Review

Progress of IL-12 in Tumor Immunotherapy

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Progress of IL-12 in Tumor Immunotherapy

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Abstract: Interleukin-12 (IL-12) is considered to be a promising cytokine for enhancing anti-tumor immune response. However, recombinant IL-12 had significant toxicity and limited efficacy in early clinical trials. Recently, many strategies for delivering IL-12 to tumor tissues have been developed, such as modifying IL-12, utilizing viral vectors, non-viral vectors and cellular vectors. Previous studies have found that IL-12 fusion with extracellular matrix proteins, collagen and immune factors is a way to enhance its therapeutic potential. In addition, studies have demonstrated that viral vectors are a good platform, and a variety of viruses such as oncolytic viruses, adenoviruses and poxviruses have been used to deliver IL-12, and testing has been conducted in various cancer models. Local expression of IL-12 in tumors based on viral delivery avoids systemic toxicity while inducing effective anti-tumor immunity and acting synergistically with other therapies without compromising safety. Also, lipid nanoparticles are currently considered to be the most mature drug delivery system. Moreover, cells are also considered to be drug carriers because they can effectively deliver therapeutic substances to tumors. In this article, we will systematically discuss the antitumor effects of IL-12 on its own or in combination with other therapies based on different delivery strategies.

Keywords: IL-12; Immunotherapy; Delivery system

1. Instruction

Cytokine is an important factor that regulates immune cells to inhibit the growth of tumor cells, which can directly affect the activity of tumor cells or indirectly enhance the cytotoxic activity of tumor cells by stimulating immune cells [1]. Several cytokines have been extensively investigated for cancer immunotherapy and shown efficacy in multiple cancer models [1,2]. Among these, IL-12 is considered to be a potent cytokine in triggering anti-tumor immune responses due to its activation of both innate and adaptive immune responses [3–5]. The therapeutic potential of IL-12 has been extensively evaluated in various preclinical models with advanced solid tumors, both alone or in combination with others, producing encouraging therapeutic outcomes and even overcoming immune checkpoint inhibitors (ICI) resistance [6–10]. However, although IL-12 has strong anticancer effects and high in vitro activity, systemic administration of therapeutic doses is limited due to severe dose-limiting toxicity, short half-life of conventional IL-12 drugs in vivo, and lethal off-target and off-tumor side effects [8,10–12]. In most cases, clinical responses have been only modest [13], which may be associated with the short serum half-life of IL-12 and its limited T-cell activation. To avoid toxic effects associated with IL-12 administration and enhance therapeutic efficacy, a number of approaches have been proposed and explored [14–16].

Recently, the development of a new generation of IL-12 drugs focuses on reducing systemic leakage of IL-12 and increasing its safety, but achieving high concentrations locally in the tumor. The modification or delivery strategies of cancer immunotherapy drugs targeting IL-12 mainly include the following categories: fusion with molecules targeting specific tumor tissues to achieve targeted delivery, application of viruses to infect tumor tissues, direct delivery of genetic material to target

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tissues through physical or chemical means, delivery through cellular carriers, etc. In this article, we will focus on discussing the safety and anti-tumor effects of modified, virally delivered, non-viral and cell-delivered IL-12 drugs as single or combination therapies.

2. Progress of IL-12 in Cancer Therapy based on Different Delivery Strategies

2.1. Modified IL-12 for Cancer Therapy

In order to increase the possibility of IL-12 in tumor therapy, several strategies have been used to modify IL-12 to develop novel IL-12 drugs. For example, IL-12 drugs are developed by fusing with extracellular matrix proteins, collagen in the tumor microenvironment (TME), and immune factors (Table 1). Most solid tumors are encapsulated in the extracellular matrix, and targeting extracellular matrix proteins can promote the accumulation of immune cytokines in tumor tissues and reduce toxicity [17,18]. Therefore, an IL-12 drug based on this was designed, namely Pro-IL-12 [17]. In preclinical animal models, intraperitoneal (i.p.) injection of low-dose Pro-IL-12 can significantly inhibit tumor growth and prolong survival of MC38, B16F10 and 4T1 tumor-bearing mice with very low toxicity [17]. The mechanism stems from the production of tumor-specific $CD8^+$ T cells and IFN-γ within the tumor [17]. In addition, the combination of Pro-IL-12 with TKI targeted therapy and ICI can further improve the therapeutic effect [17]. Similarly, IL-12-MSA-Lumican (fusion with Lumican, specific binding of type I and IV collagen) and CBD-IL-12(fusion collagen binding domain) were designed to be more effective and less toxic than unmodified IL-12 alone or in combination with PD-1 antibodies [19,20].

In addition, IL-12 binding to immune factors is also a strategy that has received much attention. For example, IL12-L19 (human IL-12 tandem L19 antibody) [21], IL12-scFv(L19)-FLAG (IL-12 derivatives fuse anti-EDB antibody fragment scFv(L19) [22], and NHS-muIL12 (fuses a DNA/ DNA-histone complex antibody (NHS76)) [23]. Taking NHS-muIL12 as an example, it subcutaneous (s.c.) injection has a longer half-life than recombinant IL-12, and whether used as a monotherapy or in combination with PD-L1 antibodies, it can stimulate anti-tumor activity, enhance cytotoxic function, and increase the production of IFN-γ and other cytokines by activating NK cells and $CD8^+$ T cells [24,25].

Table 1. Modified IL-12 for cancer therapy.

Name	manner	Cancer Model	RoA	Combination therapy	Ref
pro-IL-12	extracellular matrix proteins	MC38, B16F10, 4T1	i.p.	/	[17]
M-L-IL-12	fused a domain of the IL-12 receptor	EMT6, B16F10	intravenous (i.v.)	PD-1 antibodies	[18]
IL-12-MSA-Lumican	fuses with Lumican	B16F10	i.t.	PD-1 antibodies	[19]
CBD-IL-12	fusion collagen-binding domain	EMT6, B16F10	i.t.	PD-1 antibodies	[20]
IL12-scFv(L19)-FLAG	fuse anti-EDB antibody fragment scFv(L19)	F9	i.v.	/	[22]
mIL12-F_HAB-hIL15	fused single-chain human IL-12 and native human IL-15 in cis onto a fully human albumin binding (FHAB) domain single-chain antibody fragment (scFv)	B16F10	i.v.	/	[26]
scIL-12-B7TM	membrane-bound IL-12 containing murine single-chain	CT26	i.t.	/	[27]

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	IL-12 and B7-1 transmembrane and cytoplasmic domains				
NHS-IL12	fuses a DNA/ DNA-histone complex antibody (NHS76)	MC38, MB49, 4T1, EMT-6	s.c.	Bintrafusp alfa, PD-L1 antibodies	[23–25]

2.2. Virus-Based Delivery of IL-12 for cancer therapy

Virus-mediated cytokine therapy has been proved effective against tumors inducing local and systemic immune responses [28]. Potential viral vectors for gene delivery include herpes simplex virus, adenovirus, alphavirus, and herpes virus, semliki forest viruses, poxviruses and other viral vectors. Next, we will focus on the progress of virus-mediated IL-12 in tumor therapy.

Herpes Simplex Virus (HSV)

HSV was the first virus to be recognized as a candidate oncolytic virus (OV), ranking highly on the list of clinical trials [29,30]. Talimogene laherparepvec (T-VEC), the first HSV drug, was approved by the U.S. FDA in 2015 for the treatment of advanced melanoma [31]. With the approval of T-ECV, much research has focused on developing drugs based on HSV strategies. In preclinical studies, Intratumoral (i.t.) injections of HSV encoding IL-12 have mediated regressions of colon cancer [32–36], breast cancer [34,37], glioma [38–45], lymphoma [33,46,47], melanoma [48] and other cancers (Table 2). Indeed, most HSV-encoded IL-12 has shown good antitumor effects and prolonged survival with reduced toxicity in preclinical animal models. The mechanism is usually to change the TME, increase $CD8^+$ T cell infiltration, promote IFN-γ production, and inhibit Tregs function.

In addition, combination therapy is also of concern, with most studies in combination with ICI. For example, G47Δ-mIL12, G47Δ, G47Δ-mIL12 and R-123 have been explored for immunotherapy in combination with the anti-PD-1 or anti-CTLA-4 antibodies [38,40,41,46]. Excitingly, the G47Δ-mIL12, anti-CTLA-4 and anti-PD-1 triple therapy cured most mice in glioma models, and outperformed G47Δ-mIL12, anti-CTLA-4 and anti-PD-1 alone in prolonging survival [41].

Table 2. Viral vector delivers IL-12 for cancer therapy-HSV.

Name	Dose (pfu)	Cancer Model	RoA	Combination therapy	Ref
dvIL12-tk/tsK	2×10^5	CT26	i.t.	/	[32]
VG161	5×10^6	CT26, A20	i.t.	/	[33]
O-HSV12	10^7	MC38	i.t.	/	[36]
VG2026	10^8	A20	i.t.	/	[47]
Δ6/GM/IL12	10^7	B16-F10	i.t.	/	[48]
G47Δ-mIL2	5×10^5	005 GSC, CT-2A, GL261	i.t.	/	[45]
	9×10^5	M3 cells	i.t.	/	[49]
	2×10^6	4T1	i.t.	/	[37]
	10^6	U87	i.t.	G47Δ-mAngio	[43]
	5×10^5	005 GSCs	i.t.	/	[50]
	5×10^5	005 GSCs	i.t.	TMZ, d O6-BG	[39]
	2.5×10^5	005 GSCs, MGG123 GSCs	i.t.	Axitinib, CTLA-4 antibodies	[38]
	5×10^5	005 GSCs	i.t.	PD-1, CTLA-4 antibodies	[40]
	5×10^5	Glioma, CT-2A	i.t.	PD-1, CTLA-4 antibodies	[41]
C5252	5×10^6	U87	i.t.	/	[44]

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oHSV2-IL12	10^7	4T1, CT26	i.t.	oHSV2-PD1v, IL7 × CCL19, GM-CSF and IL15	[34]
vHsv-IL-12	8×10^3-2×10^6	Neuro2a	i.t.	vHsv-B7.1-Ig and IL-18	[42]
NV1042	5×10^7	SCC	i.v.	/	[51]
	1×10^7	CT26	i.t.	/	[35]
	5×10^5	CWr22	i.t.	Vinblastine	[52]
	2×10^7	SCC VII	i.t.	/	[53,54]
	10^7	TRAMP-C2, Pr14-2	i.p.	/	[55]
	10^7	McA-R-7777	i.t.	/	[56]
M002	10^7	Neuro-2a	i.t.	M010 (HSV expressing CCL2)	[57]
	10^7	SARC	i.t.	/	[58]
	10^7	X21415, D456, GBM-12, UAB106	i.t.	/	[59]
	1.5×10^7	Intracranial SCK	i.t.	/	[60]
	10^7	Xenograft SK-N-AS and SK-N-BE, Neuro-2a	i.t.	irradiation (XRT)	[61]
	10^7	HuH6, G401, SK-NEP-1	i.t.	irradiation (XRT)	[62]
R-115	1×10^8-2×10^9	HER2	i.p.	/	[63]
	2×10^6, 1×10^8	HER2	i.t.		[64]
R-123	10^8	HER2-LLC1	i.t.	PD-1 antibodies	[46]
T2850 T3855	10^7	A20, MFC	i.t.	/	[65]
	5×10^6 10^7 3×10^7	B16	i.t.	/	[65]

Adenovirus or Adeno-associated virus (AV or AAV)

AV or AAV is an ideal candidate platform, with genome stability, relative ease of manipulation, easy access to high titers, strong immunogenicity, and wide host range [66–70]. Preclinical studies have tested the tumor inhibitory effect of AV or AAV vectors expressing IL-12 in various tumor models such as sarcoma [71], glioblastoma [72–74], prostate cancer [28], colorectal cancer [75], melanoma [76–78], hepatocellular carcinoma [79,80] and others (Table 3). To improve efficacy, one study designed an AV-mediated co-expression of IL-12 and 4-1BB ligand (4-1BBL) that showed stronger antitumor effects in B16 tumor-bearing mice [77]. Similarly, studies of AV-encoded IL-12 are also exploring combinations with other therapies. Previous studies have shown that the combination therapy of adenovirus-encoded IL-12 with radiotherapy, cell therapy or immunoblot inhibitors has a good anti-tumor effect in prostate cancer [81], melanoma [77,78], colon cancer [82] and Lung cancer [83].

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Table 3. Viral vector delivers IL-12 for cancer therapy-AV or AAV.

Name	Dose (pfu)	Cancer Model	RoA	Combination therapy	Ref
AdmIL-12	10^8	RM-9	i.p.	/	[28]
murine IL-12	2.5×10^8	Renca cells	i.t.	/	[84]
AAV9.RS-mIL-12	2.5×10^{10} vg/kg	Hepa1-6	i.v.	/	[85]
Ad-RTS-mIL-12	5×10^9 vp	GL-261	i.t.	/	[86]
Ad-ΔB7/IL12/GMCSF	5×10^7	B16-F10	i.t.	/	[87]
AdV5-IL-12	1.5×10^8	EMT6-HER2	p.t.	/	[88]
Ad.mIL12	/	GL261	i.t.	/	[72]
AdRGD-IL12	2×10^7	Meth-A	i.t.	/	[71]
AdCMVIL-12	10^8 and 10^9	CT-26 cells	i.t.	/	[75]
ADV/mIL-12	3×10^8	MCA-26	i.t.	/	[76]
oAd+DC	2×10^{10}	LLC	i.t.	/	[83]
rAAV/IL-12	10^{11} vp	DBTRG	i.t.	/	[73]
rAAV2/IL12	1.96×10^{12}	RG2	i.t.	/	[74]
AAV8-Tetbidir-Alb-IL-12	5×10^{11} vg/kg	MC38	i.v.	/	[69]
AAV8/IL-12	10^9 - 10^{11}	BNL HCC	i.v.	/	[79]
OAV-scIL-12-TM	2.5×10^8 10^9 iu	HaP-T1	i.t.	/	[89]
Ad-DHscIL12	10^7 iu	H2T	i.t.	/	[90]
Ad.IL-12	2.5×10^{10}- 3×10^{12} vp	advanced pancreatic, colorectal, or primary liver malignancies	i.t.	/	[91]
RdB/IL-12/IL-18	10^8	B16-F10	i.t.	/	[92]
YKL-IL12/B7	5×10^8	B16-F10	i.t.	/	[93]
AdCMVIL-12	7.5×10^7	CT26	i.t.	/	[94]
Ad-IL-12	10^9	PyMidT	i.t.	/	[95]
Ad.mIL-12	3.3×10^9	7500 RM-1	i.t.	/	[96]
GL-Ad/RUhIL-12	3×10^9 iu	MC-38	i.v.	RU486	[97]
Ad/IL-12	10^9	BNL cells	i.t.	GM-CSF	[98]
AdmIL-12	10^8-10^9	178-2 BMA	i.t.	radiation therapy	[81]
AdIL-12	2.5×10^9	Hepa129	i.t.	AdK1-3	[80]
HC-Ad/RUmIL-12	2.5×10^8 iu	MC38	Intrahepatic	Oxaliplatin	[82]
Adv.mIL-12	3.2×10^8	MCA26	i.t.	4-1BB antibodies	[99]
Ad5-ZD55-CCL5-IL12	10^9	OSRC-2	i.t.	CA9-CAR-T	[100]
Ad-ΔB7/IL-12/4-1BBL	5×10^9	B16-F10	i.t.	Dendritic Cells	[77]
Ad-ΔB7/IL12/GMCSF	5×10^{10}	B16-F10	i.t.	Dendritic Cells	[78]

Vaccinia virus or modified vaccinia virus (VV or MVA)

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Currently, although OV is a promising new treatment option, they have shown limited efficacy for inaccessible and metastatic cancers that require systematic delivery of therapy. VV or MVA is a particularly strong OV candidate for the treatment of inaccessible and metastatic cancers because it has a number of intrinsic characteristics that make it superior to other viruses in clinical development, particularly the lack of need for specific surface receptors and the ability to replicate in an oxygen-deficient environment [101–104]. Moreover, most OV delivery is limited to intratumoral injection, whereas VV has been reported to reach tumors after intravenous delivery [105]. The progress of IL-12 in tumor therapy with VV or MVA delivery is listed in Table 4. In one study, i.t. injection of a tumor-selective VV encoding IL-7 and IL-12 (hIL-7/mIL-12-VV) had considerable antitumor effects in B16-F10, CT26, and LLC models, and even distant tumor suppression [106]. In addition, the combination of hIL-7/mIL-12-VV with anti-PD-L1 or CTLA4 antibodies showed a stronger anti-tumor effect in CT26 models, with complete regression in almost all mice without indications of cytokine storm despite the extent of tumor regression [106]. The combination of hIL-7/mIL-12-VV with anti-PD-L1 or CTLA4 antibodies, induced tumor regression via enhancing the $CD8^+$ T cells, and reducing the Tregs [106].

Table 4. Viral vector delivers IL-12 for cancer therapy-VV or MVA.

Name	Dose (pfu)	Cancer Model	RoA	Combination therapy	Ref
rVV–mIL-12	10^5-10^7	C6 glioma	i.t.	/	[107]
rVV-p53/rVV-2-12	2×10^7	C6 glioma	i.t.	/	[108]
VVΔTKΔN1L-IL12	10^8	LLC, LY2, DT6606,4T1, CT26, SCCVII, HCPC1	i.t.	/	[109]
VAC-2-12	10^7	CT26.CL25	i.v	/	[110]
rVVHA-IL-12	5×10^6	AE17	i.t.	/	[111]
hIL-7/mIL-12-VV	2×10^7	B16-F10, CT26, LLC, TRAMP-C2	i.t.	PD-1 or CTLA4 antibodies	[106]
VV-IL-12mCLTX-HiBiT	10^7 10^8	U2OS, ID8, 4T1.2, MC38	i.t.	PD-1 antibodies	[112]
vvDD-IL-12	10^9	MC38, B16, AB12, CT26	i.p.	PD-1 antibodies	[113]
VACV muIL-12	10^7	CT26, MC38	i.t.	PD-L1 antibodies	[114]
MVA-IL-12	6×10^5	MC38, B16F10, CT26	i.t.	PD-1 antibodies	[115]
MVA.scIL-12	5×10^7	MC38, CT26	i.p.	PD-L1 antibodies	[116]

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Other viruses

In addition to HV, AV and VV, other different viral vectors, such as Measles vaccine strain viruses (MeV), Newcastle disease virus (NDV), Semliki Forest virus (SFV), Maraba Virus (MV), Vesicular stomatitis virus (VSV), Sindbis virus (SV), Canarypox virus and Varicella-zoster virus (VZV), have also been modified to express IL-12, and their related research progress is summarized in Table 5.

Table 5. Viral vector delivers IL-12 for cancer therapy-Other viruses.

Name	Dose (pfu)	Cancer Model	RoA	Combination therapy	Ref
Measles vaccine strain viruses (MeV)					
FmIL-12	5×10^5 ciu	MC38cea, B16hCD46	i.t.	/	[117]
FmIL-12	/	MC38cea	i.t.	/	[118]
Newcastle disease virus (NDV)					
rAF-IL12	2^7 HA	CT26	i.t.	/	[119]
rClone30s-IL12	10^7	H22	i.t.	/	[120]
rAF-IL12	/	HT29	i.t.	/	[121]
Semliki Forest virus (SFV)					
SFV-IL12	10^7 iu	B16	i.t.	/	[122]
rSFV/IL12	10^6 iu	P815	i.t.	/	[123]
SFV-IL12	10^8 vp	MC38 or TC-1	i.v.	/	[124]
IL-12 VLPs	5×10^8	RG2	i.t.	/	[125]
SFV-IL12	10^8 vp	B16, MC38, 4T1 cells	i.t.	PD-1 antibodies	[126]
SFV-IL-12	10^8	B16, TC-1	i.t.	CD137 antibodies	[127]
SFV-IL12	10^8	203-glioma cells	i.t.	/	[128]
rSFV10-E-IL12	4×10^9 iu	CT26, 4T1	i.t.	/	[129]
SFV-IL-12	10^8 vp	MC38	i.t.	/	[130,131]
SFV-IL-12	10^8 vp	HCC	i.t.	/	[132]
SFV-enhIL-12	1.2×10^{10}	HCC	i.t.	/	[133]
LSFV-IL12	10^7-10^9	Panc-1	i.t.	/	[134]
SFV-IL-12	2×10^8 vp	4T1	i.t.	/	[135]
Maraba Virus (MV)					
MG1-IL12-ICV	10^5	CT26	i.p.	/	[136]
Vesicular stomatitis virus (VSV)					
rVSV-IL12	10^7	SCC	i.t.	/	[137]
rVSV-mIL12-mGMCSF	10^7 TCID50	B16F10	i.t.	/	[138]
Sindbis virus (SV)					
Sin/IL12	10^7	ES-2	i.p.	/	[139]
Sindbis/IL-12	10^7	ES-2, MOSEC	i.p.	/	[140]
SV.IgGOX40.IL-12	5×10^6 TU	MOSEC	i.p.	/	[141]
SV.IL12	5×10^6 TU	CT.26	i.p.	OX40 antibodies	[142]
Canarypox virus					

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ALVAC-IL-12	1-4 × 10^6 TCID50	Metastatic Melanoma	i.t.	/	[143,144]
ALVAC-IL12.	2.5 × 10^5 TCID50	TS/A	i.t.	/	[145]
Varicella-zoster virus (VZV)					
Ellen-ΔORF8-tet-off-scIL12	10^5	B16F10	i.t.	/	[146]

2.3. Non-Viral Delivery of IL-12 for Cancer Therapy

Many early studies used viruses as delivery vectors. However, serious clinical adverse events caused by the potential carcinogenicity and high immunogenicity of some viral vectors have affected their application in clinical trials. With the continuous development of materials and preparation technologies, non-viral vectors (such as bio-derived materials and chemical materials) with low cost, easy synthesis, easy purification, high transfection efficiency and low immunogenicity have become the main candidates for drug delivery. Among chemical-based delivery systems, polymer-based nanoparticles and lipid-based nanoparticles are most widely used due to their high potency and diversity [147]. Bioderived vectors, which mainly include exosomes (Exos) [148–150], bacterial outer membrane vesicles (Omv) [151,152], and virus-like particles (VLPs) [153,154], have been shown to be attractive in some applications. In this section, we will describe each delivery system and highlight its application in cancer therapy for delivering IL-12 (Table 6).

2.3.1. Chemical-Based Delivery Systems

Polymer-based nanoparticles

Polymers, which are generally spherical particles formed by electrostatic interactions of polymer molecules with negatively charged nucleic acids, have been extensively studied and reviewed as delivery systems for DNA and RNA-based drugs. Polymeric vectors mainly include: 1) Poly(ethyleneimine) (PEI); 2) Poly (amino acid)s, such as P(Lys), P(Orn), P(Asp); 3) Polyesters include PLGA (Poly(lactic-co-glycolic acid, PLGA), PBAEs (PBAE (poly(β-amino ester)s, PBAEs) and PACE (Polyplexes based on poly(amine-co-ester) , PACE); 4) Natural polymers are Chitosan and Protamine; 5) Other polymers and dendrimers include RAFT polymer (DMAEMA) and the dendrimer PAMAM, as well as other polymers [155–159]. Polymer-based approaches have shown capability for enhancing the delivery of different nucleic acids by protecting them from degradation and promoting cellular uptake and endosomal escape [159]. In this section, we will highlight the latest applications of polymers-delivered IL-12 in cancer therapy.

In preclinical models, polymer-based IL-12 has been used to treat melanoma, colon cancer, liver cancer, breast cancer, lung cancer, ovarian cancer, and other cancers (Table 6). Among them, PBAEs is considered a safe alternative to nucleic acid delivery due to its biodegradability. In one study, Neshat et al. engineered biodegradable lipophilic PBAE delivery co-stimulatory signaling molecules 4-1BB ligand (4-1BBL) and soluble IL-12 (4-1BBL +IL-12 NPs) which, in combination with PD-1 antibodies, effectively induced tumor regression and clearing and resistance to distant tumor reattack [160]. Besides, a study developed a co-delivery system that delivers cisplatin (CDDP) and plasmids encoding the IL-12 gene (HC/pIL-12/polyMET), acting synergistically through chemotherapy sensitization and microenvironment regulation [161]. HC/pIL-12/polyMET has ideal particle size, superior serum stability, effective intracellular CDDP release and IL-12 transfection efficiency [161]. More importantly, the long-circulating HC/pIL-12/polyMET micelle clusters promoted the accumulation of CDDP and IL-12 at the tumor site, thus significantly inhibiting the growth of tumor and prolonging the overall survival of LLC tumor-bearing mice [161]. Potential immune mechanisms suggest that HC/pIL-12/polyMET activated immune effector cells to release IFN-γ and induced M1-type differentiation of tumor-related macrophages, thereby generating synergistic chemoimmunotherapy effect [161]. In another study, a novel polymetformin (PMet)-based nanosystem that co-delivers doxorubicin (DOX) and a plasmid encoding the IL-12 gene (HA/pIL-12/DOX-PMET) was developed for the treatment of metastatic breast cancer [162]. HA/pIL-12/DOX-pmet extends its time in the blood circulation through tumor specific targeting mediated by CD44

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receptors, effectively accumulates in tumors, and is internalized in tumor cells [162]. HA/pIL-12/DOX-PMet micelle clusters synergically enhance NK cells and tumor-infiltrating cytotoxic T lymphocytes, regulate the polarization of tumor M2 macrophages to activated anti-tumor M1 macrophages, and reduce Treg cells [162]. The results showed high antitumor and antimetastatic activity in 4T1 breast cancer lung metastasis mouse models [162]. In conclusion, co-delivery nanoparticles based on multiple molecules have the dual advantages of chemotherapy and gene therapy, and co-delivery combination therapy will have great prospects in cancer therapy.

Lipid nanoparticles (LNPs)

Lipid-based delivery tools, including lipid nanoparticles (LNPs) and lipoplexes, are the most clinically advanced platforms for mRNA delivery [163]. Currently, three RNA-LNPs are currently approved by the US Food and Drug Administration (FDA) and European Medicines Agency: patisiran, a small interfering RNA (siRNA)-LNP for the treatment of hereditary transthyretin-mediated (hATTR) amyloidosis, and BNT162b2 and mRNA-1273, two mRNA-LNP-based COVID-19 vaccines [164]. In order to improve the application of mRNA-LNPs technology in cancer therapy, many studies are devoted to exploring and developing more efficient delivery methods, such as designing and screening novel lipid molecules, adjusting the proportion of lipids in LNPs, modifying the surface of LNPs, and selecting different delivery routes [165–167].

Next, we summarize the application of LNP-based IL-12 in cancer therapy (Table 6). For example, a LNP delivers IL-12 mRNA (IL-12-LNP), which can significantly reduce HCC tumor growth, delay tumor progression, and prolong survival without animal toxicity after weekly i.v. injection [168]. The mechanism is attributed to the increased infiltration of $CD3^+CD4^+CD44^+$ immune cells, but the TME has not been thoroughly explored and elaborated [168]. Compared with i.v. injection, i.t. injection of IL-12 mRNA can effectively promote the localization and sustained production of IL-12 in the TME, and reduce the systemic effect [169]. mIL-12 mRNA, an LNP formulation containing mouse IL-12 mRNA, which was well tolerated, especially with less than 10% weight loss detected at 0.05 and 0.5mg dose levels [169]. A single intratumoral dose of mIL12 mRNA induced regression of multiple tumors such as MC38-S, B16F10 and A20, and even showed good antitumor effects on MC38-R tumor models with ICI antagonism [169]. The antitumor activity of mIL12 mRNA depends on induced IFN-γ and $CD8^+$ T cells, and does not require NK and NKT cells, and its antitumor activity is also associated with TH1 TME transformation [169]. In addition, there are many studies exploring IL-12 in combination with other therapies. Local mIL12 mRNA induces a systemic anti-tumor immune response to distal lesions and exhibits a synergistic tumor suppressive effect in combination with PD-L1 antibody therapy [169]. Since IL-12 has shown promising results in combination with other therapies, direct delivery of IL-12 and other target mRNAs may also have exciting results. F-PLP/pIL12, an FRα-targeted IL-12 lipoplex, has tumor-cell targeting and IL-12 delivery functions [170]. Folate receptorα (FRα) overexpression in colon cancer, F-PLP/pIL12 treatment significantly inhibits CT26 tumor growth and is safe, accompanied by increased IL-12 expression and IFN-γ secretion in tumor tissues [170]. The anti-tumor mechanisms include inducing tumor cell apoptosis, reducing microvascular density, stimulating TNF-α secretion, and activating natural killer cells [170]. Therefore, dual-targeted or even multi-targeted IL-12 lipid nanoparticles may be a promising platform for cancer immunotherapy in the future.

Table 6. chemical-based delivery systems.

Name	Carrier description	Cancer Model	RoA	Combination therapy	Ref
chemical-based delivery systems—Polymer-based nanoparticles					
PEI:IL-12	polyethylenimine (PEI)	osteosarcoma	aerosol	/	[171]
PEI-IL12	PEI-DNA nanoparticles carrying IL12 gene	LLC, CT26	i.v.	/	[172]
mIL-12	polyethylenimine (PEI)	osteosarcoma	intranasal (i.n.)	/	[173]

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IL-12	ifosfamide (IFX) with or without intranasal polyethylenimine (PEI)	LM7 osteosarcoma	i.n.	ifosfamide	[174]
mIL-12	poly[α-(4-aminobutyl)-Lglycolic acid] (PAGA)	CT26	i.t.	/	[175]
p2CMVmIL12	poly-(D,L-lactic-co-glycolic acid) (PLGA) microspheres	CT26	s.c.	/	[176]
pmIL-12	Poly[alpha-(4-aminobutyl)-L-glycolic acid] (PAGA)	CT26	i.t.	/	[177]
4-1BBL and IL-12 mRNA	Biodegradable, lipophilic poly (beta-amino ester) (PBAE) nanoparticles	E0771, MC38	i.t.	PD-1 antibodies	[160]
HC/pIL-12/polyMET	HC/pIL-12/polyMET micelleplexes	LLC	i.v.	/	[161]
HA/pIL-12/DOX-PMet	HA/pIL-12/DOX-PMet micelleplexes	4T1	i.v.	/	[162]
p2CMVmIL-12	water-soluble lipopolymer (WSLP)	CT26	i.t.	/	[178]
p2CMVmIL-12	water soluble lipopolymer (WSLP)	4T1, EMT-6	i.t.	paclitaxel	[179]
p2CMVmIL-12	water-soluble lipopolymer (WSLP)	4T1	i.t.	paclitaxel	[180]
p2CMVmIL-12	Water soluble lipopolymers using cholesteryl chloroformate (WSLP) and PEI	CT26	i.t.	/	[181]
IL-12 plasmid	puly(N-lnethyldietheneamine sebacate) (PMDS) and cholesterol	4T1	i.t.	/	[182]
pmIL-12	Mannosylated chitosan	CT26	i.t.	/	[183]
pmIL-12	polyethylenimine covalently modified with methoxypolyethyleneglycol and cholesterol	GL261	Intracranial (i.c.)	carmustine	[184]
pCMV IL-12	Poly (D,L-lactic-co-glycolic) acid (PLGA) (50 : 50) with the cationic lipid 1,2-dioleoyl-3-(trimethylammonium) propane (DOTAP) and the ligand asialofetuin (AF)	BNL	i.t.	/	[185]
CPP-IL-12	CaCO3-polydopamine-polyethylenimine (CPP)	B16-F10	i.t.	/	[186]
Nano-IL-12	carboxydimethyl-maleic anhydride (CDM)-modified poly(ethylene glycol)-poly(L-Lysine) (PEG-pLL(CDM))	4T1 TNBC, B16F10	i.v.	CTLA4 and PD-1 antibodies	[187]
TINPs	dualtarget PLGA nanoparticles	HepG-2	/	/	[188]
chemical-based delivery systems—LNPs					
IL-12-LNP	lipid nanoparticle (LNP)	HCC	i.v.	/	[168]
IL12 mRNA	a novel lipid nanoparticle (LNP)	MC38, B16F10, A20	i.t.	PD-L1 antibodies	[169]
F-PLP/pIL12	an FRα-targeted lipoplex	CT26	i.p.	/	[170]

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DAL4-LNP-IL-12 mRNA and IL-27 mRNA	ionizable lipid materials containing di-amino groups with various head groups (DALs)-DAL4-LNP	B16F10	i.t.	/	[189]
JCXH-211	lipid nanoparticle-encapsulated self-replicating RNA (srRNA) encoding IL-12	MC38, B16F10, EMT6	i.v. i.t.	PD-1 antibodies	[190]
LNP-Rep(IL-12-alb)	lipid nanoparticles (LNP)	B16F10, CT26	i.t.	PD-1 antibodies	[191]
IL-12 mRNA	calcium carbonate nanoparticles	GL261	i.v.	ultrasound	[192]
IL12LNP	lipid nanoparticles (LNP)	HT29	i.t.	/	[193]
IL-12 circRNA LNP	ionizable lipid nanoparticles	LLC1	i.t.	PD-L1 antibodies	[194]
pCMVIL-12	transferrin (Tf)-lipoplexes	CT26	i.t.	/	[195]
DMP/IL-12	Monomethoxy poly (ethylene glycol)–poly (caprolactone) with the DOTAP lipid	C26，LL/2	i.p.	/	[196]
ATRA-cationic liposome/IL-12 pDNA	All-trans-retinoic acid (ATRA)-incorporated cationic liposome (ATRA-cationic liposome)	colon26 cells	i.v.	/	[197]

2.3.2. Bio-Derived Delivery Vector

Extracellular vesicle (EVs) are important intercellular communication systems that promote the transfer of macromolecules between cells. For example, Exos are considered natural mRNA delivery systems. In one study, an inhalable extracellular vesicle loaded with IL-12 mRNA (IL-12-Exo) was developed, which effectively controlled the development of lung cancer and enhanced systemic immunity [198]. Importantly, the specific targeting of IL-12-Exo to lung tumors is much higher than that of liposome loaded IL-12 mRNA (IL-12-Lipo), about 1.54 times [198]. IL-12-Exo significantly inhibited LL/2 and B16F10 tumor growth and progression, accompanied by moderate weight gain in mice [198]. The antitumor mechanism was attributed to the remodeling of TME, increasing the proportion of $CD8^+$ T cells, $CD4^+$ T cells, NK cells, and NKT cell populations, while decreasing the proportion of immunosuppressive Tregs and Myeloid-derived suppressor cells (MDSCs) [198]. IL-12 mRNA stimulates upregulation of a broad spectrum of inflammatory cytokines and chemokines in TME, especially the sustained secretion of high levels of IFN-γ [198]. Compared with liposome delivery systems, exosomes enhance the expression of IL-12 and greatly reduce its toxicity in vivo [198]. As a non-invasive method, inhalation is expected to lead to better patient compliance than intratumoral injection. Exos, as biocompatible vesicles, provide a universal RNA delivery scheme [198].

Table 7. Bio-derived delivery systems.

Name	Source	Dose	Cancer Model	RoA	Ref
IL-12-Exo	human embryonic kidney cell-derived exosomes	2×10^9 particles	LL/2, B16F10, 4T1	Inhal	[198]
ITGB1−mscIL12+HN3+Deg EVs	HEK293-derived EVs	5×10^{10} particles	Hepa1-6-hGPC3	i.v.	[199]
Tex MC38/IL12shTGFβ1	MC38-derived particles	2×10^6 Particles	MC38	p.t.	[200]

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exoIL-12	HEK293SF-3F6	100 ng	B16F10, MC38, CT26	i.t.	[201]
IL-12-encapsulated DEVs (DEV-IL)	mature dendritic cells (DEVs)	25 μg	GL-261	s.c.	[202]

2.4. Cells-Based Delivers IL-12 for Cancer Therapy

Recently, cells have also been proposed as drug carriers because of their effective delivery of therapeutic substances to the tumor [203–206]. Dendritic cells, T cells, Mesenchymal stromal cells and other cells have been designed to express IL-12 for cancer therapy.

Dendritic Cells (DCs)

DCs are the most powerful antigen presenting molecule (APCs) in vivo, linking innate and adaptive immunity. DCs-based therapeutic strategies have proven to be a positive approach to treating cancer by altering the TME and enhancing the systemic host immune response [207]. Following i.t. or peritumorally (p.t.) injection, DCs have been engineered to express IL-12 to induce a powerful anti-tumor immune response (Table 8).

In preclinical studies, DCs transfected with IL-12 effectively inhibited the growth of various tumors such as melanoma [208–213], Colon cancer [214–218] and other tumors [219–221]. The therapeutic benefits of DCs-expressed IL-12 are associated with induction of specific $CD8^+$ T cells and durable anti-tumor immunity. In a clinical study, the feasibility, safety, and antitumor activity of DCs-transduced IL-12 (AFIL-12) in the treatment of metastatic gastrointestinal cancer was validated. The results showed that 2 patients were stable and 8 progressed, of which 2 progressed rapidly during treatment, demonstrating that i.t. injection of AFIL-12 for the treatment of metastatic gastrointestinal malignancies is feasible and well tolerated, but further studies are needed to determine and improve clinical efficacy [222].

Table 8. Cells-Based Delivery of IL-12 for cancer therapy-DCs.

Name	Cancer Model	ROA	Ref
DC.RheoIL12	B16	i.t.	[208]
DC-mIL-12	B16F10	i.t.	[209]
mIL-12	B16	i.t.	[210]
DC+IL-12	Melanoma B6	i.t.	[211]
DC.IL12	B16	i.t.	[212]
gp100+IL12/DCs	B16BL6	i.d.	[213]
DC/IL-18+IL-12/TAg	MC38	p.t.	[215]
AdCMVmIL-12	CT26	i.t.	[214]
BM-derived DC infected with AdCMVIL-12	CT26, MC38	i.t.	[216]
AdIL12/IL18DC	CMS4, MethA	i.t.	[217]
AdIL12DC	CMS4	i.t.	[218]
mIL-12	TBJ-NB	i.t.	[219]
DC/IL-12	178-2 BMA	i.t.	[220]
DC-IL-12	RENCA	i.t.	[221]
AFIL-12	pancreatic, colorectal, primary liver, gastrointestinal cancers malignancies	i.t.	[222]

T Cells

T cells therapy is a promising therapeutic approach, but it is often hampered by the highly immunosuppressive TME, such as limited T cell trafficking, persistence and durable anti-tumor

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activity. Engineering T cells to express IL-12 has been shown to improve antitumor efficacy and reduce systemic toxicity in solid tumors (Table 9). In multiple models, injection of IL-12-expressing T cells induced regression of many tumors, including melanoma [223–227], sarcoma [226], colorectal adenocarcinoma [226,228] and other cancers [229–233]. The efficacy of T cell therapy generally depends on increasing chemokines and cytokines, promoting the proliferation of $CD8^+$ T cells, and reducing the proportion of Treg cells, which can directly promote the effective enrichment of T cells and anti-tumor effects.

Table 9. Cells-Based Delivery of IL-12 for cancer therapy-T cells.

Name	Cancer Model	ROA	Ref
OT-I-IL-12	B16-OVA, PANC02-OVA	i.p.	[223]
OT1-IL-12 mRNA	B16-OVA	i.t.	[224]
IL-12 + DRIL18	B16-OVA	i.t.	[225]
IL-12	B16 tumors	i.v.	[227]
DC101 CAR-Flexi-IL12	B16F10, MCA205, MC17–51, MC38, CT26	i.v.	[226]
T cells CAR+iIL-12	CEA^-MC38, CEA^+ C15A3	s.c.	[228]
mIL12 and mIFNα2	GL-261, CT-2A, SMA-560	i.v.	[229]
19mz/IL-12	EL4	i.v.	[230]
CAR-IL12 T-cells	A20	i.v.	[231]
4H11-28z/IL-12	SKOV3	i.p.	[232]
GPC3-28Z-NFAT-IL-12	PLC/PRF/5, Huh-7	i.v.	[233]
INS-CAR T	Raji	i.v.	[234]
RB-312	HT1080, FaDu	i.t.	[235]

Mesenchymal stromal cells (MSCs)

MSCs have been used in many trials due to their immunosuppressive properties and their tendency to target cancer cells, including as IL-12 vectors for solid tumors (Table 10). Such as, one study observed that after i.v. administration of MSCs-loaded IL-12 (MSC/IL-12) in tumor-bearing mice, tumor growth was inhibited, the number of metastases significantly decreased, blood vessel density decreased, and the number of anticancer M1 macrophages and $CD8^+$ T lymphocytes in the tumors increased, and without systemic toxicity [236]. In addition， Park et al. designed mesenchymal stem cells (MSC_IL-12) with glioblastoma propensity to secrete IL-12 and evaluated that MSC_IL-12 has a good efficacy in glioblastoma (25.0% cure rate) [237]. Tumor infiltrating lymphocytes (TILs) analysis showed that MSC_IL-12 treatment resulted in $CD4^+$ T cell and NK cell infiltration, as well as reduced Tregs frequency [237]. Moreover, the combination of PD-1 antibodies and MSC_IL-12 showed a better anti-tumor effect (50% cure rate) [237]. Excitingly, no tumor growth was observed in the cured mice after re-attack, indicating long-term immunity to treatment-induced glioblastoma [237].

Table 10. Cells-Based Delivery of IL-12 for cancer therapy-MSCs.

Name	Cancer Model	ROA	Ref
MSC/IL-12	B16-F10	i.t.	[236]
MSC/IL-12	B16-F10	i.p.	[238]
MSC(IL-12)	glioblastoma GL26	i.t.	[237]
CAd12_PD-L1 MSCs	A549, H1650	i.v.	[239]
IL-12 MSCs	4T1	s.c.	[240]
MSC-AdIL12	Ast11.9-2	/	[241]

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MSC/IL-12	786-0	i.v	[242]
MSCs/IL-12	HCa-I, Hepa 1-6	i.t.	[243]
FYD + IL-12 + BMSCs	U251	i.v	[244]
MB/IL12-MSCs	EMT6	i.v	[245]
CAR+MSC IL7/IL12	LS174T	s.c.	[246]
MSCs/IL-12M	B16F10	i.t.	[247]
UCB-MSC-IL12M	GL26	i.t.	[248]

Other cells

In fact, in addition to the commonly used cell carriers such as DC, CAR T and MSCs, there are other different cell delivery carriers, such as macrophages, NK cells, glial cells, etc., and even genetically engineered tumor cells (Table 11). In one study, autologous tumor cell vaccines via EBV/liposomes were designed to secrete IL-12 and IL-18 (B16/mIL-12+mIL-18), and repeated immunization showed strong tumor inhibition in a B16 melanoma model, accompanied by high IFN-γ production [249].

Table 11. Cells-Based Delivery of IL-12 for cancer therapy- other cells.

Name	Cancer Model	ROA	Ref
AdmIL-12	178-2BMA	i.t.	[250]
G/M//AdmIL-12	178-2BMA	i.t.	[251]
GD2.CAR(I)IL12	BV-173, CHLA-255	i.v.	[252]
B16/mIL-12+mIL-18	B16	s.c.	[249]
Neuro2a/IL-12/IL-15	neuroblastoma	i.v.	[253]
pT-mIL12 and pCMV-m7pB	B16/OVA	ACT	[254]

3. Clinical Perspectives

IL-12 with different strategic loads has been shown to have good broad-spectrum antitumor effects and safety in preclinical models, suggesting that IL-12 is an attractive therapeutic candidate. In general, safety remains the most concerned aspect when transferring results from the laboratory to the bedside, as does dose, route of administration, viral pharmacokinetics, and host cell resistance mechanisms. Currently, some IL-12 is being tested in clinical studies against various cancers, such as breast cancer, lung cancer, pancreatic cancer, ovarian cancer, colorectal cancer and melanoma, etc. (Table 12). Because some traditional recombinant human IL-12 has been associated with different degrees of adverse reactions in clinical trials, different strategies of delivery of IL-12 are under clinical study.

Safety and anti-tumor efficacy of NHS-IL12 as monotherapy or combination therapy has been demonstrated in preclinical tumor models [23–25]. Excitingly, safety and anti-tumor efficacy of human NHS-IL12 was found in a Phase I clinical trial (NCT01417546) to have good treatment tolerance, enhanced immune-related activity, and increased immune infiltration in TME [255]. And multiple clinical trials (NCT04287868, NCT04491955, NCT02994953, NCT04303117, NCT04235777) have demonstrated promising results for NHS-IL12 in Advanced HPV Associated malignancies, small bowel and colorectal cancers, kaposi's sarcoma, urothelial cancer etc. For example, the Phase II clinical trial (NCT04491955) of the NHS-IL12 combination therapy in patients with small intestine and colon cancer showed encouraging results (CR 12.5%), but was accompanied by grade 3 anemia (37.5%), grade 3 duodenal bleeding (12.5%) in some patients. And no other side effects. Similarly, virus expressing human IL-12 is also under clinical trial investigation as monotherapy or combination therapy in prostate cancer (NCT02555397, NCT00406939), pancreatic cancer (NCT03281382), breast cancer (NCT00849459, NCT00301106), melanoma (NCT01397708, NCT00003556), pediatric brain tumor (NCT03330197), glioblastoma (NCT02026271, NCT03636477, NCT05084430) and other solid tumors (NCT04613492, NCT04613492). In addition, electroporation is a non-viral gene delivery method of plasmid DNA. The plasmid gene encoding IL-12 in intratuminal metastasis has been

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proved to be safe and effective in clinical experiments, and has good local tumor control effect (Table 12). And the strategy of cells as carriers for delivering IL-12 has also been validated in clinical trials (Table 12). In our view, many preclinical and clinical studies of IL-12 delivered with different strategies have shown exciting performance in combination with other therapies. Therefore, the combined study of IL-12 and ICI is worthy of clinical investigation and may become an attractive treatment strategy for cancer patients. It is worth mentioning that many pre-clinical studies have shown that liposome loaded IL-12 mRNA has good anti-tumor effect and safety, and its related studies deserve attention and push to clinical trials.

Table 12. Research progress of IL-12 in clinical trials.

Name	Tumor type	ROA	Status	NCT Number
rhIL-12 and IL-2	Advanced Solid Tumors	i.v.+s.c.	Phase I	NCT00005604
recombinant IL-12	Primary Peritoneal Cavity Cancer Recurrent Ovarian Epithelial Cancer	i.p.	Phase II	NCT00016289
NHS-IL12	Epithelial Neoplasms, Malignant Epithelial Tumors, Malignant Malignant Mesenchymal Tumor	s.c.	Phase I	NCT01417546
NHS-IL12	Advanced HPV Associated Malignancies	s.c.	Phase I/II	NCT04287868
NHS-IL12	Small Bowel and Colorectal Cancers	s.c.	Phase II	NCT04491955
NHS-IL12	Advanced Solid Tumors	i.v.	Phase Ib	NCT02994953
NHS-IL12	Kaposi Sarcoma	i.v.	Phase I/II	NCT04303117
NHS-IL12	Urothelial Cancer Bladder Cancer Genitourinary Cancer Urogenital Cancer	i.v.	Phase I	NCT04235777
NM-IL-12	Colostomy Stoma	s.c.	Phase IIa	NCT02544061
SON-1010 (IL12-FHAB)	Platinum-resistant Ovarian Cancer	/	Phase 1b/2a	NCT05756907
Ad5-yCD/mutTKSR39rep-hIL12	Prostate Cancer	i.t.	Phase I	NCT02555397
Adv/IL-12	Prostate Cancer	i.t.	Phase I	NCT00406939
Ad5-yCD/mutTKSR39rep-hIL12	Metastatic Pancreatic Cancer	i.t.	Phase I	NCT03281382
adenovirus-mediated human interleukin-12	Breast Cancer	i.t.	Phase I	NCT00849459

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Ad.hIL-12	Radiorecurrent Prostate Cancer	i.p.	Phase I	NCT00110526
Ad-RTS-hIL-12	Melanoma	i.t.	Phase I/II	NCT01397708
Ad-RTS-hIL-12	Pediatric Brain Tumor Diffuse Intrinsic Pontine Glioma	i.t.	Phase I/II	NCT03330197
Ad-RTS-hIL-12	Glioblastoma Multiforme Anaplastic Oligoastrocytoma	i.t.	Phase I	NCT02026271
Ad-RTS-hIL-12	Glioblastoma	i.t.	Phase I	NCT03636477
Adv.RSV-hIL12	Breast Cancer Metastatic Cancer	i.t.	Phase I	NCT00301106
canarypox-hIL-12	melanoma	i.t.	Phase I	NCT00003556
MEDI9253(Recombinant Newcastle Disease Virus Encoding Interleukin-12)	Solid Tumors	i.t.	Phase I	NCT04613492
MEDI9253 + Durvalumab	Solid Tumors	i.t.	Phase I	NCT04613492
M032 (a Genetically Engineered HSV-1 Expressing IL-12)	Glioblastoma	i.t.	Phase I/II	NCT05084430
hTERT and IL-12 DNA	Breast Cancer Lung Cancer Pancreatic Cancer Head and Neck Cancer Ovarian Cancer ColoRectal Cancer Gastric Cancer Esophageal Cancer HepatoCellular Carcinoma	i.m.	Phase I	NCT02960594
IT-pIL12-EP	Triple negative breast cancer	i.t.	Phase I	NCT02531425
IL-12p DNA	Malignant Melanoma	i.t.	Phase I	NCT00323206
IL-12 DNA	Metastatic Cancer	i.t.	Phase Ib	NCT00028652
Interleukin-12 cDNA	Colorectal Cancer Metastatic Cancer	i.t.	Phase I	NCT00072098
Interleukin-12 Plasmid	Merkel Cell Carcinoma	i.t.	Phase II	NCT01440816
INO-3112 (plasmid-encoding interleukin-12/HPV DNA plasmids) and durvalumab	Recurrent/Metastatic Human Papilloma Virus Associated Cancers	i.m.	Phase II	NCT03439085
IMNN-001 (IL-12 Plasmid Formulated With PEG-PEI-	Epithelial Ovarian Cancer Fallopian Tube Cancer Primary Peritoneal Cancer	i.p.	Phase I	NCT02480374

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Cholesterol Lipopolymer)				
Egen-001 (IL-12 Plasmid Formulated With PEG-PEI-Cholesterol Lipopolymer)	Ovarian Clear Cell Cystadenocarcinoma Ovarian Endometrioid Adenocarcinoma Ovarian Seromucinous Carcinoma	i.p.	Phase I	NCT01489371
EGEN-001 (IL-12 Plasmid Formulated With PEG-PEI-Cholesterol Lipopolymer)	Fallopian Tube Carcinoma Primary Peritoneal Carcinoma Recurrent Ovarian Carcinoma	i.p.	Phase II	NCT01118052
EGEN-001 and Pegylated Liposomal Doxorubicin Hydrochloride	Ovarian Clear Cell Cystadenocarcinoma Ovarian Endometrioid Adenocarcinoma Ovarian Seromucinous Carcinoma Ovarian Serous Cystadenocarcinoma Ovarian Undifferentiated Carcinoma Recurrent Fallopian Tube Carcinoma Recurrent Ovarian Carcinoma Recurrent Primary Peritoneal Carcinoma	i.p.	Phase I	NCT01489371
phIL12 GET	basal cell carcinomas	i.t.	Phase I	NCT05077033
EGFR-IL12-CART	Metastatic Colorectal Cancer	/	Phase I/II	NCT03542799
Interleukin 12-Primed Activated T Cells (12ATC)	Melanoma	i.v.	Phase I	NCT00016055
interleukin-12-primed activated T cells (12ATC)	Colorectal Cancer Kidney Cancer	i.v.	Phase I	NCT00016042
Interleukin-12-Primed Activated T Cells in combination with 5FU, GM-CSF And Interferon Alfa-2b	Colorectal Cancer Kidney Cancer	i.v.	Phase I/II	NCT00030342
EGFRt/19-28z/IL-12 CAR T Cells	Hematologic Malignancies	i.v.	Phase I	NCT06343376
CAR-T Cells (IL7 and CCL19 or / and IL12) Targeting Nectin4/FAP	Nectin4-positive Advanced Malignant Solid Tumor	i.t.	Phase I	NCT03932565
T-Cell Membrane-Anchored Tumor	Soft Tissue Sarcoma Bone Sarcoma	i.v.	Phase 1	NCT05621668

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Targeted Il12 (Attil12)				
IL-12 gene-transduced TIL	Melanoma	i.v.	Phase I/II	NCT01236573
Dendritic and Glioma Cells Fusion Vaccine With IL-12	Glioblastoma	i.d.	phase I/II	NCT04388033
anti-ESO-1/IL-12 white blood cells	Metastatic Melanoma Metastatic Renal Cancer	i.v.	Phase I/II	NCT01457131
bacTRL-IL-12	Treatment-refractory Solid Tumours	i.v.	Phase I	NCT04025307

4. Conclusions and Future Directions

In the field of tumor therapy, although IL-12 is a high-profile molecule, its therapeutic effect on solid tumors is not ideal and even causes serious adverse reactions. The current research focus is mainly on local targeted drug delivery to reduce adverse reactions, and to play a synergistic role in combination with chemoradiotherapy or immunotherapy. For systematic administration, the focus is on reducing the off-target effect of IL-12. In order to achieve the goal of IL-12 effectively treating tumors, many strategies to modify or deliver IL-12 are under investigation. It is believed that with the in-depth study of the anti-tumor mechanism of IL-12 and the improvement of IL-12 delivery strategy, drug use, pathway and synergistic drug use, IL-12 will be successfully developed into an anticancer drug with important clinical application value.

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