ABBV-319: A CD19-targeting glucocorticoid receptor modulator antibody-drug conjugate therapy for B-cell malignancies

Tracking no: BLD-2024-023849R2

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Abstract:
Glucocorticoids are key components of the current standard-of-care regimens (e.g., R-CHOP, EPOCH-R, Hyper-CVAD) for treatment of B-cell malignancy. However, systemic glucocorticoid treatment is associated with several adverse events. CD19 displays restricted expression in normal B-cells and is up-regulated in B-cell malignancies. ABBV-319 is a CD19-targeting antibody-drug conjugate (ADC) engineered to reduce glucocorticoid-associated toxicities while possessing three distinct mechanisms of action (MOA) to increase therapeutic efficacy: (1) antibody-mediated delivery of glucocorticoid receptor modulator (GRM) payload to activate apoptosis, (2) inhibition of CD19 signaling, and (3) enhanced Fc-mediated effector function via afucosylation of the antibody backbone. ABBV-319 elicited potent GRM-driven anti-tumor activity against multiple malignant B-cell lines in vitro as well as in cell line-derived xenografts (CDXs) and patient-derived xenografts (PDXs) in vivo. Remarkably, a single-dose of ABBV-319 induced sustained tumor regression and enhanced anti-tumor activity compared to repeat dosing of systemic prednisolone at the maximum tolerated dose (MTD) in mice. The unconjugated CD19 monoclonal antibody (mAb) also displayed anti-proliferative activity on a subset of B-cell lymphoma cell lines through the inhibition of PI3K signaling. Moreover, afucosylation of the CD19 mAb enhanced Fc-mediated antibody-dependent cellular cytotoxicity (ADCC), and this activity was maintained after conjugation with GRM payloads. Notably, ABBV-319 displayed superior efficacy compared to afucosylated CD19 mAb in human CD34+ PBMC-engrafted NSG-tg(Hu-IL15) transgenic mice, demonstrating enhanced anti-tumor activity when multiple MOAs are enabled. ABBV-319 also showed durable anti-tumor activity across multiple B-cell lymphoma PDX models, including non-germinal center B-cell (GCB) DLBCL and relapsed lymphoma post R-CHOP treatment. Collectively, these data support the ongoing evaluation of ABBV-319 in Phase I clinical trial (NCT05512390).

Preprint server: No;

Author contributions and disclosures: Conception and design: CAC, EE, AD, WZ, TU, JWP Acquisition of data: CAC, AD, CC, DM, EP, AO, PE, LR, CH, LL, WA, PZ, AH, KM, ZZ, GR, ZC, OP, JC Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): CAC, EE, AD, WZ, CC, DM, RD, AH, KM, TU, JWP Writing, review, and/or revision of the manuscript: CAC wrote the manuscript and all authors reviewed the manuscript prior to submission.

Non-author contributions and disclosures: No;

Agreement to Share Publication-Related Data and Data Sharing Statement: The RNA-seq and CITE-seq data are available at GEO under accession number GSE249023 and GSE249543, respectively.

Clinical trial registration information (if any):

1 **Title:** ABBV-319: A CD19-targeting glucocorticoid receptor modulator antibody-drug conjugate
2 therapy for B-cell malignancies
3
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38 The RNA-seq and CITE-seq data are available at GEO under accession number GSE249023 and
39 GSE249543, respectively.
40
41 **Abstract word count:** 250 of maximum 250 words
42 **Word count (Introduction, Methods, Results and Discussion):** 3935 of maximum 4000 words
43 **Figure count**: 7 of maximum 7 figures
44 **References count**: 42 of maximum 100 references

1

45

46   **Data Sharing Statement:** The RNA-seq and CITE-seq data are available at GEO under accession

47   number GSE249023 and GSE249543, respectively.

48   **Key Point**

49   ABBV-319 is a CD19-targeting GRM ADC with three mechanisms that contribute to pronounced

50   anti-tumor activity across B-cell malignancy models.

51

52   **Abstract**

53   Glucocorticoids are key components of the current standard-of-care regimens (*e.g.*, R-CHO**P**,

54   E**P**OCH-R, Hyper-CVA**D**) for treatment of B-cell malignancy. However, systemic glucocorticoid

55   treatment is associated with several adverse events. CD19 displays restricted expression in

56   normal B-cells and is up-regulated in B-cell malignancies. ABBV-319 is a CD19-targeting

57   antibody-drug conjugate (ADC) engineered to reduce glucocorticoid-associated toxicities while

58   possessing three distinct mechanisms of action (MOA) to increase therapeutic efficacy: (1)

59   antibody-mediated delivery of glucocorticoid receptor modulator (GRM) payload to activate

60   apoptosis, (2) inhibition of CD19 signaling, and (3) enhanced Fc-mediated effector function via

61   afucosylation of the antibody backbone. ABBV-319 elicited potent GRM-driven anti-tumor

62   activity against multiple malignant B-cell lines *in vitro* as well as in cell line-derived xenografts

63   (CDXs) and patient-derived xenografts (PDXs) *in vivo*. Remarkably, a single-dose of ABBV-319

64   induced sustained tumor regression and enhanced anti-tumor activity compared to repeat

65   dosing of systemic prednisolone at the maximum tolerated dose (MTD) in mice. The

66   unconjugated CD19 monoclonal antibody (mAb) also displayed anti-proliferative activity on a

67   subset of B-cell lymphoma cell lines through the inhibition of PI3K signaling. Moreover,

68   afucosylation of the CD19 mAb enhanced Fc-mediated antibody-dependent cellular cytotoxicity

69   (ADCC), and this activity was maintained after conjugation with GRM payloads. Notably, ABBV-

3

70   319 displayed superior efficacy compared to afucosylated CD19 mAb in human CD34+ PBMC-

71   engrafted NSG-tg(Hu-IL15) transgenic mice, demonstrating enhanced anti-tumor activity when

72   multiple MOAs are enabled. ABBV-319 also showed durable anti-tumor activity across multiple

73   B-cell lymphoma PDX models, including non-germinal center B-cell (GCB) DLBCL and relapsed

74   lymphoma post R-CHOP treatment. Collectively, these data support the ongoing evaluation of

75   ABBV-319 in Phase I clinical trial (NCT05512390).

76

77   **Introduction**

78   Glucocorticoids exhibit clinical activity across B-cell malignancies including diffuse large B-cell

79   lymphoma (DLBCL), chronic lymphocytic leukemia (CLL), follicular lymphoma (FL) and acute

80   lymphoblastic leukemia (ALL).[6] The anti-tumorigenic effects of glucocorticoids on lymphoma

81   and leukemia were first discovered in clinical studies from the 1950s.[7,8] Glucocorticoids have

82   demonstrated single-agent and combinatorial anti-tumor activity with different

83   chemotherapeutic agents.[8,9] Glucocorticoids are thus incorporated into combination regimens

84   for B-cell malignancies, such as R-CHO**P** (rituximab, cyclophosphamide, doxorubicin, vincristine,

85   prednisone), E**P**OCH-R (etoposide, prednisone, vincristine, cyclophosphamide, doxorubicin, and

86   rituximab), and hyper CVA**D** (cyclophosphamide, vincristine, doxorubicin, dexamethasone,

87   methotrexate, and cytarabine). Mechanistically, glucocorticoids act as an agonist for

88   glucocorticoid receptors (GR) to facilitate activation or repression of downstream transcription

89   targets associated with cell cycle progression (e.g., *MYC, CCND3)* and apoptosis genes (*e.g.,*

90   *BCL2L11*, *BCL2*), which subsequently result in cell cycle arrest and/or apoptosis of malignant B-

91   cells.[11-14] However, the administration of glucocorticoids systemically is associated with a broad

4

92   range of potentially dose-limiting side effects such as hyperglycemia, diabetes mellitus,

93   osteoporosis, or psychosis,[15] and these side effects limit the full therapeutic potential of

94   glucocorticoids.

95

96   CD19 is a well-validated therapeutic target for B-cell malignancy including B-cell non-Hodgkin

97   lymphoma (NHL) and leukemia.[16] In normal development, CD19 expression is restricted to the

98   B-cell lineage and its expression increases with B-cell maturation.[17] CD19 is a co-receptor for the

99   B-cell receptor (BCR) and the phosphorylation of its cytoplasmic domain mediates recruitment

100   of phosphoinositide 3-kinase (PI3K), generation of secondary messenger phosphatidylinositol-3,

101   4,5-triphosphate (PIP3), and the activation of downstream signaling molecules including BTK,

102   PLCγ2, AKT, and mTOR.[18,19] In B-cell lymphoma and leukemia, CD19 is over-expressed and its

103   expression is often maintained in later-lines, even post CD19-targeting therapies.[20-22] Moreover,

104   genetic perturbation studies revealed that CD19 plays a critical role in the survival of Burkitt

105   lymphoma (BL) and germinal center B-cell (GCB) like DLBCL cells.[23] Therefore, CD19 is an

106   attractive therapeutic target for the development of biologics and immunotherapy.[24]

107

108   Here, we describe the preclinical characterization of ABBV-319, a CD19-targeting GRM agonist

109   ADC for the treatment of B-cell malignancy. ABBV-319 elicits anti-tumor activity through three

110   distinct mechanisms of actions (MOAs) that collectively contribute to robust anti-tumor

111   activities across B-cell lymphoma models, including non-GCB DLBCL and PDX samples from

112   relapse/refractory (R/R) lymphoma with poor clinical outcomes.[4,25] ABBV-319 is currently being

113   investigated in a clinical trial (NCT05512390) as a new therapeutic option for B-cell malignancy.

5

114

Methods

Antibodies and drug conjugates

CD19 monoclonal antibody (mAb) was discovered at AbbVie and the afucosylated monoclonal

antibody (mAb) was generated by heterologous expression of GDP-6-deoxy-D-lyxo-4-hexulose

reductase in the Chinese hamster ovary cell line that disrupts the formation of GDP-fucose, and

thus preventing addition of fucose to the mAb.

The drug substance process conjugating the mAb and linker-drug includes a phosphine-based

redox reaction and a hydrophobic interaction chromatography (HIC) purification that results in a

mix of drug-to-antibody ratio (DAR) 2/4/6 distribution with a mean DAR of approximately 4.0,

followed by buffer exchange and formulation.

Compounds and formulation

GRM small molecule was synthesized at AbbVie. Dexamethasone (S1322, Selleck Chemicals) and

prednisolone (P6004, Sigma-Aldrich) were purchased commercially. The small molecules were

dissolved in DMSO for *in vitro* studies and were formulated in 0.05% HPMC, 0.02% Tween-80 in

water for *in vivo* studies. Prednisolone sodium phosphate oral solution (44523-0182-08,

BioComp Pharma) was used for *in vivo* benchmark studies. All antibodies and ADCs were

formulated in appropriate ADC buffer containing lyoprotectant.

Cell lines

6

136   All cell lines were obtained from ATCC or DSMZ and cultured in growth media supplemented

137   with either fetal bovine serum (FBS; F4135, Sigma-Aldrich) or human serum (HS; H4522, Sigma-

138   Aldrich) at 5% CO_2 at 37°C. Growth media for each cell line is described in supplemental Table 1.

139

140   Detailed protocol for over-expression of CD19 and reporter cell line generation and assays are in

141   the supplemental Methods.

142

143   *In vitro cellular screen*

144   Cells were seeded at 1000 cells/well in 384-well tissue culture plates (Corning) in a total volume

145   of 25 µl in their respective culture media. The plates were dosed the following day with GRM

146   payload, dexamethasone, prednisolone, afucosylated (Af.) isotype mAb, Af. CD19 mAb and

147   ABBV-319 at 0.1 µM, 1 µM, 10 µM, 1 µM, 1 µM and 1 µM top doses, respectively, using ECHO

148   Liquid Handler. Each drug was dosed as a 12-point dilution series with 3-fold dilution between

149   successive concentrations and as triplicates on the same plate per dosing session. Cell

150   proliferation following 120 hours of drug treatment was determined using the Cell Titer-Glo

151   Luminescent Cell Viability Assay kit (Promega) as per manufacturer's recommendation. The EC_{50}

152   and % E_{max} for all drugs with all cell lines are relative to Staurosporine using in-house analysis

153   tools. Median EC_{50} and %E_{max} from all repeats were reported.

154

155   *RNA sequencing*

156   RNA was isolated from frozen cell pellets using Qiagen RNAeasy kit (Hilden, Germany) according

157   to manufacturer protocol. Whole-transcriptome sequencing (RNA-sequencing) was performed

7

158   using TruSeq™ Stranded Total RNA With Illumina® Ribo-Zero™ Plus rRNA Depletion kit

159   according to manufacturer protocol (Illumina, Inc. San Diego, California). The analysis workflow

160   is in supplemental Methods.

161

162   *CITE-Seq*

163   Two million peripheral blood mononuclear cells (PBMCs) were treated with vehicle, 66 nM Af.

164   CD19 mAb, and 66 nM ABBV-319 for 24 hours in IMDM (12440053, Thermo Fisher Scientific)

165   supplemented with 10% human serum (H4522, Sigma Aldrich). Viable cells were washed and

166   resuspended in cell staining buffer (420201, BioLegend). Following 100 µm strainer filtration,

167   cells were blocked with Fc block (422302, BioLegend) in cell staining buffer and stained with

168   TotalSeq Universal antibody cocktail (399904, BioLegend). Cells were washed and loaded into

169   the 10x Chromium controller at 16,000 cells per sample. Single cell encapsulation was

170   processed according to manufacturer's protocol using 3' chemistry version 3.1 (10X

171   Genomics). Gene expression libraries were sequenced at a depth of 50,000 reads per cell and

172   the surface protein/antibody tag libraries at a depth of 5,000 usable reads per cell on the

173   NextSeq and NovaSeq sequencer (Illumina). The analysis workflow is in supplemental Methods.

174

175   *Immunoblot*

176   Cells were washed twice with ice-cold PBS and then lysed in RIPA lysis buffer (R0278, Sigma-

177   Aldrich) supplemented with 1x Halt™ Protease and Phosphatase Inhibitor Cocktail (78446,

178   Thermo Fisher Scientific). The protein concentrations of the lysates were measured with BCA

179   Protein Assay Kit (23227, Thermo Fisher Scientific). Equal amounts of lysates (1-10 µg) were

8

180   resolved on 4-12% gradient gels (NW04120BOX, Thermo Fisher Scientific) and transferred onto

181   a PVDF membrane (IB24001, Thermo Fisher Scientific). The membranes were incubated with

182   primary antibodies and secondary antibody conjugated with horseradish peroxidase (HRP).

183   Enhanced chemiluminescent substrates (ECL, Thermo Fisher Scientific) were added and

184   detected with Azure Image Systems C600. Details of immunoblot detection and antibodies used

185   are in supplemental Methods.

186

187   *Glucocorticoid Response Element (GRE) reporter activation assay*

188   50,000 GRE luciferase reporter cells were treated with dose-titrated drugs in growth media

189   containing 1% charcoal stripped FBS (12676-029, Thermo Fisher Scientific) until indicated

190   endpoint. Dual-Glo Luciferase Assay System (E2920, Promega) substrate and buffer were added

191   for 10 minutes and analyzed for luminescence using the MicroBeta (PerkinElmer).

192

193   *In vitro antibody-dependent cellular cytotoxicity (ADCC) assays*

194   The ADCC Reporter Bioassay (G7010 and G9790, Promega) were performed according to the

195   manufacturer's protocol. The target lymphoma cells were labeled with CFSE (C34554, Thermo

196   Fisher Scientific) and then opsonized with antibody or ADC. PBMCs from healthy donors were

197   added at indicated effector to target (E:T) ratios. After 4-hour incubation, cells were stained with

198   Live/Dead Fixable Violet Dead Cell Stain Kit (L34955, Thermo Fisher Scientific) and fixed with 4%

199   paraformaldehyde. The fixed cells were analyzed on Stratedigm S1000EON flow cytometer. The

200   % specific lysis was calculated by subtracting the percentage of dead target cells (% Violet dye+

9

201 in CFSE+ cells) in each treated condition with the untreated control containing only effector and

202 target cells.

203

204 *In vivo efficacy study*

205 All experiments were conducted in compliance with AbbVie's Institutional Animal Care and Use

206 Committee and the National Institutes of Health Guide for Care and Use of Laboratory Animals

207 guidelines. CDX studies were conducted in-house whereas DLBCL PDX studies were conducted

208 at WuXi AppTec (Suzhou, China). Cell lines were inoculated into the flank of female CB17 SCID or

209 SCID beige mice 6 to 8 weeks of age (Charles River Laboratories) or CD34+ PBMC-engrafted NSG-

210 tg(hu-IL15) mice at 18 to 21 weeks of age (The Jackson Laboratory) with 1:1 mixture of S-MEM

211 or HBSS (Fisher Scientific, MA) and Matrigel (BD, Franklin Lakes, NJ). DLBCL PDX tumors (30 mm^3

212 tumor slices) were inoculated into the flank of 6- to 8-week-old NOD SCID mice. The DLBCL

213 tumor subtypes (GCB or non-GCB) were determined by immunohistochemical staining for CD10,

214 BCL6 and MUM1.[25] Tumors were size matched at approximately 80 to 200 mm^3, and the small

215 molecules and biologics (antibody and ADC) were dosed via oral (PO) and intraperitoneal (IP)

216 route of administration, respectively. Tail vein bleeds were taken for PK analysis (supplemental

217 Methods). Measurements of length (L) and width (W) of the tumor were obtained via electronic

218 calipers and volume was calculated according to the following equation: $V = (L \times W^2)/2$. Mice

219 were euthanized when tumor volume reached a maximum of 2,000 mm^3 or if animal health was

220 compromised, per institutional guidelines.

221

10

222   % Tumor growth inhibition (%TGI), % tumor volume change and % tumor growth delay (%TGD)

223   were determined with the equations below:

224    $\Delta\%\text{TGI max} = 1 - \left(\frac{Volume\ of\ treated\ group\ on\ day\ X - Volume\ of\ treated\ group\ at\ randomization}{Volume\ of\ control\ group\ on\ day\ X - Volume\ of\ control\ group\ at\ randomization}\right) * 100$

225

226   Δ %TGI max was determined when the difference between treatment and control groups were

227   maximal.

228

229    $\%\text{ Tumor volume change} = \left(\frac{Volume\ of\ treated\ group\ on\ day\ X - Volume\ of\ treated\ group\ at\ randomization}{Volume\ of\ treated\ group\ at\ randomization}\right) * 100$

230

231   Day X is when vehicle-treated tumors reached 1000 mm^3.

232

233    $\%\text{ TGD} = \left(\frac{TTE_t - TTE_c}{TTE_c}\right) * 100$

234   TTE_t and TTE_c are median time periods of treated and control groups to reach tumor volumes of

235   (1 cm^3) following onset of treatment.

236

237   All experiments were conducted in compliance with AbbVie's Institutional Animal Care and Use

238   Committee and the National Institutes of Health Guide for Care and Use of Laboratory Animals

239   guidelines.

240

241   **Results**

242   *Generation and characterization of ABBV-319*

11

243 Transcriptomic analysis showed that *CD19* expression is restricted to a few normal tissues (e.g.,

244 spleen, blood) whereas *NR3C1,* gene encoding GR, is expressed ubiquitously (Figure 1A). In the

245 malignant settings, *CD19* is predominantly expressed in B-cell malignancies such as FL, DLBCL,

246 CLL and Mantle Cell lymphoma (MCL) (Figure 1B), while *NR3C1* is also expressed across B-cell

247 malignancies. Importantly, both *CD19* and *NR3C1* expressions are maintained in DLBCL patients

248 following R-CHOP treatment (Figure 1C).

249

250 ABBV-319 comprises a GRM payload conjugated to an afucosylated CD19 antibody via an

251 alanine-alanine protease cleavable dipeptide linker (Figure 1D). The GRM payload was more

252 potent compared to clinical glucocorticoids (dexamethasone and prednisolone) in

253 glucocorticoid-response element (GRE) reporter and *in vitro* cell proliferation assays in a panel

254 of glucocorticoid-sensitive malignant B-cell cell lines (Figure 1E-F). By comparing the median

255 EC_{50}, the GRM payload was 25 and >300 times more potent compared to dexamethasone and

256 prednisolone, respectively.

257

258 Af. CD19 mAb and ABBV-319 were cross-reactive to both human and cynomolgus monkey CD19

259 (supplemental Figure 1A-E). Twenty-four hour treatment of ABBV-319 on KARPAS422 cells

260 resulted in CD19 internalization and lysosomal trafficking, as shown by the colocalization of

261 CD19 and Lysotracker (Figure 1G). Furthermore, there was dose-dependent activation of GRE

262 reporter activity after ABBV-319 treatment on K562 cells (Figure 1H; supplemental Figure 1D-E),

263 which confirmed the subsequent release of GRM payload in the lysosome followed by induction

264 of GR transcription. ABBV-319 treatment elicited dose-dependent cytotoxicity that was

12

265 dependent on CD19, as the isotype-GRM ADC showed a significant reduction in activity in SU-

266 DHL-6 (Figure 1I). In the screen of a panel of B-cell malignant cell lines, ABBV-319 showed

267 potent anti-proliferative activity in cell lines across a range of indications including DLBCL, MCL,

268 FL, and ALL (Figure 1J; supplemental Table 2). There was not a significant association between

269 ABBV-319 sensitivity and expression of CD19 or GR (supplemental Figure 2 A-D). B-cell

270 lymphoma with *MYC* and *BCL2* and/or *BCL6* rearrangement are known as double-hit lymphoma

271 (DHL) or triple-hit lymphoma (THL), respectively.[26] The DHL and THL are considered as high-

272 grade B-cell lymphoma with inferior survival outcomes when treated with R-CHOP.[26-28] Notably,

273 several DHL cell lines (DB, OCI-LY19, DoHH-2 and OCI-LY18) with *MYC* plus *BCL2* or *BCL6*

274 rearrangements are still responsive to ABBV-319 (Figure 1J).

275

276 *ABBV-319 engaged and activated GR in DLBCL cell lines*

277 We carried out pharmacodynamic analysis to evaluate the ability of GRM payload to engage

278 endogenous GR in malignant B-cells. GR is phosphorylated on serine 211 (S211) in response to

279 glucocorticoid treatment and this phosphorylation event is required for GR transcriptional

280 activity.[29,30] Both GRM payload and ABBV-319 treatment elicited a marked increase in S211

281 phosphorylation on GR in Farage, SU-DHL-6 and OCI-LY19 (Figure 2A-C).

282

283 We also performed RNA-seq analysis to characterize the transcriptomic changes in GRM-

284 sensitive cell lines (OCI-LY19, DoHH2, Farage, Pfeiffer, SU-DHL6, OCI-LY3, TMD-8, U-2932)

285 following ABBV-319 treatment. Compared to the vehicle control, ABBV-319 treatment elevated

286 the expression of several reported GR targets (e.g., *TSC22D3, DDIT4, FKBP5* and *KLF9*) (Figure

13

287 2D). Moreover, the meta-analysis of the differential expressed genes between ABBV-319

288 treatment versus vehicle control revealed enrichment of gene sets related to

289 steroid/corticosteroid response (Figure 2E).

290

291 To investigate the specificity of ABBV-319, we carried out CITE-seq experiments by treating

292 PMBCs with vehicle, Af. CD19 mAb and ABBV-319. We used a published 8-gene glucocorticoid

293 gene signature[31] to evaluate the pharmacodynamic effects of ABBV-319 on PBMC. ABBV-319

294 treatment for 24 hours resulted in the most prominent activation of glucocorticoid gene

295 signatures in the B-cell population (Figure 2F-G). Other immune subsets (T, NK, Monocytes)

296 displayed minimal glucocorticoid gene signature activation as compared to the B-cells.

297 Collectively, our data demonstrated that ABBV-319 can specifically deliver GRM payload to

298 CD19+ B-cells and release GRM payloads to activate GR transcriptional activity.

299

300 *ABBV-319 inhibited pro-survival signaling and induces apoptotic cell death*

301 In our *in vitro* screen, some B-cell NHL cell lines (OCI-LY2, TMD-8, Jeko-1, JVM-2, and SU-DHL-6)

302 were sensitive to the unconjugated CD19 mAb (Af. CD19 mAb) treatment (Figure 3A;

303 supplemental Table 2). We hypothesize that Af. CD19 mAb could block BCR-mediated PI3K

304 activation. Indeed, Af. CD19 mAb pre-treatment blunted anti-IgM-stimulated phosphorylation

305 on AKT (S473) (Figure 3B), suggesting that the unconjugated CD19 mAb can block BCR-mediated

306 PI3K pro-survival signaling. Notably, Af. CD19 mAb-mediated signaling effects were not observed

307 in non-responsive cell line SU-DHL-4 (supplemental Figure 3A).

308

14

309   In the meta-analysis of the RNA-seq data, we found that ABBV-319 elevated expression of genes

310   involved in apoptosis such as *BCL2L11*, *BMF*, and *TXNIP* (Figure 3C). *BCL2L11* encodes BIM and

311   has previously been shown to be involved in glucocorticoid-induced apoptosis.[13] Consistent with

312   published reports, BIM expression is up-regulated by both GRM and ABBV-319 treatment in

313   Farage, SU-DHL-6 and OCI-LY19 (Figure 3 D-F). Notably, GRM and ABBV-319 treatment led to an

314   increase in all three BIM splice isoforms (BIM_{EL}, BIM_L, BIM_S) that have been shown to be

315   involved in apoptosis.[32] The increase in BIM expression also correlated with the increase in

316   cleaved caspase 3, cleaved PARP, and sub-G1 population (dead cells) from the Western blot and

317   flow cytometric analysis (Figure 3D-I). Importantly, these GRM- and ABBV-319 driven apoptotic

318   and cytotoxic effects are specific to the responsive, but not resistant, cell lines (supplemental

319   Figure 3B-C).

320

321   *ABBV-319 elicited potent and durable anti-tumor activity in vivo*

322   In multiple DLBCL and ALL CDX, a single-dose of ABBV-319 induced dose-dependent tumor

323   regression and durable tumor control (Figure 4A-C, E; supplemental Figure 4A-C). In the RS4;11

324   tumor model where tumors were allowed to grow to large sizes (>600mm^3), a single-dose of

325   ABBV-319 at 10 mg/kg resulted in durable tumor regression >40 days (Figure 4D), suggesting

326   that ABBV-319 is efficacious in settings that are typically difficult to treat. Remarkably, the anti-

327   tumor activity of a single-dose of ABBV-319 is superior compared to multiple doses (n=15) of

328   prednisolone at 50 mg/kg (the MTD) or multiple doses of GRM (n=15) at 10 mg/kg (Figure 4A-B,

329   4E; supplemental Figure 4C).

330

15

331   The isotype-GRM ADC, the non-targeting control, displayed marginal anti-tumor activity at 10

332   mg/kg, but to a much lesser extent than the anti-tumor activity observed with ABBV-319 dosed

333   at 10 mg/kg, suggesting a CD19-dependent delivery of GRM to the tumor (Figure 4E;

334   supplemental Figure 4C). Hematological tumor models show higher levels of Fc receptors and

335   thus could potentially result in non-specific uptake of isotype-GRM ADC.

336

337   There was a dose-dependent increase in total antibody concentration and area under the curve,

338   suggesting that ABBV-319 exhibits linear pharmacokinetics in mice (Figure 4F; supplemental

339   Figure 4D). Importantly, the increase in total antibody serum concentration correlated with the

340   anti-tumor activities of ABBV-319. Moreover, the tumor control in OCI-LY19 tumors correlated

341   with the induction of selected GR targets including *FKBP5*, *TSC22D3*, and *ZBTB16* (Figure 4G-H),

342   demonstrating on-target pharmacodynamic effects of GRM *in vivo.*

343

344   ABBV-319 was also tested in DLBCL PDXs to examine its activity in clinically relevant B-cell NHL

345   models. A single-dose of ABBV-319 at 10 mg/kg elicited tumor regression in PDXs that were

346   treatment-naïve or relapsed after 4-7 rounds of R-CHOP treatments (Figure 5A-F). In the PDX

347   study, ABBV-319 elicited tumor growth inhibition compared to the vehicle control in 10/10 PDXs

348   and regression in 9/10 PDXs (Figure 5E). Robust tumor regression induced by ABBV-319 was

349   observed in 7/10 PDXs when the vehicle-treated tumors reached 1000 mm^3 (Figure 5F). Similar

350   to our *in vitro* cell line observations, Af. CD19 mAb treatment resulted in tumor growth

351   inhibition in 1/10 DLBCL PDX (Figure 5C).

352

ABBV-319 induced antibody-dependent cellular cytotoxicity in vitro and in vivo

Therapeutic antibodies can elicit anti-tumor activity via Fc-mediated effector functions such as ADCC, antibody-dependent cellular phagocytosis (ADCP), and complement-dependent cytotoxicity (CDC)[33]. We first assessed the ability of ABBV-319 to engage ADCP and CDC *in vitro*. The treatment with unconjugated Af. CD19 mAb and ABBV-319 resulted in an increase in macrophage-mediated ADCP in Raji and NU-DHL-1 cells (supplemental Figure 5A-C). Consistent with literature report[34], rituximab induced CDC in response to the addition of complements in Raji, Ramos and SU-DHL-6 (supplemental Figure 5D-F). However, neither Af. CD19 mAb nor ABBV-319 showed CDC activity in these experiments. These data therefore demonstrated that ABBV-319 can engage ADCP but not CDC *in vitro*.

The antibody backbone of ABBV-319 was afucosylated to increase Fc-mediated effector function. Af. CD19 mAb and ABBV-319 induced higher specific lysis of tumor cells compared to their fucosylated counterparts when co-cultured with PBMC (Figure 6A). ABBV-319, and Af. CD19 mAb, also activated NFAT reporter activity with similar potency in Jurkat reporter cells expressing V158 (high affinity) and F158 (low affinity) FcγRIIIa (Figure 6B-C). Moreover, ABBV-319 elicited potent specific lysis of B-cell lymphoma (ALL, BL and DLBCL) cell lines in co-cultured with PBMC (Figure 6D-F). Notably, FcγRIIIa binding and ADCC activity of ABBV-319 were comparable to the Af. CD19 mAb, indicating that conjugation of GRM payloads at DAR4 did not negatively impact ADCC activity.

17

374 Due to the differences in human and mouse FcγR network, humanized mouse models have

375 been developed to better model human NK cell biology[35-37]. In particular, NSG-Tg(Hu-IL15) is a

376 humanized NSG mice that is engineered to express human interleukin 15 (IL-15) at physiological

377 levels, which enables differentiation and development of functional human NK cells after CD34+

378 hematopoietic stem cell (HSC) engraftment[37]. A single-dose of ABBV-319 at 5 mg/kg elicited

379 deeper tumor growth inhibition and more durable anti-tumor activity in CD34+ PBMC engrafted

380 NSG-Tg(hu-IL15) compared to CB17 SCID mice (Figure 6G). The anti-tumor effects of ABBV-319

381 were dose-dependent and durable tumor regression were observed at the 5 mg/kg dose in OCI-

382 LY19 CDX in NSG-Tg(hu-IL15) (Figure 6H). Notably, flow cytometric immunophenotyping human

383 cells in the periphery showed that ABBV-319 specifically depleted normal human B- but not NK-

384 or T- cells (Figure 6I). The depletion of normal human B-cells is transient, and the peripheral B-

385 cell counts rebound over time. ABBV-319 also elicited superior anti-tumor efficacy compared to

386 Af. CD19 mAb in Raji CDX model in NSG-Tg(hu-IL15) when both agents were dosed at 5 mg/kg

387 (Figure 6J). Collectively, these data demonstrate robust ADCC activity of ABBV-319 and support

388 additive, or synergistic, anti-tumor activities from the combination of different MOAs.

389

390 **Discussion**

391 This is the first study demonstrating the therapeutic potential of a GRM ADC in oncology. Our

392 study demonstrated that ABBV-319 consists of three distinct MOAs that drive anti-tumor

393 activity in B-cell malignancy (Figure 7): (1) delivery of GRM payload via CD19 to activate

394 apoptotic cell death, (2) CD19 downstream signaling inhibition, and (3) enhanced ADCC from

395 afucosylation of the antibody backbone.

18

396

397   The GRM-mediated anti-tumor activity of ABBV-319 was demonstrated *in vitro* and in immune-

398   compromised mice, in which Af. CD19 mAb showed modest efficacy. This is further supported

399   by the pharmacodynamic analysis showing GR engagement and target gene induction following

400   ABBV-319 treatment. Remarkably, a single-dose of ABBV-319 imparts superior anti-tumor

401   efficacy compared to repeat daily dosing of prednisolone at its MTD *in vivo* (Figure 4A-B, 4E;

402   supplemental Figure 4C). The striking improvement in efficacy is likely attributed to the targeted

403   delivery of a potency-enhanced GRM payload to the CD19+ malignant cells, as well as the PK

404   and safety benefits of improved ADC exposure and lower systemic GRM payload levels relative

405   to the small molecule prednisolone. There was not a significant correlation between ABBV-319

406   sensitivity versus CD19 or GR expression *in vitro.* There are conflicting reports on the correlation

407   of CD19 ADC sensitivity to antigen expression, which may be influenced by the affinity of CD19

408   antibody, potency of the payload and uptake/processing of the ADC.[38,39] ABBV-319 consists of

409   multiple MOAs that are difficult to model simultaneously *in vitro* and in mouse model systems.

410   Thus, future studies are needed to further define predictive biomarkers for ABBV-319 using

411   clinical datasets.

412

413   A recent functional genomic study reported CD19 is essential for the survival of BL and GCB

414   DLBCL cell lines.[23] In part, this essentiality is driven by BCR-mediated phosphorylation of the

415   YXXM motif on CD19 which can result in recruitment and activation of pro-survival PI3K

416   signaling.[19] Our data demonstrated that Af. CD19 mAb is capable of blocking BCR-mediated PI3K

417   activation (Figure 3B). However, the anti-proliferative activity evoked by Af. CD19 mAb was not

19

418   restricted to BL or GCB DLBCL as shown in the functional genomic studies.[23] It is conceivable

419   that inhibitory effects of the mAb could be partial compared to complete deletion of the target.

420   Future studies are needed to better understand biomarkers that would predict response to the

421   CD19 antibody-mediated anti-tumor mechanism within ABBV-319.

422

423   Glucocorticoids combine well with chemotherapy and therapeutic antibodies because of their

424   synergistic efficacy and manageable toxicity.[6] Glucocorticoids are part of the standard-of-care

425   chemo-immunotherapy regimens (i.e., R-CHOP, hyper CVAD, or EPOCH-R) and it is anticipated

426   that ABBV-319 could be a viable combination partner in B-cell malignancies. ABBV-319 showed

427   superior anti-tumor activity compared to the unconjugated Af. CD19 mAb in the immune-

428   competent NSG-tg(hu-IL15) model (Figure 6J), demonstrating that different MOAs of ABBV-319

429   (GRM payload activity, CD19 inhibition, and ADCC) combine optimally to enhance its anti-tumor

430   activity *in vivo*. This is likely due to the ability of GRM payload to induce apoptosis, as

431   mitochondrial apoptosis has been shown to be associated with NK-mediated killing of tumor

432   cells.[40] Contrary to published reports with systemic glucocorticoids,[41-43] the total NK cell

433   numbers were not impacted by ABBV-319 treatment in the NSG-tg(hu-IL15) model,

434   demonstrating that ABBV-319 treatment did not negatively impact NK cell health.

435

436   ABBV-319 displayed remarkable efficacy in clinically relevant B-cell malignancy models. ABBV-

437   319 treatment led to tumor regression and durable tumor control in both non-GCB and GCB

438   DLBCL PDXs, with all models showing sensitivity to the ADC (Figure 5E-F). Non-GCB DLBCL

439   represents a more aggressive disease as it has worse 5-year overall survival compared to GCB

20

440 DLBCL.[25] Furthermore, PDXs with relapsed disease after R-CHOP treatment were still responsive

441 to ABBV-319, suggesting a potential role of ABBV-319 in the R/R setting. Collectively, these

442 positive preclinical data support the ongoing evaluation of ABBV-319's safety, tolerability, and

443 preliminary activity in Phase I clinical trial (NCT05512390).

444

445 **Acknowledgements**

446 We would like to thank Mandy Meng-Shan Wu of AbbVie for their analysis. No further funding

447 to disclose.

448

449 **Author Contributions**

450 **Conception and design:** CAC, EE, AD, WZ, TU, JWP

451 **Acquisition of data:** CAC, AD, CC, DM, EP, AO, PE, LR, CH, LL, WA, PZ, AH, KM, ZZ, GR, ZC, OP, JC

452 **Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational**

453 **analysis):** CAC, EE, AD, WZ, CC, DM, RD, AH, KM, TU, JWP

454 **Writing, review, and/or revision of the manuscript:** CAC wrote the manuscript and all authors

455 reviewed the manuscript prior to submission.

456

457 **Disclosure of Conflicts of Interest**

458 CAC, EE, ALD, WZ, CC, KP, DM, EP, AO, PE, LR, RD, CH, LL, WA, PZ, WL, AHJ, KM, ZZ, GR, ZC, YL, JC,

459 GZ, TC, MB, CCM, AH, MM, TU, MAP, XZ, JH, MM, KF, SML, and JWP are employees of AbbVie.

460 OP and AB were employees of AbbVie at the time of the study. The design, study conduct, and

461   financial support for this research were provided by AbbVie. AbbVie participated in the

462   interpretation of data, review, and approval of the publication.

463

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557

558   **Figure Legends**

559   **Figure 1. Characterization of ABBV-319.** (A) Analysis of *CD19* and *NR3C1* gene expression in
560   normal tissues using GTEx datasets. (B) Analysis of *CD19* and *NR3C1* gene expression across
561   different cancer indications using Aster (ORIEN) datasets. (C) Analysis of *CD19* and *NR3C1* gene
562   expression in patients that are treatment-naive or post-R-CHOP treatment using Aster (ORIEN)
563   datasets. Statistical analysis with Wilcoxon's test. ns, not significant. (D) Structure of the
564   glucocorticoid receptor modulator (GRM) linker drug. (E) The fold change in glucocorticoid
565   response element (GRE) activity compared to the untreated control after treatment of K562
566   GRE reporter cells with prednisolone, dexamethasone and GRM payload for 24 hours. Mean ±
567   SEM are depicted. (F) Summary of EC_{50} for prednisolone, dexamethasone and GRM payload
568   across 20 glucocorticoid-sensitive cell lines. Each dot represents log(EC50) of a cell line and the
569   median log(EC_{50}) is displayed. (G) Imaging analysis of CD19 localization after treatment of
570   KARPAS422 with an Alexa Fluor 647-labeled ABBV-319 for the indicated time. Brightfield,
571   LysoTracker (green), CD19 (red), and merged images are displayed. (H) The fold change in GRE
572   activity relative to the untreated control after treating K562-GRE reporter cells with ABBV-319
573   for 24 hours. (I) % viability of SU-DHL-6 cells relative to the untreated control after treatment
574   with GRM payload, isotype-GRM ADC, and ABBV-319 for 5 days. (J) EC_{50} of ABBV-319 across a
575   panel of malignant B-cell lines with a range of E_{max} from supplemental Table 2. # denotes
576   double-hit lymphoma (DHL) and * denotes triple-hit lymphoma (THL) based on published
577   annotations.[44]
578
579   **Figure 2. ABBV-319 engages and activates GR in DLBCL cell lines.** (A-C) Immunoblot analysis of
580   GR phosphorylation on Serine 211 and GR expression after treatment with 10 nM GRM and 100
581   nM ABBV-319 for indicated time in Farage (A), SU-DHL-6 (B), and OCI-LY19 (C). β-actin was used
582   as the loading control. (D) Volcano plot showing the fold change and p-value from the meta-
583   analysis of differential expressed genes (DEGs) between 24-hour ABBV-319 treatment and
584   vehicle control in GRM-sensitive cell lines. Each dot represents a DEG and selected known GR
585   targets are highlighted in red. (E) Pathways and genes enriched in the meta-analysis of the
586   DEGs between ABBV-319 and vehicle treatment. The color represents directionality of fold
587   change, and the size of circle represents log(p-value). (F) Heatmap showing the expression of
588   the 8-gene glucocorticoid gene signature in different immune subsets in PMBC after 24-hour of
589   indicated treatment. (G) UMAP projection of the immune cells within PBMC after indicated
590   treatment for 24 hours. The color indicates the expression of the 8-gene glucocorticoid gene
591   signature.
592
593   **Figure 3. ABBV-319 inhibits pro-survival signaling and induces apoptotic cell death in DLBCL.**
594   (A) The % viability relative to the untreated control after the treatment of SU-DHL-6 cells with
595   afucosylated (Af.) isotype mAb and Af. CD19 mAb. Mean ± SEM are displayed. (B) SU-DHL-6
596   cells were pre-treated with 100 nM Af. Isotype mAb or Af. CD19 mAb for an hour and then
597   stimulated with 1 µg/ml anti-IgM for indicated time. Cell lysates were resolved on SDS-PAGE
598   and immunoblot analysis for phospho-AKT (Ser473) and GAPDH are displayed. GAPDH is used
599   as the loading control. (C) Volcano plot showing the fold change and p-value from the meta-
600   analysis of differential expressed genes (DEGs) between 24-hour ABBV-319 treatment and

25

601 vehicle control in ABBV-319-sensitive cell lines. Each dot represents a DEG and genes involved
602 in apoptosis are highlighted in red. (D-F) Immunoblot analysis of BIM, caspase 3, PARP and
603 GAPDH after treating Farage (D), SU-DHL-6 (E) and OCI-LY19 (F) with 10 nM GRM payload and
604 100 nM ABBV-319 for indicated time. The arrows show the cleaved product of caspase 3 and
605 PARP. (G-I) Cell cycle analysis of Farage (G), SU-DHL-6 (H) and OCI-LY19 (I) after treatment with
606 10 nM GRM and 100 nM ABBV-319 for indicated time. The % of cells from sub-G1, G0-G1, S and
607 G2-M phases of cell cycle are displayed.
608
609 **Figure 4. ABBV-319 elicits potent and durable anti-tumor activity in cell line-derived xenograft**
610 **(CDX) models.** (A-E) Growth of xenografted KARPAS422 (A), DB (B), RS4;11 (C-D), OCI-LY19 (E)
611 tumors after the indicated treatment regimen. Drug treatments were initiated within 24-hour
612 after tumor size matching and randomization (A-C, E) whereas the large RS4;11 tumor (D) was
613 dosed at day 43 after inoculation. QD denotes once daily, and SD denotes single dose. Means ±
614 SEM of tumor volumes were plotted for each treatment group versus days from randomization
615 or days post inoculation. (F) Total antibody detected in mouse whole blood from the OCI-LY19
616 study (E). Means ± SEM are shown. (G) The volume of xenografted OCI-LY19 tumors after
617 indicated treatment for 7 days. GRM was dosed at (QDx5)x3 whereas ABBV-319 was dosed SD.
618 Means ± SEM of tumor volumes were plotted for each treatment group. QD denotes once daily,
619 and SD denotes single dose. (H) RT-qPCR analysis of *FKBP5, TSC22D3* and *ZBTB16* expression in
620 tumors after indicated treatments. Means ± SEM of fold change relative to vehicle control are
621 displayed.
622
623 **Figure 5. ABBV-319 exhibits anti-tumor activity in non-GCB DLBCL and relapsed DLBCL**
624 **patient-derived xenograft (PDX) models.** (A-D) The growth of xenografted PDX models 0395
625 (A), 0262 (B), 0207 (C), and 0016 (D) in NOD-SCID mice after indicated treatment regimen.
626 Means ± SEM of tumor volumes were plotted for each treatment group versus days from
627 randomization. SD denotes single dose. (A) and 0262 (B) were treatment naïve whereas PDX
628 models 0207 (C) and 0016 (D) were from relapsed disease following 4 R-CHOP treatments. GCB
629 and non-GCB subtyping were determined via IHC methods as described in the Methods. (E)
630 Maximal % tumor growth inhibition relative to vehicle control in each PDX model is displayed.
631 Models showing tumor regression after ABBV-319 treatment are shown on the figure. (F) %
632 tumor volume changes relative to the starting tumor volume when the vehicle control reaches
633 1000 mm^3. GCB and non-GCB DLBCL are shown as different colors. * denotes PDX samples from
634 patients with relapse disease after R-CHOP treatment.
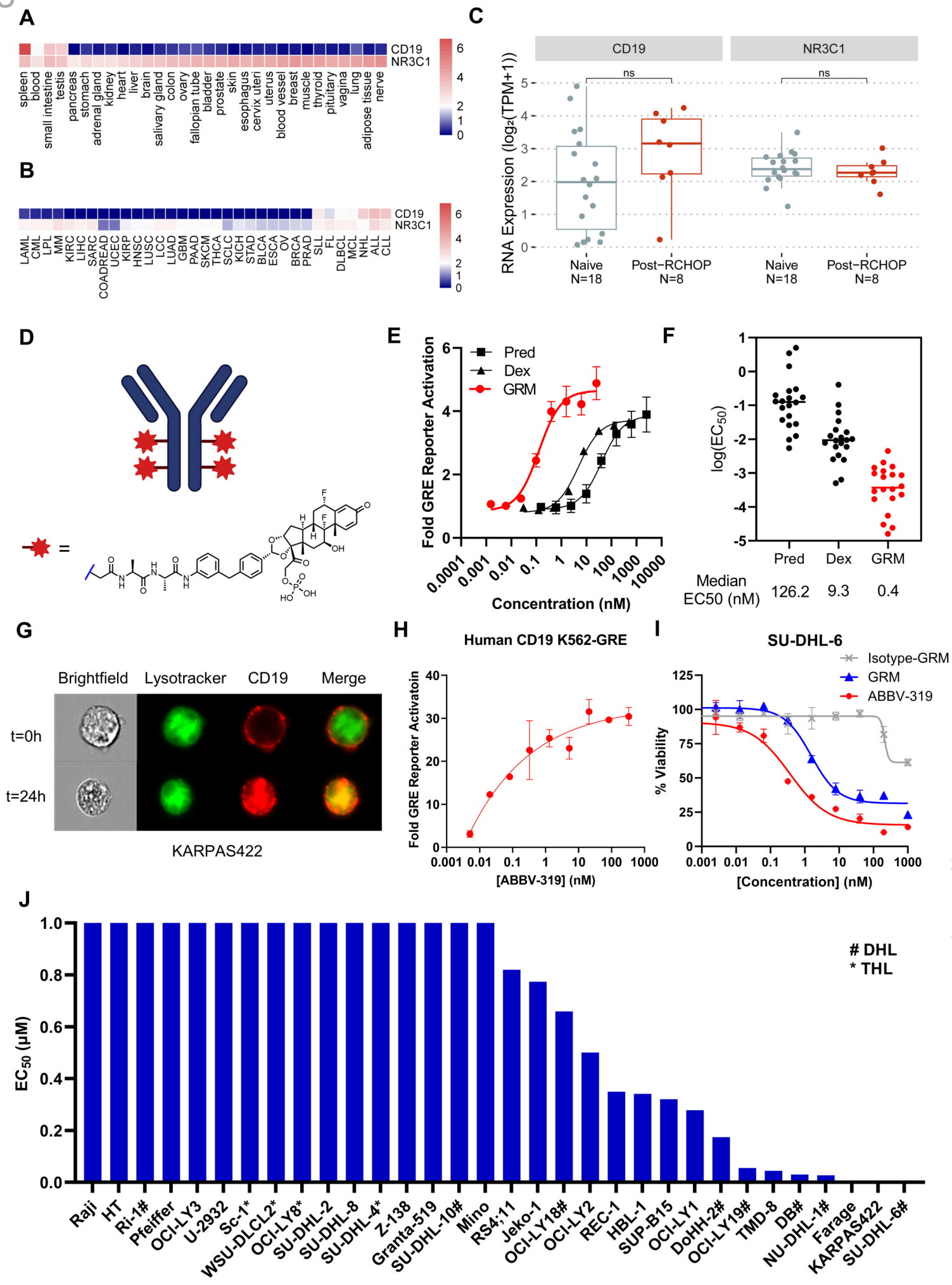635
636 **Figure 6. ABBV-319 induces antibody-dependent cellular cytotoxicity (ADCC) *in vitro* and *in***
637 ***vivo.*** (A) % specific lysis of RS4;11 cells in co-culture with PBMC at effector to target (E:T) ratio
638 of 20:1 after 4-hour treatment with indicated agents. Mean ± SEM are displayed. (B-C)
639 Luciferase reporter activation in Jurkat cells expressing V158 (B) and F158 (C) FcyRIIIa after
640 treatment with indicated agents for 4 and 16 hours, respectively. Mean ± SEM are displayed.
641 (D-F) % specific lysis of RS4;11 (D), Raji (E), and KARPAS422 (F) in co-culturing with PBMC at E:T
642 ratio of 20:1 after treatment with indicated agent for 4 hours. Mean ± SEM are displayed. (G)
643 Growth of OCI-LY19 tumors in CB17 SCID or CD34+ PBMC engrafted NSG-tg(hu-IL15) mice after
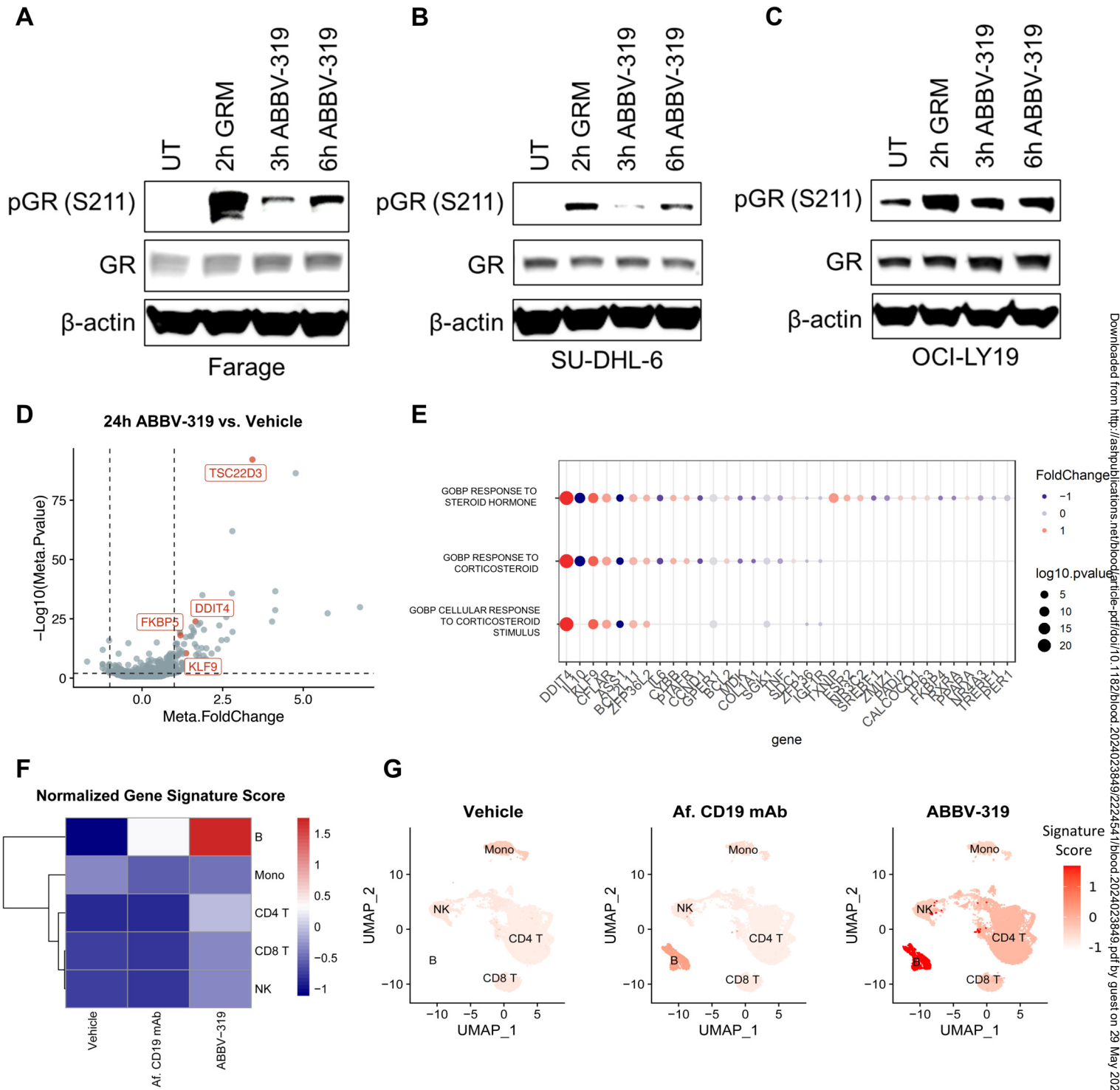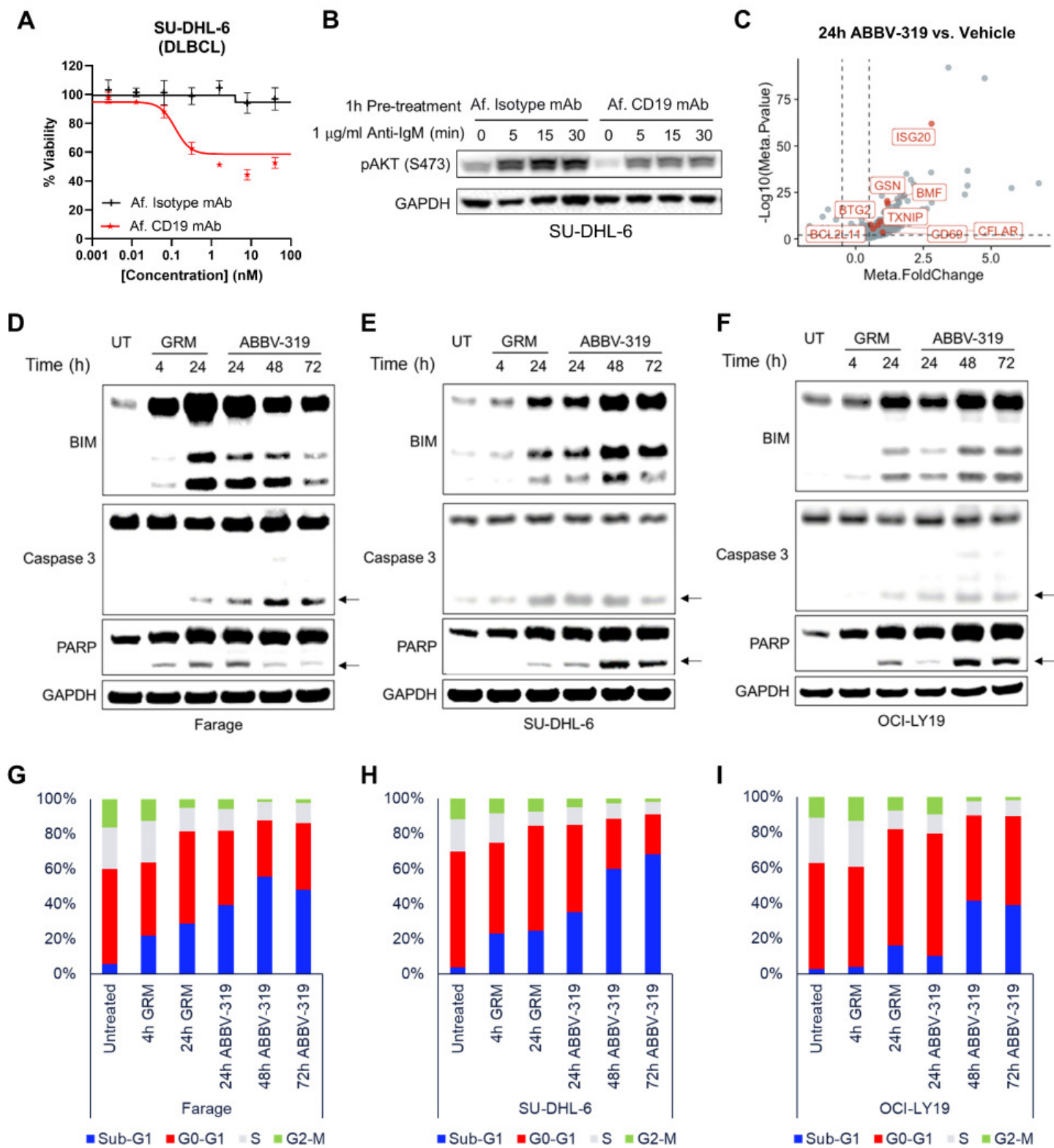644 treatment with vehicle or single-dose ABBV-319 at 5 mg/kg. Mean ± SEM of tumor volumes

26

645 were plotted for each treatment group versus days from randomization. ΔTGI_{max} and TGD_{1000}
646 were calculated as described in the Methods. (H) Growth of OCI-LY19 tumors in CD34+ PBMC
647 engrafted NSG-tg(hu-IL15) mice after treatment with vehicle or single-dose of ABBV-319 at 0.5,
648 1.5, and 5 mg/kg. Means ± SEM of tumor volumes were plotted for each treatment group
649 versus days from randomization. (I) Flow cytometric immunophenotyping analysis of tail vein
650 bleeds from OCI-LY19 tumor bearing mice (H). B cells were presented as the % of CD45+ cells
651 while T and NK cells were presented as % of CD45+ cells without B-cells. Details of the
652 immunophenotyping methods are in supplemental Methods. (J) Growth of Raji tumors in
653 CD34+ PMBC engrafted NSG-tg(hu-IL15) mice after indicated treatment regimen. Means ± SEM
654 of tumor volumes were plotted for each treatment group versus days from randomization.
655

656 **Figure 7. ABBV-319 elicits anti-tumor effects through three distinct mechanisms of action.**
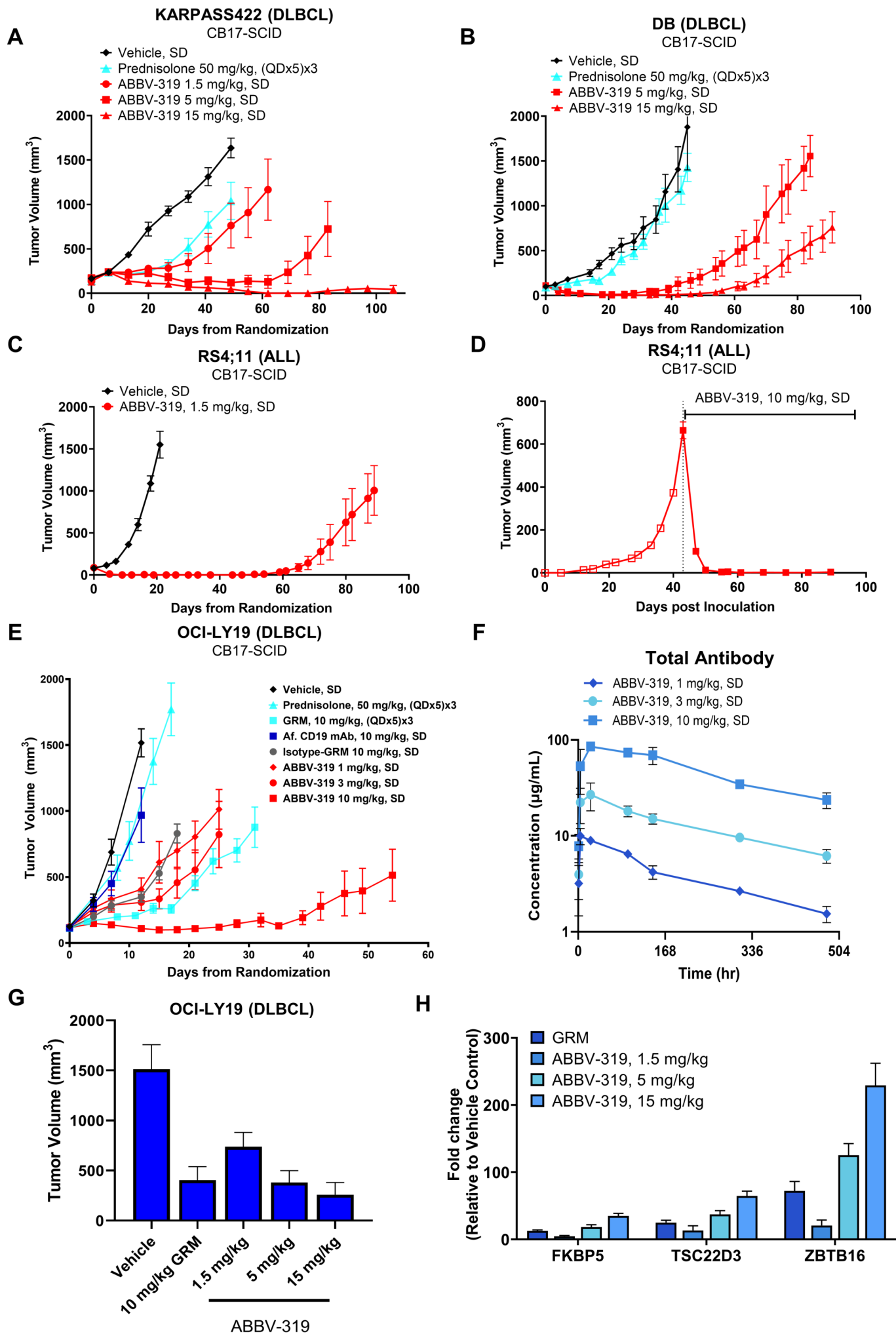657 ABBV-319 results in CD19 internalization and lysosomal trafficking to release the GRM payload.
658 GRM payload drives transcriptional activation of GR targets (e.g., *BCL2L11*) to activate apoptotic
659 cell death. ABBV-319 also blocks CD19-mediated activation of PI3K pathway. Lastly,
660 afucosylation of the fragment crystallizable (Fc) region enhances ADCC driven by effector cells.
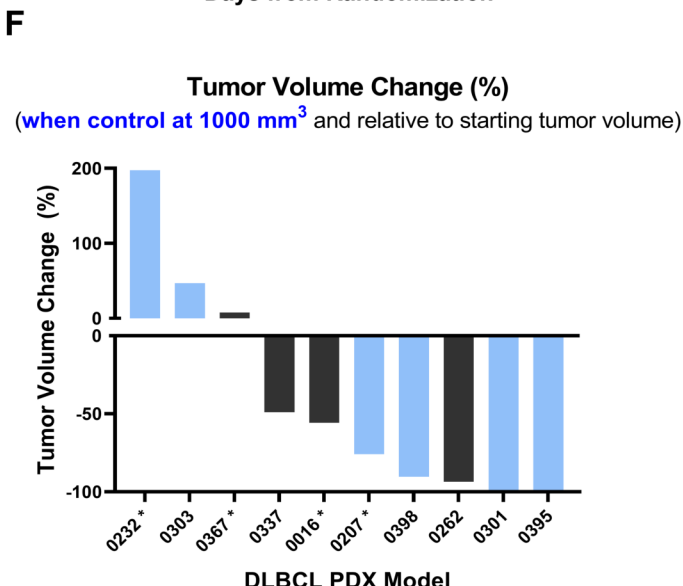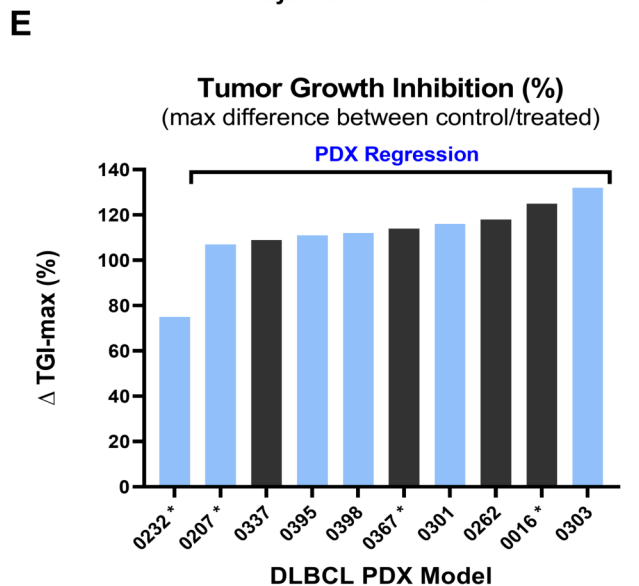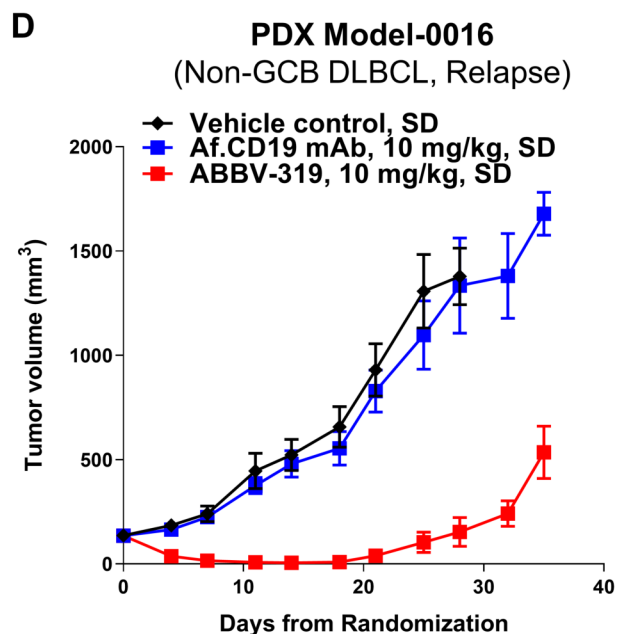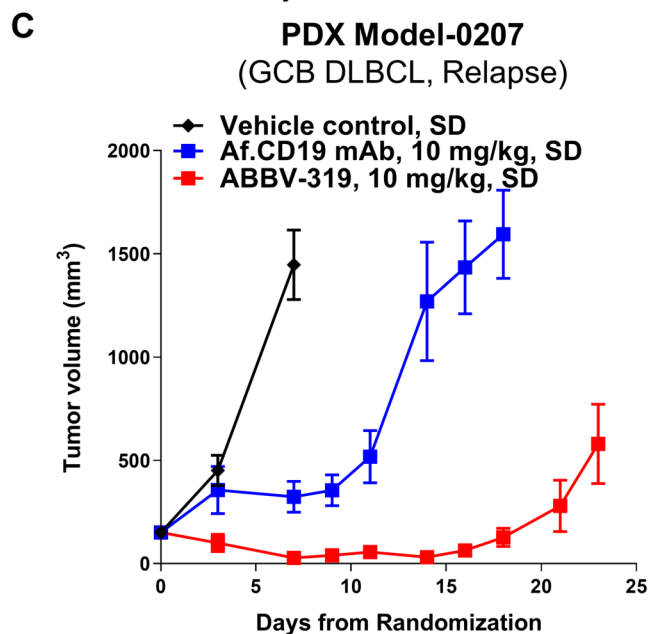
661

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

A PDX Model-0395
(GCB DLBCL, Treatment Naive)

B PDX Model-0262
(Non-GCB DLBCL, Treatment Naive)

C PDX Model-0207
(GCB DLBCL, Relapse)

D PDX Model-0016
(Non-GCB DLBCL, Relapse)

E Tumor Growth Inhibition (%)
(max difference between control/treated)

F Tumor Volume Change (%)
(**when control at 1000 mm³** and relative to starting tumor volume)

Figure 6

Figure 7

1 **GRM payload:** binds to intracellular glucocorticoid receptor

3 **Afucosylation:** increases ADCC

NK Cell or Macrophage

CD19

Growth/Survival

GRM

GR

GRE

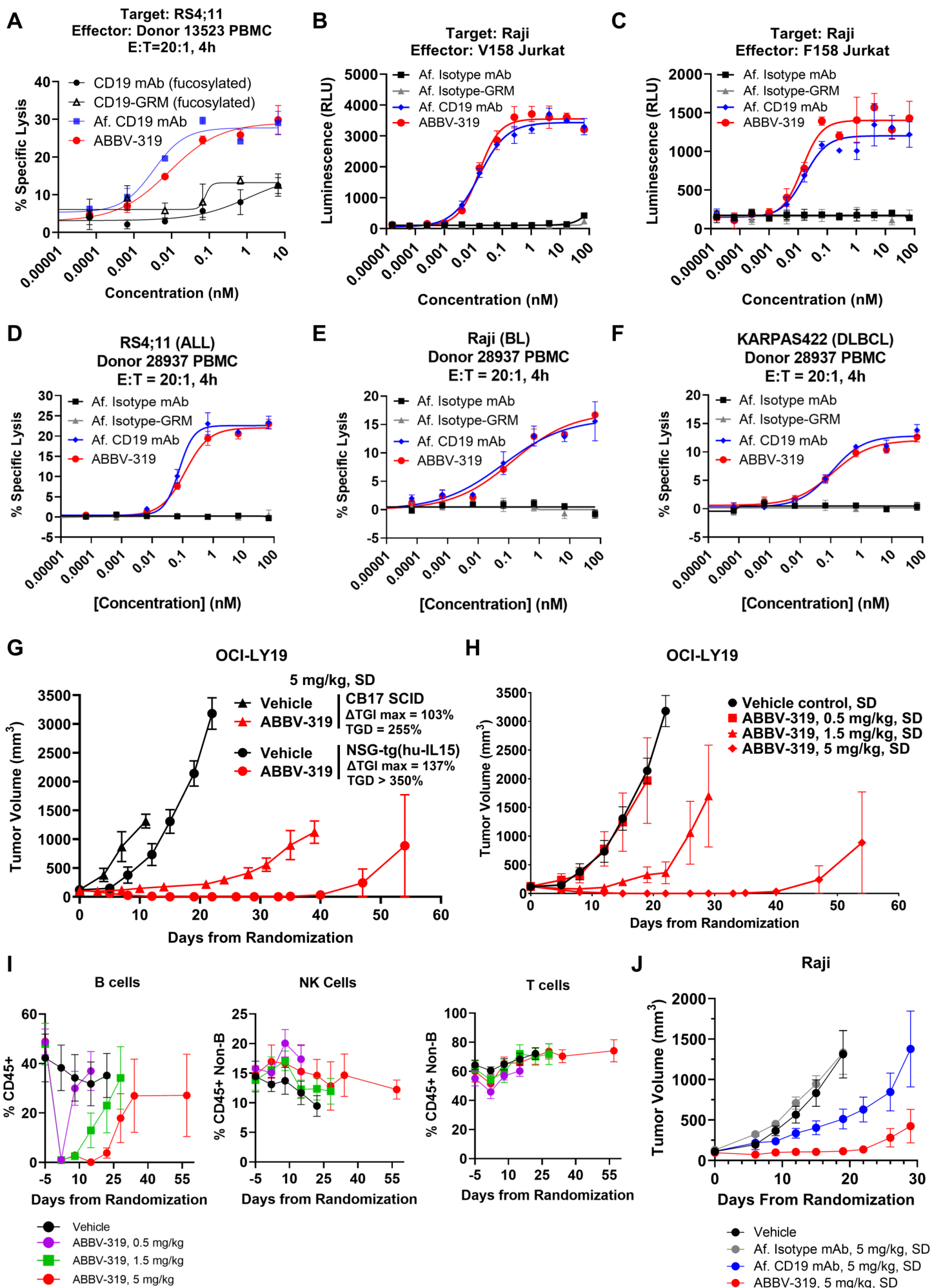Perforins, granzymes

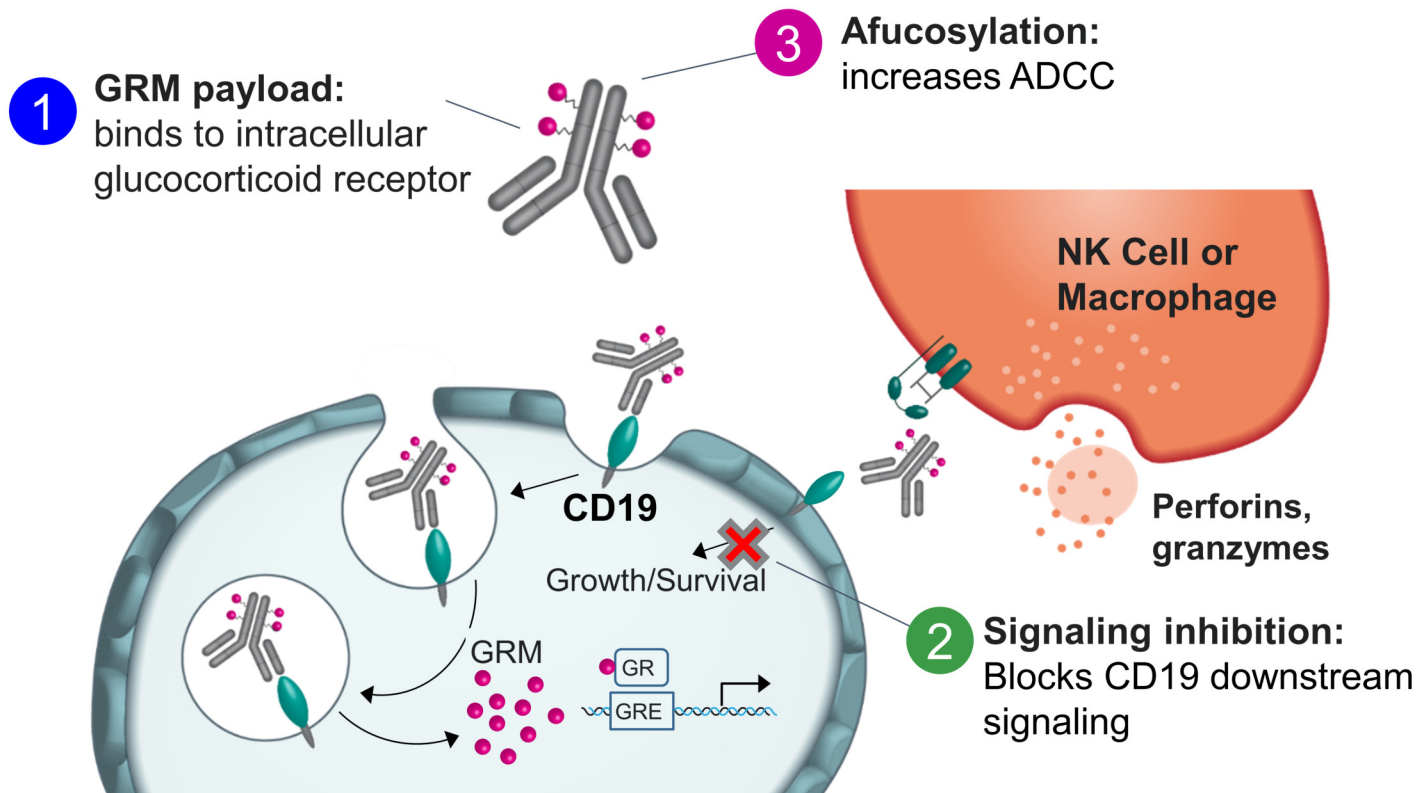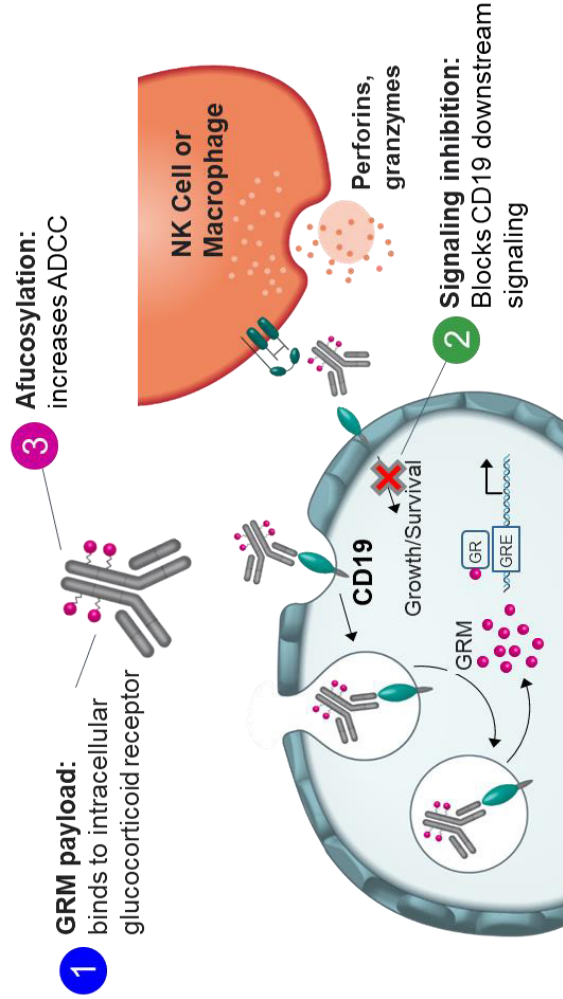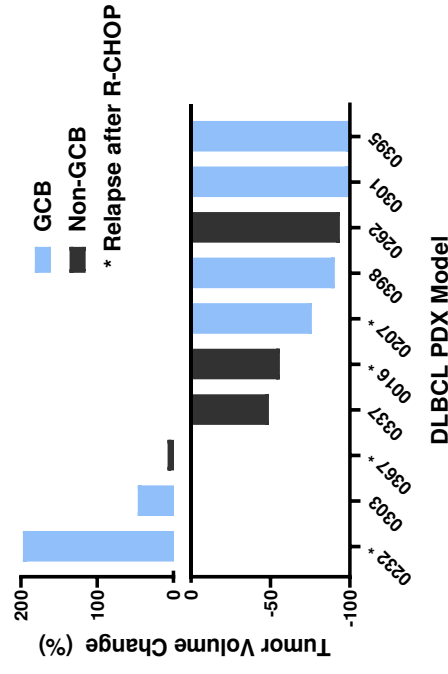**2** **Signaling inhibition:** Blocks CD19 downstream signaling

ABBV-319: A Glucocorticoid Receptor Modulator (GRM) Antibody-Drug Conjugate (ADC) For the Treatment of B-cell Malignancies

In this study, ABBV-319 was investigated as a potential treatment for B-cell malignancies.

DLBCL PDX Tumor Volume Change (%)
(when control at 1000 mm^3 and relative to starting tumor volume)

GCB
Non-GCB
* Relapse after R-CHOP

DLBCL PDX Model

Tumor Volume Change (%)

GRM payload:
binds to intracellular glucocorticoid receptor

Afucosylation:
increases ADCC

NK Cell or Macrophage

Perforins, granzymes

Signaling inhibition:
Blocks CD19 downstream signaling

CD19

Growth/Survival

GRM

GR

GRE

ABBV-319 possesses three main mechanisms of action (MOAs) including 1) GRM payload delivery, 2) CD19 signaling inhibition, 3) Enhanced ADCC via afucosylation. Collectively, these MOAs contribute to pronounced anti-tumor activity across B-cell malignancy models.