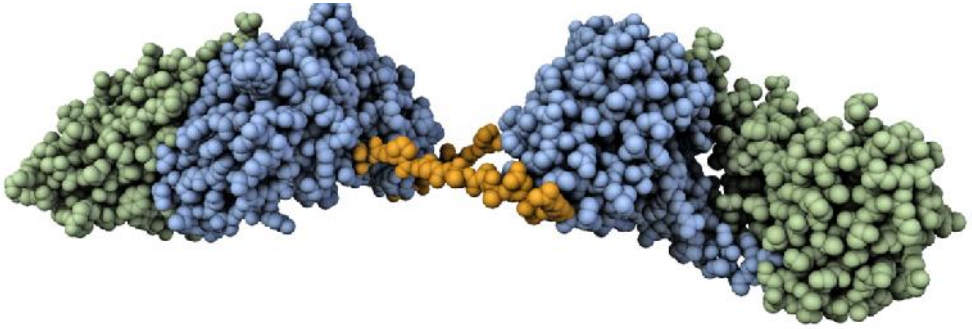
affimed
unique antibody therapeutics

RECRUIT-TandAbs: a versatile bispecific antibody platform designed for immune therapy of cancer

Eugene Zhukovsky



29th Monoclonal Antibodies Meeting, Mykonos 2012

Affimed's profile

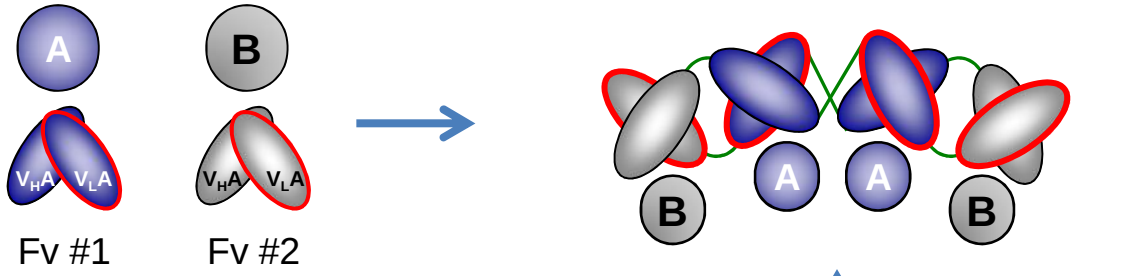
affimed
unique antibody therapeutics

- Affimed is a clinical stage company with a track record of developing antibody products from discovery to clinic
- Founded in 2000 by Prof. Melvyn Little as spin-off from DKFZ, Heidelberg
- Pipeline of potent human bi-specific therapeutic antibodies in oncology
- RECRUIT platform produces potent oncology biotherapeutics with excellent drug-like properties (production, stability, convenient dosing)
- The first therapeutic candidate, AFM13, is safe and shows activity in the clinic
- A wholly-owned subsidiary, AbCheck, is located in Check Republic:
  - is engaged in target antigen antibody generation for Affimed,
  - provides fee-for-service lead generation to clients, a key account with E. Lilly

TandAb® Platform:
Tetravalent Bispecific Antibodies

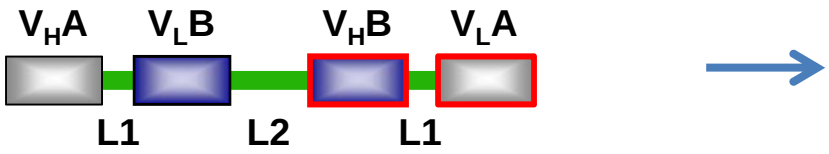
affimed
unique antibody therapeutics

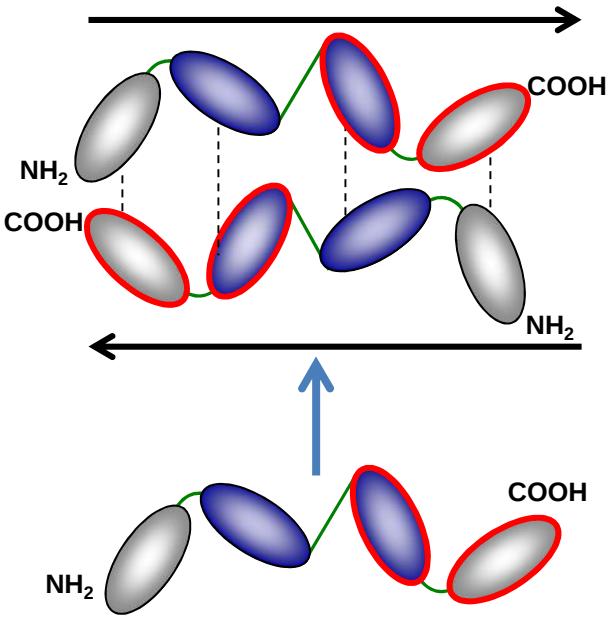
Fv derived from:
- phage display library
- native antibodies



Features:

> Based on 2 Tandem scFv
> Tetra Fv-domain antibody
> Bi-specific
> Bi-valent for each specificity
> Homodimer: single gene product

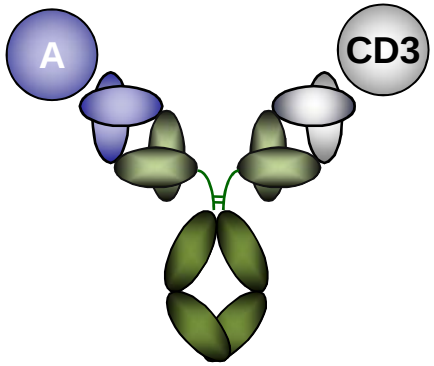




RECRUIT TandAbs possess advantages over IgG and scaffold antibodies

affimed
unique antibody therapeutics

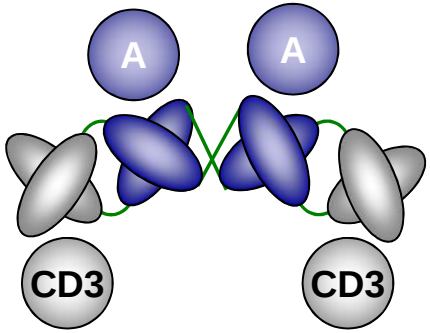
Bispecific IgG



2 binding sites

150 kD

TandAb

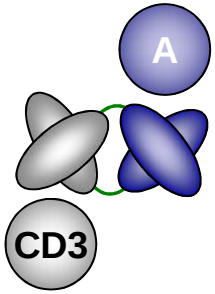


4 binding sites

105-110 kD

Diabodies

(BiTE, DART, etc)



2 binding sites

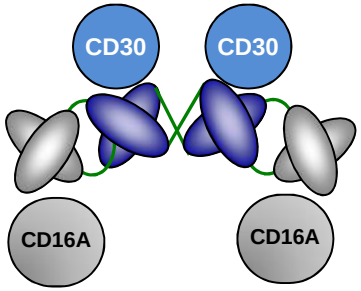
55-60 kD

affimed
unique antibody therapeutics

RECRUIT-TandAb AFM13

CD16A platform

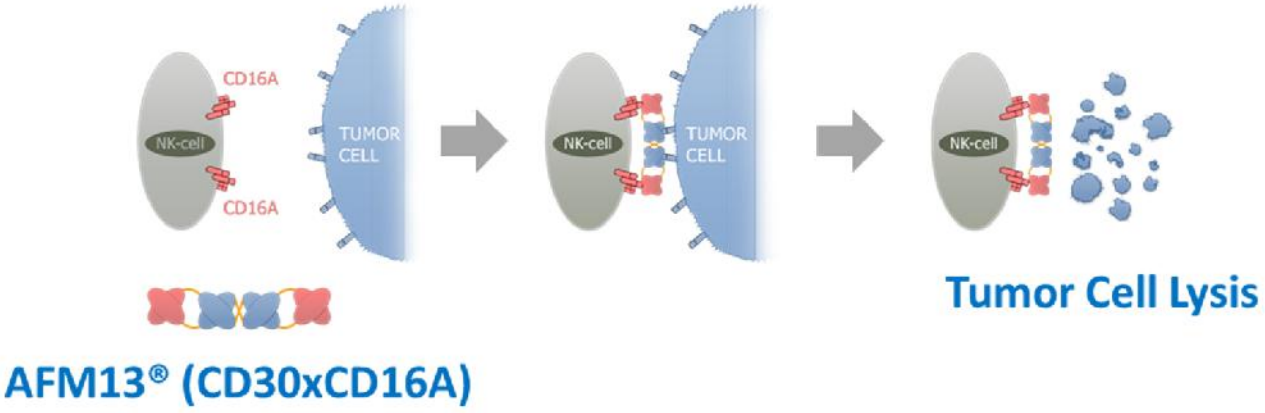
NK cell recruitment



AFM13 (CD30 x CD16A): improvement of clinical outcome through enhanced ADCC

affimed
unique antibody therapeutics

- Binds to CD16A but not to CD16B; Specifically recruits NK-cells
- Binds equally well to both CD16A V/F alleles
- Exhibits significantly higher cytotoxic activity than IgG
- Robust GMP process established; product with excellent stability
- Demonstrated to be safe and well tolerated in Phase I (Hodgkin Lymphoma)
- Demonstrated activity in 10/23 patients in dose-escalation



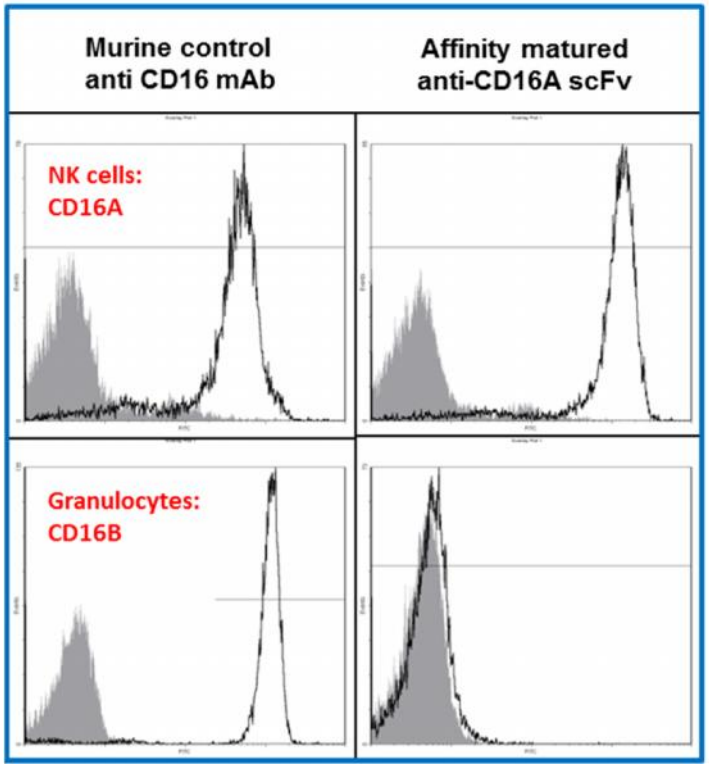
AFM13: anti-CD16A is specific for FcgRIIIA, no discrimination between F/V158 allotypes

affimed
unique antibody therapeutics

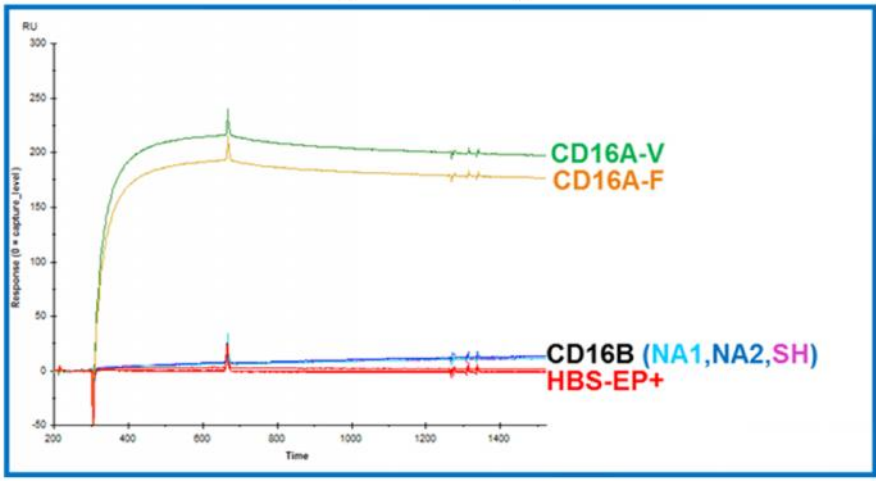
Specific targeting of CD16A but not CD16B avoids binding to non-signaling receptor

Similar binding of anti-CD16A to 158 F/V allotypes

Binding to cells (FACS)



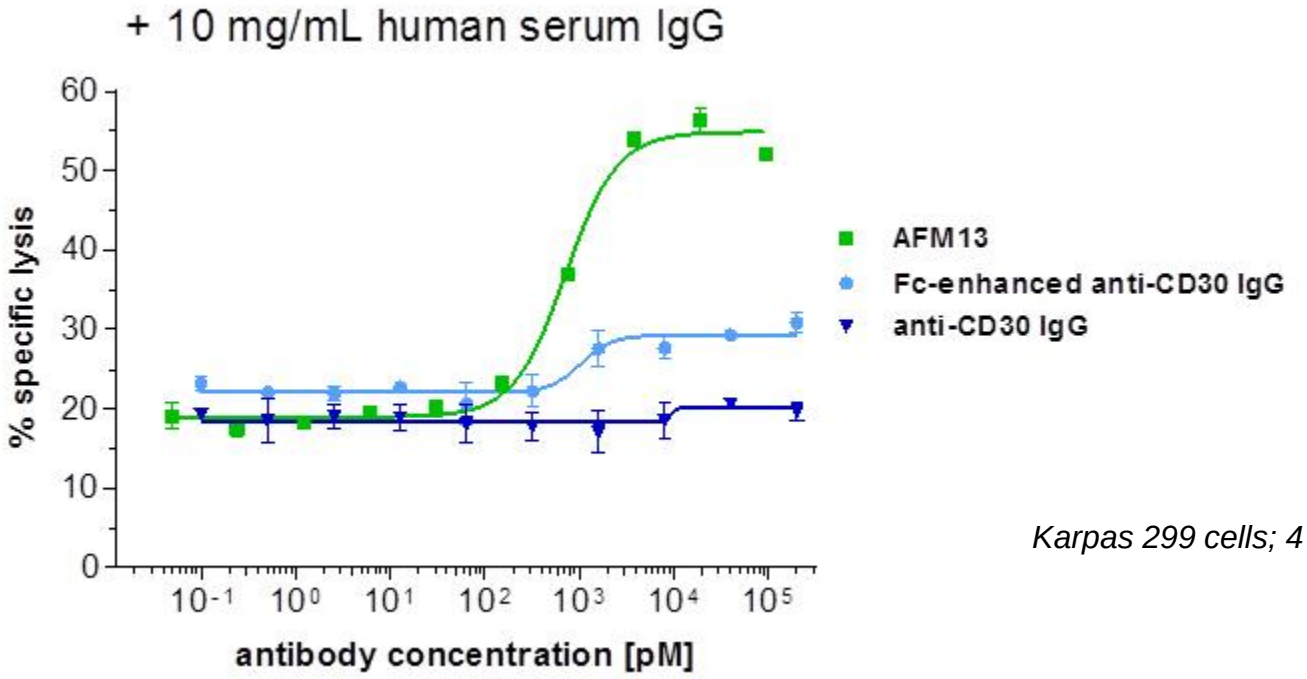
Binding measured by Biacore



	158V	158F
KD [nM]	0.4	0.8

AFM13 exhibits superior cytotoxicity in the presence of serum IgG

affimed
unique antibody therapeutics



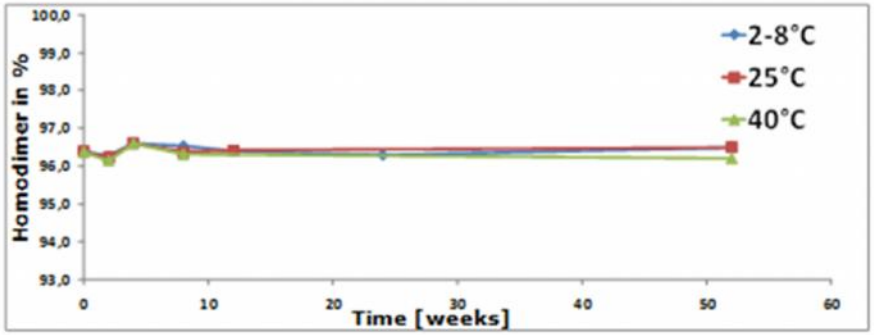
Karpas 299 cells; 4 hrs assay

AFM13 exhibits significantly higher cytotoxic potency and efficacy relative to Fc-enhanced and native IgG in the presence of serum IgG

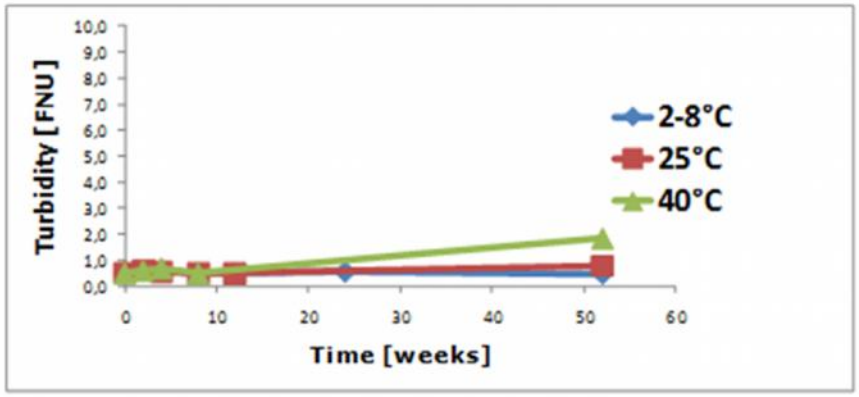
AFM13
52 weeks stability study

affimed
unique antibody therapeutics

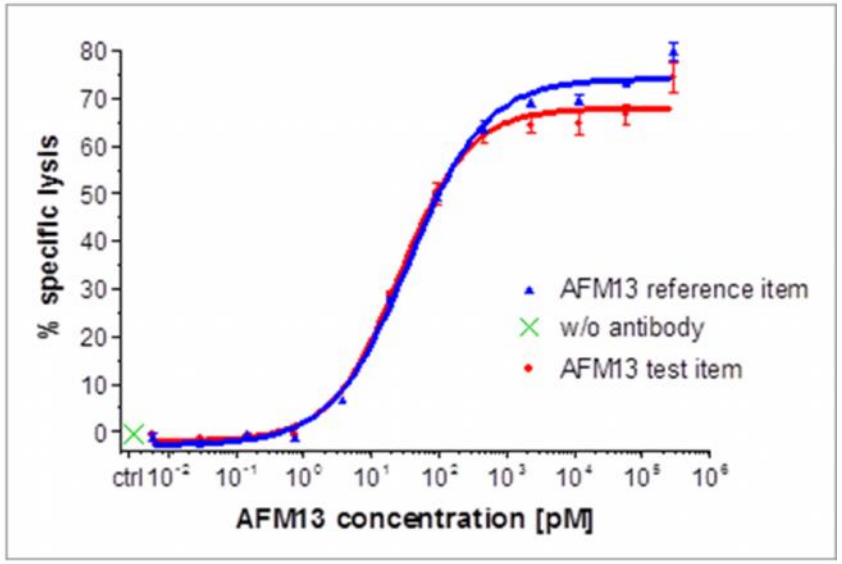
TandAb (homodimer) Content of AFM13



Turbidity in AFM13



Cytotoxicity Assay of AFM13



Formulated AFM13 demonstrates excellent stability

AFM13
Clinical study AFM13-101 preliminary status

affimed
unique antibody therapeutics

Safety

- Once-a-week dosing
- 7 dose levels completed AFM13 treatment was safe and well tolerated
- Most common non-serious AEs were fever, headache (identified as infusion related reactions) and anemia (most likely due to disease, not drug related).

Efficacy

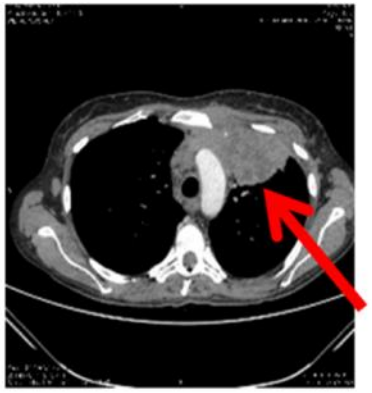
- Activity demonstrated in 10 out of 23 patients
  - 1 patient showed PR
  - 9 SD including 2 minor responses (tumor shrinkage by 10-15%)
- Clearance of B-symptoms in a dose dependent fashion
- Reduction of circulated sCD30 antigen
- 4 patients treated with AFM13 received prior treatment with SGN-35: 3/4 showed SD after AFM13 treatment

AFM13
Efficacy results

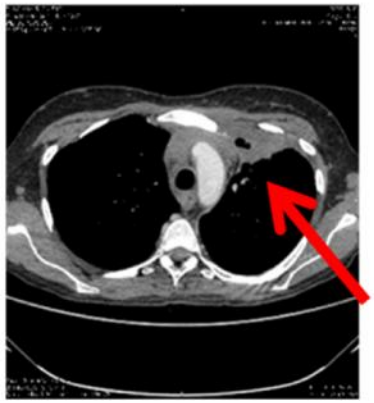
affimed
unique antibody therapeutics

CT showing a size reduction of the mediastinal lymphoma mass of 60%

Before therapy



After 2 cycles of therapy



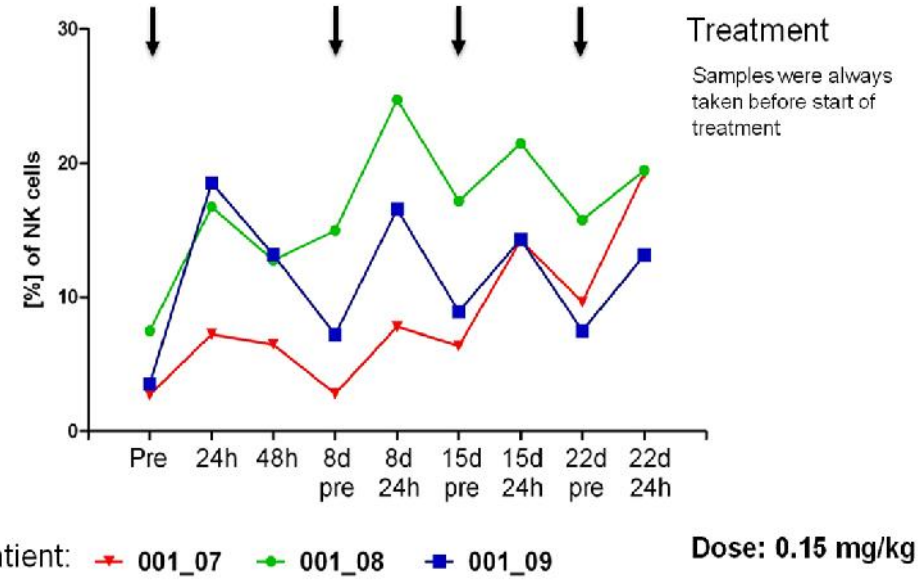
PET results show a significant reduction of tumors and elimination of 4/6 lesions

Lesion #	Screening		After cycle 1		After cycle 2	
	Visual	SUV_{max}	Visual	SUV_{max}	Visual	SUV_{max}
1	3	10.32	2	3.46	0	
2	3	9.73	3	8.72	0	
3	3	14.43	2	3.2	0	
4	3	28.61	3	23.31	3	23.31
5	3	21.38	3	14.06	3	8.16
6	3	3.92	2	3.26	0	

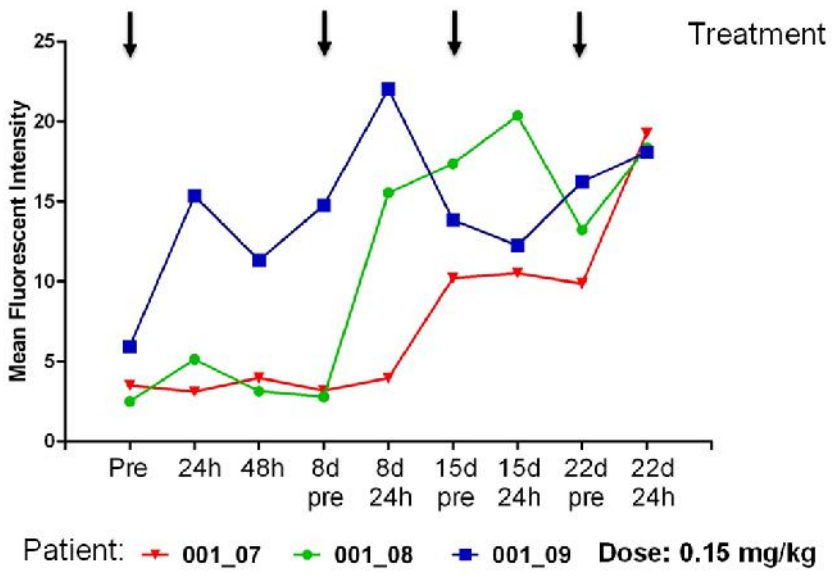
AFM13
Induction of activation markers on NK cells

affimed
unique antibody therapeutics

Expression of the Early Activation Marker CD69



Expression of the Activating Receptor NKG2D



AFM13 administration in HL patients results in sustained activation markers on NK cells

AFM13
Pharmacokinetics

affimed
unique antibody therapeutics

- PK ($t_{1/2}$) was measured in mice, cynomolgus monkeys, and humans (from the ongoing AFM13 trial):
  - **Mice**: dose and dosing regimen dependent: 5 – 9 hr
  - **Cynomolgus monkeys**: dose and dosing regimen dependent
    3 hr (single dose) and 12 – 23 hr (repeated dose); effective $t_{1/2}$ is up to 3 days
  - **Humans**: 24 hr (single dose); PK of soluble AFM13 is affected by target mediated disposition (TMD): soluble CD30, NK and malignant cells, similar to observations in cyno
  - *Human PK measurements after last dose (repeated dose) will be performed in ongoing trial*

AFM13
Summary

affimed
unique antibody therapeutics

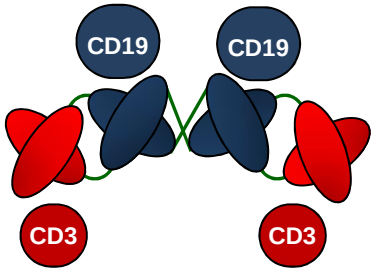
- Substantially higher efficacy and potency than Fc-enhanced IgGs
- Low competition with human serum IgG
- Specific for CD16A and displays equal binding to both CD16A alleles, 158F/V
- Specific lysis of target cells by NK cells with no bystander cell killing
- Excellent stability at 52 weeks
- Well tolerated and showed activity in HL patients
- PK profile enables weekly administration
- Overcomes the observed NK cell impairement in HL patients

affimed
unique antibody therapeutics

RECRUIT-TandAb AFM11

CD3 platform

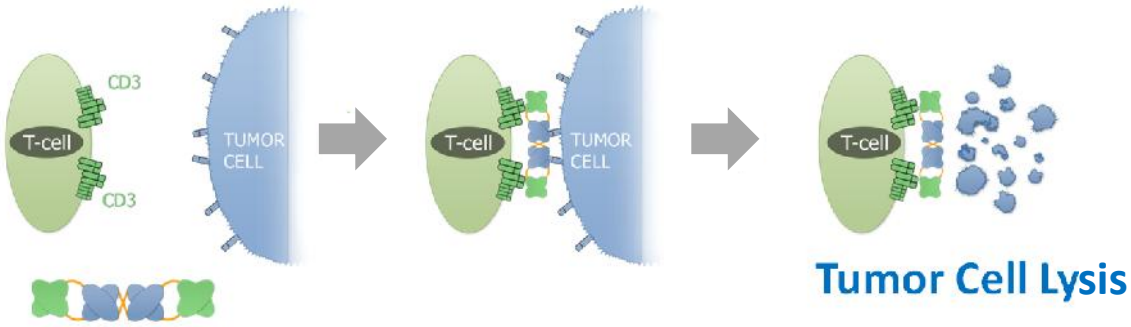
T cell recruitment



AFM11 (CD19 x CD3): therapeutic lead for the treatment of NHL, CLL, and ALL

affimed
unique antibody therapeutics

- **Validation of TandAb approach**
  - Recruitment of T cells for the killing of $CD19^+$ tumors is clinically validated
- **AFM11**
  - Possess potency in sub-picomolar range
  - TandAbs allow convenient treatment (no continuous infusion)
  - POC established *in vitro* and *in vivo*
  - Excellent drug-like properties

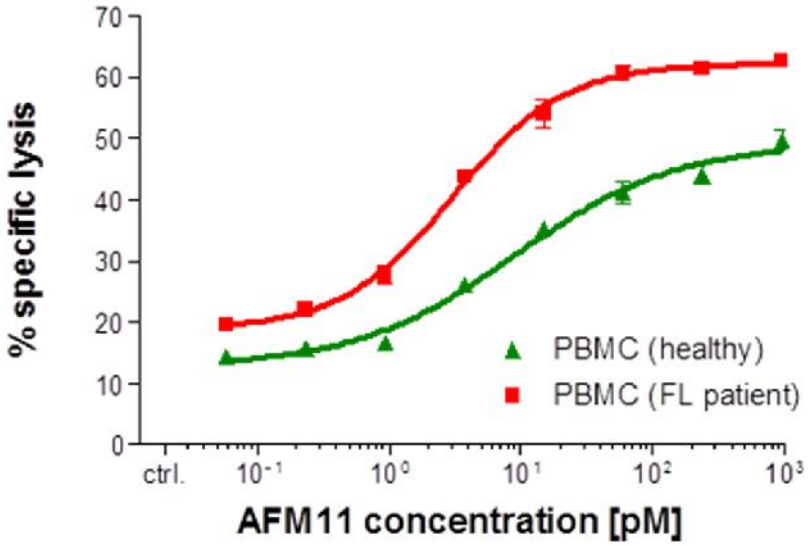


AFM11® (CD19xCD3)

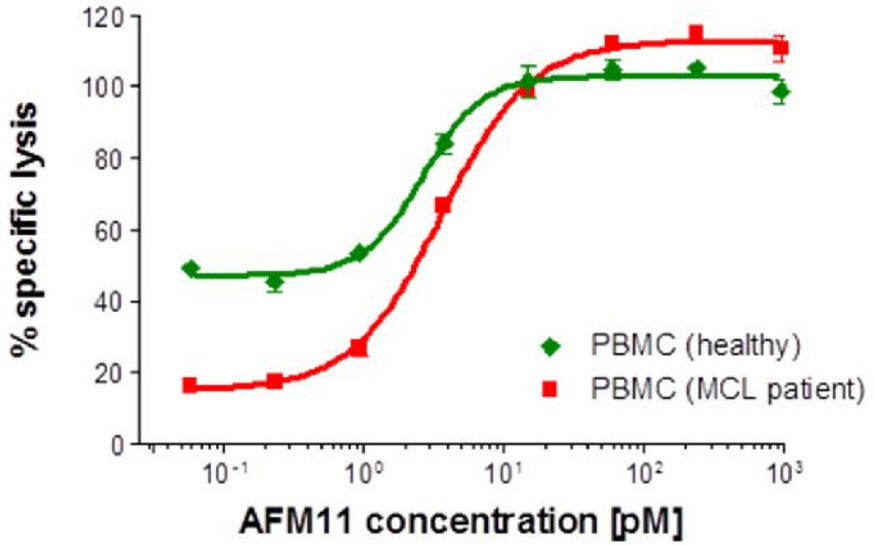
AFM11: effector cells from NHL patients efficiently facilitate cytotoxicity

affimed
unique antibody therapeutics

Follicular Lymphoma



Mantle Cell Lymphoma



Raji:PBMC=1:50; 1x10^4 targets/well; 4 hrs assay

PBMC donors	EC_{50} [pM]
healthy	9
FL patient	3
healthy	3
MCL patient	4

Comparable activity of AFM11 with effector cells from healthy individuals and NHL patients

AFM11
High activity independent of target density

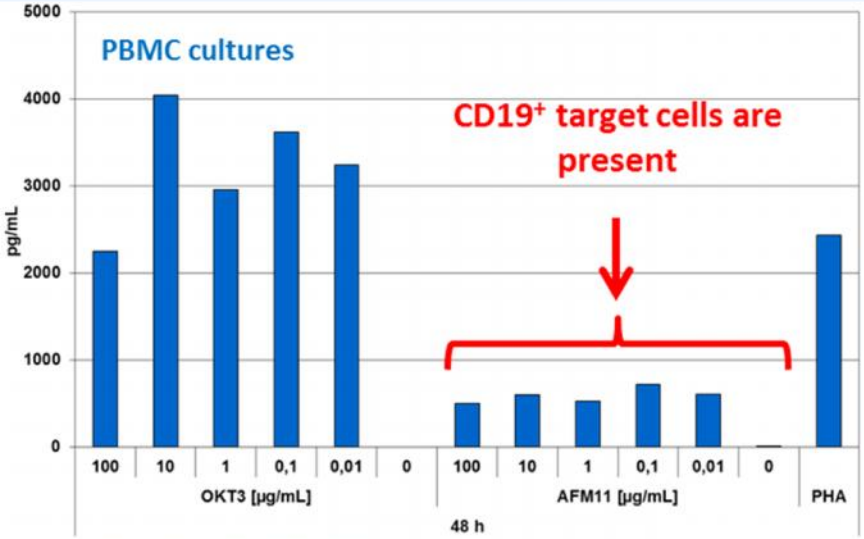
affimed
unique antibody therapeutics

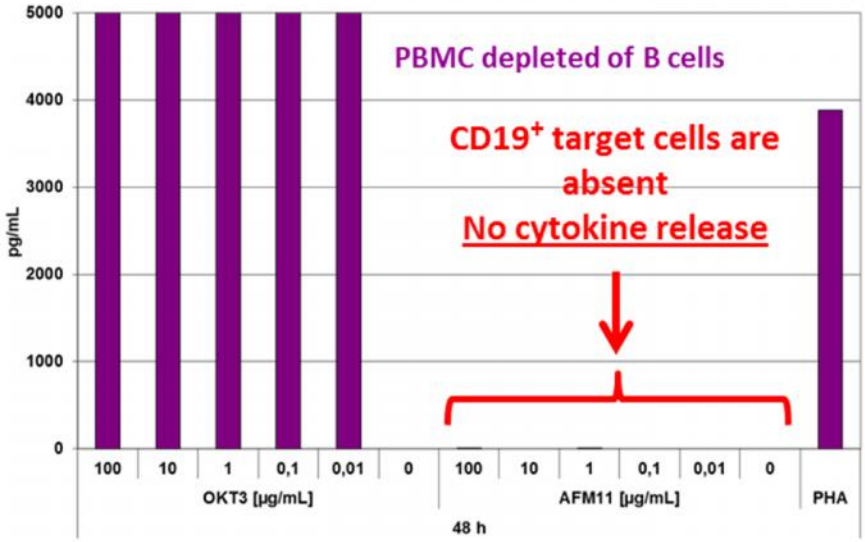
Cell line	origin	CD19 Binding Capacity	Effector cells	EC_{50} (pM)	Maximal lysis (%)
JOK-1	HCL	177,00 - 191,000	T cells (donor 1)	1.8	60-70
			PBMC (donor 2)	6.1	50-60
Raji	BL	169,000 - 177,000	PBMC (donor 2)	2.3	60-70
			PBMC (donor 3)	1.6	50-60
			T cells (donor 3)	1.6	25-30
			PBMC (donor 1)	0.7	70-80
			PBMC (donor 1)	1.1	70-80
			PBMC (donor 4)	1.0	60-70
			PBMC (donor 4)	1.0	45-55
			PBMC (donor 5)	1.3	25
MEC-1	CLL	128,000 - 141,000	T cells (donor 7)	1.0	35
VAL	ALL	104,000 - 129,000	T cells (donor 8)	0.5	30-35
NALM-6	ALL	97,000 - 126,000	PBMC (donor 5)	0.5	30
			PBMC (donor 6)	0.4	60-70
			T cells (donor 9)	0.6	15
Daudi	BL	92,000	PBMC (donor 6)	1.1	60-70
			T cells (donor 9)	0.2	15

> No correlation between AFM11 potency and CD19 density

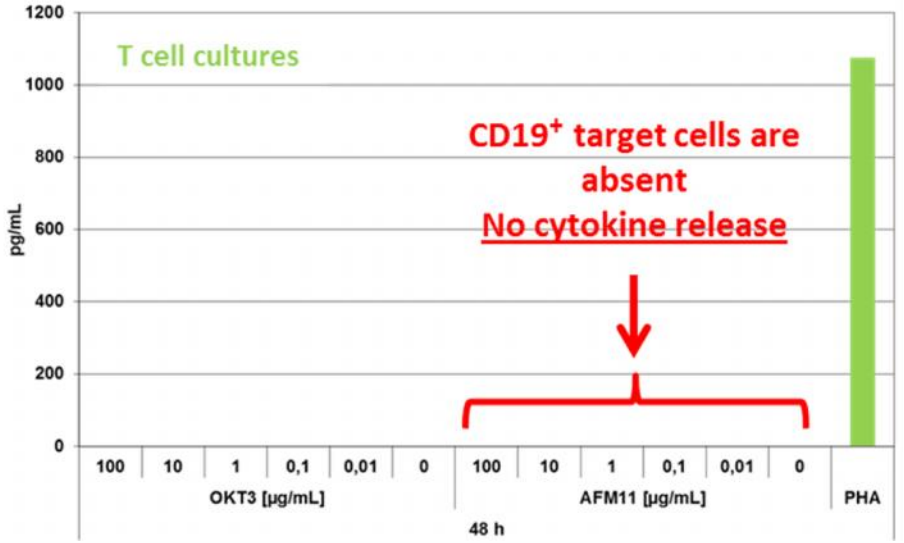
> Efficacy differences are due to donor-to-donor variability

AFM11: no T cell activation or cytokine release in the absence of target cells

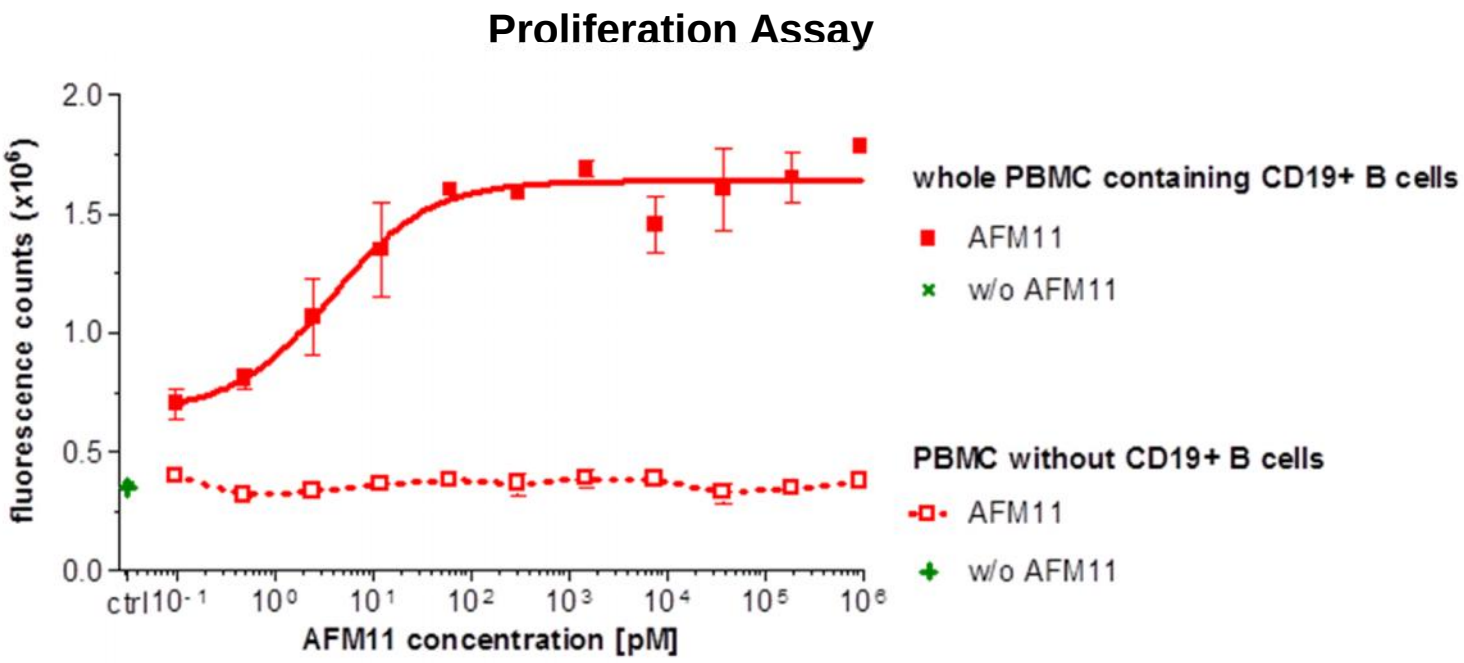




- IFN-γ release by AFM11 was assessed in the presence of CD19+ targets (PBMC) and in their absence (PBMC depleted of B cells and enriched T cells)
- Anti-CD3ε IgG (OKT3) and PHA used as controls
- AFM11 behaved similarly when TNFα, IL-2, IL-4, IL-6, and IL-10 release was evaluated



AFM11
No off-target induction of T cell proliferation

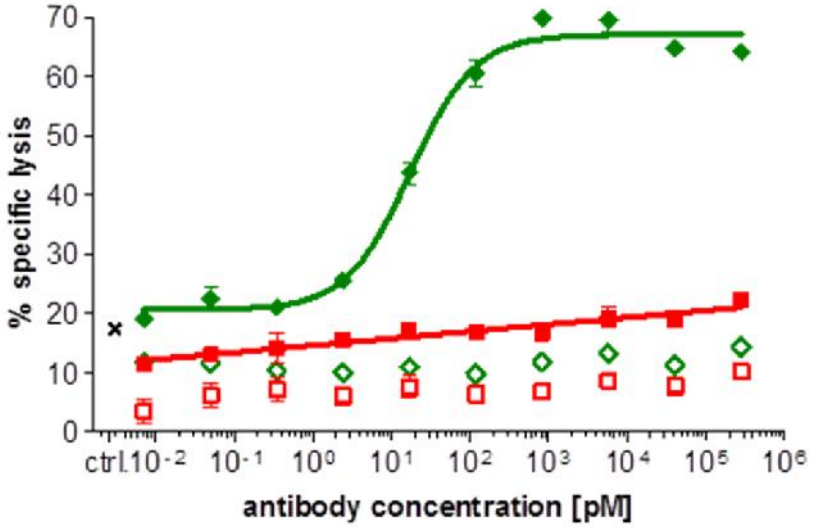


(4x10^5 PBMC/well; 3 days incubation; Alamar Blue assay)

> T cells do not proliferate in the absence of target cells

> In the presence of CD19$^+$ target cells, T cells become activated and proliferate

AFM11 targets only CD19$^+$ cells
No bystander cell killing



unlabeled CD19$^+$/CD30$^-$ JOK-1 cells mixed with calcein-labeled CD19$^-$/CD30$^+$ KARPAS-299 cells; PBMC E:T=50:1; 4 hrs assay

- Only CD19$^+$ cells are targeted by AFM11:
  - no lysis is observed when labeled CD19$^-$/CD30$^+$ KARPAS-299 cells are co-cultured with unlabeled CD19$^+$/CD30$^-$ JOK-1 target cells
  - labeled CD19$^-$/CD30$^+$ KARPAS-299 cells are lysed by CD30 directed TandAb (AFM13)
- AFM11 specifically facilitates T cell lysis of CD19$^+$ cells, and no bystander cell killing is observed

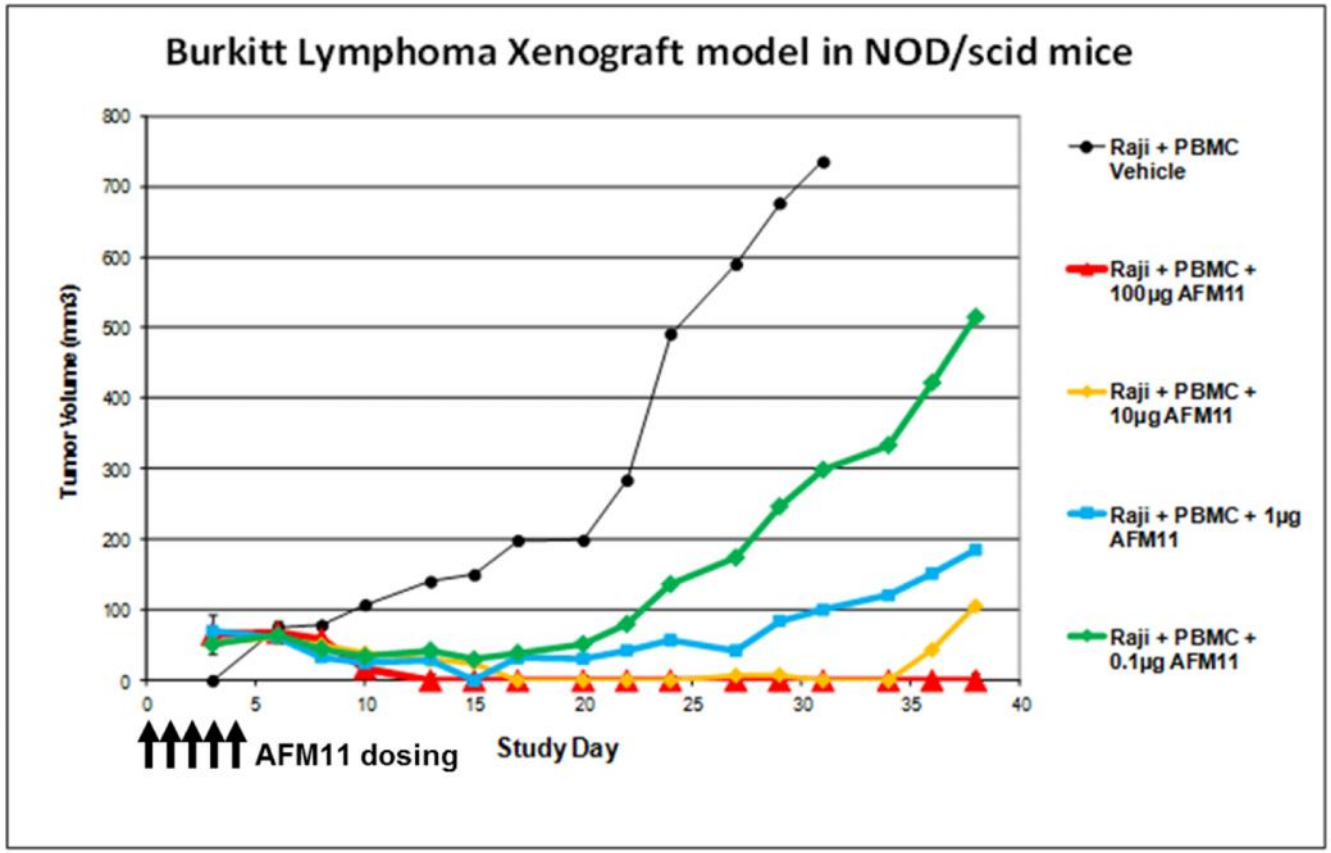
AFM11 *in vivo*
Xenograft model in NOD/scid mice

affimed
unique antibody therapeutics

- NOD/scid mice were implanted s.c. with 2.5 x10^6 Raji cells (Burkitt's lymphoma) premixed with 10^7 human PBMC (E:T = 4/1)
- Mice were randomly placed into 4 treatment groups each consisting of 3 cohorts (n=3) to address donor hPBMC variability
- Animals were dosed i.v. 100 – 0.1 ug/mouse (5 - 0.005 mg/kg) into the tail vein at 4 different dose levels of AFM11 on five consecutive days (d0 – d4)
- The following controls were used:
  - Raji only
  - Raji +PBMC
  - Raji + TandAb (5 mg/kg)

AFM11 *in vivo*
Complete suppression of Burkitt's Lymphoma

affimed
unique antibody therapeutics



- In lowest dose (0.1 µg or 5ug/kg) significant delay (~60%) in tumor growth
- In highest dose (100 µg or 5 mg/kg) complete protection

AFM11
Summary

affimed
unique antibody therapeutics

- **AFM11 (CD19 x CD3) is developed for the treatment of NHL, ALL, B-CLL**
  - A fully human antibody
  - Potency in low-sub-picomolar range
  - Effector cells from NHL patient facilitate potent cytotoxicity
  - Robust dose-dependent inhibition and eradication of tumor growth *in vivo*
  - Excellent safety profile - no T cell activation in the absence of target cells:
    - No cytokine release
    - No proliferation
    - No lysis of antigen-negative bystander cells
  - Very good stability, expression, solubility
  - IND is planned for 2013

Acknowledgements

- Fionnuala McAleese (Lead Discovery)
- Markus Eser (Lead Development)
- Kristina Ellwanger (Mammalian Cell Expression)
- Uwe Reusch (Cell Culture)
- Stefan Knackmuss (Preclinical Development)
- Christian Hücke (Clinical Development)
- Adi Hoess
- Rolf Günther
- Miroslav Ravic
- Erich Rajkovic
- Claudia Wall
- Melvyn Little
- Volker Lang and Vera Molkenthin (AbCheck)