

# Validation of *Cynomolgus* Monkeys as Relevant Species for Safety Assessment of a Novel Human BiTE Antibody Platform for Cancer Therapy

B. Rattel, R. Kischel, O. Thomas, M. Friedrich, D. Rau, E. Ebert, T. Raum, A. Wolf, S. Mangold, M. Kvesic, P. A. Baeuerle and P. Kufer

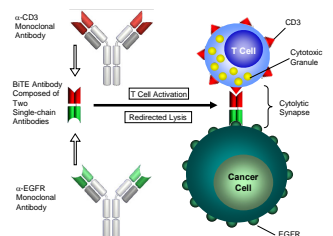
Micromet, Inc., 6707 Democracy Boulevard, Bethesda, 20817 Maryland, USA; Staffelsestr. 2, 81477 Munich, Germany;

## Background

Non-clinical safety assessment of antibodies for clinical use critically relies on their cross-reactivity with respective antigens of non-human animal species. The absence of binding to orthologous antigens precludes toxicology and safety pharmacology testing in commonly used, well-established and extensively characterized animal models. Accordingly, antigen cross-reactivity greatly influences the non-clinical developmental path taken for an antibody.

BiTE® antibodies (bisppecific T cell engager) are designed to transiently connect the CD3 subunit of the T cell receptor with an antigen on target cells. This creates a cytolytic synapse between the two cell types that very effectively activates the T cell leading to potent redirected and serial lysis of target cells.

Limited cross-reactivity has been seen with a first generation of BiTE antibodies. For example, for CD19/CD3-bispecific blinatumomab, which currently is in phase 2 clinical development, the sole pharmacologically-relevant animal species is the chimpanzee (*Pan troglodytes*). Because this animal is endangered, safety studies comprising a very small number of non-naïve animals can provide only very limited data sets, with no necropsy and histopathology evaluations possible.



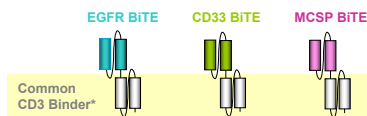
Generation and Mode of Action of EGFR-specific BiTE Antibody

One consequence of a limited cross-reactivity may be the need to invest in the generation and characterization of surrogate antibodies for safety studies with no guarantee that regulatory requirements will be satisfied.

As an alternative to surrogate antibodies or solely relying on *in vitro* pharmacology data, we have generated a series of novel human anti-CD3 antibodies that cross-react with non-human primate species, including *Cynomolgus* (Cyno) monkeys (*Macaca fascicularis*).

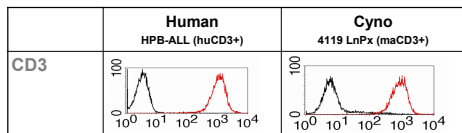
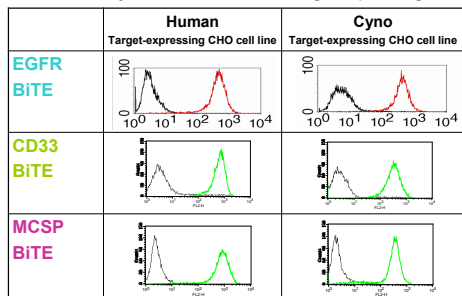
A selected human, primate cross-reactive anti-CD3 single-chain antibody has been used to generate three new BiTE antibodies specific for:

- (1) Endothelial growth factor receptor (EGFR), a validated target for treatment of colorectal cancer;
- (2) CD33, a target antigen for treatment of acute myelogenous leukemia (AML)
- (3) Melanoma-associated chondroitin sulfate proteoglycan (MCSP, NG2, HMW-MAA), a potential target for treatment of melanoma



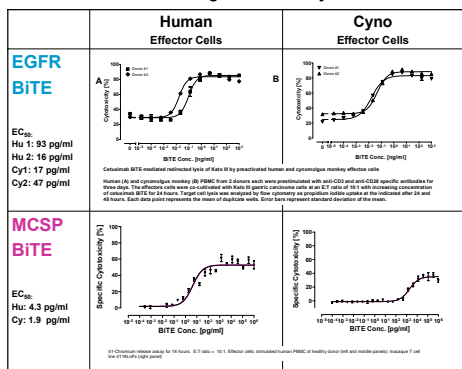
\*Fully human and cross-reactive with non-human primates

## Cross-reactivity of BiTE antibodies to target-expressing cells



FACS-based binding assays show comparable binding of BiTE to human and cynomolgus (Cyno) effector and target cell systems

## Redirected lysis of human antigen\* target cells by cross-reactive BiTEs using human and cyno effector cells

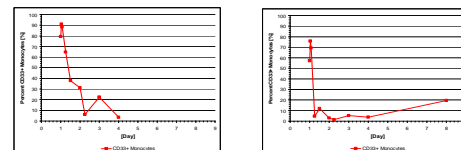


## Continuous intravenous infusion studies in *Cynomolgus* monkeys with BiTE antibodies

	EGFR BiTE (3-week infusion)	CD33 BiTE (1- or 2-week infusion)	MCSP BiTE (1-week infusion)
Dose Levels (µg/kg/d)	0 6.2 12.4 31.0 154.0	0 2.5 5.0 10.0 20.0 83.3	0 2.5 5.0 10.0 20.0 83.3 416.6

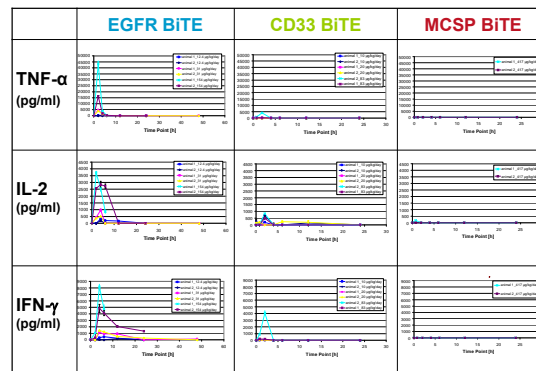
Two weeks before start of treatment, a catheter was implanted for continuous infusion into the posterior vena cava of animals via a femoral vein and tunneled subcutaneously to exit at the inter-scapular region. The catheter was attached to the delivery system via a tether system and a swivel joint. Groups of two male animals were administered either BiTE or vehicle as stated above.

## *Cynomolgus* monkeys are pharmacologically responsive to CD33 BiTE antibody



Depletion of circulating CD33-expressing monocytes by CD33 BiTE

## Distinct cytokine release patterns are induced by EGFR, CD33 and MCSP BiTE antibodies in *Cynomolgus* monkeys



## Distinct tolerability of EGFR, CD33 and MCSP BiTE antibodies in *Cynomolgus* monkeys

EGFR BiTE		CD33 BiTE		MCSP BiTE	
Dose (µg/kg/d)	Severe toxicity	Dose (µg/kg/d)	Severe toxicity	Dose (µg/kg/d)	Severe toxicity
0	0/2	0	0/2	0	0/2
6.2	0/2	2.5	0/2	2.5	0/2
12.4	0/2	5.0	0/2	5.0	0/2
31.0	2/2	10.0	0/2	10.0	0/2
154.0	2/2	20.0	1/2	20.0	0/2
		83.3	2/2	83.3	0/2
				416.6	0/2

Animals were observed at least daily during the study for mortality/morbidity as well as clinical signs/reaction to treatment. Other evaluations conducted both pre-test and on one or more occasions during the study included body temperature, ophthalmology, body weight, food consumption, cardiovascular function, haematology, coagulation, serum chemistry, urine analysis, lymphocyte subtyping (FACS), cytokine release and toxicokinetics. Serum was also prepared pre-test and at necropsy for possible immunogenicity evaluation. Animals were also subjected, at this latter occasion, to a thorough macroscopic assessment of external and internal surfaces, orifices and cavities, and tissues and organs as well as the implantation site(s). Selected organs were weighed and an extensive list of tissues sampled for subsequent histopathological examination

## EGFR, CD33 and MCSP BiTE antibodies show target-dependent toxicities in *Cynomolgus* monkeys

<b>EGFR BiTE</b>	<ul style="list-style-type: none"> <li>• 6.2 or 12.4 µg/kg/day were well tolerated during 3-week treatment</li> <li>• MTD was higher than pharmacologically active dose either obtained in <i>in vitro</i> experiments with <i>Cynomolgus</i> monkey effector cells, or needed in murine xenograft experiments for complete tumor inhibition</li> <li>• Pattern of target organ toxicity matches with TCR data of EGFR expression from cetuximab, i.e., kidney, gastrointestinal, liver, lung, etc.</li> <li>• No skin toxicity seen after 3 weeks of treatment</li> </ul>
<b>CD33 BiTE</b>	<ul style="list-style-type: none"> <li>• Depletion of circulating CD33-expressing target cells shows <i>in vivo</i> efficacy</li> <li>• MTD higher than pharmacologically active dose levels</li> </ul>
<b>MCSP BiTE</b>	<ul style="list-style-type: none"> <li>• No MTD reached</li> <li>• No T cell-related toxicities observed from high levels of human/Cyno cross-reactive CD3-arm of MCSP BiTE</li> </ul>

## Conclusions

- EGFR, CD33 and MCSP BiTE antibodies show cross-reactive binding to CD3 and the respective human or *Cynomolgus* orthologous antigens on target cells
- The three antibodies mediate redirected lysis of respective target cells with effector cells of human as well as *Cynomolgus* origin
- In *Cynomolgus* monkeys, EGFR, CD33 and MCSP BiTE antibodies show a distinct pharmacology with respect to cytokine release, MTD, and toxicity pattern, indicating that their unique, target-specific binding arms are responsible for such differences
- The anti-CD3 binding arm of the new BiTE antibody platform has little pharmacological activity on its own as is evident from the high tolerability of MCSP BiTE antibody in *Cynomolgus* monkeys
- There is evidence that the MTD of EGFR and CD33 BiTE antibodies exceeds the dose level needed for efficient target cell lysis, indicating a therapeutic window
- *Cynomolgus* monkeys are validated as a relevant species for non-clinical safety assessment of BiTE antibodies based on the new human BiTE platform