AFM13: a first-in-class tetravalent bispecific anti-CD30/CD16A antibody for NK cell-mediated immunotherapy

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Abstract

Monoclonal antibodies against CD20 molecule have been leading the revolution of lymphoma treatment. In addition to monoclonal antibodies against CD20 and CD30, novel agents of immunotherapeutics in clinical development are being developed and are rapidly migrating to clinical application. One area of active development is NK cell activators, such as AFM13. This review will highlight the latest development of AFM13 as the first-in-class tetravalent bispecific anti-CD30/CD16A antibody for NK cell-mediated immunotherapy.

Precision therapy with targeted agents against tyrosine kinases and cancer-associated molecules has been leading the revolution of cancer treatment [1–7]. Cancer immunotherapy represents another wave of revolution in cancer therapy [8–13]. In addition to monoclonal antibodies against CD20 and CD30 [14, 15], the main agents of immunotherapeutics in clinical development include the following: (1) immunomodulators (e.g., lenalidomide) [16]; (2) immune checkpoint blockers (e.g., pembrolizumab, nivolumab, ipilimumab) [10, 11, 17]; (3) T cell activators (e.g., CAR-T19; blinatumomab, AFM11) [18–20]; (4) inhibitors of B cell receptor signaling (e.g., ibrutinib) [2, 21]; and (5) NK cell activators (e.g., AFM13) [22]. AFM13 is a first-in-class tetravalent bispecific anti-CD30/CD16A antibody for NK cell-mediated immunotherapy.

AFM13 AFM13 is a tetravalent bsAb against CD30 and CD16A produced from the mammalian CHO cells by Reusch et al. [27]. Initially, a human anti-CD16A antibody with no binding to 16B isoform was isolated. The variable anti-CD16A-specific human scFv was then derived. The anti-CD30 Fv domain was derived from the murine HRS-3 IgG. The heavy and light chain DNA sequences of CD30 and CD16A were then molecularly engineered in a special order (Fig. 1) [27]. The CD30 and CD16A peptide domains were linked by a 9-amino acid linker peptide to form a bispecific diabody [28]. A tandem diabody with four domains was engineered to form a single

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polypeptide (nonfunctional monomer) (Fig. 2). A fully functional tetravalent bispecific chimeric antibody construct (TandAb) is formed by homodimerization of the single polypeptide monomer through non-covalent interactions of the domains in the Ig heavy (\(V_H\)) and light (\(V_L\)) variable chains. The TandAb has a molecular weight of 104 kDa. One arm of AFM13 binds to the CD30 antigen on lymphoma cells, whereas the other arm binds to the CD16A antigen on the NK cells (Fig. 3). The anti-CD30/CD16A tetravalent bsAb AFM13 was shown to have an IC\(_{50}\) value of 35.8 nM for CD30 antigen. Cytotoxicity assays showed that the AFM13-mediated activation of NK cells was strictly CD30-dependent. In the absence of CD30 target cells, neither cytotoxicity nor NK cell activation was elicited by the TandAb [27].

AFM13 was studied by the groups in Germany and in MD Anderson Cancer Center in a phase I dose-escalation study in 28 patients who have been heavily pretreated for their relapsed/refractory CD30+ HL (AFM13-101, NCT01221571) [22]. AFM13 was infused weekly for 4 weeks as one cycle at doses ranged between 0.01 and 7 mg/kg body weight. The MTD was not reached. The only dose-limiting toxicity reported in the study was hemolytic anemia in a patient who received 3 infusions at 0.5 mg/kg. Significant NK cell activation and reduction of soluble CD30 in peripheral blood were reported, though the best clinical response was only PR (11.5 %, 3/26 evaluable patients). In patients who received AFM13 at a dose of \(\geq\) 1.5 mg/kg, the overall response rate was 23 % and the overall disease control rate was 77 % in the heavily pretreated subjects. Brentuximab vedotin (BV) is an antibody-drug conjugate which also binds to CD30 and delivers a chemotherapeutic agent to the CD30+ cells [15]. In this study, AFM13 was found to be active in BV-refractory patients. Since the effector mechanism of

![Gene structure of tetravalent bispecific AFM13 antibody domains. The heavy and light chain DNA sequences of CD30 and CD16A were molecularly engineered in the special order as shown. This figure was modified from Rothe et al. and Reusch et al. [22,27]](image1)

![Protein structure and antibody formation pathway of the tetravalent bispecific AFM13 antibody. The CD16A (domain A, diamond shape) and CD30 (domain B, oval shape) peptide domains were linked by a 9-amino acid linker (L) to form a single polypeptide (nonfunctional monomer). A fully functional tetravalent bispecific chimeric antibody construct (TandAb) is formed by homodimerization of the single polypeptide monomer in a head-to-tail fashion through non-covalent interactions (dotted lines) of the domains in the Ig heavy (\(V_H\)) and light (\(V_L\)) variable chains. The AFM13 TandAb has a molecular weight of approximately 104 kDa. This figure was modified from Rothe et al. and Reusch et al. [22, 27]](image2)
these two antibodies is entirely different, AFM13 represents a novel agent for HL refractory to BV.

Issues and future directions
AFM13 is a chimeric antibody with a murine anti-CD30 domain. Antidrug antibodies (ADA) were detected in 15 out of 28 patients. Half of the detected ADAs had neutralizing potential. This remains an issue for further investigation in a large cohort. The half-life of AFM13 was 19 h, which is longer than that of the smaller bispecific BiTE antibody, blinatumomab, which is administered as a continuous infusion [19, 29]. Dosage, dosing-schedules, such as twice-weekly dosing, and duration of treatment are the issues for further investigations. Future studies are also needed for correlation of clinical activity with biomarkers, such as NK cell numbers, and soluble CD30 molecules. Evaluation of these biomarkers in the biopsy specimens will also be informative. A phase II study is underway for this novel tetravalent bsAb AFM13 as the first-in-class NK cell-specific agent for cellular immunotherapy targeting CD30+ malignancies (GHSG-AFM13, NCT02321592). Additional tetravalent bsAbs are also being engineered for targeting other malignancies [20, 30].

Competing interests
The authors declare that they have no competing interest

Author contributions
DL designed and drafted the manuscript. JW and JF assisted on the reference and data preparation. DL and JF designed and finalized the figures. JW and JF contributed equally to this work. All authors were involved in the manuscript preparation and revisions. All authors read and approved final manuscript.

Acknowledgements
Jiapeng Fu received a fellowship grant from Shaoxing People’s Hospital, Zhejiang Province, China. Jingjing Wu is a recipient of the Henan Provincial Grant for Overseas Research for Young Leaders of Medical Technology (No. 2014041). The grants supported their research training at the Division of Hematology and Oncology, New York Medical College, USA.

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Received: 4 July 2015 Accepted: 16 July 2015
Published online: 01 August 2015

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Fig. 3 AFM13-mediated activation of NK cells. One arm of AFM13 binds to the CD30 antigen on lymphoma cells, whereas the other arm binds to the CD16A antigen on the NK cells. The activated NK cells destroy the lymphoma cells. The NK cell activation and lymphoma destruction mediated by AFM13 are CD30-dependent
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