

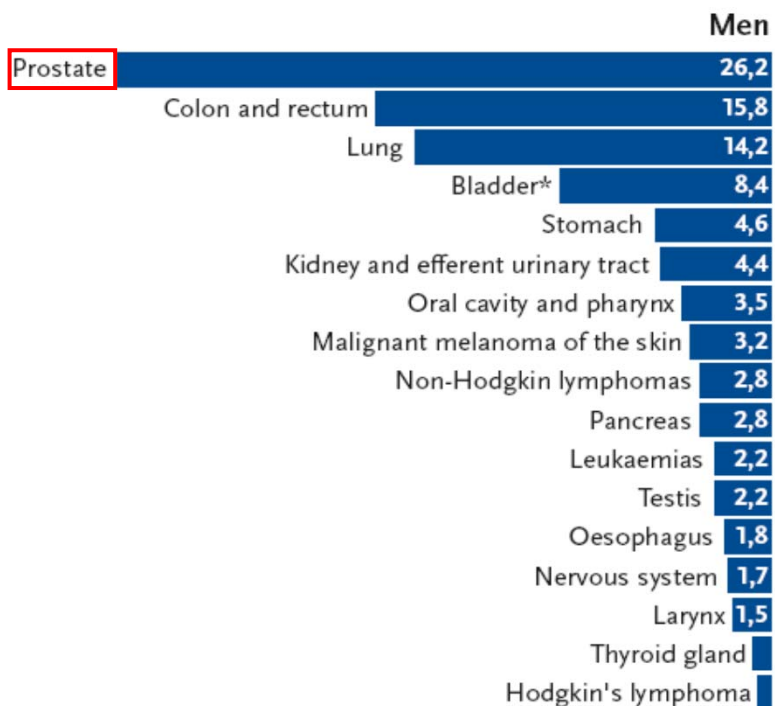
Targeted therapy of prostate cancer with a PSMAxCD3 bispecific single-chain diabody

Kerstin Fortmüller

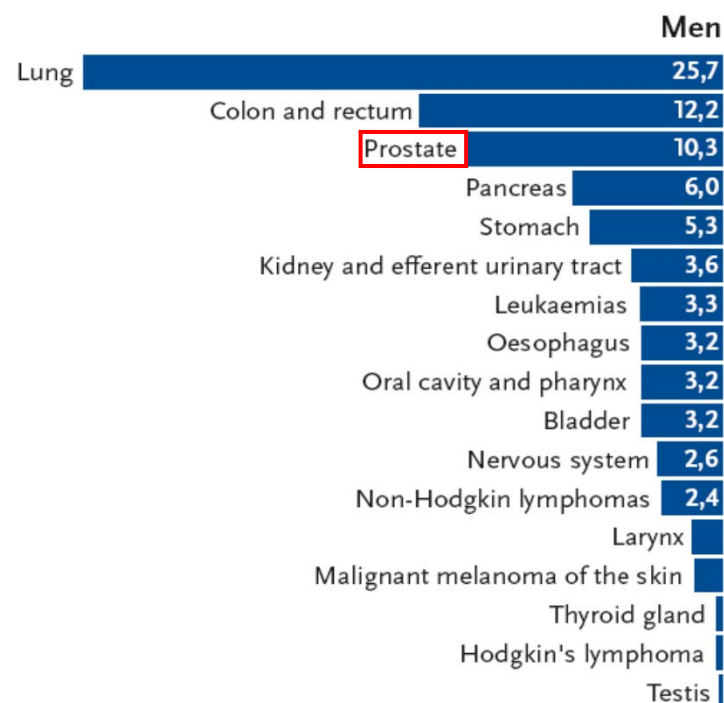
University Hospital Freiburg

Prostate cancer statistics in Germany 2006

% of all new cancer cases
(excluding non-melanoma skin cancer)

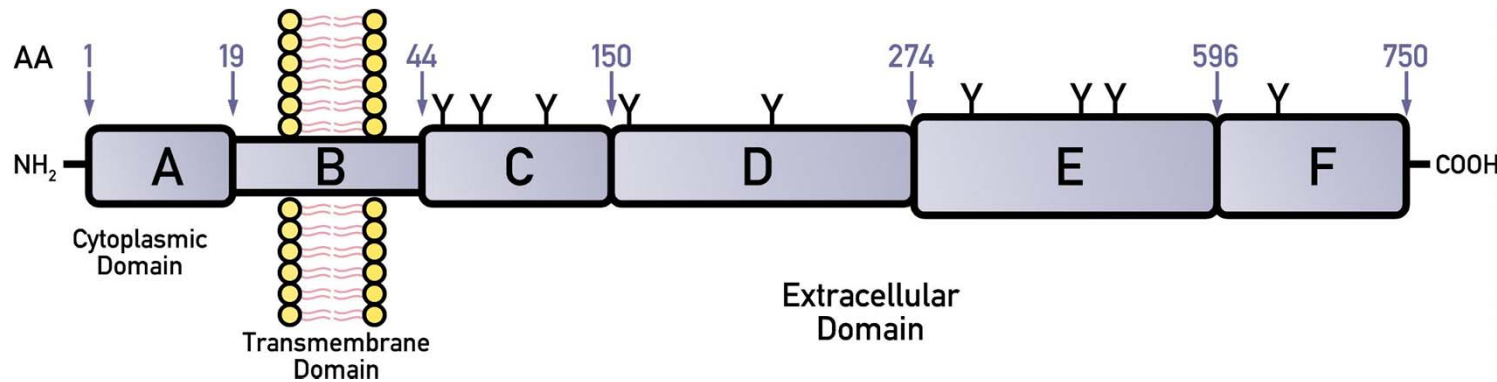


% of all new cancer-related deaths



Source: estimate by the Federal Cancer Surveillance Unit at the Robert Koch Institute (2010)

The prostate specific membrane antigen



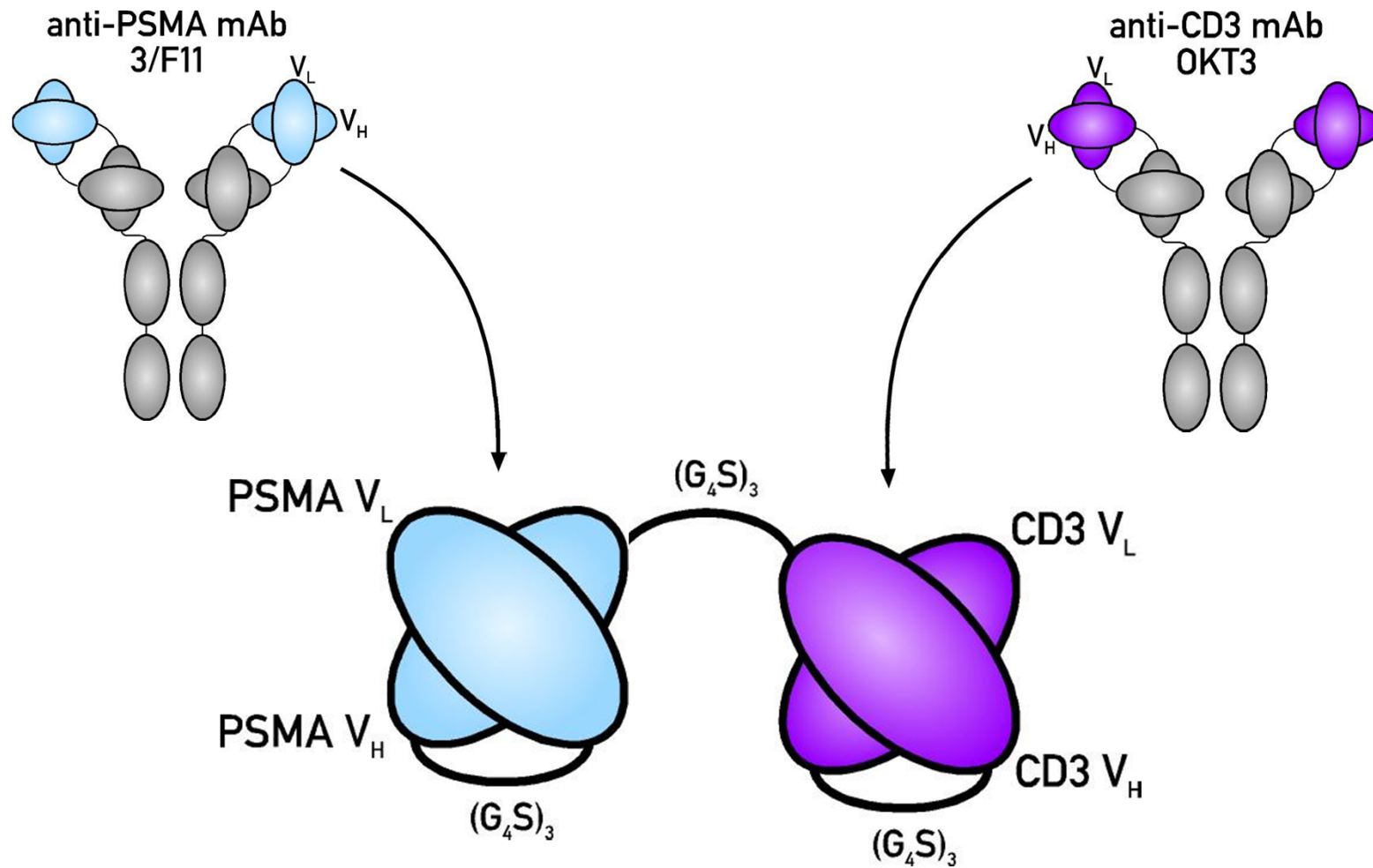
- Type II membrane glycoprotein
- Approximately 100 kDa
- Small intracellular domain
- Small transmembrane domain
- Large and glycosylated extracellular domain

The prostate specific membrane antigen

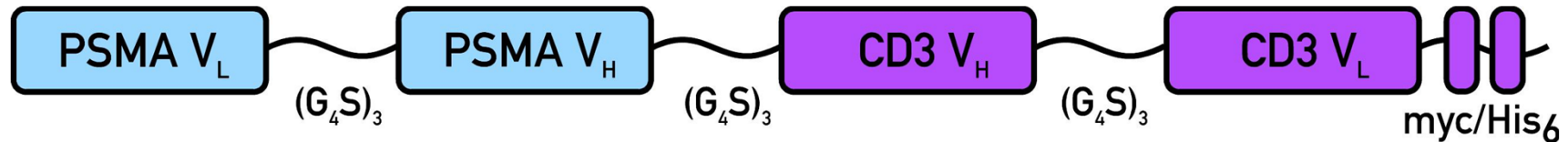
- Selectively expressed as a homodimer
- Upregulated in all stages of prostate cancer
- Not released into the circulation

This makes PSMA an excellent target for immunotherapy

Construction of the PSMAxCD3 diabody

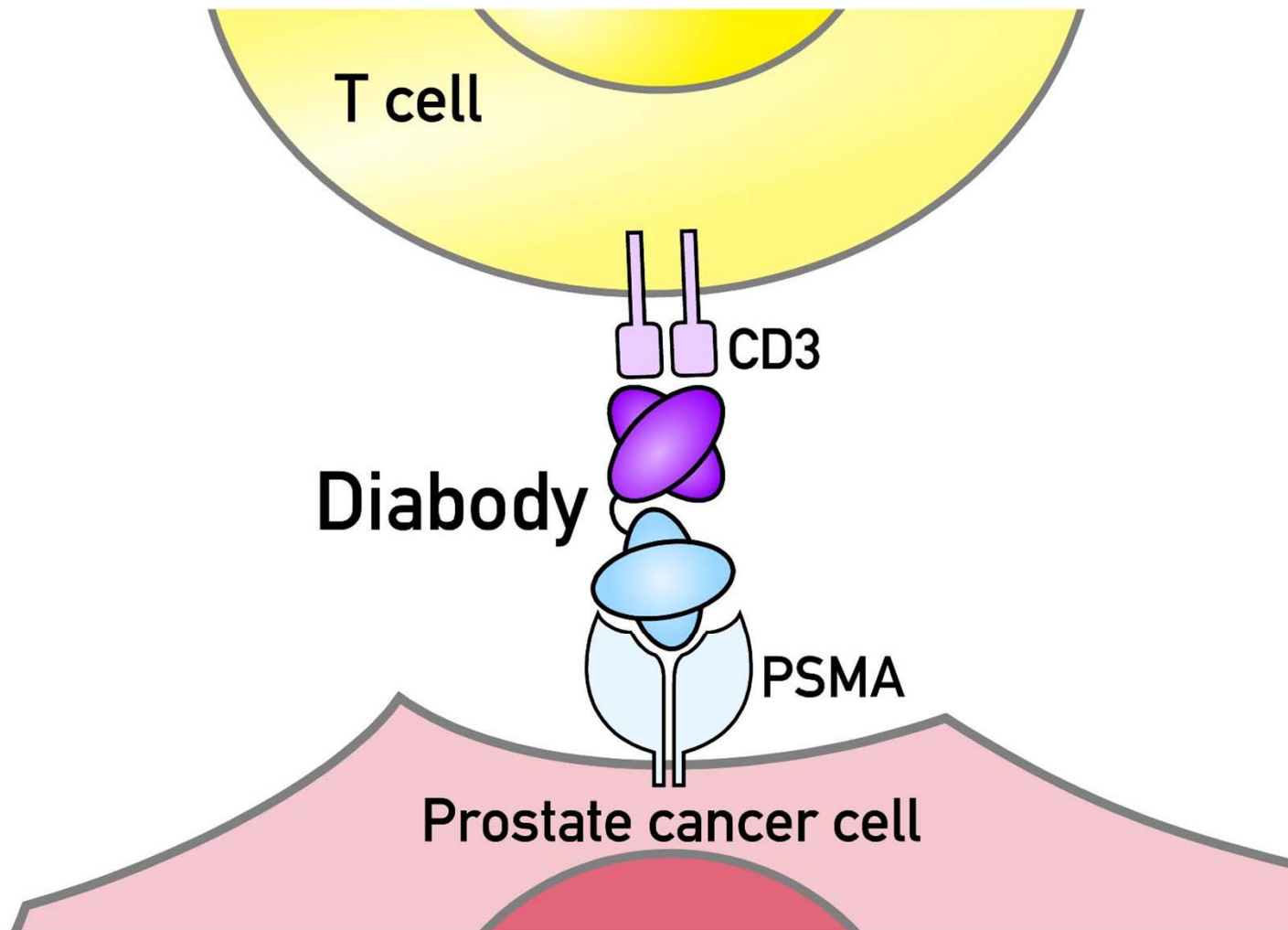


Expression of the diabody in *E.coli*

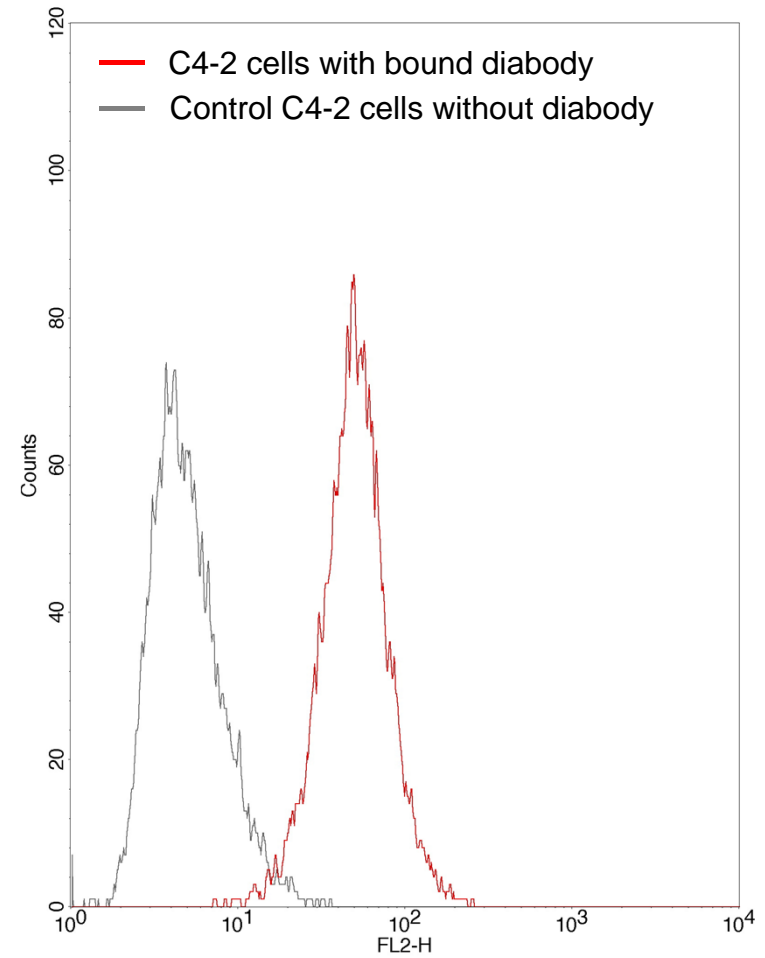
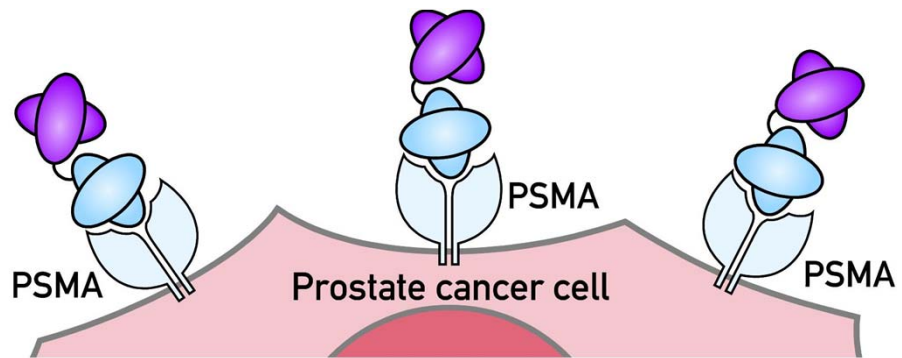


- Recombination of gene segments into a bacterial expression vector
- Expression in *E.coli*
- Purification from periplasm and culture supernatant via His-Tag
- Yield about 100-150 µg/l

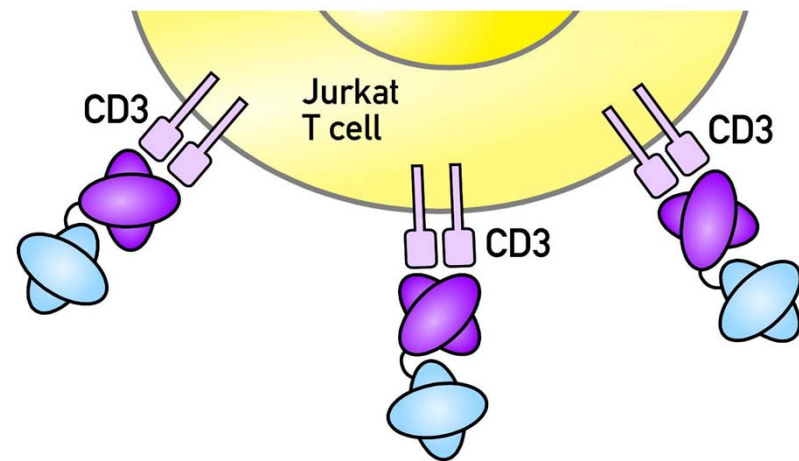
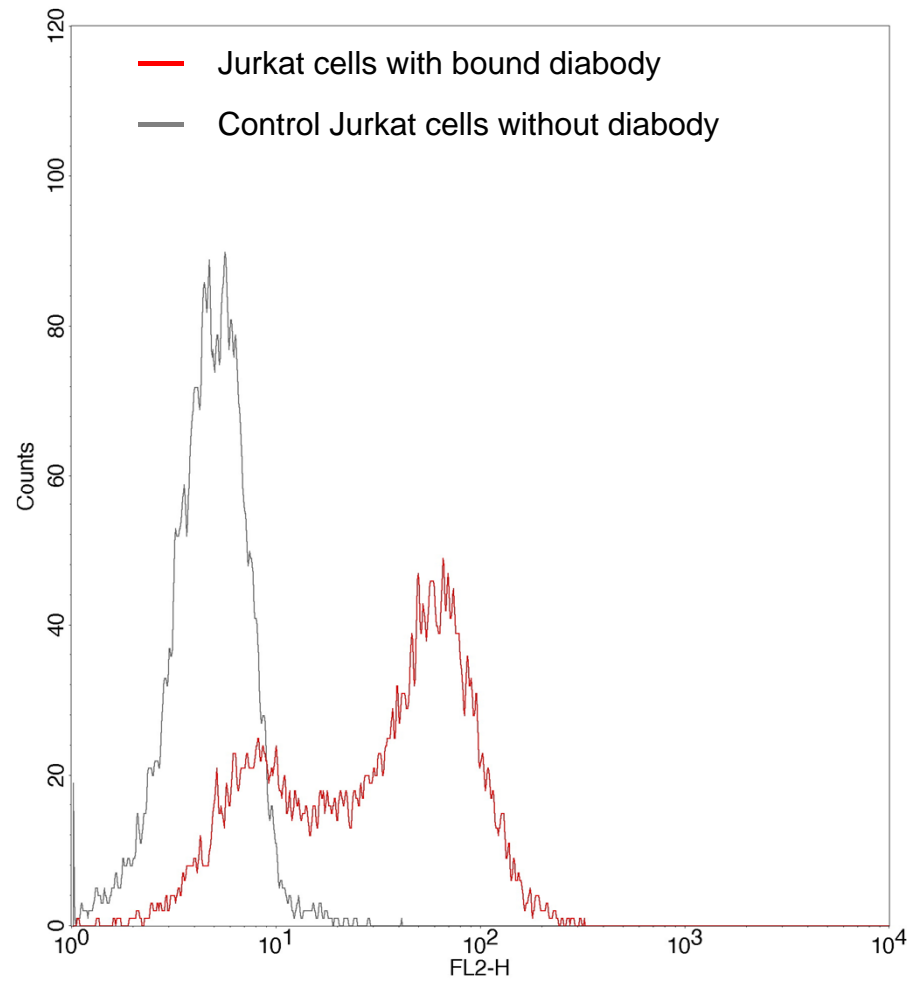
Diabody: Linking a T cell with a tumor cell



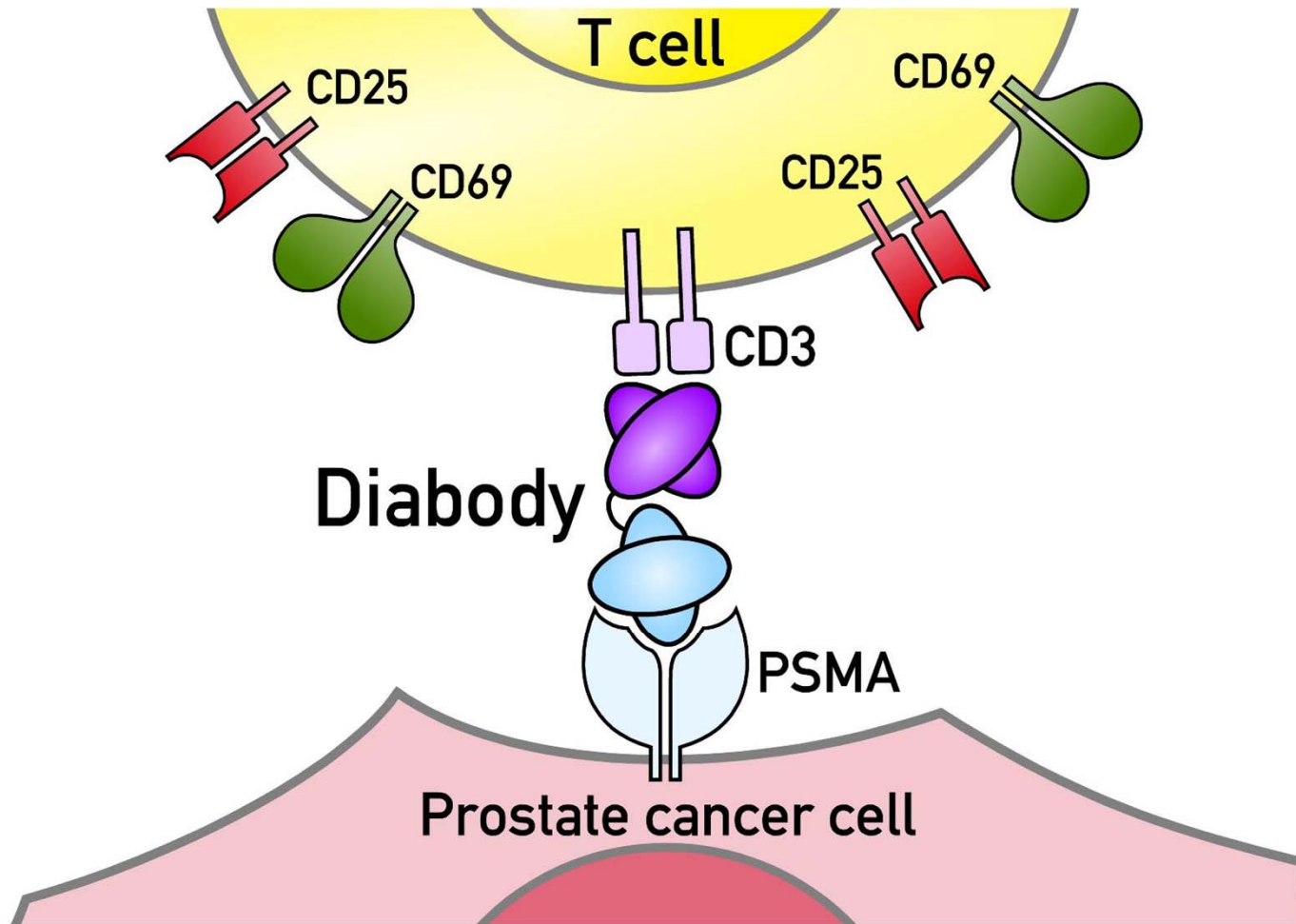
Evaluation of binding properties: PSMA



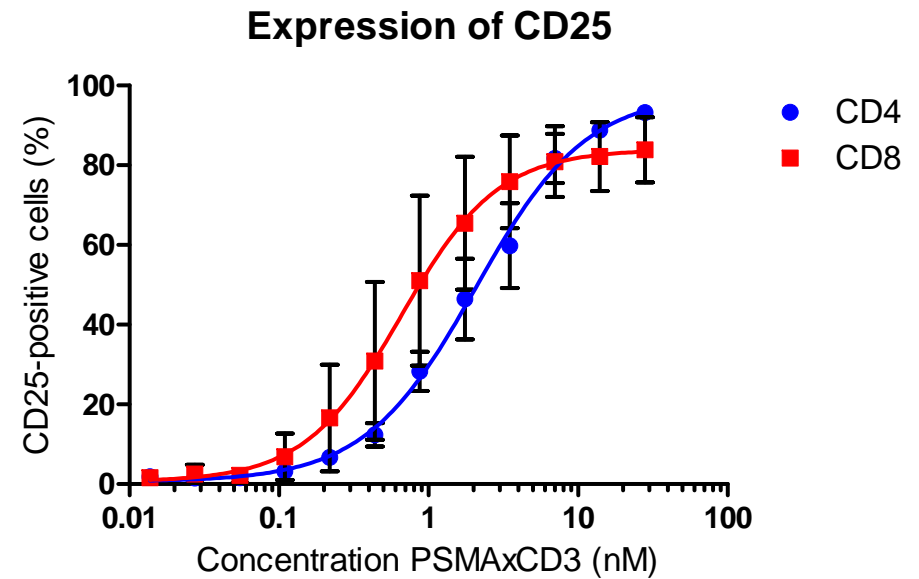
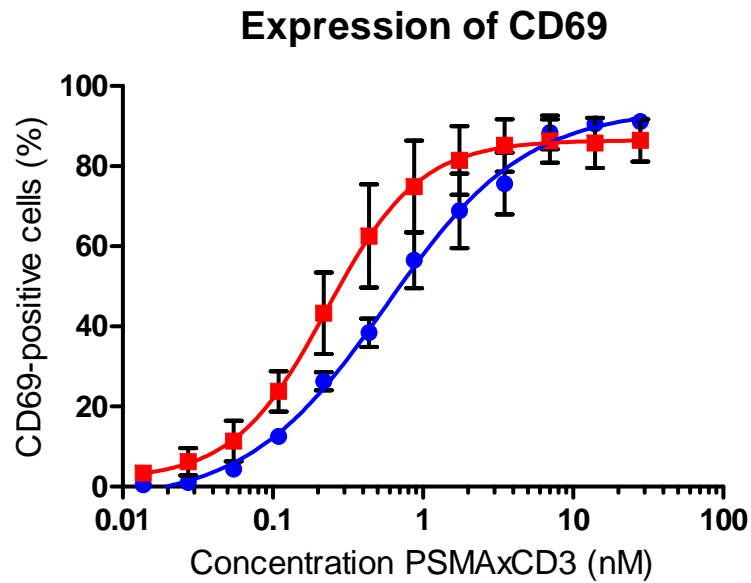
Evaluation of binding properties: CD3



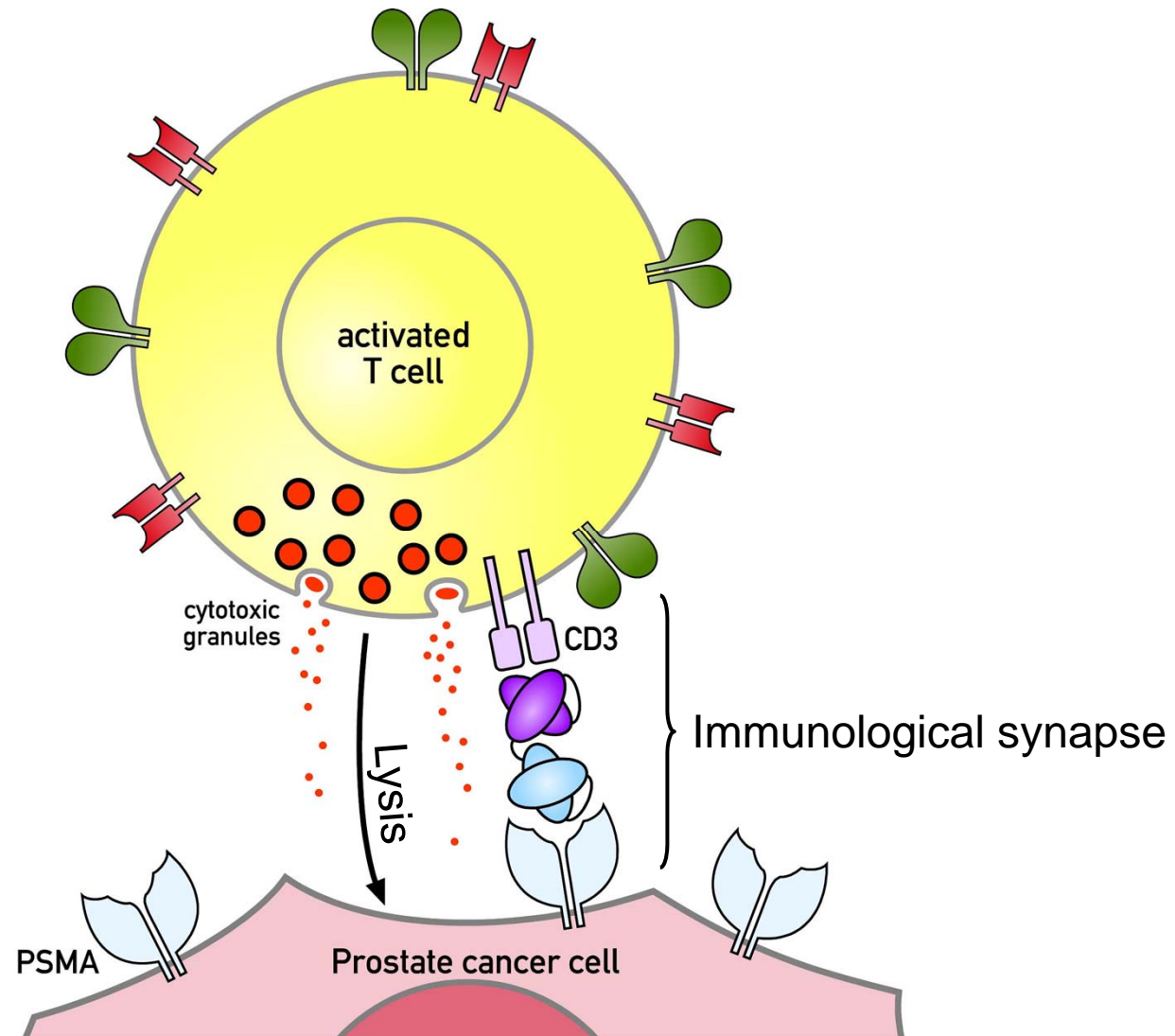
Diabody: Induction of T cell activation



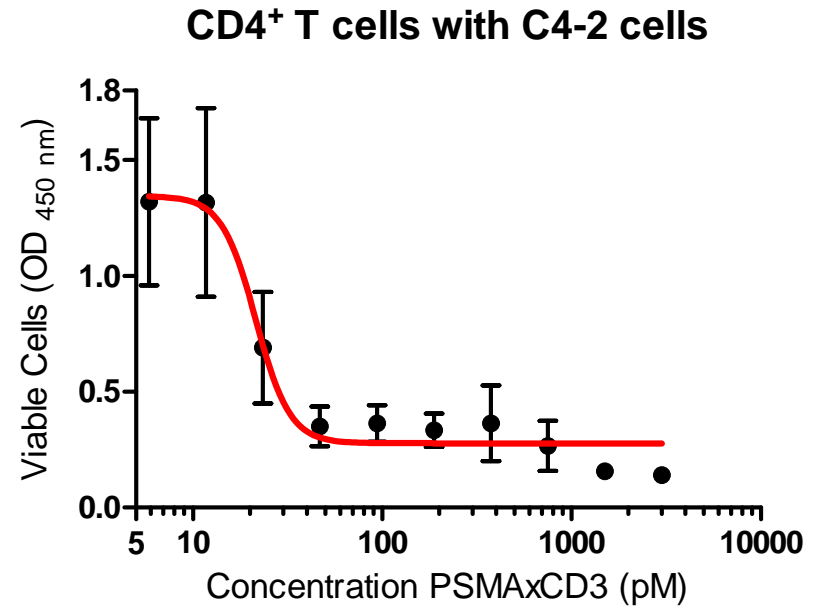
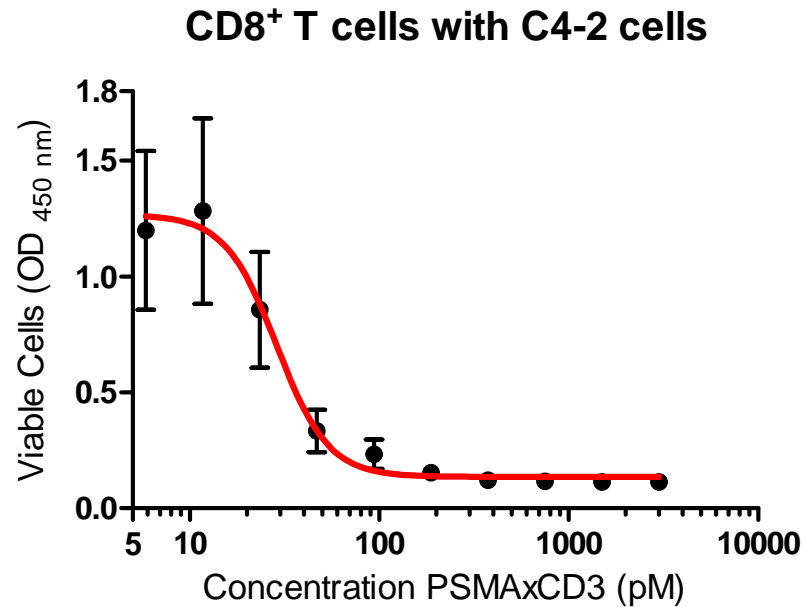
Upregulation of T cell activation markers



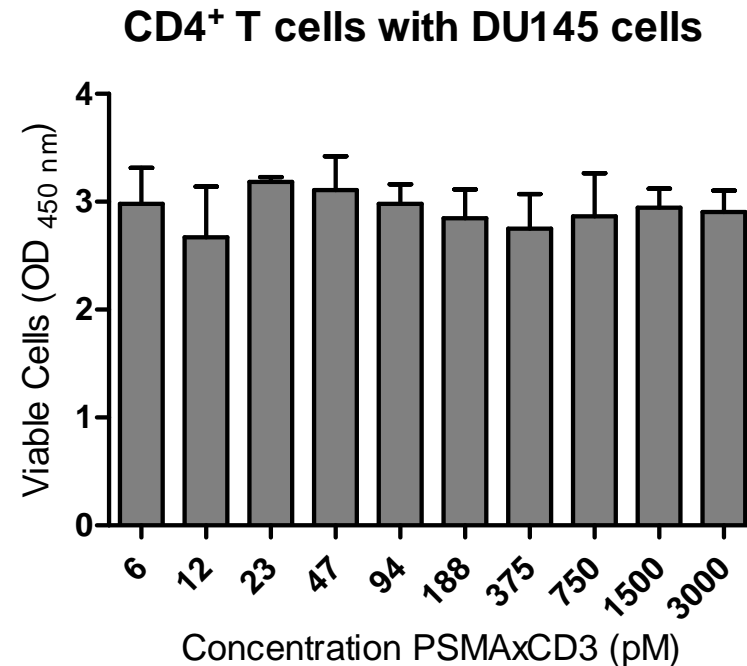
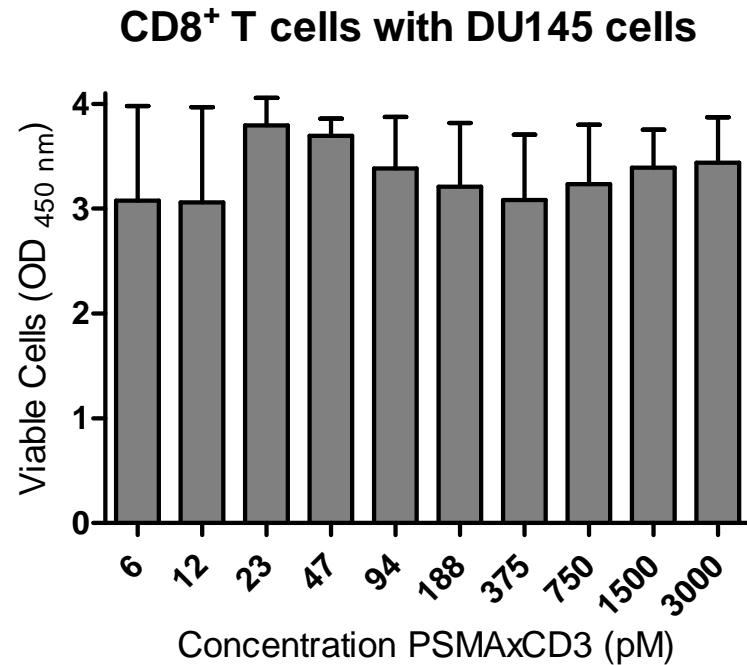
Diabody: Induction of cell lysis



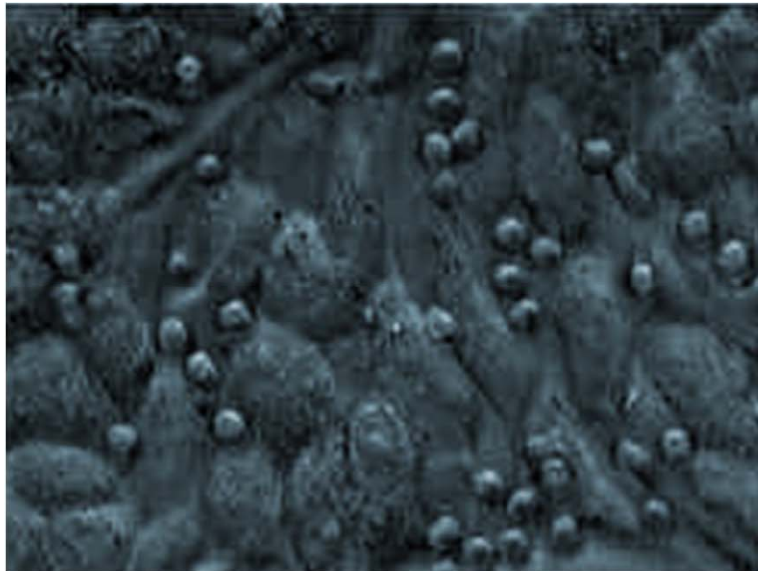
Lysis of PSMA-positive prostate cancer cells *in vitro*



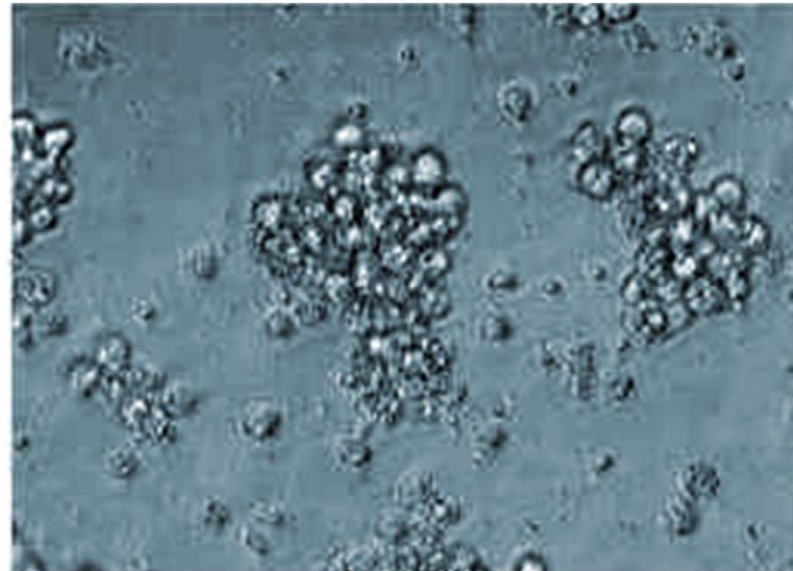
Lysis of PSMA-negative prostate cancer cells *in vitro*



Lysis of PSMA-positive prostate cancer cells *in vitro*



30 min after diabody and lymphocyte addition



48 hours after diabody and lymphocyte addition

Therapeutic approach: SCID mouse xenograft model

s.c. injection of
human prostate
cancer cells
(n=24)



Development
of tumors



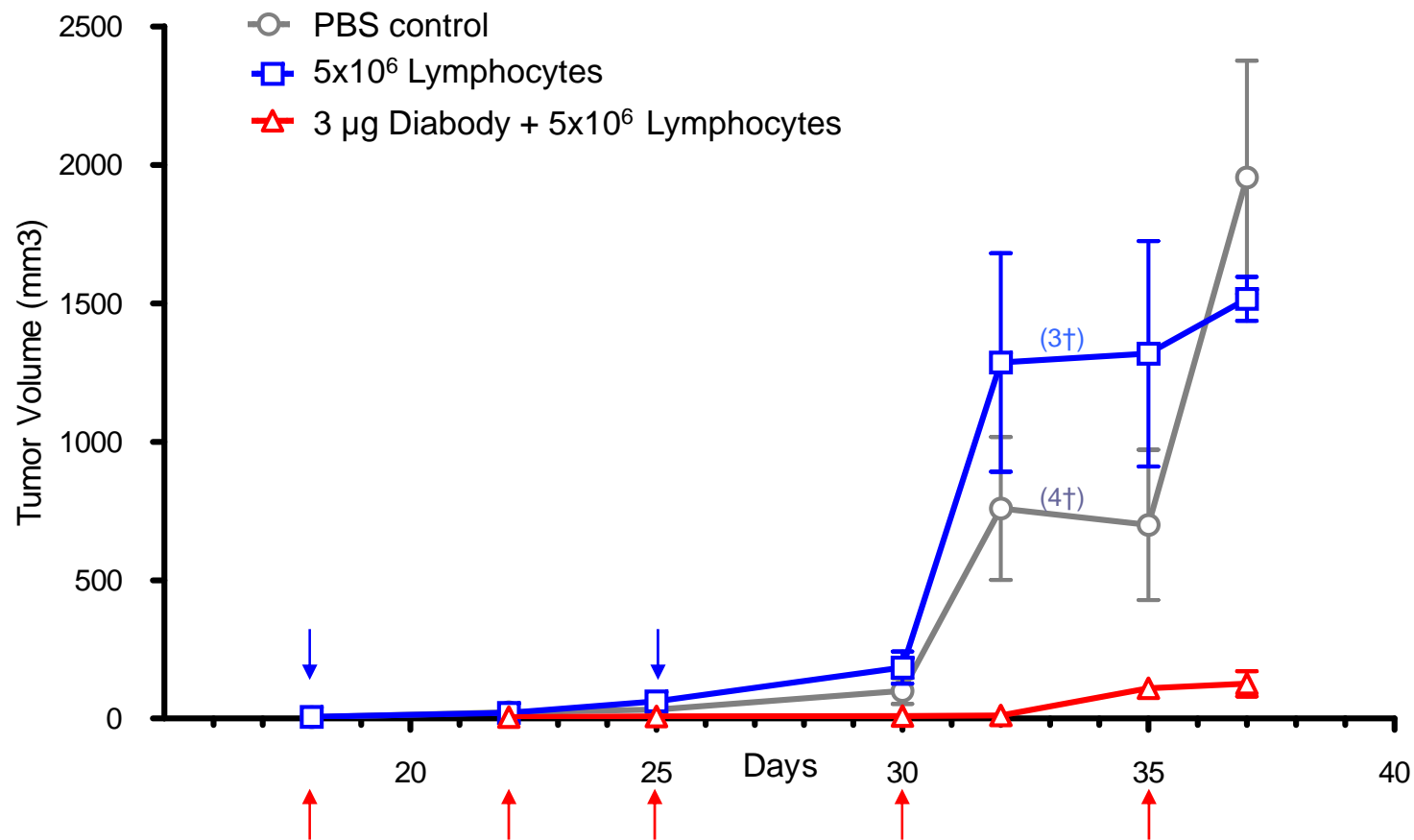
Lymphocytes only (n=8) or
vehicle control PBS (n=8) i.v.



3 μ g diabody
and **human**
lymphocytes
i.v. (n=8)



Treatment of SCID mice bearing prostate cancer xenografts with PSMa α CD3



Conclusion

The PSMAxCD3 diabody

- specifically binds to the cell surface marker PSMA
- induces target-dependent T cell activation
- induces highly efficient lysis of prostate cancer cells *in vitro* and *in vivo* by retargeting human T cells
- thus bears a high potential for immunotherapy of prostate cancer.

We thank our sponsor



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