3083 AGI-134, a fully synthetic α-Gal-based cancer immunotherapy that shows synergy with anti-PD-1 and favorable pre-clinical pharmacokinetic and toxicity profiles

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INTRODUCTION

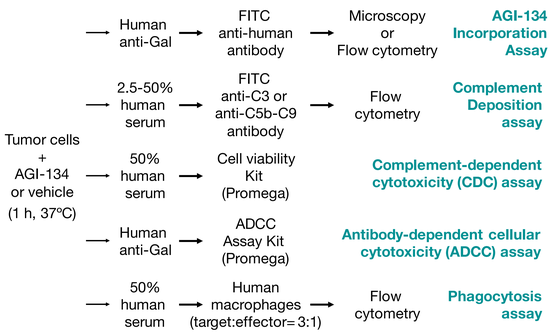
Natural anti-Gal antibodies to the α-Gal epitope (Gal-α-1,3-Gal-β-1,4-GlcNAc-R) are present in high titers in humans and are responsible for the hyperacute rejection of non-primate mammalian xenografts [1,2,3]. We propose that intratumorally administered α-Gal glycolipids will insert into the plasma membranes of tumor cells resulting in anti-Gal IgG and IgM binding. This will initiate an immune response that attacks the injected tumor and, through uptake of IgG and C3b complement opsonized tumor debris/cells by antigen presenting cells, will create a patient-specific, systemic T cell anti-tumor activity [4,5].

AGI-134 is a fully synthetic Function-Spacer-Lipid (FSL) glycolipid based on Kode Biotech technology. It is composed of an α-Gal sugar moiety attached via an adipate linker to a lipid tail.

Here we present the *in vitro* and *in vivo* characterization of the anti-cancer activity, mechanism of action, and efficacy of AGI-134. Moreover, we show pharmacokinetic data in mice and the results of a non-GLP dosage-range finding toxicokinetic study in cynomolgus monkeys (*Macaca fascicularis*).

METHODS

***In vitro* experiments**

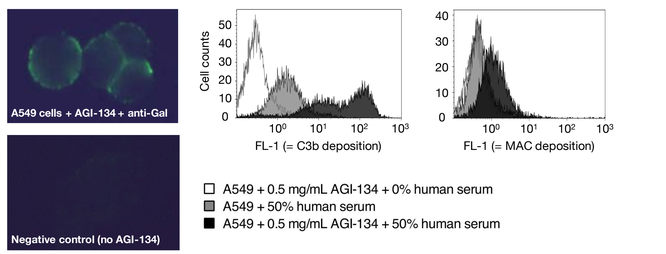


***In vivo* experiments**

1. Immunization of α-Gal-deficient α-1,3-galactosyltransferase knockout (GT-KO) mice with pig kidney homogenate to induce anti-Gal IgG/IgM [4,5].
2. Day 0: Subcutaneous injection of α-Gal negative B16 melanoma cells: 10^6 on right flank → primary tumor; 10^4 on left flank → secondary tumor
3. Day 4-6: Intratumoral (IT) injection of primary tumors (~5 mm in diameter) with AGI-134, control glycolipid, or PBS.
4. Monitor tumor growth. Ablate primary tumors ≥10 mm IT with ethanol
5. In checkpoint inhibitor combination experiments, mice were treated IT with AGI-134, as above, followed by administration of four intraperitoneal (IP) anti-PD-1 (Clone RMP1-14, BioXCell) doses starting at Day 8 or 10.

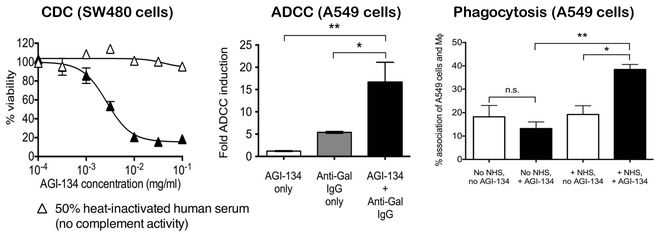
RESULTS

AGI-134 incorporates into plasma membranes of human cancer cells*, attracts human anti-Gal antibodies, and induces complement deposition



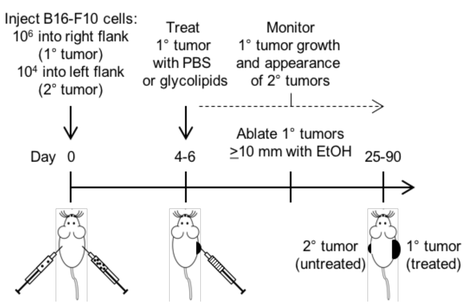
*, anti-Gal antibodies bound to all tested AGI-134-labeled human and mouse cancer cell lines

AGI-134 promotes complement-dependent cytotoxicity (CDC), antibody-dependent cellular cytotoxicity (ADCC), and macrophage (MΦ) phagocytosis of human cancer cells

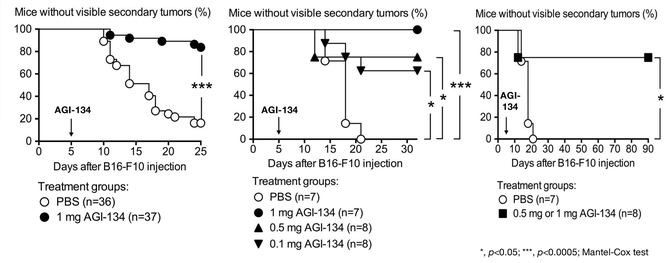


NHS, normal human serum;
*, $p<0.05$; **, $p<0.005$; n.s., not significant; one-way ANOVA

GT-KO mouse B16 melanoma model as read-out for adaptive anti-tumor immunity

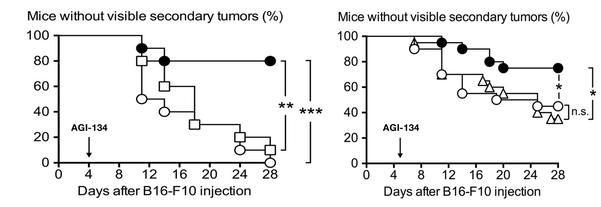


A single intratumoral AGI-134 dose strongly protects GT-KO mice from distal B16 melanoma lesion formation



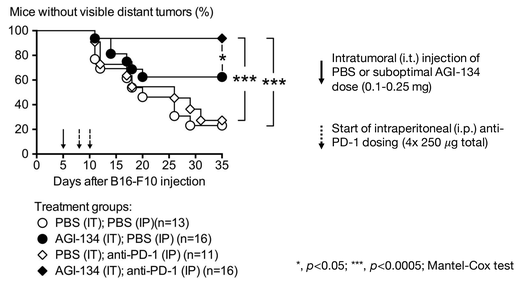
*, $p<0.05$; ***, $p<0.0005$; Mantel-Cox test

The activity of AGI-134 is dependent on anti-Gal and α-Gal interaction



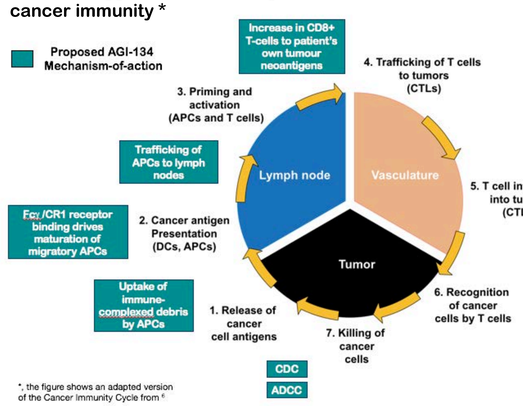
*, $p<0.05$; **, $p<0.005$; ***, $p<0.0005$; n.s., not significant; Mantel-Cox test

AGI-134 synergizes with an anti-PD-1 antibody



*, $p<0.05$; ***, $p<0.0005$; Mantel-Cox test

AGI-134 triggers an immunological cascade that induces anti-cancer immunity *



*, the figure shows an adapted version of the Cancer Immunity Cycle from *

AGI-134 pharmacokinetics in wild-type and GT-KO mice

- AGI-134 dosing: 10-40 mg/kg intravenously (IV), subcutaneously (SC), intramuscularly (IM) in wild-type mice or SC or intratumorally (IT) in B16 melanomas in GT-KO mice.
- AGI-134 had low systemic bioavailability after SC, IM, or IT administration
- AGI-134 in B16 tumors declined from ~3 mg/g tumor weight at 15 min post-dosing to ~10 μg/g at 24 hours post-dosing, with an estimated 6-hour elimination half-life.

Mouse Strain	Dose Route	Dose (mg/kg)	C_{max} (μg/mL)	T_{max} (hrs)	Half-Life (hrs)	AUC (μg.h/mL)	Bioavailability (%)
C57BL6	IV	10	119	0	15.6	81.9	n/a
	SC	40	6.34	2	11.4	25.8	7.87
	IM	40	15.8	1	9.3	44.7	13.6
GT-KO	IT	40	8.56	0.25	10.4	26.6	8.12
	SC	40	1.91	2	4.98	17.2	5.25

Vss: Volume of distribution at steady-state; Cmax: Maximal observed concentration; Tmax: Time of the observed maximum concentration; AUC: Area Under the Curve between time 0 and extrapolated to infinity; n/a, not applicable.

Dose range finding toxicokinetic study in cynomolgus monkeys

- AGI-134 dosing: 2, 10, or 50 mg/kg s.c. or 2 mg/kg i.v. on Days 1, 5, 8, 12, and 15
- AGI-134 had low bioavailability
- No morbidity, mortality or effects on body weight or food consumption were observed
- No significant AGI-134 related effects on haematological and coagulation parameters
- Potential mechanism-related effects were observed:
  - Haemorrhage, mixed cell inflammatory cell infiltrates, myofiber necrosis and regeneration seen at injection sites for highest s.c. dose
  - Non-significant increases in creatine kinase, LDH, AST, ALT and C-reactive protein, notably in i.v. group and 50 mg/kg s.c. group

Dose Route	Dose (mg/kg)	C_{max} (μg/mL)	T_{max} (hrs)	Half-Life (hrs)	AUC (μg.hrs/mL)	Bioavailability (%)
IV	2	40.3	0	19.0	213	n/a
SC	2	0.44	6	5.75	7.99	3.75
SC	10	3.06	6	11.9	48.0	4.50
SC	50	19.7	6	12.0	302	5.67

Vss: Volume of distribution at steady-state; Cmax: Maximal observed concentration; Tmax: Time of the observed maximum concentration; AUC: Area Under the Curve between time 0 and extrapolated to infinity; n/a, not applicable.

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 clonal and subclonal tumor neoantigens.
- AGI-134 inserts into cell membranes of all human cancer cells tested. Anti-Gal IgG and IgM antibodies bind to the cells, leading to CDC, ADCC and effective Fcγ and complement receptor mediated uptake by antigen-presenting cells.
- For *in vivo* testing, 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 in a dose-dependent fashion for at least 90 days and synergizes with an anti-PD-1 antibody.
- AGI-134 has low systemic exposure after intratumoral and subcutaneous administration in mice and monkeys.
- AGI-134 showed a favorable safety and PK profile in a DRF toxicokinetic study in cynomolgus monkeys. The no-observed-adverse-effect level (NOAEL) is considered to be 50 mg/kg SC.
- We believe that 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.

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We thank Profs. Stephen Henry and Nicolai Bovin (Kode Biotech Ltd.) for initial AGI-134 supply. We thank Drs. Adriana Manzi and Stanley Roberts (Atheln Inc.) for helping to plan and the Charles River Laboratories in Reno, NV for excellent execution of the toxicokinetic study. We are thankful to Fay Davidson, Dr. Iain Beattie (both XenoGesis Ltd.), and Dr. Andy Gray (Drug Development Consultants Ltd.) for PK data acquisition and analysis. Finally, we greatly appreciate the support of and helpful discussions with Damian Marron (CEO Agalimmune Ltd.) and Graham Griffiths (COO Agalimmune Ltd.).