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Poster 159

Redirected Optimized Cell Killing (ROCK®): A novel multispecific antibody platform for innate immune cell engagement to fight cancer

Antibody-dependent cellular cytotoxicity (ADCC) has been described as a potent mechanism to destroy cells with high target antigen expression. Malignant cells can downregulate tumor-specific/-associated antigens (TSA/TAA) rendering ADCC of classical or Fc-enhanced antibodies less potent. To overcome these limitations, we have developed multivalent, bispecific innate cell engagers (ICEs) which bind the TSA/TAA on tumor cells and CD16A (Fc gamma receptor IIIA) on NK cells and macrophages with high affinity, leading to enhanced potency and efficacy in target cell killing compared to other ADCC-based approaches.

Recently, we used our ROCK® platform to generate a gallery of multivalent, multispecific ICEs based on different scaffolds. We show that these ICEs bind with high affinity and selectivity to an epitope on CD16A distinct from the Fc binding site, reducing competition with plasma IgG and mediating potent and efficacious killing of malignant cells with a detection threshold of <1000 copies of TSA/TAA per cell. The modularity of the ROCK® platform allows the design of antibodies with different pharmacokinetic (PK) properties and tailoring of properties to specific disease settings in hematological or solid tumors. Moreover, ICEs are engineered to prevent NK cell fratricide, despite their bivalent CD16A engagement.

An update on our product and development candidates will be provided. Phase 1 and 2 clinical studies of our lead product AFM13 have shown evidence of safety and clinical efficacy both as monotherapy and in combination with anti-PD-1 in patients with CD30+ lymphomas.

Our ICEs are highly differentiated and designed to potentially overcome the limitations of other approaches through improved potency, efficacy, safety and reduced plasma IgG competition. Thus, ICEs based on our ROCK® platform have the potential to expand treatment options for hematologic and solid tumors, both as monotherapy and in combination with other agents or adoptive NK cell transfer.

High affinity CD16A-specific engagement of innate immune cells

Optimized CD16A binding antibody domains

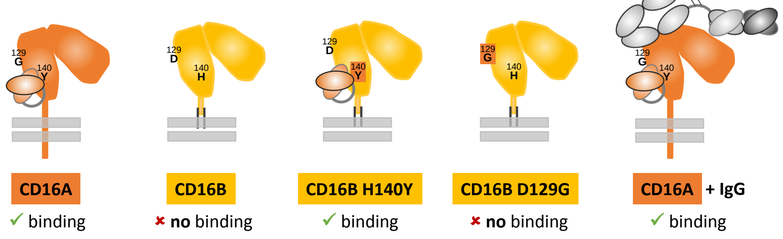
- CD16A-specific binding (NK cells and macrophages) and no binding to CD16B (neutrophil granulocytes)
- Affinity-matured scFv reach single digit nM K_D for monovalent binding to CD16A
- High affinity binding of both CD16A alleles 158V and 158F

Unique epitope on CD16A enables binding in the presence of serum IgG

- CD16A and CD16B differ in their membrane anchorage and few amino acids in the extracellular domains: Tyrosine (Y140) but not Glycine (G129) is crucial for CD16A selectivity of scFvs, as binding is induced by mutating CD16B H140Y but not CD16B D129G
- In contrast to anti-CD16 control 3G8, our scFv binding site on CD16A is distinct from the Fc binding site, resulting in low competition with plasma IgG in CD16A binding

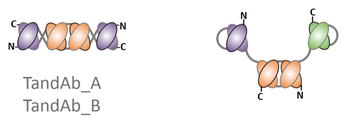
CD16 binding moiety	K_D [nM] to rec. antigen in SPR		K_D [nM] on NK cells at 37°C		
	CD16A-158V	CD16A-158F	in buffer	+10mg/mL IgG	fold loss
scFv Ab16^{lo}	308	286	n.t.	n.t.	n.t.
scFv Ab16^{mid}	21.0	19.8	52.3	138.3	2.7
scFv Ab16^{hi}	1.96	3.13	7.9	37.2	7.7
scFv 3G8	8.6	n.t.	3.8	64.6	21.4
IgG1 Fc	424	2720	n.t.	n.t.	n.t.
IgG1 Fc enh*	13.5	n.t.	n.t.	n.t.	n.t.

*Fc enhancing mutations S239D/I332E ; n.t.: not tested



ROCK® platform – modular architecture of antibody formats with bivalent CD16A binding

Variable fragment-based (symmetric) trispecific



TandAb_A
TandAb_B
TandAb_C

aTriflex

anti-CD16A (scFv, scDb or Db modules)

anti-TSA/TAA1

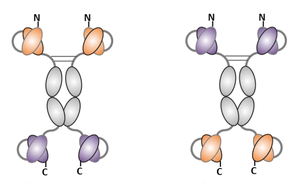
anti-TSA/TAA2

Knob-into-hole (KiH) asymmetric CH3 pair

Engineering variables

- Bivalent Ab16^{mid} or Ab16^{hi} variable domains as scFv, scDb or Db modules with different domain orders, or in Fab
- Fc domains containing effector function silencing mutations
- Versatility demonstrated with different target (TSA/TAA) binding moieties (e.g. EGFR, BCMA, CD19, CD20, CD30, RSV and undisclosed) in bivalent or monovalent form
- Two-in-one or trispecific formats

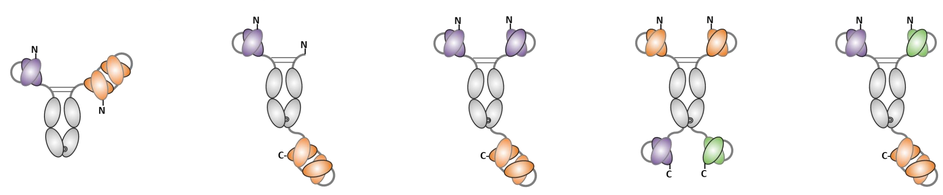
Fc fusion (symmetric)



Bi-scFv-Fc_A
Bi-scFv-Fc_D

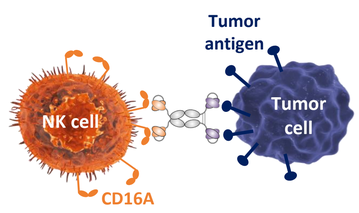
Bi-scFv-Fc_B
Bi-scFv-Fc_C

Fc fusion (asymmetric) trispecific



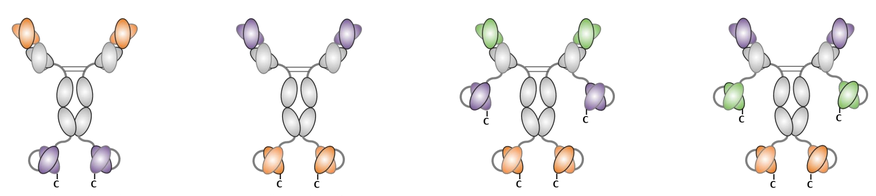
KiH-scFv-Fc_A

ROCK® mode of action



- NK cell redirection
- Tumor cell recognition
- NK cell activation and target lysis

IgG fusion (symmetric) trispecific



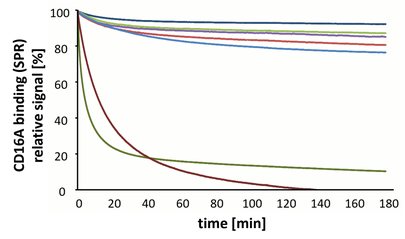
scFv-IgAb_A
scFv-IgAb_C
scFv-IgAb_E

scFv-IgAb_B
scFv-IgAb_D
scFv-IgAb_F
scFv-IgAb_G

ROCK® antibodies exploit avidity to maximize innate cell engagement via CD16A

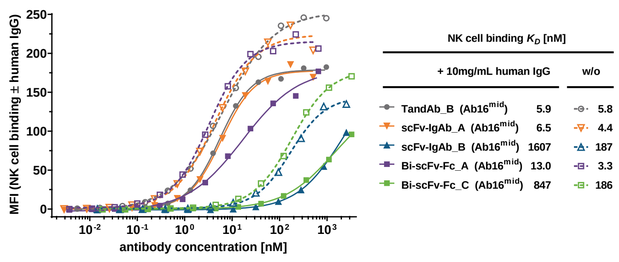
Bivalency substantially reduces dissociation from CD16A

- ROCK® antibodies show superior retention on CD16A compared with IgG Fc or monovalent anti-CD16 scFv



% molecule bound to receptor after 3h dissociation	human CD16A-158V	cyno. CD16
scFv-IgAb_C	92.5	97.9
Db-Fc_A	87.4	95.4
scFv-IgAb_D	85.3	90.0
KiH-scDb-Fc_A	80.8	90.3
TandAb_C	76.6	84.9
IgAb_E	10.3	10.5
scFv Ab16^{hi}	0.0	2.4

ROCK® antibodies bind to primary human NK cells with K_D values in the nM range even in presence of competing IgG

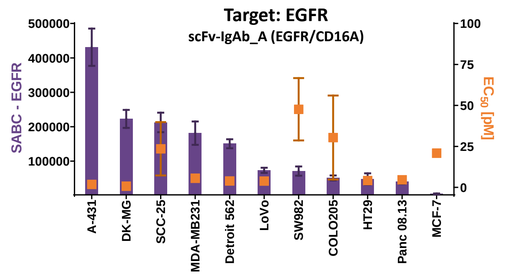


NK cell binding K_D [nM]	+ 10mg/mL human IgG	w/o
TandAb_B (Ab16^{mid})	5.9	5.8
scFv-IgAb_A (Ab16^{mid})	6.5	4.4
scFv-IgAb_B (Ab16^{mid})	1607	187
Bi-scFv-Fc_A (Ab16^{mid})	13.0	3.3
Bi-scFv-Fc_C (Ab16^{mid})	847	186

- Effects on binding affinity of different anti-CD16A modules, formats and domain order were also observed with recombinant antigen (Ellwanger et al., MAbs. 2019 Jul;11(5):899-918)

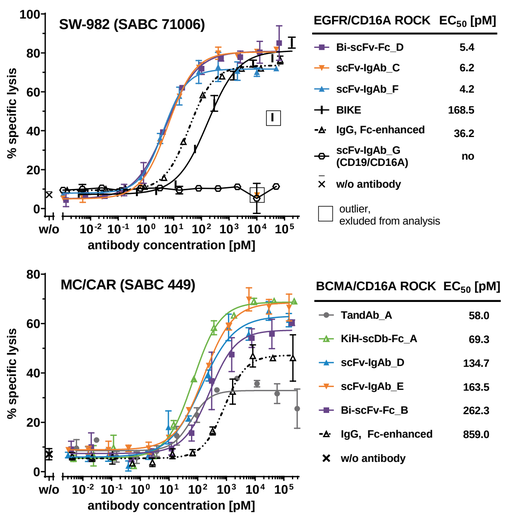
Superior *in vitro* cytotoxicity of ROCK®

Potent killing of target cells with high or low antigen density



- Highly potent and efficacious tumor cell lysis *in vitro* mediated by ROCK® antibodies on various cell lines, including cells with very low target expression levels (SABC)

In vitro potency is superior to comparators – NK cell binding strength translates into higher cytotoxic activity



EGFR/CD16A ROCK	EC_{50} [pM]
Bi-scFv-Fc_D	5.4
scFv-IgAb_C	6.2
scFv-IgAb_F	4.2
BIKE	168.5
IgG, Fc-enhanced	36.2
scFv-IgAb_G (CD19/CD16A)	no
w/o antibody	
outlier, exluded from analysis	

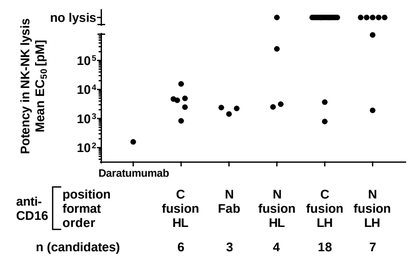
BCMA/CD16A ROCK	EC_{50} [pM]
TandAb_A	58.0
KiH-scDb-Fc_A	69.3
scFv-IgAb_D	134.7
scFv-IgAb_E	163.5
Bi-scFv-Fc_B	262.3
IgG, Fc-enhanced	859.0
w/o antibody	

- EGFR/CD16A ROCK® engager-induced NK cell-mediated lysis of EGFR$^+$ tumor cells *in vitro* demonstrates superior potency to comparators
- High *in vitro* potency and efficacy of BCMA/CD16A ROCK® engager even on BCMA^{low} target cells
- High affinity NK cell engagement by CD16A-ROCK® engager translates into highly potent and efficacious target cell lysis *in vitro*

ROCK® design prohibits NK cell fratricide

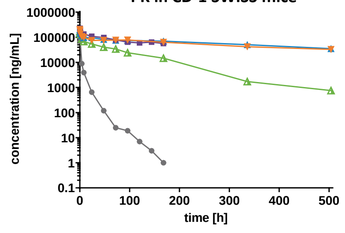
Risk of NK cell fratricide upon multivalent CD16A crosslinking is mitigated by using validated ROCK® modules

- Avoid depletion of effector cell population despite high affinity bivalent binding
- Daratumumab targeting CD38 induces NK cell crosslinking and fratricide
- Preferred ROCK® design ameliorates risk of NK cell fratricide
  - Variable heavy (VH) and light (VL) chain order VL-VH (LH)
  - Avoid N-terminal position of anti-CD16A moieties



ROCK® formats offer different PK profiles

PK properties confirmed in different mouse studies



PK parameters	$t_{1/2}$ [days]	Cl [mL/kg/days]	
scFv-IgAb_A	13.7	5.6	(1)
scFv-IgAb_D	15.2	4.8	(1)
Bi-scFv-Fc_A	4.0	11.2	(2)
KiH-scDb-Fc_A	3.1	32.0	(1)
TandAb_B	0.8	982.4	(2)

(1) in CD-1 SWISS mice (3 weeks study)
(2) in CD-1 SWISS mice (1 week study)

- IgG-like PK properties of scFv-IgAb format in CD-1 SWISS mice
- scDb- and Fc fusion constructs exhibit slightly shorter half-life and faster clearance in mice than typical IgG
- Fc-less constructs have short half-life in mice

Key attributes

- CD16A-specific antibody domains for innate cell redirection
- High affinity and avidity binding to a unique epitope on CD16A enables robust effector cell engagement in the presence of competing serum IgG
- Versatile platform of bispecific or multispecific innate cell engagers for targeting different tumor entities
- Potent and efficacious killing of tumor cells with high or low antigen densities
- Evidence of safety and efficacy from Phase 1 and 2 clinical studies with AFM13

Clinical stage ROCK® AFM13 (CD30/CD16A) to treat r/r HL and CD30+ lymphoma

Phase 1: Safety and clinical activity in heavily pre-treated HL patients

- Dose escalation study: 0.01 – 7.0 mg/kg
- No MTD reached, favorable safety profile determined
- Tumor shrinkage in 62 % (8/13) and PRs in 23% (3/13) of patients at doses of ≥1.5 mg/kg

Phase 1b/2a: IST in r/r CD30+ lymphoma (Columbia University, ongoing)

- Preliminary efficacy data: 10 patients treated to date in 3 dose cohorts: 50% ORR including 1 CR (10%) and 4 PRs (40%)

Phase 1b: Combination with pembrolizumab in r/r HL

- Recruitment completed into dose expansion cohort; total of 24 patients evaluable in highest AFM13 dose cohort
- Preliminary efficacy data for 24 patients treated at highest dose level of AFM13
  - ORR of 88% (21/24) and CR rates of 42% (10/24) and 46% (11/24) by local and independent assessments
- Well-tolerated with adverse events generally mild to moderate in nature and manageable

In preparation:

- Phase 2 registration-directed study of AFM13 in r/r CD30+ T cell lymphoma or transformed mycosis fungoides (REDIRECT)
- Phase 1 IST of adoptive NK cells in combination with AFM13 in r/r CD30+ non-Hodgkin or Hodgkin lymphoma (MD Anderson Cancer Center)