

# T cell engagers targeting CD19 to treat B cell malignancies, and beyond

Xiaoqiang Yan, Ph.D\*

ITabMed Ltd. Shanghai, China

\*Author for correspondence:  
Email: xiaoqiang.yan@itabmed.com

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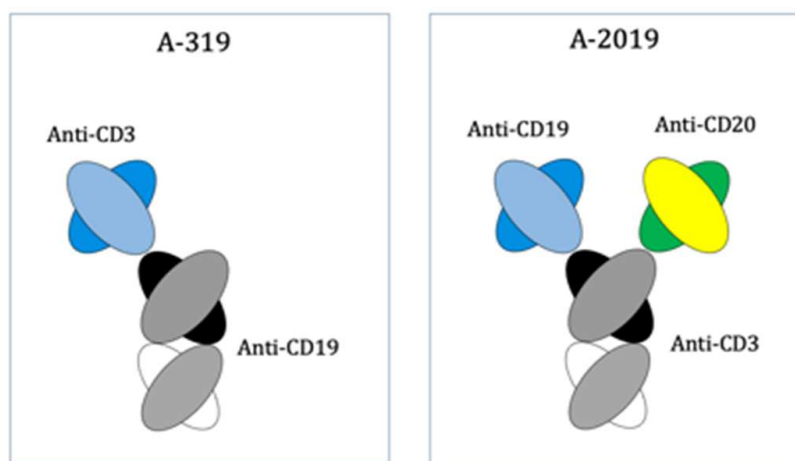
In the past decade, a tremendous progress has been made in developing T cell engaging bi-specific antibodies (BsAbs) targeting CD19 to treat B cell malignancies. The T cell engaging bi-specific molecule binds to CD3 on a T cell and a tumor-associated antigen (TAA) on a target cell and redirects the T cell to a specific target cell bypassing the MHC restriction mechanism, leading to the formation of immune synapse between the T cell and target cell and target cell lysis [1,2].

To date, Blinatumomab, a scFv CD3/CD19 BiTE™, is the only T cell engaging BsAb approved by regulatory agencies including US FDA, EMA and China NPMA to treat B cell precursor acute lymphoblastic leukemia (B-ALL) [3]. In the development of blinatumomab to treat non-Hodgkin's lymphoma (NHL) patients, a significant long-term survival benefit was reported at the maximum tolerated dose (MTD) [4]. No further clinical development was conducted might be due to the insufficient therapeutic index, or a low safety window to treat NHL. In addition to the continuous intravenous (IV) infusion and significant side effects of blinatumomab treatment, the loss of CD19 surface marker post-blinatumomab treatment was reported in some patients (12% to 30% in B-ALL), a phenomenon described as antigen escape [5]. Antigen escape was also reported in CD19 CART treated patients (7% to 25% in ALL, and 27% in NHL) [6]. Antigen escape appears to be the key factor in disease relapse or resistance to the CD19 targeted immunotherapies. Therefore, to develop a CD3 T cell engager targeting CD19 and CD20 simultaneously may evade the antigen escape hurdle thus to bring improved clinical benefits to patients with B cell malignancies.

To overcome the loss of CD19 antigen in the CD19 targeted immunotherapy, Wang et al. reported preclinical study results with a CD3/CD19/CD20 tri-specific antibody [7]. The reported two CD3/CD19 bi-specific antibodies, namely A-319 and A-2019, were generated from the immunotherapy antibody platform (ITab™) with a series of structure optimization [8]. The CD3 antibody was a humanized SP34 antibody with monkey cross-reactivity. It has reduced T cell activation and cytokine release compared to OKT3 antibody [9,14]. The Fab containing structure improved the chemistry, manufacture and control (CMC) process with superior drug stability in comparison to BiTE™, or BiTE-like molecules (Figure 1). A-319 and A-2019 has molecular weight of 75 Kd, and 100 Kd, respectively, thus with reduced clearance and without the need for continuous IV infusion to reach an expected drug exposure in patients. The lack of a Fc fragment in both A-319 and A-2019 molecules aimed to evade the Fc effector function-associated off-target toxicities [10]. ITab™ design and dose regiment would provide patients with a drug-free interval and may have the potential to avoid T-cell exhaustion which is likely due to the extended or continuous drug exposure [15,16]. A-319 is currently in phase I clinical development to treat NHL and B-ALL patients.

The report by Wang et al. [7] demonstrated that both A-319 and A-2019 exhibited BiTE-like mechanism of action, including the formation of synapse between autologous B cell and T cell, B-cell depletion and T cell activation and proliferation, as well as cytokine release in primary B-ALL patient samples. A-2019 is slightly less efficient in the synapse formation, and T cell activation, and targeting killing and in tumor growth inhibition in the Raji Xenograft model when compared to those of A-319. It appears that A-319 has a CD3 in the scFv format, while A-2019 has CD3 in a Fab format with the addition of CD20 binding domain which could result in a weaker CD3 engaging activity. A-319 has a CD19 Fab, while A-2019 has a CD19 in the scFv format, although the biological effect of this difference has not been evaluated. The dose-dependent and potent *in vitro* CD19 (EC<sub>50</sub> at 1.2 pM) and CD20 (EC<sub>50</sub> at 3.4 pM) target cell killing, and Raji cell killing (EC<sub>50</sub> at 0.5 pM). The *in vivo* efficacy of A-2019 demonstrated that targeting CD19 and CD20 simultaneously is a valid approach

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**Figure 1:** Illustration of A-319 and A-2019 design. A CD19 binding Fab domain is shown in A-319, while a CD3 binding Fab domain is shown in A-2019.

to design a tri-specific T cell engager to treat B cell malignancies.

CD3/CD19 T cell engaging bispecific antibodies have obvious advantages over CAR-T based cell therapies due to its convenience, off-the-shelf and easy access for patients. The long-term overall clinical benefits and relapses rate of the two therapies appears to be comparable [11,12]. In the clinical development of CD19 targeted immunotherapies, a great deal of acknowledge have been accumulated to manage cytokine release syndrome (CRS) and neurotoxicity in patients. It remains a great challenge to have a large enough clinical safety window for CD3 T cell engagers in clinical development.

A recent study reported that CD19 is expressed in human brain mural cells using single cell-seq and confirmed by IHC staining, suggesting that the treatment-related neurotoxicity is CD19 target related [13]. In addition, tissue/cell damage caused by the immune cell over activation involving T cells, monocyte/macrophages etc. requiring sufficient time to repair and to reestablish a homeostasis, a consideration that may have been ignored or overlooked during the early drug development stage. It is reasonable to believe that maintaining a constant level of blinatumomab in circulation for weeks, or a long-acting CD3 BsAb, or CART cells would narrow the safety window in clinical development, as reported in the blinatumomab NHL trials, in which the long-term survival benefit was shown at MTD level [4]. The design of A-2019 may have advantages to overcome some of the safety issues and remains to be further investigated in preclinical and clinical studies.

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