Poster #: 1906

XXI IWCLL International Workshop on CLL

Fibroblast mediated Modulation of bispecific Antibody Treatment in Chronic Lymphocytic Leukemia

Anton von Lom[1,2,3], Alexander vom Stein[1,2,3], Hendrik Jestrabek[1,2,3], Phuong-Hien Nguyen[1,2,3], Michael Hallek[1,2,3]

[1]University of Cologne, Faculty of Medicine and University Hospital Cologne
[2]Cluster of Excellence Cellulahr Stress Responses in Aging-associated Diseases (CECAD)
[3]Center for Integrated Oncology Aachen Bonn Cologne Düsseldorf, Germany

OBJECTIVES

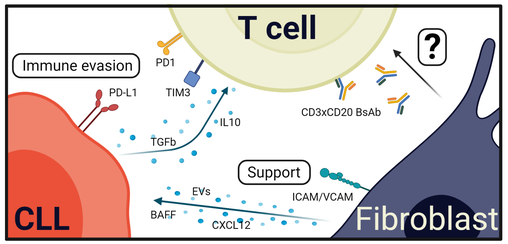
- **Does the stromal tumor microenvironment influence bispecific antibody (BsAb) efficacy in Chronic Lymphocytic Leukemia (CLL)?**
- **What are possible therapeutic strategies for enhancing BsAb responsiveness by targeting the stromal compartment?**

CONCLUSIONS

- **Suprisingly, the presence of fibroblasts enhances BsAb mediated T cell killing of CLL cells *in vitro.***
- **Fibroblasts increase expression of T cell activation markers and T cell cytotoxicity.**
- **Modulation of stromal tumor microenvironment represents a potential target for enhancing immunotherapy in CLL.**

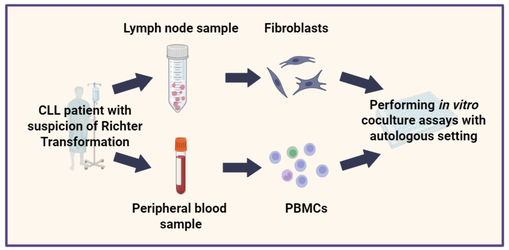
Contact: anton.von-lom@uk-koeln.de

INTRODUCTION



- CLL cell survival strongly depends on interactions within the tumor microenvironment (TME).[1]
- Fibroblasts in lymphoid homing sites can adopt a lymphoma induced CAF phenotype, promoting immune evasion and therapy resistance.[1,2]
- Due to their immunomodulative effects, fibroblasts may influence bispecific antibody (BsAb) efficacy, which is reduced in CLL compared to other B cell malignancies.[2,3]

METHODS



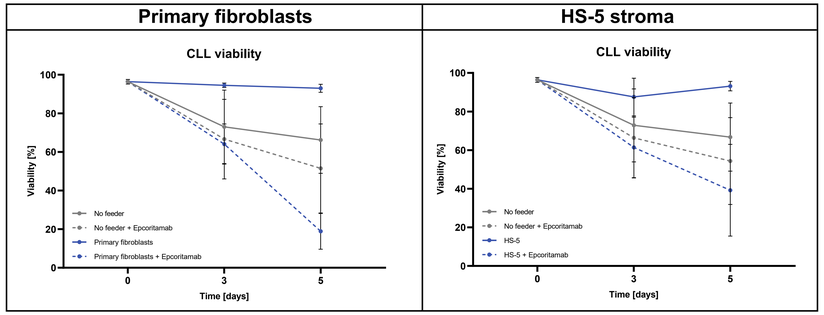
- An ex vivo model with primary lymph node fibroblasts and autologous CLL PBMCs was established.
- Cocultures of stromal cell line HS-5 with PBMCs from healthy donors and CLL patients were performed in addition.
- CD3xCD20 bispecific antibodies (Epcoritamab and Glofitamab) were applied to assess effects on CLL viability and T cell phenotype
- Stromal modulators (TGFb and TGFb inhibitor) were applied to our coculture systems.
- Flow cytometry was used to analyze CLL cell survival, T cell viability, activation and exhaustion.

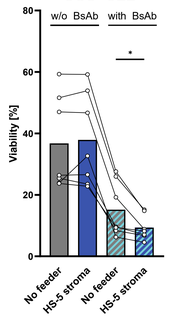
REFERENCES

1. vom Stein, A. F., et al. (2023). "LYN kinase programs stromal fibroblasts to facilitate leukemic survival via regulation of c-JUN and THBS1." Nature Communications 14(1): 1330.
2. Apollonio, B., et al. (2023). "Tumor-activated lymph node fibroblasts suppress T cell function in diffuse large B cell lymphoma." J Clin Invest **133**(13).
3. Cheson, B. D. and J. P. Sharman (2024). "Current Approaches and Novel New Agents in the Treatment of Chronic Lymphocytic Leukemia." JCO Oncol Pract: Op2300770.

RESULTS

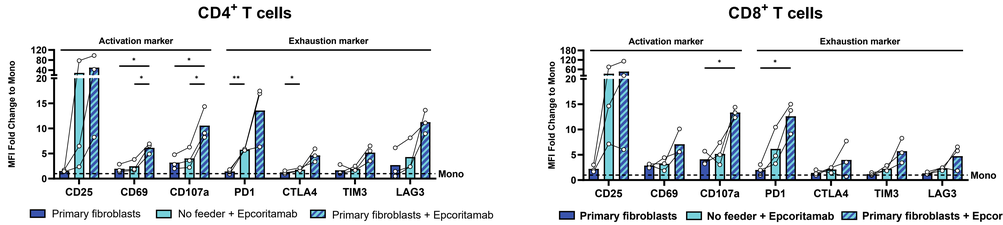
1. Stroma cells support bispecific antibody immunotherapy *ex vivo* and *in vitro*





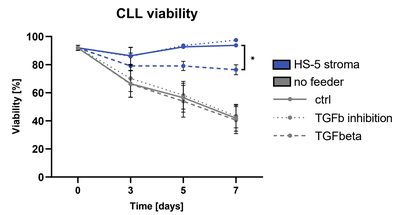
- Stroma cells show a strong feeder effect on CLL cells when cocultured together, resulting in an increased viability (solid blue line), while CLL cells in monoculture undergo spontaneous apoptosis (solid grey line).
- Adding CD3xCD20 bispecific antibody to the coculture reduces CLL cell viability (grey dotted line) with the strongest effect seen in the presence of stromal cells, especially with primary lymph node fibroblasts (blue dotted line).
- This effect can be recapitulated using HD PBMCs with OSU CLL cell line that is not depending on feeder cells as primary CLL cells are. (right graph)

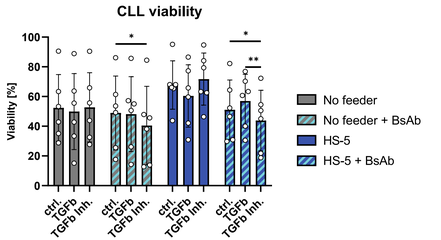
2. Fibroblasts potentiate T cell activation and cytotoxicity upon BsAb treatment



- Combination of CD3xCD20 BsAb and fibroblasts induces stronger upregulation of activation markers CD25 and CD69 on both $CD4^+$ and $CD8^+$ T cells.
- Degranulation marker CD107a is markedly increased upon addition of fibroblasts, indicating enhanced T cell cytotoxicity.
- Immune checkpoint receptors are also upregulated, likely reflecting strong activation rather than functional exhaustion.

3. TGF beta modulates CLL viability in a stroma dependent manner and influences BsAb therapy response





- TGF beta reduces CLL cell viability in the presence of HS-5 stroma cells but not in the monoculture. TGF beta inhibition shows no significant effect on CLL viability (left graph).
- Upon CD3xCD20 BsAb treatment, TGF beta increases CLL viability in the presence of stromal cells, whereas TGF beta inhibition slightly decreases it, indicating an interaction between TGF-β signaling and immunotherapy response (right graph).
- The modulatory effects of TGF beta are more pronounced in stromal coculture than in CLL monoculture, supporting the hypothesis of an indirect effect mediated by TGF beta responsive stromal cells.