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 multispecific, bispecific innate cell engagers (ICEs) which bind the TSA/TAA on tumor cells and CD16A (Fc gamma receptor IIa) 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.

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 clearance. 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 C16F and 16B

Unique epitope on CD16A enables binding in the presence of serum IgG
- CD1A and CD14B differ in their membrane anchorage and few amino acids in the extracellular domains. Tyr526 (14B) but not Glu586 (CD1A) is crucial for CD16A selectivity of scFv, as binding is induced by mutating CD14B H526Y but not CD16B E519G
- 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

Table 1: Potency of CD16A ROCK cocktail.

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<thead>
<tr>
<th>Antibody</th>
<th>EC50[pM]</th>
<th>n (%)</th>
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<tbody>
<tr>
<td>Daratumumab</td>
<td>163.5</td>
<td>99</td>
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<tr>
<td>MC/CAR (SABC 449)</td>
<td>50</td>
<td>80</td>
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Variable fragment-based (symmetric) trispecific

Fc fusion (symmetric) trispecific

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

Biavility substantially reduces dissociation from CD16A
- ROCK® antibodies show superior retention on CD16A compared with IgG Fc or monovalent anti-CD16 scFv

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

<table>
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<tr>
<td>ROCK®</td>
<td>&lt;1</td>
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Superior in vitro cytotoxicity of ROCK®

Potent killing of target cells with high or low antigen density

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

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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 binding
- Quantitatively targeting CD38 induces NK cell crosslinking and fratricide
- Preferred ROCK® design ameliorates risk of NK cell fratricide

- Variable hinge (H1a and H1b) clean-out

- No intermolecular avidity to CD16A receptors

ROCK® formats offer different PK profiles

PK properties confirmed in different mouse studies

- IgG-like PK profiles of scFv-igG fusions in CD1-1 Swiss mice
- scFv-FC fusion constructs exhibit slightly shorter half-life and faster clearance in mice than parental IgG
- Fc-less constructs have short half-life in mice

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

Phase 1a: Safety and clinical activity in heavily pre-treated HL patients
- Dose escalation study: 0.1 – 7.5 mg/kg
- No MTD reached, favorable safety profile determined
- Tumor shrinkage in 62 % (4/13) and PRs in 23 % (3/13) of patients at doses of ≤5.5 mg/kg

Phase 1b: Combination with pembrolizumab in rHL
- 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 48% (2/4) and CRs of 42% (2/4) and 46% (2/4) by local and independent assessments
- Well-tolerated with adverse events generally mild to moderate in nature and manageable

In preparation:
- Phase 1 registration-directed study of AFM13 in rHL + CD16+ T cell lymphomas or transformed mycosis fungoides (REDBRICK)
- Phase 1/2 of adoptive NK cells in combination with AFM13 in rHL + CD16+ non-Hodgkin or Hodgkin lymphomas (MD Anderson Cancer Center)