



A One-Shot Cure for Malaria: Preclinical Efficacy of VhH-Based Antibodies Against *Plasmodium falciparum* Blood Stage Infection

Vidal F. de la Cruz^{1*}, Sawyer Llewellyn¹, Benjamin Koch¹, and Nirbhay Kumar²

¹ Ibex Biosciences, Inc.

² George Washington University

*Correspondence to Vidal F. de la Cruz (vdelacruz@ibex.bio)

Abstract

This study evaluates the efficacy of camelid-derived (VhH-Fc full-length) antibodies in preventing *Plasmodium falciparum* merozoite entry into red blood cells and treatment of malaria infection in a human red blood cell (RBC) chimeric mouse model. These antibodies (lbx13, lbx11) target the CD147 molecule on human erythrocytes, a receptor utilized by the *P. falciparum* malaria parasite for invasion. *In vitro* malaria invasion assays using human RBCs showed the lbx13 antibody has potent invasion-blocking potential. Experiments in a human RBC chimeric mouse model demonstrated that lbx13 also had invasion blocking activity *in vivo*. A single intravenous dose of lbx13-G1 (lbx13 VhH genetically fused to human IgG1 Fc) or lbx13-G2 (lbx13 VhH genetically fused to human IgG2 Fc) at a dose <150 mcg/mouse resulted in rapid parasite clearance in less than 5 days (i.e., two invasion cycles) and complete cure (i.e., no recrudescence up to Day 60). There was no evidence of significant adverse effects. By binding to a human receptor protein rather than targeting the parasite directly, these antibodies virtually eliminate the possibility of developing drug resistance, a major limitation of current antimalarial therapies. Our findings suggest these camelid-based antibodies can provide a single-dose treatment for malaria, overcoming current challenges associated with drug resistance and multi-dose treatment regimens.

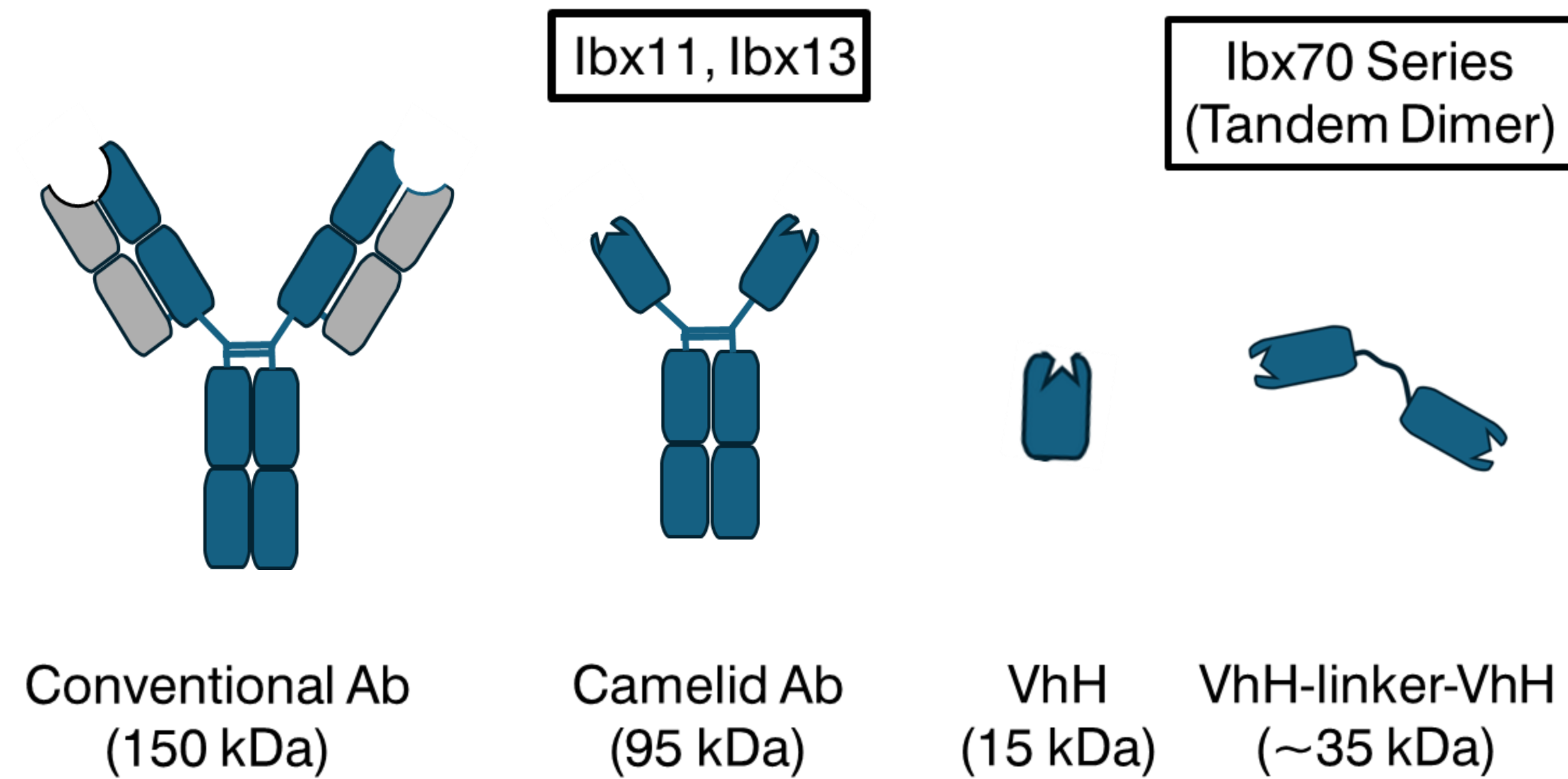
Introduction

Malaria remains a significant global health challenge and extensive efforts to control malaria are hampered by the emergence of drug-resistant strains and the limited effectiveness of current vaccines. Traditional antimalarial therapies primarily target the parasite itself, leading to rapid development of resistance. Our approach targets the human receptor for RBC invasion, thus much less likely to lead to drug resistance. Our VhH-based antibody therapeutics, derived from camelids, offer a promising alternative due to several advantages (e.g., smaller size, enhanced stability in high temperature and chemically challenging environments, and less dependence on cold chain logistics).

The camelid-based lbx13 antibodies specifically target the human CD147 receptor that *P. falciparum* merozoites use to invade red blood cells. By blocking this receptor, the antibodies disrupt the parasite's life cycle, preventing invasion and leading to rapid parasite clearance. This study aimed to assess the efficacy of lbx13 *in vitro* and in a humanized mouse model to explore potential as a single-dose cure for malaria. By targeting a host receptor rather than the parasite, these antibodies could eliminate the problem of resistance development and provide a robust solution to one of the most pressing issues in malaria treatment today.

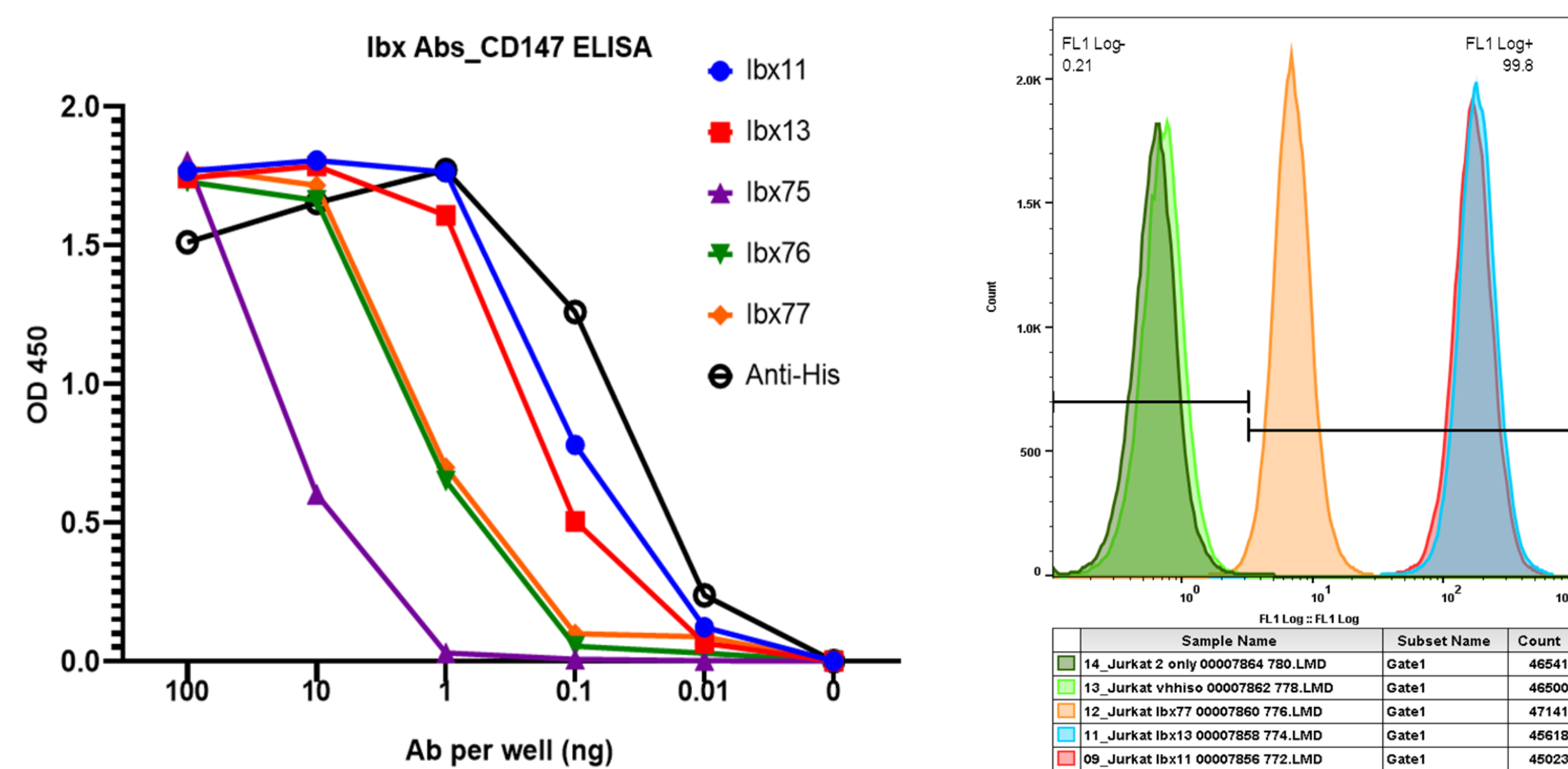
Antibody Generation & Characterization

Anti-CD147 antibody was generated using a llama-immunized yeast display platform. Selected VhH sequences were selected and expressed as recombinant antibody in formats that included full-length VhH-Fc genetic fusions and tandem VhH dimers ("tandemers").



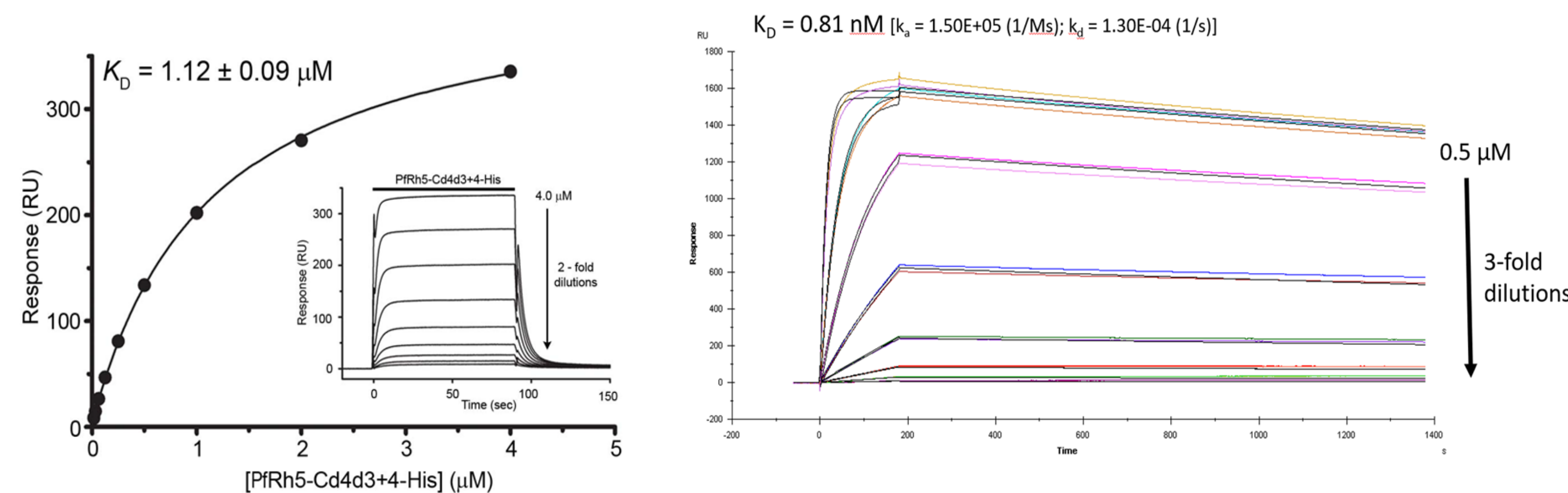
Anti-CD147 Antibody Binding Profiles

The anti-CD147 camelid antibodies were demonstrated to bind recombinant human CD147 protein by ELISA (left) and flow cytometry on Jurkat cells (right). With confirmation of hCD147 binding, we next assessed the affinity of binding by SPR.



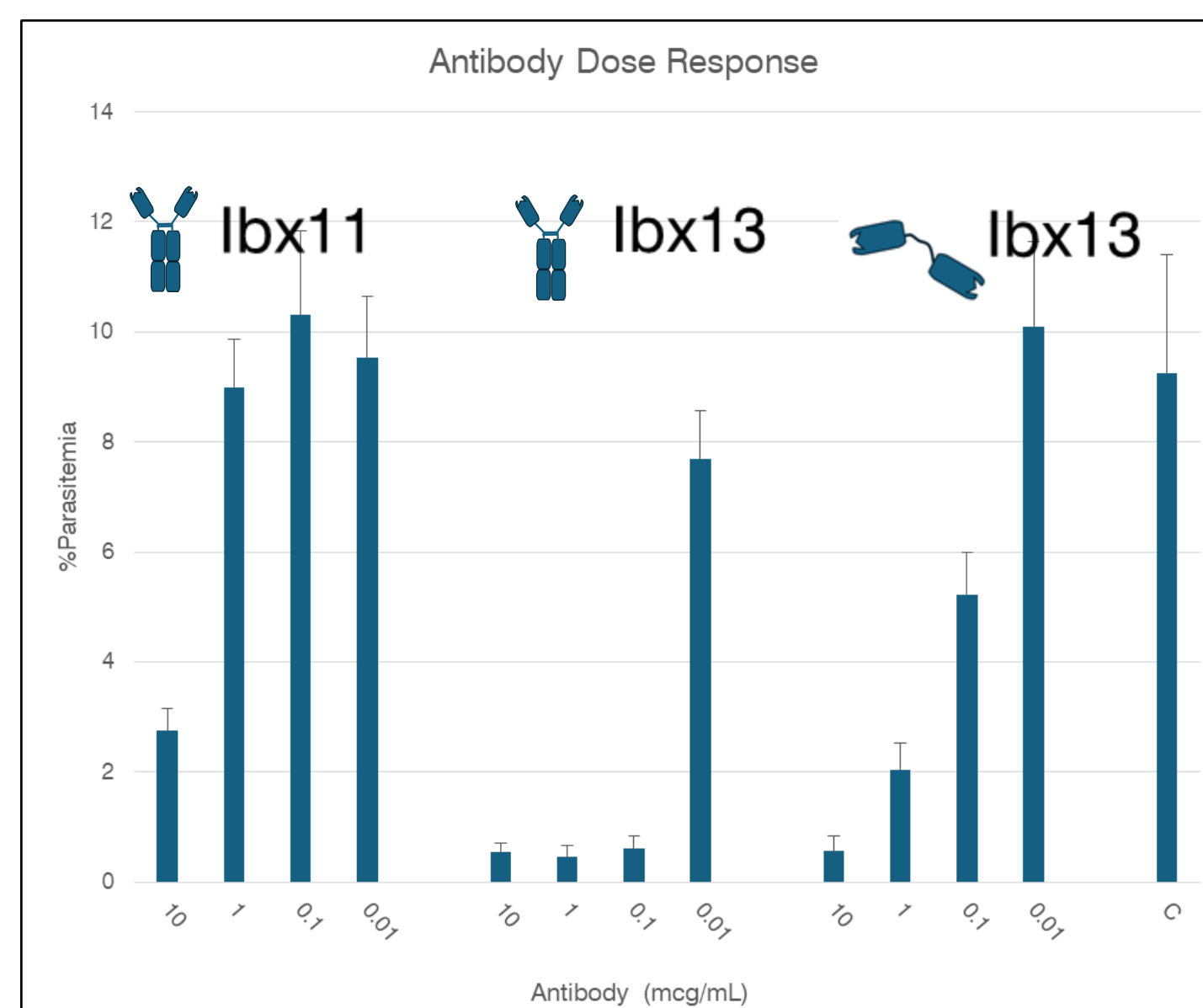
lbx13 Affinity Determination

SPR binding studies demonstrated that the anti-CD147 antibodies bound human CD147 protein with high affinity. The affinity of the *P. falciparum* RH5 protein for human CD147 has been reported to be on the order of ~1 μ M (Crosnier et al., 2012; left), while the affinity of the lbx13 antibody was demonstrated to be ~1 nM (right). With a strong CD147 binding advantage of the antibody over the parasite RH5 protein, we then explored the ability of the antibodies to inhibit malaria invasion of RBCs.



Inhibition RBC Invasion *In Vitro*

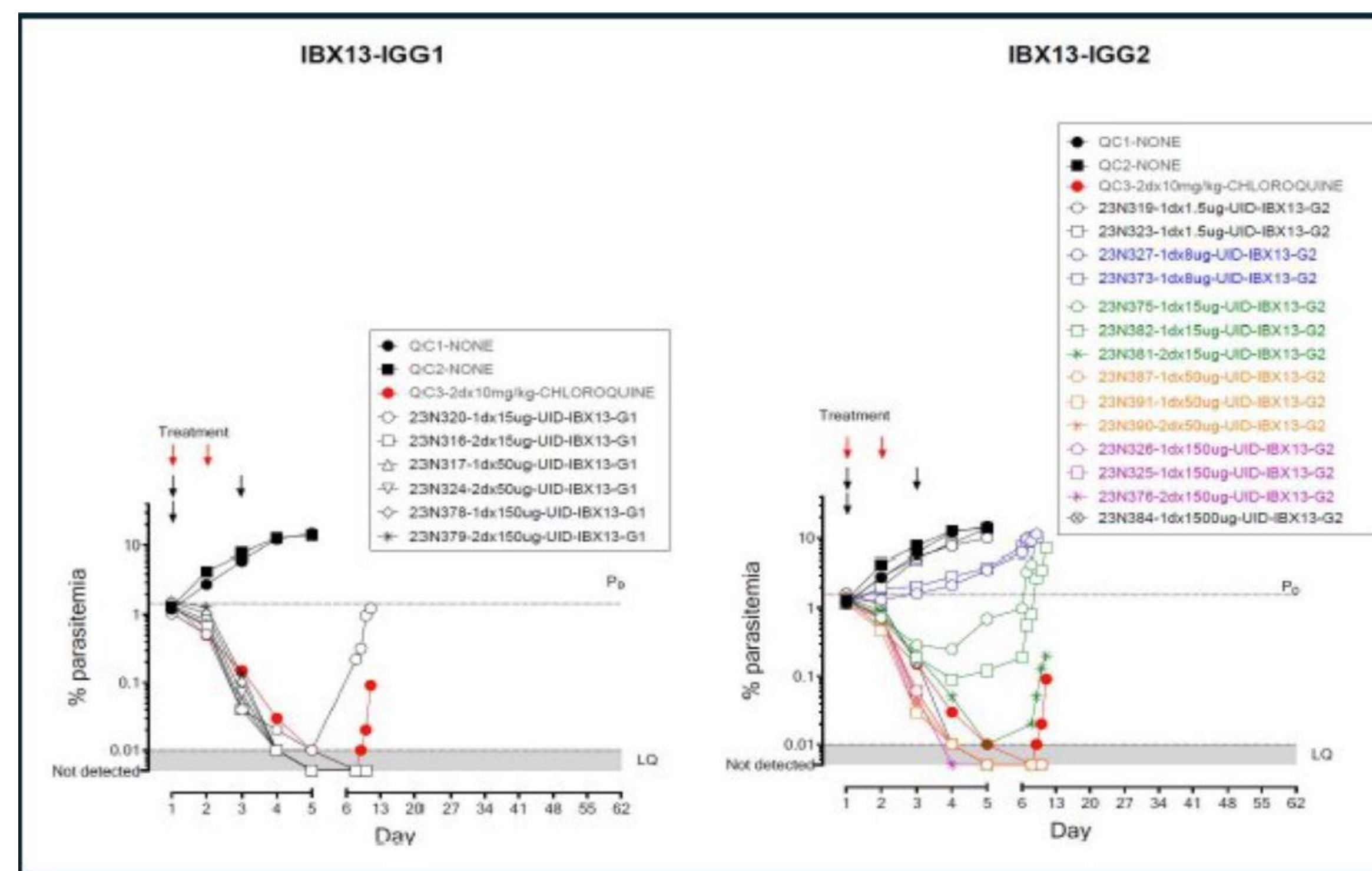
The antibodies were tested for blocking activity in an *in vitro* human red blood cell malaria invasion assay using the parasite strain NF54. Initial parasitemia was ~1.3% and cultured at ~2% hematocrit. Parasites without any antibody ("C") were cultured and used as a negative control, and parasites cultured with chloroquine (CQ; not shown) were used as a positive control. Blood smears were prepared at 48 and 96 hours of treatment, stained and read by microscopy in a blinded manner. The number of infected RBCs were scored out of approximately 1000 total RBCs and expressed as % parasitemia. As demonstrated below, the different antibodies had different capacity to block malaria RBC invasion *in vitro*. Based on these results, lbx13 was chosen for testing in an *in vivo* malaria infection mouse model.



In Vivo Animal Model and Infection

Preclinical efficacy studies were conducted using a humanized mouse model (PfalcHuMice) infected with *P. falciparum* (Angulo-Barturen et al., 2008; Jiménez-Díaz et al., 2009). Two VhH-based antibodies, lbx13-G1 (lbx13 VhH fused to human IgG1 Fc) and lbx13-G2 (lbx13 VhH fused to human IgG2 Fc), were tested for their ability to prevent merozoite invasion and cure malaria infection. The studies included various dosage regimens to evaluate the minimum effective dose and compare the efficacy of single versus double dosing.

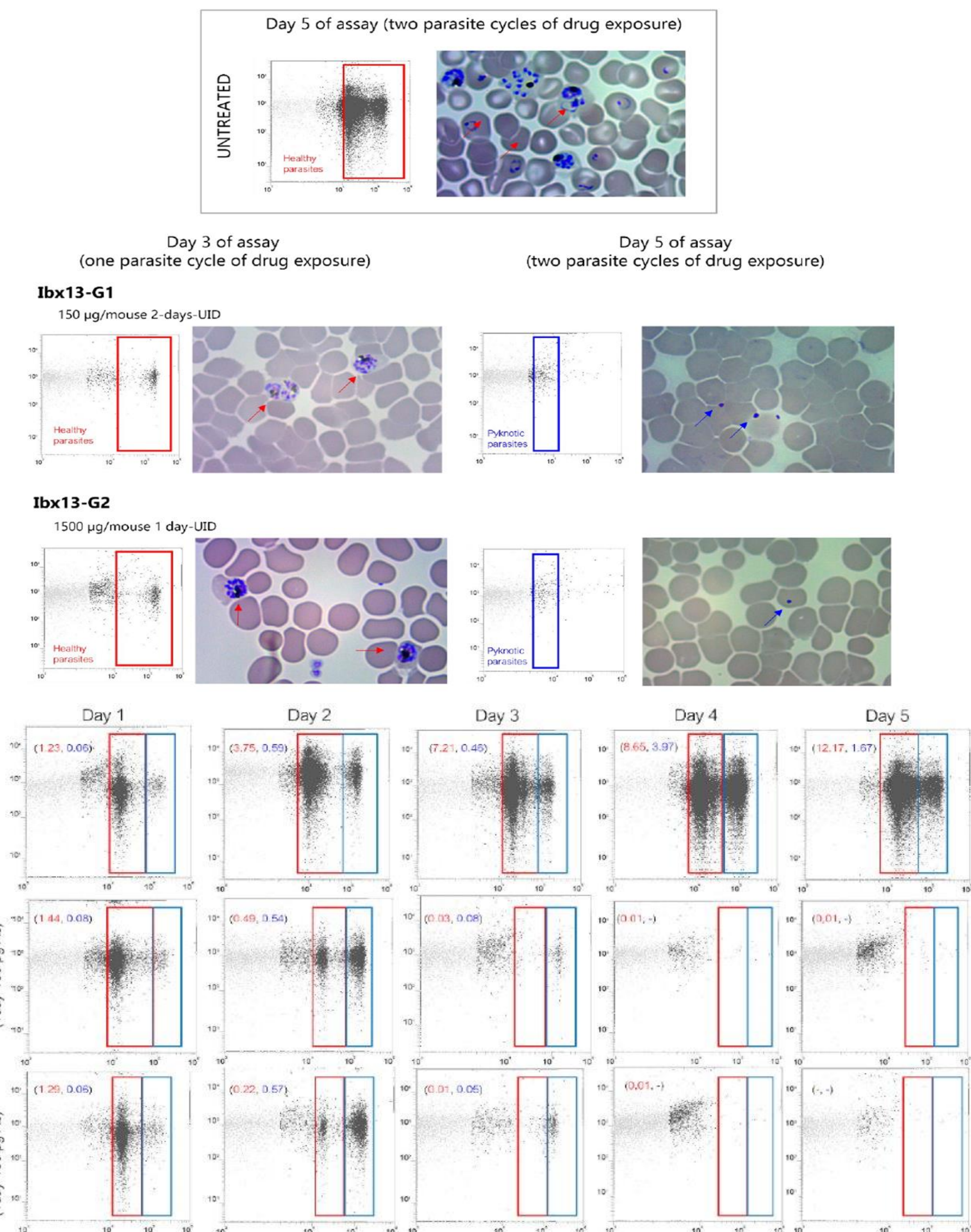
Immunodeficient female NSG female mice (NOD-*scid*-IL-2R γ^{null}) were engrafted with human erythrocytes and then infected with peripheral blood from donor mice harboring 5-10% parasitemia with *P. falciparum* merozoites. Mice were randomized into treatment groups once parasitemia reached 1-2% and antibodies were administered intravenously at indicated doses and regimens. Control drug treatment (chloroquine) was started on Day 1 of the study when infected mice had ~1-2% patent parasitemia (denoted as P0). Before drug administration, each infected mouse was randomly assigned to its corresponding treatment. The treatment was administered by intravenous route and volume of administration was 10 mL $\cdot$ kg⁻¹ bodyweight⁻¹ for the intravenous route. Parasitemia was measured using flow cytometry, and the morphological effects of treatment on the parasite were assessed by microscopy. Data on pharmacodynamics were collected to evaluate the dose-response relationship and the efficacy of different dosing regimens.



The *in vivo* results show:

- A single dose as low as 8 mcg is sufficient to decrease parasitemia growth rate;
- Increased doses progressively decreased parasitemia growth rate;
- Two 50 mcg doses 48 hr apart resulted in complete cure of infection;
- A single dose of 150 mcg resulted in complete cure of infection.

Flow cytometry and microscopy confirmed the mechanism of action was compatible with inhibition of invasion of new erythrocytes. As demonstrated by flow cytometry, human RBCs from untreated mice show that early-stage parasites (i.e., rings and trophozoites, left red box) develop into later-stage forms (schizonts; right blue box) that then are released from the RBC into the bloodstream to initiate a new round of RBC invasion and replication. In contrast, in animals treated with anti-CD147 antibody the rings in infected RBCs develop into trophozoites and schizonts that are then released from the RBC, but the new merozoites do not initiate a new round of RBC invasion and replication. This was further confirmed microscopically by the lack of detection of infected RBCs and the observance of merozoites attached to the RBC surface.



Conclusions

We have generated novel camelid (VhH) antibodies to human CD147. Expression of these antibodies in different formats (e.g., as human IgG Fc fusions and tandemers) binding human CD147 with high affinity sufficient to block malaria merozoite invasion of RBCs *in vitro* and *in vivo*. The present results predict that the lbx13 antibodies can be an effective single dose treatment against uncomplicated *P. falciparum* malaria in adults at predicted dose levels of ≥ 300 mg by the intravenous route. Anticipated dosing in children will be significantly lower.

References

Angulo-Barturen et al. (2008) A Murine Model of *falciparum*-Malaria by *In Vivo* Selection of Competent Strains in Non-Myelodepleted Mice Engrafted with Human Erythrocytes. *PLoS ONE* 3(5): e2252. doi.org/10.1371/journal.pone.0002252

Crosnier et al. (2011) BASIGIN is a receptor essential for erythrocyte invasion by *Plasmodium falciparum*. *Nature* 480(7378):534-537. 2011. doi:10.1038/nature10606

Jiménez-Díaz et al. (2009) Improved murine model of malaria using *Plasmodium falciparum* competent strains and non-myelodepleted NOD-*scid* IL2R γ^{null} mice engrafted with human erythrocytes. *Antimicrob. Agents Chemother.* 53(10):4533-4536. doi:10.1128/AAC.00519-09