

Bispecific PSMAxCD3 Diabody

Novel Immunotherapeutic Agent for Prostate Cancer

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Technology

In the field of cancer immunotherapy, the retargeting of T-cells to tumor cells by bispecific antibodies or diabodies is an appealing new therapeutic concept. Since the Prostate Specific Membrane Antigen (PSMA) represents an excellent tumor target for prostate cancer immunotherapy, we have generated a diabody specific for human PSMA and the T-cell antigen CD3 ϵ . Specific binding of the PSMAxCD3 diabody both to CD3-expressing Jurkat-cells and PSMA-expressing C4-2 cells was shown by flow cytometry. In vitro the PSMAxCD3 diabody proved to be a potent agent for retargeting human lymphocytes to lyse C4-2 prostate cancer cells. In vivo, treatment of C4-2 xenografts bearing SCID mice with the diabody and lymphocytes efficiently inhibited tumor growth.

Innovation

- The PSMAxCD3 diabody represents the first recombinant diabody against PSMA
- The PSMAxCD3 diabody is highly effective in retargeting human lymphocytes for specific lysis of PSMA expressing prostate cancer cells in vitro
- Application of the PSMAxCD3 diabody together with human lymphocytes inhibits the growth of prostate cancer in a xenograft model in vivo

Application

- The PSMAxCD3 diabody represents a novel tool for the immunotherapy of prostate cancer
- The PSMAxCD3 diabody bears the potential for the elimination of minimal residual disease

Market Potential

Considering that prostate cancer is the most frequent malignant disease in men in the Western world, the PSMAxCD3 diabody as a potent novel therapeutic agent has the potential for significant sales worldwide.

Responsible Scientist

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A Bispecific PSMAxCD3 Diabody as Immunotherapeutic Agent for Prostate Cancer

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Background

Cross-linking of tumor antigens with T-cell associated antigens by bispecific monoclonal antibodies or diabodies has been shown to increase antigen-specific cytotoxicity in T-cells. These retargeted T-cells range among the most potent effector cells of the immune system.

For a diabody based immunotherapy the Prostate Specific Membrane Antigen (PSMA) has been shown to represent an excellent target, because it is expressed on all prostate cancer cells as dimer with multiple binding sites.

Materials & Methods

Generation of the PSMAxCD3 diabody

A heterodimeric diabody specific for human PSMA and the T-cell antigen CD3 was constructed from the DNA of an anti-CD3 and an anti-PSMA single chain Fv fragment (scFv). It was expressed in *E. coli* from a vector containing a bicistronic operon for co-secretion of the hybrid scFv V_H CD3- V_L PSMA and V_H PSMA- V_L CD3. The resulting PSMAxCD3 diabody was purified from the periplasmic extract by immobilized metal affinity chromatography.

Binding

The binding properties were tested on PSMA-expressing C4-2 prostate cancer cells and PSMA-negative cell lines (DU 145, PC3) as well as on CD3+ Jurkat cells by flow cytometry.

Tumor cell lysis *in vitro*

For *in vitro* functional analysis PSMA-expressing C4-2 cells were coincubated with human CD3+, CD4+ or CD8+ lymphocytes and diabody for 48 h. Then, a cell viability test (WST) was performed to determine the number of remaining viable tumor cells.

Antitumor activity *in vivo*

For *in vivo* evaluation, the diabody was applied together with human peripheral blood lymphocytes (PBL) in a C4-2 xenograft SCID mouse model. A single injection of 5×10^6 lymphocytes was given at day 6 after tumor inoculation together with 10 μ g diabody, followed by four additional diabody doses of 10 μ g at days 7, 10, 11, and 12.

Results

→ We have generated a bispecific PSMAxCD3 diabody, that shows specific binding to cell-adherent PSMA and the T-cell antigen CD3.

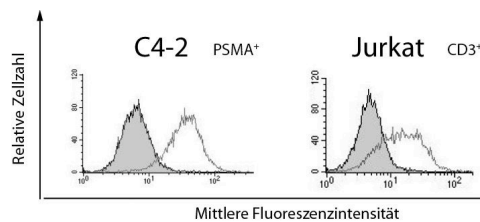


Fig.1: Binding of the PSMAxCD3 diabody to PSMA-positive C4-2 cells and CD3+ Jurkat cells as shown by flow cytometry.

→ *In vitro* the PSMAxCD3 diabody proved to be a potent agent for re-targeting human lymphocytes to lyse C4-2 prostate cancer cells. This effect was seen with CD3+, CD4+ and CD8+ lymphocytes, which were equally activated upon coincubation with tumor cells and diabody.

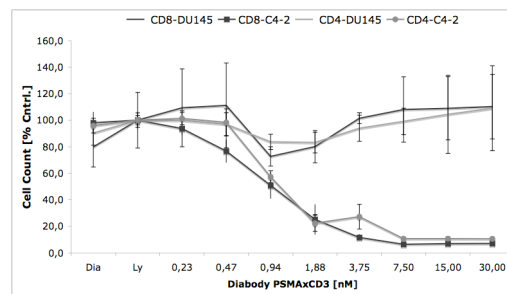


Fig.2: Diabody-mediated tumor cell lysis. WST assays of PSMA-positive C4-2 cells and PSMA-negative DU 145 cells after coincubation with human CD4+ or CD8+ T-lymphocytes and diabody.

→ Treatment of SCID mice inoculated with PSMA-positive C4-2 prostate cancer xenografts with the PSMAxCD3 diabody and human lymphocytes efficiently inhibited tumor growth.

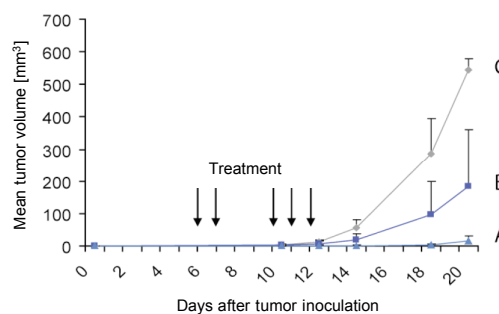


Fig. 3: Treatment of C4-2 xenografted SCID mice with (A) lymphocytes plus diabody, (B) lymphocytes alone and (C) PBS vehicle.

Conclusions

Due to its high anti-tumor effectivity the PSMAxCD3 diabody is suggested as a potent novel therapeutic agent for prostate cancer

References

- Elsässer-Beile, U. et al. (2006). *Prostate* 66(13):1359-70.
- Bühler, P. et al. (2008). *Cancer Immunol Immunother* 57(1):43-52