

# A CD19/CD3 bispecific TandAb, AFM11, recruits T cells to potently and safely kill CD19<sup>+</sup> tumor cells in pre-clinical models

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## 1. Introduction

We engineered a humanized, bispecific tetravalent antibody with two binding sites for CD19 and two binding sites for CD3, the CD19/CD3 RECRUIT-TandAb® AFM11, for the treatment of CD19<sup>+</sup> B cell malignancies, such as Non Hodgkin Lymphoma (NHL) and Acute Lymphoblastic Leukemia (ALL).

CD19 is expressed from early B cell development through terminal differentiation into plasma cells, and is therefore an attractive target for the development of therapeutic antibodies to treat B cell malignancies. Clinical proof of concept for T cell recruitment was demonstrated by the bispecific CD19/CD3 BiTE blinatumomab in ALL.

The advantages of the TandAb® technology, relative to other approaches, include: (i) a sub/low-pM potency due to high affinity binding to both, T cells and tumor cells, (ii) a stable, off-the shelf product, and (iii) a half-life allowing for bolus infusion. We evaluated *in vitro* efficacy and safety of AFM11 using CD19<sup>+</sup> cell lines, and *in vivo* efficacy in a murine NOD/scid xenograft model reconstituted with human PBMC. Further, we used standard preclinical IND enabling assays to evaluate tissue cross reactivity, PK and toxicological profile.

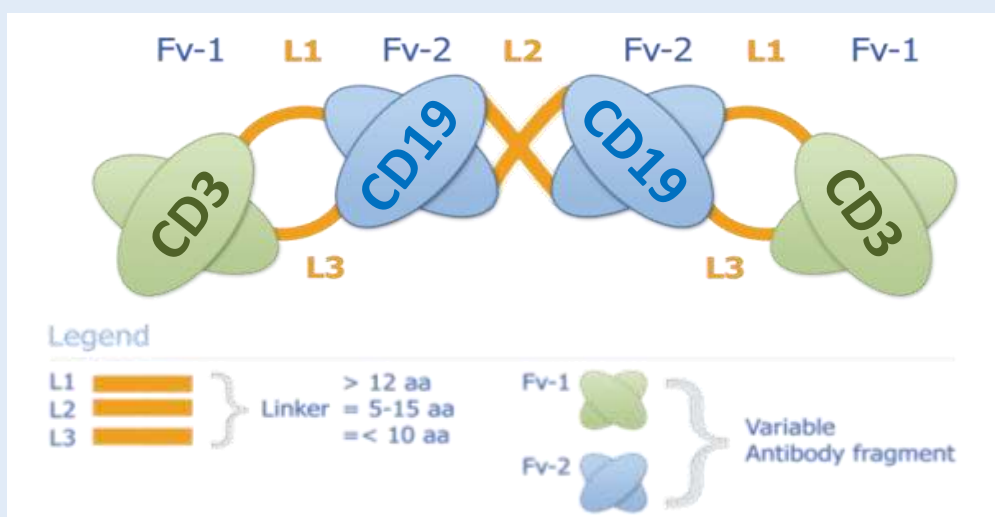
## 2. RECRUIT-TandAbs – tetravalent, bispecific antibodies

### a. TandAb Features

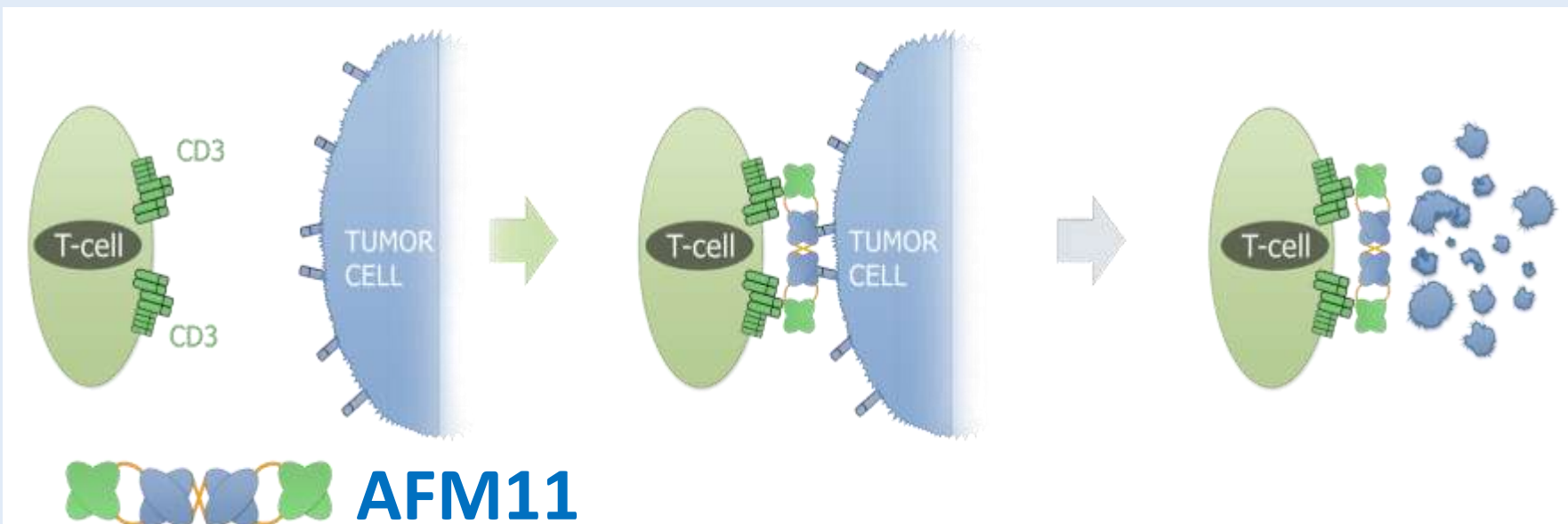
- comprised of scFv domains
- expressed as a single gene product
- linkers favor intermolecular head-to-tail homodimerization

### b. TandAb Properties

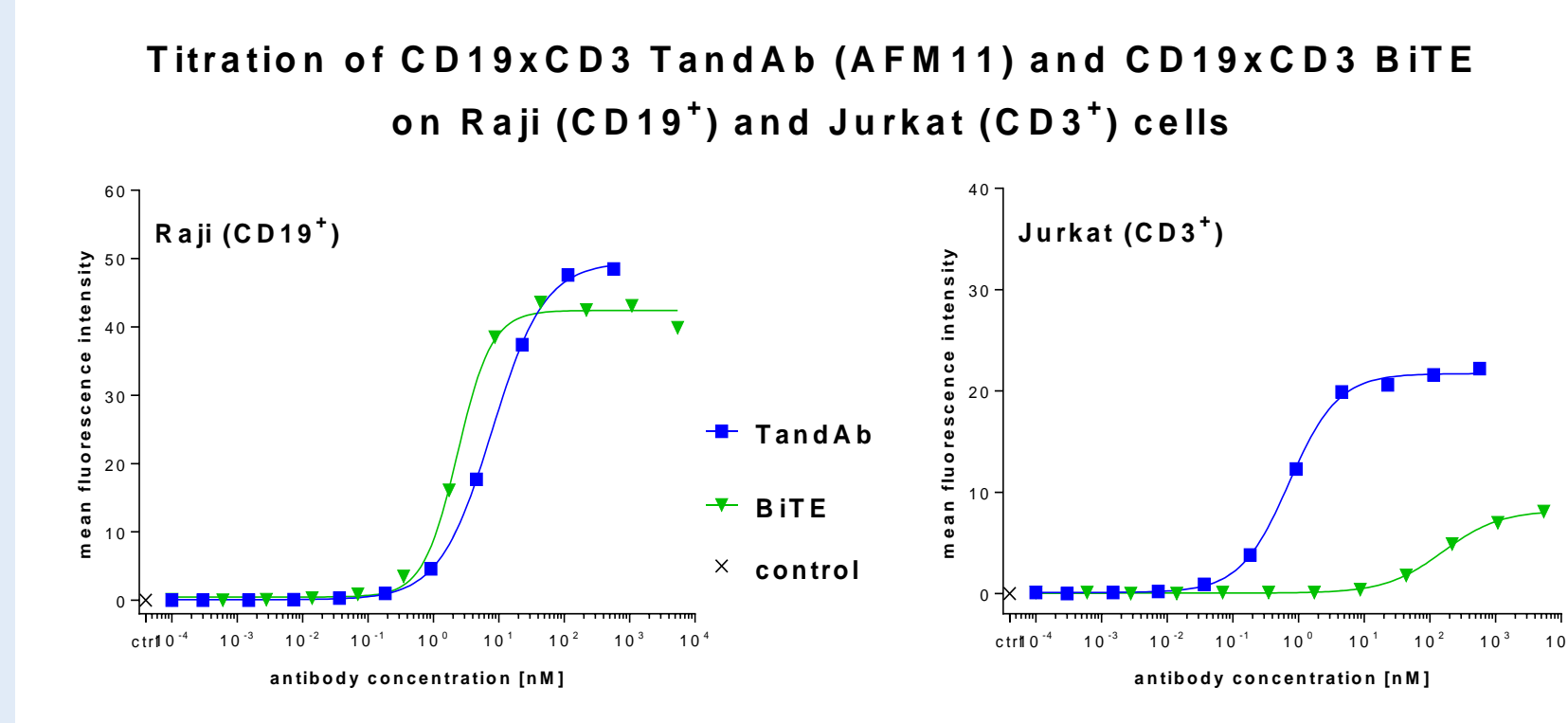
- recruitment of NK or T cells as effector cells
- potent cytotoxicity against target cells
- no off-target cytotoxicity
- no Fc-associated side effects
- bivalent binding to each target
- drug-like properties (production and stability)
- favorable half-life



## 3. AFM11 is a CD3 RECRUIT-TandAb engaging T cells

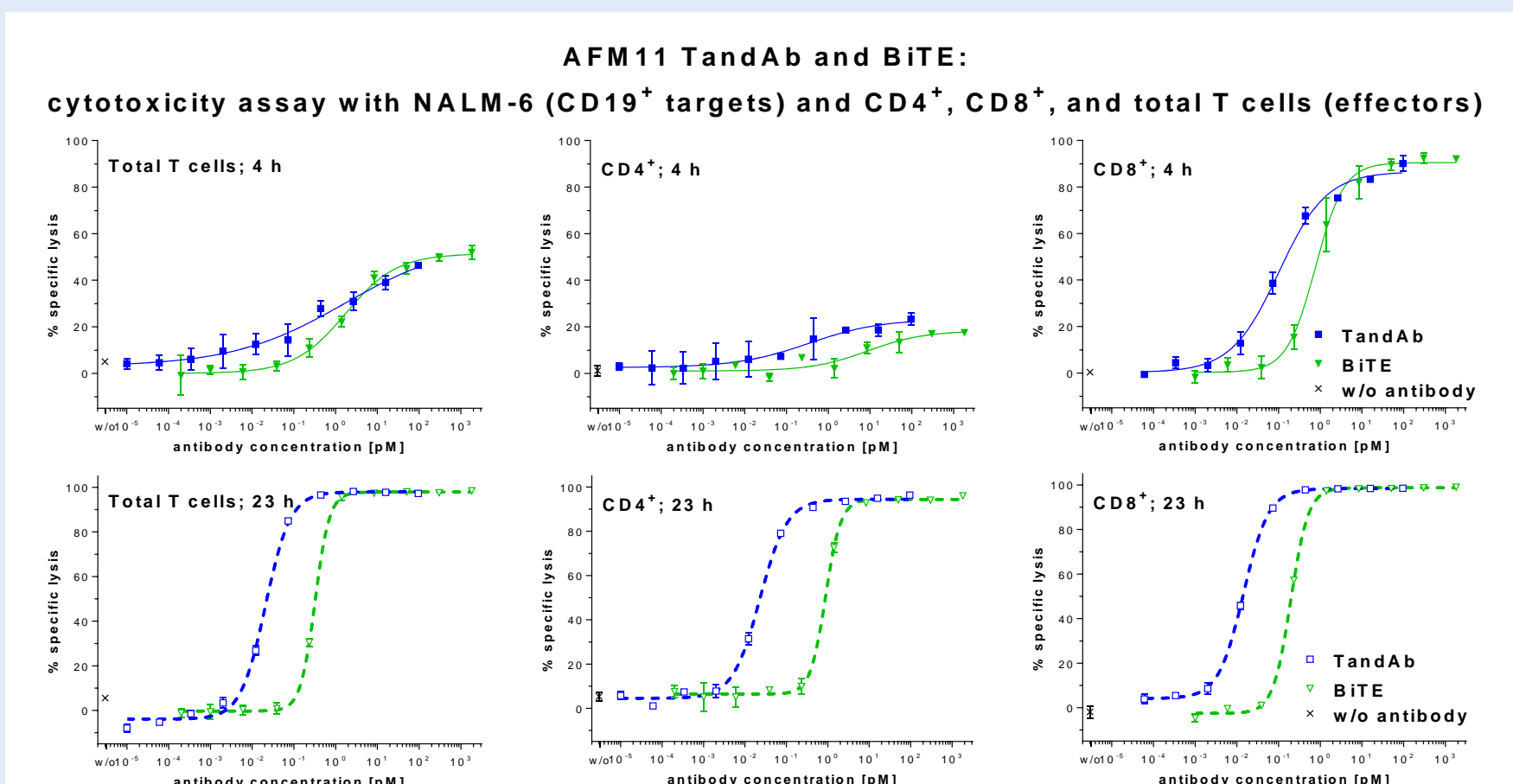


## 4. AFM11 TandAb displays high affinity to CD3 and CD19



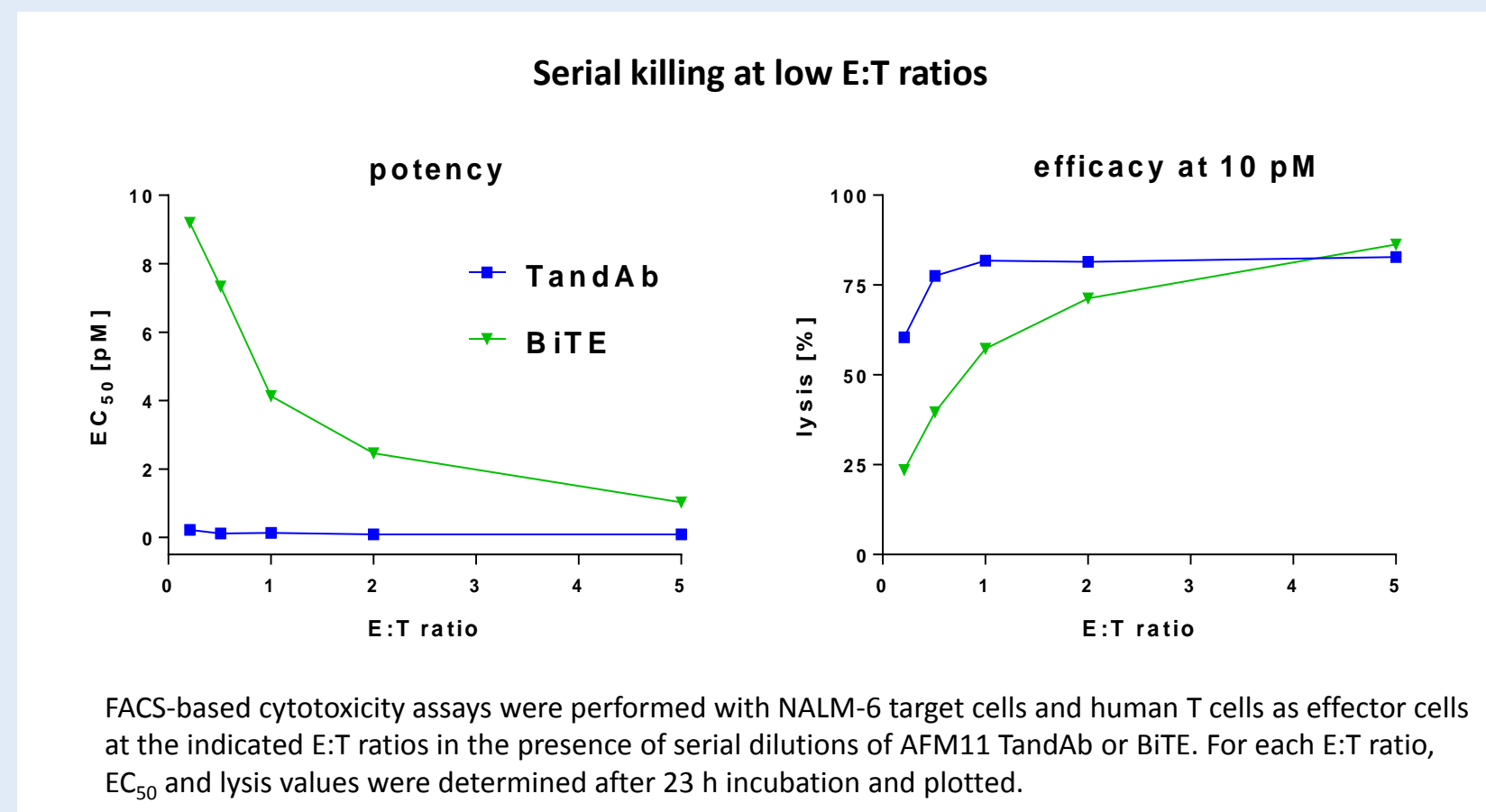
- High affinity to CD3<sup>+</sup> ( $K_D$ : 0.7 nM) and CD19<sup>+</sup> ( $K_D$ : 7 nM) cells
- Similar affinity of the AFM11 TandAb and the BiTE to CD19
- Affinity to CD3<sup>+</sup> cells of AFM11 TandAb is 2 orders of magnitude higher compared to BiTE

## 5. AFM11 TandAb facilitates cytotoxicity via CD4<sup>+</sup> and CD8<sup>+</sup> T cells



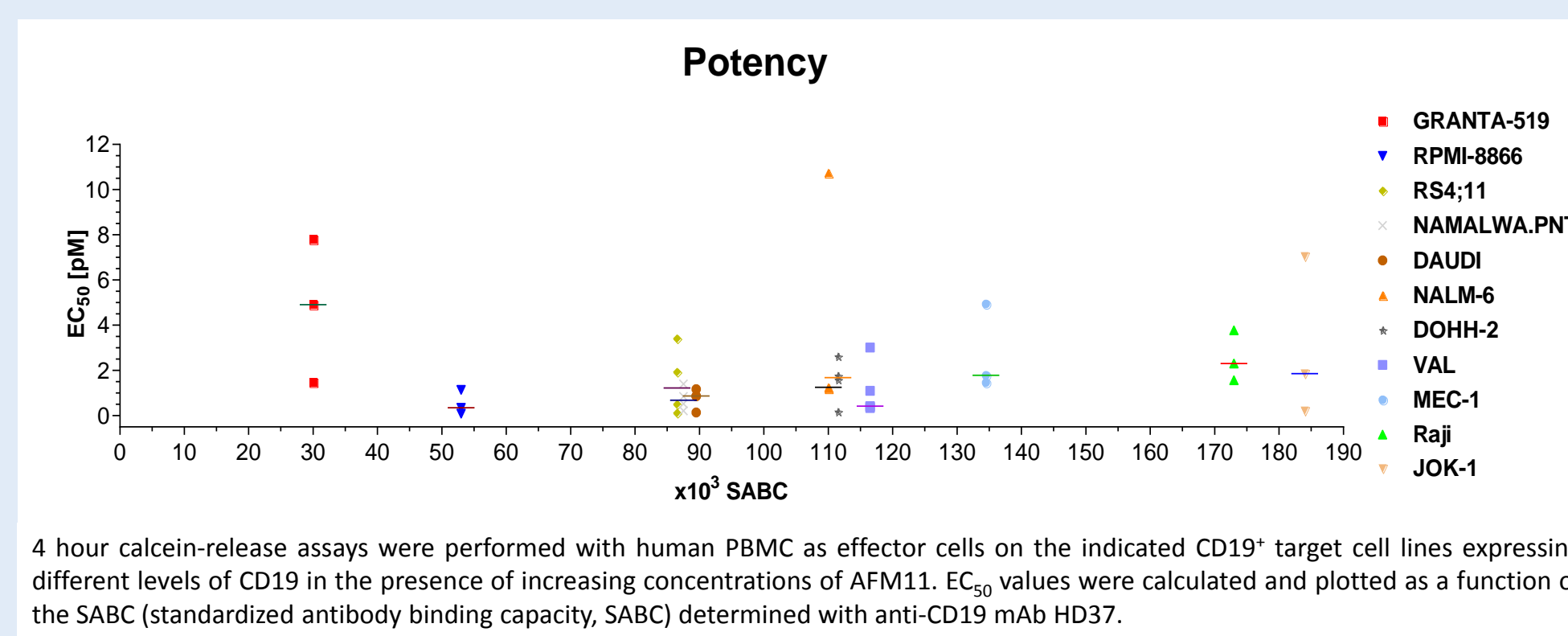
- CD8<sup>+</sup> T cells facilitate AFM11-mediated cytotoxicity with faster kinetics than CD4<sup>+</sup> T cells
- Both CD8<sup>+</sup> and CD4<sup>+</sup> T cells facilitate cytotoxicity of AFM11
- AFM11 is more potent than CD19/CD3 BiTE

## 6. AFM11 TandAb displays high cytotoxicity and serial killing

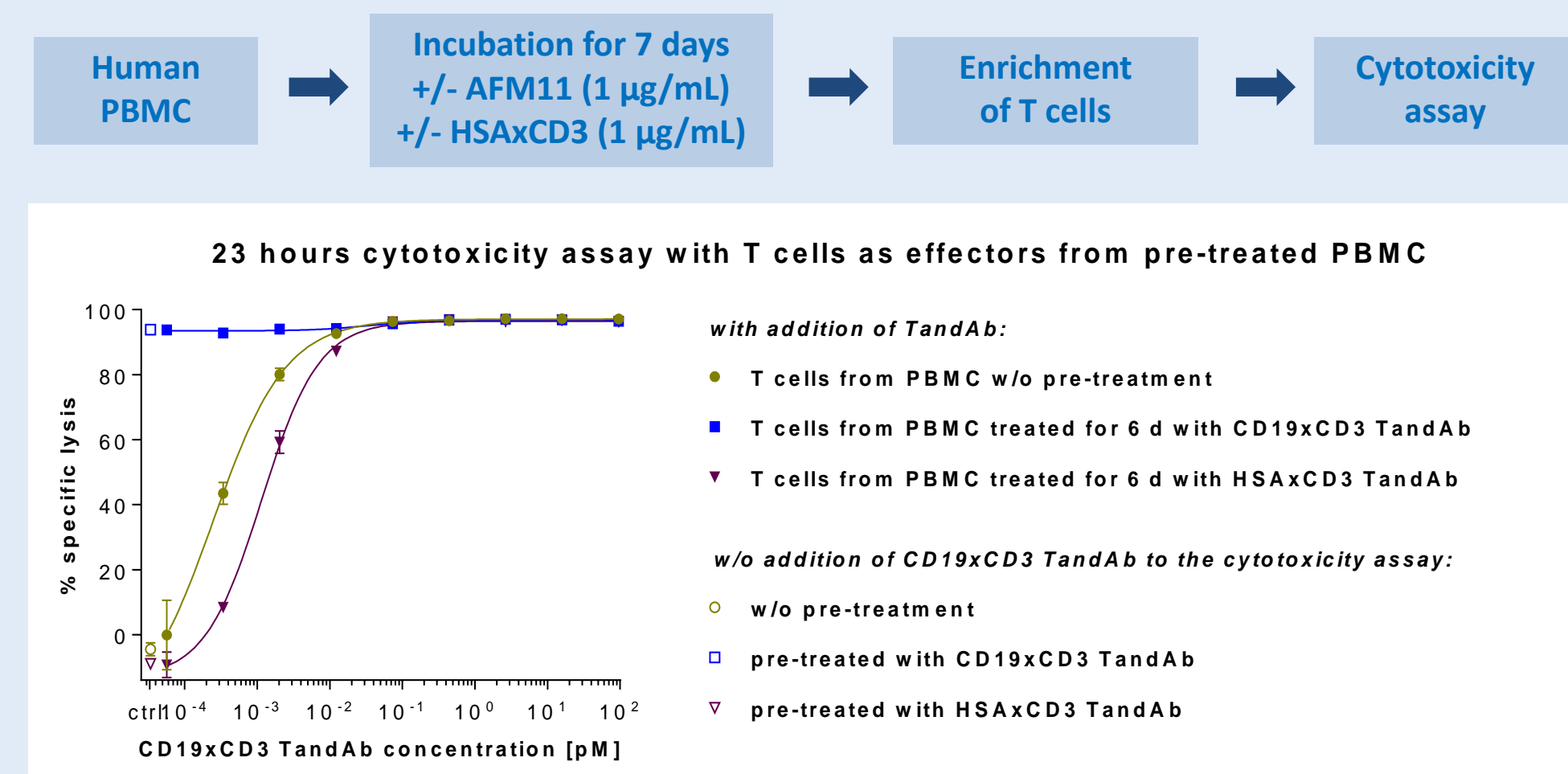


- AFM11 TandAb exhibits higher efficacy and potency than BiTE in the course of the cytotoxicity assay and at different E:T ratios
- Similar efficacy and potency at various E:T ratios suggests that AFM11 TandAb facilitates serial killing of tumor target cells by T cells

## 7. AFM11 TandAb potency does not correlate with CD19 density

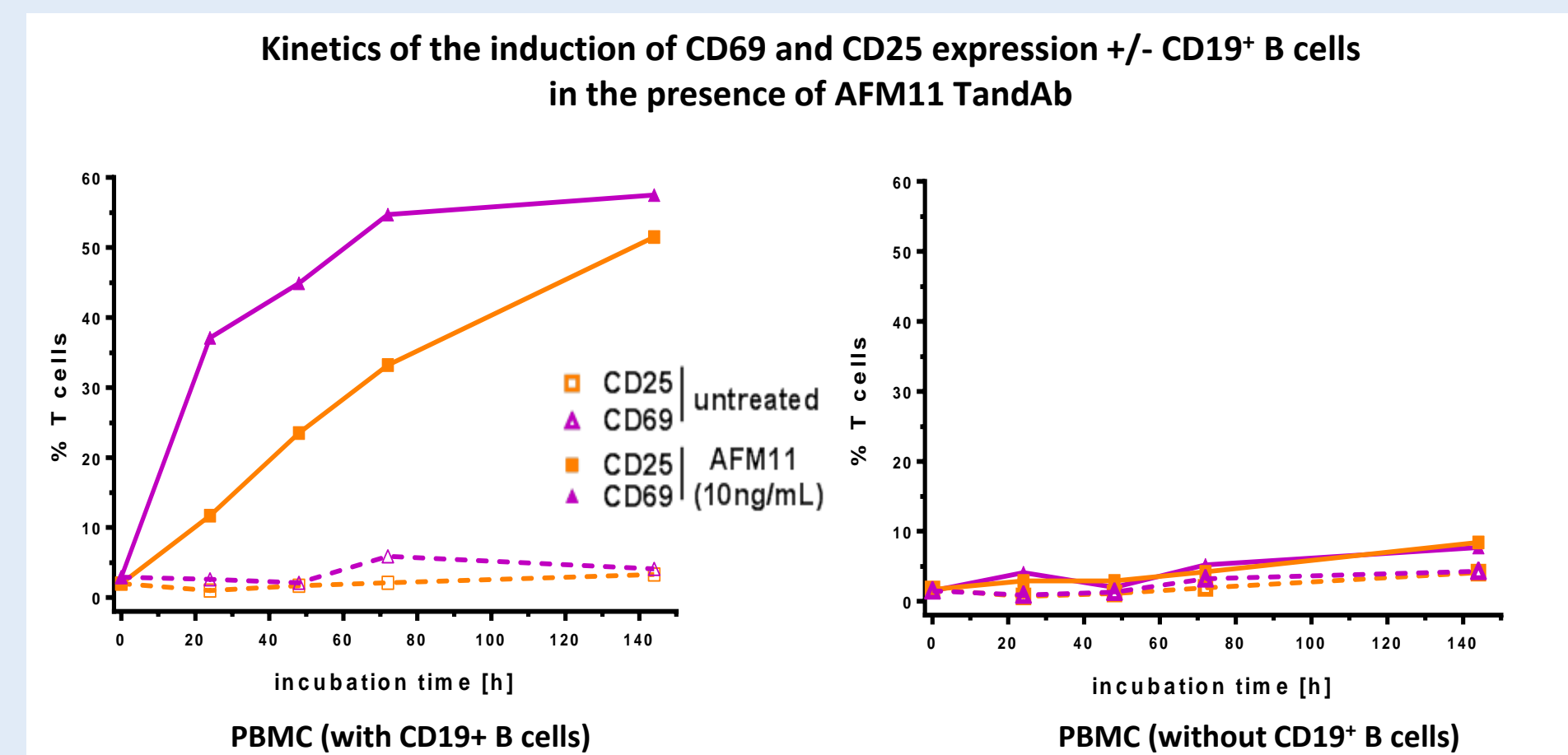


## 8. T cells retain cytotoxicity after stimulation of PBMC with AFM11

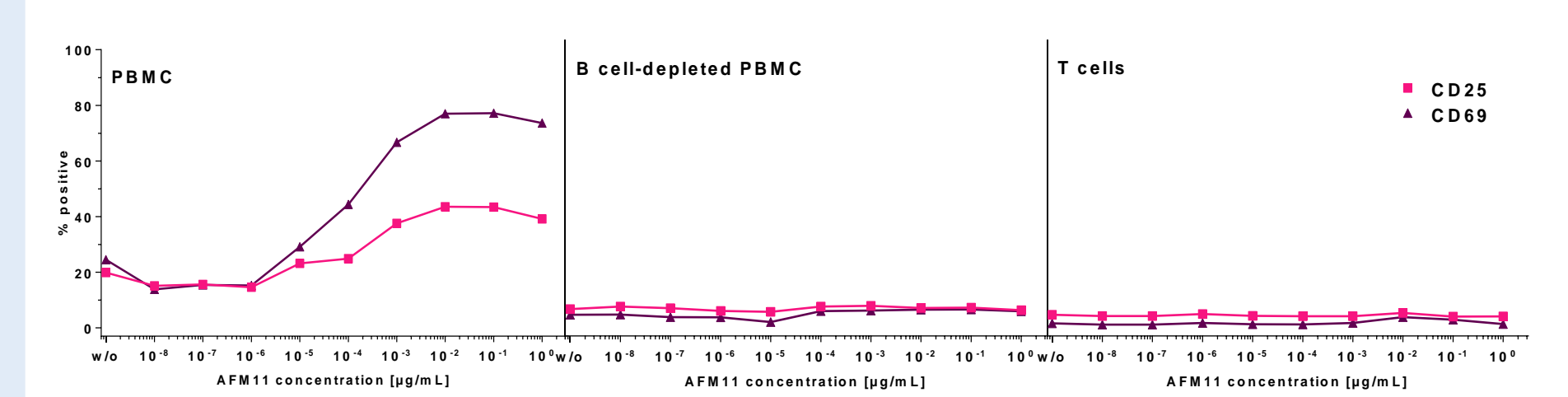


## 9. No off-target activity

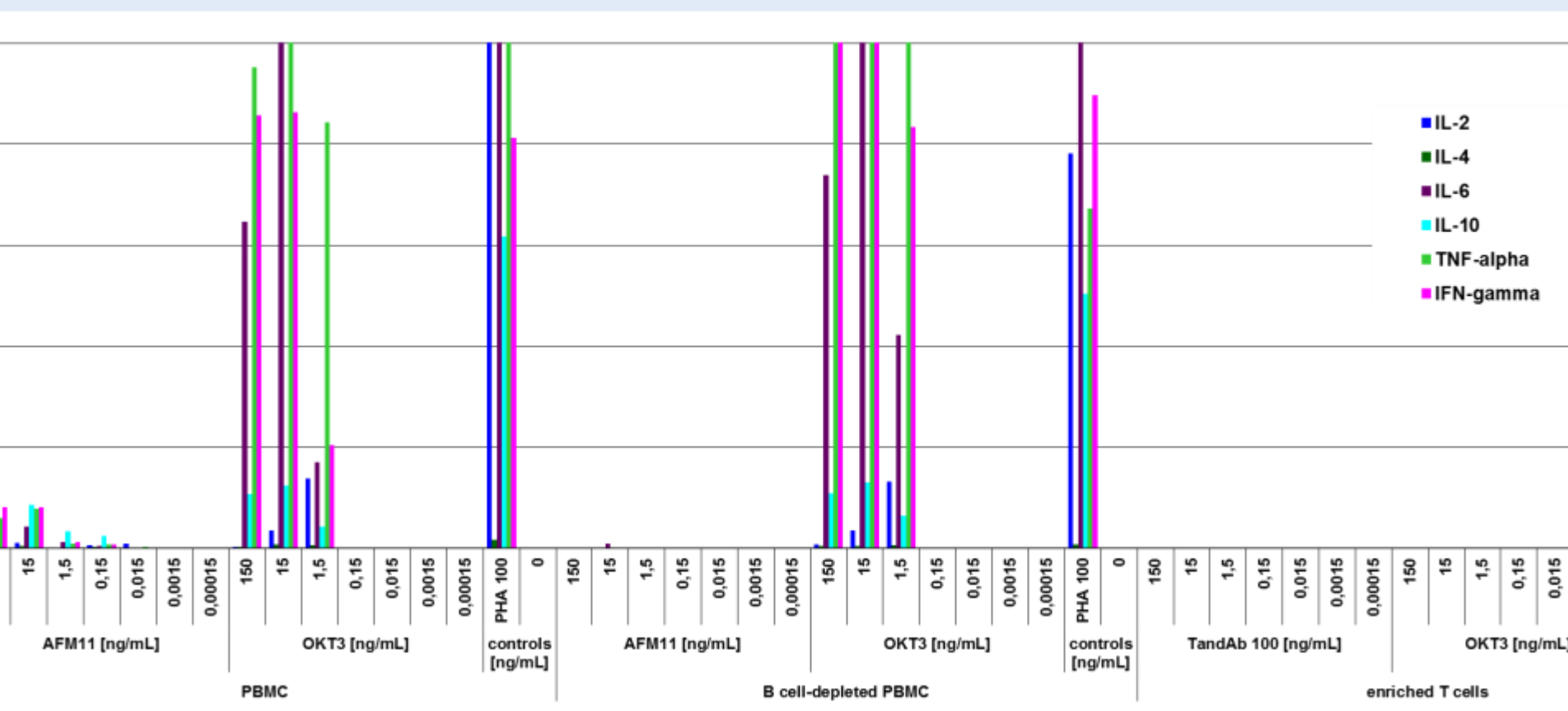
### a. No induction of activation markers on T cells in the absence of CD19<sup>+</sup> target cells



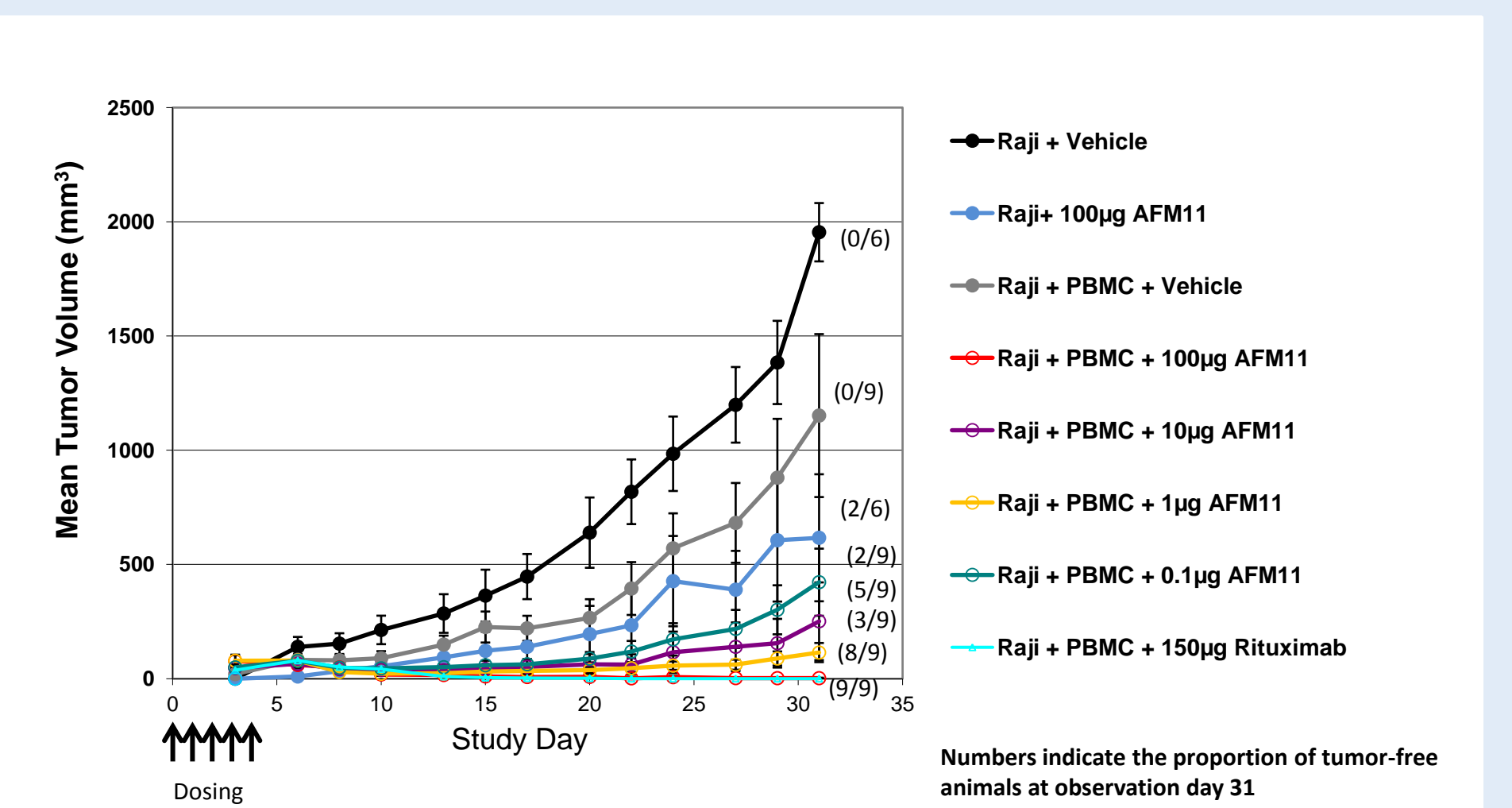
### b. No induction of cytokine release in the absence of CD19<sup>+</sup> target cells



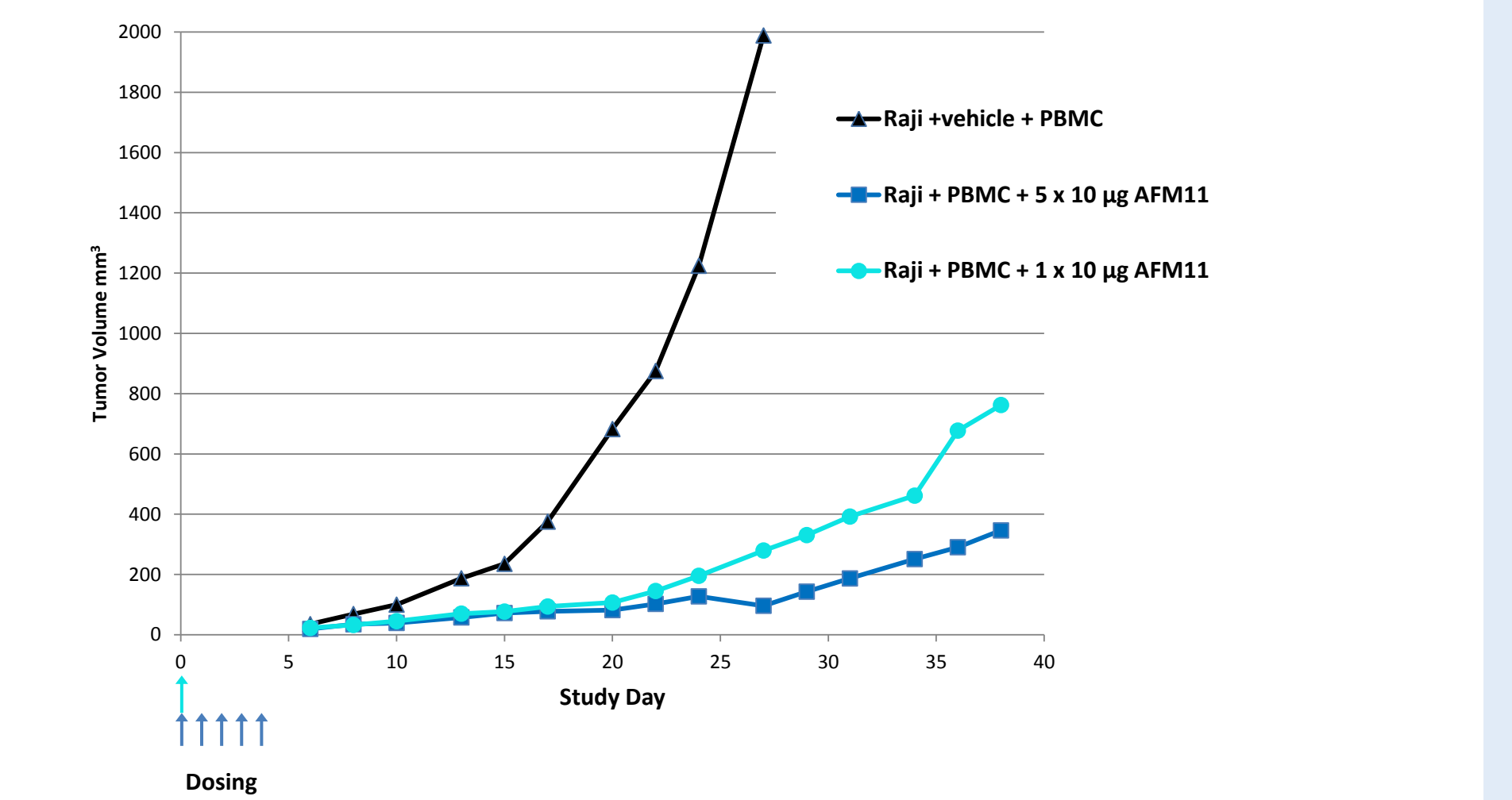
### c. No induction of cell proliferation in the absence of CD19<sup>+</sup> target cells



## 10. Xenograft Burkitt Lymphoma model: highly efficacious protection by AFM11 TandAb

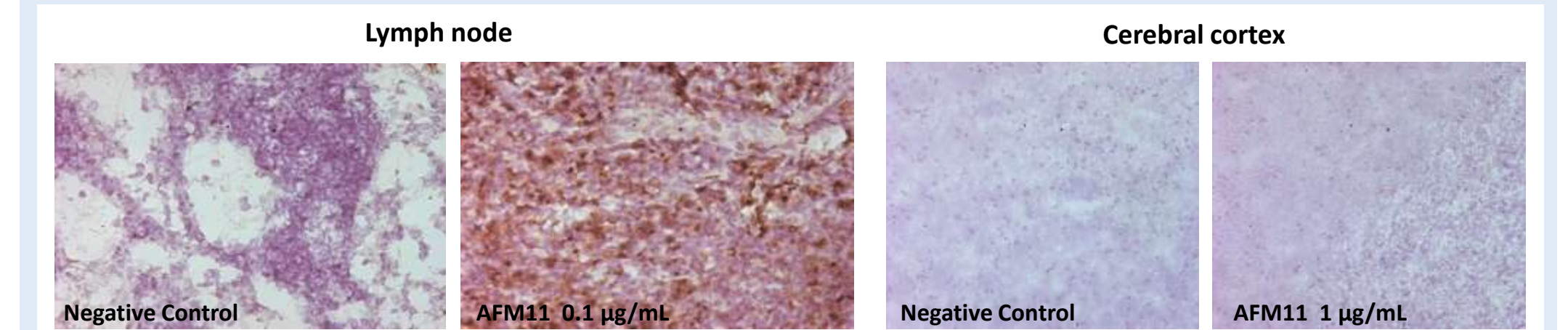


### 5 daily doses vs a single dose



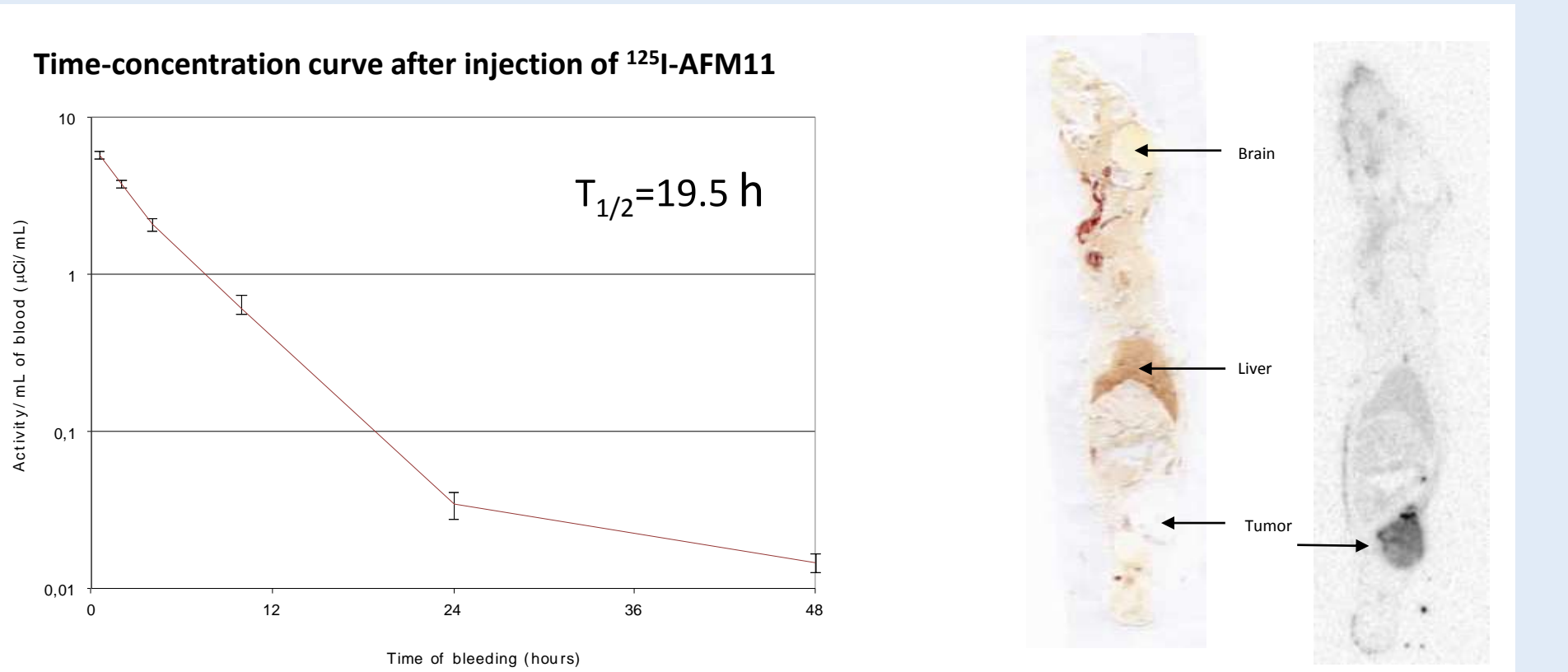
- Potent, dose-dependent growth inhibition of CD19<sup>+</sup> Raji tumors in an *in vivo* xenograft model
- Similar efficacy of a single 0.5 mg/kg dose and 5 daily injections

## 11. Tissue crossreactivity study



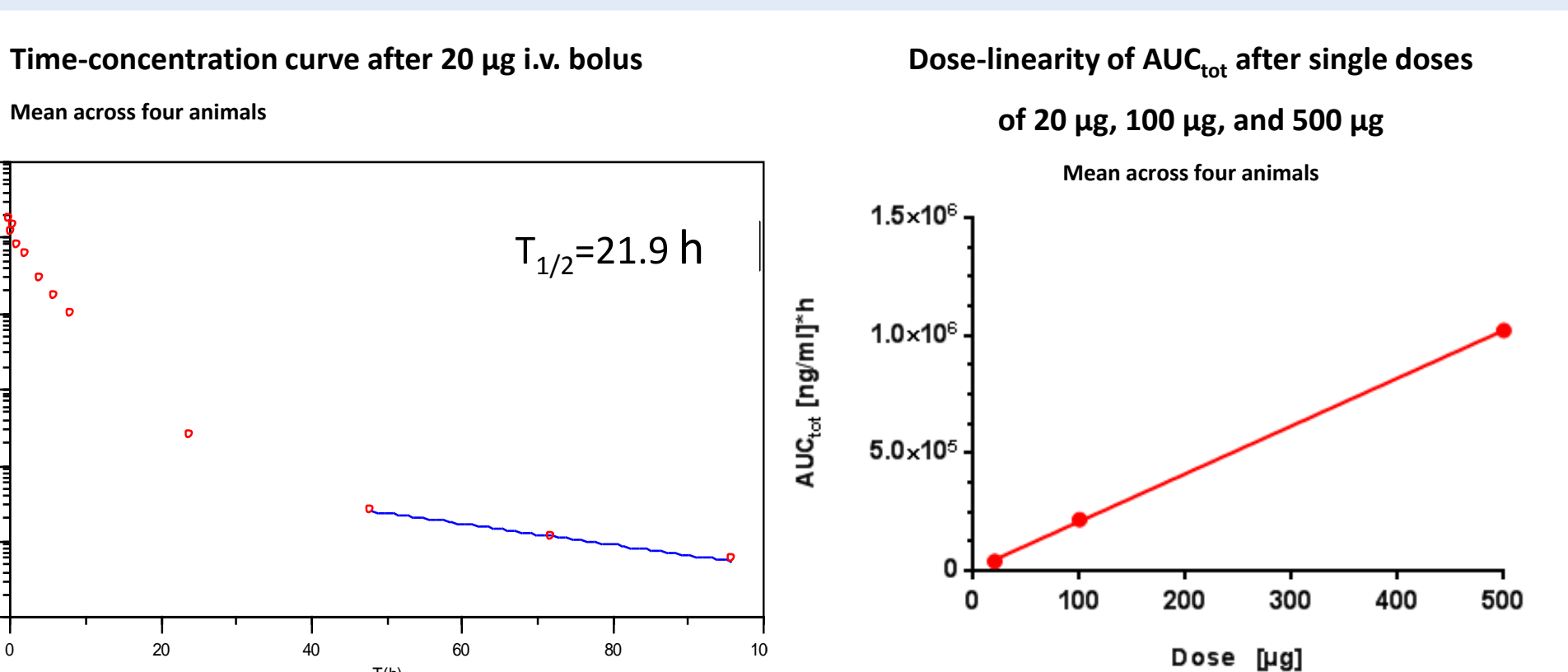
- Strong specific cell surface staining of lymphoid cells in lymph nodes
- No staining of CD3/CD19<sup>+</sup> tissues

## 12. Autoradiography study with <sup>125</sup>I-AFM11 in a Raji xenograft model in NOD/scid mice



- Autoradiography of animals from the 24 h time point
- Accumulation of <sup>125</sup>I-AFM11 in the tumor

## 13. Excellent PK properties of AFM11 in CD1 mice



## 14. Summary/Conclusion

- Excellent potency and efficacy characteristics *in vitro*
- Potent anti-tumor activity *in vivo*
- Promising safety characteristics:
  - no off-target activity *in vitro*
  - no unspecific accumulation in a xenograft model in mice
  - specific staining of positive tissues in a tissue crossreactivity study
- PK properties that do not require administration by continuous infusion
- Excellent drug-like properties (data not shown)
- Phase I study initiated in patients with relapsed and/or refractory Non-Hodgkin Lymphoma (NHL)