Nano Today 50 (2023) 101872

Contents lists available at ScienceDirect

Nano Today

journal homepage: www.elsevier.com/locate/nanotoday

Exogenous CD38 upregulation enables high-efficacy dually cascade targeted molecular therapy of leukemia

Jianwei Du [a,1], Shujing Yue [a,1], Chenming Li [a], Jiaying Li [b], Songsong Zhao [a], Yangyang Dong [a], Yifan Zhang [a], Ru Cheng [a], Huanli Sun [a,*], Zhiyuan Zhong [a,c,**]

[a] Biomedical Polymers Laboratory, College of Chemistry, Chemical Engineering and Materials Science, and State Key Laboratory of Radiation Medicine and Protection, Soochow University, Suzhou 215123, PR China
[b] Department of Orthopaedic Surgery, Orthopaedic Institute, The First Affiliated Hospital, Soochow University, Suzhou 215007, PR China
[c] College of Pharmaceutical Sciences, Soochow University, Suzhou 215123, PR China

ARTICLE INFO

Article history:
Received 5 November 2022
Received in revised form 10 April 2023
Accepted 3 May 2023
Available online 10 May 2023

Keywords:
Acute myeloid leukemia
Nanomedicines
Exogenous stimulation
Molecular targeted drugs
Targeted therapy

ABSTRACT

Acute myeloid leukemia (AML) patients face poor prognosis with high mortality rate, mainly due to the lack of suitable antigens and shortage of effective targeted therapeutics. Here, we utilize exogenous all-trans retinoic acid (ATRA) to upregulate CD38 level on AML cells and therefore boost the AML-targeting of daratumumab-directed polymersomal volasertib molecular targeted drugs (DPVol), for potent and safe AML depletion via cascade targeting. DPVol with a small size of ~ 30 nm had tailored daratumumab density, efficient and robust Vol loading, as well as reduction-triggered intracellular Vol release. ATRA stimulation substantially increased CD38 level on AML cell lines and primary cells isolated from AML patients as much as 20-fold, and accordingly enhanced the cellular uptake and anti-AML activity of DPVol, displaying a low IC_{50} of 16.3 nM in MV-4-11 cells. Intriguingly, ATRA + DPVol combination treatment dramatically reduced the leukemia burden in orthotopic $CD38^{low}$ MV-4-11 and Molm-13-Luc AML bearing mice without causing toxicity, resulting in 4.3–5.8-fold prolonged survival with 20–40 % of mice cured, superior to DPVol and ATRA + PVol. The exogenous stimulation mediated CD38 upregulation in combination with DPVol may provide a safe and cascade targeting strategy for potent treatment of leukemia.

Introduction

Ligand-targeted cancer nanotherapeutics that can selectively bind to receptors or antigens overexpressed on tumor cells have rapidly grown as a promising strategy to overcome limitations of traditional chemotherapy and to revolutionize cancer treatment [1–4]. However, unlike antibody-drug conjugates (ADCs) with high specificity [5,6], ligands of most targeted systems are less specific, which may compromise the safety and efficacy of chemotherapy. In this case, delivery of a more specific drug, such as molecular targeted drugs, is anticipated to augment tumor selectivity and avoid detrimental effects to normal tissues via cascade targeting [7]. In addition, the development of targeted therapeutics for some tumor types is largely restricted by the lack of suitable antigens, leading to poor therapeutic outcomes.

Acute myeloid leukemia (AML), as one of the most common malignancies lacking mature antigens, bears a 5-year survival rate as low as 27 % [8,9]. Due to the limited development of targeted therapeutics, for instance, antibodies and chimeric antigen receptor T cell (CAR-T) therapy which are well established for other malignancies, daunorubicin + cytarabine (7 + 3) chemotherapy has been the standard care for AML patients in recent four decades [10–12]. The response rate though was high for newly diagnosed patients, intolerance and drug resistance generally emerged for most patients, resulting in high rate of relapse and mortality [13,14]. With the discovery of small molecular targeted drugs that possessing higher specificity and lower toxicity, the treatment landscape and outcome have been improved for many malignancies including AML [15–18]. For example, polo-like kinase 1 (PLK1) inhibitor volasertib (Vol), which targets to PLK1 oncogene overexpressed in AML and many

* Corresponding author.
** Corresponding author at: Biomedical Polymers Laboratory, College of Chemistry, Chemical Engineering and Materials Science, and State Key Laboratory of Radiation Medicine and Protection, Soochow University, Suzhou 215123, PR China.
E-mail addresses: sunhuanli@suda.edu.cn (H. Sun), zyzhong@suda.edu.cn (Z. Zhong).
[1] These authors contributed equally.

https://doi.org/10.1016/j.nantod.2023.101872
1748-0132/

other malignancies, has been recognized as a breakthrough therapy for AML by FDA as a result of its good clinical effect [19–21]. Despite this, Vol is typically perplexed with fast clearance in vivo, low tumor enrichment, dose-limiting toxicity and susceptibility to drug resistance, impeding its clinical utility and efficacy.

Targeted delivery of Vol may elegantly overcome its imperfections and increase its specificity, boosting the efficacy on one hand and reducing dosage as well as toxic effects on the other hand. This is, however, critically challenged in AML treatment by the lack of mature and effective targetable antigens [22–24]. CD38, a clinically validated target for multiple myeloma [25,26], has recently gained increasing attention in developing targeted strategies for AML, as evidenced by the undergoing clinical trials of CD38-targeting antibodies, ADCs and CAR-T in treating AML [27–29]. It should be noted, however, that CD38 though expressed on most AML blasts is in general with low and heterogeneous levels and not conducive to targeted treatment [30,31]. All-trans retinoic acid (ATRA) is recently shown to upregulate CD38 levels on multiple myeloma and AML cells, which can somehow augment the efficacy of CD38-targeting daratumumab [32,33].

Herein, we report on a potent anti-AML cascade targeting strategy via exogenous ATRA stimulation and daratumumab-polymersomes mediated CD38-targeted delivery of volasertib (ATRA + DPVol) (Fig. 1**a**). DPVol with disulfide-crosslinking in the hydrophobic polymersomal membrane and tunable Dar density on the surface not only possessed high stability but also showed fast Vol release in a reductive condition mimicking the cytosol. Interestingly, exogenous ATRA stimulation upregulated the CD38 level on both AML cell lines and primary AML cells isolated from patients by up to 20-fold, consequently boosted the cellular uptake and anti-AML effect of CD38-targeting DPVol. In orthotopic MV-4-11 and Molm-13-Luc AML bearing mice, ATRA + DPVol combination treatment displayed good safety, potent inhibition of AML cells, and exceptional survival benefit compared to DPVol and all the other control groups.

Results and discussion

DPVol was fabricated via co-assembly of poly(ethylene glycol)-*b*-poly(trimethylene carbonate-*co*-dithiolane trimethylene carbonate)-poly(aspartic acid) (PEG-P(TMC-DTC)-KD_n) and N_3-PEG-P(TMC-DTC) block copolymers at a weight ratio of 98:2 with synchronized Vol encapsulation and disulfide-crosslinking to form N_3PVol, and subsequent click reaction with dibenzocyclooctyne-functionalized daratumumab (Dar-DBCO) (Fig. S1). PEG-P(TMC-DTC)-KD_n (n = 5, 10, 15, 5.0-(15.1–2.0)-0.7/1.3/1.9 kg/mol) and N_3-PEG-P(TMC-DTC) (7.9-(15.1–2.0) kg/mol, M_w/M_n: 1.1) copolymers were synthesized according to our previous reports [34,35]. The longer PEG chain in N_3-PEG-P(TMC-DTC) polymer can be beneficial to the surface exposure of N_3 groups and post-modification with Dar. In light of the electrostatic interaction mediated Vol encapsulation, the effect of aspartic acid length of PEG-P(TMC-DTC)-KD_n on Vol loading was firstly studied at a theoretical drug loading content (DLC) of 10 wt%. It was found that with an increase of KD_n length (n from 5 to 10 and 15), N_3PVol exhibited increasing Vol loading from 6.5 to 6.9 wt%, while similar sizes (~ 26 nm) and PDI (~ 0.1) (Table S1). In addition, with an increase of the theoretical DLC from 0 to 30 wt%, N_3PVol based on PEG-P(TMC-DTC)-KD_{15} polymer showed incremental sizes from 25.8 to 30.5 nm and increased Vol loading with a maximum DLC of 25.7 wt% (Table S2 **and** Fig. S2a). The highest drug loading efficiency (DLE, 84.2 %) was observed at the theoretical DLC of 20 wt%, which showed a spherical vesicular structure (Fig. S2b). All N_3PVol maintained narrow size distribution (PDI: 0.08–0.10) and slightly negative zeta potential (− 2.5 to − 5.8 mV). N_3PVol had much smaller size than previously reported Vol nano-formulations but with higher Vol loading [36–38]. Hereinafter, N_3PVol prepared from PEG-P(TMC-DTC)-KD_{15} and N_3-PEG-P(TMC-DTC) copolymers at a theoretical DLC of 20 wt% was concentrated to a polymersome concentration of 20 mg/mL for constructing DPVol. The concentrated N_3PVol with a size of ~ 28.0 nm and PDI of ~ 0.12 was stable during 120 days storage at 4 °C, showing negligible size change and less than 2.5 % Vol leakage (Fig. 1**b**).

To click Dar onto the surface of N_3PVol, Dar-DBCO with an average of 2.8 DBCO groups on each Dar was prepared via reaction of Dar with NHS-OEG_4-DBCO at a molar ratio of 1:3 (Fig. S2c). Via tuning the Dar-DBCO/N_3 molar ratio from 0.25:1, 0.5:1, 1:1, to 2:1, D_xPVol with an average of 0.6, 1.3, 2.5 to 3.0 Dar on each polymersome was obtained, respectively, as calculated based on the molecular weight of N_3P (4.11×10^6 g/mol) determined by static light scattering (SLS, Table 1 **and** Fig. S2d). Regardless of Dar densities, D_xPVol all displayed a small size (28.0–30.5 nm) and narrow size distribution (PDI: 0.11–0.12) (Fig. 1**c**). Moreover, after incubation with APC anti-human IgG Fc antibody, DPVol emitted strong fluorescence at 660 nm while N_3PVol not, supporting the successful Dar conjugation for DPVol (Fig. S2e). DPVol possessed high stability against extensive dilution or incubation with 10 % FBS (Fig. S2f), showed only 8.8 % of Vol release within 24 h in a non-reductive condition while released over 72.5 % in 12 h under the trigger of 10 mM GSH (Fig. 1**d**).

One major hurdle for effective AML therapy lies in the lack of specific and mature targets [39,40]. CD38 is overexpressed in a majority of AML patients, but with highly heterogeneous expression levels [30], hindering the development and application of CD38-targeted anti-AML therapy. ATRA was previously reported to be a potential inducer to enhance CD38 expression on AML cells, boosting the cytotoxic effect of anti-CD38 CAR-T in vitro [41]. Herein, CD38 expression levels of MV-4-11 and Molm-13-Luc AML cell lines as well as that after ATRA stimulation were measured via flow cytometry. As shown in Fig. 2**a**, MV-4-11 and Molm-13-Luc AML cells both showed relatively low CD38 expression, which was however sharply upregulated by 20 and 17-fold after ATRA stimulation, respectively. Of note, ATRA stimulation only slightly increased the CD38 expression of T cells isolated from normal human donors (Fig. S3). Consequently, the cellular uptake of DPCy5 with different Dar densities in ATRA-stimulated MV-4-11 and Molm-13-Luc cells was enhanced compared to either PCy5 or DPCy5 in original cells. $D_{2.5}$PCy5 with the best targetability showed 4.5 and 3.9-fold higher cellular uptake than PCy5 in ATRA stimulated MV-4-11 and Molm-13-Luc cells, respectively (Fig. 2**b**). In addition, 4-fold higher cellular uptake of $D_{2.5}$PCy5 was also observed in ATRA stimulated MV-4-11 cells in comparison to that without ATRA treatment. However, in original MV-4-11 and Molm-13-Luc cells, negligible cellular uptake increase was observed for $D_{2.5}$PCy5 compared to PCy5. Confocal laser scanning microscopy (CLSM) images further revealed that ATRA stimulation boosted the cellular uptake of $D_{2.5}$PCy5 in MV-4-11 and Molm-13-Luc cells, showing stronger intracellular Cy5 fluorescence, while without influence for PCy5 (Fig. 2**c,d and** Fig. S4). Furthermore, ATRA-stimulated CD38 upregulation was also detected in primary AML cells (P1 and P2) that isolated from fresh bone marrow aspirates of two patients diagnosed with AML (Fig. 2**e**). P1 with endogenous lower CD38 expression exhibited 6.2-fold enhancement in CD38 level upon ATRA treatment, and P2 with 3.6-fold higher CD38 expression than P1 also displayed 2.5-fold CD38 upregulation. Accordingly, ATRA stimulation enhanced the cellular uptake of $D_{2.5}$PCy5 in P1 and P2 as well, resulting in 5.0–5.7-fold higher cellular interaction than non-targeted PCy5 (Fig. 2**f**).

In line with cellular uptake studies, ATRA stimulation also boosted the in vitro anti-AML activity of DPVol with different Dar surface densities in MV-4-11 and Molm-13-Luc cells. Thereinto, $D_{2.5}$PVol was most potent in ATRA stimulated MV-4-11 cells with a half-maximal inhibitory concentration (IC_{50}) of 16.3 nM, which was 3.9 and 4.9-fold lower than PVol (IC_{50}: 64.3 nM) and free Vol (IC_{50}: 80.5 nM), respectively (Fig. 2**g**). Similarly, in ATRA-stimulated Molm-

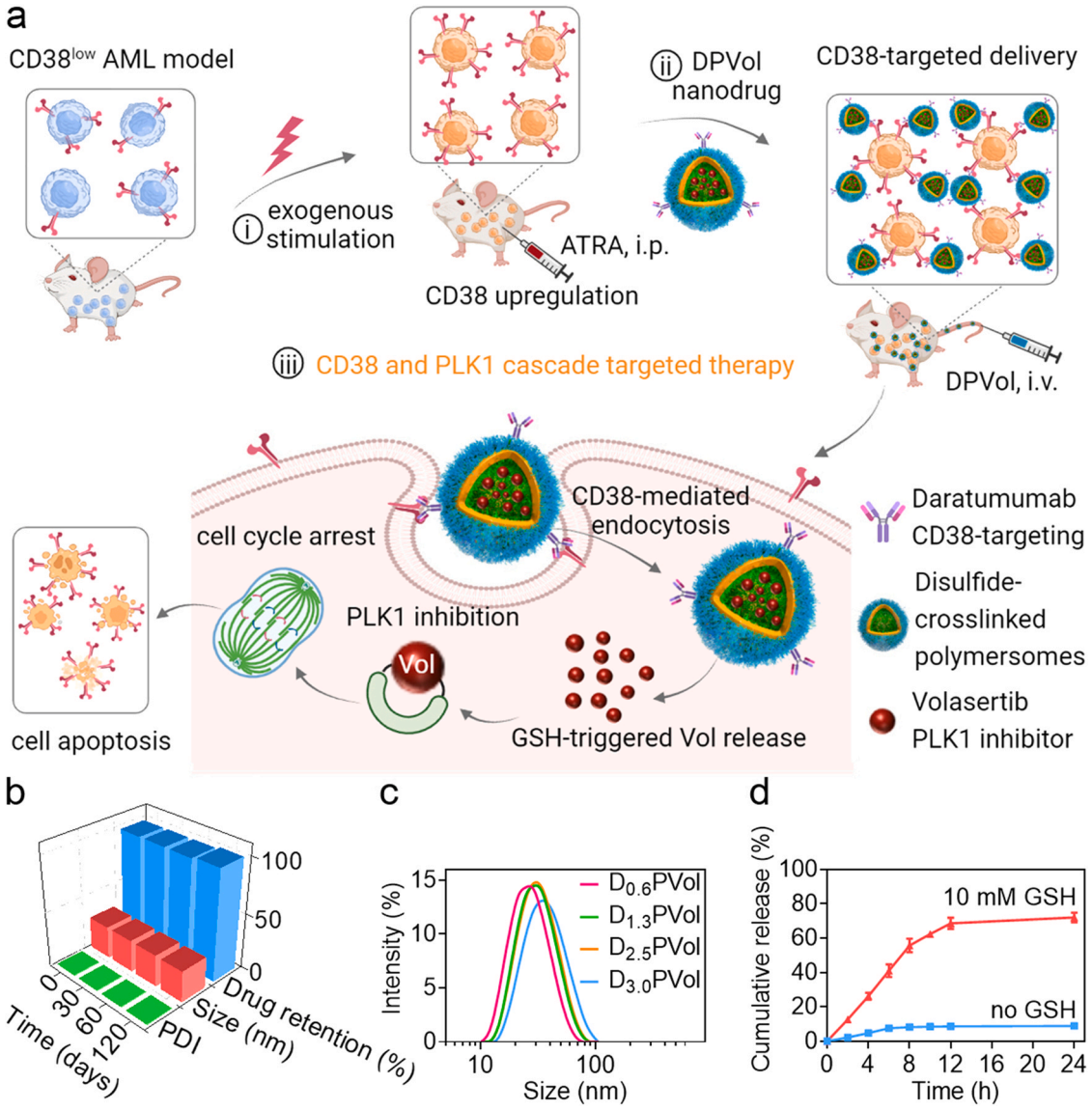

Fig. 1. The combination strategy for cascade targeted molecular therapy of leukemia and characterizations of DPVol. (a) Schematic showing the combination of exogenous ATRA stimulation with daratumumab directed polymersomal volasertib (DPVol) nanodrug for CD38 and PLK1 cascade targeted therapy of AML. (b) Long-term stability of azide-functionalized polymersomal volasertib (N_3PVol) during 120 days storage at 4 °C. (d) Size distribution of DPVol with different daratumumab densities. (e) In vitro Vol release of $D_{2.5}$PVol either with or without GSH at pH 7.4.

Table 1
Characterization of DPVol with different Dar surface densities.

Polymersomes	Feed molar ratio Dar-DBCO:N_3	Size (nm)[a]	PDI[a]	Number of Dar per PVol[b]
$D_{0.6}$PVol	0.25:1	28.0	0.12	0.6 ± 0.1
$D_{1.3}$PVol	0.5:1	29.2	0.11	1.3 ± 0.1
$D_{2.5}$PVol	1:1	30.3	0.11	2.5 ± 0.3
$D_{3.0}$PVol	2:1	30.5	0.12	3.0 ± 0.2

[a] determined by DLS
[b] determined by HPLC.

13-Luc cells, $D_{2.5}$PVol was also 2.3 and 3.0-fold more potent compared to PVol and free Vol (IC_{50}: 47.0 versus 109.4 and 141.4 nM), respectively (Fig. 2**h**). In contrast, for original MV-4-11 and Molm-13-Luc cells without ATRA stimulation, $D_{2.5}$PVol exhibited comparable anti-AML effect to non-targeted PVol and showed a 3.9 and 2.1-fold higher IC_{50} than $D_{2.5}$PVol in corresponding ATRA stimulated cells (Fig. 2**i**,**j**). Notably, $D_{2.5}$PVol, hereinafter referred to as DPVol and PVol caused negligible toxicity against normal L929 fibroblast cells even at a Vol concentration up to 485 nM (Fig. S5a), likely due to the inefficient cellular uptake and low PLK1 level in L929 cells. More importantly, ATRA and blank polymersomes were non-toxic toward MV-4-11 and/or Molm-13-Luc cells at 5 μM ATRA (Fig. S5b) and a polymersome concentration of 100 μg/mL (Fig. S5c,d).

Vol is known to specifically inhibit PLK1, inducing cell cycle arrest at G2/M phase and cell apoptosis [36,42,43]. MV-4-11 cells were herein utilized to study the effect of DPVol in combination with ATRA (ATRA + DPVol) on PLK1 protein regulation, cell cycle arrest and apoptosis. Western blot results (Fig. 3**a**,**b**) showed that ATRA + DPVol combination treatment significantly down-regulated PLK1 protein by 75 %, which was in sharp contrast to DPVol, PVol, free Vol and ATRA + PVol groups that showing slight effect on PLK1 protein. Consistently, ATRA + DPVol combination treatment increased arrest of cell cycle at G2/M phase from 10.2 % of PBS group to 63.4 %, which was ca. 2-fold higher than ATRA + PVol (32.4 %) (Fig. 3**c**,**d**). However, DPVol itself similar as PVol and free Vol, only induced 35.1 % of G2/M phase cell arrest. In addition, ATRA + DPVol effectively promoted the apoptosis of MV-4-11 cells at 32.3 nM Vol,

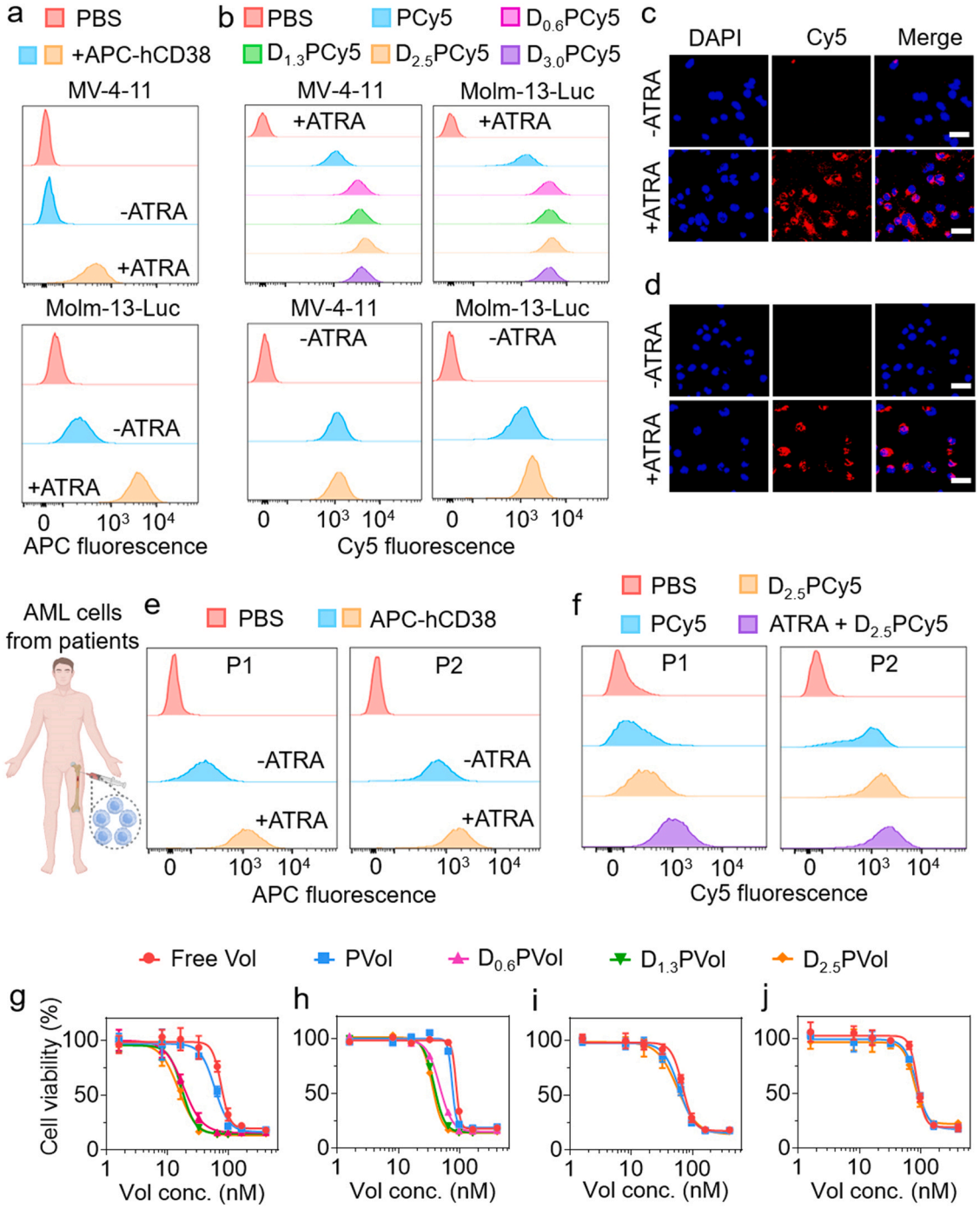

Fig. 2. ATRA-mediated CD38 upregulation enhanced AML-targeting and potency of DPVol. (a) CD38 expression levels of MV-4-11 and Molm-13-Luc cells before and after ATRA stimulation. (b) Flow cytometry analysis showing the cellular uptake of DPCy5 and PCy5 in MV-4-11 and Molm-13-Luc cells either with or without ATRA stimulation. CLSM images of (c) MV-4-11 and (d) Molm-13-Luc cells with or without ATRA stimulation following 4 h incubation with $D_{2.5}$PCy5 (scale bars: 25 μm). (e) CD38 levels of primary AML cells isolated from patient samples either with or without ATRA stimulation. (f) Cellular uptake of $D_{2.5}$PCy5 and PCy5 in primary AML cells and that pretreated with ATRA. The potency of DPVol against ATRA-stimulated (g) MV-4-11 and (h) Molm-13-Luc cells, as well as original (i) MV-4-11 and (j) Molm-13-Luc cells. Free Vol and PVol were used as controls.

showing a total apoptosis rate of 45.4 %, over 3-fold higher than DPVol, ATRA + PVol and PVol (11.6–14.9 %) (Fig. 3**d**). Importantly, ATRA itself did not cause cell apoptosis. Similarly, increased apoptosis of primary AML cells was also observed for ATRA + DPVol treated cells in comparison to that treated with DPVol or ATRA + PVol (Fig. 3**e**). Taken together, these results confirmed that ATRA stimulation upregulated CD38 levels on AML cells, hence boosted the cellular uptake of DPVol, inhibited PLK1 protein, increased G2/M phase cell cycle arrest and cell apoptosis, resulting in significantly enhanced anti-AML activity in vitro.

In vivo acute toxicity tests revealed that DPVol was well tolerated in Kunming mice at a Vol dosage of 9, 27 and 45 mg/kg, wherein, mice constantly gained weight and all survived during the 14-day observation period (Fig. S6a,b). At an even higher Vol dosage of 54 mg/kg, mice though suffered weight loss on day 3 post administration, recovered gradually from day 8, showing a survival rate of

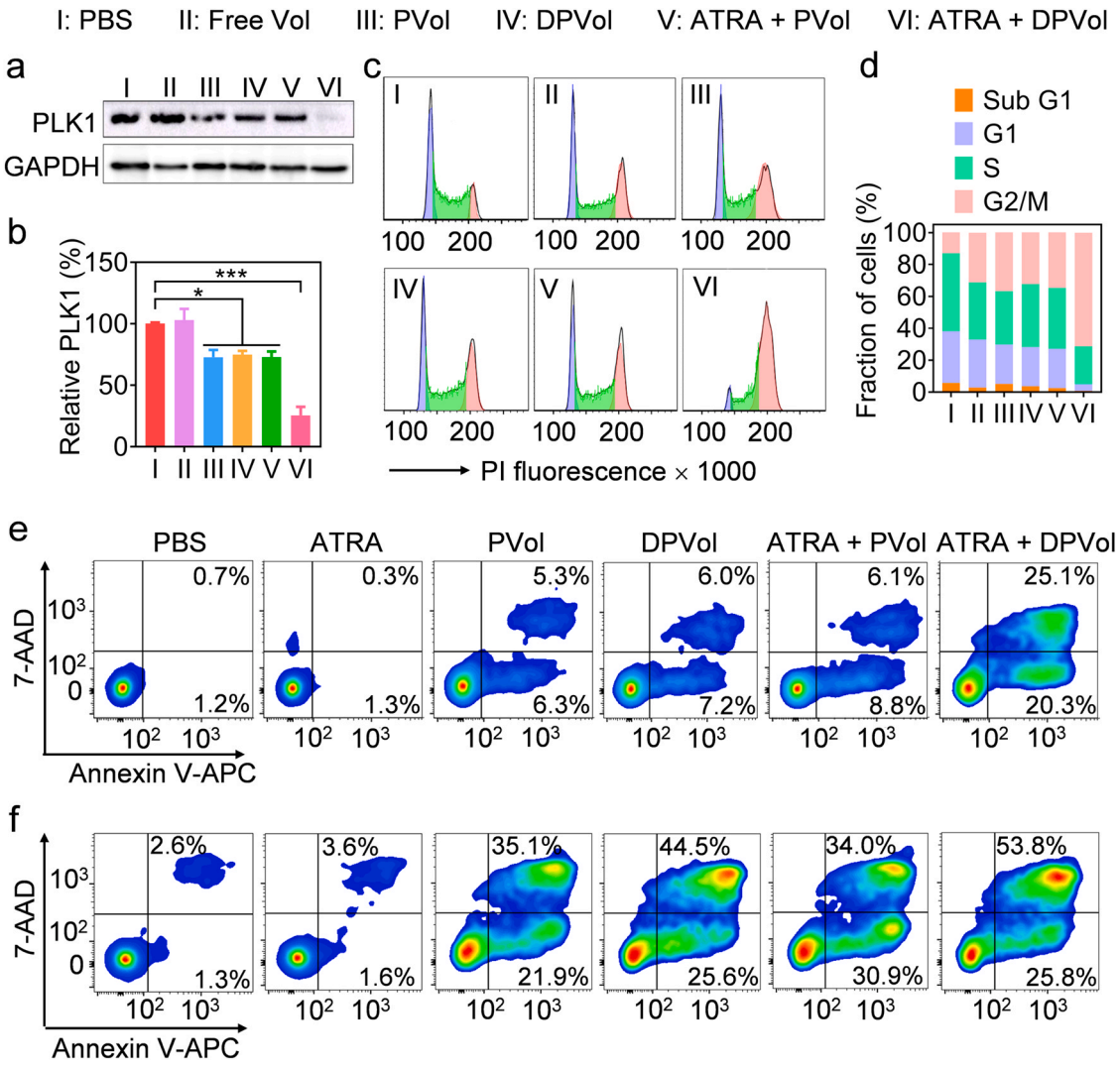

Fig. 3. In vitro PLK1 inhibition, cell cycle arrest and proapoptotic ability of ATRA + DPVol. (a) Western blot and (b) semi-quantification analysis of PLK1 protein in MV-4-11 cells treated with different formulations (n = 3, $^{*}p < 0.05$, $^{***}p < 0.001$). (c) Cell cycle analysis of MV-4-11 cells following different treatments and (d) quantitative data of c. Cell apoptosis analysis of (e) MV-4-11 cells and (f) primary AML cells isolated from bone marrow samples of patient with different treatments.

83.3 % and similar blood routine parameters to the healthy mice (Fig. S6c). To investigate the anti-AML activity of DPVol in combination with ATRA in vivo, orthotopic MV-4-11 AML model was firstly established by direct injection of MV-4-11 cells into B-NDG mice through tail vein [44,45]. Three days later, treatment was initiated with intravenous injection of different Vol formulations at 9 mg Vol equiv./kg every three days for four times (Fig. 4**a**). For the combination groups, ATRA (10 mg/kg) was intraperitoneally injected daily for two days prior to each administration. On day 25 post inoculation, MV-4-11 cells disseminated and infiltrated to the liver, spleen, bone marrow and peripheral blood of PBS treated mice, showing a leukemia burden of 45.0 %, 19.0 %, 16.7 % and 12.4 %, respectively (Fig. 4**b,c**), and symptoms of weight loss, slow-motion, and hind limb paralysis etc., which is consistent with previous report [46]. Notably, ATRA + DPVol combination strategy dramatically depleted the leukemia cells and completely inhibited leukemia infiltration in different organs. However, ATRA + free Vol and ATRA + PVol treatments only slightly retarded the leukemia progression and remained with obvious leukemia infiltration in liver, spleen, bone marrow and peripheral blood, which was significantly higher than that in ATRA + DPVol group. Accordingly, ATRA + DPVol combination regimen exceptionally extended the survival of mice with orthotopic MV-4-11 AML model, showing a median survival time (MST: 140 days) of 5.2, 4.4 and 3.3-fold longer than PBS (27 days), ATRA + free Vol (32 days) and ATRA + PVol (43 days) groups, respectively (Fig. 4**d**). Moreover, 40 % of mice receiving ATRA + DPVol treatment were cured and still alive at 170 days. In comparison, DPVol alone only slightly prolonged the MST of mice to 46 days, which was comparable to that of ATRA + PVol, evidencing that ATRA stimulation can substantially improve the CD38-targeted anti-AML effect of DPVol. Of note, all regimens were well tolerated with no significant side effects (Fig. 4**e**).

Hematoxylin and eosin (H&E) stained images further presented solid evidence, wherein, ATRA + DPVol treated mice showed no obvious leukemia infiltration in different organs and hind limbs with normal histomorphology (Fig. 5**a and** Fig. S7). However, visible leukemia cells were observed in the spleen and bone marrow of mice following treatment with PBS, ATRA + free Vol or ATRA + PVol. The infiltration and proliferation of leukemia cells inside bone marrow is known to impair hematopoiesis and cause bone marrow failure, a common sign for AML patients [47–49]. As shown in the H&E stained images of hind limbs (Fig. 5**a**), PBS, ATRA + free Vol and ATRA + PVol

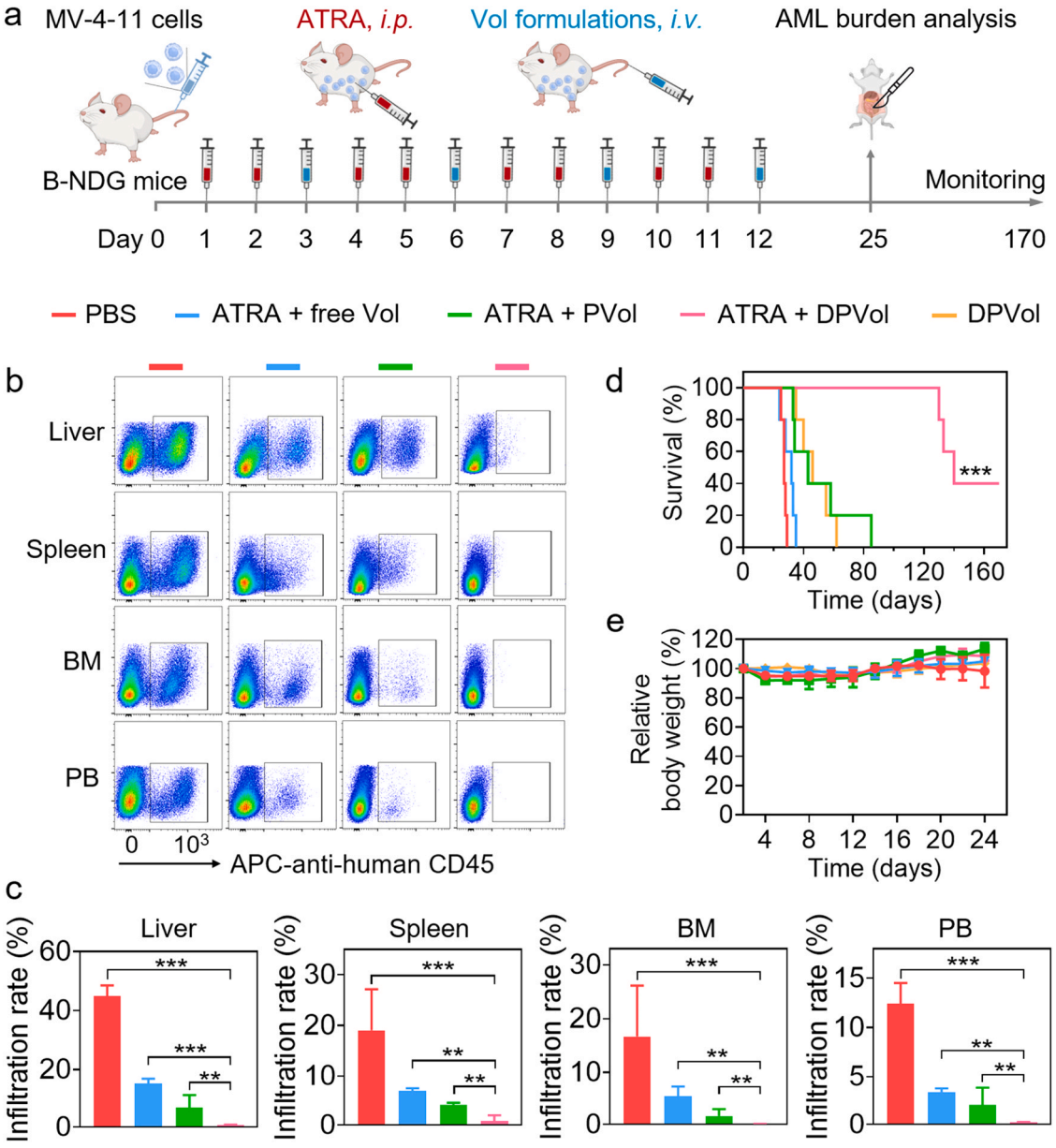

Fig. 4. In vivo therapeutic efficacy of DPVol in combination with ATRA (ATRA + DPVol) in orthotopic MV-4-11 AML model. PBS, ATRA + free Vol, ATRA + PVol and DPVol were utilized as controls. (a) Schematic illustration of the treatment schedule for orthotopic MV-4-11 model. (b) Representative scattered flow cytometry pattern and (c) quantitative analysis of infiltration of MV-4-11 cells in liver, spleen, bone marrow (BM) and peripheral blood (PB) on day 25 after tumor inoculation. (n = 3, $^{**}p < 0.01$, $^{***}p < 0.001$). (d) Survival and (e) body weight changes of mice in different treatment groups (n = 5, ATRA + DPVol versus all control groups, $^{***}p < 0.001$).

treated mice suffered significant loss of hematopoietic cells in the bone marrow cavity, which was completely relieved after treatment with ATRA + DPVol. In addition to impaired hematopoiesis, osteolysis represents another common consequence of tumor invasion into the bone [50–52]. We therefore further analyzed the structure of femur and tibia collected from mice in different groups using micro-computer tomography (micro-CT). The images revealed severe osteolytic bone destruction and loss of trabecular bone for PBS treated mice, while ATRA + DPVol treatment alleviated such symptoms to the largest extent and showed normal bone structure (Fig. 5**b**).

In order to further verify the anti-AML effect of ATRA + DPVol combination regimen, orthotopic Molm-13-Luc AML model was constructed similarly via tail vein injection of Molm-13-Luc cells into B-NDG mice (Fig. 5**c**). Treatment was also initiated on day 3 post model establishment, wherein, PBS, ATRA + PVol and ATRA + DPVol were administrated in the same way as MV-4-11 AML model. PBS treated mice developed symptoms on day 17 as a result of the rapid proliferation of Molm-13-Luc cells in vivo, displaying weight loss, hind leg paralysis and a MST of only 21 days (Fig. 5**d**). The survival benefit of ATRA + PVol was limited with all mice died in 27 days. In sharp contrast, ATRA + DPVol essentially suppressed the leukemia progression and significantly prolonged the mice survival with 20 % remained alive after 100 days (Fig. 5**e**). Moreover, ATRA + DPVol regimen caused negligible weight loss of mice (Fig. 5**f**). To sum up, ATRA stimulation largely upregulated the CD38 level of AML cells, thus boosted CD38-targeted delivery of PLK1 targeted drugs by DPVol, leading to potent cascade targeted anti-AML therapy and exceptional survival benefit.

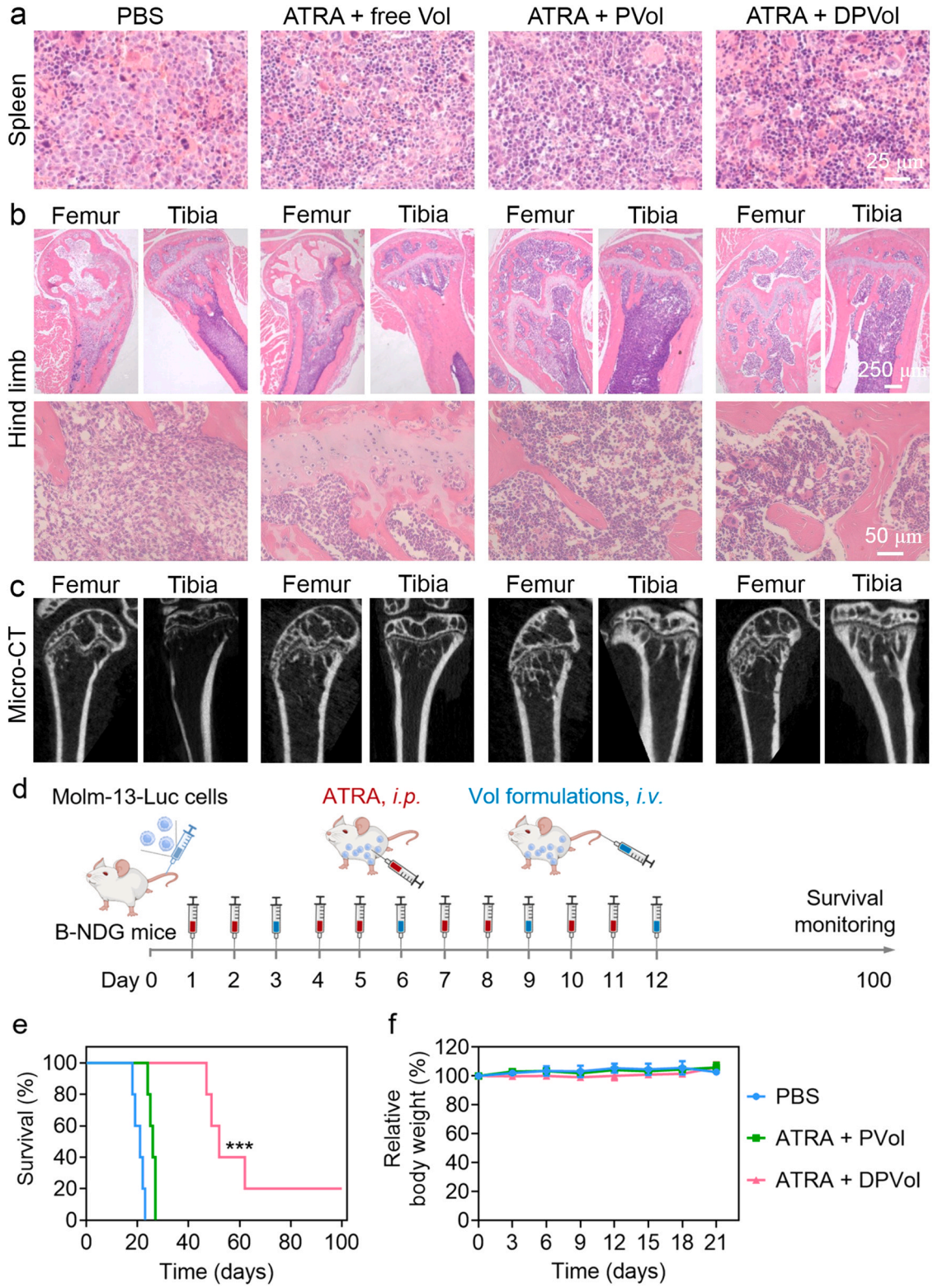

Fig. 5. Anti-AML effect of ATRA + DPVol in vivo. Representative H&E stained images of (a) spleen and (b) hind limbs, as well as (c) micro-CT images of femur and tibia isolated from orthotopic MV-4-11 AML bearing mice following different treatments. (d) Schematic illustration of the treatment and monitoring schedule for orthotopic Molm-13-Luc AML model. (e) Survival curves and (f) body weight changes of orthotopic Molm-13-Luc AML bearing mice following treatment with ATRA + DPVol, ATRA + PVol and PBS (n = 5, $^{***}p < 0.001$).

Conclusion

In summary, we have demonstrated that exogenous ATRA stimulation created a valuable target for AML cells and enabled CD38-targeted delivery of molecular targeted drug, volasertib, via daratumumab polymersomes (DPVol), leading to potent leukemia depletion in orthotopic AML models with endogenous low CD38 expression. The combination of ATRA with DPVol not only provided exceptional survival benefit but also improved systemic safety via cascade targeting. This represents the first report on exogenous

stimulation boosting targeted delivery of molecular targeted drugs for leukemia treatment, which may offer an effective and unique targeted regimen for AML patients.

CRediT authorship contribution statement

Z.Z., H.S. J.D. and S.Y. conceived the project and designed the experiments. **J.D. and S.Y.** performed the experiments and analyzed the data. **C.L., S.Z., Y.D., and Y.Z.** helped to conduct the experiments. **J.L.** performed the micro-CT analysis. **R.C.** provided valuable suggestions and discussions. **J.D.** wrote the original draft. **H.S. and Z.Z.** supervised the project and revised the manuscript.

Data Availability

Data will be made available on request.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

This work was supported by the National Natural Science Foundation of China (52073196, 52233007, 52273251, and 52073195), the National Key R&D Program of China (2021YFB3800900), the Natural Science Foundation of the Jiangsu Higher Education Institutions of China (21KJA150007) and Qinglan Program of the Jiangsu Higher Education Institutions. We thank Dr. Jingnan An and Prof. Yang Xu from The First Affiliated Hospital of Soochow University for providing patient samples. We thank Dr. Fengtao You from Persongen Bio Therapeutics (Suzhou) Co., Ltd. for providing Molm-13-Luc cells.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.nantod.2023.101872.

References

[1] J. Shi, P.W. Kantoff, R. Wooster, O.C. Farokhzad, Cancer nanomedicine: progress, challenges and opportunities, Nat. Rev. Cancer 17 (2017) 20–37.
[2] P. Mi, H. Cabral, K. Kataoka, Ligand-installed nanocarriers toward precision therapy, Adv. Mater. 32 (2020) 29.
[3] D. Rosenblum, N. Joshi, W. Tao, J.M. Karp, D. Peer, Progress and challenges towards targeted delivery of cancer therapeutics, Nat. Commun. 9 (2018) 1410.
[4] C.Y. Zhang, L. Yan, X. Wang, S. Zhu, C.Y. Chen, Z.J. Gu, Y.L. Zhao, Progress, challenges, and future of nanomedicine, Nano Today 35 (2020) 101008.
[5] C.H. Chau, P.S. Steeg, W.D. Figg, Antibody–drug conjugates for cancer, Lancet 394 (2019) 793–804.
[6] C. Criscitiello, S. Morganti, G. Curigliano, Antibody-drug conjugates in solid tumors: a look into novel targets, J. Hematol. Oncol. 14 (2021) 20.
[7] M.T. Manzari, Y. Shamay, H. Kiguchi, N. Rosen, M. Scaltriti, D.A. Heller, Targeted drug delivery strategies for precision medicines, Nat. Rev. Mater. 6 (2021) 351–370.
[8] C. Sheridan, First new drug approval for aml in 15 years, Nat. Biotechnol. 35 (2017) 696–698.
[9] X. Xie, Y. Hu, T. Ye, Y. Chen, L. Zhou, F. Li, X. Xi, S. Wang, Y. He, X. Gao, W. Wei, G. Ma, Y. Li, Therapeutic vaccination against leukaemia via the sustained release of co-encapsulated anti-PD-1 and a leukaemia-associated antigen, Nat. Biomed. Eng. 5 (2021) 414–428.
[10] J. Schaefer, S. Cassidy, R.M. Webster, The acute myeloid leukaemia market, Nat. Rev. Drug Discov. 19 (2020) 233–234.
[11] V. Trujillo-Alonso, E.C. Pratt, H. Zong, A. Lara-Martinez, C. Kaittanis, M.O. Rabie, V. Longo, M.W. Becker, G.J. Roboz, J. Grimm, M.L. Guzman, FDA-approved ferumoxytol displays anti-leukaemia efficacy against cells with low ferroportin levels, Nat. Nanotechnol. 14 (2019) 616–622.
[12] H. Dombret, C. Gardin, An update of current treatments for adult acute myeloid leukemia, Blood 127 (2016) 53–61.
[13] Q. Hu, W. Sun, J. Wang, H. Ruan, X. Zhang, Y. Ye, S. Shen, C. Wang, W. Lu, K. Cheng, G. Dotti, J.F. Zeidner, J. Wang, Z. Gu, Conjugation of haematopoietic stem cells and platelets decorated with anti-PD-1 antibodies augments anti-leukaemia efficacy, Nat. Biomed. Eng. 2 (2018) 831–840.
[14] D.T. Johnson, J. Zhou, A.V. Kroll, R.H. Fang, M. Yan, C. Xiao, X. Chen, J. Kline, L. Zhang, D.-E. Zhang, Acute myeloid leukemia cell membrane-coated nanoparticles for cancer vaccination immunotherapy, Leukemia 36 (2022) 994–1005.
[15] P.L. Bedard, D.M. Hyman, M.S. Davids, L.L. Siu, Small molecules, big impact: 20 years of targeted therapy in oncology, Lancet 395 (2020) 1078–1088.
[16] W.C. Hahn, J.S. Bader, T.P. Braun, A. Califano, P.A. Clemons, B.J. Druker, A.J. Ewald, H. Fu, S. Jagu, C.J. Kemp, W. Kim, C.J. Kuo, M.T. McManus, G.B. Mills, X. Mo, N. Sahni, S.L. Schreiber, J.A. Talamas, P. Tamayo, J.W. Tyner, B.K. Wagner, W.A. Weiss, D.S. Gerhard, V. Dancik, S. Gill, B. Hua, T. Sharifnia, V. Viswanathan, Y. Zou, F. Dela Cruz, A. Kung, B. Stockwell, J. Boehm, J. Dempster, R. Manguso, F. Vazquez, L.A.D. Cooper, Y. Du, A. Ivanov, S. Lonial, C.S. Moreno, Q. Niu, T. Owonikoko, S. Ramalingam, M. Reyna, W. Zhou, C. Grandori, I. Shmulevich, E. Swisher, J. Cai, I.S. Chan, M. Dunworth, Y. Ge, D. Georgess, E.M. Grasset, E. Henriet, H. Knútsdóttir, M.G. Lerner, V. Padmanaban, M.C. Perrone, Y. Suhail, Y. Tsehay, M. Warrier, Q. Morrow, T. Nechiporuk, N. Long, J. Saultz, A. Kaempf, J. Minnier, C.E. Tognon, S.E. Kurtz, A. Agarwal, J. Brown, K. Watanabe-Smith, T.Q. Vu, T. Jacob, Y. Yan, B. Robinson, E.F. Lind, Y. Kosaka, E. Demir, J. Estabrook, M. Grzadkowski, O. Nikolova, K. Chen, B. Deneen, H. Liang, M.C. Bassik, A. Bhattacharya, K. Brennan, C. Curtis, O. Gevaert, H.P. Ji, K.A.J. Karlsson, K. Karagyozova, Y.-H. Lo, K. Liu, M. Nakano, A. Sathe, A.R. Smith, K. Spees, W.H. Wong, K. Yuki, M. Hangauer, D.S. Kaufman, A. Balmain, S.R. Bollam, W.-C. Chen, Q. Fan, K. Kersten, M. Krummel, Y.R. Li, M. Menard, N. Nasholm, C. Schmidt, N.K. Serwas, H. Yoda, A. Ashworth, S. Bandyopadhyay, T. Bivona, G. Eades, S. Oberlin, N. Tay, Y. Wang, J. Weissman, An expanded universe of cancer targets, Cell 184 (2021) 1142–1155.
[17] J. Westermann, L. Bullinger, Precision medicine in myeloid malignancies, Semin. Cancer Biol. 84 (2022) 153–169.
[18] H. Kantarjian, N.J. Short, C. DiNardo, E.M. Stein, N. Daver, A.E. Perl, E.S. Wang, A. Wei, M. Tallman, Harnessing the benefits of available targeted therapies in acute myeloid leukaemia, Lancet Haematol. 8 (2021) e922–e933.
[19] T. Otto, P. Sicinski, Cell cycle proteins as promising targets in cancer therapy, Nat. Rev. Cancer 17 (2017) 93–115.
[20] K. Strebhardt, Multifaceted polo-like kinases: drug targets and antitargets for cancer therapy, Nat. Rev. Drug Discov. 9 (2010) 643–660.
[21] B.T. Gjertsen, P. Schoffski, Discovery and development of the polo-like kinase inhibitor volasertib in cancer therapy, Leukemia 29 (2015) 11–19.
[22] N. Daver, A.S. Alotaibi, V. Bücklein, M. Subklewe, T-cell-based immunotherapy of acute myeloid leukemia: current concepts and future developments, Leukemia 35 (2021) 1843–1863.
[23] N.J. Short, M. Konopleva, T.M. Kadia, G. Borthakur, F. Ravandi, C.D. DiNardo, N. Daver, Advances in the treatment of acute myeloid leukemia: new drugs and new challenges, Cancer Discov. 10 (2020) 506–525.
[24] A. Khwaja, M. Bjorkholm, R.E. Gale, R.L. Levine, C.T. Jordan, G. Ehninger, C.D. Bloomfield, E. Estey, A. Burnett, J.J. Cornelissen, D.A. Scheinberg, D. Bouscary, D.C. Linch, Acute myeloid leukaemia, Nat. Rev. Dis. Prim. 2 (2016) 16010.
[25] M. D'Agostino, R. Mina, F. Gay, Anti-CD38 monoclonal antibodies in multiple myeloma: another cook in the kitchen? Lancet Haematol. 7 (2020) e355–e357.
[26] N.W.C.J. van de Donk, P.G. Richardson, F. Malavasi, CD38 antibodies in multiple myeloma: back to the future, Blood 131 (2018) 13–29.
[27] M. Böhme, S. Kayser, Immune-based therapeutic strategies for acute myeloid leukemia, Cancers 14 (2022) 105.
[28] B.A. Williams, A. Law, J. Hunyadkurti, S. Desilets, J.V. Leyton, A. Keating, Antibody therapies for acute myeloid leukemia: unconjugated, toxin-conjugated, radioconjugated and multivalent formats, J. Clin. Med. 8 (2019) 1261.
[29] Q. Cui, C. Qian, N. Xu, L. Kang, H. Dai, W. Cui, B. Song, J. Yin, Z. Li, X. Zhu, C. Qu, T. Liu, W. Shen, M. Zhu, L. Yu, D. Wu, X. Tang, CD38-directed CAR-T cell therapy: a novel immunotherapy strategy for relapsed acute myeloid leukemia after allogeneic hematopoietic stem cell transplantation, J. Hematol. Oncol. 14 (2021) 82.
[30] M. Gurney, A. Stikvoort, E. Nolan, L. Kirkham-McCarthy, S. Khoruzhenko, R. Shivakumar, S. Zweegman, N.W.C.J. van de Donk, T. Mutis, E. Szegezdi, S. Sarkar, M. O'Dwyer, CD38 knockout natural killer cells expressing an affinity optimized CD38 chimeric antigen receptor successfully target acute myeloid leukemia with reduced effector cell fratricide, Haematologica 107 (2022) 437–445.
[31] J. Naik, M. Themeli, R. de Jong-Korlaar, R.W.J. Ruiter, P.J. Poddighe, H. Yuan, J.D. de Bruijn, G.J. Ossenkoppele, S. Zweegman, L. Smit, T. Mutis, A.C.M. Martens, N. van de Donk, R.W.J. Groen, CD38 as a therapeutic target for adult acute myeloid leukemia and T-cell acute lymphoblastic leukemia, Haematologica 104 (2019) e100–e103.
[32] N.J. Buteyn, K. Fatehchand, R. Santhanam, H. Fang, G.M. Dettorre, S. Gautam, B.K. Harrington, S.E. Henderson, G. Merchand-Reyes, X. Mo, D.M. Benson, W.E. Carson III, S. Vasu, J.C. Byrd, J.P. Butchar, S. Tridandapani, Anti-leukemic effects of all-trans retinoic acid in combination with daratumumab in acute myeloid leukemia, Int. Immunol. 30 (2018) 375–383.
[33] I.S. Nijhof, R.W.J. Groen, H.M. Lokhorst, B. van Kessel, A.C. Bloem, J. van Velzen, R. de Jong-Korlaar, H. Yuan, W.A. Noort, S.K. Klein, A.C.M. Martens, P. Doshi, K. Sasser, T. Mutis, N.W.C.J. van de Donk, Upregulation of CD38 expression on multiple myeloma cells by all-trans retinoic acid improves the efficacy of daratumumab, Leukemia 29 (2015) 2039–2049.
[34] N. Yu, Y. Zhang, J. Li, W. Gu, S. Yue, B. Li, F. Meng, H. Sun, R. Haag, J. Yuan, Z. Zhong, Daratumumab immunopolymersome-enabled safe and CD38-targeted

chemotherapy and depletion of multiple myeloma, Adv. Mater. 33 (2021) 2007787.
[35] C. Zhou, Y. Xia, Y. Wei, L. Cheng, J. Wei, B. Guo, F. Meng, S. Cao, J.C.M. van Hest, Z. Zhong, GE11 peptide-installed chimaeric polymersomes tailor-made for high-efficiency EGFR-targeted protein therapy of orthotopic hepatocellular carcinoma, Acta Biomater. 113 (2020) 512–521.
[36] X. Xin, F. Lin, Q. Wang, L. Yin, R.I. Mahato, Ros-responsive polymeric micelles for triggered simultaneous delivery of PLK1 inhibitor/mir-34a and effective synergistic therapy in pancreatic cancer, ACS Appl. Mater. Interfaces 11 (2019) 14647–14659.
[37] Q. Fan, Y. Liu, G. Cui, Z. Zhong, C. Deng, Brain delivery of Plk1 inhibitor via chimaeric polypeptide polymersomes for safe and superb treatment of orthotopic glioblastoma, J. Control. Release 329 (2021) 1139–1149.
[38] M. Reda, W. Ngamcherdtrakul, M.A. Nelson, N. Siriwon, R. Wang, H.Y. Zaidan, D.S. Bejan, S. Reda, N.H. Hoang, N.A. Crumrine, J.P.C. Rehwaldt, A. Bindal, G.B. Mills, J.W. Gray, W. Yantasee, Development of a nanoparticle-based immunotherapy targeting PD-L1 and PLK1 for lung cancer treatment, Nat. Commun. 13 (2022) 4261.
[39] J. Marvin-Peek, B.N. Savani, O.O. Olalekan, B. Dholaria, Challenges and advances in chimeric antigen receptor therapy for acute myeloid leukemia, Cancers 14 (2022) 497.
[40] F.S. Lichtenegger, C. Krupka, S. Haubner, T. Kohnke, M. Subklewe, Recent developments in immunotherapy of acute myeloid leukemia, J. Hematol. Oncol. 10 (2017) 142.
[41] T. Yoshida, K. Mihara, Y. Takei, K. Yanagihara, T. Kubo, J. Bhattacharyya, C. Imai, T. Mino, Y. Takihara, T. Ichinohe, All-trans retinoic acid enhances cytotoxic effect of T cells with an anti-CD38 chimeric antigen receptor in acute myeloid leukemia, Clin. Transl. Immunol. 5 (2016) e116.
[42] J. Van den Bossche, F. Lardon, V. Deschoolmeester, I. De Pauw, J.B. Vermorken, P. Specenier, P. Pauwels, M. Peeters, A. Wouters, Spotlight on volasertib: preclinical and clinical evaluation of a promising Plk1 inhibitor, Med. Res. Rev. 36 (2016) 749–786.
[43] U. Tontsch-Grunt, D. Rudolph, I. Waizenegger, A. Baum, D. Gerlach, H. Engelhardt, M. Wurm, F. Savarese, N. Schweifer, N. Kraut, Synergistic activity of BET inhibitor BI 894999 with PLK inhibitor volasertib in AML in vitro and in vivo, Cancer Lett. 421 (2018) 112–120.
[44] C. Larrue, E. Saland, H. Boutzen, F. Vergez, M. David, C. Joffre, M.-A. Hospital, J. Tamburini, E. Delabesse, S. Manenti, J.E. Sarry, C. Récher, Proteasome inhibitors induce FLT3-ITD degradation through autophagy in AML cells, Blood 127 (2016) 882–892.
[45] X.N. Qiao, J. Ma, T. Knight, Y.W. Su, H. Edwards, L. Polin, J. Li, J. Kushner, S.H. Dzinic, K. White, J. Wang, H. Lin, Y. Wang, L.P. Wang, G. Wang, J.W. Taub, Y.B. Ge, The combination of CUDC-907 and gilteritinib shows promising in vitro and in vivo antileukemic activity against FLT3-ITD AML, Blood Cancer J. 11 (2021) 111.
[46] W. Gu, T. Liu, D. Fan, J. Zhang, Y. Xia, F. Meng, Y. Xu, J.J.L.M. Cornelissen, Z. Liu, Z. Zhong, A6 peptide-tagged, ultra-small and reduction-sensitive polymersomal vincristine sulfate as a smart and specific treatment for CD44+ acute myeloid leukemia, J. Control. Release 329 (2021) 706–716.
[47] H. Cai, M. Kondo, L. Sandhow, P. Xiao, A.-S. Johansson, T. Sasaki, J. Zawacka-Pankau, K. Tryggvason, J. Ungerstedt, J. Walfridsson, M. Ekblom, H. Qian, Critical role of lama4 for hematopoiesis regeneration and acute myeloid leukemia progression, Blood 139 (2022) 3040–3057.
[48] M. Yamashita, P.V. Dellorusso, O.C. Olson, E. Passegué, Dysregulated haematopoietic stem cell behaviour in myeloid leukaemogenesis, Nat. Rev. Cancer 20 (2020) 365–382.
[49] F. Kong, H.Y. Bai, M. Ma, C. Wang, H.Y. Xu, N. Gu, Y. Zhang, Fe_3O_4@Pt nanozymes combining with CXCR4 antagonists to synergistically treat acute myeloid leukemia, Nano Today 37 (2021) 101106.
[50] Y. Yan, X. Gao, S. Zhang, Y. Wang, Z. Zhou, J. Xiao, Q. Zhang, Y. Cheng, A carboxyl-terminated dendrimer enables osteolytic lesion targeting and photothermal ablation of malignant bone tumors, ACS Appl. Mater. Interfaces 11 (2019) 160–168.
[51] Q. Qiao, L. Xu, Q. Li, Y. Wang, H. Lu, N. Zhao, Y. Pu, L. Wang, Y. Guo, C. Guo, Bone morphogenetic protein receptor 1α promotes osteolytic lesion of oral squamous cell carcinoma by SHH-dependent osteoclastogenesis, Cancer Sci. 113 (2022) 1639–1651.
[52] G.R. Mundy, Metastasis to bone: causes, consequences and therapeutic opportunities, Nat. Rev. Cancer 2 (2002) 584–593.