4862 AGI-134: 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 (Galα1-3Galβ1-4GlcNAc-R) sugar moiety attached via a linker to a lipid tail. AGI-134 is a Function-Spacer-Lipid (FSL) molecule (Kode Biotech, Auckland, NZ).

Natural anti-Gal antibodies to the alpha-Gal epitope are responsible for the hyperacute rejection of non-primate mammal xenografts in humans[1,2,3]. It has been proposed that intratumorally administered alpha-Gal glycolipids 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 injected tumor and, through uptake of immune-complexed tumor antigens by antigen presenting cells, will create patient-specific, systemic anti-tumor activity[4,5].

Here we present the *in vitro* and *in vivo* characterization of the mechanism of action and efficacy of AGI-134.

METHODS

In vitro

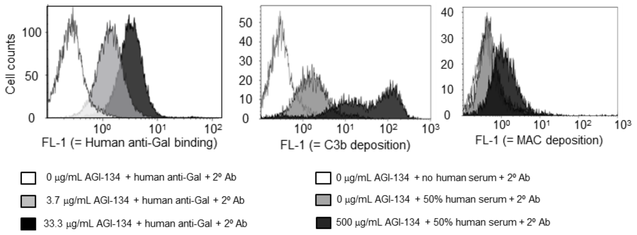
Cells were incubated with AGI-134 in PBS for 1 hour at 37ºC prior to downstream analyses. In complement deposition experiments, cells were incubated with 2.5% human serum for 20 minutes before staining and analysis by flow cytometry. In complement-dependent cytoxicity (CDC) experiments, cells were incubated 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 ADCC reporter assay (Promega). For phagocytosis assays, human monocytes were differentiated into macrophages using M-CSF. A549 cells were labelled with AGI-134 and then incubated with macrophages in the presence of human serum (NHS).

In vivo

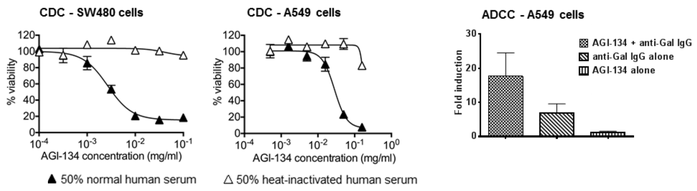
α1,3-galactosyltransferase knockout mice (GT-KO) were immunised with pig kidney homogenate to induce anti-Gal antibodies prior to experimentation. B16-F10 cells (alpha-Gal negative) were grafted onto the right ($1x10^6$ cells) and left ($1x10^4$ cells) flanks to create “primary” and “secondary” tumors, 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 (Bio X Cell) are depicted. Statistical differences in secondary tumor development over time were calculated by Mantel-Cox test (*, p<0.05; **, p<0.005; ***, p<0.0005; n.s., not significant).

RESULTS

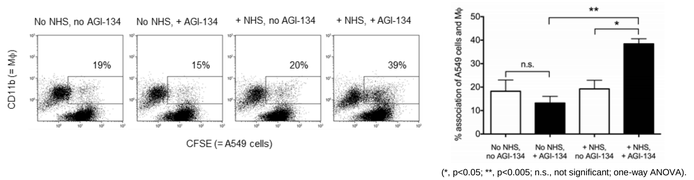
AGI-134 binds anti-Gal antibodies to labelled A549 cells and stimulates complement deposition from human serum



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

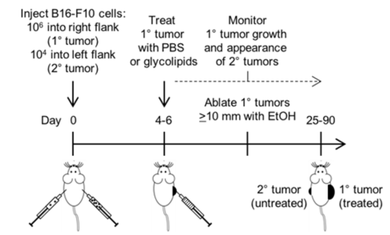


AGI-134 promotes phagocytosis of A549 cells by human macrophages

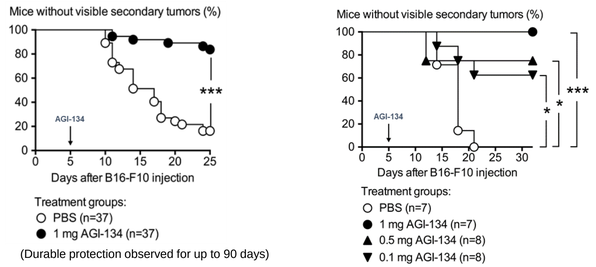


(*, p<0.05; **, p<0.005; n.s., not significant; one-way ANOVA).

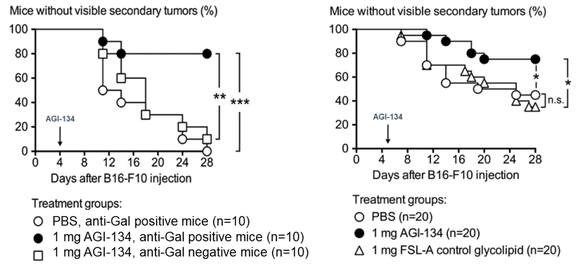
The B16 melanoma model in GT-KO mice as a model of adaptive anti-tumor immunity



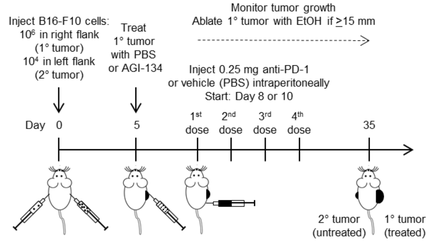
A single intratumoral dose of AGI-134 strongly protects GT-KO mice from the development of secondary lesions

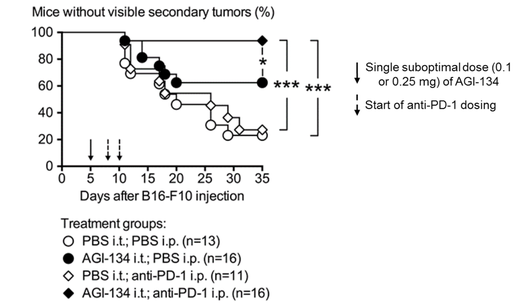


The efficacy of AGI-134 is dependent upon the interaction between anti-Gal and alpha-Gal

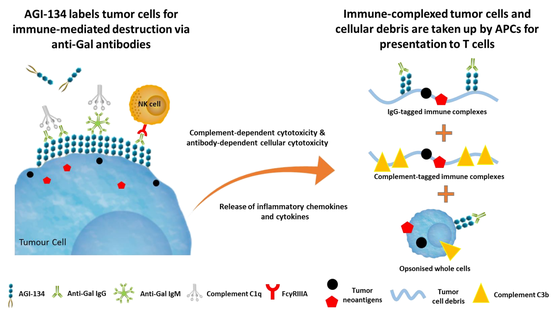


When AGI-134 is combined with an anti-PD-1 antibody, the efficacy of both molecules is enhanced





Proposed mechanism of action of AGI-134



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 cells tested, binds anti-Gal antibodies to the cells, leading to complement-dependent cytotoxicity, antibody-dependent cellular cytotoxicity and effective FcγR/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.

REFERENCES & ACKNOWLEDGEMENTS

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