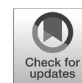
Translational Cancer Mechanisms and Therapy

Clinical Cancer Research

Targeting Multiple Myeloma with AMG 424, a Novel Anti-CD38/CD3 Bispecific T-cell–recruiting Antibody Optimized for Cytotoxicity and Cytokine Release



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Abstract

Purpose: Despite advances in the treatment of multiple myeloma, new therapies are needed to induce more profound clinical responses. T-cell–redirected lysis triggered by bispecific antibodies recruiting T cells to cancer cells is a clinically validated mechanism of action against hematologic malignancies and CD38 is a tumor-associated antigen with near-universal expression in multiple myeloma. Thus, an anti-CD38/CD3 bispecific T-cell–recruiting antibody has the potential to be an effective new therapeutic for multiple myeloma.

Experimental Design: Anti-CD38/CD3 XmAb T-cell–recruiting antibodies with different affinities for CD38 and CD3 were assessed *in vitro* and *in vivo* for their redirected T-cell lysis activity against cancer cell lines, their lower levels of cytokine release, and their potency in the presence of high levels of soluble CD38. Select candidates were further tested in cynomolgus monkeys for B-cell depletion and cytokine release properties.

Results: AMG 424 was selected on the basis of its ability to kill cancer cells expressing high and low levels of CD38 *in vitro* and trigger T-cell proliferation, but with attenuated cytokine release. *In vivo*, AMG 424 induces tumor growth inhibition in bone marrow–invasive mouse cancer models and the depletion of peripheral B cells in cynomolgus monkeys, without triggering excessive cytokine release. The activity of AMG 424 against normal immune cells expressing CD38 is also presented.

Conclusions: These findings support the clinical development of AMG 424, an affinity-optimized T-cell–recruiting antibody with the potential to elicit significant clinical activity in patients with multiple myeloma.

Introduction

Multiple Myeloma results from the neoplastic transformation of antibody-producing plasma cells and is characterized by multifocal malignant lesions throughout the bone marrow (1). It is the second most common hematologic malignancy, causing more than 12,000 deaths per year in the United States alone (2). The outcome for patients with multiple myeloma has greatly improved over the last decade (3), but even the most promising therapeutic regimens with targeted agents fail to prevent disease relapse in the vast majority of patients (4). Relapses are less frequent and manifest later when minimal residual disease (MRD) is greatly reduced (5, 6), thus further advances against multiple myeloma will require new therapeutic options that reduce tumor load below stringent MRD detection limits (4).

The recruitment of cytotoxic T cells to cancer cells expressing tumor-associated antigens by bispecific antibodies is a highly promising therapeutic mode of action (MOA) for treating hematologic malignancies (7, 8). This approach was first clinically validated with blinatumomab, a bispecific T-cell engager (BiTE) antibody construct targeting CD3 and CD19, approved for the treatment of B-cell acute lymphoblastic leukemia and capable of inducing MRD negativity in a majority of patients (9, 10).

CD38 is a transmembrane glycoprotein (11) universally expressed (12) and clinically validated as a therapeutic target (13, 14) in multiple myeloma. Although lymphocytes, natural killer (NK) cells, dendritic cells and bone marrow progenitor cells (11) express CD38, plasma cells and malignant multiple myeloma cancer cells express CD38 at higher levels (12, 15).

This study describes AMG 424, a novel humanized bispecific XmAb T-cell–recruiting antibody directed against CD38 and CD3 that aims to leverage the universal expression of CD38 on multiple myeloma cells to trigger T-cell–mediated cancer cell cytotoxicity. Although functionally related, AMG 424 is structurally distinct from the FDA-approved blinatumomab. It is based on the

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Note: Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

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Translational Relevance

Existing therapies for multiple myeloma lead to relapse in most patients due to the persistence of minimal residual disease (MRD) posttherapy. Eliminating MRD or resistant disease to existing targeted therapies will likely require a more potent mechanism of action. Bispecific T-cell–engager antibody therapy has proven to eliminate MRD in other hematologic malignancies. AMG 424, a novel bispecific T-cell–recruiting antibody targeted at the multiple myeloma surface cell marker, CD38, triggers complete killing of cancer cells expressing high and low levels of CD38 *in vitro*. AMG 424 also induces antitumor activity in bone marrow–invasive cancer models in mice and the depletion of peripheral B cells in cynomolgus monkeys, indicating potent *in vivo* activity. AMG 424 possesses the required attributes to yield potentially significant clinical activity in patients with multiple myeloma and is currently in clinical development.

XmAb bispecific platform consisting of a hetero-Fc domain devoid of Fcγ receptor and complement binding, an anti-CD3 single chain variable fragment (scFv) domain and a humanized anti-CD38 Fab domain derived from the OKT10 antibody (16).

High-affinity binding to CD38 or CD3 is associated with inherent challenges. An anti-CD38/CD3 XmAb carrying a high-affinity CD3 binder was previously reported to induce non-tolerated cytokine release in cynomolgus monkeys (17). In addition, the large CD38 target load in patients with multiple myeloma is associated with the short half-life of the CD38-targeted antibody daratumumab at nonsaturating doses due to target-mediated drug disposition (TMDD; refs. 18, 19). This study details how AMG 424 was selected from a panel of candidates with varying affinities for CD3 and CD38 to achieve complete killing of target cells expressing high or low levels of CD38 *in vitro* and *in vivo*, while demonstrating lower and tolerated cytokine release.

Materials and Methods

Cell lines

Cell lines were obtained from ATCC (CHO-K1, HEL 92.1.7, NCI-H929, and RPMI-8226) or DSMZ (SKM-1, OPM-2, MOLM-13, and KMS-12-BM) and grown as recommended. Luciferase-expressing variants were obtained by transduction with lentivirus pLV417-expressing firefly luciferase and selection in blasticidin (10 μg/mL). Cell lines were passaged 10 to 30 times and tested for CD38 expression two to nine times during the project. Cell lines were *Mycoplasma*-tested with PCR-based testing (IMPACT testing, IDEXX Bioresearch) and authenticated using short tandem repeat (STR) profile (CellCheck Profile, IDEXX Bioresearch).

Affinity measurements

To calculate the constant of dissociation (K_D) between XmAbs and soluble CD38 (sCD38), a Fortebio Octet RED384 and an Octet HTX instrument were used with streptavidin (SAX) fiber optic biosensor tips. To quantitate the K_D between XmAbs and soluble CD3e, a Biacore T200 instrument was used with CM5 chips (details in Supplementary Materials and Methods).

Quantification of CD38 expression, serum sCD38 levels, and serum anti-CD38/CD3 XmAb levels

Surface CD38 was quantified with Qifikit (Dako), using antibody AT1 (Ancell). Maximum antibody-binding values and calibration beads were used to quantify the number of CD38 antibody-binding sites (ABS) per cell. Human and cynomolgus soluble CD38 were measured by electro-chemiluminescence (MSD Quickplex SQ 120) using anti-human or -cynomolgus CD38 capture and detection antibodies (Amgen) and recombinant human CD38-His (R&D Systems) and cynomolgus CD38-His (Sino Biologicals) as standards. XmAbs in cynomolgus serum were measured by electro-chemiluminescence (MSD Quickplex SQ 120) using an anti-CD3 scFv monoclonal capture antibody (Xencor), biotinylated recombinant CD38 capture protein (Sino Biologicals) or biotinylated anti-human Fc monoclonal capture antibody (Amgen), and a ruthenylated anti-human Fc detection antibody (Amgen).

Redirected cytotoxicity by flow cytometry or luciferase measurement

Redirected T-cell cytotoxicity was evaluated by flow cytometry tracking carboxyfluorescein diacetate succinimidyl ester (CFSE)-labeled cells or Steady-Glo measurement. Frozen human T cells (AllCells) or frozen peripheral blood mononuclear cells (PBMC) from humans or cynomolgus (CepheusBio) were cocultured with target cells at an effector cell (E) to target cell (T) ratio (E:T) of 10:1 or 1:1 and serial dilutions of CD38 XmAb molecules. After incubation for 48 hours at 37°C, cells were analyzed using a BD LSR-II instrument. For luciferase-tagged cells, Steady-Glo Luciferase (Promega) was used. Cytotoxicity curves were generated with Prism7.04 software (GraphPad).

Immune cell depletion from PBMCs and T-cell activation

Immune cell depletion and T-cell activation were measured by flow cytometry. PBMCs were transferred to 96-well V-bottom plates (BD 353263) and centrifuged at 300 × *g* for 5 minutes at 4°C, then washed with cold Stain Buffer (BD 554656). Human Fc Block (BD 564220; 2.5 μg) was added prior to staining. Antibodies for CD3, CD8, CD14, CD69 (BD Bioscience), CD4, CD16, CD25, CD20, CD56 (BioLegend), and CD38 (StemCell) were mixed as needed and incubated for 30 minutes on ice (antibody panels in Supplementary Materials and Methods). Cells were washed 3× with Stain Buffer and resuspended in cold Stain Buffer containing 7-AAD (BD Biosciences). Flow cytometry was performed on an LSR-II instrument (BD Biosciences) and data processed with FlowJo10 software (TreeStar).

Cytokines detection

Cytokines from cell culture supernatants were analyzed with the Human ProInflammatory I 4 Plex Tissue Culture Kit (MSD). Cytokines from cynomolgus serum were analyzed with the Milliplex Non-Human Primate Cytokine Magnetic Kit (Millipore).

In vivo efficacy studies in mouse models

Mice were cared for following the Guide for the Care and Use of Laboratory Animals (eight edition; ref. 20). Animals were housed at facilities accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) and all study protocols were approved by the Amgen Institutional Animal Care and Use Committee (IACUC). On Day 0, 10^6 OPM2-Luc cells were injected intravenously in 7-week-old female NOD scid γ

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(NSG) mice (Jackson Laboratories). On Day 6, mice were imaged and randomized ($n = 10$/group) and 2×10^7 expanded human T cells were injected intraperitoneally. On Day 7, 2 hours prior to AMG 424 administration, anti–mu-FcRII antibody 2.4G2 (Amgen) and human IgG (KIOVIG, Baxalta) were injected intraperitoneally at 8 mg/kg and 400 mg/kg, respectively, to block Fc receptor binding. AMG 424 was then administered intraperitoneally [once weekly (QW) for 2 weeks] at 0.1, 1, or 10 mg/kg. On Day 0, 5×10^4 MOLM13-Luc cells were injected intravenously in 8-week-old female NSG mice. On Day 2, 2×10^7 expanded human T-cells were injected intraperitoneally. On Day 4, mice were imaged and randomized ($n = 10$/group). Vehicle or AMG 424 at 0.01, 0.1, or 1.0 mg/kg was administered intravenously once weekly for 4 weeks starting on day 4, starting 2 hours after Fc block. Bioluminescence imaging (BLI) of whole-body tumor burden and body weight of each animal was measured twice weekly.

Studies in cynomolgus monkeys

Cynomolgus monkey studies were conducted at Charles River Laboratories using purpose-bred, naïve Mauritian cynomolgus monkeys. Animals were housed at an AAALAC-accredited facility and the study protocol was approved by the Charles River Laboratories IACUC. XmAb-4, AMG 424, XmAb-5, and XmAb-7 were administered to 3 animals per dose group via bolus intravenous injection. Whole blood was collected for the assessment of immune cell counts and T-cell activation by flow cytometry. Monocyte counts in blood smears were obtained using an Advia hematology analyzer (Siemens). Serum was collected and stored at $-70°C$ for pharmacokinetic (PK) and cytokine measurements.

Statistical analysis

Student *t* tests were used to assess statistically significant differences between experimental groups. For the mouse MOLM-13 study, a Kaplan–Meier analysis followed by Wilcoxon rank test and Bonferroni correction for multiple comparisons was performed.

Results

Affinity and potency of anti-CD38/CD3 XmAb panel

A panel of anti-CD38/CD3 XmAb antibodies was created through pair-wise combination of three highly related anti-CD38 Fab domains and three anti-CD3 scFv domains with variable affinities for each target (High, Hi; Medium, Med; Low, Lo; Fig. 1A). Binding affinities for human targets, expressed by their K_D, ranged from 0.9 to 17 nmol/L for human CD38 and from 1.9 to 170 nmol/L for human CD3 (Table 1). XmAb antibodies bound to recombinant cynomolgus monkey CD38 and CD3 with comparable affinity to human orthologs (Table 1; Supplementary Figs. S1 and S2; Supplementary Tables S1 and S2). MOLM-13-luc target cells, which express relatively low levels of CD38 compared with multiple myeloma cells (~29,000 binding sites per cell; Fig. 1B) and are minimally sensitive to daratumumab-mediated antibody-dependent cell-mediated cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC) killing (21), were assessed for their sensitivity to the XmAb panel. MOLM-13-luc cells were cocultured with human T cells at an E:T ratio of 10:1 and incubated in the presence of increasing concentrations of XmAb antibodies for 48 hours. Except for XmAb-8, all XmAb constructs demonstrated complete MOLM-13-luc cell lysis (Fig. 1C and D) with half-maximal effective concentration for target cell lysis (EC_{50}) values ranging from 0.9 pmol/L for XmAb-1 to 9,275 pmol/L for XmAb-5 (Table 1). The potency of XmAb antibodies, expressed by their EC_{50} values, was directly related to their affinity for CD3 and CD38.

Interference of soluble CD38 with high-affinity CD38 XmAb candidates

Serum levels of soluble CD38 (sCD38) in cynomolgus monkeys are much higher (up to 80.4 ng/mL) than those observed in healthy human volunteers (up to 0.1 ng/mL) or patients with multiple myeloma (up to 2.8 ng/mL; Supplementary Fig. S3A). The impact of recombinant cynomolgus monkey sCD38 (200 ng/mL) on the redirected T-cell lysis activity of several XmAb candidates against MOLM-13-luc target cells was assessed, using cynomolgus monkey PBMCs as effector cells. The EC_{50} of the $CD38^{Hi}$ XmAb molecules were increased 8- to 9-fold in the presence of sCD38 (Supplementary Fig. S3B and S3C; Supplementary Table S3), but the EC_{50} values of the $CD38^{Med}$ (Supplementary Fig. S3D and S3E) and $CD38^{Lo}$ XmAb molecules were decreased only 2-fold or less (Supplementary Table S3). Because of the meaningful potency shift in the presence of soluble CD38 *in vitro* for both $CD38^{Hi}$-containing XmAb molecules, $CD38^{Hi}$-containing XmAb candidates were deprioritized, as their activity would be considerably shifted in cynomolgus monkeys, the key preclinical species in which *in vivo* activity was assessed.

Decreased maximal cytokine release through CD3 affinity modulation

In a previous study, administration of XmAb-1 (containing a high-affinity anti-CD3 domain) to cynomolgus monkeys resulted in unacceptably high cytokine release (17). Therefore, cytokine release was analyzed at the end of a redirected lysis assay with XmAb molecules carrying CD3 binders of different affinities to identify candidates that release lower levels of cytokines.

Using NCI-H929-luc multiple myeloma target cells cocultured with purified human T cells at an E:T ratio of 10:1, all XmAb antibodies tested achieved complete target cell lysis (Fig. 2A). However, in cell supernatants, IFNγ (Fig. 2B) and TNFα (Fig. 2C) C_{max} levels were associated with the affinity for CD3 in each molecule. Lowering CD3 affinity from high (XmAb-4) to medium (AMG 424) led to a 39% reduction in IFNγ C_{max} ($P < 0.05$) and a 55% reduction in TNFα C_{max} ($P < 10^{-3}$); lowering CD3 affinity further from medium (AMG 424) to low (XmAb-5) led to an additional 64% reduction in IFNγ ($P < 10^{-4}$) and 54% reduction in TNFα release ($P < 10^{-3}$; Fig. 2B and C; Supplementary Table S4).

The observed difference in cytokine production was not due to differences in potencies because two molecules with nearly identical potencies but different CD3 affinities (Fig. 2A; XmAb-2 vs. XmAb-4) demonstrated significantly different maximal cytokine release (Fig. 2B and C). IL6 levels tracked those of IFNγ and TNFα closely but were very low (data not shown), reflecting the limited release of IL6 by isolated T cells *in vitro* (22).

These data indicate that maximal cytokine release can be decreased by lowering CD3 affinity, without reducing the ability of XmAb molecules to trigger complete target cell lysis. On the basis of the higher levels of cytokine release from $CD3^{Hi}$-containing XmAb antibodies *in vitro*, regardless of potency, $CD3^{Hi}$-containing XmAb candidates were deprioritized.

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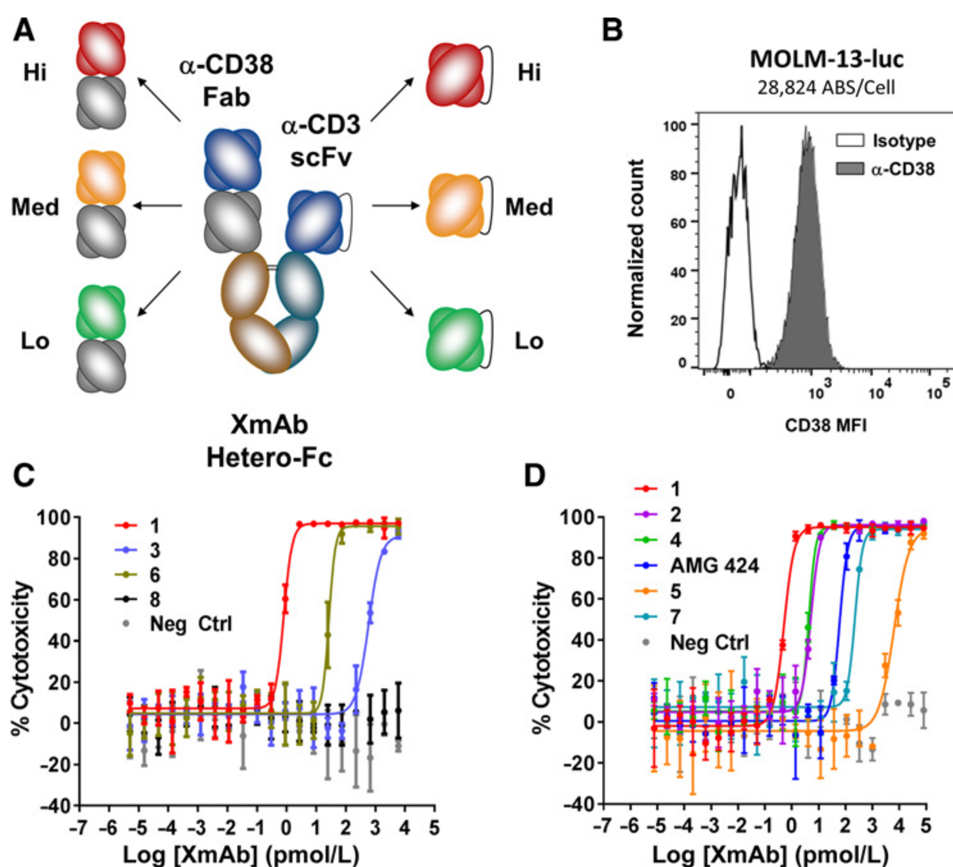

Figure 1.
Assessment of the redirected lysis potency of a panel of anti-CD38/CD3 XmAb antibodies. **A,** Schematic representation of the XmAb bispecific hetero-Fc antibody scaffold with three anti-CD38 Fab domains and three anti-CD3 single chain Fv domains combined pairwise to form a panel of nine anti-CD38/CD3 bispecific XmAb molecules. **B,** MOLM-13-luc target cells, stained with an isotype control antibody (white histogram) or an anti-CD38 monoclonal antibody (grey histogram). **C–D,** MOLM-13-luc cells (target cells, T) cocultured with purified human T cells (effector cells, E) at an E:T ratio of 10:1 in the presence of increasing concentrations of individual anti-CD38/CD3 XmAb antibodies for 48 hours. Target cell lysis was monitored by luciferase activity measurement. Cytotoxicity percentages at any concentration represent the mean of triplicate measurements; error bars, SE.

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Decreased human T-cell activation and proliferation with lower CD3 affinity

AMG 424 and XmAb-5 were further characterized, as representative $CD3^{Med}$ and $CD3^{Lo}$ molecules, for their ability to trigger T-cell activation and expansion, while depleting target cells and other immune cells *in vitro*. Human PBMCs were cocultured with CFSE-labeled NCI-H929 cells at an E:T ratio of 1:1 and increasing concentrations of AMG 424 or XmAb-5. After 72 hours of incubation, the cells were subjected to flow cytometry analysis (gating strategy, Supplementary Fig. S4). AMG 424 ($CD3^{Med}$) triggered the pronounced depletion of NCI-H929 cell ($CFSE^+$) and normal B cells ($CFSE^-$, $CD20^+$), an approximately 2-fold increase in T-cell numbers per well ($CFSE^-/CD3^+$), and a significant reduction in the number of non-B, non-T immune cells ($CFSE^-/CD3^-/CD20^-$; Fig. 2D). At the concentration where target cell killing started to plateau (Fig. 2D, red arrow), AMG 424 also triggered a robust induction of the CD25 activation marker on T cells (Fig. 2E) and T-cell numbers increased (Fig. 2D). In contrast, at a concentration at which near-complete target cell killing was obtained for XmAb-5 (Fig. 2F, red arrow), a much more limited induction of CD25 (Fig. 2G) with no accompanying T-cell expansion (Fig. 2F) was observed.

Thus, AMG 424 (CD3 $K_D \sim 15$ nmol/L) induces near-complete target cell killing accompanied by robust T-cell activation and increased T-cell numbers, but for XmAb-5 (CD3 $K_D \sim 170$ nmol/L),

Table 1. Affinity and potency of CD38/CD3 XmAb panel

CD38 XmAb	Human CD38 K_D (nmol/L)	Cynomolgus monkey CD38 K_D (nmol/L)	Human CD3 K_D (nmol/L)	Cynomolgus monkey CD3 K_D (nmol/L)	MOLM-13 TDCC EC_{50}[a] (pmol/L)
1	0.9 (Hi)	0.9 (Hi)	1.9 (Hi)	1.9 (Hi)	0.9
2	[b]0.9 (Hi)	[b]0.9 (Hi)	7 (Med)	18 (Med)	4.5
3	[b]0.9 (Hi)	[b]0.9 (Hi)	80 (Lo)	150 (Lo)	808
4	8.9 (Med)	7.4 (Med)	3.7 (Hi)	4.4 (Hi)	3.6
AMG 424	7.7 (Med)	6.6 (Med)	15 (Med)	34 (Med)	88.3
5	12 (Med)	10 (Med)	[b]170 (Lo)	[b]150–230 (Lo)	9,275
6	17 (Lo)	n/a	3 (Hi)	3.3 (Hi)	39.8
7	[b]17 (Lo)	n/a	[b]7–15 (Med)	[b]18–34 (Med)	307.6
8	[b]17 (Lo)	n/a	170 (Lo)	[b]230 (Lo)	n/a

[a]Average EC_{50} of assays performed with 3 to 12 human T-cell donors.
[b]Affinity inferred from other XmAb in the panel with the same binding domain.

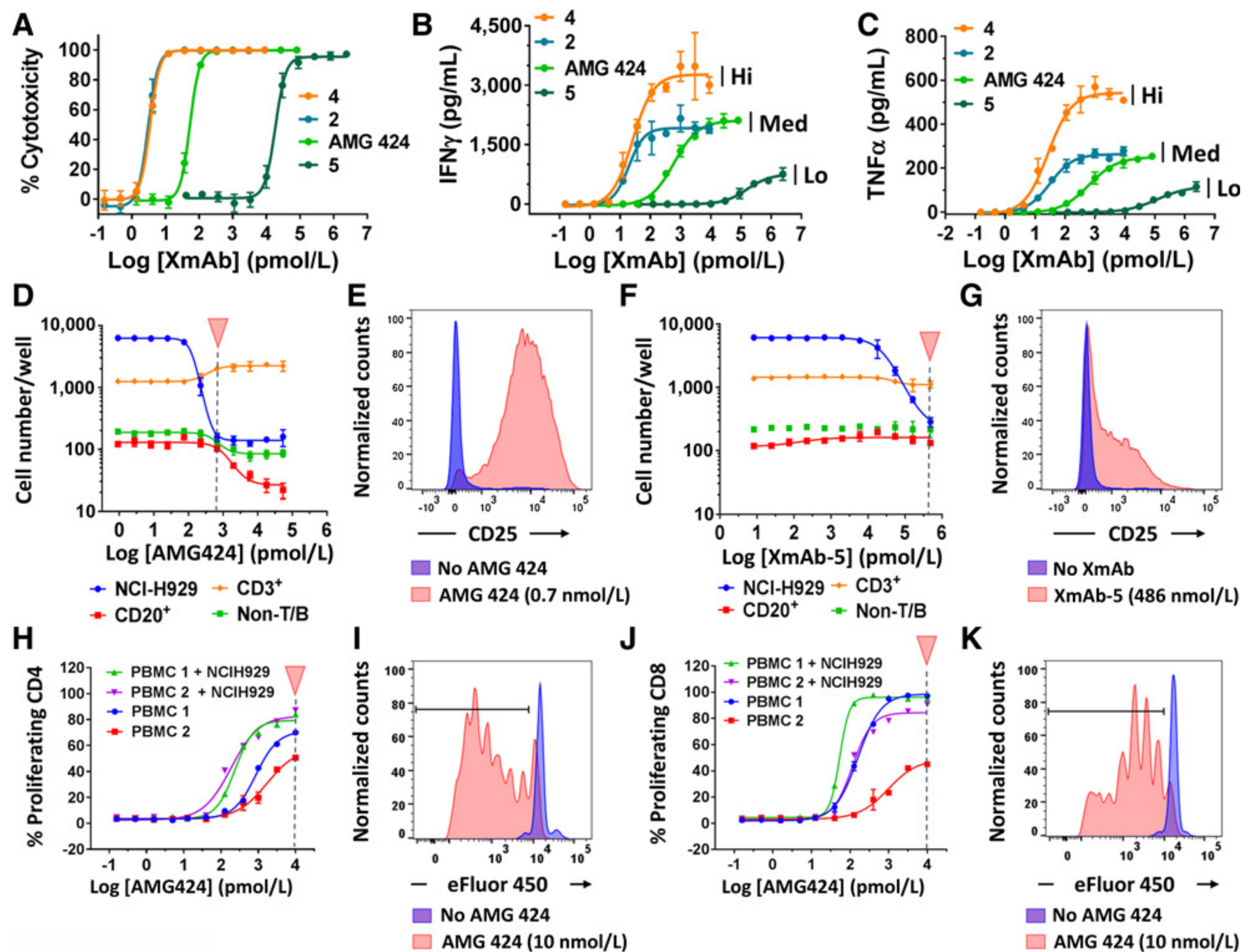

Figure 2.

Decreased maximal cytokine release through CD3 affinity modulation and maintenance of T-cell expansion and proliferation in AMG 424–mediated redirected lysis assays. **A–C,** CD38$^+$ NCI-H929-luc target cells were cocultured with human T cells at an E:T cell ratio of 10:1 with increasing concentrations of CD38/CD3 XmAb antibodies for 48 hours. Target cell lysis was monitored by luciferase activity measurement (**A**) and concentrations of IFNγ (**B**) and TNFα (**C**) were measured in cell culture supernatants, demonstrating that cytokine C_{max} is lower for XmAb candidates carrying a lower affinity anti-CD3 domain. CFSE-labeled NCI-H929-luc target cells were then cocultured with human PBMCs at an E:T ratio of 1:1 and increasing concentrations of AMG 424 (**D**) or XmAb-5 (**F**) for 72 hours. Numbers of CFSE$^+$ NCI-H929, CFSE$^-$/CD20$^+$ B cells, CFSE$^-$/CD3$^+$ T cells, and CFSE$^-$/CD3$^-$/CD20$^-$ (non-T/B) immune cells were monitored by flow cytometry at the end of the assay, demonstrating target cell, B-cell, and non-T/B immune cell killing, but T-cell expansion for AMG 424. Expression of the CD25 T-cell activation marker at the surface of T cells was determined by flow cytometry after treatment with AMG 424 (**E**) or lower CD3 affinity XmAb-5 (**G**). At a concentration at which a comparable cytotoxicity of target cells is achieved (**D, F**/red arrows), T-cell expansion and T-cell activation are decreased with the lower CD3 affinity molecule. Proliferation dye (eFluor 450)-labeled PBMCs from 2 human donors were cultured alone or with NCI-H929-luc target cells at an E:T ratio of 1:1 and increasing concentrations of AMG 424 for 96 hours and T-cell proliferation was measured (**H**, **K**). The proportion of CD4$^+$ T cells (**H**) and CD8$^+$ T cells (**K**) with decreased eFluor 450 fluorescence intensity compared with cells not treated with AMG 424 (aka proliferating T cells) was plotted for each AMG 424 concentration tested. For the maximal AMG 424 concentration tested (**H, J**/red arrows), representative histograms demonstrating eFluor 450 proliferation dye dilution for CD4$^+$ T cells (**I**) and CD8$^+$ T cells (**J**) in the presence (red histogram) but not in the absence (purple histograms) of AMG 424 are presented, demonstrating active T-cell proliferation triggered by AMG 424 at 96 hours. Because of CD38 expression on immune cells, T-cell proliferation is also triggered by AMG 424 in the absence of NCI-H929 target cells (**H, J**/blue and red curves). Each data point in the plots represents the mean of triplicate measurements; error bars, SE.

target cell killing coincides with greatly reduced T-cell activation and expansion.

T-cell proliferation upon AMG 424 stimulation

To further characterize the increase in T-cell numbers observed with AMG 424, PBMCs from 2 human donors were labeled with a proliferation dye (eFluor 450) and cultured alone or in the presence of NCI-H929 target cells for 96 hours at an E:T ratio of 1:1 and with increasing concentrations of AMG 424. The dilution of the proliferation dye was tracked in the CD4 and CD8 T cells by flow cytometry at the end of the assay and the percentage of proliferating CD4$^+$ T cells (Fig. 2H) and CD8$^+$ T cells (Fig. 2J) demonstrating eFluor 450 signal dilution was plotted for each AMG 424 concentration. At the highest AMG 424 concentration and in the presence of target cells (Fig. 2H–J, red arrows), representative histograms of the

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eFluor 450 channel for $CD4^+$ (Fig. 2I, red histogram) or $CD8^+$ (Fig. 2K, red histogram) indicate robust T-cell proliferation compared with T cells cultured in the absence of AMG 424, where no proliferation is detected (Fig. 2I–K, purple histograms; detailed gating strategy in Supplementary Fig. S5).

Thus, AMG 424 induces T-cell proliferation in the presence as well as the absence of NCI-H929 target cells (Fig. 2H–J), indicating that $CD38^+$ cells present in PBMCs are sufficient to trigger AMG 424-mediated T-cell proliferation.

Because of its relatively modest affinity for CD38 (K_D = 7.7 nmol/L), the presence of RPMI-8226 multiple myeloma target cells expressing much higher levels of CD38 than T cells (Supplementary Fig. S6A) has a limited impact on AMG 424 binding to T cells. The T-cell binding EC_{50} of AMG 424 presents a modest shift from 3.6 to 7.1 nmol/L in the presence of RPMI-8226 cells (E:T = 1:1) after 1 hour on ice (Supplementary Fig. S6B). In the RPMI-8226/T-cell mixture, the T-cell binding EC_{50} (7.1 nmol/L) is close to the RPMI-8226 cell binding EC_{50} (13.8 nmol/L; Supplementary Fig. S6C), indicating a balanced engagement of both targets, a key feature for the tumor biodistribution of BiTE antibodies (23).

Immune cell depletion by AMG 424 in human and cynomolgus monkey PBMCs

To better understand the potential toxicities of AMG 424 in human and cynomolgus monkeys, given the partial depletion of human B cells and the partial depletion of non-B, non-T immune cells depicted in Fig. 2D, the expression of CD38 at the surface of human and cynomolgus monkey B cells, CD8 T cells, monocytes, and NK cells was measured by flow cytometry (Fig. 3A) using lineage-specific markers (gating strategy, Supplementary Fig. S7). Cynomolgus monkey lymphocytes and monocytes expressed substantially higher levels of CD38 compared with their human counterparts, and human NK cells expressed high levels of CD38 (Fig. 3A). Unlike their human counterparts (Supplementary Fig. S7A), cynomolgus monkeys' $CD14^-/CD4^-/CD8^-/CD20^-/CD16^+$ PBMCs did not express the CD56 NK marker and could not be unequivocally identified as NK cells in this analysis (Supplementary Fig. S8A); although the phenotype of these $CD16^+$ cells is equivocal, they express CD16, the only marker reported to correlate with NK-cell activity in cynomolgus monkey peripheral lymphocytes (24) and are efficiently depleted by AMG 424 (Supplementary Fig. S8B).

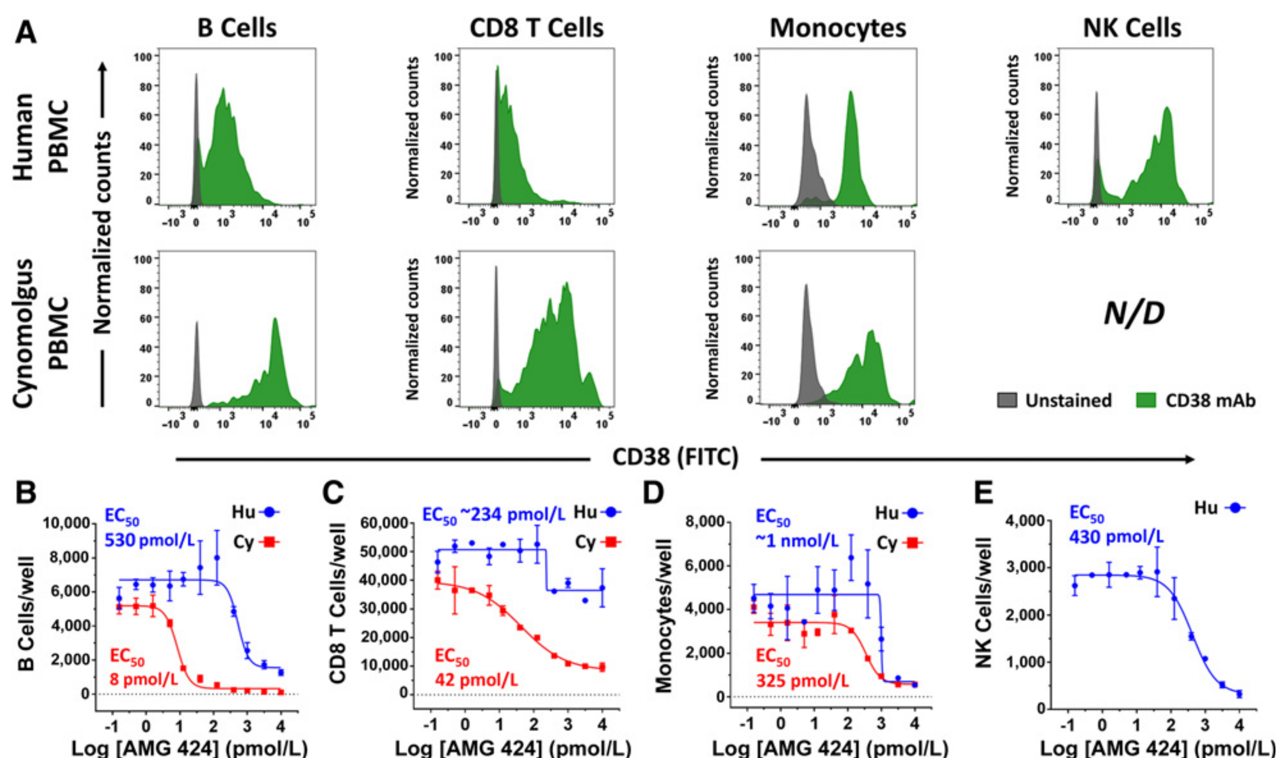

Figure 3.

Relative sensitivity of B cells, T cells, monocytes, and NK cells to AMG 424–mediated cell lysis. Human and cynomolgus monkey PBMCs were stained with a panel of antibodies (CD14, CD4, CD8, CD20, CD16, CD38, and CD56) to separate B cells, CD8 T cells, monocytes, and NK cells (gating strategy in Supplementary Figs. S7A and S8A) and CD38 expression was visualized by plotting the CD38-FITC fluorescence intensity (**A,** green histograms) alongside the same sample lacking the CD38-FITC antibody (**A,** grey histograms). Cynomolgus monkey B cells, CD8 T cells, and monocytes express higher levels of CD38 than their human counterparts. Human NK cells express CD38 and CD56 (Supplementary Fig. S7A) but cynomolgus monkey NK cells couldn't be identified unequivocally through CD56 staining (Supplementary Fig. S8A). Human and cynomolgus monkey PBMCs were then incubated with increasing concentrations of AMG 424 for 48 hours and analyzed by flow cytometry using the same panel of antibodies, alongside a viability dye (eFluor 780) and counting beads (Supplementary Figs. S7 and S8). Absolute counts of human (blue curves) or cynomolgus monkey (red curves) B cells (**B**), CD8 T cells (**C**), monocytes (**D**), and NK cells (**E**) for each concentration of AMG 424 were plotted and the AMG 424 EC_{50} value for each cell population indicated on each graph. Each data point in the plots represents the mean of duplicate measurements; error bars, SE.

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After incubating human or cynomolgus PBMCs with AMG 424 for 48 hours, B cells, CD8 T cells, monocytes, and NK cells were counted by flow cytometry. At 48 hours, AMG 424 caused the near complete autologous depletion of B cells from cynomolgus PBMCs with an EC_{50} more than 65-fold lower than the EC_{50} for human B-cell depletion (Fig. 3B). Thus, B cells are a valuable pharmacodynamic (PD) response marker in cynomolgus monkey, given that tracking plasma cells in cynomolgus blood samples proved technically challenging. In addition, at 48 hours, AMG 424 also caused a decrease in CD8 T cells, monocytes, and NK cells, at substantially higher concentrations.

Higher resting levels of CD38 on cynomolgus monkey T cells compared with human T cells were confirmed in additional donors (Supplementary Fig. S9A and S9B), the likely cause of the differential effect of AMG 424 on human and cynomolgus monkey T cells. At 72 hours, AMG 424 depleted both B and T cells in cynomolgus PBMCs, but selectively targeted B cells in human PBMCs, while causing a 2-fold expansion of the T-cell compartment (Supplementary Fig. S9C and S9D).

Thus, although B-cell depletion in cynomolgus monkey PBMCs occurs at an AMG 424 concentration comparable with the concentration needed for multiple myeloma target cell depletion, the depletion of T cells, monocytes, and NK cells requires significantly higher AMG 424 concentrations.

Depletion of peripheral B cells in cynomolgus monkeys with AMG 424, XmAb-5, and XmAb-7

The PD effect on peripheral B cells and the PK properties of several anti-CD38/CD3 XmAb antibodies were assessed in cynomolgus monkeys. XmAb-4, AMG 424, XmAb-5, and XmAb-7 were administered intravenously (n = 3/group) on days 0, 1, 4, 7, and 10 in a step-wise fashion (Supplementary Table S5). XmAb-4 ($CD3^{Hi}$) was not tolerated beyond the day 1 dose and animals were removed from the study without further treatment. For the remaining XmAb candidates, after an initial decrease and rebound in B-cell counts following the day 1 dose, the number of peripheral B cells decreased steadily on days 7 and 10 and B cells were nearly depleted on day 11 (Fig. 4A). The percentage of T cells expressing the activation marker CD69 rose sharply following the day 1 dose. AMG 424 and XmAb-7, which contained the $CD3^{Med}$ binder, triggered comparable or higher T-cell activation responses, respectively, than XmAb-5, which contained the $CD3^{Lo}$ binder (Fig. 4B). Induction of CD25 on T cells following AMG 424 administration was not informative (Supplementary Fig. S10). *In vivo* cytokine release was assessed 4 hours postdose on day 1. Consistent with prior *in vitro* results and *in vivo* clinical observations, serum samples from cynomolgus monkeys administered XmAb-4 ($CD3^{Hi}$) exhibited markedly higher MCP-1 cytokine levels than animals administered AMG 424, XmAb-7 ($CD3^{Med}$), or XmAb-5 ($CD3^{Lo}$; Fig. 4C). Comparable trends were observed for IFNγ, TNFα, and IL6 (Supplementary Table S6).

In individual animals, AMG 424 caused the pronounced depletion of B cells (Fig. 4D) and T cells (Fig. 4E), assessed by flow cytometry. Monocyte counts, measured by automated peripheral blood smear analysis, revealed a similar level of depletion in response to AMG 424 treatment (Fig. 4F).

The comparison of individual monkey B-cell depletion curves (Fig. 4D) with the serum concentration curves of AMG 424 in the corresponding animals (Fig. 4G) indicates an indirect PK/PD relationship with consistent B-cell depletion being observed (>98% depletion in 2 of 3 animals and 91% in the third animal) with high variability in AMG 424 exposures arguing for activity at subsaturating CD38 occupancy. Throughout the study, the AMG 424 serum concentration remained ~approximately 7-fold above the *in vitro* B-cell depletion EC_{10} concentration (Fig. 4G, dotted lines).

AMG 424 was selected as a therapeutic candidate based on the observations of the near-complete B-cell depletion in cynomolgus monkeys at subsaturating CD38 concentrations, decreased cytokine release compared with high-affinity CD3-containing XmAb candidates, and robust T-cell activation, proliferation, and expansion *in vitro*.

Specific activity of AMG 424 against high and low CD38-expressing cells

In a cell-binding assay, AMG 424 specifically stained CHO-K1 cells transfected with human or cynomolgus monkey CD38 (Supplementary Fig. S11) and triggered the redirected lysis of CHO-K1 cells transfected with human or cynomolgus monkey CD38 by human pan T cells with comparable potency (Supplementary Fig. S12). AMG 424 also triggered the redirected lysis of MOLM-13-luc cancer cells with human or cynomolgus monkey PBMCs with comparable potency (Supplementary Fig. S13).

In redirected lysis assays, AMG 424 triggered the complete lysis of human cancer cell lines with CD38 surface expression ranging from very low (<1,000 ABS/cell in SKM-1 cells), to intermediate (65,500 ABS/cell in NCI-H929 luc cells), to very high (177,000 ABS/cell in RPMI-8226 cells; Fig. 5A), without inducing lysis of the CD38-negative cell line, HEL92.1.7 (Fig. 5B). Thus, AMG 424 induces the specific and complete redirected lysis of cell lines expressing high and low levels of CD38 and with comparable potencies in TDCC assays using human and cynomolgus PBMCs or target cells expressing human and cynomolgus CD38.

AMG 424 inhibits tumor growth in two *in vivo* bone marrow–invasive cancer models

In vivo antitumor growth activity of AMG 424 was tested in the OPM2-luc multiple myeloma xenograft model, an established orthotopic model obtained from the intravenous injection of OPM2-luc cells in NSG mice homing to the mouse bone marrow. OPM2-luc cells express levels of surface CD38 comparable with NCI-H929 cells and are killed *in vitro* by AMG 424 in the presence of human effector cells at an EC_{50} of 276 pmol/L (Supplementary Fig. S14). Six days after injection of OPM2-luc cells, mice were imaged, randomized, and injected with human T cells intraperitoneally. The following day, mice received the first of two AMG 424 doses, which were provided weekly at 0.1, 1, or 10 mg/kg. AMG 424 induced tumor regression at all doses tested (Fig. 5C). In a parallel experiment, serum concentration of AMG 424 in NSG mice 1 week after AMG 424 intravenous dosing at 0.1 and 1 mg/kg was 3.2 and 44.2 nmol/L, respectively (data not shown). Thus, serum concentration remained 10-fold above the OPM2-luc *in vitro* cytotoxic EC_{50} for the entire duration of the experiment at the lowest AMG 424 dose tested. Whole body imaging was performed on mice from the vehicle or the AMG 424 group on day 7, just before AMG 424 dosing and on day 18, demonstrating the localization of OPM2 luciferase signal in the hind-limbs of implanted mice and complete tumor regression upon AMG 424 treatment (Fig. 5D). Femoral bone marrow from 5 mice was analyzed by flow cytometry 4 days after vehicle or AMG 424

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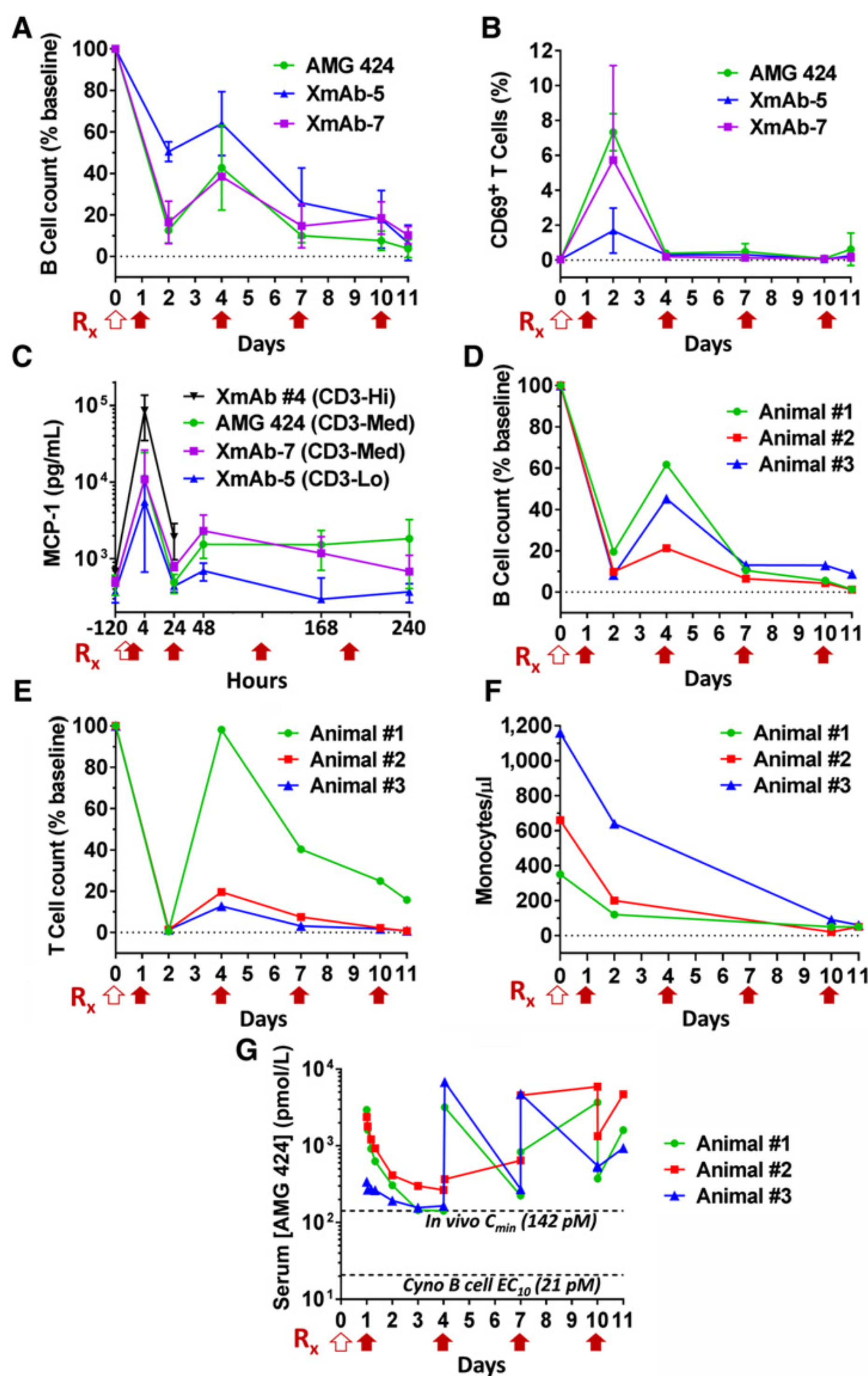

Figure 4.

Depletion of cynomolgus monkey B cells, T cells, and monocytes and activation of T cells and induction of cytokine release *in vivo* with anti-CD38/CD3 XmAb antibodies. **A–C,** In each plot, XmAb administration (Rx) time points are indicated by the open red arrow (first lower dose) and the solid red arrows (repeat higher doses). Pharmacodynamic effects of AMG 424, XmAb-5, and XmAb-7 after bolus intravenous injection to cynomolgus monkeys. Average changes in B-cell counts expressed as % depletion from baseline and the average proportion of activated peripheral T cells were obtained by flow cytometry from blood samples using mAbs against CD20 (**A**) or CD3 and CD69 (**B**). Average serum MCP-1 cytokine levels 120 hours prior to study initiation and at the 4-, 24-, 48-, 168-, and 240-hour time points are displayed on a log scale as a function of time, indicating peak-release at the 4-hour time point and a relationship between higher MCP-1 release and higher CD3 affinity (**C**). Separate PD effects (**D–F**) and PK (**G**) of AMG 424 in the blood of 3 animals. Peripheral B-cell and T-cell counts expressed as % depletion from baseline were obtained by flow cytometry from blood samples using mAbs against CD20 (**D**) or CD3 (**E**). Absolute blood monocyte counts were measured by automated analysis of blood smears (**F**). Serum AMG 424 concentration was measured over time (**G**). The minimal AMG 424 concentration (C_{min}) and the *in vitro* EC_{10} B-cell depletion values are plotted (dotted lines). All data points in the plots represent the mean of triplicate measurements; error bars, SE.

administration, demonstrating that CD8 T cells significantly induced activation markers CD69 and PD1 upon AMG 424 administration (Fig. 5E). The substantial decrease in the total number of CD4 and CD8 bone marrow–infiltrating T cells was also noted (Supplementary Fig. S15A), indicating possible *in vivo* fratricide T-cell activity, but this T-cell depletion is accompanied by the maintenance of the *in vivo* E:T ratio, when T-cell numbers are normalized for tumor BLI, resulting from the near-complete eradication of cancer cells mediated by AMG 424 (Supplementary Fig. S15B).

Antitumor activity of AMG 424 was also confirmed in the orthotopic MOLM-13-luc model, expressing lower levels of CD38. AMG 424 administration resulted in prolonged median survival at all doses tested (37.5, 36, and 37 days, respectively)

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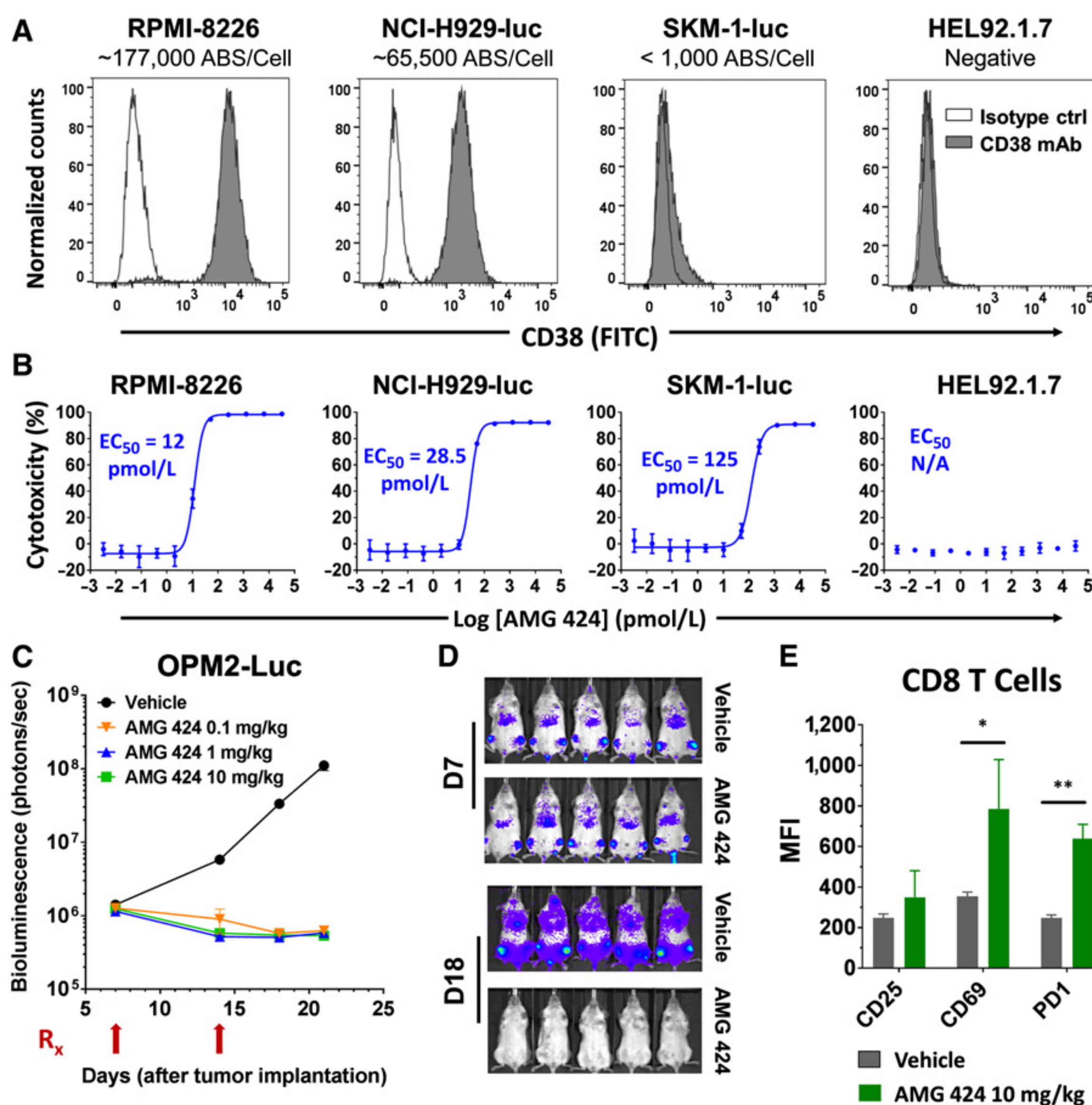

Figure 5.

Specific and complete redirected lysis of target cells expressing high and low levels of CD38 and *in vivo* activity of AMG 424 in the OPM2-luc orthotopic xenograft multiple myeloma model. **A,** Flow cytometry detection of CD38 expression at the surface of cell lines RPMI-8226, NCI-H929-luc, SKM-1-luc, and HEL92.1.7; fluorescence intensity of the FITC channel is plotted for cells stained with a CD38 mAb (gray histograms) or an isotype control mAb (white histograms); the number of ABS per cell using Qifikit are displayed. RPMI-8226 and HEL92.1.7 were CFSE-labeled then cocultured with human T cells, whereas NCI-H929-luc and SKM-1-luc cells were directly cocultured with human T cells, at an E:T cell ratio of 10:1 and increasing concentrations of AMG 424 for 48 hours. Target cell lysis was monitored by luciferase activity for the luciferase-labeled cells or flow cytometry measurement of the viable $CFSE^+$ cell fraction for nonluciferase-labeled cells. **B,** Specific cytotoxicity was plotted for each concentration of AMG 424, indicating complete and specific lysis of CD38+ cells. Each data point represents the mean of triplicate measurements. Error bars, SD. Female NSG mice were injected intravenously on day 0 with 10^6 OPM2-luc cells. On day 6, mice were imaged and randomized into four groups, then injected intraperitoneally with 2×10^7 human T cells ($n = 10$/group). AMG 424 was administered intraperitoneally once weekly on days 7 and 14 at 0.1, 1, and 10 mg/kg doses. **C,** Total body bioluminescence reflecting tumor burden was plotted over time. AMG 424 administration resulted in a profound and statistically significant regression in tumor burden in all three treated groups compared with the vehicle-treated group ($P < 0.0001$). **D,** Representative ventral BLI images were obtained from 5 mice per group on day 7, prior to AMG 424 dosing and on day 18, 4 days after administration of the second dose of AMG 424. **E,** Increases in the fluorescence intensity of the CD25, CD69 and PD1 activation markers detected by flow cytometry at the surface of CD8 T cells sorted from the mouse femoral bone marrow 4 days after the first AMG 424 administration (10 mg/kg) demonstrates a significant T-cell PD response *in vivo* compared with vehicle-treated mice (*, $P < 0.005$; **, $P < 0.0001$).

compared with vehicle-treated mice (22 days, $P < 10^{-4}$; Supplementary Fig. S16A), and BLI imaging demonstrated a significant decrease in tumor burden in the hind-limbs of AMG 424-treated mice (Supplementary Fig. S16B).

Discussion

Despite great progress in recent years, approved therapies for multiple myeloma fail to cure most patients with multiple

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myeloma due to lack of initial clinical response (intrinsic resistance), adaptation of malignant multiple myeloma cells to the therapy (acquired resistance), or persistence of MRD leading to subsequent relapse. Although, in combination, daratumumab greatly improves survival in patients with multiple myeloma (6, 25), a significant proportion of patients fail to respond to the antibody and nearly all patients eventually relapse after treatment (26).

CD3 bispecific antibodies trigger T-cell redirected lysis, a potent mechanism of action clinically proven to eliminate MRD in acute lymphoblastic leukemia (10). CD38/CD3-targeting XmAb molecules present an opportunity to overcome the limitations of current therapies in patients with multiple myeloma by triggering the complete elimination of malignant multiple myeloma cells in patients, including low-expressing cells that may evade daratumumab (21), with the potential to induce profound clinical responses.

Our evaluation of new anti-CD38/CD3 XmAb antibodies indicates that the affinity of the two target arms contributes meaningfully to antibody function and is likely to be critical for clinical tolerability and efficacy.

Attenuated cytokine release with maintenance of target cell killing through CD3 affinity modulation

XmAb-1 (17) and XmAb-4 carrying the $CD3^{Hi}$ binder ($K_D = 1.9$ and 3.7 nmol/L, respectively) triggered excessive cytokine release even before B-cell depletion could be assessed in cynomolgus monkeys. AMG 424, which carries the $CD3^{Med}$ binder ($K_D =$ 15 nmol/L), was selected as a clinical candidate based on evidence of attenuated cytokine release and near-complete target cell depletion *in vivo*. Leong and colleagues (2017) recently compared CLL1/CD3 bispecific antibodies carrying a high-affinity ($K_D = 0.5$ nmol/L) or low-affinity ($K_D = 50$ nmol/L) CD3 binder (27), and observed a decrease in cytokine release without impairment of complete CLL1-expressing target cell killing *in vitro* and *in vivo* by lowering the affinity of the CD3 arm. Our study goes further in demonstrating that additional lowering of the CD3 affinity (e.g., XmAb-5, $K_D = 170$ nmol/L) further reduces maximal cytokine release *in vitro* (Fig. 2B and C). In cynomolgus monkeys, the decrease in T-cell activation (Fig. 4B) and cytokine release (Fig. 4C) did not impair the ability of XmAb-5 to deplete B cells (Fig. 4A). The cytolytic response, in the hierarchy of T-cell functions, is achieved at the lowest threshold of T-cell receptor (TCR) signal strength, not even requiring the formation of a stable synapse between T cells and target cells (28). Cytokine production and proliferation, on the other hand, require a more sustained TCR receptor signal (29) typically achieved by higher affinity peptide/MHC binding (30). We hypothesize that lowering CD3 affinity maintains the cytotoxic potential (lowest threshold response) without engaging TCR signaling sufficiently to induce the higher-threshold responses, resulting in lower cytokine release.

AMG 424 is active against low CD38-expressing cells and triggers antitumor cytokine production

Daratumumab significantly extends the overall survival of patients with multiple myeloma, but most patients relapse after treatment, in part, because MRD negativity is only achieved in a small subset of daratumumab-treated patients even in combination therapy (7.2% and 22.4%, respectively, in the CASTOR (6) and POLLUX (25) Phase III trials).

Within hours of daratumumab infusion, the surface expression of CD38 on multiple myeloma cells decreases by 80%, due to the rapid elimination of high CD38-expressing cells and the transfer of CD38/daratumumab complexes from multiple myeloma cells to immune effector cells by trogocytosis (31). Because this CD38 decrease is observed equally in patients who do and who do not respond to daratumumab, lower CD38 levels alone may not be sufficient to drive daratumumab resistance (31). However, (i) levels of CD38 expression prior to treatment initiation are associated with lower clinical response rates (26), (ii) daratumumab is markedly less potent against cell lines (21) and patient-derived multiple myeloma cells expressing lower levels of CD38 (32), and (iii) daratumumab demonstrates a much lower response rate in non-Hodgkin lymphoma, where CD38 is expressed at lower levels than in multiple myeloma (33), suggesting that low CD38 levels may contribute to daratumumab evasion. Daratumumab may also stimulate CD8 T-cell immunity against multiple myeloma by decreasing the CD38-dependent production of immune-suppressive adenosine (34, 35) and depleting CD38-expressing immune-suppressive cells such as myeloid-derived suppressor cells (MDSC) and regulatory T cells (Treg; 36), suggesting that evasion mechanisms could include yet-uncovered immune suppressive pathways.

Because of a different MOA, AMG 424 has the potential to achieve lasting clinical responses and overcome limitations of other therapeutics by triggering the complete T-cell–redirected lysis of CD38-expressing cells, including those expressing low CD38 levels such as MOLM-13 and SKM1 cells (Fig. 5) and inducing antitumor TH1 cytokines (Fig. 2B and C) that could antagonize the immune-suppressive activity of Tregs (37) and MDSCs (38).

CD38 is expressed on T cells

Because CD38 is also expressed at the surface of T cells (Fig. 3), fratricide T-cell activity could interfere with the activity of AMG 424 by depleting effector cells. In the cynomolgus monkey study, in addition to B cells, T cells were also depleted after AMG 424 dosing (Fig. 4E), and T-cell numbers also decreased when cynomolgus monkey PBMCs were exposed to AMG 424 *in vitro* for 48 hours (Fig. 3C) or 72 hours (Supplementary Fig. S9D). However, resting cynomolgus monkey T cells express much higher levels of CD38 than their human counterparts (Fig. 3A). This cross-species difference in T-cell CD38 expression may explain why in human *in vitro* cell assays, AMG 424 only induces a modest T-cell number decrease at 48 hours (Fig. 3C) and triggers their expansion (Fig. 2D; Supplementary Fig. S9C) and their proliferation (Fig. 2H–K) at 72 or 96 hours. Although the number of bone marrow–infiltrating CD8 T cells decreases noticeably 4 days after AMG 424 treatment in the OPM2-luc xenograft study (Supplementary Fig. S15A), this effect is accompanied by a profound antitumor activity (Fig. 5C and D) and a maintenance of the E:T ratio of CD8 cells, because tumor cells are simultaneously eliminated (Supplementary Fig. S15B). Thus, T-cell fratricide activity does not preclude profound and potent AMG 424 activity *in vitro* and *in vivo* against multiple myeloma cancer cells.

Safety implications of normal CD38 expression for AMG 424

CD38 is also expressed on dendritic cells (39), B cells, monocytes, and NK cells (40) (Fig. 3A). AMG 424 triggers the redirected lysis of these cells *in vitro* (Fig. 3B) and the lysis of lymphocytes and monocytes in cynomolgus monkeys *in vivo* (Fig. 4). Evaluation of

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the safety profile in cynomolgus monkeys indicated that lymphoid/hematopoietic tissues were primarily affected by AMG 424, with all changes being both monitorable and reversible. Elimination of normal immune cells may not occur in human subjects to the same extent as in cynomolgus monkeys whose immune cells express higher CD38 levels than their human counterparts and who do not harbor malignant cells expressing high levels of CD38. In addition, depletion of the immune system is a recognized consequence of cytotoxic chemotherapy that is clinically manageable and is not expected to hinder the clinical development of AMG 424. Even with the lower affinity CD3 binder engineered in AMG 424, cytokine release may occur in patients and this will be managed in the clinic through a variety of strategies, including the use of "step-up" dosing (41) and codosing with dexamethasone and anti-IL6 antibody therapy (42).

CD38 target load in patients with multiple myeloma

The average patients with multiple myeloma at diagnosis carries an average of 2×10^{12} malignant cells (43) that express high levels of CD38 (12). This represents a large CD38 target sink with implications for the PK profile of CD38-binding antibodies, because TMDD causes the rapid clearance of CD38 binders at subsaturating concentrations (18, 19). Daratumumab, for instance, demonstrates a short serum half-life at nonsaturating doses (18) and must be administered at a dose of 16 mg/kg in patients with multiple myeloma to ensure target saturation. Cynomolgus monkey lymphocytes express much higher levels of CD38 than their human counterparts (Fig. 3A), and therefore, model a large CD38 target sink. Although daratumumab is dosed to target saturation in patients with multiple myeloma (18), we observed near-complete B-cell depletion in cynomolgus monkeys with AMG 424 (Fig. 4D) at serum concentrations that were highly variable between animals receiving the same dose of the drug (Fig. 4G). This indicates that target saturation was likely not achieved and may not be required for AMG 424 T-cell–redirected lytic activity *in vivo*, an observation supported by other studies demonstrating that BiTEs exert their activity at receptor occupancy well below 1% (44).

Future immunotherapies for multiple myeloma

Numerous targeted immunotherapies are currently in development for the treatment of multiple myeloma with mechanisms of action that may offer significant advantages over (or complementarity with) available treatments (45). Notable amongst them are chimeric antigen receptor T-cell therapies targeted at the B-cell maturation antigen (BCMA) multiple myeloma antigen that have demonstrated remarkable responses in heavily pretreated relapsed/refractory patients, including high proportions of patients with complete clinical responses accompanied by MRD negativity (46, 47), as well as BCMA-directed T-cell engager therapeutics with promising clinical potential (48–50).

In this study, we have characterized a novel anti-CD38/CD3 bispecific T-cell–recruiting antibody, AMG 424, which aims to combine the relative ease of use of an antibody-based therapy with the potent T-cell–mediated cytotoxicity MOA. AMG 424 was affinity-optimized to decrease cytokine release without compromising complete target cell killing or T-cell proliferation and to maintain its potency in the presence of soluble CD38. AMG 424 demonstrated activity *in vitro* and *in vivo* against high and low CD38-expressing cells and induced a robust *in vivo* B-cell depletion response in cynomolgus monkeys at nonsaturating concentrations, in the face of a large CD38 target sink. Affinity tuning of AMG 424 using the XmAb antibody platform demonstrates that antibody engineering can overcome target-associated challenges to maximize therapeutic index and antitumor activity in the preclinical setting.

Disclosure of Potential Conflicts of Interest

F. Fajardo holds ownership interest (including patents) in Amgen. W. Zhong holds ownership interest (including patents) in Amgen. M.J. Bernett holds ownership interest (including patents) in Xencor, Inc. G.L. Moore holds ownership interest (including patents) in Xencor, Inc. J. Stevens holds ownership interest (including patents) in Amgen. R. Case holds ownership interest (including patents) in Amgen. P.L. McElroy holds ownership interest (including patents) in Amgen. J.R. Desjarlais is an employee of and holds ownership interest (including patents) in Xencor, Inc. A. Coxon holds ownership interest (including patents) in Amgen. O. Nolan-Stevaux holds ownership interest (including patents) in Amgen. No potential conflicts of interest were disclosed by the other authors.

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