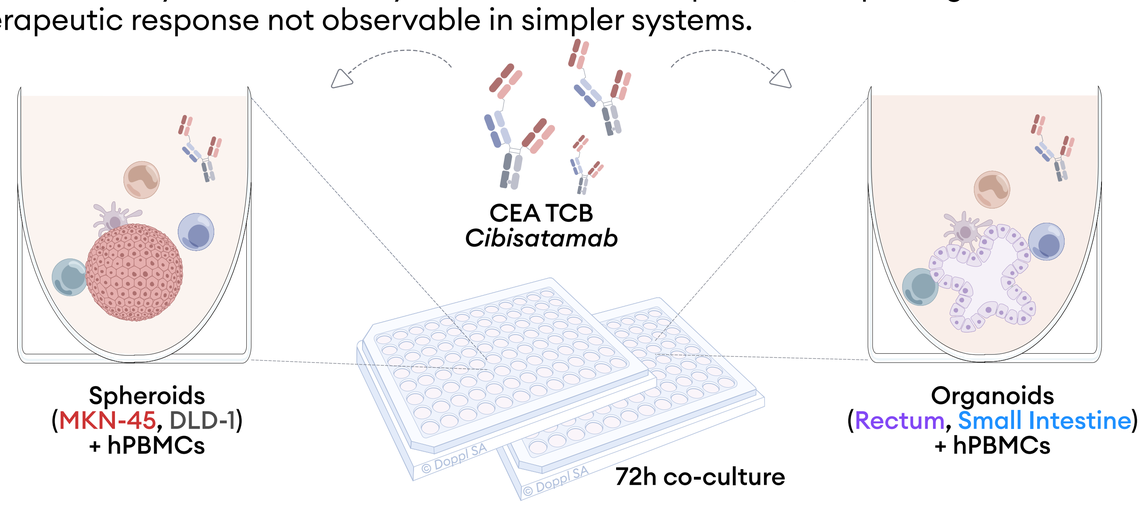

Fig. 2: Advanced 3D redirected lysis assay of human PBMCs, healthy human organoids and colorectal cancer spheroids to evaluate the efficacy and safety of *Cibisatamab*, a bispecific antibody targeting carcinoembryonic antigen (CEA) on cancer cells and CD3 on T cells.

POTENCY & SAFETY STUDIES

Our model integrates live imaging and cytotoxicity analyses to enable precise, real-time tracking of antibody effects on CEA-expressing tumor cells compared to healthy cells.

Additionally, Flow cytometry and Granzyme B release profiling further allowed us to characterize immune cell activation, revealing distinct T cell activation markers and Granzyme B release patterns tied to *Cibisatamab* treatment.

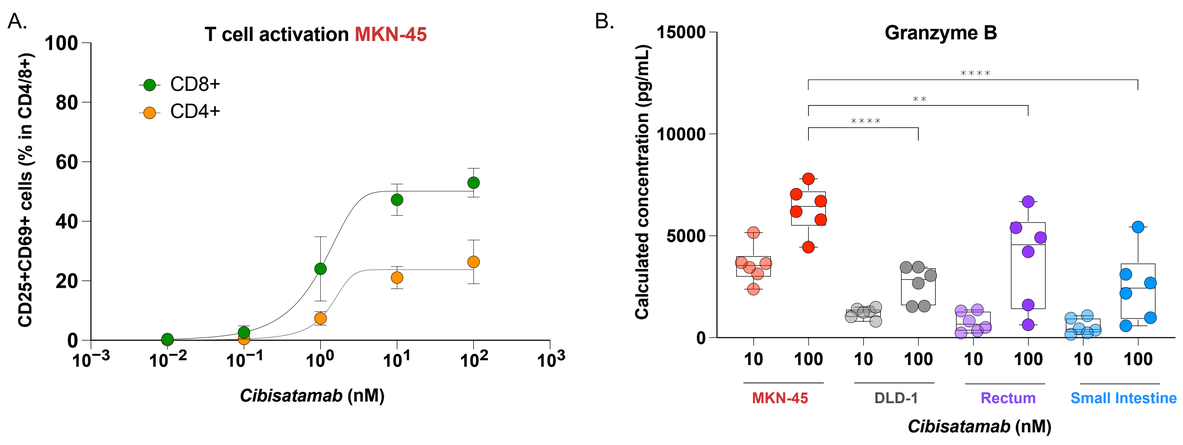

Fig. 3: Potency and safety studies. A. T cell activation is shown for MKN-45 with the percentage of live CD8 cells that express CD25 and CD69 upon CEA-TCB treatment. B. Granzyme B quantification after 72h co-culture of rectal/intestinal healthy organoids and 3D grown MKN-45/DLD-1 spheroids in the presence of hPBMCs treated at different doses of CEA TCB.

CONCLUSION

- **Organoid biobank** enables candidate selection for on-target, on-tumor studies.
- Preclinical pipeline assesses immune-modulatory agents with **RNA-seq, flow cytometry, cytotoxicity assays** and **imaging-based readouts**.
- *Cibisatamab* induced cancer cell death in high-CEA spheroids while showing **dose-dependent off-tumor effects in healthy organoids**, demonstrating the model's ability to predict immunotherapy outcomes.
- Supports therapies like **checkpoint inhibitors, NK cell engagers**, and **combination treatments**.

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RESULTS

The cell-surface glycoprotein CEA is highly expressed in gastrointestinal tissues (Fig. 4A). RNA and protein analysis of **Doppl SA's** organoid biobank shows the highest CEA levels in rectal and colon organoids, with lower levels in small intestinal organoids (Fig. 4B,C). Organoids thus provide an alternative platform for assessing antibody potency compared to CEA_{high} (MKN-45) and CEA_{low} (DLD-1) adenocarcinoma cell lines.

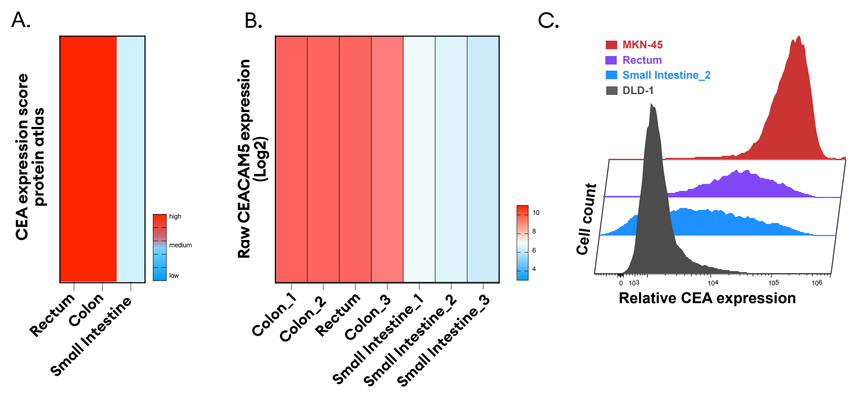

Fig. 4: CEA protein and RNA expression in human, organoids and cell lines. A. CEA protein expression scores of rectum, colon and small intestine from the human protein atlas. B. RNA expression values of CEACAM5 in rectum, colon and small intestinal organoids. C. FACS analysis of surface-expressed CEA in cell lines MKN-45, DLD-1, as well as small intestinal and rectal organoids.

We evaluated antibody potency and safety in 3D by combining our image-based workflow with viability analyses and specific lysis determination in a high-throughput manner. *Cibisatamab* induced cancer cell death in high-CEA spheroids and dose-dependent off-tumor effects in healthy organoids co-cultured with human PBMCs.

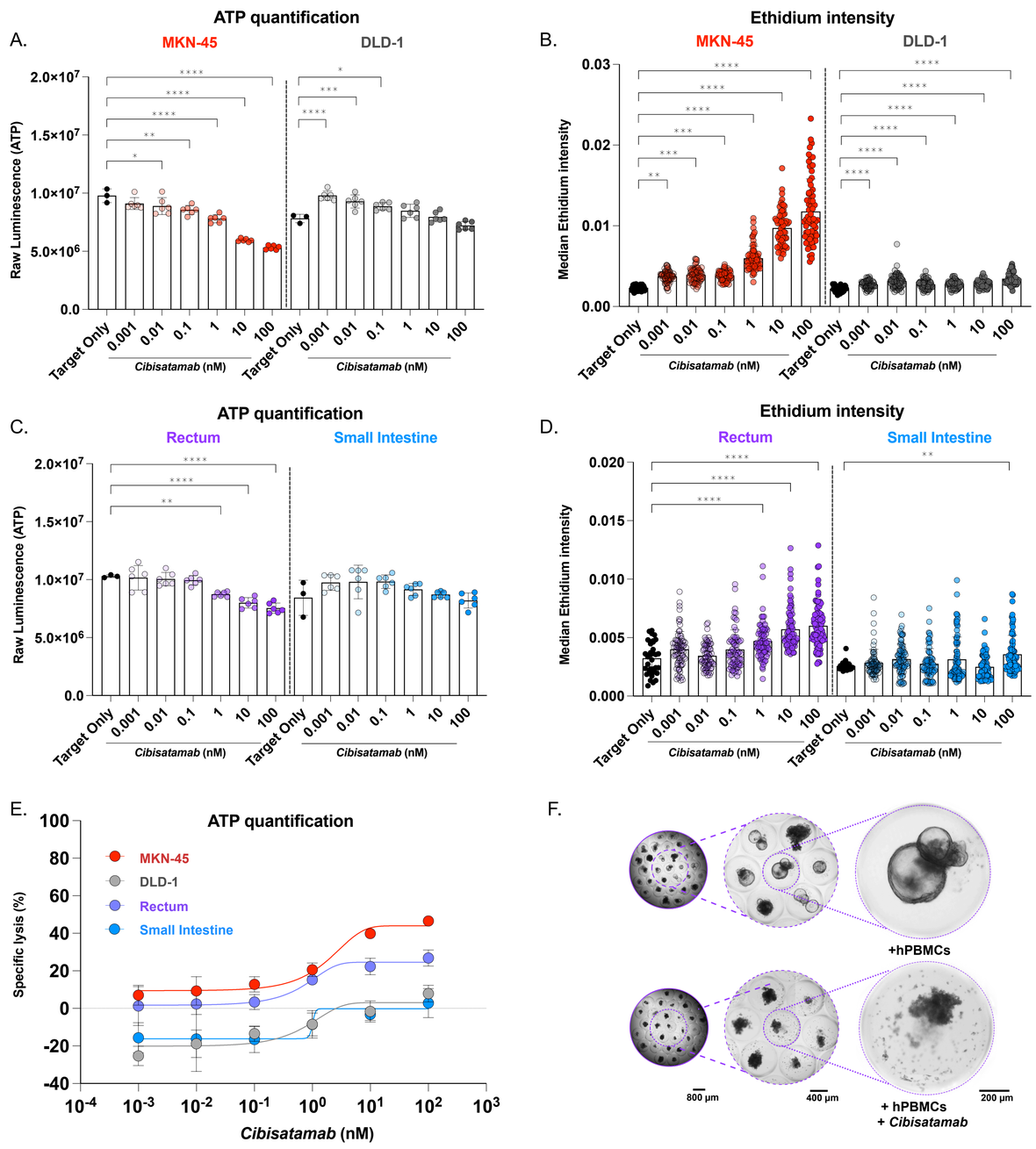

Fig. 5: Antibody potency. Spheroid and organoid viability was measured by quantifying ATP content (A.,C.) and Ethidium median intensity (B., D.). Specific lysis (E.) was calculated as follow: ((Sample - Target Only)/(TritonX - Target Only)). All results were obtained after 72h co- culture of PBMC's isolated from 3 different healthy volunteers tested in duplicates at 5:1 (E:T) effector-to-target ratio. The effect of the *Cibistatmab* treatment in rectal organoids was also detected in Bright-field images (F).Each well is composed by 31 microwells containing one organoid or spheroid each. Mean values +/- SD. One-way ANOVA with Sidak multiple comparisons test. Mean comparison of each column with the mean of control column (target only).

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