ADG153, an Anti-CD47 Monoclonal Antibody Prodrug, Has Strong *In Vivo* Anti-Tumor Activity, Minimal RBC-Related and Antigen Sink Liabilities, and Extended Half Life in Comparison with Benchmark Clinical Antibodies of the Same IgG Subclass

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ADAGENE

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BACKGROUND AND SIGNIFICANCE

Therapies targeting the CD47/SIRPα axis have shown promising success in various preclinical models and in clinical trials for hematologic malignancies. However, the fundamental issue for effective CD47 targeting remains: an active Fc effector function for strong anti-tumor effects without the safety (e.g., anemia) and pharmacokinetic (e.g., antigen sink) liabilities. We set out to resolve this paradox by designing anti-CD47 antibodies that can decouple the efficacy from the safety and PK liabilities. Using our NEObody™ technology, we designed the ADG153 anti-CD47 antibodies, in IgG1 and IgG4 formats, that target a novel epitope on CD47. Our SAFEbody® technology was then used for precision masking of the binding sites on these antibodies (Fig. 1).

Our data show that the ADG153 parental anti-CD47 antibody targets a novel epitope of CD47 that does not lead to human RBC hemagglutination. The ADG153 SAFEbodies are highly masked (with masking efficiencies >690-fold) and are conditionally activated to bind, equivalent to its parental and reference antibodies, to CD47. In monkeys, the anti-CD47 SAFEbodies in both IgG1 and IgG4 formats show minimal RBC depletion and much longer half-lives than their parental antibodies with significant safety margin for potent anti-CD47 therapies. Although ADG153 in IgG4 format shows anti-tumor efficacy, the ADG153 in IgG1 format with an active effector function for stronger ADCC and ADCP activities shown here is anticipated to realize the full potential for more efficacious and safer single agent and combination anti-CD47 therapies in both hematological and solid malignancies.

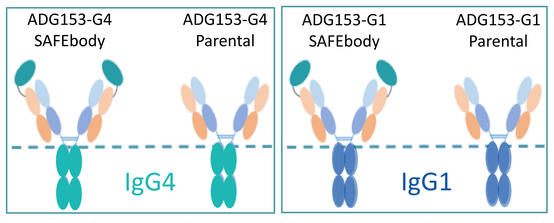
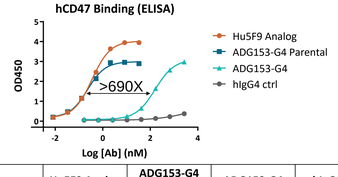


Fig 1. An Adagene SAFEbody, masked by covalently linked peptides, can be conditionally activated to bind its target. Shown are two sets of ADG153 (anti-CD47) SAFEbodies and their Parental or Activated forms in hIgG4 (in teal) and hIgG1 (in blue) isotypes.

RESULTS

The high masking efficiency of anti-CD47 SAFEbody in comparison with its parental and reference antibodies in binding to CD47 *in vitro*

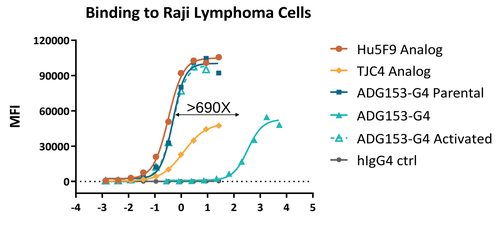


	Hu5F9 Analog	ADG153-G4 Parental	ADG153-G4	hIgG4 ctrl
EC_{50} (nM)	0.35	0.23	160	ND

Fig 2. ADG153-G4 Parental and Hu5F9 Analog showed overall comparable *in vitro* binding to hCD47 by ELISA. ADG153-G4 demonstrated >690-fold masking efficiency for binding to hCD47.

RESULTS

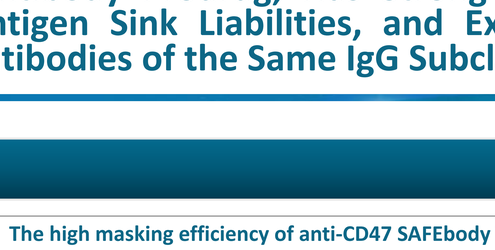
The high masking efficiency of anti-CD47 SAFEbody in comparison with its parental, activated, and reference antibodies in binding to Raji cells *in vitro*



	Hu5F9 Analog	ADG153-G4 Parental	ADG153-G4	ADG153-G4 Activated	hIgG4 ctrl
EC_{50} (nM)	0.33	0.48	335	0.48	ND

Fig 3. ADG153-G4 SAFEbody demonstrated >690-fold masking efficiency for binding to Raji cells. ADG153-G4 Parental and activated form (*in vitro* MMP cleavage), together with Hu5F9 Analog, showed comparable binding to $CD47^+$ Raji lymphoma cells.

The high masking efficiency of anti-CD47 SAFEbody prevents its binding to human RBCs



	Hu5F9 Analog	ADG153-G4 Parental	ADG153-G4	hIgG4 ctrl
EC_{50} (nM)	0.76	0.37	482	ND

Fig 4. ADG153-G4 demonstrated >1300-fold masking efficiency for binding to human RBCs, while ADG153-G4 Parental and Hu5F9 Analog showed comparable binding to human RBCs.

ADG153 targets a novel epitope and does not induce human RBC hemagglutination *in vitro*

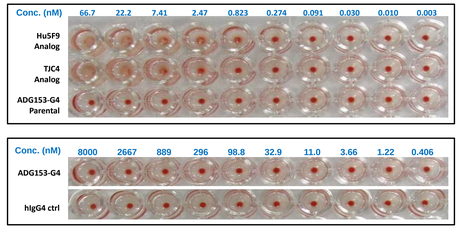


Fig 5. Hu5F9 and TJC4 Analogs showed *in vitro* human RBC hemagglutination (HA) at concentrations ≥ 7.41 nM. ADG153-G4 Parental and SAFEbody molecules did not induce HA. Results for 1 donor are shown; similar results were observed for a second donor (not shown).

Parental and activated ADG153-G1 induce stronger ADCP for efficacy than ADG153-G4 and reference antibodies

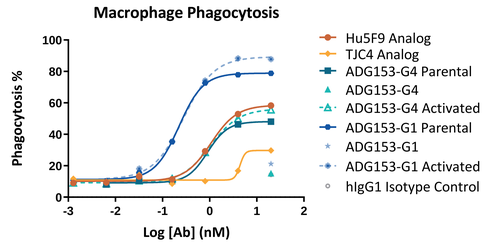


Fig 6. ADG153-G1 parental and activated forms had more potent and higher max induction of phagocytosis than ADG153-G4 Parental, ADG153-G4 Activated,, Hu5F9 Analog, and TJC4 Analog. SAFEbodies had no to minimal activity before its activation *in vitro* by MMP cleavage.

Parental ADG153-G1, but none of the anti-CD47 Abs of the IgG4 subclass, induces NK-mediated ADCC activity *in vitro*

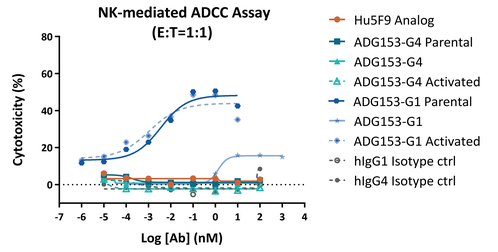
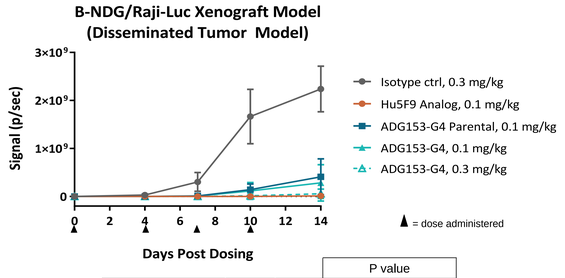


Fig 7. ADG153-G1 Parental induced potent NK-mediated ADCC activity *in vitro*. Anti-CD47 Abs of the IgG4 subclass did not have this activity. The ADG153-G1 SAFEbody demonstrated minimal activity *in vitro*, and induction of NK-mediated ADCC activity can be fully restored with *in vitro* MMP cleavage (Activated).

ADG153-G4 SAFEbody shows strong *in vivo* anti-tumor activity in the Raji-Luc disseminated tumor model



Ab ID	Dose (mg/kg)	TGI	P value vs Isotype ctrl	P value vs Hu5F9 Analog
Hu5F9 Analog	0.1	99.6%	<0.0001	
ADG153-G4 Parental	0.1	81.8%	<0.0001	*ns*
ADG153-G4	0.1	87.4%	<0.0001	*ns*
	0.3	97.5%	<0.0001	*ns*

Fig 8. Strong anti-tumor activity was observed in all 0.1 mg/kg treatment groups; there was no statistical significance among these groups.

ADG153-G1 and -G4 SAFEbodies demonstrate significantly reduced RBC-related liabilities in cynomolgus monkeys

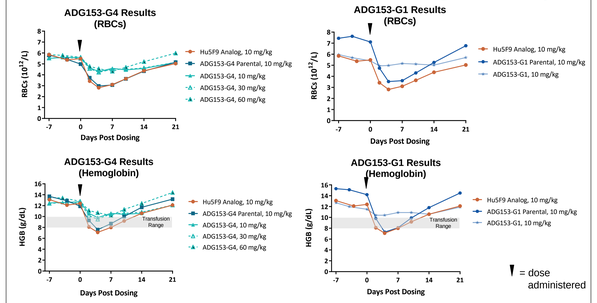
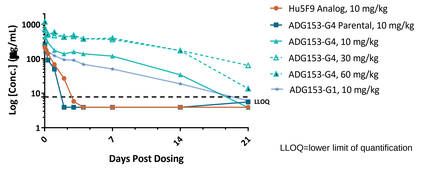


Fig 9. In exploratory toxicology studies in cynomolgus monkeys, the ADG153-G4 SAFEbody showed significantly less reduction than Hu5F9 Analog in RBC-related parameters such as RBC counts and hemoglobin. Hu5F9 Analog at 10 mg/kg caused ~49% maximum decrease in RBCs, while ADG153-G4 SAFEbody at 60 mg/kg showed ~23% maximum decrease in RBCs. ADG153-G1 SAFEbody at 10 mg/kg demonstrated only 8% maximum decrease in RBCs.

ADG153-G1 and -G4 SAFEbodies demonstrate favorable PK properties in cynomolgus monkeys



LLOQ=lower limit of quantification

Fig 10. Pharmacokinetic (PK) studies of single intravenous dose of ADG153-G1 and ADG153-G4 SAFEbodies compared to Hu5F9 Analog in monkeys demonstrated ~8-fold longer apparent half-lives and ~9-fold (G4) or 5-fold (G1) higher Area Under the Curve (AUC) at 10 mg/kg.

SUMMARY

- ADG153 anti-CD47 SAFEbodies, in IgG1 and IgG4 formats, were developed using the precision masking technology from the fully human anti-CD47 NEObody/parental antibody targeting a novel epitope of CD47 with similar cross-reactivity against human and monkey CD47.
- ADG153 parental and SAFEbody target a novel epitope of CD47. They did not induce human RBC hemagglutination, unlike reference anti-CD47 antibodies.
- ADG153-G4 SAFEbody had high masking efficiencies (at least 690-fold) and were conditionally activated to bind strongly (equivalent to its parental and reference antibodies) to CD47 protein, Raji lymphoma cells, and human RBCs.
- ADG153 antibodies showed effector function dependent activities. The parental and activated ADG153 induced stronger ADCP in the IgG1 than IgG4 format; ADCC effect was observed only in the IgG1 NOT IgG4 format.
- ADG153-G4 SAFEbody showed reduced RBC-related liabilities in exploratory monkey tox studies even at 60mg/kg. ADG153-G4 SAFEbody also demonstrated favorable PK properties in monkeys.
- ADG153-G1 SAFEbody showed significantly less RBC and hemoglobin decreases in monkeys at the same 10 mg/kg dose level compared to parental ADG153-G1 and Hu5F9 Analog, while still maintaining favorable PK properties in monkeys.
- The preclinical safety and efficacy profiles for anti-CD47 SAFEbody in IgG1 and IgG4 formats provide a strong rationale for advancing anti-CD47 ADG153 SAFEbody into clinical development.

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