The anti-CD20 monoclonal antibody GA101 displays more robust anti-tumor activity versus Rituximab or Ofatumumab in Waldenstrom's Macroglobulinemia (WM).

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Background: Rituximab is an IgG class CD20-directed monoclonal antibody used in the treatment of B-cell malignancies, including WM. Ofatumumab represents a novel CD20-directed monoclonal antibody for the treatment of indolent NHL. We and others have previously demonstrated dependence for IgG class therapeutic antibodies on polymorphisms at FcγRIIIA-158. Approximately half of WM patients express V/V or V/F, and the remainder half express F/F at this polymorphic locus. Patients with WM expressing FcγRIIIA-158 V/V or V/F show improved rituximab single agent activity, as well as attainment of deeper responses (VGPR or CR) with combination rituximab therapy. GA101 is a novel humanized anti-CD20 antibody with a glyco-engineered Fc domain that exhibits increased Fcγ receptor binding and ADCC activity. **Methods:** In this study, we examined the *in vitro* activity of GA101, Rituximab and Ofatumumab against WM cells, and also examined the activity of these antibodies in context of FcγRIIIA-158 polymorphisms. ADCC activity was assessed using genotyped healthy donor derived NK cells against BCWM.1 WM cells, as well autologous NK cells against the patient's own lymphoplasmacytic cells. *In vitro* B-cell depletion and direct cell death induction assays were also performed. **Results:** We observed significantly greater ADCC activity against WM cells for GA101 versus Rituximab or Ofatumumab in both healthy donor, as well as autologous NK cell assays. GA101 mediated ADCC activity was particularly more robust versus Rituximab or Ofatumumab in patients expressing FcγRIIIA-158 F/F versus V/V or V/F (**Figure 1**). In addition, GA101 induced significant direct cell death against WM lymphoplasmacytic cells, as well as *in vitro* B-cell depletion assays in comparison to Rituximab or Ofatumumab, which exhibited little or no direct activity. Nuclear translocation of apoptosis inducing factor (AIF) was observed following GA101 by immunofluorescence microscopy. **Conclusions:** GA101 is associated with enhanced ADCC activity relative to Rituximab or Ofatumumab by NK cells, particularly for those subjects expressing FcγRIIIA-158 F/F. In addition, GA101 demonstrated direct cell death in WM lymphoplasmacytic cells through an AIF mediated caspase-independent pathway. These studies provide the framework for the investigation of GA101 in WM, and suggest particular benefit for those patients who express FcγRIIIA-158 F/F.

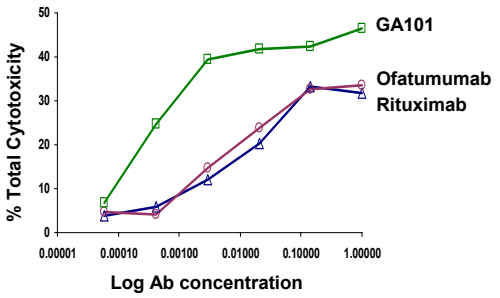

Figure 1: The comparation of ADCC activities for GA101 vs. Rituximab and Ofatumumab using autologous NK cells from WM patients (the FcγRIIIA-158: V/F) as effecters and WM LPCs as target cells. V: valine; F: phenylalanine.

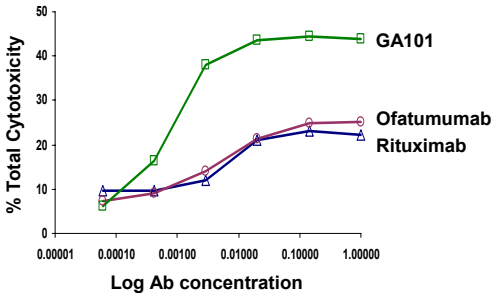

Figure 2: The comparation of ADCC activities for GA101 vs. Rituximab and Ofatumumab using healthy donor NK cells (the FcγRIIIA-158: F/F) as effecters and WM cells, BCWM.1 as target cells. F: phenylalanine.