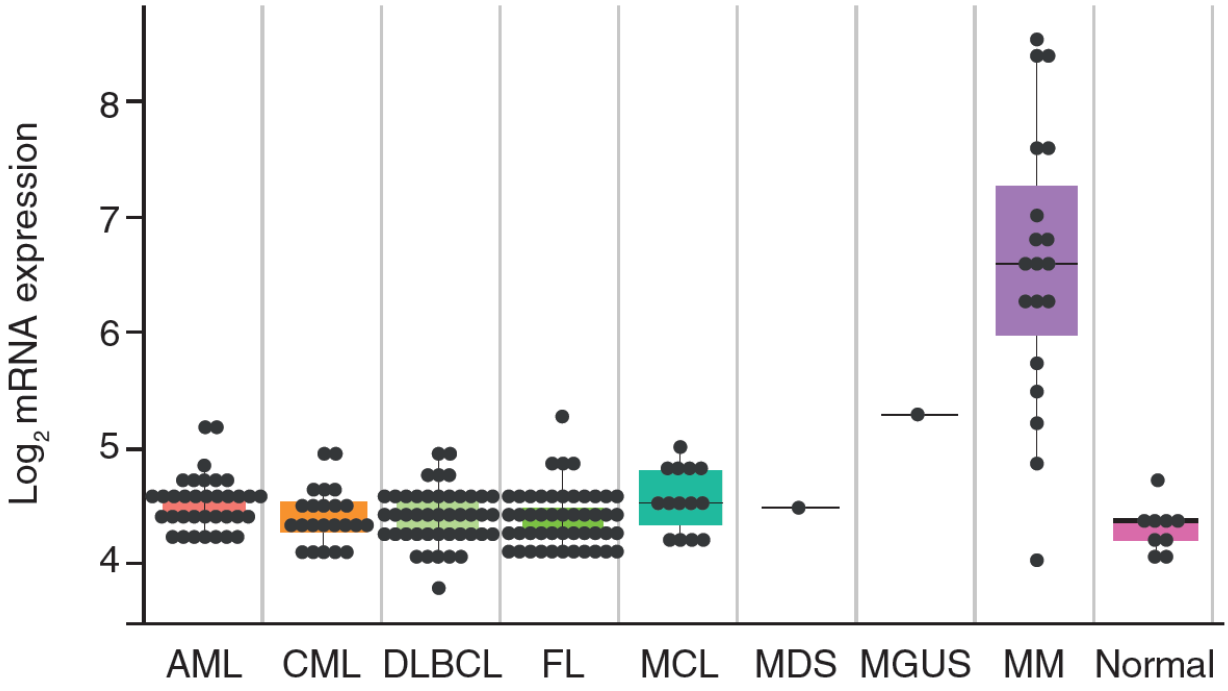
Supplementary Materials

Supplemental Figure S1.

Expression of GPRC5D mRNA in various heme malignancies. Affymetrix GeneChip CEL files were obtained for the Gene Logic data set from Occimum Biosolutions, and raw data were processed and normalized using robust multichip averaging method in the Affy Bioconductor R package and visualized using the *ggplot2* R package. GPRC5D mRNA is clearly overexpressed in MM compared with the other heme malignancies depicted. AML: Acute myeloid leukemia; CML: Chronic myelogenous leukemia; DLBCL: Diffuse large B-cell lymphoma; FL: Follicular lymphoma; MCL: Mantle cell lymphoma; MDS: Myelodysplastic syndrome; MGUS: Monoclonal gammopathy of undetermined significance; MM: Multiple myeloma. Normal: samples from various tissues of healthy donors.

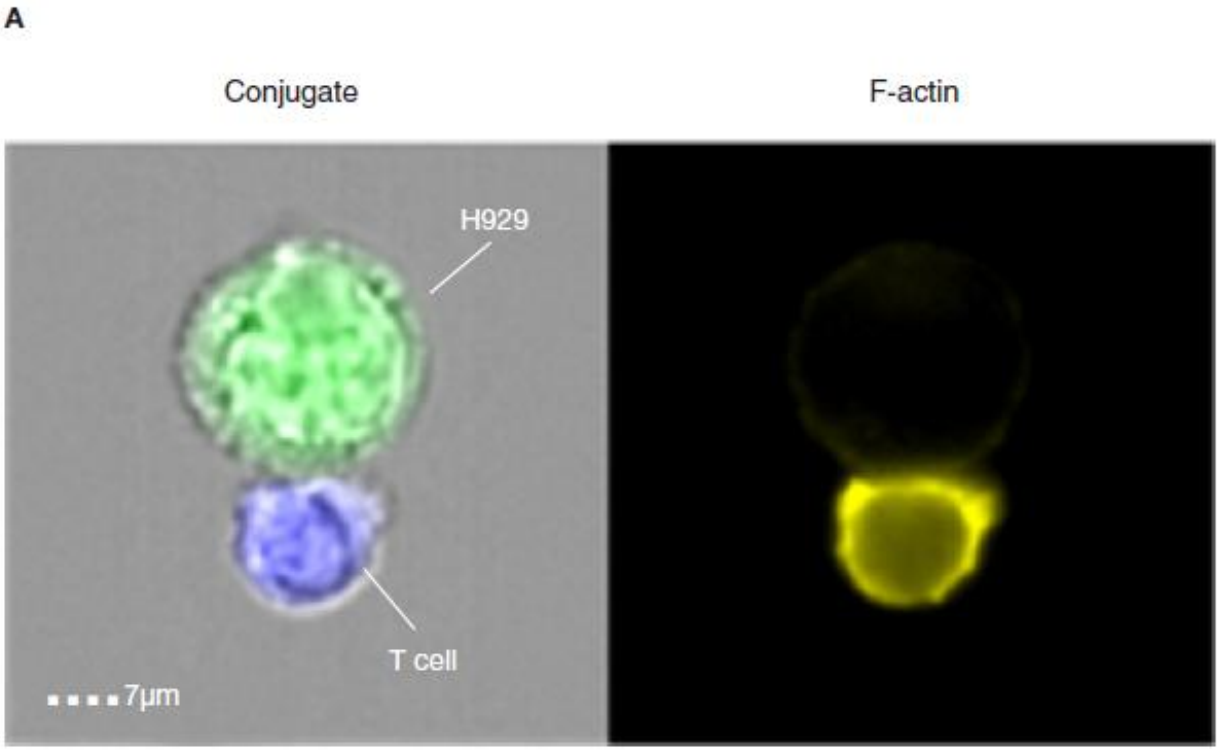
Figure S1

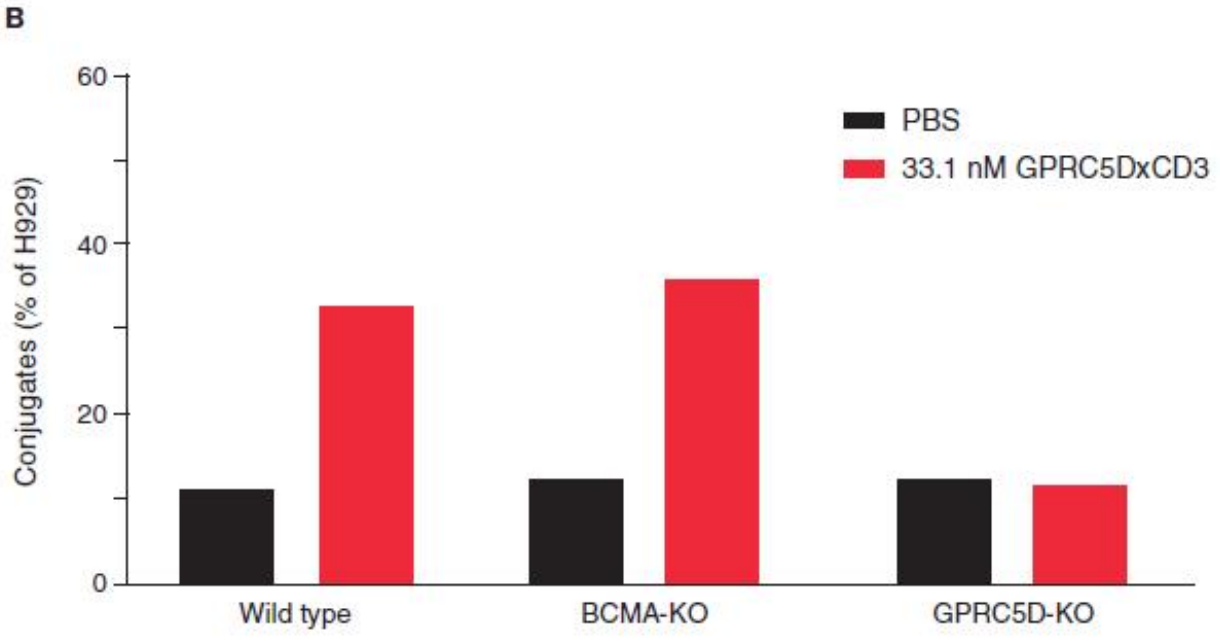

Log2 mRNA expression
8
7
6
5
4
AML
CML
DLBCL
FL
MCL
MDS
MGUS
MM
Normal

Supplemental Figure S2.

Formation of T cell / tumor cell conjugates with JNJ-64407564. (A) Representative picture of T cell recruited to H929 tumor cells *in vitro*. Actin filaments can be seen on the right and are consistent with T cell activation. (B) JNJ-64407564 led to an increase of T cell / tumor cell conjugates when using wild type H929 and BCMA KO cells but not in GPRC5D KO cells.

Figure S2

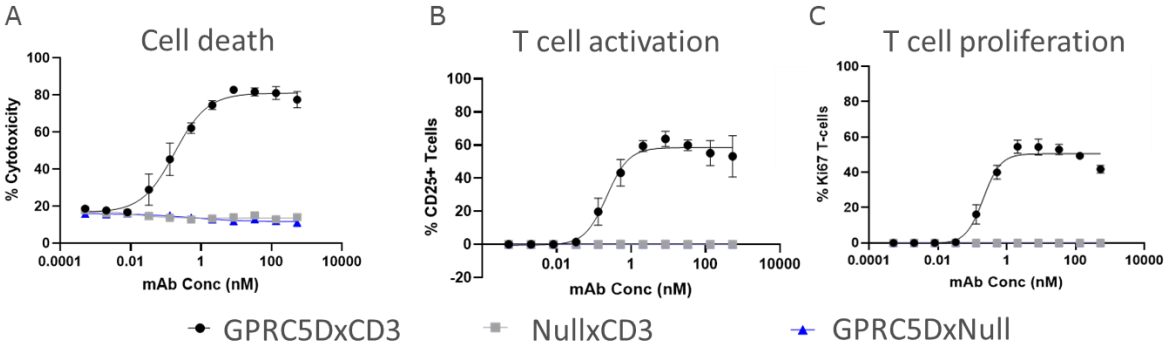

A
Conjugate
F-actin
H929
T cell
7μm

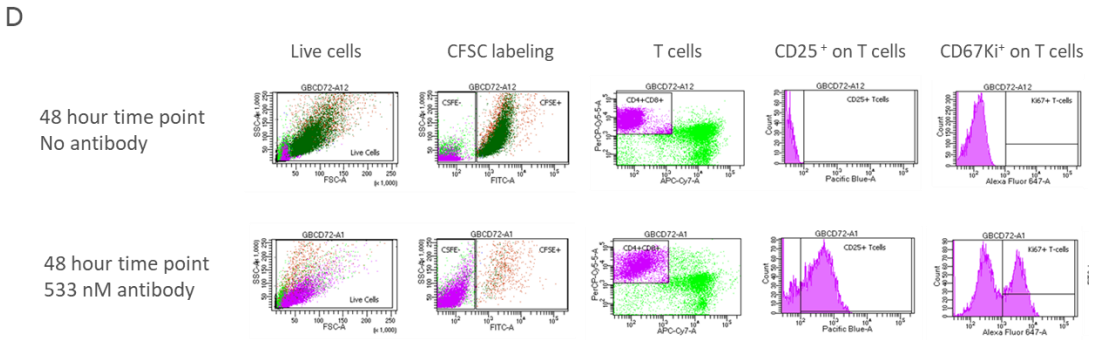

B
Conjugates (% of H929)
60
40
20
0
PBS
33.1 nM GPRC5DxCD3
Wild type
BCMA-KO
GPRC5D-KO

Supplemental Figure S3.

Cytotoxicity correlates with T cell activation and T cell proliferation in MM.1R cells at 48 h. (A) JNJ-64407564-mediated cytotoxicity (measured by enumerating CFSC labeled cells). (B) T cell activation as measured by %$CD25^+$ T cells and (C) T cell proliferation as measured by Ki67 positivity. (D) Histograms showing LIVE/DEAD cells (1^{st} column), CFSC labeled cells from the LIVE cell gate (2^{nd} column) and live T cells (3^{rd} column), followed by $CD25^+$ T cells (4^{th} column) and $Ki67^+$ T cells (last column). Top row is in the absence of antibody and the bottom row is in the presence of JNJ-64407564. Treatment with JNJ-64407564 led to an increase of T cell proliferation in a dose-dependent manner in the presence of MM.1R cells (panel C and last column of panel D).

Figure S3

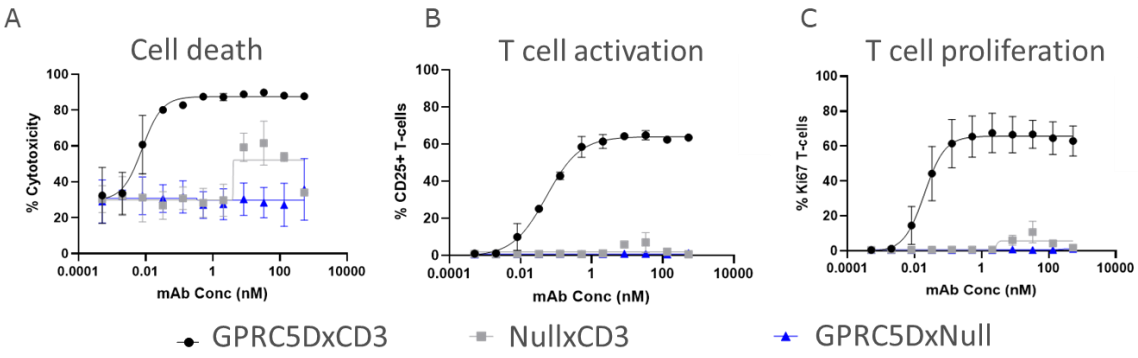

A
Cell death
% Cytotoxicity
mAb Conc (nM)
B
T cell activation
% CD25+ Tcells
mAb Conc (nM)
C
T cell proliferation
% Ki67 T-cells
mAb Conc (nM)
GPRC5DxCD3
NullxCD3
GPRC5DxNull

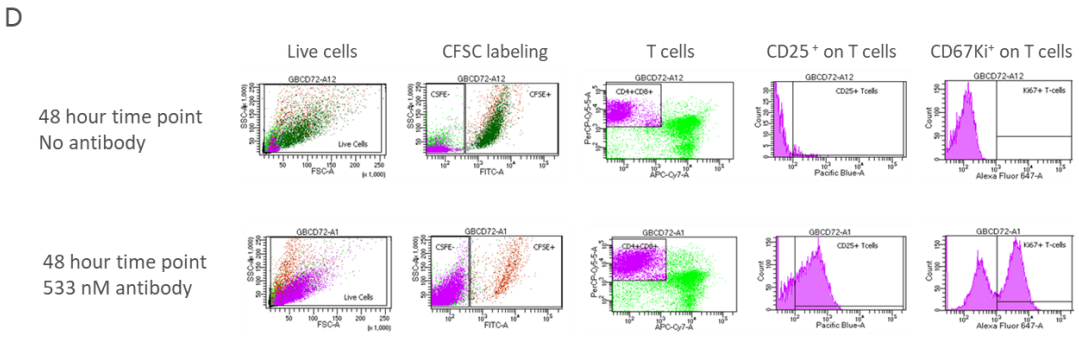

D
Live cells
CFSC labeling
T cells
CD25 + on T cells
CD67Ki+ on T cells
48 hour time point
No antibody
48 hour time point
533 nM antibody

Supplemental Figure S4.

Cytotoxicity correlates with T cell activation and T cell proliferation in H929 cells at 48 h. (A) JNJ-64407564-mediated cytotoxicity (measured by enumerating CFSC labeled cells). (B) T cell activation as measured by %$CD25^+$ T cells and (C) T cell proliferation as measured by Ki67 positivity. (D) Histograms showing LIVE/DEAD cells (1^{st} column), CFSC labeled cells from the LIVE cell gate (2^{nd} column) and live T cells (3^{rd} column), followed by $CD25^+$ T cells (4^{th} column) and $Ki67^+$ T cells (last column). Top row is in the absence of antibody and the bottom row is in the presence of JNJ-64407564. Treatment with JNJ-64407564 led to an increase of T cell proliferation in a dose-dependent manner in the presence of H929 cells (panel C and last column of panel D).

Figure S4


A
Cell death
% Cytotoxicity
mAb Conc (nM)
B
T cell activation
% CD25+ T-cells
mAb Conc (nM)
C
T cell proliferation
% Ki67 T-cells
mAb Conc (nM)
GPRC5DxCD3
NullxCD3
GPRC5DxNull

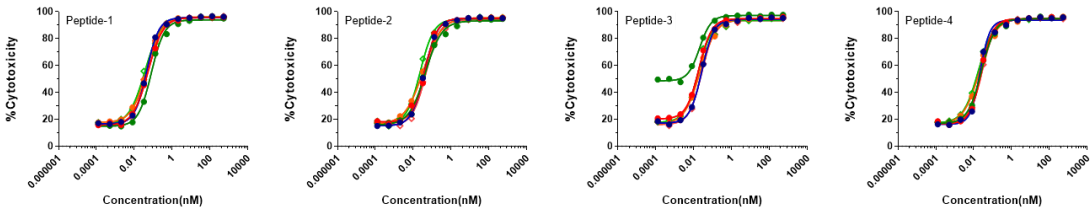

D
Live cells
CFSC labeling
T cells
CD25 + on T cells
CD67Ki+ on T cells
48 hour time point
No antibody
48 hour time point
533 nM antibody

Supplemental Figure S5.

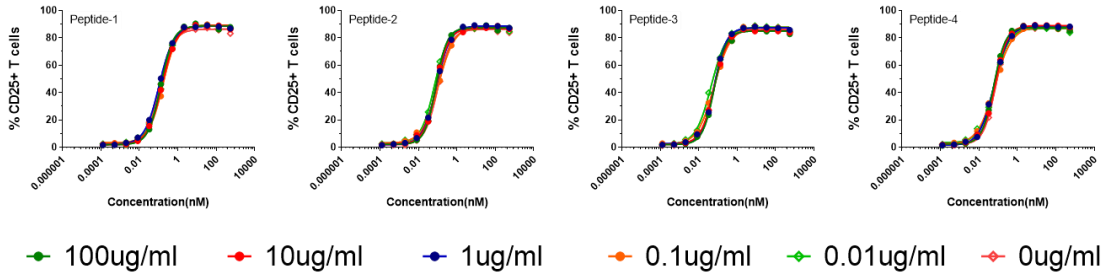
Effect of GPRC5D extracellular peptides on the activity of JNJ-64407564. The influence of various peptides (1-4) on the JNJ-64407564 cytotoxicity (A) as measured by enumeration of CFSC labeled MM.1R cells and T cell activation (B) as measured by $CD25^{+}$ T cells. None of the peptides interfered with JNJ-64407564 cytotoxicity or T cell activation up to 100 ug/ml (highest concentration). (C) Peptide sequences of the extracellular (ECL) domains of the GPRC5D protein.

Figure S5

A



B



C

GPRC5D External domains:

Location	Peptide	Sequence
N-term	1	MYKDCIESTGDYFLLCDAEGPWGIILEGGGGSK (Biotin)-NH2
ECL-1	2	NQQTAPVRYGGGGSK(Biotin)-NH2
ECL-2	3	TEYVTLIMTRGMMFVNMTPCQLNGGGGSK(Biotin)-NH2
ECL-3	4	RGNPQFQRQPQWDDGGGGSK(Biotin)-NH2

Table 1S. *In vitro* bioactivity of the clinical candidate JNJ-64407564 and the surrogate molecule JNJ-64024701 in HEK cells expressing human or cyno GPRC5D

Antibody	Assay	EC_{50} (nM)		
		hGPRC5D HEK cells and human T cells	cGPRC5D HEK cells and human T cells	cGPRC5D HEK cells and cyno T cells
JNJ-64407564	Cytotoxicity	**0.03**	3.41	4.01
	T cell activation	**0.03**	3.33	1.85
JNJ-64024701	Cytotoxicity	0.04	0.07	**0.12**
	T cell activation	0.03	0.14	**0.19**

JNJ-64024701 = surrogate molecule containing GC5B673 (GPRC5D arm #2); JNJ-64407564 = clinical candidate containing GC5B596 (GPRC5D arm #1). Both antibodies were generated with identical CD3 parental antibody and share the IgG4-PAA isotype. Bold values highlight that the surrogate molecule had similar activity with cyno GPRC5D as the clinical candidate with human GPRC5D. HEK 293 cells were transfected with human or cynomolgus monkey GPRC5D plasmids and selected for clones that were neomycin resistant. Cytotoxicity and T cell activation was measured after 48 hours incubation of target cells with human or cynomolgus monkey T cells as described in Material and Methods.

Additional notes for toxicology assessments in cynomolgus monkey

JNJ-64407564 bound to human GPRC5D (hGPRC5D), however it bound weakly to cynomolgus monkey GPRC5D (cGPRC5D) (data not shown) and demonstrated reduced cytotoxicity and T cell activation in cGPRC5D expressing HEK cells compared to hGPRC5D expressing HEK cells. Therefore, a surrogate molecule which had comparable in vitro bioactivity to the clinical candidate in both hGPRC5D and cGPRC5D expressing HEK cells was used for safety profiling (hazard identification) in cynomolgus monkeys in accordance with ICH S6(R1) and ICH S9. The in vitro bioactivity of the clinical candidate JNJ-64407564 and the surrogate molecule JNJ-64024701 are summarized in Table 1S.

Cynomolgus monkey studies were conducted according to the guidelines of the site IACUC, using naïve, cynomolgus monkeys sourced from China. For the pivotal study with the surrogate molecule JNJ-64024701, cynomolgus monkeys (3/sex/group) were administered IV bolus doses of 0, 10, or 30 mg/kg once weekly for 4 weeks (Days 1, 8, 15, and 22) and necropsied on Day 29. The general study parameters included clinical, physical and ophthalmic examinations; qualitative food consumption; body weight; body temperature; clinical pathology (coagulation, clinical chemistry, hematology, and urinalysis); safety pharmacology (blood pressure, heart rate, electrocardiology); gross pathology, organ weights, and microscopic pathology.

JNJ-64024701 was well tolerated up to 30 mg/kg in the pivotal toxicology study in cynomolgus monkeys. There were no JNJ-64024701-related effects on clinical observations, food consumption, body weight, veterinary physical examination (including respiration), ophthalmic examination, blood pressure, heart rate, body temperature, electrocardiology, urinalysis, gross pathology, organ weights, or microscopic pathology. JNJ-64024701-related changes in clinical pathology parameters included transient, mildly to moderately decreased lymphocytes at 10 and 30 mg/kg on Day 2 (24 hours after the first dose [males: 0.36x- 0.45x; females: 0.33x-0.58x of day -7 baseline mean values]), which were considered consistent with the expected mechanism of action of JNJ-64024701. Other clinical pathology changes (relative to day -7 baseline mean values) consisted of minimally increased globulins at 10 and 30 mg/kg on Day 28 (males: 1.18x-1.21x; females: 1.17x and increased large unstained cells at 30 mg/kg on Days 23 and 28 (males: 1.76x; females: 2.56x-2.70x) without any microscopic correlates. An increased incidence of minimally increased fibrinogen was also noted at both dose levels; however, these changes were of uncertain relationship to JNJ-64024701 administration as the absolute values and magnitudes of change generally overlapped with control and/or baseline values, and no other changes supportive of an acute phase response were noted at these time points. JNJ-64024701-related changes in cytokines were limited to transient increases in IL-10 seen in the 10 and 30 mg/kg groups on Day 1 at 2 hours post dose. Systemic drug exposure (mean Cmax and AUC within one dose interval) following the IV doses on Day 1 increased in an approximately dose-proportional manner in the dose range from 10 to 30 mg/kg. Mean (standard deviation [SD]) JNJ-64024701 exposure after 4 doses at 30 mg/kg was 758.31 (134.66) µg/mL for Cmax (Day 22) and 1,471.16 (1,074.52) µg/mL for $AUC_{Day\ 22\text{-}29}$. Some animals at both dose levels demonstrated fast concentration decreases before the dose on Day 22. There was no drug accumulation in general following 4 weekly dose administrations of 10 and 30 mg/kg. The decrease in drug exposure following repeated-dose administrations was attributed to the due to development of anti-drug antibodies.

Overall, the robust pharmacodynamic effects often observed with this class of molecule (e.g. polycytokine release, acute phase response) in cynomolgus monkey were not observed with JNJ-64024701. The limited pharmacodynamic activity of JNJ-64024701 in the cynomolgus monkey is possibly due to limited GPRC5D target in a healthy monkey. CD3 bispecifics require formation of a trimolecular complex involving a T cell, the CD3 bispecific and a target (GPRC5D expressing) cell in order to mediate an effect. Thus, limited pharmacodynamic activity is consistent with limited GPRC5D expression given that bioactivity was demonstrated in vitro (Table 1S). There could be limited translatability of these nonclinical toxicity findings to tumor-bearing patients who carry a greater target cell burden.

Reference:

ICH S6 (R1) Preclinical safety evaluation of biotechnology-derived pharmaceuticals.ICH S9 Nonclinical Evaluation for Anticancer Pharmaceuticals