AGI-134 is a fully synthetic alpha-Gal glycolipid that prevents the development of distal lesions and is synergistic with an anti-PD-1 antibody in a mouse melanoma model

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BACKGROUND
AGI-134 is a fully synthetic glycolipid-like molecule, composed of an alpha-Gal (Galα1,3GalNAc-R) sugar moiety attached via a linker to a lipid tail. AGI-134 is a Function-Spacer-Lipid (FSL) molecule (Kode Bovin, Auckland, NZ). Natural anti-Gal antibodies to the alpha-Gal epitope are responsible for the hyperacute rejection of non-primate mammalian xenografts in humans. AGI-134 has been proposed that intratumorally administered alpha-Gal glycolipid will incorporate into the plasma membranes of tumor cells, presenting the alpha-Gal epitope for recruitment of anti-Gal antibodies to the tumor. This will initiate a hyperacute immune response that attacks the infected tumor and, through uptake of immune-complexed tumor antigens by antigen presenting cells, will create patient-specific, systemic antitumor activity. Here we present the in vitro and in vivo characterization of the mechanism of action and efficacy of AGI-134.

METHODS
In vitro
Cells were incubated with AGI-134 in PBS for 1 hour at 37°C prior to downstream analysis. In complement depletion experiments, cells were incubated with 2.5% human serum for 30 minutes before staining and analysis by flow cytometry. In complement-dependent cytotoxicity (CDC) experiments, cells were incubated with 50% normal or heat-inactivated human serum for 1 hour before cell viability was determined using Cell Titre Glo reagent (Promega). Antibody-dependent cellular cytotoxicity (ADCC) experiments were performed with an ADCR reporter assay (Promega). For phagocytosis assays, human monocytes were differentiated into macrophages using M-CSF. A49 cells were labelled with 111I and then incubated with macrophages in the presence of human serum (NH9L). In vivo
s1.3-galactosyltransferase knockout mice (GT-KO) were immunized with pig kidney homogenate to induce anti-Gal antibodies prior to experimentation. B16-F10 cells (alpha-Gal negative) were grafted onto the right (1×10⁶ cells) and left (1×10⁶ cells) flanks to create ‘primary’ and ‘secondary’ tumors, as depicted. The primary tumor was injected with AGI-134 and the growth of the secondary tumor monitored for up to 90 days. The design of the combination experiments using the anti-PD-1 antibody RMP1-14 (Boehringer) are depicted. Statistical differences in secondary tumor development over time were calculated using Mantel-Cox test (*, p<0.05; **, p<0.005; ***, p<0.0005; n.a., not significant).

RESULTS
AGI-134 binds anti-Gal antibodies to labelled A49 cells and stimulates complement deposition from human serum

AGI-134 induces complement-dependent cytotoxicity and antibody-dependent cellular cytotoxicity of labelled human cancer cells

AGI-134 promotes phagocytosis of A49 cells by human macrophages

The B16 melanoma model in GT-KO mice as a model of adaptive anti-tumor immunity

When AGI-134 is combined with an anti-PD-1 antibody, the efficacy of both molecules is enhanced

REFERENCES & ACKNOWLEDGEMENTS

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SS and SK contributed equally to this work.

SUMMARY & CONCLUSIONS

• To combat challenges associated with tumor antigen heterogeneity, treatments that target the diversity of patient tumor neoantigens are urgently required.
• Our hypothesis is that intratumoral administration of AGI-134 will drive adaptive anti-tumor immunity to a diverse panel of patient-specific tumor neoantigens.
• AGI-134 inserts into cell membranes of all human cancer lines tested, binds anti-Gal antibodies to the cells, leading to complement-dependent cytotoxicity, antibody-dependent cellular cytotoxicity and effective FcγRI-CR uptake by antigen presenting cells.

• We have used the challenging B16 melanoma model, a poorly immunogenic and highly immunosuppressive tumor that is often predictive of immunotherapeutic efficacy in man.
• A single intratumoral dose of AGI-134 strongly protects mice from distal B16 tumor development for up to 90 days.
• AGI-134 and an anti-PD-1 antibody are synergistic when combined in the B16 model.
• AGI-134 holds promise as a new immunotherapy for solid tumors, alone and in combination with checkpoint inhibitors. AGI-134 will be entering clinical trials shortly.

• SS and SK contributed equally to this work.