

Improved Kinetics and Potency of DXL625 (CD20) Compared to Rituximab

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ABSTRACT

The use of monoclonal antibodies (Mab) as therapeutic agents for a variety of cancers is well established. Expanding the therapeutic range of existing Mabs and inclusion of new antibodies as therapeutic agents may be possible in many cases through enhancement of their binding kinetics and of their ability to mediate effector functions (potency). A unique approach to increase the potency of monoclonal antibodies involves conferring “autophilic” or self-binding activity to the biologic agent. Antibodies photo-chemically conjugated to novel, self-associating DXL™ peptides remain monomeric in solution however, following engagement with their epitope the DXL-antibodies form clusters at the target site. Data reported here delineates improved kinetics and potency of the DXL-conjugated preclinical candidate antibody, DXL625 (CD20), compared to a commercially available therapeutic CD20 antibody (rituximab). Surface plasmon resonance (SPR, BIAcore) sensorgrams illustrate that although the two antibodies have similar association kinetics with immobilized CD20-peptidomimetics [$K_{on(DXL625)} = 1.0 \text{ mM s}^{-1}$ vs. $K_{on(rituximab)} = 1.2 \text{ mM s}^{-1}$], DXL625 dissociates more slowly from immobilized CD20 compared to rituximab [$K_{off} = 0.004 \text{ s}^{-1}$ for DXL625 vs. $K_{off} = 0.02 \text{ s}^{-1}$ for rituximab]. Flow cytometry revealed that DXL625 also “sees” more surfaced-expressed antigen on established B-cell tumor cell lines ($MFI_{(DXL625@10\text{mg/ml})} = 25,150$ vs. $MFI_{(rituximab@10\text{mg/ml})} = 2,621$) and is superior to rituximab in mediating *in vitro* apoptosis of established B-cell tumor cell lines. DXL625 was more effective than rituxan at inhibition of B-cell proliferation and was inhibitory with lines selected for resistance to rituxan. Furthermore, recent *in vivo* data indicate that DXL625 is superior to rituximab in a B-cell depletion model in rabbits, while displaying no significant toxicity or specific immunogenicity. In conclusion, there is full agreement using both *in vitro* and *in vivo* model systems that DXL625 significantly outperformed a commercially available anti-CD20 antibody (rituximab).