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REVIEW ARTICLE

Emerging immunologic approaches as cancer anti-angiogenic therapies

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Abstract

Targeting tumor angiogenesis, the formation of new blood vessels supporting cancer growth and spread, has been an intense focus for therapy development. However, benefits from anti-angiogenic drugs like bevacizumab have been limited by resistance stemming from activation of compensatory pathways. Recent immunotherapy advances have sparked interest in novel immunologic approaches that can induce more durable vascular pruning and overcome limitations of existing angiogenesis inhibitors. This review comprehensively examines these emerging strategies, including modulating tumor-associated macrophages, therapeutic cancer vaccines, engineered nanobodies and T cells, anti-angiogenic cytokines/chemokines, and immunomodulatory drugs like thalidomide analogs. For each approach, the molecular mechanisms, preclinical/clinical data, and potential advantages over conventional drugs are discussed. Innovative therapeutic platforms like nanoparticle delivery systems are explored. Moreover, the importance of combining agents with distinct mechanisms to prevent resistance is evaluated. As tumors hijack angiogenesis for growth, harnessing the immune system's specificity to disrupt this process represents a promising anti-cancer strategy covered by this review.

Keywords Immunotherapy · Immune checkpoint inhibitors · Nanoparticles · Chimeric antigen receptor T cells (CART) · Antiangiogenic vaccines

Introduction

The formation of new blood vessels, a process known as angiogenesis, plays a critical role in tumor growth, survival, and metastasis. In the majority of malignancies, angiogenesis is not only accelerated but also aberrant in structure and function. As tumors outgrow their existing blood supply,

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they secrete pro-angiogenic factors that stimulate the sprouting of new vessels from nearby capillaries. This "angiogenic switch" creates a disorganized network of leaky, tortuous tumor blood vessels that facilitate tumor expansion by providing nutrients and oxygen while also providing routes for metastatic dissemination. [1]. Through this event, endothelial cells (ECs) will begin to proliferate and migrate through the extracellular matrix, utilizing matrix metalloproteinases (MMPs). Detached ECs will then begin to rearrange and form tube-like structures, which will ultimately turn into mature functional vessels. Newly formed vessels are commonly leaky owing to enhanced permeability and insufficient stabilization and/or maturation due to the absence of pericytes [1], and are frequently more intricate, inflamed, and tortuous [1, 2].

Targeting angiogenesis has been an intense area of focus for cancer therapy over the past two decades. The rationale is that depriving tumors of their blood supply can inhibit their growth and even cause regression. To this end, over 40 angiogenesis inhibiting drugs have been evaluated clinically, including monoclonal antibodies, soluble receptor decoys, and tyrosine kinase inhibitors that target key pro-angiogenic pathways like vascular endothelial growth factor (VEGF) signaling. However, the clinical benefit of anti-angiogenic monotherapies has been limited, in part due to activation of compensatory pro-angiogenic mechanisms that allow tumors to regrow their vasculature and develop resistance [3]. Unfortunately, the main drawback associated with the application of these therapies is the development of a more invasive form of the cancer, demonstrating an aggravated rate of growth and metastasis which is referred to as the "rebound effect" [4], occurring partly as a consequence of the activation of compensatory angiogenic pathways by the activity of other members of the VEGF superfamily and numerous secreted cytokines and angiogenesis promoting factors [2]. Along with these pathways, other mechanisms, including vessel cooption, vessel intussusception, and/or vasculogenic mimicry are responsible for instant compensation of angiogenesis suppression mediated by anti-angiogenic drugs [2].

Considering this brief preface in mind, the importance of developing an anti-angiogenic approach capable of yielding a prolonged beneficial outcome will become much bolder. Today, immunotherapies are of paramount concern for treatment of solid tumors and novel anti-cancer immunologic approaches are progressively introduced into clinic. The rate of progress has been accelerated even more by the discovery of checkpoint inhibitors and their ability to bring on long-term remission and possibly cure in specific cases. This breakthrough has also encouraged immunologists to develop several novel immunologic approaches for achieving anti-angiogenic effects with the hope of overcoming the failure associated with bevacizumab monotherapy. Presently, some of these approaches including modulators of tumor-associated macrophages (TAMs), peptide vaccines against tumor associated ECs, bispecific nanobodies, anti-angiogenic cytokines and chemokines, engineered T cells (CART cells) and specific immunomodulatory small molecules like thalidomides have demonstrated interesting preclinical outcomes and have been successfully passed their way into clinical settings (Fig. 1). In this review, we will concisely review this set of novel immunologic efforts dedicated to cancer antiangiogenic therapy and mention the clinical outcomes associated with them.

Targeting tumor-associated macrophages (TAMs)

Role of TAMs in promoting angiogenesis

As reported by several studies, one of the key components of the tumor microenvironment (TME) capable of promoting angiogenesis is TAM [5]. Primarily, the pivotal role of TAMs in the induction of an angiogenic switch was discovered in a breast cancer mouse model [6]. Since then, ongoing studies have demonstrated that TAMs are capable of secreting numerous proangiogenic growth factors, most importantly VEGF, and accelerating degradation rate of perivascular ECM through upregulation of secretion of a range of enzymes from MMP superfamily [7]. The proangiogenic molecules and ECM degrading enzymes secreted by TAMs which have been discovered so far, range from VEGF, epidermal growth factor (EGF), platelet derived growth factor (PDGF), transforming growth factor alpha (TGF-α) and beta (TGF-β), angiopoietin 1 and 2 (Ang-1 and -2) up to different MMPs (including MMP-2, MMP-9 and MMP-12) and serine/cysteine proteinases (including cathepsins and plasminogen activator) [8]. Moreover, other proangiogenic factors comprising the S100 superfamily members, SEMA family members, cyclo-oxygenase 2 enzyme, osteopontin 1, osteonectin, Tie-2, and several members of chitinase-like proteins such as YKL-39, YKL-40 have also been discovered to be produced by TAMs in various in vitro studies. Hence, inhibitors of TAMs may have great potential for targeting angiogenesis.

Moreover, the ratio between M1 and M2 phenotypes of macrophages (M1/M2 ratio) also plays a pivotal role in the acceleration of tumor associated angiogenesis and expansion. Overall, the M1 phenotype is commonly considered to demonstrate anti-tumoral effects while the M2-polarized one is often deemed the tumor-associated macrophage, which contributes to the above-mentioned tumorigenic outcomes through upregulating angiogenic, and lymphangiogenic events, promoting immunosuppressive events, inducing, hypoxia and promoting cancer cells' proliferation and

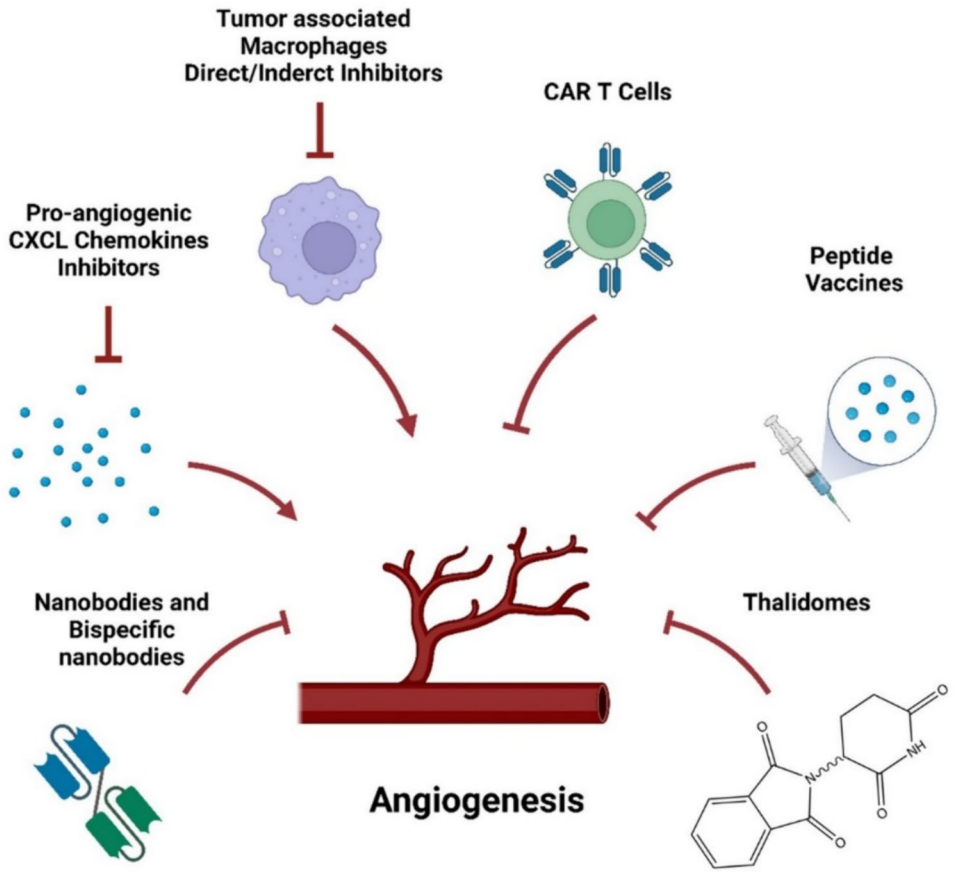

Fig. 1 Novel immunologic approaches with anti-angiogenic effects against tumor associated angiogenesis. So far, modulators of TAMs, peptide vaccines against tumor associated ECs, bispecific nanobodies, anti-angiogenic cytokines and chemokines, engineered T cells (CART cells) and specific immunomodulatory small molecules like thalidomides have demonstrated interesting preclinical outcomes and have been successfully passed their ways in to clinical settings

dissemination. TME and its components are the main modulators of macrophage recruitment and polarization, hence, promoting tumorigenic outcomes [9].

TAM inhibitors can be broadly classified as direct and indirect inhibitors. Direct inhibitors often aim at reprograming TAMs toward M1 phenotype, eradicating currently existing TAMs, and/or inhibiting the recruitment of new TAMs, while indirect inhibitors are mostly associated with suppression of endothelial cell recruitment and formation of new blood vessels or attenuating the action of angiogenic growth factors secreted by both cancer and stromal cells (Fig. 2).

Direct inhibitors of TAMs

So far, the most pivotal tumor-secreted biomolecule capable of recruiting monocytes is colony-stimulating factor 1 (CSF-1), which does so by interacting with the CCL2/CCR2 axis, where CCL2 (chemokine (C–C motif) ligand 2) is a chemokine that attracts monocytes, and CCR2 (C–C chemokine receptor type 2) is the receptor on monocytes that mediates this recruitment. Hence, blockade of CCR2 may be an effective way of inhibiting TAM recruitment to the TME [10]. In this context, the application of anti-CCL2 mAbs as well as CCR2 inhibitors in preclinical mouse models of cancer has shown promising results both alone and together with other anti-neoplastic agents [11]. Despite this, using a breast cancer mouse model, the rebound effect was spotted following cessation of anti-CCL2 mAB administration, ending in an enhanced recruitment of bone marrow-derived monocytes into the TME and promoting development of lung metastasis [12]. The other pivotal route responsible for infiltration of monocytes into the TME and promotion of M2 polarization in tumor site is the CXCL12/CXCR4 axis. In this context, enhanced secretion of CXCL12 by breast cancer cells have shown to enhance the number of residing macrophages in tumor niche, increase the number of blood vessels in tumor site and accelerate metastasis [13]. Application of AMD3100, a selective inhibitor of CXCR4, in this case was combined with a reduction of metastasis to secondary sites [14].

The second group of direct inhibitors are those demonstrating TAM depleting capacity. As mentioned in the previous paragraph, since CSF-1 and CSF-1R upregulation in the TME is often associated with poor prognosis and considering the fact that CSF-1 is a pivotal factor in proliferation and survival of both monocytes and macrophages, blocking the CSF-1/CSF-1R axis is also an effective way of eradicating TAMs and reducing their accumulation in TME. Using a mouse model of cancer, it was shown that application of emactuzumab, a mAB specific to CSF-R1, could effectively

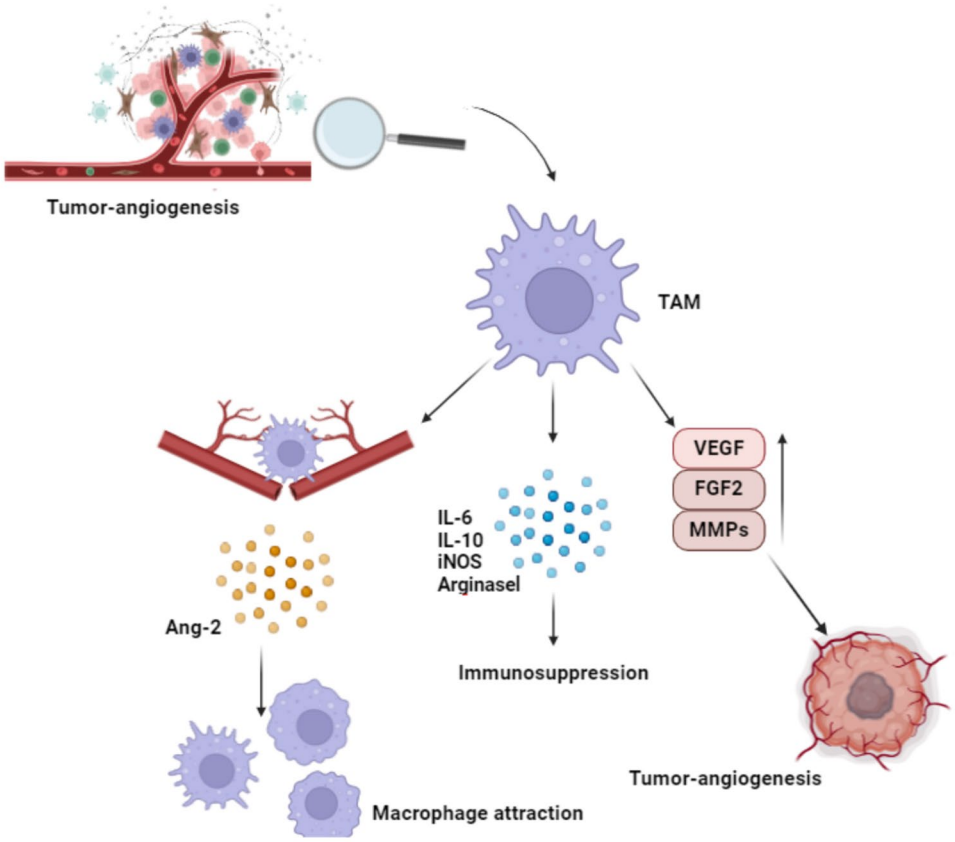

Fig. 2 The multifaceted role of Tumor-Associated Macrophages (TAMs) in fostering tumor growth and angiogenesis. TAMs can secrete pro-angiogenic factors including, VEGF (Vascular Endothelial Growth Factor), FGF2 (Fibroblast Growth Factor 2), and MMPs (Matrix Metalloproteinases) that promote further tumor angiogenesis. In addition, TAMs release immunosuppressive factors such as IL-6, IL-10, iNOS, and Arginase. In addition, TAMs can secrete Ang-2 (Angiopoietin-2) attract more macrophages to the tumor site

reduce TAM population, while enhancing the $CD8^+$/$CD4^+$ T-cell ratio, which is also an advantageous immunologic event in fighting against tumors [15]. Moreover, application of PLX3397, a small molecule inhibitor of CSF-R1, was associated with an enhancement in $CD8^+$ T-cell recruitment and an enhanced therapeutic response in different preclinical in vivo studies [16]. Unfortunately, another drawback of targeting CSF-1/CSF-R pathway is that long term blockade of this axis will alternatively end in induction of the phosphoinositide 3-kinase (PI3K) pathway and the gradual development of resistance to the therapy. Hence, administration of a PI3K pathway inhibitor together with CSF-1R blockers is necessary and has been shown to have extra advantageous results in pre-clinical settings [17].

The third group of direct TAM inhibitors are the ones capable of redirecting existing M2 macrophages into an anti-cancer M1 phenotype. It has been shown that the activation of toll-like receptors (TLRs) by their specific ligands is the key event in polarization to M1 phenotype [18]. So far, numerous activators of TLR subclasses, including TLR-3, TLR-7, TLR-8, and TLR-9 have been discovered. However, the only TLR ligand with Food and Drug Administration (FDA) approval for the treatment of squamous and basal cell carcinomas is imiquimod, which interacts with TLR-7 [19]. Recently, stimulating TLR-3 with poly I:C has been shown to be more potent compared to imiquimod in redirecting M2 phenotype to M1 [20] but further studies are necessary for confirmation of this statement. In this context, application of poly I:C loaded nanoparticles in vitro resulted in enhanced secretion of nitric oxide (NO) and upregulation of TNF-α, all of which are hallmarks of the enhancement of the number of M1 macrophages [21].

Indirect inhibitors of TAMs

As mentioned previously, one of the major ways to indirectly reduce the number of TAMs is to inhibit the recruitment and proliferation of ECs at the site of TME. Endostatin, canstatin, and tumstatin are the main molecules that do so by targeting the signaling pathways in ECs [22]. As a recombinantly expressed protein, endostatin is the 20 kDa C-terminal moiety of the human collagen type XVIII protein, [23] which suppresses ECs proliferation and induces apoptosis via dysregulation of ATPase activity in these cells [24]. In this context, application of endostatin in a A549-GFP expressing xenograft mouse tumor model could effectively attenuate tumor growth rate by promoting the number of apoptotic cancer cells and suppressing tumor associated angiogenesis, as evident from a statistically significant reduction in the number of CD31 + cells, compared to the wild type control group [23]. Similarly, canstatin is a non-collagenous C-terminal moiety of the

α2 chain of collagen type IV, which has been shown to effectively suppress proliferation and migration of ECs and to interfere with normal tube formation assays [25]. Research has demonstrated that canstatin can inhibit AKT phosphorylation and promote FASL expression on the surface of human umbilical vein endothelial cells (HUVECs) [26]. In addition, when applied to a mouse model of cancer, canstatin could reduce angiogenesis, as evident by a reduction in the number of CD31-expressing cells [27].

The other way indirect inhibitors of TAM do their action is through suppression of angiogenic factors secreted by cancer cells and stromal cells presented in TME. A list of these molecules, as well as direct inhibitors of TAMs has been provided in Table 1.

Peptide vaccine approaches

Advantages of peptide vaccines

Vaccination against tumor-associated ECs is one of the most promising ones, considering the fact that immune system's components can directly get in contact with the ECs at the surface of the tumor vessels and breach the physical barrier around TME. The ultimate goal of vaccination is the induction of immune responses toward specifically selected antigens on tumor ECs while passing those cross-reacting with normal vessels' ECs to prevent induction of unwanted deleterious autoimmune responses. So far, a range of biomolecules with the mentioned characteristics have been spotted and have been used for the development of a range of traditional anti-angiogenic therapies, including mABs.

Table 1 Direct and indirect inhibitors of TAM for targeting cancer-associated angiogenesis

Group of action	Name of inhibitor	Site of action	Mechanism of action	Ref
Direct inhibitors				
A. Suppressors of chemokine pathways	Gefitinib	CCL5 chemokine	Decreasing production of CCL5	[28]
	Zoledronic acid	CCL2 chemokine	Decreasing expression of CCL2	[29]
B. TAM-depleting approaches	PLX3397	CSF-1R	Downregulation of CSF-1R expression	[30]
	GW2580	CSF-1	Inhibiting expression of CSF-1	[31]
	Wortmannin	PI3K pathway	Suppressing production of cytokines	[32]
C. M2 to M1 redirecting approaches	GHI/75	LILRB1	Inhibiting immunosuppressive activity	[33]
	Trabectedin	M2 macrophage	Inhibiting Immunosuppressive activity	[28]
	lenalinomide	M2 macrophage	Block the macrophage activity on angiogenesis	[28]
	5-Azacytidine	M2 macrophage	M2 to M1 polarizer	[34]
	Difluoromethylornithine	M2 macrophage	M2 to M1 polarizer	[28]
Indirect inhibitors				
A. EC proliferation and recruitment inhibitors	Endostatin	Endothelial cells	Downregulating secretion of proangiogenic factors/ suppressing proliferation of endothelial cells	[35]
	Angiostatin	Endothelial cells	Downregulating secretion of proangiogenic factors/ suppressing proliferation of endothelial cells	[35]
	Arresten	Endothelial cells	Suppress tumor growth, inhibit EC proliferation and migration; induce EC apoptosis	[35]
	2-Methoxyestradiol	Endothelial cells	Inhibits the proliferation, migration and invasion of endothelial cells	[36]
	Pigment epithelia derived factor (PEDF)	Endothelial cells	Inhibits the proliferation, migration and invasion of endothelial cells	[37]
	Platelet factor 4 (PF4)	Endothelial cells	Suppress tumor growth, inhibit EC proliferation and migration; induce EC apoptosis	[38]
	Thrombospondin- 1	Endothelial cells	Suppress tumor growth, inhibit EC proliferation and migration; induce EC apoptosis	[39]
	Tumstatin	Endothelial cells	suppress tumor growth, inhibit EC proliferation and migration; induce EC apoptosis	[35]
	Terahydrocortisol	Endothelial cells	altering basement membrane turnover in proliferating capillary blood vessels	[40]

Nevertheless, the improvement in clinical outcomes of patients associated with these therapies was unsatisfactorily low [41]. In contrast, vaccination against tumor-associated ECs is assured to fulfill drawbacks (including adverse effects, low potency, and the development of resistance) associated with previously developed therapeutic modalities.

Peptide vaccines elicit active immunity, inducing vigorous immune responses and durable memory that are crucial for preventing tumor recurrence. Compared to mABs, the application of peptide vaccines is often simpler, more specific, cheaper, acquire less complex manufacturing steps and is safe and associated with lower toxicity. Unfortunately, regardless of peptide vaccines capability of eliciting powerful immune reactions, advantageous responses associated with their application are limited in clinical settings. This has been mostly attributed, from one side, to the existence of central and/or peripheral tolerance events, limiting self-antigen recognizing T-cell population only to low-affinity ones; and from the other side, to the immunosuppressive behavior of the TME [42]. Other mechanisms that are also involved in this process include tumor cells' bypassing mechanisms from immune detection consisting of reducing major histocompatibility type 1 (MHC-1) [43] or IFNAR expression [44].

Antigen selection strategies

One of the most pivotal steps in the successful manufacturing of peptide vaccines is choosing the right antigen. Optimally, the antigen should be selectively expressed at extremely high levels only on cancer cells, ensuring detection even by low-affinity effector T and B cells and the beginning of a powerful immune response. In this context, antigens can be categorized to be either tumor specific antigens (TSAs) or tumor associated antigens (TAAs). Viral antigens are the best examples of TSAs, which are completely unique to tumors, and originated as the consequence of viral transformation. Recent studies have highlighted the role of human papillomavirus (HPV) and Epstein–Barr virus (EBV) in the pathogenesis of various cancers. Nevertheless, in most cases, tumors arise as a consequence of different genetic instabilities and/or mutations, creating a protein with new structural characteristics, a truncated one, or exposing previously crypted epitopes, dissimilar to the existing physiologic ones. Consequently, these neoantigens are considered as a secondary group of TSAs that are recognized by the immune system. Based on previous studies, a direct correlation exists between the high number of tumor-associated mutations and developed anti-tumoral responses, positive clinical outcomes, including survival, and positively responding to checkpoint inhibitor mABs, which strongly strengthens this opinion [45].

In the abovementioned opinion, encouraging the development of vaccines against neoantigens is a very promising one that can be categorized as a specific subcategory in "personalized medicine" approaches. Nevertheless, its translation to clinical practice is currently very challenging and requires complex processes [46]. For this purpose, the whole tumor exome must initially be mapped, the immunogenicity of the products of the existing mutations assessed in silico, the predicted most reactive and matched peptide(s) with patients HLA class I and II molecules recognized and synthesized under the Good Manufacturing Practice (GMP) standards and finally, injected into the patients [45]. Although tailoring peptide vaccines for each patient can enhance the observed rate of responses, this method is highly time consuming, expensive, and labor intensive.

Contrary to TSAs, TAAs are physiologically expressed on specific normal cells but in the case of tumor cells an aberrant overexpression is replaced with the normal physiologic one. For instance, cancer testis antigens are physiologically expressed by male gametes but are not expressed in normal mature tissues. In the case of cancer cells, expression of these antigens, including MAGE-A, NY-ESO-1, and SSX-2 again becomes activated and can be spotted on cancer cell surfaces. As another example, differentiation antigens, including Melan-A/MART-1, gp100, and tyrosinase, are precisely expressed by a certain cell lineage or organ. In case expression of these antigens in tumors exceeds a specific immunologic threshold, they will be detected by TCRs and will also result in the activation of $CD4^+$ T helper cells. The presence of antibodies against these TAAs in the sera of patients is suggestive of the fact that such recognition takes place even in the absence of any treatments [47].

So far, most of the currently existing peptide vaccines are designated in a way to interact with TAA, including CT antigen 1B (CTAG1B), MAGE family member 3 (MAGE-3), TTK protein kinase (TTK), Wilms tumor 1 (WT1) and so on [42]. Despite this, the mentioned antigens are not critically essential for the survival of tumor cells and their expression will become highly suppressed upon the initiation of therapy, eventually ending in the development of resistance. To overcome this drawback, targeting proteins involved in regulation of angiogenesis and/or stromal-cancer cell interaction, which promotes pathologic angiogenesis may be of high benefit. This is mostly due to the fact that anti-angiogenic peptide vaccines will indirectly eradicate tumor cells. More importantly, the production of these types of peptide vaccines is not very complex and time-consuming and the associated production associated cost is usually low [46]. A list of peptide vaccines targeting angiogenesis, as well as their antigens and mechanisms of action, is provided in Table 2.

Table 2 Peptide vaccines targeting cancer-associated angiogenesis

Antigen	Peptide vaccine's characteristics	Cancer type	Outcome	Ref
VEGF	79 aa long peptide chain which two cysteine residues are substituted with alanine	Melanoma	Up to 50% tumor growth inhibition	[48]
VEGFR2	Epitope screen of 38 short in length peptides (each about 10 aa)	A2/K2 transgenic mice bearing different mouse origin cancer cells	Fivefolds decrease in tumor growth rate	[49]
VEGFFR1	Epitope screen of 40 short in length peptides (each about 10 aa)	A2/K2 transgenic mice bearing different mouse origin cancer cells	Twofolds decrease in tumor growth rate	[50]
Fibronectin	Recombinantly expressed Fusion peptide linked to thioredoxin (length shorter than 100 aa)	MMTV-PyMT	Up to 40% tumor growth inhibition	[51]
Heparanase	Octa branched MAP with a 15 aa long peptide	Hepatocellular ccarcinoma	threefold reduction in tumor volume	[52]
FGF-2	The heparin binding section of the FGF-2 with a length of 44 aa long	B16BL6 and long metastasis model of cancer	96% reduction in metastasis	[53]
CD147	Octa branched MAP with a 15 aa long peptide	A498, CTT26 and TTRAMP-C2 cells	72 to 94% reduction in tumor growth rate	[54]

Nanobody & bispecific nanobody therapies

Benefits of nanobody format candidates

mABs, as a specific group of passive immunity-activating therapeutics, can selectively target TSAs and inhibit activation of several biological pathways in relation to the survival and/or proliferation of cancer cells [55]. In this context, a variety of mAbs have also been developed for targeting proteins involved in the angiogenesis pathway, some of which have also been successful in acquiring FDA approval, like bevacizumab (Avastin®), which targets VEGF. Despite this, the huge molecular structure of mABs mostly limits their free infiltration into the TME. [56]. Moreover, the specific three-dimensional structure of the recognition compartment of the mABs, composed of two variable domains which have been non-covalently bound through hydrophobic interactions, makes their manipulation and bioengineering extremely hard. Hence, development of a newer version of mABs, possessing same biological characteristics with smaller dimensions, less structural complexity, better stability and longer biological half-life and pharmacokinetic profile is of great importance in achievement of a more potent anti-angiogenic response [57]. So far, multiple new formats of mABs including antigen-binding fragment (Fab), variable fragment (Fv) and single-chain variable fragment (scFv) have been developed to overcome drawbacks associated with mABs in targeted cancer therapy. However, under desirable efficacy, as well as unsatisfactorily low antigen binding affinity of these modalities have in large part restricted application of these newly engineered targeted therapies in clinical setting [58]. Moreover, recently bioengineered synthetic protein scaffolds comprising affibodies, DARPins, and minibodies are yet in their infancy and require further preclinical and clinical studies to confirm their superior effectiveness in comparison to mABs in eradication of tumor cells or suppression of angiogenesis [57].

Serendipitously discovered in the beginning years of the twenty century, camelid heavy-chain antibodies, missing the light chains of the mABs, have revolutionized the field of cancer targeted therapy and seem to be the answer to the abovementioned challenge [59]. Occupying only one tenth of the volume filled by mABs, the heavy-chain variable domain of Camelidae antibodies, referred to as "VHH" or "nanobody®", preserves their complete functionality toward their targeted antigen with the same affinity or even more than those associated with mAB [60]. These characteristics have made them an optimal candidate for application in clinical settings. Most importantly, since nanobodies do not undergo post-translational amendments and are expressed by only one specific gene, they can be readily and cost effectively expressed in different microorganisms as recombinant proteins [61]. Finally, owing to their higher hydrophilicity in comparison to conventional mABs, their tendency for aggregation with nanobodies is much less in aqueous solutions [62].

Nanobodies targeting angiogenic pathways

So far, numerous nanobodies targeting components of the angiogenesis pathway have been developed and their efficacy has been investigated in preclinical and/or clinical settings. In the study performed by Ghavamipour et al., a group of nanobodies against VEGF with binding affinities ranging between 0.1 and 60 nM were developed that could effectively inhibit endothelial cells' growth and interfere with HUVEC's tube formation capacity [63]. In another study performed by Behdani et al., the nanobody

was raised against VEGF receptor-2 (VEGFR-2) and demonstrated to effectively bind with HUVECs cell surface VEGFR2 antigen and interfere with tube formation assay in vitro [64]. The list of several other nanobodies targeting angiogenesis pathway's components has been provided in Table 3.

Capable of targeting two types of antigens, bispecific nanobodies are a novel promising group of immunotherapies, capable of introducing novel functionalities that are not observed with the mixture of the parental or reference antibodies. In this context, the bispecific nanobody takes on the role of a linker, which brings together the so-called the effector cell and target cell either temporally (i.e., sequentially binding to the effector and target antigen) or spatially (i.e., linking with effector and antigen bearing cells at the same time) and accelerates the induction of desired effects. In the case of angiogenesis, Barzaman et al. demonstrated that application of a bispecific nanobody against epithelial cell adhesion molecule (EPCAM) and VEGF could synergistically enhance apoptosis, migration, and invasion of the MDA-MB-231 breast cancer cell line compared to those observed with either anti-EPCAM or anti-VEGF nanobodies alone. A developed bispecific nanobody could interfere with tube formation of HUVECs at concentrations as low as 100 nM [69].

Cytokine and chemokine therapy

Role of cytokines/chemokines in angiogenesis

Classified as 8–10 kDa in molecular weight heparin binding proteins, chemokines were basically discovered for their action of recruiting leukocytes and accelerating their infiltration inside inflammatory site. CXC chemokines are specific members of this superfamily, playing a pivotal role in regulating angiogenesis in both physiologic and pathological states, such as malignancies, fibrosis, and disorders associated with chronic inflammation. The structural hallmark of CXC chemokines is presence of four cysteine amino acids next to the N-terminal position of the chain among which the initial two cysteines are spaced by a non-conserved amino acid, hence, being termed Cys-X-Cys or easily "CXC" moiety. CXC chemokines are further subdivided into two subgroups based on the existence or lack of a glutamic acid-leucine-arginine or "ELR" moiety, proximally to the CXC moiety. In this context, it has been shown that containing the ELR motif, which were initially recognized for their neutrophil-attracting potency, are commonly promoted by angiogenesis, while those lacking the ELR motif, known for their potent mono-nuclear leukocyte attracting capacity, are most often anti-angiogenic (Table 4) [70].

Table 3 Nanobodies and bi-specific nanobodies targeting cancer-associated angiogenesis

Antigen	Name of nanobody	Model or investigated cell	Outcome	Ref
VEGF	ZFR-5	HUVECs	Suppression of HUVECs response to VEGF	[63]
	Nb22, 23, 35, 42	HUVECs	Tube formation assay interference	
	V12	CAM of egg	Substantial antiangiogenic effects	
VEGFR2	3VGR19	HUVECs	Tube formation inhibition and recognition of antigen on HUVECs surface	[64]
PGF	NB-C18	CAM model, HUVECs	Inhibition of HUVECs proliferation, migration and 3D capillary formation; inhibition of vascular formation	[65]
HER2	5F7GGC	BT474M1, MT474M1 xenografts	Successful targeting of HER2 + malignancies	[66]
	2Rs15d	CHO, LS174T, SKBR3, BT474, SKOV3; Xenograft mice model	Successful imaging of HER2 + cells in vivo	
HGF	1E2, 6E10	U-87, PC3, A549	Successful PET imaging of HGF expressing tumors	[67]
EGFR	7C12, 7D12	A431 and R1M xenografts	Rapid clearance and poor pharmacokinetic	[68]
	8B6	A431, DU145, MCF7, NIH3T3	High selectivity for EGFR + cells	
	^{99m}Tc-7C12	A431; ICR/CD1 mice, megalin deficient mice	Selective accumulation in tumor site, candidate for early recognition and therapy of EEGFR + tumors	
EPCAM × VEGFR2	Anti-EPCAM × VEGFR2	MDA-MB-231, JURKAT, HUVECs	Suppressing tube formation assay, inhibiting migration and invasion of HUVECs	[69]

Table 4 Chemokine induced modulatory effects on cancer-associated angiogenesis

Chemokine subfamily	Name of chemokine	Other names	Mechanism of action	Ref
Proangiogenic ELR motif containing CXCs	CXCL1	Growth related oncogene alpha (GRO-a)	Acts as a growth factor, promotes inflammation	[71]
	CXCL2	Growth related oncogene beta (GRO-β)	Acts as a growth factor, promotes inflammation	[71]
	CXCL3	Growth related oncogene beta (GRO-γ)	Acts as a growth factor, promotes inflammation	[71]
	CXCL5	Epithelial neutrophil-activating protein 78 (ENA-78)	Promoting neovascularization by recruiting neutrophils and enhancing VEGF secretion	[72]
	CXCL6	Granulocyte chemotactic protein 2 (GCP-2)	Enhanced MMP9 expression and endothelial cell recruitment	[73]
	CXCL7	Neutrophil activating protein 2 (NAP-2)	Induces expression of VEGF and Flt-1, activation of NF-kB; promotion of EC proliferation and migration	[74]
	CXCL8	IL-8	Promoting EC proliferation and tube formation	[75]
Antiangiogenic ELF motif lacking CXCs	CXCL4	Platelet factor 4 (PF4)	Interact with FGF and VEGF and interfere with receptor binding; interfere with the proteoglycan-bystander effect on growth factor action; activate cell surface receptors on EC and promote inhibitory signals	[38]
	CXCL9	Monokine induced by interferon-γ	Chemoattraction of activated T cells Inhibiting ECs chemotaxis; inhibiting growth factor-induced angiogenesis	[76]
	CXCL10	Interferon-γ inducible protein (IP-10)	Chemoattraction of activated T cells Inhibiting ECs chemotaxis; inhibiting growth factor-induced angiogenesis	[76]
	CXCL11	Interferon-inducible T-cell alpha chemoattractant (ITAC)	Chemoattraction of activated T cells Inhibiting ECs chemotaxis; inhibiting growth factor-induced angiogenesis	[77]
	CXCL12	Stromal cell-derived factor 1 (SDF-1)	Recruitment and retention of CXCR4 + BM cells to the neoangiogenic niches	[78]

IL-23–IL-17 immune pathway

IL-23 is a cytokine made up of two different subunits: a common p40 subunit, which it shares with IL-12, and a unique p19 subunit [79, 80]. IL-23 attaches to a heterodimeric IL-23 receptor (IL23R), which triggers the activation of STAT3 and other signaling pathways [79]. IL-23 is primarily synthesized by activated M1 macrophages in response to the activation of Toll-like receptors (TLRs), which stimulate its expression via the NF-κB and STAT3 transcription factors [81, 82]. IL23 has a crucial function in promoting the release of IL-17A, a cytokine, by stabilizing and encouraging the growth of Th17 cells (a kind of T cells that produce IL-17) or by activating innate lymphoid cells (iLC) and γδ T cells in conjunction with IL-1. The IL-17 family consists of six members, specifically IL-17A, B, C, D, E, and F [83]. IL-17A and F are the most closely related members of this family. They both attach to IL-17 receptors A (IL-17RA) and C, which triggers the activation of mitogen activated protein kinases (MAPK), NF-κB, and C/EBP signaling pathways. This activation occurs through the involvement of adaptor proteins Act1 and TRAF6 [84]. Th17 cells, γδT cells, NKT cells, and other types of iLCs are responsible for the production of IL-17A and F [84–86].

IL-23 and IL-17 have historically been investigated for their involvement in immune response, autoimmune diseases, and chronic inflammation. However, increased levels of these cytokines and their receptors have also been observed in several types of human cancers, such as colon, ovarian, lung [87, 88], breast, stomach, skin, liver, and head

and neck cancers [89, 90]. Interestingly, El-Gedamy et al. indicated that some variants of IL-23 (Rs-1884444 G/T) is correlated with the risk of bladder urothelial carcinoma by regulating IL-23/IL-17 inflammatory pathway [91, 92]. Significantly, increased levels of IL-23, IL-17, and IL-6 in stages 1 to 4 of colorectal cancer have been associated with a negative prognosis and a more aggressive form of the illness [93]. A separate investigation identified an IL-23-Th17 gene signature, which was found to have increased expression in stage 1 and 2 early colorectal cancer. This increased expression was associated with a higher likelihood of rapid progression to incurable metastatic disease [94].

Research has shown that there is a complex interaction between the IL-23-IL-17 pathway and TAMs. Interleukin-17, which is produced by T-helper 17 cells, can increase the recruitment of TAMs to the tumor microenvironment [87]. The process of recruiting cells is facilitated by several chemokines, such as CCL2 and CXCL12, which are released by tumor cells and other stromal cells in the tumor microenvironment. IL-17 can enhance the production of these chemokines, so attracting a greater number of monocytes that undergo differentiation into TAMs. Furthermore, the presence of IL-23 and IL-17 can influence the orientation of macrophages towards the M2 phenotype by creating an inflammatory milieu [95]. This polarization is crucial for facilitating angiogenesis and sustaining tumor proliferation. TAMs in addition release IL-10 and TGF-β, which have the ability to inhibit immune responses against tumors and promote tumor growth. Furthermore, there exists a feedback mechanism through which TAMs have the ability to exert influence on the IL-23-IL-17 pathway. TAMs have the ability to generate IL-23, which enhances the activity of Th17 cells and maintains the inflammatory environment that supports the growth of tumors and the formation of new blood vessels (angiogenesis) [96].

Directing therapeutic efforts on the IL-23-IL-17 pathway and its interaction with TAMs has great potential as a treatment strategy. Possible strategies encompass the suppression of IL-23 or IL-17, the reprogramming of TAMs, and the utilization of combination therapies. Inhibiting IL-23 or IL-17 can decrease the recruitment and activation of TAMs, which may lead to a decrease in tumor angiogenesis and development [97]. Implementing tactics focused on transforming M2 (TAMs) into M1-like macrophages has the potential to bolster the body's ability to fight against tumors and impede the formation of new blood vessels. This may entail utilizing TLR agonists that stimulate M1 polarization. In addition, the combination of IL-23/IL-17 inhibitors with current anti-angiogenic medicines has the potential to overcome resistance mechanisms and enhance treatment outcomes.

The IL-23-IL-17 pathway has a substantial impact on the function of TAMs in the TME, by stimulating the growth of new blood vessels (angiogenesis) and facilitating the advancement of tumors [98]. Gaining a comprehensive understanding of this interaction offers vital insights into possible therapeutic approaches that try to interrupt this route, so enhancing the body's ability to fight against tumors and improving the overall outcomes for patients undergoing cancer treatment. By specifically focusing on the inflammatory cytokines and the macrophages they attract, it could be feasible to create more potent therapies that tackle the intricacies of tumor biology (Fig. 3).

Pro-Angiogenic cytokine/chemokine inhibitors

With this in mind, numerous researchers have used chemokines as a therapeutic modality for achieving antiangiogenic responses in cancer therapy. For instance, the application of an anti-IL-8 mAB has demonstrated promising effects in preclinical and in vitro settings and a satisfactorily broad safety profile in phase I studies. Nevertheless, unable to fulfill the desired outcomes in the phase II clinical trial for the treatment of psoriasis, the study was abandoned [99]. Currently, a range of other compounds capable of targeting the CXCR4-CXCL12 pathway have been established and are undergoing phase I and II clinical studies with the purpose of treating different types of cancers [100]. Nevertheless, as described previously, this pathway is responsible for modulating cellular characteristics other than angiogenesis. Interfering with the CCR2-CCL2 pathway has been shown to produce anti-angiogenic effects but is also associated with numerous undesirable effects, most importantly infiltration of inflammatory leukocytes and exaggeration of Th1-induced immunity. Consistently, modulating the CXCR3-non-ELR CXC pathway through systemic administration of IL-2 or other related ligands could be an effective way of suppressing angiogenesis while enhancing the rate of tumor rejection [101].

Engineered T-Cell approaches

CAR T-cell therapy is one of the most recent approaches in immunotherapy and is based on the application of bioengineered T cells that have been fortified with chimeric antigens and possess verified specificity [102]. This method has shown to be very effective against B cell acute lymphoblastic leukemia (ALL) and when targeting CD19 antigen, inducing complete remission in more than 80% of cases. This has been mainly attributed to the free engagement of leukemic cells circulating in the vasculature by CAR T cells. Despite this, CAR T cell therapy for solid tumors has been shown to be much more difficult [103] since extraversion from the bloodstream is extremely challenging for these therapeutic agents. This is mostly due to the awkward nature of the vessels and development of anergy in endothelial cells, which

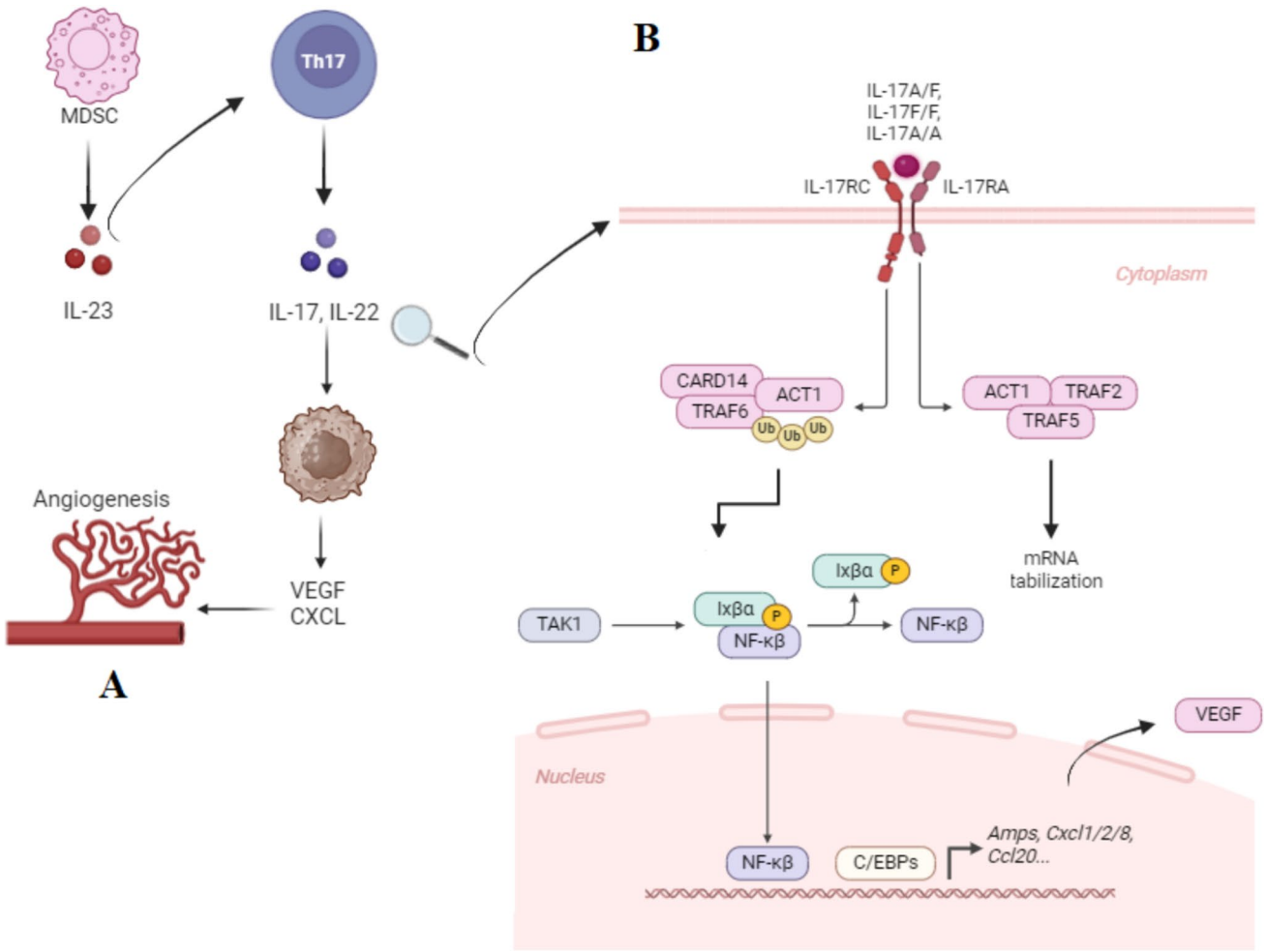

Fig. 3 IL-23/IL-17/VEGF signaling pathway in tumor angiogenesis. A.: MDSC (Myeloid-Derived Suppressor Cell) produces IL-23, which stimulates Th17 cells to produce IL-17 and IL-22. These cytokines induce VEGF and CXCL production, promoting angiogenesis. B. Intracellular signaling pathway: IL-17 family cytokines (IL-17A/F, IL-17F/F, IL-17A/A) bind to IL-17RC and IL-17RA receptors. Receptor activation leads to two signaling branches: CARD14-TRAF6-ACT1 complex formation and ACT1-TRAF2-TRAF5 complex formation. TRAF6 pathway activates TAK1, leading to NF-κB activation that translocates to the nucleus and induces gene expression, including VEGF, Amos, Cxcl1/2/8, Ccl20, and other genes (via C/EBPs). TRAF2-TRAF5 pathway leads to mRNA stabilization. This pathway demonstrates how inflammatory signals can promote tumor angiogenesis through the production of pro-angiogenic factors like VEGF, highlighting potential targets for cancer therapy

ends in endothelial cells non-stickiness to these cells [104]. Overcoming the intense anti-inflammatory nature of TME is the next challenge for CAR T cells to induce a proper response. In this case, abundant concentrations of IL-10, TGFβ, and VEGF, as well as regulatory T cells and M2 macrophages in TME result in the suppression of even invasive activated cytotoxic T cells.

Regardless of the fact that application of checkpoint inhibitors can overcome the immune suppression mentioned above, directing CAR T cells toward vasculature can overcome both of the abovementioned challenges at the same time. In this context, from one side, the targeting molecule is accessible from inside vasculature and from the other side, there is no need for infiltration of the engineered T cell into the anti-inflammatory and immunosuppressive TME. CAR T cells targeting angiogenesis pathway components have been the subject of numerous preclinical and clinical studies. In a number of studies, CAR T cells were engineered in a way to target VEGFR2 receptor [105]. Although this method was shown to be efficient in different murine models, the possibility of developing resistance to this therapeutic approach still exists. In another approach, PSMA was used for developing CAR T cells and was shown to be effective in treating ovarian cancer. The same study also demonstrated that prostate-specific membrane antigen (PSMA) overexpressing patients respond more effectively to CAR T cell therapy [106]. Tumor endothelial marker (TEM)-8 has also been used for the development of CAR T cells for the treatment

of triple-negative breast cancer (TNBC) [107]. Based on the results, application of TEM-8 directed CAR T cells could sharply decrease the speed of tumor growth instantly after infusion of the engineered cells. whereas CAR T-cell therapy could not induce complete remission and eradication of xenografts, the size of tumors was yet smaller and they possessed fewer densified blood vessels following a 2-month period [107]. Another promising approach has been introduced by Xie et al., directing CAR T cells against the EIIIB domain containing splice variant of fibronectin [108]. Whereas this specific variant form of fibronectin has been reported to be expressed in different types of cancer, it has also been shown to be expressed in tumor associated vessels undergoing angiogenesis. Based on the results, the method was only effective in immunocompetent mice but not in immunodeficient ones, suggesting the involvement of endogenous immunity in the effects induced by this specific type of CAR T cell.

Immunomodulatory small molecules

Thalidomides are a group of anti-inflammatory and immunomodulatory agents, with thalidomide being the prototype and lenalidomide and pomalidomide being its derivatives. These therapeutic compounds have been successfully applied in the treatment of patients suffering from multiple myeloma, as well as pancreatic, prostate, and lung cancers. Despite the great teratogenicity and maternal concerns associated with thalidomides, the promising anti-cancer effects of thalidomides have been recognized and contribute to this agent's growth inhibitory, anti-angiogenic, and immunomodulatory activities. Based on the literature, there are several biologic pathways maintaining cellular characteristics and behavior that can be affected by thalidomides (Fig. 4). Among them, anti-angiogenic effects, anti-proliferative effects and apoptosis inducing effects are the most important ones in relation to cancer treatment. For instance, it has been shown that thalidomides can effectively attenuate secretion of a range of chemokines involved in production of an immunologically misfunctioning TME and help in restoring the indigenous physiologic immune system [109].

In the face of angiogenesis, thalidomide has shown to effectively interfere with VEGF- and basic fibroblast growth factor (bFGF-)-promoted angiogenic events in vitro[110]. Based on the study performed by Lu and colleagues, lenalidomide, by interfering with the PI3K/protein kinase B (AKT) signaling pathway, is capable of suppressing the induction of angiogenic effects mediated by ECs in vitro[111]. Moreover, application of lenalidomide in vivo was associated with a reduction in the rate of B16-F10 cells' metastasis to the lung. In another study, considering the pivotal role of angiogenesis in the pathology of plasma cell myeloma, Medinger and colleagues investigated the efficacy of coadministration of

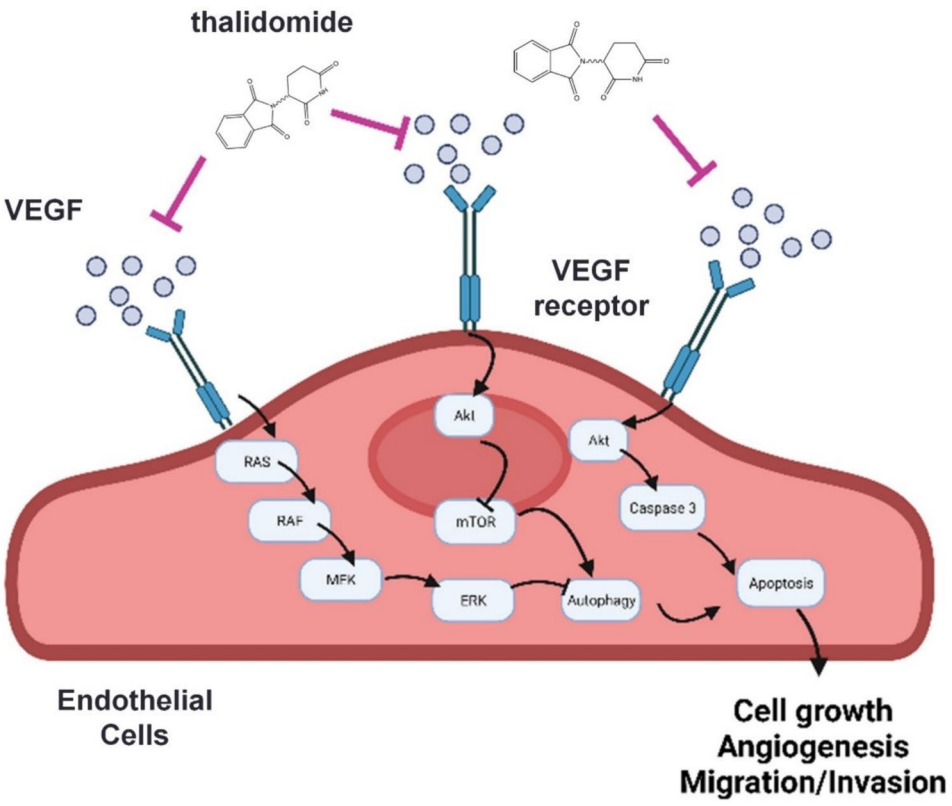

Fig. 4 Pathways associated with anti-angiogenic effects, anti-proliferative effects and apoptosis inducing effects of thalidomide's in relation to cancer treatment. the inhibitory activity of thalidomides on PI3K/AKT/ mammalian target of rapamycin (mTOR) signaling pathway is directly linked to the induction of apoptosis and suppression of ECs proliferation. Moreover, inhibiting interaction of VEGF with its related receptors will interfere with EC's proliferation capacity

thalidomides with bortezomib, an angiogenesis non-affecting proteasome inhibitory drug, in complete remission of the disease. Overall, patients with advanced disease stages were those with high baseline levels of VEGF, hepatocyte growth factor (HGF), tumor necrosis factor-α (TNFα), and angiopoietin-2 (ANG2). Interestingly, the application of thalidomides together with bortezomib could effectively enhance the therapeutic effects associated with each drug alone. Examining levels of proangiogenic factors, including VEGF, soluble VEGFR2, bFGF, placental-derived growth factor (PGF), ANG2, HGF and neuropilin-1 (NRP1) in samples derived from thalidomides and combination treated groups demonstrated a statistically significant decline compared to those obtained from bortezomib treated ones [112]. This finding is highly representative of thalidomides effective anti-angiogenic properties.

One of the main pathways through which thalidomides are capable of inducing antiangiogenic effects is interfering with NOTCH1/DELTA4 or the PI3K/AKT pathways [113]. Moreover, the nuclear factor-κB (NF-κB) transcription factor is the other factor involved in anti-angiogenic activities of thalidomide [114]. In this context, Keifer et al. have shown that thalidomide is capable of inhibiting I kappa B Kinase (IKK), as well as a number of NF-κB associated target genes including IL-8, interleukin signal transducer tumor necrosis factor receptor associated factor 1 (TRAF1) and cellular inhibitor of apoptosis protein 2 (IAP2). Based on Liu et al., thalidomides are capable of simultaneously inhibiting NF-κB pathway and enhancing the BCL-2 associated X (BAX)/BCL2 ratio while the effect on apoptosis associated genes was more noticeable than the NF-κB inhibition [115]. At the end, the inhibitory activity of thalidomides on PI3K/AKT/ mammalian target of rapamycin (mTOR) signaling pathway is directly linked to the anti-angiogenic effects associated with these agents [109].

Immunological-based drugs for *cancer* treatment

Pembrolizumab (Keytruda) and Nivolumab (Opdivo) are monoclonal antibodies that act as immune checkpoint inhibitors by obstructing the PD-1 receptor on T cells. The blockage hinders the interaction between PD-1 and PD-L1 on cancer cells, hence boosting the immune response against cancer by eliminating the inhibitory signal that typically restrains T cell activation. Pembrolizumab has demonstrated effectiveness in the treatment of several cancer types, including as melanoma, non-small cell lung cancer (NSCLC), and head and neck squamous cell carcinoma [116]. Nivolumab is indicated for the treatment of various types of cancer, including melanoma, renal cell carcinoma, NSCLC, and Hodgkin lymphoma. Both medications function by utilizing similar processes to enhance the body's inherent immunologic defenses against cancer cells [117, 118].

In addition, Tisagenlecleucel (Kymriah) is an innovative form of CAR T-cell treatment that modifies a patient's own T cells to express a chimeric antigen receptor that specifically targets the CD19 protein found on B cells. These altered immune cells are subsequently reintroduced into the patient's body to target and eradicate cancer cells. This treatment has received approval for specific forms of B-cell lymphoma and acute lymphoblastic leukemia [119]. Trastuzumab (Herceptin), a type of antibody called a monoclonal antibody, specifically binds to the HER2 receptor found on cancer cells. By doing so, it inhibits the signaling of this receptor and enhances the process of antibody-dependent cellular cytotoxicity. Trastuzumab is mainly employed in the treatment of breast and gastric cancers that are positive for the HER2 receptor [120, 121].

Moreover, using cytokine therapy such as IL-2 enhances the immune system's ability to fight cancer and is specifically used to treat metastatic melanoma and renal cell carcinoma [122]. Moreover, Blinatumomab (Blincyto) is a bispecific T-cell engager that attaches to both CD19 on B cells and CD3 on T cells. This helps T cells to effectively destroy cancer cells. Blinatumomab is specifically approved for the treatment of B-cell precursor acute lymphoblastic leukemia [123]. Talimogene laherparepvec (T-VEC) is a type of therapy that uses a virus to specifically infect and destroy cancer cells. It also triggers an immune response against the tumor by releasing GM-CSF. T-VEC is primarily utilized in the treatment of melanoma [124]. Lenalidomide (Revlimid) is an immune modulator that has several methods, such as improving the activity of T cells and NK cells, preventing the growth of new blood vessels, and regulating the generation of cytokines [125]. It is proven to be beneficial in the treatment of multiple myeloma, mantle cell lymphoma, and myelodysplastic syndromes.

Several of these immunotherapies indirectly affect the growth of new blood vessels by altering the surrounding environment of the tumor. For example, drugs called immune checkpoint inhibitors such as pembrolizumab and nivolumab can decrease the signaling that promotes the growth of new blood vessels (angiogenesis) by boosting the immune system's capacity to identify and eliminate cancer cells. As a result, the production of molecules that promote angiogenesis is reduced. CAR T-cell treatments and monoclonal antibodies have the ability to selectively target antigens on tumor cells, resulting in their elimination and subsequently decreasing angiogenic signaling. In addition, cytokine therapies such as IL-2 can augment the function of T cells and NK cells, which can target tumor-associated endothelial cells, therefore hindering the development of new blood vessels. T-VEC, an oncolytic viral therapy, has the ability to both directly destroy tumor cells and activate

an immune response against the tumor, which can interfere with the angiogenesis. Immune modulators, such as thalidomide analogs, exert direct anti-angiogenic actions by suppressing the growth of endothelial cells and decreasing the release of pro-angiogenic substances. Therefore, these immunologic methods not only directly attack cancer cells but also interfere with the formation of new blood vessels that are crucial for tumor development and survival. This provides a comprehensive approach to cancer treatment.

Combinational therapy

Since the approval of the first angiogenic inhibitor, bevacizumab, combination therapy utilizing anti-angiogenic drugs has become prevalent in the field of anti-tumor treatment [126]. Combination therapy is a treatment approach that seeks to improve the effectiveness of anti-tumor treatment by combining two or more therapeutic agents. These agents can include anti-angiogenic therapy, surgery, immunotherapy, chemotherapy, radiation, genetherapy, or other targeted anti-tumor therapies [127]. The combination of anti-tumor medications enhances therapeutic efficacy compared to using a single treatment, either by acting synergistically or additively, and targeting crucial signaling pathways. The implementation of diverse approaches in anti-cancer therapy offers a wider range of choices for clinical treatment and facilitates the formation of robust collaborations.

Recently, there has been a growing focus on combining angiogenic inhibitors and immune checkpoint inhibitors in research. It has been observed that HCC and RCC patients treated with a combination of programmed cell death 1 (PD-1) and VEGFR-2 inhibitors experience more therapeutic improvements compared to those treated with only one type of inhibitor [128, 129]. Tumors can promote immunologic tolerance and restrict the proliferation and activation of T cells during their growth and spread using immune checkpoints (ICs) expressed on T cells to evade the immune response. Using various immune checkpoint inhibitors can stimulate the immune system and reduce the suppression of the immune response in the TME. This can be achieved by enhancing the activation and multiplication of T cells, such as PD-1, PD-L1, and CTLA-4 inhibitors, which target tumor cells. The tumor microenvironment consists of tumor cells, cancer stem cells, immune cells, fibroblasts, and other cells together with their secretions. It also includes non-cellular components such the extracellular matrix. High levels of VEGF in the tumor microenvironment play a multifaceted role in suppressing anti-tumor immunity. First, VEGF directly impairs the function of immune effector cells like dendritic cells and cytotoxic T cells by inhibiting their maturation and promoting apoptosis. Second, it enhances the recruitment and activation of immunosuppressive cell populations such as regulatory T cells, myeloid-derived suppressor cells, and M2 tumor-associated macrophages. In addition, by upregulating endothelial adhesion molecules and immune checkpoints, VEGF creates an endothelial barrier that selectively restricts cytotoxic T-cell infiltration while allowing regulatory T-cell entry. Moreover, excessive VEGF secretion by tumor cells leads to the formation of disordered, leaky tumor vasculature, which hinders effective delivery of chemotherapeutics and immunotherapeutic agents. Collectively, these effects of VEGF establish an immunosuppressive microenvironment that protects tumors from immune attack and impairs response to therapy [129–131]. When immunotherapy is used with anti-angiogenic medications that target the VEGF pathway, it counteracts the immunosuppressive effects generated by VEGF and improves the immunologic function of patients. Simultaneously, it has the ability to counteract surplus VEGF, rebuild the vascular system of tumor tissue, restore the normal functioning of blood vessels, enhance the transportation of immunosuppressant through the bloodstream, hinder excessive formation of new blood vessels, decrease the density of small blood vessels, and restrict the growth, invasion, and spread of tumors [132]. In addition, ICIs stimulate the activity of T cells within the tumor, modify the TME, enhance the immune response of the host, and increase the expression of γ-interferon. These effects all contribute to the normalization of blood vessels. In a phase III clinical trial (NCT03434379), the combination of bevacizumab and PD-1 inhibitor atezolizumab shown a substantial improvement in overall survival (OS) and progression-free survival (PFS) rates for patients with unresectable hepatocellular carcinoma (HCC) compared to sorafenib [133]. In many clinical trials evaluating combination therapy, the efficacy of PD-1 inhibitors (such as nivolumab and pembrolizumab) when used in conjunction with cabozantinib, axitinib, or bevacizumab was significantly superior than the use of sunitinib alone in patients with renal cell carcinoma (RCC), NSCLC, colorectal cancer (CRC), and gastrointestinal stromal tumor (GIST) [129].

The concurrent use of anti-angiogenic and immunologic therapy has had a favorable impact on the treatment of cancer, as indicated by the majority of clinical trials. This is particularly true for patients with advanced malignant tumors who do not respond well, are unwilling, or have low tolerance to chemotherapy [129]. However, several issues such as the efficacy, toxicity, and tolerability of this combination treatment need to be improved by additional research on the appropriate therapeutic dosage, timing, and sequence for different individuals [134–136]. An in-depth and interconnected analysis of the mechanisms behind the positive loops between angiogenic inhibitors and ICIs should be conducted. This will aid in the development of new formulations and the design of clinical studies, ultimately promoting the integration of this promising

strategy as a standard therapeutic approach for cancer. As previously stated, chemotherapy, although causing harm to normal cells, blood vessels, and the immune system due to the use of high doses and lack of tissue selectivity, remains an indispensable treatment for advanced cancer patients with metastases, as it effectively extends their survival [137]. The progress in medical technology, clinical medicine, and pharmacy has demonstrated that incorporating anti-angiogenic therapy or developing immunotherapy alongside chemotherapy can yield more advantages for patients. Angiogenic inhibitors restore the normal structure of blood vessels in tumors, decrease osmolality, alleviate local oxygen deficiency, enhance the penetration and delivery of drugs into tumor cells, and also decrease the required dosage of medication and enhance patient tolerance during effective chemotherapy. On the other hand, ICIs enhance the immune system of patients and prevent tumors from evading the immune response. An instance of a phase III clinical trial (NCT02366143) demonstrated that the inclusion of atezolizumab (an anti-PD-L1 drug) significantly prolonged the OS period (19.2 vs. 14.7 months), PFS duration (8.3 vs. 6.8 months), and objective response (OR) rates (63.5% vs. 48.0%) of NSCLC patients who were treated with bevacizumab, carboplatin, and paclitaxel [138]. While numerous positive outcomes have been documented, it is important to acknowledge certain instances of failure. These failures highlight the significance of appropriate drug compatibility, selection of primary or auxiliary drugs, dosage and order of administration, individual variations among patients, and the varying stages and types of tumors [135].

Another noteworthy therapeutic approach is a developing supplementary strategy called neoadjuvant chemotherapy (NACT). The goal of NACT is to diminish the tumor size and eliminate undetectable metastatic tumor cells by administering systemic chemotherapy. This is done to aid following surgical procedures, radiation, and other treatments. Thus far, different NACT treatments such as SOX, XELOX, and FOLFOX have been proposed, yielding favorable clinical outcomes in both early and advanced tumors, as well as reducing the risk of disease progression. However, recent research has also revealed dismal clinical outcomes associated with NACT, particularly in cases of breast cancer [139–141]. Perelmuter et al. conducted a review that outlined several possible mechanisms of chemoresistance in NACT. The review reported that NACT has the potential to promote the spread of cancer through the stimulation of angiogenesis, lymphangiogenesis, and inflammatory infiltration. In addition, NACT can alter immune responses and worsen the TME, which may lead to secondary chemoresistance [142]. Can the combination of angiogenic inhibitors and immune checkpoint inhibitors be effective against this resistance? In theory, there is promise, but substantial endeavors are also required. Several clinical trials are currently in progress (NCT05202314, NCT04606108, NCT04294511, NCT05554276, NCT05468242).

Summary and future perspective

As discussed in this paper, anti-angiogenic treatments were primarily developed with the purpose of suppressing tumor associated neovascularization. Nevertheless, soon it was understood that monotherapy with anti-angiogenic agents would, in most cases, end in the development of resistance due to the activation of compensatory proangiogenic pathways. Encouragingly, the discovery of immune checkpoint inhibitors and their great success in achieving prolonged disease-free survival episodes resulted in the implication of emerging immunologic approaches as novel anti-angiogenic therapies in the treatment of cancer. Despite the great beneficial effects associated with the application of these agents in preclinical studies, their application in clinical settings was poor to moderately efficacious in most cases, restricting the free administration of agents at the bedside. Moreover, severe adverse effects, including the development of autoimmune reactions to normal ECs, associated with specific treatment modalities are also of great concern for translation into clinics. For instance, CAR T cell therapy against tumor vasculature in rare cases has resulted in the achievement of complete remission. Or, in the case of peptide vaccines, the achievement of poor clinical outcomes is the most important limiting factor in the manufacturing of new peptide vaccines. Therefore, it is necessary to perform a range of amendments in each category before paving the path to the bedside. The main challenge associated with direct and indirect inhibitors of TAMs is the restricted accessibility of the drug cargo to the TAMs located at the hypoxic, low-nutrient core site of the immunosuppressive TME as a consequence of abnormal tumor-associated vasculature and surrounded by a densified ECM. Hence, the application of indirect EC proliferation inhibitors such as endostatin and canastatin in this context is of greater interest. With the emerging role of nanotechnology in the field of drug delivery and cancer therapy, future studies should be directed toward the development of nanocarriers, capable of enhancing the delivery potency of desired encapsulated drugs to the central compartment of the tumor niche. The main challenges facing nanobody and bispecific nanobody therapies are their poor pharmacokinetics and rapid clearance from the bloodstream. So far, numerous methods, (including their ligation to bloodstream proteins such as albumin) have been proposed for improving their bioavailability. Nevertheless, all these methods are in their infancy and future studies are highly warranted for evaluating their biologic effects in this new format. For peptide vaccines, despite their almost safe profile, the possibility

of autoimmune disorder development against ECs exists, which must be overcome by better selection of antigens and applying improved manufacturing methods. In addition, the efficacy of peptide vaccines as adjuvant therapies has not been fully studied yet and this should be carefully addressed in future studies. For CAR T cell therapy, improving the efficacy of therapy against solid tumors through the selection of more specifically and abundantly expressed antigens on EC is highly warranted, as this approach can effectively interfere with vascular integrity and promote bystander immunity. Moreover, in future studies, the development of CARs with the capability of more than one target recognition for enhancement of specificity, affinity, and consequently safety profiles are highly recommended. At the end, the main perspective of the study predictable for thalidomides is the development of a less teratogenic derivative of the family members based on the analysis of the main signaling pathways affected by the drug.

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Data availability No datasets were generated or analyzed during the current study.

Declarations

Conflict of interest The authors hereby declare that there exists no conflicting interest.

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Patient consent statement Not applicable.

Permission to reproduce material from other sources Not applicable.

Clinical trial registration Not applicable.

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