Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplementary Appendix

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Key Patient Enrollment Criteria

Key Inclusion Criteria

- B-cell NHL expressing CD20 by immunohistochemistry or flow cytometry, relapsed or refractory to at least 2 prior lines of therapy
- Eastern Cooperative Oncology Group (ECOG) score 0 – 2
- Disease that is measurable or assessable for response per Lugano Classification for lymphomas
- Hemoglobin $\geq$ 9.5 g/dL
- Absolute neutrophil count $\geq$ 1.0 x $10^{9/}$mL
- Platelets $\geq$ 50 x 10^9/mL
- Serum creatinine $\leq$ 1.5 x upper limit of normal or calculated glomerular filtration rate > 40 mL/min/1.73m^2

Key Exclusion Criteria

- Patients with active brain metastases
- Red blood cell transfusion dependence
- History of hemolytic anemia or Evans syndrome in the last 3 months
- Prior treatment with CD47 or signal regulatory protein alpha targeting agents
- Second malignancy, except indolent cancers not on active anti-cancer therapy
- Prior anti-cancer therapy within 2 weeks or at least 4 half-lives prior to dosing

Lugano Classification for Efficacy Evaluation

Response and Site	PET-CT–Based Response	CT-Based Response
Complete	Complete metabolic response	Complete radiologic response (all of the following)
Lymph nodes and extra lymphatic sites	Score 1, 2, or 3* with or without a residual mass on 5PS†	Target nodes/nodal masses must regress to < 1.5 cm in LDi
	It is recognized that in Waldeyer's ring or extranodal sites with high physiologic uptake or with activation within spleen or marrow (e.g., with chemotherapy or myeloid colony-stimulating factors), uptake may be greater than normal mediastinum and/or liver. In this circumstance, complete metabolic response may be inferred if uptake at sites of initial involvement is no greater than surrounding normal tissue even if the tissue has high physiologic uptake	No extralymphatic sites of disease
Nonmeasured lesion	Not applicable	Absent
Organ enlargement	Not applicable	Regress to normal
New lesions	None	None
Bone marrow	No evidence of FDG-avid disease in marrow	Normal by morphology; if indeterminate, IHC negative
Partial	Partial metabolic response	Partial remission (all of the following):
Partial Lymph nodes and extralymphatic sites	Score 4 or 5† with reduced uptake compared with baseline and residual mass(es) of any size	> 50% decrease in SPD of up to 6 target measurable nodes and extranodal sites
	At interim, these findings suggest responding disease	When a lesion is too small to measure on CT, assign 5 mm x 5 mm as the default value
	At end of treatment, these findings indicate residual disease	When no longer visible, 0 x 0 mm
		For a node > 5 mm x 5 mm, but smaller than normal, use actual measurement for calculation
		Absent/normal, regressed, but no increase
		Spleen must have regressed by > 50% in length beyond normal
Nonmeasured lesions	Not applicable	
Organ enlargement	Not applicable	
New lesions	None	None
Bone marrow	Residual uptake higher than uptake in normal marrow but reduced compared with baseline (diffuse uptake compatible with reactive changes from chemotherapy allowed). If there are persistent focal changes in the marrow in the context of a nodal response, consideration should be given to further evaluation with MRI or biopsy or an interval scan	Not applicable
No response or stable disease	No metabolic response	Stable disease
Target nodes/nodal masses, extranodal lesions	Score 4 or 5 with no significant change in FDG uptake from baseline at interim or end of treatment	< 50% decrease from baseline in SPD of up to 6 dominant, measurable nodes and extranodal sites; no criteria for progressive disease are met
Nonmeasured lesions	Not applicable	No increase consistent with progression
Organ enlargement	Not applicable	No increase consistent with progression
New lesions	None	None
Bone marrow	No change from baseline	Not applicable

Response and Site	PET-CT–Based Response	CT-Based Response
Progressive disease Individual target nodes/nodal masses Extranodal lesions	Progressive metabolic disease Score 4 or 5 with an increase in intensity of uptake from baseline and/or New FDG-avid foci consistent with lymphoma at interim or end-of-treatment assessment	Progressive disease requires at least 1 of the following PPD progression: An individual node/lesion must be abnormal with: LDi > 1.5 cm and Increase by > 50% from PPD nadir and An increase in LDi or SDi from nadir 0.5 cm for lesions < 2 cm 1.0 cm for lesions > 2 cm In the setting of splenomegaly, the splenic length must increase by > 50% of the extent of its prior increase beyond baseline (e.g., a 15-cm spleen must increase to > 16 cm). If no prior splenomegaly, must increase by at least 2 cm from baseline New or recurrent splenomegaly New or clear progression of preexisting nonmeasured lesions
Nonmeasured lesions	None	Regrowth of previously resolved lesions
New lesions	New FDG-avid foci consistent with lymphoma rather than another etiology (e.g., infection, inflammation). If uncertain regarding etiology of new lesions, biopsy or interval scan may be considered	A new node _ 1.5 cm in any axis A new extranodal site _ 1.0 cm in any axis; if _ 1.0 cm in any axis, its presence must be unequivocal and must be attributable to lymphoma Assessable disease of any size unequivocally attributable to lymphoma
Bone marrow	New or recurrent FDG-avid foci	New or recurrent involvement

Abbreviations: 5PS = 5-point scale; CT = computed tomography; FDG = fluorodeoxyglucose; IHC = immunohistochemistry; LDi = longest transverse diameter of a lesion; MRI = magnetic resonance imaging; PET = positron emission tomography; PPD = cross product of the LDi and perpendicular diameter; SDi = shortest axis perpendicular to the LDi; SPD = sum of the product of the perpendicular diameters for multiple lesions.
Footnotes: *A score of 3 in many patients indicates a good prognosis with standard treatment, especially if at the time of an interim scan. However, in trials involving PET where de-escalation is investigated, it may be preferable to consider a score of 3 as inadequate response (to avoid undertreatment).

Measured dominant lesions: Up to six of the largest dominant nodes, nodal masses, and extranodal lesions selected to be clearly measurable in two diameters. Nodes should preferably be from disparate regions of the body and should include, where applicable, mediastinal and retroperitoneal areas. Non-nodal lesions include those in solid organs (e.g., liver, spleen, kidneys, lungs), GI involvement, cutaneous lesions, or those noted on palpation.

Nonmeasured lesions: Any disease not selected as measured, dominant disease and truly assessable disease should be considered not measured. These sites include any nodes, nodal masses, and extranodal sites not selected as dominant or measurable or that do not meet the requirements for measurability but are still considered abnormal, as well as truly assessable disease, which is any site of suspected disease that would be difficult to follow quantitatively with measurement, including pleural effusions, ascites, bone lesions, leptomeningeal disease, abdominal masses, and other lesions that cannot be confirmed and followed by imaging.
In Waldeyer's ring or in extranodal sites (e.g., GI tract, liver, bone marrow), FDG uptake may be greater than in the mediastinum with complete metabolic response, but should be no higher than surrounding normal physiologic uptake (e.g., with marrow activation as a result of chemotherapy or myeloid growth factors).
†PET 5PS: 1, no uptake above background; 2, uptake ≤ mediastinum; 3, uptake > mediastinum but ≤ liver; 4, uptake moderately > liver; 5, uptake markedly higher than liver and/or new lesions; X, new areas

of uptake unlikely to be related to lymphoma. Source: Cheson BD, Fisher RI, Barrington SF, et al. Recommendations for initial evaluation, staging, and response assessment of Hodgkin and non-Hodgkin lymphoma:
the Lugano Classification. J Clin Oncol. 2014;32(27):3059-3068 (Cheson 2014).

Reference:
Cheson BD, Fisher RI, Barrington SF, et al. Recommendations for initial evaluation, staging, and response assessment of Hodgkin and non-Hodgkin lymphoma: the Lugano classification. J Clin Oncol. 2014;32(27):3059-3068.

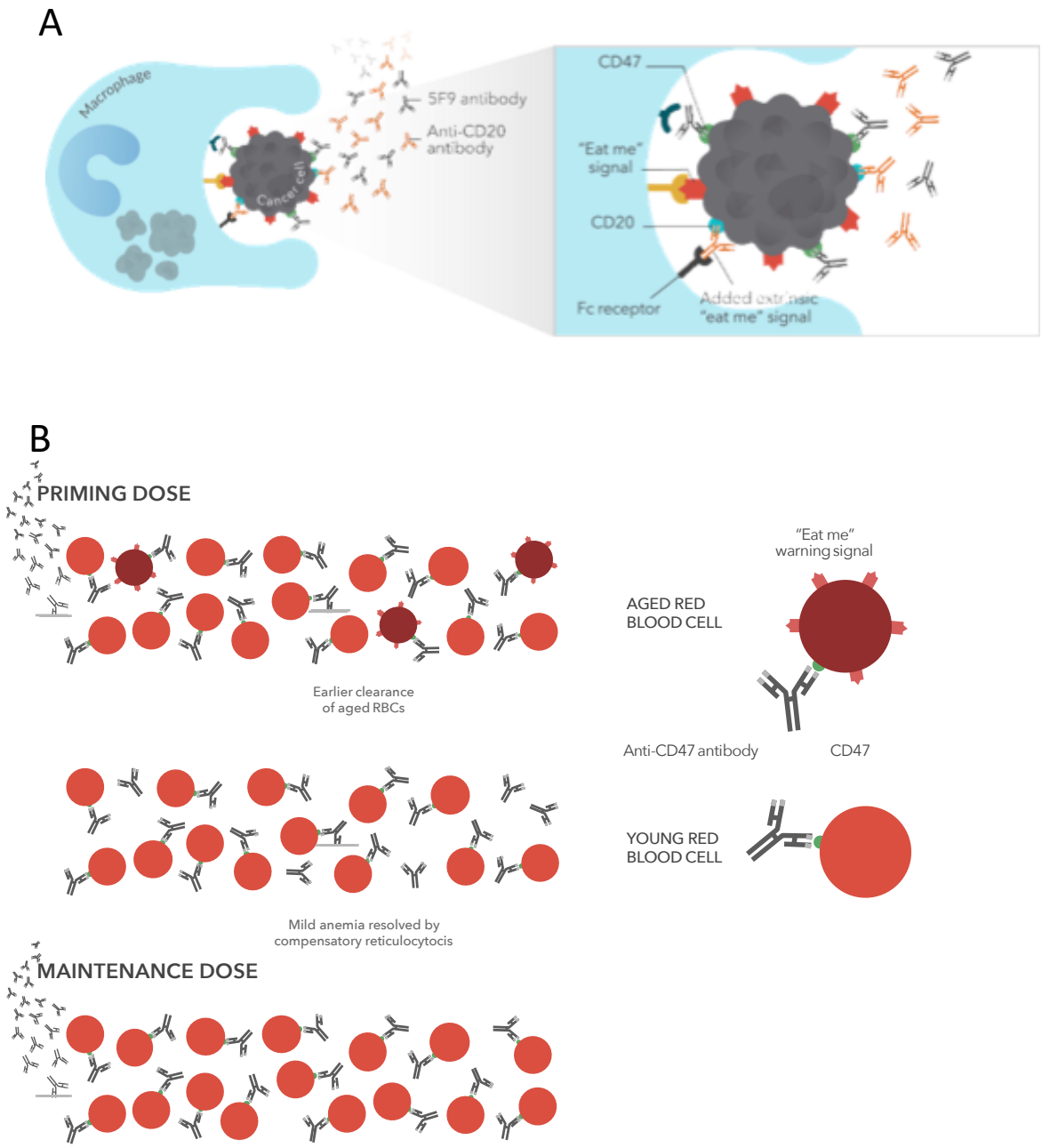


Figure S1. Mechanism of Action for 5F9 + Rituximab Anti-Tumor Synergy and 5F9 Priming/Maintenance Dose Impact on Anemia

A. Diagram showing mechanism of anti-tumor synergy combining 5F9 with rituximab. Synergy occurs through supply of an extrinsic "eat me" signal by rituximab through the Fc-Fc receptor interaction combined with blockade of the "do not eat me" CD47 signal by 5F9. B. Diagram showing the impact of a low priming dose of 5F9 eliminating only aged RBCs which express eat me signals whereas younger RBCs do not (top). The priming dose leads to a mild anemia resolved by a compensatory reticulocytosis as 5F9 shifts the pool of RBCs from old to young (middle). Higher maintenance doses can then be administered which leads to no further anemia (bottom).

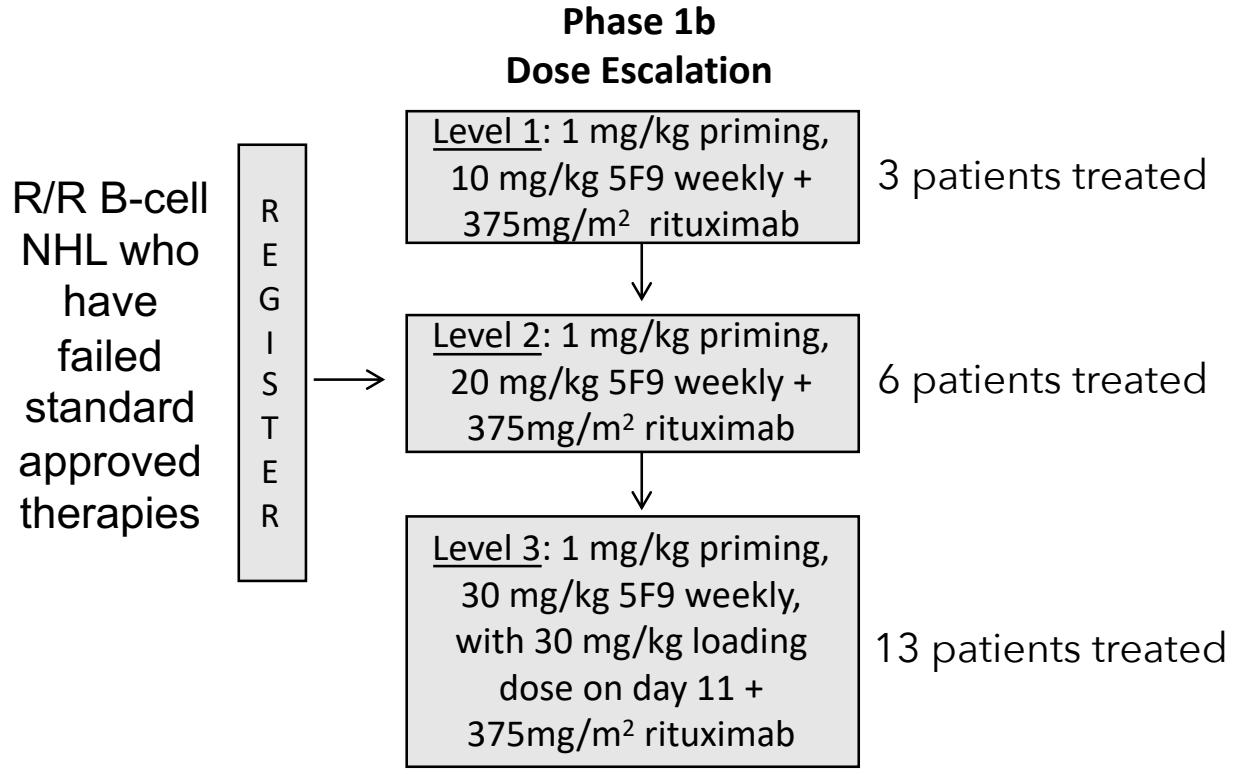
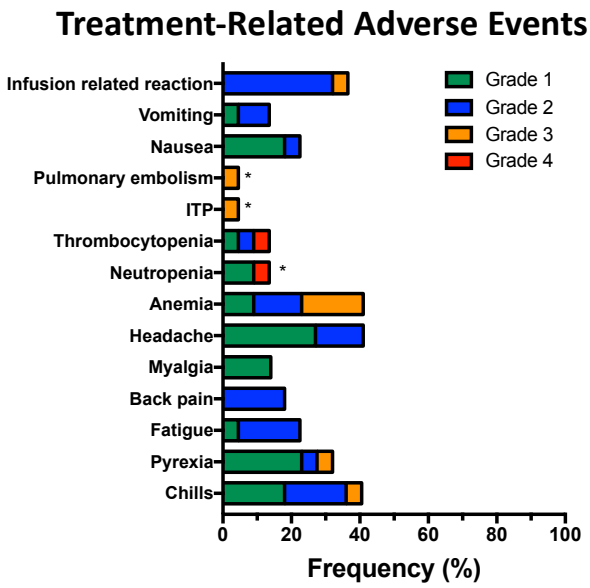
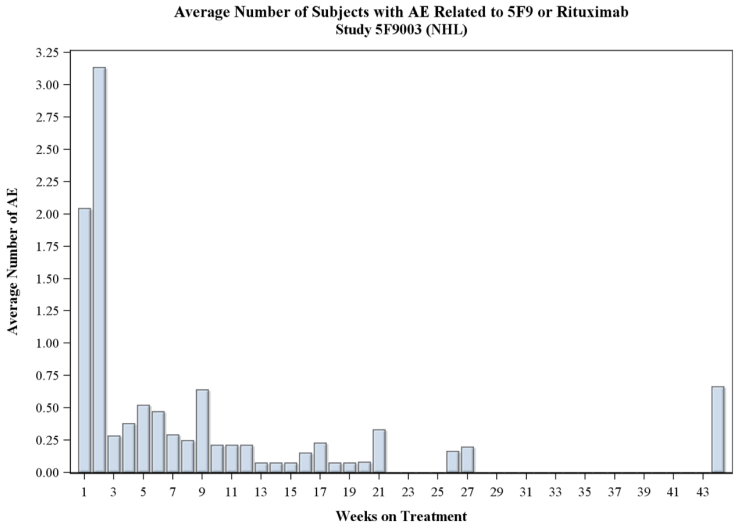


Figure S2. Study Schema
For all dose cohorts rituximab was administered at 375 mg/m^2 intravenously on days 8, 15 and 22 of cycle 1 and then day 1 in cycles 2-6.

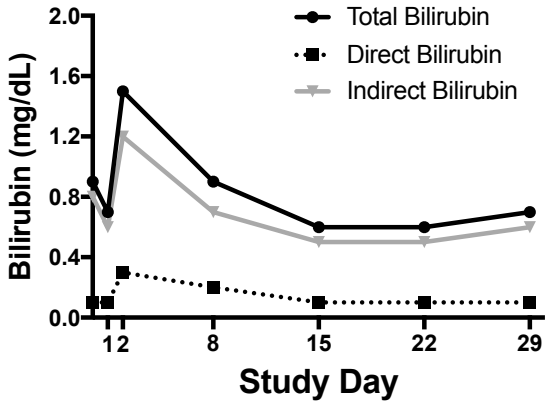
A


Treatment-Related Adverse Events
Infusion related reaction
Vomiting
Nausea
Pulmonary embolism
ITP
Thrombocytopenia
Neutropenia
Anemia
Headache
Myalgia
Back pain
Fatigue
Pyrexia
Chills
*
*
*
Grade 1
Grade 2
Grade 3
Grade 4
0
20
40
60
80
100
Frequency (%)

B


Average Number of Subjects with AE Related to 5F9 or Rituximab
Study 5F9003 (NHL)
Average Number of AE
Weeks on Treatment

C


Total Bilirubin
Direct Bilirubin
Indirect Bilirubin
Bilirubin (mg/dL)
Study Day

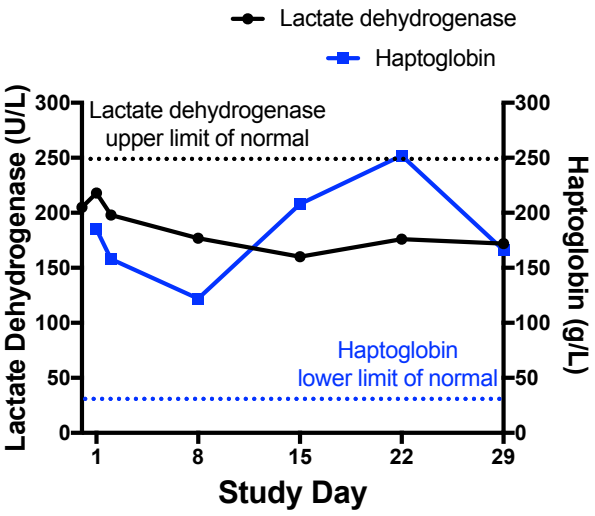

Lactate dehydrogenase
Haptoglobin
Lactate dehydrogenase
upper limit of normal
Haptoglobin
lower limit of normal
Lactate Dehydrogenase (U/L)
Haptoglobin (g/L)
Study Day

Figure S3. Treatment-Related Adverse Events and Treatment-Related Adverse Events Over Time

A. Treatment-related AEs observed in ≥ 10% of patients are shown as well as all dose limiting toxicities (*) regardless of frequency. B. Average treatment-related AEs to 5F9 and/or rituximab per patient is shown across all patients over time. C. Labs evaluating hemolysis (bilirubin, lactate dehydrogenase and haptoglobin) are shown for a typical patient, treated at a 5F9 dose of 30 mg/kg weekly.

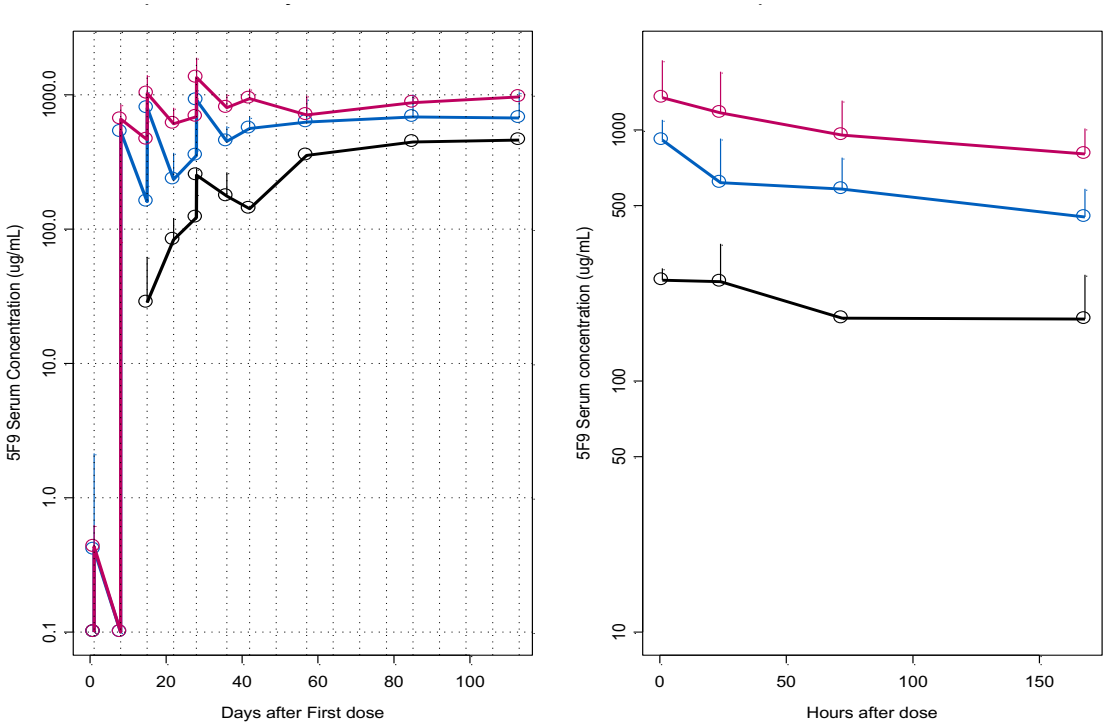


Figure S4. Median (+SD) 5F9 Serum Concentration–Time Profile in NHL Patients Over the Dose Range 10-30 mg/kg Q Weekly

Data represent serum 5F9 (median + standard deviation) for each of 10, 20, and 30 mg/kg treatment groups. Doses were given on Day 0 (priming dose of 1 mg/kg) and subsequently on Days 7-113 on a weekly basis. 5F9 concentrations in peak and trough serum samples over the duration of treatment are plotted on the left. The pharmacokinetic profile during week 5 (Days 28-36) vs time in hours is shown on the right.

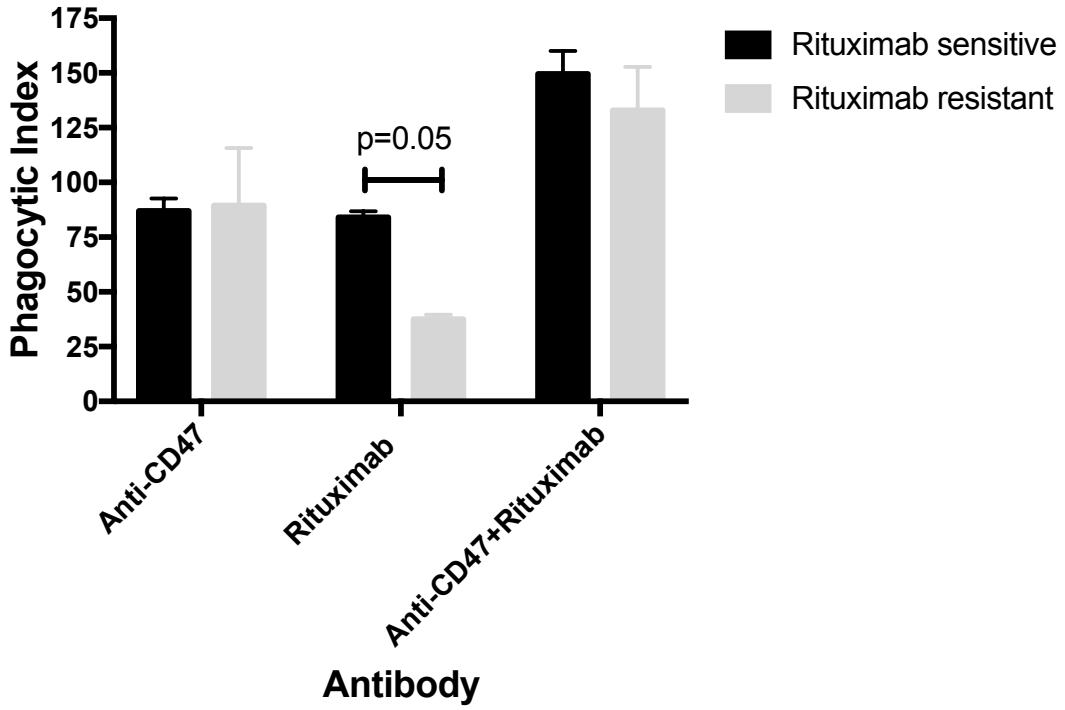


Figure S5. Anti-CD47 Antibody Synergizes with Rituximab to Induce Phagocytosis of a Rituximab-Resistant Large Cell Lymphoma Cell Line
Raji cells (a human aggressive large cell lymphoma cell line) were rendered rituximab resistant through serial passaging and exposure of rituximab to Raji cells in the presence of effector cells. Anti-CD47 antibody enabled phagocytosis in both rituximab sensitive and resistant Raji cells, whereby rituximab monotherapy was unable to induce phagocytosis of resistant cells. However, the combination of anti-CD47 antibody + rituximab enabled significantly increased phagocytosis compared to monotherapy alone, with no difference observed in the rituximab sensitive or resistant cell lines. P values are shown using a paired student's t-test.

Supplemental Tables

Table S1. DLBCL Subtype Patient Characteristics of 15 patients Treated

Characteristic	N (%)	Objective Response (%)
De novo DLBCL	8 (53%)	2/8 (25%)
Transformed DLBCL	7 (47%)	4/7 (57%)
Cell of Origin		
Activated B-cell	3 (20%)	2/3 (67%)
Germinal Center B-cell	6 (40%)	1/6 (17%)
Unknown	6 (40%)	3/6 (50%)
Double hit lymphoma[1]	3 (20%)	1/3 (33%)

[1]Defined as high grade B-cell lymphomas with MYC and BCL2 and/or BCL6 rearrangement per WHO classification of lymphoid neoplasms[26].

Table S2. Disposition of Patients on Study

Characteristic	Total (n=22)	DLBCL (n=15)	FL (n=7)
Study discontinuation due to adverse event	1 (4.5%)	1 (6.7%)	0 (0%)
Study discontinuation due to death	0 (0%)	0 (0%)	0 (0%)
Study discontinuation due to disease progression	9 (41%)	7 (47%)	2 (29%)
Median treatment duration in weeks (range)	22 (1.7 – 70.7)	19 (1.7 – 59.3)	24 (5.1 – 70.7)
Median number of Hu5F9-G4 infusions (range)	18.5 (3 – 67)	18 (3 – 50)	19 (3 – 67)
Median number of Rituximab infusions (range)	7 (1 – 9)	7 (1 – 8)	8 (4 – 9)
Duration of follow-up in months (range)	6.2 (1.9 – 16.4)	6.2 (1.9 – 13.9)	8.1 (1.9 – 16.4)

Table S3. Serious Adverse Events of all 22 Patients

SAE (Any Grade)	Frequency (%)
Infections	4 (18)
Anemia	1 (4.5)
Dyspnea	1 (4.5)
Pyrexia	1 (4.5)
Lactic acidosis	1 (4.5)
Retroperitoneal mass	1 (4.5)
Pulmonary Embolism	1 (4.5)
Infusion-related Reaction	1 (4.5)

Notes: All SAEs are shown. MeDRA version 19.0