Targeting Wnt/β-catenin signaling using XAV939 nanoparticles in tumor microenvironment-conditioned macrophages promote immunogenicity

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ABSTRACT

The aberrant activation of Wnt/β-catenin signaling in tumor cells and immune cells in the tumor microenvironment (TME) promotes malignant transformation, metastasis, immune evasion, and resistance to cancer treatments. The increased Wnt ligand expression in TME activates β-catenin signaling in antigen (Ag)-presenting cells (APCs) and regulates anti-tumor immunity. Previously, we showed that activation of Wnt/β-catenin signaling in dendritic cells (DCs) promotes induction of regulatory T cell responses over anti-tumor CD4$^+$ and CD8$^+$ effector T cell responses and promotes tumor progression. In addition to DCs, tumor-associated macrophages (TAMs) also serve as APCs and regulate anti-tumor immunity. However, the role of β-catenin activation and its effect on TAM immunogenicity in TME is largely undefined. In this study, we investigated whether inhibiting β-catenin in TME-conditioned macrophages promotes immunogenicity. Using nanoparticle formulation of XAV939 (XAV-Np), a tankyrase inhibitor that promotes β-catenin degradation, we performed *in vitro* macrophage co-culture assays with melanoma cells (MC) or melanoma cell supernatants (MCS) to investigate the effect on macrophage immunogenicity. We show that XAV-Np-treatment of macrophages conditioned with MC or MCS significantly upregulates the cell surface expression of CD80 and CD86 and suppresses the expression of PD-L1 and CD206 compared to MC or MCS-conditioned macrophages treated with control nanoparticle (Con-Np). Further, XAV-Np-treated macrophages conditioned with MC or MCS significantly increased IL-6 and TNF-α production, with reduced IL-10 production compared to Con-Np-treated macrophages. Moreover, the co-culture of MC and XAV-Np-treated macrophages with T cells resulted in increased CD8$^+$ T cell proliferation compared to Con-Np-treated macrophages. These data suggest that targeted β-catenin inhibition in TAMs represents a promising therapeutic approach to promote anti-tumor immunity.

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1. Introduction

Melanoma is a highly metastatic and fatal tumor responsible for most skin cancer-associated deaths [1,2]. Melanoma can be treated successfully with an improved survival rate if diagnosed in the early stages [3–7]. Conversely, highly aggressive melanomas with metastasis to distant secondary locations are very difficult to treat, leading to a significantly diminished median survival rate [3–7]. Conventional melanoma therapies include surgical dissection, chemotherapy, and radiation therapy [3–5]. However, these therapeutic strategies cause severe systemic side effects, fail to induce protective long-term anti-tumor immunity, and are moderately successful in late-stage metastatic melanomas [3–5,7]. Recent advances in cancer immunotherapy, particularly immune checkpoint blockade (ICB) therapies targeting co-inhibitory receptors and ligands such as PD-1 (nivolumab), CTLA4 (ipilimumab), and PD-L1 (atezolizumab), have transformed melanoma treatment with a significant increase in median survival rate [5,7–11]. However, only a subset of patients with active T cell infiltration in the TME benefits from these immunotherapies [5,8–10,12,13]. The partial effectiveness of ICB immunotherapies in a subgroup of patients is attributed to the reduced infiltration and induction of tolerance in TME DCs, which results in reduced activation of effector T cell responses [12–16]. Thus, there is an urgent need to develop novel combinatorial ICB therapeutic approaches that can promote APC immunogenicity, T-cell infiltration and activation, anti-tumor immunity, and effectiveness in a broad pool of patients, refractory to mono/combination ICB therapies.

Tumors employ multiple molecular and cellular mechanisms to evade anti-tumor immunity. These include apoptotic over necrotic immunogenic cell death (ICD), production of immunoregulatory molecules, suppression of APCs and T cell-mediated effector anti-tumor immune responses [15,17–22]. Our past studies have shown that melanoma activates Wnt/β-catenin signaling in tumor resident DCs to induce tumor immune tolerance and suppresses host anti-tumor immunity [23,24]. The Wnt/β-catenin signaling pathway is essential for normal cell growth, survival, proliferation, and immune cell function [25,26]. Wnts are the secretary glycoproteins that bind to the Frizzled receptors (Fzd) and activate the canonical β-catenin signaling pathway [27]. Wnt-mediated canonical signaling promotes β-catenin accumulation in the cytoplasm, followed by its nuclear translocation to initiate the targeted gene expression [25, 27]. Our past studies have shown that increased Wnt ligands expression in TME and tumor-draining lymph nodes (TDLNs) activates β-catenin in DCs, causing increased retinoic acid (RA) and IL-10 production, whereas diminished inflammatory cytokine production (IL-6, IL-23p19, IL-12p35, and TNF-α) [23,24,28]. This differential cytokine production by DCs in TME/TDLNs promotes the induction of Treg over cytotoxic effector CD4$^+$ and CD8$^+$ T cells responses [24]. However, whether increased Wnt ligands in TME regulate TAMs-mediated anti-tumor responses is still poorly defined.

TAMs promote tumor progression through numerous mechanisms, including angiogenesis, metastasis, the release of growth factors, and suppression/evasion of anti-tumor immune responses [29,30]. Indeed, the increased infiltration of TAMs in TME of many different types of tumors is associated with poor prognosis [31–34]. Tumors promote an alternatively activated M2 phenotype in TME macrophages to promote angiogenesis and inhibit anti-tumor immunity [35,36]. Due to their plastic nature, TAMs can be reprogrammed to an immunogenic phenotype, which represents a promising therapeutic target in cancer management, including melanomas [30,37–39]. Thus, understanding the molecular and cellular mechanisms in TAMs that promote tumor malignancy and suppression of anti-tumor immunity can identify novel therapeutic targets to develop better, safe, and more effective anti-tumor therapies against many deadly cancers. Our past studies demonstrated that β-catenin signaling in TME APCs suppresses the induction of CD4$^+$ and CD8$^+$ T cell-mediated anti-tumor immunity [23,24]. Further, a recent study showed that activation of β-catenin signaling in tumor cells suppresses the expression of CCL4, a chemokine that promotes the migration of CD103$^+$ DCs, monocytes, and T cells in TME [16]. Thus, active β-catenin signaling in tumor cells regulates APCs and T cell migration and is associated with resistance to ICB therapies [40,41]. Although the role of Wnt/β-catenin signaling in DCs in the induction of immune tolerance and CD8$^+$ T-cell exclusion in TME is known, whether β-catenin signaling regulates the immunogenicity of TAMs and T-cell mediated immunity remains poorly understood.

In this study, we used a nanoparticle-based formulation of XAV939, a small molecule inhibitor of tankyrase, to investigate whether inhibiting β-catenin in macrophages from TME can promote immunogenicity. We used FDA-approved ᴅ-α-tocopheryl polyethylene glycol 1000 succinate (TPGS), a non-ionic surfactant synthesized from Vitamin E and PEG 1000 esterification [42], to generate XAV-Np. TPGS is a potent solubilizer and emulsifier, enhances cellular uptake, acts as an adjuvant, and is commonly used to formulate anti-cancer drugs for nanoparticle-based delivery approaches [43–47]. Using bone-marrow-derived macrophages (BMDMs) co-culture with MC or MCS, we show that XAV-Np promotes immunogenicity in TME-conditioned macrophages by suppressing the cell surface expression of PD-L1 and CD206 and increased expression of CD80 and CD86 compared to Con-Np-treated TME-conditioned macrophages. Further, XAV-Np-treatment significantly increased the production of TNF-α and IL-6, whereas it suppressed IL-10 production by TME-conditioned macrophages. Further, co-culture of XAV-Np-treated TME-conditioned macrophages with T cells showed increased CD8$^+$ T cell proliferation compared to Con-Np-treated TME-conditioned macrophages. Collectively, these data suggest that β-catenin inhibition in TAMs can promote anti-tumor immunity by promoting macrophage immunogenicity and increased CD8$^+$ T cell-mediated anti-tumor responses. Since, active β-catenin signaling in TME (melanoma cells and DCs) is linked with T cell-deficient phenotype and resistance to ICB therapies [16], combining ICB with targeted delivery of XAV-Np to TAMs and DCs could promote immunogenicity, T-cell infiltration, and promote anti-tumor immunity in patients showing resistance to currently approved ICB therapies.

2. Material and methods

2.1. Materials: cell lines & mouse

B16F10 mouse melanoma cell line was cultured in complete DMEM (Dulbecco's Modified Eagle's Medium, with 4.5 g/L Glucose,

10% heat-inactivated FBS, 2 mM L-glutamine, HEPES, Sodium pyruvate, Sodium bicarbonate, 2-ME, MEM Non-essential amino acid, strepto-penicillin) at 37 °C in CO_2 (5%) incubator. C57BL/6 mice (6–8 weeks) were purchased from Jackson Laboratory (Bar Harbor, ME). Mice were maintained at the animal facility in the College of Veterinary medicine, Auburn University, Auburn, AL.

2.2. Drugs, reagents, and antibodies

XAV939 was purchased from Selleckchem (Catalog #S1180). The following dyes/conjugated antibodies were used for the flow cytometry analysis. LIVE/DEAD™ Fixable Yellow Dead Cell Stain Kit (Cat: L34959; Invitrogen) was used to exclude dead cells. Cell surface staining was performed using Biolegend® antibodies such as CD11b-PerCPCy5.5 (M1/70), CD80-BV421 (16-10A1), CD86-APC (GL-1), CD206-FITC (C068C2) and PDL1-PECy7 (10F.9G2) or Isotypes, Mouse IgG1, κ - PECy7 or BV421, and Rat IgG2a, κ conjugated with FITC or APC. Anti-mouse CD16/CD32 monoclonal antibody (clone 93) (eBioscience™) was used as an Fc blocker.

2.3. Preparation and characterization of XAV-Np and Con-Np

XAV939-loaded micelles were prepared using the film-dispersion method described previously [48]. At first, 10 mg XAV939 was dissolved in the 4 mL mixed solvent (1 mL acetone and 3 mL DCM), then sonicated for 10 minutes to fully dissolve the drug. 1 mL of drug mixed with 1 mL of 37.5 mg/mL tocopheryl polyethylene glycol 1000 succinate (TPGS). Then the mixture was rotor evaporated in a round-bottom flask at 42 °C. After that, PBS was used to hydrate the formed homogeneous thin drug−polymer film. The loading concentrations of XAV939 in the micelles were quantified by UV-VIS absorbance using Nanodrop. Dynamic laser scattering (DLS) (Zetasizer, Malvern) was used to measure the size of XAV-Np and Con-Np. The morphology of the XAV-Np and Con-Np was analyzed using a high-resolution transmission electron microscope (HRTEM) (FEI Tecnai F-20). Further, the size change of XAV-Np and Con-Np in PBS containing FBS (10%) was determined at 25 °C and 37 °C at different time points.

2.4. Bone marrow-derived macrophages (BMDMs) culture

We isolated and differentiated the bone marrow (BM) cells as described previously with slight modifications [49]. Briefly, bone marrow from mouse femurs and tibia was flushed with PBS using a syringe with a 27-gauge needle. A single-cell suspension was prepared by passing the suspension several times through a 70-μm cell strainer using a syringe with a 23-gauge needle. ACK lysis buffer was used to lyse red blood cells in the suspension. BM single cells were resuspended with L929 conditioned medium (20% L929 cell supernatant, RPMI supplemented with 10% heat-inactivated FBS), counted, and seeded into the cell culture flasks. The flasks were maintained for 7 days at 37 °C in a CO_2 (5%) incubator. L929 conditioned media was replenished every 3 days. On day 7 post culture, adhered macrophages were harvested using 5 mM EDTA in PBS solution.

2.5. TME-conditioned macrophage co-culture assays

Past studies have shown that tumor, stromal, and other immune and non-immune cells in TME secrete Wnt ligands which act in an autocrine and paracrine manner to activate β-catenin signaling [26,50]. To mimic *in vivo* TME and TAMs, we treated BMDMs with MC or MCS and performed co-culture assays using widely-used and well-accepted *in vitro* co-culture assays as described previously with slight modifications [51,52]. Briefly, BMDMs (1×10^5 cells/well) were seeded in a 96-well low-binding tissue culture plate. BMDM were treated with XAV-Np or Con-Np for 1–2 hours. Next, the MC or the MCS (obtained from melanoma cells cultured for 7 days) was added to the BMDMs for 3 hours to generate TME-conditioned macrophages. The BMDMs were further stimulated with lipopoly-saccharide (LPS) (100 ng/mL) for 20 hours to promote the expression of co-stimulatory/co-inhibitory ligands on the cell surface and stimulate cytokine production. After incubation, the cell culture supernatants were collected to measure the cytokines, and the cells were used for flow cytometry analysis.

In a separate experiment, MCs (5×10^4 cells/well) were treated with XAV-Np or Con-Np for 20 hours. The MCs were washed 4 times with PBS and co-incubated with BMDMs (1×10^5 cells/well). Cells were further stimulated with LPS and incubated for another 20 hours in L929 conditioned media at a 37 °C incubator supplemented with 5% CO_2. Co-culture assay supernatants were collected and stored at −20 °C until further use for cytokine analysis by ELISA. Next, cells were washed with PBS and stained with LIVE/DEAD stain. TME-conditioned macrophages were blocked using anti-mouse CD16/CD32 monoclonal antibody (clone 93) (eBioscience™). The cell surface staining was performed using CD11b-PerCPCy5.5 (M1/70), CD80-BV421 (16-10A1), CD86-APC (GL-1), CD206-FITC (C068C2) and PDL1-PECy7 (10F.9G2) or isotypes, mouse IgG1, κ - PECy7 or BV421, and Rat IgG2a, κ antibodies conjugated with FITC or APC. Cells were acquired using a BD LSR II flow cytometer, and the data analysis was performed using FlowJo software (Tree Star, Ashland, OR).

2.6. ELISA

Cell culture supernatants from the TME-conditioned macrophage co-culture assays were analyzed for immunogenic (IL-6 and TNF-α) and immunoregulatory (IL-10) cytokines using commercially available ELISA kits according to the manufacturer's protocols (Invitrogen).

2.7. T cells proliferation assay

BMDMs (2×10^4 cells/well) were co-cultured with MC (4×10^4 cells/well) in the presence or absence of XAV-Np (2 μM) and Con-Np for 16 hours. Next, co-cultured cells were stimulated with LPS (5 ng/mL) for 4 hours, followed by four times washing with PBS. The total T cells were isolated from the mouse spleen and lymph nodes per the manufacturer's protocol (STEMCELL technologies, CA). Next, carboxyfluorescein succinimidyl ester (CFSE; Invitrogen)-labeled T cells (1×10^5 cells/well) were co-cultured with the TME-conditioned macrophages treated with Con-Np or XAV-Np for 96 hours at 37 °C. Finally, cells from co-culture assays were washed and stained with CD4-APC (GK1.5) and CD8-PE-Cy7 (53–6.7) antibodies (Invitrogen) followed by acquisition using a BD LSR II flow cytometer and data analysis to characterize T-cell proliferation using FlowJo software (Tree Star, Ashland, OR).

2.8. Statistics

GraphPad Prism (GraphPad Software Inc., San Diego, CA) was used for statistical analysis. Statistical significance was calculated using a non-parametric student's *t*-test or one-way ANOVA with Tukey's multiple comparisons post hoc test. All experiments were repeated at least 2 times independently. The error bars show mean ± SEM. The differences between different groups were considered statistically significant at *p < 0.05, **p < 0.01, ***p < 0.001, and ****p < 0.0001.
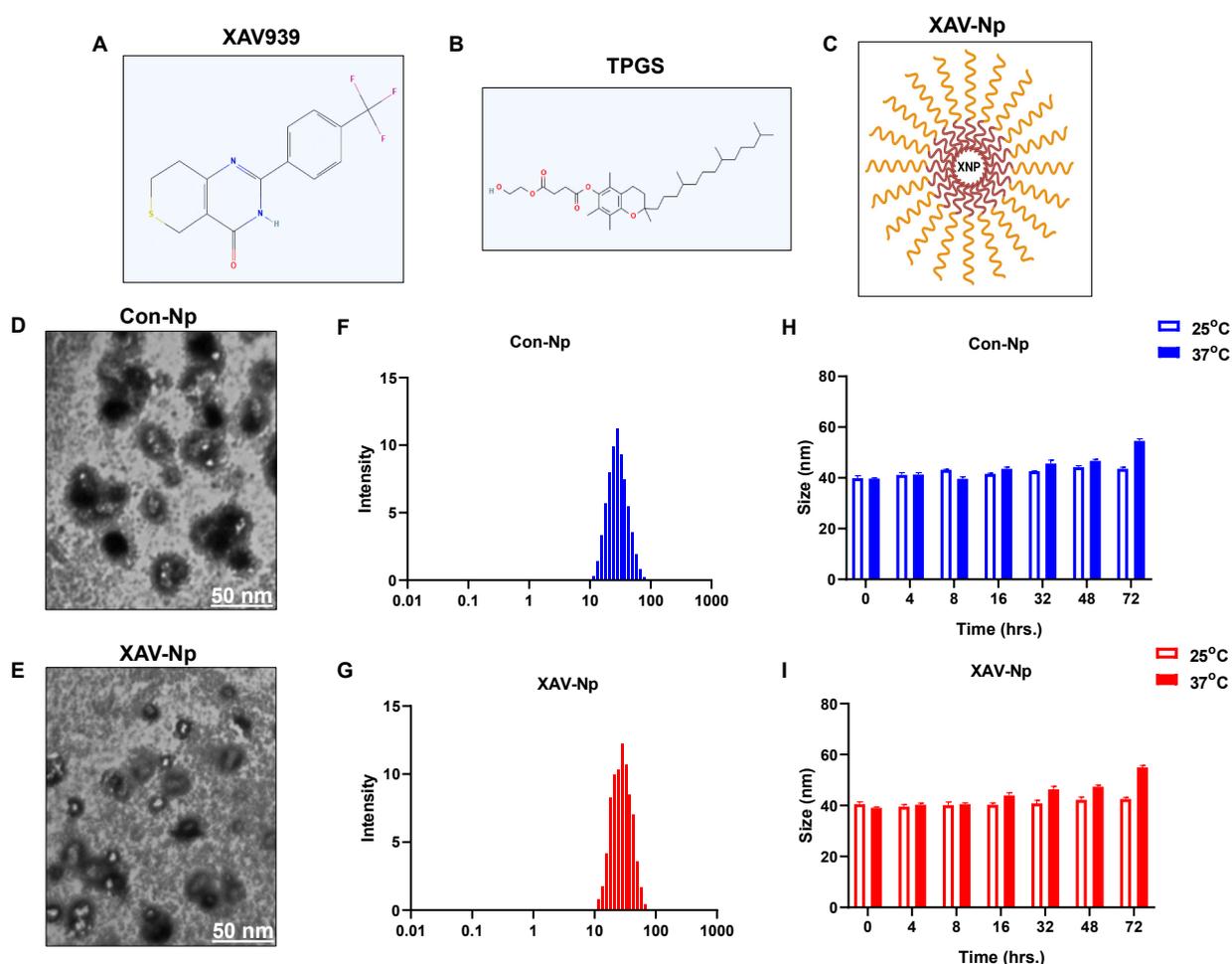
Fig. 1. Synthesis and the characterization of XAV-Np and Con-Np. (A) Chemical structure of XAV939. **(B)** Chemical structure of TPGS. **(C)** Structure of XAV-Np. Morphological analysis of the Con-Np **(D)** and XAV-Np **(E)** by HRTEM (magnification- 10000). Dynamic light scattering analysis of Con-Np **(F)** and XAV-Np **(G)** showing the size of 40.6 ± 0.13 nm and 41.7 ± 0.18 nm, respectively. The stability of Con-Np **(H)** and XAV-Np **(I)** in serum-containing PBS was determined by the change in particle size (nm) at 25 °C and 37 °C for different time intervals. *n* = 3, Error bar shown mean ± SEM.

3. Results

3.1. Formulation and characterization of XAV-Np and Con-Np

Our recent studies demonstrated that increased Wnt ligands expression in TME promotes immune tolerance by activating β-catenin signaling in DCs [23,24]. We showed that the pharmacological inhibition of β-catenin during melanoma progression using XAV939 significantly suppresses tumor growth through increased induction of effector $CD4^+$ and $CD8^+$ T cell responses over immunoregulatory Treg responses [24]. The XAV939 is a small molecule inhibitor of tankyrase (Fig. 1A), an enzyme involved in stabilizing Axin in Wnt/β-catenin signaling [53]. Axin is a scaffolding protein critical for assembling the destruction complex that promotes the degradation of the β-catenin [53]. XAV939 induces apoptosis in cancer cells and has been used as an anti-cancer agent [54–60]. Due to low water solubility, XAV939 is dissolved in organic solvents such as DMSO for various *in vitro* and in *vivo* studies [24,61–63]. However, these organic solvents are toxic and cause many systemic side effects [64,65]. Past studies have shown that TPGS-based nanoparticle formulation of small molecule drugs enhances cellular uptake by macrophages and tumor cells and can be synthesized using cell-specific ligands for targeted delivery to minimize off-target side effects [66,67]. TPGS is amphiphilic and promotes intracellular uptake through endocytosis [43–47]. Thus, we used a TPGS-based nanoparticle (Fig. 1B–C) micelle formulation as described in our previous studies [68,69]. We formulated XAV-Np and empty nanoparticles (Con-Np) micelles using a film-dispersion method, as described in our recent publications [48,60,68]. We analyzed the morphology of the Con-Np and XAV-Np using HRTEM (Fig. 1D and E). Next, we used dynamic laser scattering (DLS) to measure the size of Con-Np and XAV-Np. As shown in Fig. 1F and G, Con-Np had a size of 40.6 ± 0.13 nm, and XAV-Np had a size of 41.7 ± 0.18 nm. Further, we measured the serum stability of both Con-Np and XAV-Np at 25 °C and 37 °C for 72 hours in 10% serum-containing media. Both nanoparticle formulations were stable with no significant change in size over 72 hours (Fig. 1H and I). These data confirmed the size and stability of XAV-Np and Con-Np, which was used in subsequent TME-conditioned macrophage immunogenicity analysis experiments.
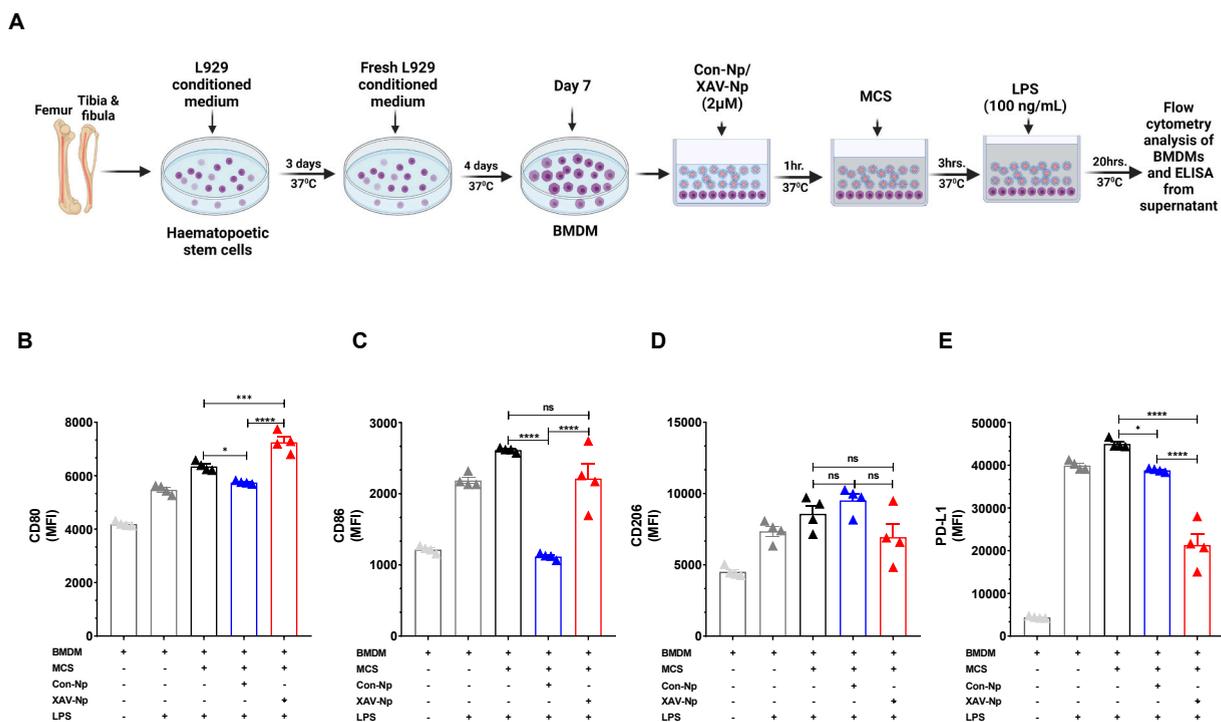
Fig. 2. XAV-Np promotes TME-conditioned macrophage immunogenicity. (A) Schematic diagram of the experiment plan (Created with BioRender.com). Bone marrow stem cells collected from C57BL/6 mice were cultured in L929 conditioned media for 7 days. Differentiated BMDMs were treated with 2 μM of XAV-Np, or Con-Np in the presence or absence of MCS, followed by LPS stimulation (100 ng/mL) for 20 hours. The mean fluorescence intensities (MFI) of co-stimulatory and co-inhibitory molecules on BMDMs surface were analyzed by flow cytometry. Bar graphs indicate the MFIs of CD80 **(B)**, CD86 **(C)**, CD206 **(D)**, and PD-L1 **(E)** in unstimulated macrophages compared to different stimulatory conditions. Data are derived from two independent experiments. $n = 4$, Error bar shown mean $\pm$ SEM. ns, non-significant, *P < 0.05, ***P < 0.001, and ****p < 0.0001 (One-way ANOVA with multiple comparisons).

3.2. XAV-Np treatment differentially regulates the cell surface expression of co-stimulatory and co-inhibitory ligands in TME-conditioned macrophages

For efficient induction of Ag-specific effector CD4^{+} and CD8^{+} T cell responses, activated or mature APCs deliver three signals to naïve T cells. These include i) Ag presentation on MHC molecules (MHC-I for CD8^{+} and MHC-II for CD4^{+} T cells), ii) co-stimulation through increased co-stimulatory ligands (CD80/CD86) expression that bind to co-stimulatory receptors (CD28) on T cells, and iii) increased production of cytokines (IL-6, IL-12, IL-23, IL-4) that defines the differentiation and effector function of CD4^{+} and CD8^{+} T cells [70,71]. Further, APCs regulate the effector functions of activated T cells in the TME [72,73]. However, tumors promote immune evasion through the induction of immunoregulatory over immunogenic phenotype in TDLN and TME APCs [74,75]. Tolerogenic APCs in TME are characterized by reduced co-stimulatory ligands (CD80, CD86) expression, increased co-inhibitory ligands (PD-L1, PD-L2) expression, secretion of immunoregulatory (IL-10, TGF-β, RA) over immunogenic (IL-1β, IL-6, IL-12, IL-23) cytokines, activation of immunoregulatory signaling pathways (IDO, β-catenin), diminished tumor Ag uptake and presentation, and increased Treg over cytotoxic CD4^{+}/CD8^{+} T cell responses [24,76]. In addition to DCs, TAMs also serve as APCs and regulate anti-tumor immunity [72, 77]. Moreover, melanoma promotes CD206 and PD-L1 expression on the TAMs as a part of immune evasion [78,79]. Although our and others' past studies demonstrated that β-catenin inhibition during melanoma progression regulates DCs to promote anti-tumor immunity, the role of β-catenin signaling in TAMs and its effect on TAM immunogenicity is poorly understood [23,24,76]. Thus, to investigate the impact of β-catenin inhibition on macrophage immunogenicity, we performed *in vitro* studies using TME-conditioned macrophages in the presence or absence of XAV-Np and Con-Np. These *in vitro* TME-conditioned macrophages acquire an immuno-suppressive phenotype closely resembling *in vivo* TAMs compared to M2 macrophages generated or stimulated using IL-4/IL-10 [80, 81]. First, we generated BMDMs for 7 days using L929 media and pre-treated these macrophages with XAV-Np or Con-Np for 1 hour in 96 well in a 37 °C incubator (Fig. 2A). Next, to investigate the effect of melanoma cell-secreted Wnt ligands on macrophage immunogenicity and the impact of β-catenin inhibition, we added MCS to these nanoparticle-treated macrophages followed by LPS stimulation for 20 hours. We did not observe detectable cytokine production without LPS stimulation in no treatment and MCS-conditioned macrophages treated with or without XAV-Np or Con-Np (data not shown). Thus, we stimulated TME-conditioned macrophages with toll-like receptor 4 (TLR4) ligand (LPS) to measure cytokine production and phenotypic characterization of cell surface markers using ELISA and flow cytometry, respectively. As shown in Fig. 2B and S1A, we noted a significantly increased CD80 (co-stimulatory ligand) on MCS-conditioned macrophages treated with XAV-Np compared to MCS-conditioned macrophages or MCS-conditioned-Con-Np-treated macrophages. Although we noted a significantly increased expression of CD86 on XAV-Np-treated MCS-conditioned macrophages compared to Con-Np-treated MCS-conditioned macrophages, there was no significant change compared to the control MCS-conditioned macrophage group (Fig. 2C, S1B). Further, we noted a slight decrease in CD206 (mannose receptor, M2 TAM marker) expression in XAV-Np-treated MCS-conditioned macrophages compared to control groups [82]. However, the differences were not statistically significant compared to Con-Np-treated TME-conditioned macrophage or TME-conditioned macrophage groups (Fig. 2D, S1C). In contrast to co-stimulatory ligand expression, we noted a substantial decrease in PD-L1 (co-inhibitory ligand) expression on XAV-Np-treated MCS-conditioned macrophages compared to Con-Np-treated or no treatment MCS-conditioned macrophage groups (Fig. 2E, S1D). Collectively, these data suggest that cancer cell-secreted immunoregulatory factors in TME can act in a paracrine manner to regulate macrophage cell surface phenotype, and the selective inhibition of β-catenin in
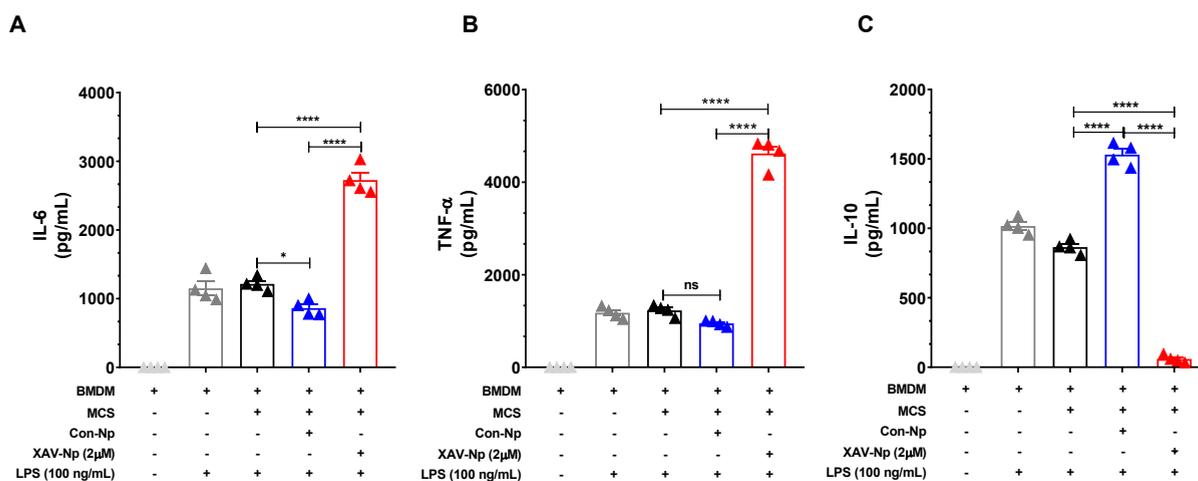
Fig. 3. XAV-Np treatment differentially regulates cytokine production by TME-conditioned macrophages. Differentiated BMDMs were treated with 2 μM of XAV-Np, or Con-Np in the presence or absence of MCS, followed by LPS stimulation (100 ng/mL) for 20 hours. The culture supernatant was used to determine cytokine levels by sandwich ELISA. Bar graphs show the protein levels of IL-6 **(A)**, TNF-α **(B)**, and IL-10 **(C)** in unstimulated BMDM culture supernatant compared to different stimulatory conditions. Data are derived from two independent experiments. $n = 4$, Error bar shown mean ± SEM. ns, non-significant, *P < 0.05, and ****P < 0.0001 (One-way ANOVA with multiple comparisons).

TME macrophages can differentially promote co-stimulatory ligand (CD80) and inhibit co-inhibitory ligand (PD-L1) expression.

3.3. XAV-Np treatment differentially regulates immunoregulatory and immunogenic cytokine production by macrophages conditioned with tumor supernatants

In addition to differential expression of co-stimulatory and co-inhibitory ligands on APCs [15], TME promotes the production of immunoregulatory molecules such as IL-10, RA, and TGF-β to selectively induce Treg responses over effector T cell responses [83]. Recently, we showed Wnt ligands in TME activate β-catenin signaling in DCs to differentially regulate effector versus regulatory T cell responses through increased cell surface expression of co-inhibitory molecules on APCs, increased production of RA, TGF-β, and decreased production of TNF-α, IL-6, and IL-12 by DCs [24]. Therefore, we investigated whether XAV-Np treatment in MCS-conditioned macrophages promotes inflammatory cytokine production over regulatory molecules. As shown in Fig. 3A–B, XAV-Np treatment of MCS-conditioned macrophages significantly promoted the production of IL-6 (Fig. 3A) and TNF-α (Fig. 3B) compared to MCS-conditioned macrophages treated with or without Con-Np. In contrast, we noted a complete inhibition of IL-10 production by XAV-Np-treated MCS-conditioned macrophages compared to control groups (Fig. 3C). These data suggest that
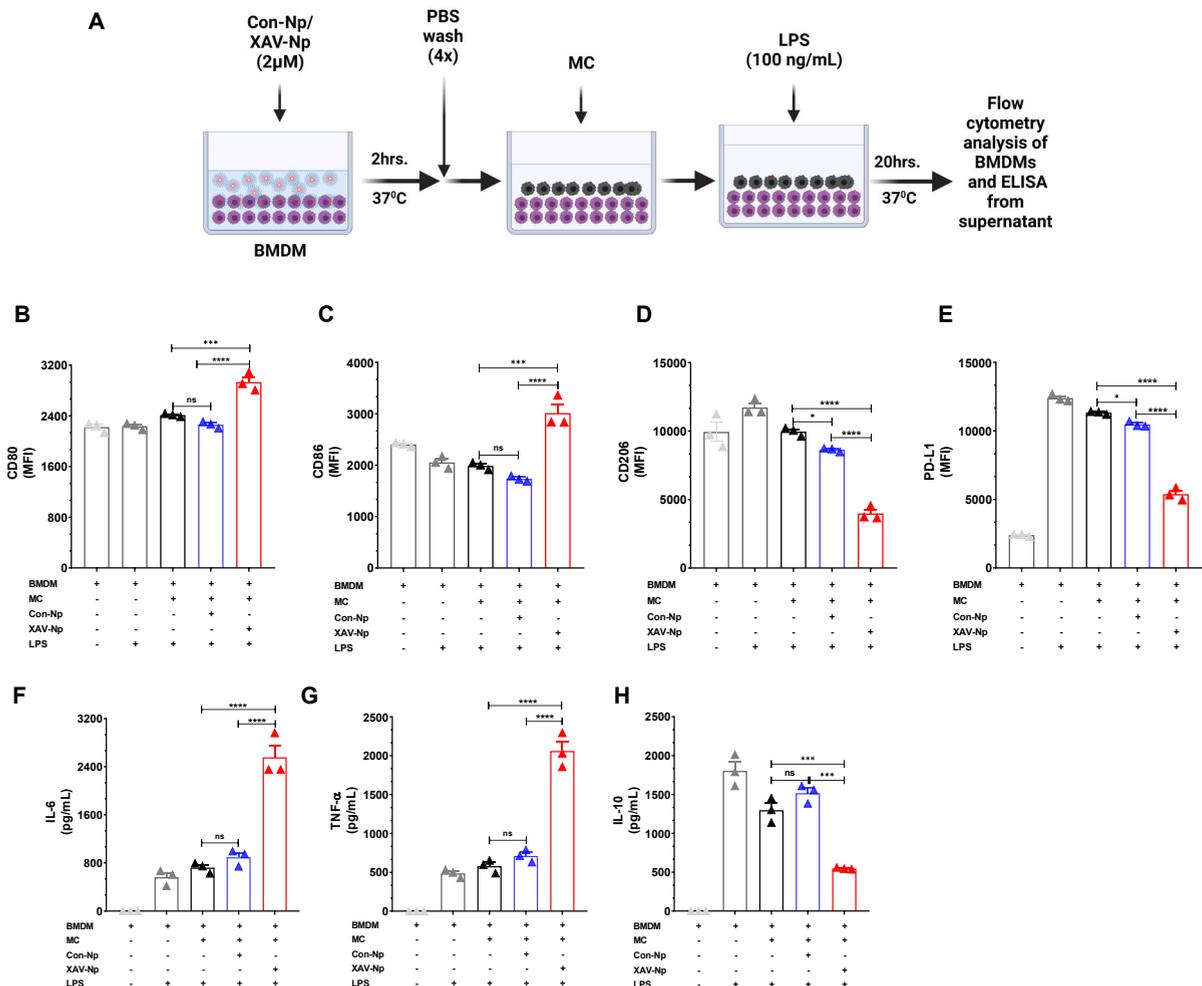
Fig. 4. XAV-Np treatment induces immunogenicity in macrophages co-cultured with MCs. On day 7, after bone marrow stem cells were cultured in L929 conditioned media, differentiated BMDMs were harvested and treated with 2 μM XAV-NPs or Con-NPs for 2 hours. BMDMs were washed with PBS, and MCs were co-cultured with BMDMs, followed by LPS stimulation (100 ng/mL) for another 20 hours. The expression of co-stimulatory and co-inhibitory molecules on the BMDMs was analyzed by flow cytometry. **(A)** Schematic diagram of experiment plan (Created with BioRender.com). Bar graphs indicate the MFIs of CD80 **(B)**, CD86 **(C)**, CD206 **(D)**, and PD-L1 **(E)** in unstimulated macrophages compared to different stimulatory conditions. Culture supernatants were collected from the co-culture assay and were analyzed for cytokine levels by sandwich ELISA. Graphs indicate the protein level of IL-6 **(F)**, TNF-α **(G)**, and IL-10 **(H)** in unstimulated BMDM culture supernatant compared to different stimulatory conditions. Data are derived from two independent experiments. $n = 3$, Error bar shown mean ± SEM. ns, non-significant, *P < 0.05, ***P < 0.001, and ****p < 0.0001 (One-way ANOVA with multiple comparisons).

targeted β-catenin inhibition in TME macrophages using XAV-Np can promote immunogenic cytokine production and overcome TME-induced immunoregulatory cytokine production.

3.4. XAV-Np treatment promotes immunogenic phenotype in macrophages co-cultured with tumor cells

In Figs. 2 and 3 experiments, we investigated the effects of MCS-mediated paracrine β-catenin activation and XAV-Np-mediated β-catenin inhibition in TME macrophage. Macrophages in TME also perform phagocytosis and engulf rapidly proliferating apoptotic tumor cells [84]. Thus, the phagocytosis of tumor cells, Ag processing and presentation on MHC molecules, and cytokine production define the magnitude of tumor-specific effector T-cell responses [85]. Thus, we performed the next series of experiments to elucidate these functions using MC-macrophage co-culture assays. For selective inhibition of β-catenin in macrophages, we pre-treated BMDMs with XAV-Np or Con-Np for 2 hours, followed by multiple washes with PBS to remove nanoparticles. Next, we added MC to macrophages and stimulated co-cultures with LPS for 20 hours (Fig. 4A). We measured macrophage cell surface expression of CD80, CD86, CD206, and PD-L1 by flow cytometry and analyzed cytokine production by ELISA. As shown in Fig. 4B–C, the co-culture of macrophages with MC in the presence of XAV-Np resulted in significantly increased expression of CD80 and CD86 compared to control macrophages co-cultured with MC in the presence or absence of Con-Np. In contrast, we noted a significant reduction in CD206 and PD-L1 expression on XAV-Np-treated macrophages co-cultured with MC compared to no treatment and Con-Np-treated macrophages co-cultured with MC (Fig. 4D–E). The XAV-Np treatment not just differentially regulated the cell surface expression of co-stimulatory
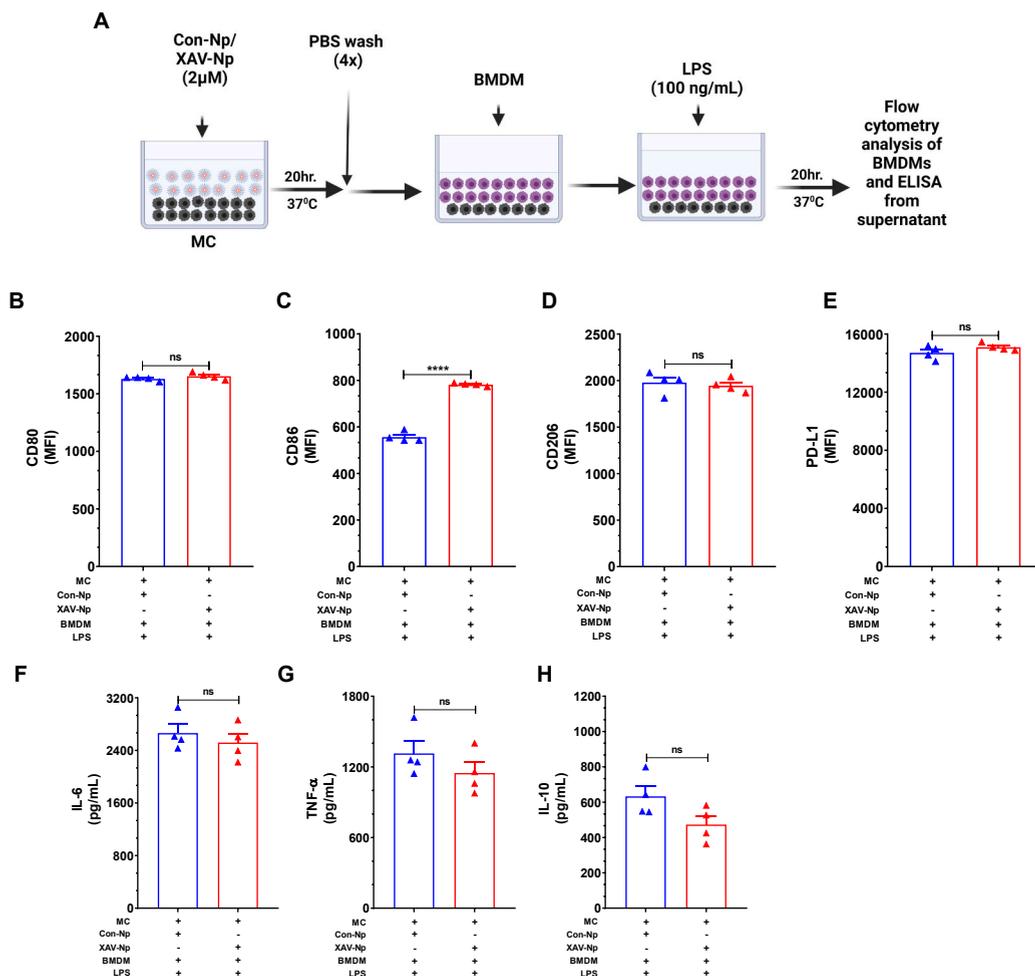
Fig. 5. XAV-Np treatment in MCs regulates macrophage immunogenicity. The MCs were treated with 2 μM XAV-Np or Con-Np. After 20 hours of the treatment, MCs were washed with PBS. Differentiated BMDMs were co-cultured with B16F10 cells, followed by LPS stimulation (100 ng/mL) for 20 hours. The expression of co-stimulatory and co-inhibitory molecules on BMDMs were analyzed by flow cytometry. **(A)** Schematic diagram of experiment plan (Created with BioRender.com). Graphs indicate the MFIs of CD80 **(B)**, CD86 **(C)**, CD206 **(D)**, and PD-L1 **(E)** in macrophages co-cultured with MCs treated with Con-Np or XAV-Np. The culture supernatant obtained from the co-culture assay was analyzed for cytokine levels. Graphs indicate the protein levels of IL-6 **(F)**, TNF-α **(G)**, and IL-10 **(H)** in macrophages co-cultured with MCs treated with Con-Np or XAV-Np. Data are derived from two independent experiments. $n = 4$, Error bar shown mean ± SEM. ns, non-significant, ****$P < 0.0001$ (Student's t-test).

and co-inhibitory molecules but also showed differential effector cytokine production. Similar to earlier MCS co-culture studies, we noted a significantly increased production of IL-6 and TNF-α and reduced IL-10 secretion by XAV-Np-treated macrophages co-cultured with MC compared to no treatment or Con-Np-treated macrophages co-cultured with MC (Fig. 4D–E). Since monocytes from circulation continuously infiltrate the TME, selective β-catenin inhibition in circulatory monocytes/TME macrophages can promote immunogenic phenotype and enhance anti-tumor immunity when macrophages come in direct contact with tumor cells in TME.

3.5. XAV-Np treatment in melanoma cells regulates macrophage immunogenicity

XAV939 inhibits tankyrase 1, which is a member of poly (ADP-ribose) polymerase (PARP) superfamily [53]. Tankyrase 1-mediated poly ADP-ribosylation of Axin promotes Axin-1 degradation and increases intracellular β-catenin levels [53]. Many tumor cells promote the expression of tankyrase 1, resulting in increased β-catenin activation and tumor progression [86]. Furthermore, previous studies have shown that XAV939 treatment inhibits tumor cell proliferation and promotes tumor cell death [60,87,88]. Thus, we explored whether selective inhibition of β-catenin in tumor cells regulates macrophage effector functions in TME. To investigate this, we pre-treated MC with XAV-Np or Con-Np for 20 hours, followed by multiple washes to remove nanoparticles. Next, we co-cultured these nanoparticle-treated MC with BMDMs and analyzed the macrophage cellular phenotype and effector cytokine production post 20 hours of LPS stimulation (Fig. 5A). As shown in Fig. 5C, there was significantly increased CD86 expression on macrophages co-cultured with XAV-Np-treated MC compared to macrophages co-cultured with Con-Np-treated MC. We did not observe any significant differences in the expression of other cell surface markers (CD80, CD206, and PD-L1) or cytokine production (IL-6, TNF-α, and IL-10) by macrophages co-cultured with XAV-Np or Con-Np-treated MC (Fig. 5B, D, E, F, G, and H). These data suggest that although XAV939 has direct anti-cancer properties, inhibiting β-catenin in melanoma cells alone may not be sufficient to increase the macrophage immunogenicity in the TME.
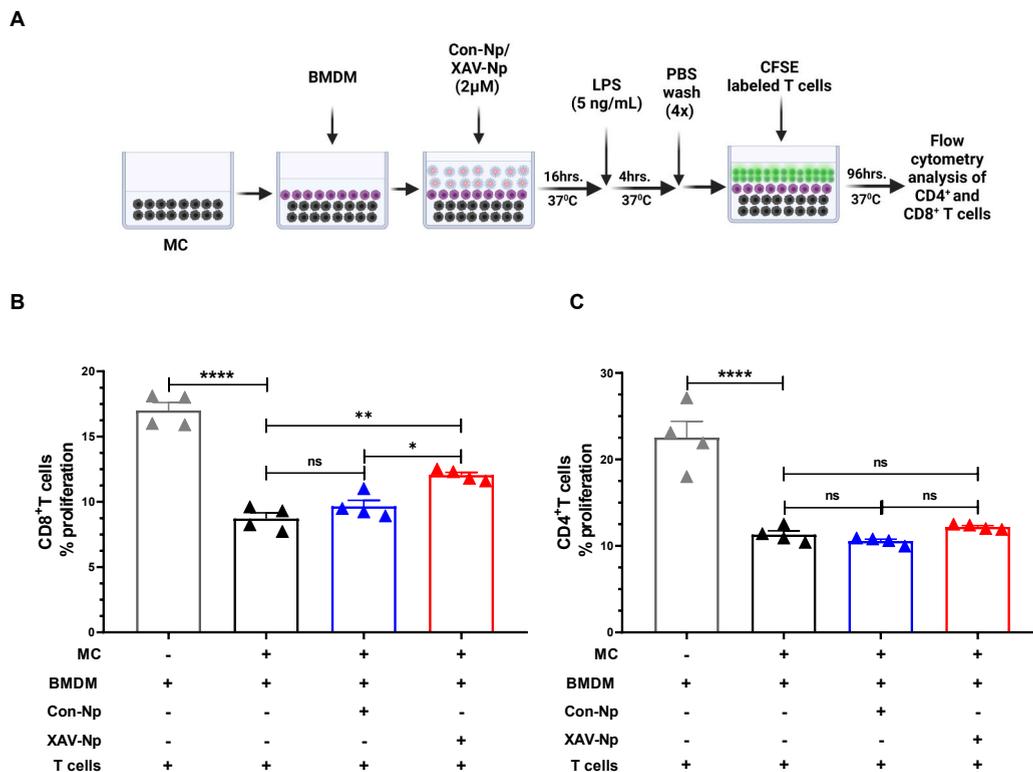
Fig. 6. XAV-Np treatment in TME-conditioned macrophages promotes T-cells proliferation. On day 7 post-culture, differentiated BMDMs were cultured with MCs and treated with XAV-NPs or Con-NPs for 16 hours, Followed by LPS stimulation (5 ng/mL) for another 4 hours. Cells were washed with PBS and incubated with purified CFSE-labeled T cells for 96 hours. The CD4$^+$ and CD8$^+$ T cell proliferation was analyzed by flow cytometry. **(A)** Schematic diagram of the experimental plan (Created with Biorender.com). Bar graphs indicate the percent proliferation of CD8$^+$ T cells **(B)** and CD4$^+$ T cells **(C)**. Data are derived from two independent experiments. $n = 4$, Error bar shown mean ± SEM. ns, non-significant, *P < 0.05, **P < 0.01, and ****P < 0.0001 (One-way ANOVA with multiple comparisons).

3.6. XAV-Np treatment in macrophages promotes T-cell proliferation

Since APCs, including TAMs, in TME capture tumor Ags and present to naïve T cells in TDLNs [89–92], we investigated whether β-catenin inhibition in macrophage-MC co-culture assays affect macrophage-mediated naïve T cell proliferation. To investigate, we co-cultured macrophages (2×10^4 cells/well) with MC (4×10^4 cells/well) in the presence or absence of XAV-Np or Con-Np for 16 hours, followed by LPS stimulation for 4 hours. Next, we added CFSE-labeled naïve T cells (1×10^5 cells/well) and measured T cell proliferation after 4 days (Fig. 6A). As shown in Fig. 6B-C and S2A-B, we noted a significantly diminished T cell proliferation of both CD4$^+$ and CD8$^+$ T cells in the macrophage-MC co-culture group. However, XAV-Np treatment of macrophage-MC co-cultures resulted in significantly increased CD8$^+$ T cell proliferation compared to the Con-Np-treated group (Fig. 6B, S2A). We did not observe any significant differences in CD4$^+$ T cell proliferation between XAV-Np-treated versus Con-Np-treated groups (Fig. 6C, S2B). Collectively, these data indicate that XAV-Np treatment of macrophages during tumor progression could promote anti-tumor CD8$^+$ T cell responses through increased cell surface expression of co-stimulatory ligands, increased immunogenic cytokine production, and tumor Ag uptake and presentation. However, further studies are needed to investigate the impact of XAV-Np on macrophage-mediated tumor Ag uptake, processing, and cross-presentation to CD8$^+$ T cells.

4. Discussion

The involvement of deregulated Wnt/β-catenin signaling in the tumor progression of many cancers has been extensively studied [93,94]. Over the last 10 years, several studies have demonstrated that tumors also activate β-catenin signaling in TME-infiltrating immune cells to evade anti-tumor immunity [16,95,96]. Recent studies from our and other groups indicate that tumors promote
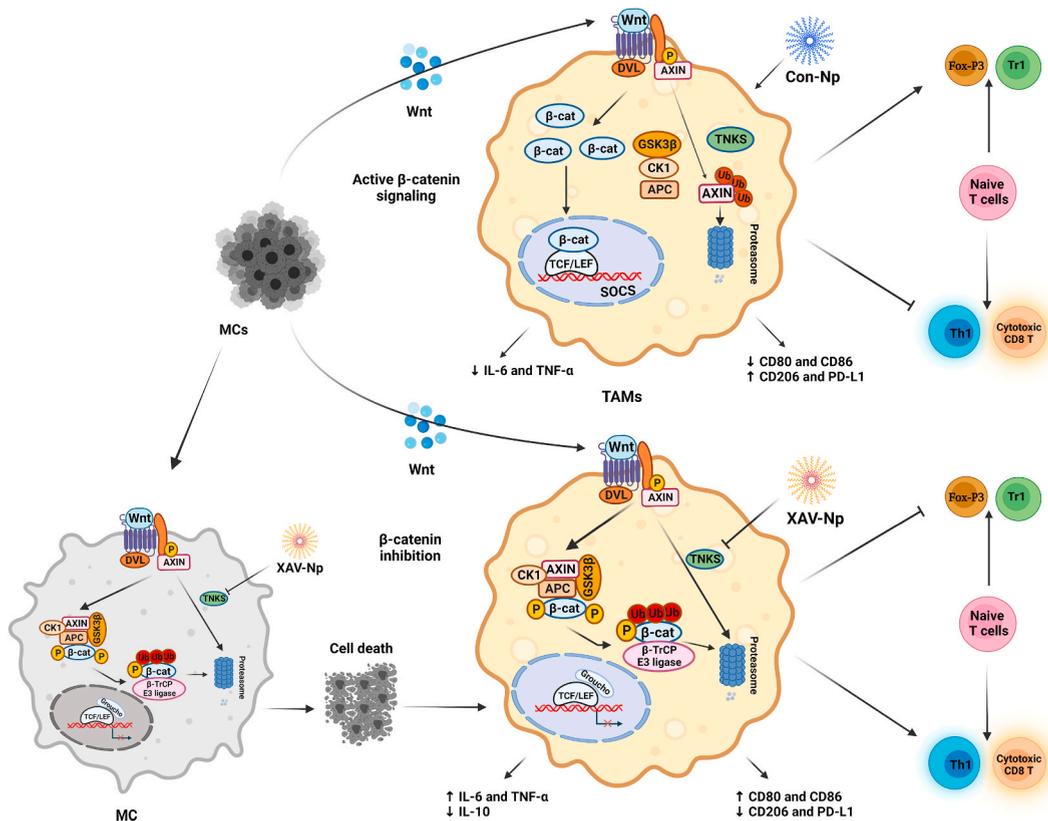
Fig. 7. Schematic illustration depicting the role of XAV-Np in promoting macrophage immunogenicity. In the TME, Wnt secreted by the MCs activates the canonical β-catenin signaling by binding to the Fzd/LRP5/LRP6 receptor complex. Axin, a part of the β-catenin destruction complex, is ubiquitinated by TNKS and degraded in the proteasome. As a result, β-catenin accumulates in the cytoplasm and translocates to the nucleus to transcribe targeted genes. β-catenin promotes an immunosuppressive environment and helps differentiate T regulatory cells (Tr1 and FoxP3$^+$ Treg), inhibiting cytotoxic CD8$^+$ T cells and Th1 cells. It also enhances the expression of the co-inhibitory molecule (PD-L1) and mannose receptor (CD206) and downregulates the co-stimulatory molecules (CD80 and CD86) expression on TAMs (top panel). However, XAV-Np treatment inhibits TNKS and promotes the formation of β-catenin destruction complex leading to β-catenin degradation. Inhibition of β-catenin in TAMs promotes expression of CD80 and CD86, inhibits expression of PD-L1 and CD206, and promotes secretion of IL-6 and TNF-α, leading to increased effector T cell proliferation. Further, XAV-Np treatment can induce melanoma cells death, which further enhances macrophage immunogenicity (Created with BioRender.com).

β-catenin activation in TME and TDLN DCs to induce Treg responses over cytotoxic effector T cell responses [23,24,76]. Further, these studies showed that pharmacological inhibition of the Wnt/β-catenin pathway using small molecule inhibitors suppresses tumor progression [24,[60]76]. In addition to DCs, TAMs also serve as APCs, and the increased infiltration of TAMs in TME is associated with poor patient prognosis. TAMs play a critical role in many solid cancers' tumor progression, angiogenesis, metastasis, and immune evasion [97,98]. However, the role of β-catenin activation in TAMs and its effect on tumor Ag uptake, presentation, and activation of effector CD4^{+} and CD8^{+} T cells responses is poorly defined. Thus, we investigated whether β-catenin suppression can regulate TAM immunogenicity and anti-tumor immunity. Our data demonstrate that the selective inhibition of β-catenin using XAV-Np in TME macrophages can promote immunogenicity and anti-tumor immunity (Fig. 7). We show that XAV-Np treatment of MC or MCS-conditioned macrophages differentially regulates co-stimulatory and co-inhibitory ligand expression on the cell surface and stimulates the production of IL-6 and TNF-α over immunoregulatory IL-10. Further, co-culture assays showed increased CD8^{+} T cell proliferation when macrophages and melanoma cells were treated with XAV-Np. Currently, numerous small molecule regulators of β-catenin signaling have been identified, with several being tested in pre-clinical and clinical trials for many cancer indications [40,99, 100]. Our data suggest that the targeted inhibition of β-catenin using these molecules in TME APCs by employing novel selective drug delivery approaches can overcome tumor-induced immune evasion and represents a promising therapeutic target to promote robust anti-tumor immunity.

The Wnt/β-catenin signaling pathway is an evolutionarily conserved pathway that plays a crucial role in cellular differentiation/ proliferation/survival, normal epithelial cell regeneration and tissue homeostasis, embryogenesis, wound healing, and immune cell functions [101,102]. On the contrary, numerous malignant tumors hijack these Wnt/β-catenin-mediated normal cellular processes and physiological homeostatic responses to promote malignant transformation, metastasis, and immune evasion [50]. Indeed, Wnt/β-catenin signaling is dysregulated in many tumors and is associated with poor prognosis [93,99]. Further, recent studies indicate active β-catenin signaling in tumor cells/TME causes resistance to recently developed and highly effective ICB therapies [16,96]. Wnts are secreted lipid-modified cysteine-rich glycoproteins and act as autocrine and paracrine morphogens to activate canonical β-catenin signaling [103]. Wnt ligands bind to Fzd receptors and recruit co-receptors such as low-density lipoprotein receptor-related proteins (LRP) 5/6 to initiate downstream multiple signaling cascades, including canonical and non-canonical β-catenin activation [103]. In TME, many cells, including malignant tumor cells, stromal cells, and immune cells (DCs and TAMs), secrete Wnt ligands to promote tumorigenesis and immunosuppression [26,50]. Although Wnt/β-catenin signaling in tumor cells is directly involved in malignant transformation, recent studies from our and several other groups suggest that Wnt/β-catenin activation in TME immune cells also promotes tumor growth indirectly through suppression of anti-tumor immunity [23,24,76]. Our past studies showed increased Wnt ligands in TME and local TDLNs reprogram DCs to promote Treg over anti-tumor cytotoxic CD4^{+} and CD8^{+} T cell responses. Mechanistically, these studies identified the critical role of canonical Wnt-LRP5/6-mediated β-catenin activation in DCs, inducing the expression of RA synthesizing enzymes (retinaldehyde dehydrogenase), TGF-β, and IL-10. Further, this study showed that canonical β-catenin signaling in DCs suppresses tumor Ag uptake and cross-presentation to naïve CD8^{+} cells [23]. Our data indicate that selective inhibition of β-catenin in TME-conditioned macrophages differentially regulates the cell surface expression of co-stimulatory and co-inhibitory ligands and promotes immunogenic cytokines (IL-6, TNF-α) over immunoregulatory cytokine (IL-10). Since TME actively suppresses the expression of co-stimulatory ligands (CD80/CD86) and promotes the expression of co-inhibitory ligands (PD-L1/PD-L2) to evade anti-tumor immunity [15], targeted inhibition of β-catenin in TAMs and DCs can enhance anti-tumor immunity through increased CD80/CD86-mediated co-stimulation of naïve T cells in TDLNs and activation of effector T cells in TME.

Currently approved ICB therapies are targeted to block co-inhibitory receptors (blocking CTLA4 binding to CD86 and PD-1 binding to PD-L1) to boost effector T cells-mediated anti-tumor immunity [104]. However, only a subset of patients with active T-cell infiltration in TME benefits from these ICB immunotherapies [8–10,12,13]. In this context, a recent study indicates active β-catenin signaling in melanoma cells suppresses the expression of CCL4, a chemokine critical for infiltration of CD103^{+} DCs and T cells in TME [16]. Past studies have shown a positive correlation between increased expression of CCL4 and other chemokine signatures in melanoma metastases with increased infiltration of immune cells (mature DCs, B, and T cells) and favorable patient prognosis [12,105]. Since CCL4 is produced by many cells, including melanoma cells, epithelial cells, monocytes, T cells, fibroblasts, DCs, etc., further studies are needed to explore the impact of β-catenin inhibition in TME APCs (DCs and TAMs), chemokine production, and effect on CD8^{+} T cell infiltration and resistance to ICB therapies. Our *in vitro* data indicate that selective β-catenin inhibition in MC or MCS-conditioned macrophages not only differentially regulates CD80, CD86, and PD-L1 but also increases the production of IL-6 and TNF-α and suppresses IL-10. Further, the optimum induction of anti-tumor effector cytotoxic CD4^{+} and CD8^{+} T cell responses depends on efficient tumor Ag uptake by APCs, migration to local TDLNs, and presentation to naïve T cells [70,85]. Although the role of Wnt/β-catenin signaling in Ag presentation by DCs and activation of effector T cell responses during tumor progression is well defined [83], the role of β-catenin activation in TAMs and effect on T cell responses is poorly understood. Moreover, the macrophages can perform cross-Ag presentation to CD8^{+} T cells [106]. Accordingly, our *in vitro* data demonstrate that β-catenin inhibition in MC-macrophage co-culture promotes naïve CD8^{+} T cell proliferation. However, we did not observe any significant changes in CD4^{+} T cell proliferation in this assay. This may be explained partly by caveats (discussed in a later section) with our *in vitro* assay and the dominant role of DCs over macrophages in the priming of CD4^{+} T cells [107]. Collectively, our data demonstrate that selective β-catenin inhibition promotes macrophage immunogenicity through increased cell surface expression of CD80/CD86 over PD-L1, increased IL-6 and TNF-α production over IL-10 and enhanced priming of CD8^{+} T cell responses (Fig. 7). This increased immunogenicity in TAMs will promote further activation of effector T cell responses and complement ICB therapies in the patient population refractory to current ICB therapies. Currently, we are exploring a combinatorial therapeutic approach targeting selective β-catenin inhibition in TME APCs in combination with *anti*-PD-1/*anti*-CTLA4 ICB therapies using different mouse models of melanoma progression.

The tumor cell-specific delivery of cytotoxic drugs is a significant clinical challenge for chemotherapy treatment approaches, as off-target delivery results in severe side effects [108–110]. With recent advances in nanotechnology, a wide variety of nanoparticles have been developed for the targeted delivery of anti-cancer drugs [111–114]. Nanoparticles-based delivery of anti-cancer drugs has several advantages, including increased stability and half-life in the serum, high solubility, limited systemic side effects, and extended drug release, etc. [115–119]. Several different types of nanoparticles (gold, polymeric, liposomes, cationic, carbon-based, mannose sugar-coated, CD206 targeting peptide-based) have been developed for TAM-specific targeted delivery of anti-tumor therapies [120–125]. TAMs, particularly M2 macrophages, express a very high-level CD206 (mannose receptor), a scavenger receptor involved in the phagocytosis of pathogens expressing cell surface mannosylated glycoproteins [125,126]. Several recent studies have identified the critical role of CD206$^+$ macrophages in tumor progression through the regulation of innate and adaptive immune responses [78, 82]. Moreover, several peptides, biologics, and nanoparticle-based approaches targeting CD206 have been developed to promote macrophage-specific drug delivery and anti-tumor immunity [78,125,127–131]. CD206, upon ligand engagement (terminal mannosylated glycans or antibody), promotes endocytosis of the target ligand and is continuously shuffled between the cell membrane and endosomal compartments [132]. This CD206-mediated endocytosis represents an ideal target for nanoparticle-mediated delivery of anti-cancer drugs to TAMs. Our *in vitro* studies using TPGS-based XAV-NPs showed a marked downregulation of CD206 on macrophage cell surface compared to control nanoparticle-treated groups, possibly due to endocytosis and the direct effect of β-catenin inhibition on CD206 expression. Mannosylated nanoparticles are widely used to target CD206$^+$ macrophages and promote anti-tumor immunity [127–131]. Based on our studies, we propose a targeted delivery of β-catenin inhibitor to TAMs using mannosylated nanoparticles to revert pro-tumor phenotype and promote anti-tumor activities in macrophages. Further studies are undergoing in our lab to evaluate the efficacy of targeted β-catenin inhibition in a mouse model of melanoma progression using mannosylated nanoparticles in combination with ICB immunotherapy.

Although our data show a strong rationale for targeted inhibition of β-catenin in TAMs, there are several caveats with *in vitro* co-culture studies that need to be explored further using more robust assays or *in vivo* studies. *First*, most *in vitro* assays, including our MC/MCS-conditioned macrophage co-culture assays, are contrived experimental systems and rarely mimic the complex and dynamic interplay of different cells and factors in TME. We pre-treated macrophages or melanoma cells to exclude the effect of β-catenin inhibition on other cell populations. The inhibition of β-catenin in tumor cells inhibits proliferation and promotes cell death [87,88]. Similarly, recently we showed that XAV-Np directly promotes immunogenic cell death (with the extracellular release of DAMPs such as HMGB1 and ATP) and could promote anti-tumor immunity in TME [60]. However, when we pre-treated melanoma cells with XAV-Np followed by co-culture with macrophages, we did not observe a significant increase in macrophage immunogenicity (Fig. 5). This can be partly explained by our rationale to wash XAV-Np-treated melanoma cells (thus washing away DAMPs) before co-culturing with macrophages to exclude the direct effect of XAV-Np on macrophages in the co-culture assay. *Second*, we used BMDM cells for co-culture assays. Although co-culturing BMDMs with tumor cells or tumor cell supernatants mimics *in vivo* immunosuppressive phenotype of TAMs compared to M2 condition (IL-4/IL-10) polarized macrophages [80,81], these cells do not completely mimic primary macrophages or monocytes infiltrating TME. Thus, further *in vivo* studies targeting selective β-catenin inhibition in TAMs needs to be performed for detailed understanding and further confirmation of these data. *Third*, we used LPS to promote maturation and cytokine production by macrophages. LPS binds to TLR4 to activate NF-κB signaling and dominantly induce an inflammatory phenotype in macrophages [133]. Although some rapidly proliferating tumor cells undergo cell death and release DAMPs, this DAMP-mediated APC activation is mild. Thus, further studies are warranted using a mild agonist. *Finally*, we used total T cells to measure the effect of β-catenin inhibition in macrophages-melanoma cell co-culture assay. Although macrophages can cross-present Ags to naïve CD8$^+$ T cells [106], DCs are more efficient and act as professional APCs [134,135]. Recent studies have shown the role of macrophages in TDLNs in cross-priming CD8$^+$ T-cells [92,136]. However, further detailed *in vitro* and *in vivo* studies using naïve Ag-specific CD4$^+$ T cells (OT-II), CD8$^+$ T cells (OT-I), and the effect of selective β-catenin inhibition in macrophages on direct and cross-presentation of tumor Ag (tumor cells expressing Ova) need to be performed.

In summary, our study indicates that targeted inhibition of β-catenin in TME can promote macrophage immunogenicity through differential regulation of CD80, CD86, CD206, and PD-L1 markers on the cell surface (Fig. 7). Also, we show that β-catenin inhibition in MC or MCS-conditioned macrophages suppresses IL-10 production, whereas it increases TNF-α and IL-6 production (Fig. 7). Since CD206$^+$ TAMs can cross-present tumor Ags and promote anti-tumor immunity [137], macrophage-targeted β-catenin inhibition using mannosylated nanoparticles can promote immunogenic phenotype and activate CD4$^+$ and CD8$^+$ T cell-mediated immune responses in TME. Finally, since the activation of Wnt/β-catenin signaling in tumor cells and TME infiltrating immune cells is associated with the suppression of anti-tumor immunity and resistance to current ICB therapies, our data support the notion that selective inhibition of β-catenin in TME APCs (TAMs and DCs) in combination with current ICB therapies can promote anti-tumor immunity, overcome resistance to ICB therapies, and effective in a broad pool of melanoma patients.

Author contribution statement

Chetan Pundkar: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Ferrin Antony; Xuejia Kang: Performed the experiments; Analyzed and interpreted the data.

Amarjit Mishra; R. Jayachandra Babu: Contributed reagents, materials, analysis tools or data.

Pengyu Chen; Feng Li: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Amol Suryawanshi: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Data availability statement

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.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e16688.

References

[1] A.J. Miller, M.C. Mihm, Melanoma, N. Engl. J. Med. 355 (2006) 51–65, https://doi.org/10.1056/NEJMra052166.
[2] B. Bandarchi, C.A. Jabbari, A. Vedadi, R. Navab, Molecular biology of normal melanocytes and melanoma cells, J. Clin. Pathol. 66 (2013) 644–648, https://doi.org/10.1136/jclinpath-2013-201471.
[3] S. Bhatia, S.S. Tykodi, J.A. Thompson, Treatment of metastatic melanoma: an overview, Oncol. Williston Park N. 23 (2009) 488–496.
[4] C.K. Bichakjian, A.C. Halpern, T.M. Johnson, A. Foote Hood, J.M. Grichnik, S.M. Swetter, H. Tsao, V.H. Barbosa, T.-Y. Chuang, M. Duvic, V.C. Ho, A.J. Sober, K.R. Beutner, R. Bhushan, W. Smith Begolka, American Academy of Dermatology, Guidelines of care for the management of primary cutaneous melanoma. American Academy of Dermatology, J. Am. Acad. Dermatol. 65 (2011) 1032–1047, https://doi.org/10.1016/j.jaad.2011.04.031.
[5] H. Sadozai, T. Gruber, R.E. Hunger, M. Schenk, Recent successes and future directions in immunotherapy of cutaneous melanoma, Front. Immunol. 8 (2017) 1617, https://doi.org/10.3389/fimmu.2017.01617.
[6] A.M.M. Eggermont, A. Spatz, C. Robert, Cutaneous melanoma, Lancet Lond. Engl. 383 (2014) 816–827, https://doi.org/10.1016/S0140-6736(13)60802-8.
[7] E. Maverakis, L.A. Cornelius, G.M. Bowen, T. Phan, F.B. Patel, S. Fitzmaurice, Y. He, B. Burrall, C. Duong, A.M. Kloxin, H. Sultani, R. Wilken, S.R. Martinez, F. Patel, Metastatic melanoma - a review of current and future treatment options, Acta Derm. Venereol. 95 (2015) 516–524, https://doi.org/10.2340/00015555-2035.
[8] F.S. Hodi, S.J. O'Day, D.F. McDermott, R.W. Weber, J.A. Sosman, J.B. Haanen, R. Gonzalez, C. Robert, D. Schadendorf, J.C. Hassel, W. Akerley, A.J.M. van den Eertwegh, J. Lutzky, P. Lorigan, J.M. Vaubel, G.P. Linette, D. Hogg, C.H. Ottensmeier, C. Lebbé, C. Peschel, I. Quirt, J.I. Clark, J.D. Wolchok, J.S. Weber, J. Tian, M.J. Yellin, G.M. Nichol, A. Hoos, W.J. Urba, Improved survival with ipilimumab in patients with metastatic melanoma, N. Engl. J. Med. 363 (2010) 711–723, https://doi.org/10.1056/NEJMoa1003466.
[9] S.L. Topalian, M. Sznol, D.F. McDermott, H.M. Kluger, R.D. Carvajal, W.H. Sharfman, J.R. Brahmer, D.P. Lawrence, M.B. Atkins, J.D. Powderly, P.D. Leming, E.J. Lipson, I. Puzanov, D.C. Smith, J.M. Taube, J.M. Wigginton, G.D. Kollia, A. Gupta, D.M. Pardoll, J.A. Sosman, F.S. Hodi, Survival, durable tumor remission, and long-term safety in patients with advanced melanoma receiving nivolumab, J. Clin. Oncol. Off. J. Am. Soc. Clin. Oncol. 32 (2014) 1020–1030, https://doi.org/10.1200/JCO.2013.53.0105.
[10] J.D. Wolchok, H. Kluger, M.K. Callahan, M.A. Postow, N.A. Rizvi, A.M. Lesokhin, N.H. Segal, C.E. Ariyan, R.-A. Gordon, K. Reed, M.M. Burke, A. Caldwell, S.A. Kronenberg, B.U. Agunwamba, X. Zhang, I. Lowy, H.D. Inzunza, W. Feely, C.E. Horak, Q. Hong, A.J. Korman, J.M. Wigginton, A. Gupta, M. Sznol, Nivolumab plus ipilimumab in advanced melanoma, N. Engl. J. Med. 369 (2013) 122–133, https://doi.org/10.1056/NEJMoa1302369.
[11] A. Ribas, A. Algazi, P.A. Ascierto, M.O. Butler, S. Chandra, M. Gordon, L. Hernandez-Aya, D. Lawrence, J. Lutzky, W.H. Miller, K.M. Campbell, B. Delafont, S. Marshall, N. Mueller, C. Robert, PD-L1 blockade in combination with inhibition of MAPK oncogenic signaling in patients with advanced melanoma, Nat. Commun. 11 (2020) 6262, https://doi.org/10.1038/s41467-020-19810-w.
[12] H. Harlin, Y. Meng, A.C. Peterson, Y. Zha, M. Tretiakova, C. Slingluff, M. McKee, T.F. Gajewski, Chemokine expression in melanoma metastases associated with CD8+ T-cell recruitment, Cancer Res. 69 (2009) 3077–3085, https://doi.org/10.1158/0008-5472.CAN-08-2281.
[13] R.-R. Ji, S.D. Chasalow, L. Wang, O. Hamid, H. Schmidt, J. Cogswell, S. Alaparthy, D. Berman, M. Jure-Kunkel, N.O. Siemers, J.R. Jackson, V. Shahabi, An immune-active tumor microenvironment favors clinical response to ipilimumab, Cancer Immunol. Immunother. CII. 61 (2012) 1019–1031, https://doi.org/10.1007/s00262-011-1172-6.
[14] I. Mellman, G. Coukos, G. Dranoff, Cancer immunotherapy comes of age, Nature 480 (2011) 480–489, https://doi.org/10.1038/nature10673.
[15] G.A. Rabinovich, D. Gabrilovich, E.M. Sotomayor, Immunosuppressive strategies that are mediated by tumor cells, Annu. Rev. Immunol. 25 (2007) 267–296, https://doi.org/10.1146/annurev.immunol.25.022106.141609.
[16] S. Spranger, R. Bao, T.F. Gajewski, Melanoma-intrinsic β-catenin signalling prevents anti-tumour immunity, Nature 523 (2015) 231–235, https://doi.org/10.1038/nature14404.
[17] D.S. Chen, I. Mellman, Oncology meets immunology: the cancer-immunity cycle, Immunity 39 (2013) 1–10, https://doi.org/10.1016/j.immuni.2013.07.012.
[18] R.A. Flavell, S. Sanjabi, S.H. Wrzesinski, P. Licona-Limón, The polarization of immune cells in the tumour environment by TGFbeta, Nat. Rev. Immunol. 10 (2010) 554–567, https://doi.org/10.1038/nri2808.
[19] D.R. Green, T. Ferguson, L. Zitvogel, G. Kroemer, Immunogenic and tolerogenic cell death, Nat. Rev. Immunol. 9 (2009) 353–363, https://doi.org/10.1038/nri2545.
[20] G. Kroemer, L. Galluzzi, Combinatorial immunotherapy with checkpoint blockers solves the problem of metastatic melanoma-An exclamation sign with a question mark, OncoImmunology 4 (2015) e1058037, https://doi.org/10.1080/2162402X.2015.1058037.

[21] M. Obeid, A. Tesniere, F. Ghiringhelli, G.M. Fimia, L. Apetoh, J.-L. Perfettini, M. Castedo, G. Mignot, T. Panaretakis, N. Casares, D. Métivier, N. Larochette, P. van Endert, F. Ciccosanti, M. Piacentini, L. Zitvogel, G. Kroemer, Calreticulin exposure dictates the immunogenicity of cancer cell death, Nat. Med. 13 (2007) 54–61, https://doi.org/10.1038/nm1523.

[22] K. Palucka, J. Banchereau, Cancer immunotherapy via dendritic cells, Nat. Rev. Cancer 12 (2012) 265–277, https://doi.org/10.1038/nrc3258.

[23] Y. Hong, I. Manoharan, A. Suryawanshi, A. Shanmugam, D. Swafford, S. Ahmad, R. Chinnadurai, B. Manicassamy, Y. He, A.L. Mellor, M. Thangaraju, D. H. Munn, S. Manicassamy, Deletion of LRP5 and LRP6 in dendritic cells enhances antitumor immunity, OncoImmunology 5 (2016), e1115941, https://doi.org/10.1080/2162402X.2015.1115941.

[24] Y. Hong, I. Manoharan, A. Suryawanshi, T. Majumdar, M.L. Angus-Hill, P.A. Koni, B. Manicassamy, A.L. Mellor, D.H. Munn, S. Manicassamy, β-catenin promotes regulatory T-cell responses in tumors by inducing vitamin A metabolism in dendritic cells, Cancer Res. 75 (2015) 656–665, https://doi.org/10.1158/0008-5472.CAN-14-2377.

[25] H. Clevers, R. Nusse, Wnt/β-catenin signaling and disease, Cell 149 (2012) 1192–1205, https://doi.org/10.1016/j.cell.2012.05.012.

[26] A. Suryawanshi, R.K. Tadagavadi, D. Swafford, S. Manicassamy, Modulation of inflammatory responses by Wnt/β-catenin signaling in dendritic cells: a novel immunotherapy target for autoimmunity and cancer, Front. Immunol. 7 (2016) 460, https://doi.org/10.3389/fimmu.2016.00460.

[27] B.T. MacDonald, K. Tamai, X. He, Wnt/beta-catenin signaling: components, mechanisms, and diseases, Dev. Cell 17 (2009) 9–26, https://doi.org/10.1016/j.devcel.2009.06.016.

[28] A. Suryawanshi, S. Manicassamy, Tumors induce immune tolerance through activation of β-catenin/TCF4 signaling in dendritic cells: a novel therapeutic target for cancer immunotherapy, OncoImmunology 4 (2015), e1052932, https://doi.org/10.1080/2162402X.2015.1052932.

[29] B.-Z. Qian, J.W. Pollard, Macrophage diversity enhances tumor progression and metastasis, Cell 141 (2010) 39–51, https://doi.org/10.1016/j.cell.2010.03.014.

[30] Z. Duan, Y. Luo, Targeting macrophages in cancer immunotherapy, Signal Transduct. Targeted Ther. 6 (2021) 127, https://doi.org/10.1038/s41392-021-00506-6.

[31] M. Yu, R. Guan, W. Hong, Y. Zhou, Y. Lin, H. Jin, B. Hou, Z. Jian, Prognostic value of tumor-associated macrophages in pancreatic cancer: a meta-analysis, Cancer Manag. Res. 11 (2019) 4041–4058, https://doi.org/10.2147/CMAR.S196951.

[32] X. Zhao, J. Qu, Y. Sun, J. Wang, X. Liu, F. Wang, H. Zhang, W. Wang, X. Ma, X. Gao, S. Zhang, Prognostic significance of tumor-associated macrophages in breast cancer: a meta-analysis of the literature, Oncotarget 8 (2017) 30576–30586, https://doi.org/10.18632/oncotarget.15736.

[33] X.L. Wang, J.T. Jiang, C.P. Wu, Prognostic significance of tumor-associated macrophage infiltration in gastric cancer: a meta-analysis, Genet. Mol. Res. GMR. 15 (2016), https://doi.org/10.4238/gmr15049040.

[34] P. Wu, D. Wu, L. Zhao, L. Huang, G. Chen, G. Shen, J. Huang, Y. Chai, Inverse role of distinct subsets and distribution of macrophage in lung cancer prognosis: a meta-analysis, Oncotarget 7 (2016) 40451–40460, https://doi.org/10.18632/oncotarget.9625.

[35] Z. Zhu, H. Zhang, B. Chen, X. Liu, S. Zhang, Z. Zong, M. Gao, PD-L1-Mediated immunosuppression in glioblastoma is associated with the infiltration and M2-polarization of tumor-associated macrophages, Front. Immunol. 11 (2020), 588552, https://doi.org/10.3389/fimmu.2020.588552.

[36] Z. JiaWei, D. ChunXia, L. CunDong, L. Yang, Y. JianKun, D. HaiFeng, Y. Cheng, H. ZhiPeng, W. HongYi, L. DeYing, L. ZhiJian, X. Xiao, Z. QiZhao, X. KangYi, G. WenBing, X. Ming, Z. JunHao, B. JiMing, Z. ShanChao, C. MingKun, M2 subtype tumor associated macrophages (M2-TAMs) infiltration predicts poor response rate of immune checkpoint inhibitors treatment for prostate cancer, Ann. Med. 53 (2021) 730–740, https://doi.org/10.1080/07853890.2021.1924396.

[37] E. Cendrowicz, Z. Sas, E. Bremer, T.P. Rygiel, The role of macrophages in cancer development and therapy, Cancers 13 (2021) (1946), https://doi.org/10.3390/cancers13081946.

[38] C. Ceci, M.G. Atzori, P.M. Lacal, G. Graziani, Targeting tumor-associated macrophages to increase the efficacy of immune checkpoint inhibitors: a glimpse into novel therapeutic approaches for metastatic melanoma, Cancers 12 (2020) E3401, https://doi.org/10.3390/cancers12113401.

[39] H. Wang, L. Zhang, L. Yang, C. Liu, Q. Zhang, L. Zhang, Targeting macrophage anti-tumor activity to suppress melanoma progression, Oncotarget 8 (2017) 18486–18496, https://doi.org/10.18632/oncotarget.14474.

[40] M. Haseeb, R.H. Pirzada, Q.U. Ain, S. Choi, Wnt signaling in the regulation of immune cell and cancer therapeutics, Cells 8 (2019) 1380, https://doi.org/10.3390/cells8111380.

[41] N.C. DeVito, M. Sturdivant, B. Thievanthiran, C. Xiao, M.P. Plebanek, A.K.S. Salama, G.M. Beasley, A. Holtzhausen, V. Novotny-Diermayr, J.H. Strickler, B. A. Hanks, Pharmacological Wnt ligand inhibition overcomes key tumor-mediated resistance pathways to anti-PD-1 immunotherapy, Cell Rep. 35 (2021), 109071, https://doi.org/10.1016/j.celrep.2021.109071.

[42] C. Yang, T. Wu, Y. Qi, Z. Zhang, Recent advances in the application of vitamin E TPGS for drug delivery, Theranostics 8 (2018) 464–485, https://doi.org/10.7150/thno.22711.

[43] X. Bao, M. Gao, H. Xu, K.-X. Liu, C.-H. Zhang, N. Jiang, Q.-C. Chu, X. Guan, Y. Tian, A novel oleanolic acid-loaded PLGA-TPGS nanoparticle for liver cancer treatment, Drug Dev. Ind. Pharm. 41 (2015) 1193–1203, https://doi.org/10.3109/03639045.2014.938081.

[44] P. Agrawal, R.P. Singh, null Sonali, L. Kumari, G. Sharma, B. Koch, C.V. Rajesh, A.K. Mehata, S. Singh, B.L. Pandey, M.S. Muthu, TPGS-chitosan cross-linked targeted nanoparticles for effective brain cancer therapy, Mater. Sci. Eng. C Mater. Biol. Appl. 74 (2017) 167–176, https://doi.org/10.1016/j.msec.2017.02.008.

[45] M.K. Viswanadh, N. Agrawal, S. Azad, A. Jha, S. Poddar, S.K. Mahto, M.S. Muthu, Novel redox-sensitive thiolated TPGS based nanoparticles for EGFR targeted lung cancer therapy, Int. J. Pharm. 602 (2021), 120652, https://doi.org/10.1016/j.ijpharm.2021.120652.

[46] W. Wang, M. Zhou, Y. Xu, W. Peng, S. Zhang, R. Li, H. Zhang, H. Zhang, S. Cheng, Y. Wang, X. Wei, C. Yue, Q. Yang, C. Chen, Resveratrol-loaded TPGS-resveratrol-solid lipid nanoparticles for multidrug-resistant therapy of breast cancer: in vivo and in vitro study, Front. Bioeng. Biotechnol. 9 (2021), 762489, https://doi.org/10.3389/fbioe.2021.762489.

[47] W. Tao, X. Zeng, T. Liu, Z. Wang, Q. Xiong, C. Ouyang, L. Huang, L. Mei, Docetaxel-loaded nanoparticles based on star-shaped mannitol-core PLGA-TPGS diblock copolymer for breast cancer therapy, Acta Biomater. 9 (2013) 8910–8920, https://doi.org/10.1016/j.actbio.2013.06.034.

[48] X. Kang, Y. Cai, Q. Wang, C. Wang, W. Chen, W. Yang, A. Suryawanshi, G. Zhou, P. Chen, F. Li, Near-infrared light triggered activation of pro-drug combination cancer therapy and induction of immunogenic cell death, Int. J. Pharm. 607 (2021), 120972, https://doi.org/10.1016/j.ijpharm.2021.120972.

[49] F.M. Marim, T.N. Silveira, D.S. Lima, D.S. Zamboni, A method for generation of bone marrow-derived macrophages from cryopreserved mouse bone marrow cells, PLoS One 5 (2010), e15263, https://doi.org/10.1371/journal.pone.0015263.

[50] L. Galluzzi, S. Spranger, E. Fuchs, A. López-Soto, WNT signaling in cancer immunosurveillance, Trends Cell Biol. 29 (2019) 44–65, https://doi.org/10.1016/j.tcb.2018.08.005.

[51] V.-F. Rauca, E. Licarete, L. Luput, A. Sesarman, L. Patras, P. Bulzu, E. Rakosy-Tican, M. Banciu, Combination therapy of simvastatin and 5, 6-dimethylxanthenone-4-acetic acid synergistically suppresses the aggressiveness of B16.F10 melanoma cells, PLoS One 13 (2018), e0202827, https://doi.org/10.1371/journal.pone.0202827.

[52] W. Yin, Y. Li, Y. Song, J. Zhang, C. Wu, Y. Chen, Y. Miao, C. Lin, Y. Lin, D. Yan, J. Chen, R. He, CCRL2 promotes antitumor T-cell immunity via amplifying TLR4-mediated immunostimulatory macrophage activation, Proc. Natl. Acad. Sci. U. S. A. 118 (2021), e2024171118, https://doi.org/10.1073/pnas.2024171118.

[53] S.-M.A. Huang, Y.M. Mishina, S. Liu, A. Cheung, F. Stegmeier, G.A. Michaud, O. Charlat, E. Wiellette, Y. Zhang, S. Wiessner, M. Hild, X. Shi, C.J. Wilson, C. Mickanin, V. Myer, A. Fazal, R. Tomlinson, F. Serluca, W. Shao, H. Cheng, M. Shultz, C. Rau, M. Schirle, J. Schlegl, S. Ghidelli, S. Fawell, C. Lu, D. Curtis, M. W. Kirschner, C. Lengauer, P.M. Finan, J.A. Tallarico, T. Bouwmeester, J.A. Porter, A. Bauer, F. Cong, Tankyrase inhibition stabilizes axin and antagonizes Wnt signalling, Nature 461 (2009) 614–620, https://doi.org/10.1038/nature08356.

[54] D. Stakheev, P. Taborska, Z. Strizova, M. Podrazil, J. Bartunkova, D. Smrz, The WNT/β-catenin signaling inhibitor XAV939 enhances the elimination of LNCaP and PC-3 prostate cancer cells by prostate cancer patient lymphocytes in vitro, Sci. Rep. 9 (2019) 4761, https://doi.org/10.1038/s41598-019-41182-5.

[55] D. Kim, B. Koh, K.R. Kim, K.Y. Kim, W.H. Jung, H.Y. Kim, S. Kim, S. Dal Rhee, Anticancer effect of XAV939 is observed by inhibiting lactose dehydrogenase A in a 3-dimensional culture of colorectal cancer cells, Oncol. Lett. 18 (2019) 4858–4864, https://doi.org/10.3892/ol.2019.10813.

[56] J. Zhang, J. Si, L. Gan, M. Guo, J. Yan, Y. Chen, F. Wang, Y. Xie, Y. Wang, H. Zhang, Inhibition of Wnt signalling pathway by XAV939 enhances radiosensitivity in human cervical cancer HeLa cells, Artif, Cells Nanomed. Biotechnol. 48 (2020) 479–487, https://doi.org/10.1080/21691401.2020.1716779.

[57] M.M. Afifi, L.A. Austin, M.A. Mackey, M.A. El-Sayed, From small inhibitor to a potent drug bioconjugate when delivered by gold nanoparticles, Bioconjugate Chem. 25 (2014) 207–215, https://doi.org/10.1021/bc400271x.

[58] C. Li, X. Zheng, Y. Han, Y. Lv, F. Lan, J. Zhao, XAV939 inhibits the proliferation and migration of lung adenocarcinoma A549 cells through the WNT pathway, Oncol. Lett. 15 (2018) 8973–8982, https://doi.org/10.3892/ol.2018.8491.

[59] X.-H. Tian, W.-J. Hou, Y. Fang, J. Fan, H. Tong, S.-L. Bai, Q. Chen, H. Xu, Y. Li, XAV939, a tankyrase 1 inhibitior, promotes cell apoptosis in neuroblastoma cell lines by inhibiting Wnt/β-catenin signaling pathway, J. Exp. Clin. Cancer Res. CR. 32 (2013) 100, https://doi.org/10.1186/1756-9966-32-100.

[60] F. Antony, X. Kang, C. Pundkar, C. Wang, A. Mishra, P. Chen, R.J. Babu, A. Suryawanshi, Targeting β-catenin using XAV939 nanoparticle promotes immunogenic cell death and suppresses conjunctival melanoma progression, Int. J. Pharm. 640 (2023), 123043, https://doi.org/10.1016/j.ijpharm.2023.123043.

[61] R.C. Dregalla, J. Zhou, R.R. Idate, C.L.R. Battaglia, H.L. Liber, S.M. Bailey, Regulatory roles of tankyrase 1 at telomeres and in DNA repair: suppression of T-SCE and stabilization of DNA-PKcs, Aging 2 (2010) 691–708, https://doi.org/10.18632/aging.100210.

[62] S. Di Micco, L. Pulvirenti, I. Bruno, S. Terracciano, A. Russo, M.C. Vaccaro, D. Ruggiero, V. Muccilli, N. Cardullo, C. Tringali, R. Riccio, G. Bifulco, Identification by Inverse Virtual Screening of magnolol-based scaffold as new tankyrase-2 inhibitors, Bioorg, Med. Chem. 26 (2018) 3953–3957, https://doi.org/10.1016/j.bmc.2018.06.019.

[63] D. Shetti, B. Zhang, C. Fan, C. Mo, B.H. Lee, K. Wei, Low dose of paclitaxel combined with XAV939 attenuates metastasis, angiogenesis and growth in breast cancer by suppressing Wnt signaling, Cells 8 (2019) E892, https://doi.org/10.3390/cells8080892.

[64] E.G. Worthley, C.D. Schott, The toxicity of four concentrations of DMSO, Toxicol. Appl. Pharmacol. 15 (1969) 275–281.

[65] G. Cavaletti, N. Oggioni, F. Sala, G. Pezzoni, E. Cavalletti, P. Marmiroli, M.G. Petruccioli, L. Frattola, G. Tredici, Effect on the peripheral nervous system of systemically administered dimethylsulfoxide in the rat: a neurophysiological and pathological study, Toxicol. Lett. 118 (2000) 103–107, https://doi.org/10.1016/s0378-4274(00)00269-1.

[66] D.M. Pawde, M.K. Viswanadh, A.K. Mehata, R. Sonkar, null Narendra, S. Poddar, A.S. Burande, A. Jha, K.Y. Vajanthri, S.K. Mahto, V.N. Azger Dustakeer, M. S. Muthu, Mannose receptor targeted bioadhesive chitosan nanoparticles of clofazimine for effective therapy of tuberculosis, Saudi Pharm. J. SPJ Off. Publ. Saudi Pharm. Soc. 28 (2020) 1616–1625, https://doi.org/10.1016/j.jsps.2020.10.008.

[67] S. Rathod, P. Bahadur, S. Tiwari, Nanocarriers based on vitamin E-TPGS: design principle and molecular insights into improving the efficacy of anticancer drugs, Int. J. Pharm. 592 (2021), 120045, https://doi.org/10.1016/j.ijpharm.2020.120045.

[68] W. Chen, W. Yang, P. Chen, Y. Huang, F. Li, Disulfiram copper nanoparticles prepared with a stabilized metal ion ligand complex method for treating drug-resistant prostate cancers, ACS Appl. Mater. Interfaces 10 (2018) 41118–41128, https://doi.org/10.1021/acsami.8b14940.

[69] Y. Chang, J. Jiang, W. Chen, W. Yang, L. Chen, P. Chen, J. Shen, S. Qian, T. Zhou, L. Wu, L. Hong, Y. Huang, F. Li, Biomimetic metal-organic nanoparticles prepared with a 3D-printed microfluidic device as a novel formulation for disulfiram-based therapy against breast cancer, Appl. Mater. Today 18 (2020), 100492, https://doi.org/10.1016/j.apmt.2019.100492.

[70] S.J. Gaudino, P. Kumar, Cross-talk between antigen presenting cells and T cells impacts intestinal homeostasis, bacterial infections, and tumorigenesis, Front. Immunol. 10 (2019) 360, https://doi.org/10.3389/fimmu.2019.00360.

[71] N. Pishesha, T.J. Harmand, H.L. Ploegh, A guide to antigen processing and presentation, Nat. Rev. Immunol. 22 (2022) 751–764, https://doi.org/10.1038/s41577-022-00707-2.

[72] R.J. Stopforth, E.S. Ward, The role of antigen presentation in tumor-associated macrophages, Crit. Rev. Immunol. 40 (2020) 205–224, https://doi.org/10.1615/CritRevImmunol.2020034910.

[73] A.R. Sánchez-Paulete, A. Teijeira, F.J. Cueto, S. Garasa, J.L. Pérez-Gracia, A. Sánchez-Arráez, D. Sancho, I. Melero, Antigen cross-presentation and T-cell cross-priming in cancer immunology and immunotherapy, Ann. Oncol. Off. J. Eur. Soc. Med. Oncol. 28 (2017), https://doi.org/10.1093/annonc/mdx237 xii44–xii55.

[74] S.K. Kim, S.W. Cho, The evasion mechanisms of cancer immunity and drug intervention in the tumor microenvironment, Front. Pharmacol. 13 (2022), 868695, https://doi.org/10.3389/fphar.2022.868695.

[75] D.S. Vinay, E.P. Ryan, G. Pawelec, W.H. Talib, J. Stagg, E. Elkord, T. Lichtor, W.K. Decker, R.L. Whelan, H.M.C.S. Kumara, E. Signori, K. Honoki, A. G. Georgakilas, A. Amin, W.G. Helferich, C.S. Boosani, G. Guha, M.R. Ciriolo, S. Chen, S.I. Mohammed, A.S. Azmi, W.N. Keith, A. Bilsland, D. Bhakta, D. Halicka, H. Fujii, K. Aquilano, S.S. Ashraf, S. Nowsheen, X. Yang, B.K. Choi, B.S. Kwon, Immune evasion in cancer: mechanistic basis and therapeutic strategies, Semin. Cancer Biol. 35 (Suppl) (2015), https://doi.org/10.1016/j.semcancer.2015.03.004. S185–S198.

[76] A. Holtzhausen, F. Zhao, K.S. Evans, M. Tsutsui, C. Orabona, D.S. Tyler, B.A. Hanks, Melanoma-derived Wnt5a promotes local dendritic-cell expression of IDO and immunotolerance: opportunities for pharmacologic enhancement of immunotherapy, Cancer Immunol. Res. 3 (2015) 1082–1095, https://doi.org/10.1158/2326-6066.CIR-14-0167.

[77] Y. Pan, Y. Yu, X. Wang, T. Zhang, Tumor-associated macrophages in tumor immunity, Front. Immunol. 11 (2020), 583084, https://doi.org/10.3389/fimmu.2020.583084.

[78] J.M. Jaynes, R. Sable, M. Ronzetti, W. Bautista, Z. Knotts, A. Abisoye-Ogunniyan, D. Li, R. Calvo, M. Dashnyam, A. Singh, T. Guerin, J. White, S. Ravichandran, P. Kumar, K. Talsania, V. Chen, A. Ghebremedhin, B. Karanam, A. Bin Salam, R. Amin, T. Odzorig, T. Aiken, V. Nguyen, Y. Bian, J.C. Zarif, A.E. de Groot, M. Mehta, L. Fan, X. Hu, A. Simeonov, N. Pate, M. Abu-Asab, M. Ferrer, N. Southall, C.-Y. Ock, Y. Zhao, H. Lopez, S. Kozlov, N. de Val, C.C. Yates, B. Baljinnyam, J. Marugan, U. Rudloff, Mannose receptor (CD206) activation in tumor-associated macrophages enhances adaptive and innate antitumor immune responses, Sci. Transl. Med. 12 (2020), https://doi.org/10.1126/scitranslmed.aax6337 eaax6337.

[79] G.P. Hartley, L. Chow, D.T. Ammons, W.H. Wheat, S.W. Dow, Programmed cell death ligand 1 (PD-L1) signaling regulates macrophage proliferation and activation, Cancer Immunol. Res. 6 (2018) 1260–1273, https://doi.org/10.1158/2326-6066.CIR-17-0537.

[80] A.J. Boutilier, S.F. Elsawa, Macrophage polarization states in the tumor microenvironment, Int. J. Mol. Sci. 22 (2021) 6995, https://doi.org/10.3390/ijms22136995.

[81] B. Benner, L. Scarberry, L.P. Suarez-Kelly, M.C. Duggan, A.R. Campbell, E. Smith, G. Lapurga, K. Jiang, J.P. Butchar, S. Tridandapani, J.H. Howard, R. A. Baiocchi, T.A. Mace, W.E. Carson, Generation of monocyte-derived tumor-associated macrophages using tumor-conditioned media provides a novel method to study tumor-associated macrophages in vitro, J. Immunother. Cancer. 7 (2019) 140, https://doi.org/10.1186/s40425-019-0622-0.

[82] A. Bobrie, O. Massol, J. Ramos, C. Mollevi, E. Lopez-Crapez, N. Bonnefoy, F. Boissière-Michot, W. Jacot, Association of CD206 protein expression with immune infiltration and prognosis in patients with triple-negative breast cancer, Cancers 14 (2022) 4829, https://doi.org/10.3390/cancers14194829.

[83] A. Suryawanshi, M.S. Hussein, P.D. Prasad, S. Manicassamy, Wnt signaling cascade in dendritic cells and regulation of anti-tumor immunity, Front. Immunol. 11 (2020) 122, https://doi.org/10.3389/fimmu.2020.00122.

[84] X. Zhou, X. Liu, L. Huang, Macrophage-mediated tumor cell phagocytosis: opportunity for nanomedicine intervention, Adv. Funct. Mater. 31 (2021), 2006220, https://doi.org/10.1002/adfm.202006220.

[85] E.M. Muntjewerff, L.D. Meesters, G. van den Bogaart, Antigen cross-presentation by macrophages, Front. Immunol. 11 (2020) 1276, https://doi.org/10.3389/fimmu.2020.01276.

[86] E. Zamudio-Martinez, A.B. Herrera-Campos, A. Muñoz, J.M. Rodríguez-Vargas, F.J. Oliver, Tankyrases as modulators of pro-tumoral functions: molecular insights and therapeutic opportunities, J. Exp. Clin. Cancer Res. CR. 40 (2021) 144, https://doi.org/10.1186/s13046-021-01950-6.

[87] D. Stakheev, P. Taborska, Z. Strizova, M. Podrazil, J. Bartunkova, D. Smrz, The WNT/β-catenin signaling inhibitor XAV939 enhances the elimination of LNCaP and PC-3 prostate cancer cells by prostate cancer patient lymphocytes in vitro, Sci. Rep. 9 (2019) 4761, https://doi.org/10.1038/s41598-019-41182-5.

[88] F. Pan, F. Shen, L. Yang, L. Zhang, W. Guo, J. Tian, Inhibitory effects of XAV939 on the proliferation of small-cell lung cancer H446 cells and Wnt/β-catenin signaling pathway in vitro, Oncol. Lett. 16 (2018) 1953–1958, https://doi.org/10.3892/ol.2018.8790.

[89] C.W. Kim, K.-D. Kim, H.K. Lee, The role of dendritic cells in tumor microenvironments and their uses as therapeutic targets, BMB Rep 54 (2021) 31–43, https://doi.org/10.5483/BMBRep.2021.54.1.224.

[90] Y.H. Gupta, A. Khanom, S.E. Acton, Control of dendritic cell function within the tumour microenvironment, Front. Immunol. 13 (2022), 733800, https://doi.org/10.3389/fimmu.2022.733800.

[91] S. Singhal, J. Stadanlick, M.J. Annunziata, A.S. Rao, P.S. Bhojnagarwala, S. O'Brien, E.K. Moon, E. Cantu, G. Danet-Desnoyers, H.-J. Ra, L. Litzky, T. Akimova, U.H. Beier, W.W. Hancock, S.M. Albelda, E.B. Eruslanov, Human tumor-associated monocytes/macrophages and their regulation of T cell responses in early-stage lung cancer, Sci. Transl. Med. 11 (2019), https://doi.org/10.1126/scitranslmed.aat1500 eaat1500.

[92] K. Asano, A. Nabeyama, Y. Miyake, C.-H. Qiu, A. Kurita, M. Tomura, O. Kanagawa, S. Fujii, M. Tanaka, CD169-positive macrophages dominate antitumor immunity by crosspresenting dead cell-associated antigens, Immunity 34 (2011) 85–95, https://doi.org/10.1016/j.immuni.2010.12.011.

[93] R. Nusse, H. Clevers, Wnt/β-Catenin signaling, disease, and emerging therapeutic modalities, Cell 169 (2017) 985–999, https://doi.org/10.1016/j.cell.2017.05.016.

[94] S. Spranger, T.F. Gajewski, Impact of oncogenic pathways on evasion of antitumour immune responses, Nat. Rev. Cancer 18 (2018) 139–147, https://doi.org/10.1038/nrc.2017.117.

[95] S. Muto, Y. Ozaki, H. Yamaguchi, H. Mine, H. Takagi, M. Watanabe, T. Inoue, T. Yamaura, M. Fukuhara, N. Okabe, Y. Matsumura, T. Hasegawa, J. Osugi, M. Hoshino, M. Higuchi, Y. Shio, H. Nanamiya, J.-I. Imai, T. Isogai, S. Watanabe, H. Suzuki, Tumor β-catenin expression is associated with immune evasion in non-small cell lung cancer with high tumor mutation burden, Oncol. Lett. 21 (2021) 203, https://doi.org/10.3892/ol.2021.12464.

[96] J.J. Luke, R. Bao, R.F. Sweis, S. Spranger, T.F. Gajewski, WNT/β-catenin pathway activation correlates with immune exclusion across human cancers, Clin. Cancer Res. Off. J. Am. Assoc. Cancer Res. 25 (2019) 3074–3083, https://doi.org/10.1158/1078-0432.CCR-18-1942.

[97] G. Solinas, G. Germano, A. Mantovani, P. Allavena, Tumor-associated macrophages (TAM) as major players of the cancer-related inflammation, J. Leukoc. Biol. 86 (2009) 1065–1073, https://doi.org/10.1189/jlb.0609385.

[98] Q. Yang, N. Guo, Y. Zhou, J. Chen, Q. Wei, M. Han, The role of tumor-associated macrophages (TAMs) in tumor progression and relevant advance in targeted therapy, Acta Pharm. Sin. B. 10 (2020) 2156–2170, https://doi.org/10.1016/j.apsb.2020.04.004.

[99] T. Zhan, N. Rindtorff, M. Boutros, Wnt signaling in cancer, Oncogene 36 (2017) 1461–1473, https://doi.org/10.1038/onc.2016.304.

[100] W.N. Goldsberry, A. Londoño, T.D. Randall, L.A. Norian, R.C. Arend, A review of the role of Wnt in cancer immunomodulation, Cancers 11 (2019) 771, https://doi.org/10.3390/cancers11060771.

[101] R. van Amerongen, R. Nusse, Towards an integrated view of Wnt signaling in development, Dev. Camb. Engl. 136 (2009) 3205–3214, https://doi.org/10.1242/dev.033910.

[102] J. Liu, Q. Xiao, J. Xiao, C. Niu, Y. Li, X. Zhang, Z. Zhou, G. Shu, G. Yin, Wnt/β-catenin signalling: function, biological mechanisms, and therapeutic opportunities, Signal Transduct. Targeted Ther. 7 (2022) 3, https://doi.org/10.1038/s41392-021-00762-6.

[103] D. Routledge, S. Scholpp, Mechanisms of intercellular Wnt transport, Dev. Camb. Engl. 146 (2019) dev176073, https://doi.org/10.1242/dev.176073.

[104] P. Darvin, S.M. Toor, V. Sasidharan Nair, E. Elkord, Immune checkpoint inhibitors: recent progress and potential biomarkers, Exp. Mol. Med. 50 (2018) 1–11, https://doi.org/10.1038/s12276-018-0191-1.

[105] J.L. Messina, D.A. Fenstermacher, S. Eschrich, X. Qu, A.E. Berglund, M.C. Lloyd, M.J. Schell, V.K. Sondak, J.S. Weber, J.J. Mulé, 12-Chemokine gene signature identifies lymph node-like structures in melanoma: potential for patient selection for immunotherapy? Sci. Rep. 2 (2012) 765, https://doi.org/10.1038/srep00765.

[106] A. Vermare, M.V. Guérin, E. Peranzoni, N. Bercovici, Dynamic CD8+ T cell cooperation with macrophages and monocytes for successful cancer immunotherapy, Cancers 14 (2022) 3546, https://doi.org/10.3390/cancers14143546.

[107] J.M.H. de Jong, D.H. Schuurhuis, A. Ioan-Facsinay, M.M. Welling, M.G.M. Camps, E.I.H. van der Voort, T.W.J. Huizinga, F. Ossendorp, J.S. Verbeek, R.E. M. Toes, Dendritic cells, but not macrophages or B cells, activate major histocompatibility complex class II-restricted CD4+ T cells upon immune-complex uptake in vivo, Immunology 119 (2006) 499–506, https://doi.org/10.1111/j.1365-2567.2006.02464.x.

[108] O. Hamid, C. Robert, A. Daud, F.S. Hodi, W.-J. Hwu, R. Kefford, J.D. Wolchok, P. Hersey, R.W. Joseph, J.S. Weber, R. Dronca, T.C. Gangadhar, A. Patnaik, H. Zarour, A.M. Joshua, K. Gergich, J. Elassaiss-Schaap, A. Algazi, C. Mateus, P. Boasberg, P.C. Tumeh, B. Chmielowski, S.W. Ebbinghaus, X.N. Li, S.P. Kang, A. Ribas, Safety and tumor responses with lambrolizumab (anti-PD-1) in melanoma, N. Engl. J. Med. 369 (2013) 134–144, https://doi.org/10.1056/NEJMoa1305133.

[109] G. Graziani, L. Tentori, P. Navarra, Ipilimumab: a novel immunostimulatory monoclonal antibody for the treatment of cancer, Pharmacol. Res. 65 (2012) 9–22, https://doi.org/10.1016/j.phrs.2011.09.002.

[110] L. Zimmer, S.M. Goldinger, L. Hofmann, C. Loquai, S. Ugurel, I. Thomas, M.I. Schmidgen, R. Gutzmer, J.S. Utikal, D. Göppner, J.C. Hassel, F. Meier, J.K. Tietze, A. Forschner, C. Weishaupt, M. Leverkus, R. Wahl, U. Dietrich, C. Garbe, M.C. Kirchberger, T. Eigentler, C. Berking, A. Gesierich, A.M. Krackhardt, D. Schadendorf, G. Schuler, R. Dummer, L.M. Heinzerling, Neurological, respiratory, musculoskeletal, cardiac and ocular side-effects of anti-PD-1 therapy, Eur. J. Cancer Oxf. Engl. 60 (2016) (1990) 210–225, https://doi.org/10.1016/j.ejca.2016.02.024.

[111] L. Liu, Q. Chen, C. Ruan, X. Chen, Y. Zhang, X. He, Y. Zhang, Y. Lu, Q. Guo, T. Sun, H. Wang, C. Jiang, Platinum-based nanovectors engineered with immuno-modulating adjuvant for inhibiting tumor growth and promoting immunity, Theranostics 8 (2018) 2974–2987, https://doi.org/10.7150/thno.24110.

[112] J. He, S. Duan, X. Yu, Z. Qian, S. Zhou, Z. Zhang, X. Huang, Y. Huang, J. Su, C. Lai, J. Meng, N. Zhou, X. Lu, Y. Zhao, Folate-modified chitosan nanoparticles containing the IP-10 gene enhance melanoma-specific cytotoxic CD8(+)CD28(+) T lymphocyte responses, Theranostics 6 (2016) 752–761, https://doi.org/10.7150/thno.14527.

[113] M. Kong, J. Tang, Q. Qiao, T. Wu, Y. Qi, S. Tan, X. Gao, Z. Zhang, Biodegradable hollow mesoporous silica nanoparticles for regulating tumor microenvironment and enhancing antitumor efficiency, Theranostics 7 (2017) 3276–3292, https://doi.org/10.7150/thno.19987.

[114] X. Liu, Y. Li, X. Sun, Y. Muftuoglu, B. Wang, T. Yu, Y. Hu, L. Ma, M. Xiang, G. Guo, C. You, X. Gao, Y. Wei, Powerful anti-colon cancer effect of modified nanoparticle-mediated IL-15 immunogene therapy through activation of the host immune system, Theranostics 8 (2018) 3490–3503, https://doi.org/10.7150/thno.24157.

[115] L. Palanikumar, S. Al-Hosani, M. Kalmouni, V.P. Nguyen, L. Ali, R. Pasricha, F.N. Barrera, M. Magzoub, pH-responsive high stability polymeric nanoparticles for targeted delivery of anticancer therapeutics, Commun. Biol. 3 (2020) 95, https://doi.org/10.1038/s42003-020-0817-4.

[116] C. Wang, C. Zhang, Z. Li, S. Yin, Q. Wang, F. Guo, Y. Zhang, R. Yu, Y. Liu, Z. Su, Extending half life of H-ferritin nanoparticle by fusing albumin binding domain for doxorubicin encapsulation, Biomacromolecules 19 (2018) 773–781, https://doi.org/10.1021/acs.biomac.7b01545.

[117] S. Guo, M. Vieweger, K. Zhang, H. Yin, H. Wang, X. Li, S. Li, S. Hu, A. Sparreboom, B.M. Evers, Y. Dong, W. Chiu, P. Guo, Ultra-thermostable RNA nanoparticles for solubilizing and high-yield loading of paclitaxel for breast cancer therapy, Nat. Commun. 11 (2020) 972, https://doi.org/10.1038/s41467-020-14780-5.

[118] H. Wang, J. Yu, X. Lu, X. He, Nanoparticle systems reduce systemic toxicity in cancer treatment, Nanomed 11 (2016) 103–106, https://doi.org/10.2217/nnm.15.166.

[119] S.F. Gad, J. Park, J.E. Park, G.N. Fetih, S.S. Tous, W. Lee, Y. Yeo, Enhancing docetaxel delivery to multidrug-resistant cancer cells with albumin-coated nanocrystals, Mol. Pharm. (2018), https://doi.org/10.1021/acs.molpharmaceut.7b00783.

[120] S. Bancos, D.L. Stevens, K.M. Tyner, Effect of silica and gold nanoparticles on macrophage proliferation, activation markers, cytokine production, and phagocytosis in vitro, Int. J. Nanomed. 10 (2015) 183–206, https://doi.org/10.2147/IJN.S72580.

[121] L. Ye, L. Zhang, B. Yang, X. Zhou, J. Li, Z. Qin, D. Dong, Y. Cui, F. Yao, Zwitterionic-modified starch-based stealth micelles for prolonging circulation time and reducing macrophage response, ACS Appl. Mater. Interfaces 8 (2016) 4385–4398, https://doi.org/10.1021/acsami.5b10811.

[122] R. Rajan, M.K. Sabnani, V. Mavinkurve, H. Shmeeda, H. Mansouri, S. Bonkoungou, A.D. Le, L.M. Wood, A.A. Gabizon, N.M. La-Beck, Liposome-induced immunosuppression and tumor growth is mediated by macrophages and mitigated by liposome-encapsulated alendronate, J. Control. Release Off. J. Control. Release Soc. 271 (2018) 139–148, https://doi.org/10.1016/j.jconrel.2017.12.023.

[123] Z. Huang, Y. Yang, Y. Jiang, J. Shao, X. Sun, J. Chen, L. Dong, J. Zhang, Anti-tumor immune responses of tumor-associated macrophages via toll-like receptor 4 triggered by cationic polymers, Biomaterials 34 (2013) 746–755, https://doi.org/10.1016/j.biomaterials.2012.09.062.

[124] M. VanHandel, D. Alizadeh, L. Zhang, B. Kateb, M. Bronikowski, H. Manohara, B. Badie, Selective uptake of multi-walled carbon nanotubes by tumor macrophages in a murine glioma model, J. Neuroimmunol. 208 (2009) 3–9, https://doi.org/10.1016/j.jneuroim.2008.12.006.

[125] R.A. Ortega, W.J. Barham, B. Kumar, O. Tikhomirov, I.D. McFadden, F.E. Yull, T.D. Giorgio, Biocompatible mannosylated endosomal-escape nanoparticles enhance selective delivery of short nucleotide sequences to tumor associated macrophages, Nanoscale 7 (2015) 500–510, https://doi.org/10.1039/c4nr03962a.

[126] Q. Taban, P.T. Mumtaz, K.Z. Masoodi, E. Haq, S.M. Ahmad, Scavenger receptors in host defense: from functional aspects to mode of action, Cell Commun. Signal. CCS. 20 (2022) 2, https://doi.org/10.1186/s12964-021-00812-0.

[127] P. Scodeller, L. Simón-Gracia, S. Kopanchuk, A. Tobi, K. Kilk, P. Säälik, K. Kurm, M.L. Squadrito, V.R. Kotamraju, A. Rinken, M. De Palma, E. Ruoslahti, T. Teesalu, Precision targeting of tumor macrophages with a CD206 binding peptide, Sci. Rep. 7 (2017) 14655, https://doi.org/10.1038/s41598-017-14709-x.

[128] E.B. Glass, A.A. Hoover, K.K. Bullock, M.Z. Madden, B.I. Reinfeld, W. Harris, D. Parker, D.H. Hufnagel, M.A. Crispens, D. Khabele, W.K. Rathmell, J. C. Rathmell, A.J. Wilson, T.D. Giorgio, F.E. Yull, Stimulating TAM-mediated anti-tumor immunity with mannose-decorated nanoparticles in ovarian cancer, BMC Cancer 22 (2022) 497, https://doi.org/10.1186/s12885-022-09612-2.

[129] K. Movahedi, S. Schoonooghe, D. Laoui, I. Houbracken, W. Waelput, K. Breckpot, L. Bouwens, T. Lahoutte, P. De Baetselier, G. Raes, N. Devoogdt, J.A. Van Ginderachter, Nanobody-based targeting of the macrophage mannose receptor for effective in vivo imaging of tumor-associated macrophages, Cancer Res. 72 (2012) 4165–4177, https://doi.org/10.1158/0008-5472.CAN-11-2994.

[130] X.-Y. He, B.-Y. Liu, J.-L. Wu, S.-L. Ai, R.-X. Zhuo, S.-X. Cheng, A dual macrophage targeting nanovector for delivery of oligodeoxynucleotides to overcome cancer-associated immunosuppression, ACS Appl. Mater. Interfaces 9 (2017) 42566–42576, https://doi.org/10.1021/acsami.7b13594.

[131] H. Peng, B. Chen, W. Huang, Y. Tang, Y. Jiang, W. Zhang, Y. Huang, Reprogramming tumor-associated macrophages to reverse EGFRT790M resistance by dual-targeting codelivery of gefitinib/vorinostat, Nano Lett. 17 (2017) 7684–7690, https://doi.org/10.1021/acs.nanolett.7b03756.

[132] U. Gazi, L. Martinez-Pomares, Influence of the mannose receptor in host immune responses, Immunobiology 214 (2009) 554–561, https://doi.org/10.1016/j.imbio.2008.11.004.

[133] L. Swanson, G.D. Katkar, J. Tam, R.F. Pranadinata, Y. Chareddy, J. Coates, M.S. Anandachar, V. Castillo, J. Olson, V. Nizet, I. Kufareva, S. Das, P. Ghosh, TLR4 signaling and macrophage inflammatory responses are dampened by GIV/Girdin, Proc. Natl. Acad. Sci. U. S. A. 117 (2020) 26895–26906, https://doi.org/10.1073/pnas.2011667117.

[134] L. Chaperot, M. Chokri, M.C. Jacob, P. Drillat, F. Garban, H. Egelhofer, J.P. Molens, J.J. Sotto, J.C. Bensa, J. Plumas, Differentiation of antigen-presenting cells (dendritic cells and macrophages) for therapeutic application in patients with lymphoma, Leukemia 14 (2000) 1667–1677, https://doi.org/10.1038/sj.leu.2401888.

[135] C. Théry, S. Amigorena, The cell biology of antigen presentation in dendritic cells, Curr. Opin. Immunol. 13 (2001) 45–51, https://doi.org/10.1016/s0952-7915(00)00180-1.

[136] T. Shiota, K. Miyasato, K. Ohnishi, M. Yamamoto-Ibusuki, Y. Yamamoto, H. Iwase, M. Takeya, Y. Komohara, The clinical significance of CD169-positive lymph node macrophage in patients with breast cancer, PLoS One 11 (2016), e0166680, https://doi.org/10.1371/journal.pone.0166680.

[137] M. Modak, A.-K. Mattes, D. Reiss, W. Skronska-Wasek, R. Langlois, N. Sabarth, R. Konopitzky, F. Ramirez, K. Lehr, T. Mayr, D. Kind, C. Viollet, L.K. Swee, J. Petschenka, K.C. El Kasmi, E. Noessner, K. Kitt, S. Pflanz, CD206+ tumor-associated macrophages cross-present tumor antigen and drive antitumor immunity, JCI Insight 7 (2022), e155022, https://doi.org/10.1172/jci.insight.155022.