Lab on a Chip

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Engineering perfusion to meet tumor biology: are vascularized tumor-on-a-chip models ready to drive therapy innovation?

Ines Poljak,[ab] Ciro Chiappini [ac] and Giulia Adriani *[bde]

The development of effective cancer therapies remains constrained by the complex and dynamic nature of the tumor microenvironment (TME), with tumor vasculature representing a critical barrier and modulator of treatment response. This review critically examines recent advances in the generation of vascularized tumor models using organ-on-a-chip (OoC) microfluidic technologies, emphasizing their capacity to recapitulate key interactions between tumor cells, stroma, and vasculature *in vitro*. We outline the mechanistic roles of tumor vasculature in therapy resistance, metastatic dissemination, and immune modulation, and highlight current strategies targeting vasculature for improved therapeutic outcomes. State-of-the-art biomaterials and engineering approaches, including template-based fabrication, self-organization, and the integration of patient-derived organoids, are discussed regarding their efficacy in constructing physiologically relevant vasculature. The review critically assesses findings from drug testing studies and discusses the translational potential of microfluidic platform capabilities, such as real-time monitoring, precise flow control, and functional assessment of vessel permeability and drug delivery, while identifying key limitations for clinical implementation. Challenges in standardization, scalability, and clinical translation are discussed, and recommendations are proposed to enhance the human-relevance and impact of vascularized OoC models in preclinical oncology research. These advanced platforms represent a transformative approach for bridging the translational gap between preclinical research and clinical oncology, offering opportunities to advance personalized cancer therapeutics and improve patient outcomes.

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Introduction

Cancer remains a leading cause of mortality worldwide, driving continued efforts to develop more effective treatments that can overcome intrinsic and acquired resistance mechanisms.[1] Remarkable therapeutic advances have transformed cancer treatment, including multimodal approaches combining surgery, radiotherapy, chemotherapy, and immunotherapy.[2] Breakthrough immune checkpoint therapies demonstrated survival improvements in some types of cancers, such as advanced melanoma,[3] metastatic non-small-cell lung cancer,[4] and renal cancer,[5] while ongoing challenges persist in immunotherapy-resistant malignancies like pancreatic cancer, advanced ovarian cancer, glioblastoma (GBM) and microsatellite stable (MSS) colorectal cancer (CRC). Cell therapy approaches, particularly chimeric antigen receptor (CAR) T cells, tumor-infiltrating lymphocytes (TILs), and T cell receptor (TCR) engineered T cells, have also demonstrated significant clinical benefits across multiple cancer types. The Food and Drug Administration (FDA)-approved CAR-T cell therapy has achieved improved survival in hematological malignancies, such as multiple myeloma, and large B-cell lymphoma as recently reviewed.[6] Notably, both TIL and TCR-engineered T cell therapies have also achieved FDA approval for unresectable or metastatic melanoma and synovial sarcoma. However, translation of T cell therapy to solid tumors remains challenging due to adverse effects, antigen heterogeneity and limited efficacy.[7,8]

Central to these therapeutic challenges is the tumor microenvironment (TME), a complex network of cellular, molecular, and structural components. It comprises cancer cells, immune cells, stromal cells, blood and lymphatic vasculature, extracellular matrix (ECM), and various soluble factors, that collectively promote therapy resistance.[9] While each TME component contributes to therapeutic resistance,

[a] *Centre for Craniofacial and Regenerative Biology, King's College London, London, UK*
[b] *Singapore Immunology Network (SIgN), Agency for Science, Technology and Research (A*STAR), 8A Biomedical Grove, Immunos, Singapore 138648, Republic of Singapore. E-mail: giulia_adriani@a-star.edu.sg*
[c] *London Centre for Nanotechnology, King's College London, London, UK*
[d] *Department of Biomedical Engineering, National University of Singapore (NUS), 4 Engineering Drive 3, Singapore 117583, Republic of Singapore*
[e] *Lee Kong Chian School of Medicine, Nanyang Technological University, Singapore*

the tumor vasculature, as both a structural TME component and functional gatekeeper, determines treatment outcomes through multiple interconnected mechanisms. Abnormal vessel structure represents the primary barrier to effective therapy delivery, as tumor vessels exhibit chaotic organization, irregular branching, heterogeneous diameters, leaky endothelium and aberrant basement membrane that create regions of poor perfusion and elevated interstitial fluid pressure.[10] This abnormal architecture impedes uniform drug distribution, resulting in subtherapeutic concentrations and impedes therapeutic cell infiltration, limiting their trafficking to tumor sites.[11] Endothelial cells (ECs) further promote resistance through angiocrine signaling: they produce inhibitory mediators, including Interleukin-10 (IL-10) and Transforming growth factor-β (TGF-β), and upregulate checkpoint ligands that actively suppress immune effector cells, whilst simultaneously secreting factors such as Angiopoietin-1 (Ang-1) that can maintain cancer stem cell niches and promote tumor dormancy, enabling relapse at distant sites.[12] Additionally, the poor perfusion creates hypoxic conditions that induce cellular adaptations reducing chemotherapy, radiotherapy and immunotherapy efficacy.[13–15] Cancer cells also exploit the vasculature for metastatic dissemination through multiple mechanisms, forming new vessels *via* vasculogenesis or angiogenesis, hijacking existing vessels through vascular co-option, or creating vessel-like channels *via* vasculogenic mimicry, enabling therapy escape and colonization in distant organs.[16]

Current preclinical models fail to adequately capture these complex biological features that drives vascular-mediated resistance mechanisms. Conventional two-dimensional (2D) cell cultures cannot sustain the oxygen gradients, perfusion or matrix-mediated mechanical cues that drive hypoxic signaling and vascular dysfunction, creating significant translational gaps in preclinical-to-clinical cancer therapy. On the other hand, animal models, while informative for systemic safety and multi-organ interactions, demonstrate limitations in precisely control and measure specific human vascular defects, such as poor perfusion, leakiness, tortuous architecture and pericyte loss, that directly influence therapeutic outcomes.[17] Although humanized mouse models have advanced significantly, limitation persists in replicating the human vascular system, often focusing on small and/or fast-growing tumors that lack the complexity, heterogeneity and maturity of human tumor vasculature.[18] These limitations, combined with time, cost, and ethical considerations, have prompted regulatory agencies to actively promote alternative methodologies for therapy assessment, including microphysiological systems (MPSs).[19,20] To address this fundamental engineering challenge, MPSs must recreate the pathological vascular features and resulting microenvironmental heterogeneity described above: abnormal vessel geometry, hypoxic cores, elevated interstitial pressure, and the angiocrine signaling that lead to a therapy-resistant state.

MPSs integrate engineering with cell biology to create three-dimensional (3D) models featuring cell–cell and cell–ECM interactions, fluid flow, and mechanical cues.[21] These microfluidic systems replicate human-relevant tissue functions more accurately than conventional 2D or even 3D cell culture methods while overcoming main challenges of animal models such as time, cost, and ethical concerns.[22] By incorporating perfusable vascular networks adjacent to tumor organoids or spheroids, vascularized MPSs enable the establishment of signaling gradients of angiogenic factors, including Vascular endothelial growth factor (VEGF), Platelet derived growth factor (PDGF) and Ang-1, that govern vessel maturation and dysfunction. These platforms can reproduce the pathological vascular features described above, such as leaky endothelium, tortuous architecture, poor perfusion, elevated interstitial pressure and hypoxic cores, permitting functional assessment of therapy delivery, immune cell trafficking, and vascular-mediated resistance mechanisms for functional oncology screening.[23–27] A comprehensive overview of cancer-on-chip platforms for TME modelling with emphasis on integration of patient-derived components and advanced sensors has been reviewed in this same collection.[28] This review takes instead a vascular-focused approach, discussing how vascularized MPSs are addressing the fundamental engineering challenge of recapitulating tumor vascular biology, the latest advancements in vascularized tumor-on-chip models and their translational potential to transform preclinical cancer research. We introduce tumor vasculature biology to understand the multifaceted role of vasculature in therapy resistance, and how microfluidic approaches are enabling tumor vascularization strategies. We discuss clinical evidence of vascular-targeting therapies and comment on current challenges, providing recommendations for enhancing model clinical relevance and integration into therapeutic development pipelines.

Integrated barriers to therapy: the TME–vasculature axis

The complex TME creates a protective niche for cancer cells and comprises multiple interdependent barriers to therapy. Immunosuppressive stromal cells, particularly tumor-associated macrophages (TAMs) and cancer-associated fibroblasts (CAFs), secrete cytokines (*e.g.*, IL-10, TGF-β, VEGF) and remodel the ECM, creating physical obstacles to drug and immune cell penetration while promoting tumor cell survival and stemness.[29–32] In addition to TAMs and CAFs as players in therapy resistance, other immunosuppressive cells in TME like regulatory T cells and myeloid-derived suppressor cells (MDSCs) have shown overproduction of molecular factors, such as IL-6, TGF-β and VEGFA, promoting tumor immune evasion, metastasis and therapy resistance.[9] Furthermore, enhanced glycolysis in tumor cells drives lactate accumulation and acidification, thereby reducing the efficacy of weakly basic chemotherapeutics, such as cisplatin.[33,34] Tumor-derived reactive oxygen species (ROS) induce oxidative stress and mitochondrial dysfunction in neighboring cells like CAFs

and TAMs, further promoting the lactate buildup in the TME.[35] While an acidic microenvironment can initially trigger cancer cell apoptosis, persistent acidity leads to tumor-cell adaptation and the development of more aggressive tumor phenotypes reducing treatment response.[36] Additionally, acidic TME facilitates activation of TAMs and pro-tumorigenic neutrophils and dendritic cells (DCs) while simultaneously inhibiting TILs cytotoxic activity.[37] Importantly, extracellular acidification is tightly linked to another defining hallmark of the TME, chronic hypoxia, which arises from poor vascular perfusion and increased metabolic demand. Hypoxia activates Hypoxia-inducible factor 1-α (HIF-1α) signaling, which drives drug resistance gene expression, upregulates immune-suppressive checkpoint proteins, *i.e.*, Programmed death-1(PD-1)/Programmed death-ligand 1 (PD-L1), and triggers ECM remodeling that further limits drug diffusion and immune infiltration.[38–40] These microenvironmental features, including stromal activation, acidosis and hypoxia, are not independent; rather, they converge on a central regulator: the tumor vasculature. Tumor endothelial cells (TECs) display abnormal behavior due to the influence of the TME, generating vessels that are not passive conduits for blood flow but active orchestrators of therapy resistance, operating through both structural abnormalities and dynamic signaling. Structurally, tumor vessels exhibiting chaotic organization, irregular diameters, incomplete basement membrane, gaps between ECs, and deficient pericyte coverage, resulting in critically reduced perfusion.[41–44] This perfusion failure directly drives hypoxia, limits convective drug transport, and impairs immune cell extravasation, while simultaneously enabling the leakiness and instability that promote metastatic dissemination.[45–48] Beyond their structural role, TECs actively shape the TME through angiocrine signaling, the secretion of bioactive factors, including Ang-1, Ang-2, Neurogenic locus notch homolog (Notch) ligands such as Jagged1 (JAG1) and Delta-like ligand 4 (DLL4), Hepatocyte growth factor (HGF), and periostin, that shape the behavior of neighboring cancer, stromal and immune cells.[12] Notably, Notch1 receptor activation on tumor endothelium promotes endothelial senescence and tumor cell intravasation in primary tumors, simultaneously priming the pre-metastatic niche endothelium to enhance disseminated tumor cell homing at secondary sites.[12] In solid tumors, such as breast cancer, TECs secrete Tumor necrosis factor-α (TNF-α) that amplifies the CXCL1-mediated myeloid recruiting loop, driving chemoresistance through enhanced myeloid-derived immunosuppression.[49] These cell-intrinsic effects are amplified by stromal crosstalk: CAF-derived extracellular vesicles enriched with VEGF promote angiogenesis, contributing to resistance to bevacizumab in colon cancer,[50] while CAF-derived HGF sustained angiogenesis through endothelial c-MET activation despite anti-VEGF blockade.[51] Collectively, these findings highlight that TECs are dynamic regulators of tumor progression, microenvironmental remodeling and therapy response, supporting the importance of studying tumor vasculature as an integrated, functional compartment in preclinical studies.

Because these resistance mechanisms, *i.e.*, stromal activation, metabolic stress, hypoxia, and vascular dysfunction, are tightly integrated and spatially organized, faithful *in vitro* recapitulation requires vascular model systems that capture their interdependence. Conventional 2D endothelial monolayers do not sustain oxygen or nutrient gradients, while non-vascularized 3D spheroids develop hypoxia in their cores due to limited oxygen diffusion but lack perfusion control, preventing dissection of how vascular function specifically drives resistance. This is where MPS offer a distinct engineering advantage: by combining perfused vascular networks with 3D tumor spheroids and defined stromal populations, these platforms enable recapitulation and perturbation of oxygen gradients, drug diffusion, mechanical cues and angiocrine signaling, permitting to analyze the bidirectional molecular exchange and potentially predict clinical endpoints like dormancy, metastatic risk and vascular-mediated therapy resistance.

Formation of the tumor vasculature *in vivo*

Although tumor cells were recognized early to secrete factors supporting vascular growth[52] it was Judah Folkman's seminal work in the 1970s that established angiogenesis as a critical step in malignant progression.[53] Nowadays we know that tumor vascularization may occur through mechanistically distinct pathways associated with angiogenesis, vasculogenesis or non-angiogenic strategies. Angiogenesis is the growth of new vessels from pre-existing capillaries *via* sprouting, where endothelial cells migrate and proliferate to form branches, and *via* intussusception, a splitting mechanism where existing vessels partition into daughter vessels.[54] Vasculogenesis involves *de novo* vessel formation from bone marrow-derived progenitor cells or cancer stem cell differentiation. Non-angiogenic strategies, including vascular co-option by exploiting pre-existing vasculature and vasculogenic mimicry of tumor cells forming vascular channels without ECs, represent fundamentally different mechanisms insensitive to anti-angiogenic drugs.[54] Because these pathways are tumor-type dependent and distinct mechanisms often emerge as resistance following therapy, understanding which vascularization strategy a tumor employs is critical for rational design of vascularized MPS and therapy development. However, angiogenesis is currently the most targeted vascularization mechanism, as the "angiogenic switch" occurs early in tumorigenesis and marks the initiation of new vessel formation, representing an important hallmark of cancer progression.[55,56] Multiple factors contribute to this switch, including well-known players, such as VEGFA, a potent pro-angiogenic factor, and its endogenous inhibitor thrombospondin-1 (TSP-1).[52] Hypoxic and acidic TME promotes the release of VEGFA and other pro-angiogenic signals, activating ECs and stromal

populations to remodel the ECM and support tumor vascularization.[38,57–61] The dysfunctional architecture of tumor vasculature reflects the molecular imbalance between pro-angiogenic and vessel-maturation signals, such as PDGF and Ang-1 (ref. 62) that are essential for pericyte recruitment and stabilization of the endothelial monolayer during physiological angiogenesis.[63] The resulting VEGFA-high/PDGF-low/Ang-1-low signaling imbalance leads to: (i) EC hyperproliferation and migration without vessel stabilization, (ii) failure of pericyte recruitment and coverage, (iii) loss of VE-cadherin junctional integrity, and (iv) excessive vascular permeability. This molecular imbalance is the primary driver of the characteristic tortuous, leaky, and poorly perfused vasculature observed in tumors.[64–66] The abnormal tumor vasculature perpetuates a vicious cycle: hypoxia promotes VEGFA secretion, but the resulting vessels are functionally deficient due to impaired maturation signaling, leading to further hypoxia and a hostile tumor microenvironment.[39] Faithfully replicating these aberrant vascular features *in vitro* is essential for understanding the key cellular and molecular contributors, whose interactions govern vessel formation, remodeling, and function, providing the basis for physiologically relevant *in vitro* models.

Stromal and immune cells as drivers of vascular dysfunction

TAMs and CAFs are the dominant stromal populations driving abnormal tumor vascularization. TAMs, derived from circulating monocytes and tissue-resident macrophages,[67] respond to hypoxia by secreting VEGFA, Placental growth factor (PlGF), IL-6, TNF-α and Fibroblast growth factor 2 (FGF2), which collectively promote rapid but disorganized angiogenesis, affecting the response to cytotoxic treatment.[45,68–72] In hepatocellular carcinoma (HCC), TECs induce $CD163^+$ TAM polarization through IL-4 secretion, promoting immune suppression and cancer progression[71] contrary to pancreatic ductal adenocarcinoma, where TAM-derived exosomes stimulate TEC proliferation and increase vascular density of the tumors.[72] Perivascular TAMs further destabilize vessels by secreting microvesicles and exosomes that disrupt endothelial barrier function.[73] These inter-tumoral signaling loops between TAMs and TECs are absent in monocellular tumor spheroids or not fully replicated when using blood-derived monocytes as TAM models *in vitro*, lacking the tissue resident population. Furthermore, CAFs contribute to vascular dysfunction through both paracrine and mechanical mechanisms, playing a key role in vascular remodeling in cancer. CAFs are plastic cells primarily derived from activated resident fibroblasts,[74] such as pancreatic[75] and hepatic stellate cells,[76] pericytes,[77] and mesenchymal stem cells[78,79] or even from ECs through endothelial-to-mesenchymal transition,[80] and macrophages *via* macrophage–myofibroblast transition.[81,82] Once formed, CAFs are commonly divided into three subsets: 1) myofibroblast-like CAFs (myCAFs) with SMA^{high}, $IL6^{low}$, $MHCII^-$; 2) inflammatory CAFs (iCAFs) with SMA^{low}, $IL6^{high}$, $MHCII^-$; and 3) antigen presenting CAFs (apCAFs) specifically $MHCII^+$. MyCAFs are mostly associated with vascular remodeling: they upregulate VEGFA whilst downregulating VE-cadherin, destabilizing endothelial junctions and promoting leakiness.[76,83] CAFs also promote angiogenesis independently of VEGFA through PDGF-C, HGF, FGF2 and CXCL12/CXCR4 signaling, and mechanically compress vessels *via* ECM contraction and proteolytic remodeling.[84,85] This CAF-driven desmoplasia increases solid stress, collapses fragile capillaries and impairs perfusion, a key mechanism in pancreatic and breast tumors. CAFs also attract ECs and myeloid cells from bone marrow *via* the CXCL12/CXCR4 axis.[86] Despite their functional heterogeneity, CAFs subtype identity is often neglected in vascularized tumor MPSs, as detailed in Table 2.

Beyond TAMs and CAFs, other TME cells modulate tumor vascular remodeling: neutrophils rapidly release VEGF, immature myeloid cells degrade ECM to create physical space for sprouting, while lymphocytes can suppress angiogenesis *via* IFN-γ, and decidual-like NK cells secrete pro-angiogenic VEGF and PlGF.[87–91] Pericytes contribute through PDGF-B/PDGFR-β signaling, adipocytes enhance EC proliferation and tube formation synergistically with tumor-derived factors,[92–94] and platelets have the potential to secrete either pro- or anti-angiogenic factors.[95] This intricate stromal interplay generates the heterogeneous, leaky, poorly perfused vasculature that supports tumor growth, facilitates metastasis and reinforces therapy resistance, all features that vascularized MPSs must recapitulate through inclusion of multiple, appropriately activated stromal populations.

Heterogeneity of tumor endothelial cells (TECs)

ECs are the main component of tumor vasculature and, during angiogenesis, they are commonly divided in "tip cells" responsible for vascular expansion by migrating towards VEGF signal, and "stalk cells" responsible for proliferation.[41,96] ECs from different tissues and anatomical locations in the vascular hierarchy express unique molecular signatures that reflect their specialized functions. For example, high endothelial venules in lymphoid tissues express lymphocyte-recruiting adhesion molecules, while brain endothelium expresses tight junction proteins essential for the blood–brain barrier (BBB) function.[43,97] Similarly, tumor vasculature is not uniform but profoundly shaped by organ context. Brain tumors encounter the restrictive BBB, which limits drug penetration and immune infiltration[98] while liver and bone tumors develop within a highly fenestrated or permeable vasculature that supports distinct angiogenic programs and facilitates circulating tumor cell seeding.[83] These organ-specific vascular phenotypes determine drug accessibility, immune cell trafficking, and metastatic colonization, emphasizing why vascularized MPSs must incorporate tissue-specific endothelial characteristics to accurately predict therapy responses in organ-specific contexts. However, among many tumor types, universal

markers specific for TECs have been identified: ACKR1, PLVAP and IGFBP3.[97] A comprehensive single-cell transcriptomic study analyzing 437 tumor samples representing 31 cancer types identified 5 different EC subtypes: lymphatic ECs (LECs) (LYVE1$^+$ and PROX1$^+$), vascular ECs (VECs), arterial ECs (SEMA3G$^+$), venous ECs (ACKR1$^+$) and capillary-like ECs (RGCC$^+$), and 2 subtypes of mural cells (MCs): pericytes (RGS5$^+$ and PDGFRB$^+$) and smooth muscle cells (ACTA2$^+$ and TAGLN$^+$), revealing substantial heterogeneity within tumor endothelial populations and demonstrating the presence various stromal cells surrounding tumor vasculature.[99] A similar bioinformatic analysis in CRC samples from 97 patients identified 5 subsets of ECs, with some overlapping: venous ECs (ACKR$^+$), ESM1$^+$, IGFBP3$^+$, STMN1$^+$, and lymphatic ECs (TFF3$^+$, PROX1$^+$).[100] Interestingly, the venous ECs and IGFBP3$^+$ subclusters were identified as immune modulating ECs (IMECs) with potential to recruit mature CD4$^+$ T cells, reinforcing the importance of ECs in shaping the immune landscape in tumors.[100] Furthermore, analysis of renal cell carcinoma (RCC) revealed 5 subclusters of ECs, with a slight difference than previous studies: 1) afferent/efferent arterioles/descending vasa recta (CLDN5$^+$/AQP1$^+$), 2) ascending vasa recta (PLVAP$^+$), 3) pericytes (PDGFB$^+$, RGS5$^+$), 4) glomerular capillaries (ITGA8$^+$) and 5) unidentified cluster with general EC markers like PECAM1$^+$.[101] A single cell study from two different regions of GBM tissue of 4 patients also revealed 5 clusters of ECs with distinct spatial organization: 1) peripheral EC type I (KLF2$^+$, SLC2A1$^+$), 2) tumor core EC type I (COL4A1$^+$, CD93$^+$, KDR$^+$), 3) tumor core EC type II (FABP1A$^+$), 4) peripheral EC type II (CCL4$^+$, HLA-DR$^+$) and 5) tumor core EC type III (ACKR1$^+$), suggesting that anatomical position, not just intrinsic EC identity, drives functional specialization.[102] Altogether these single-cell studies revealed the existence of both intra- and intertumoral heterogeneity of TECs, reflecting diverse functional roles and phenotypes across tumor types and regions that must be taken in account within engineered tumor models. Yet most MPSs lack EC subtype specification and spatial characterization of phenotypic diversity. Achieving physiological vascular architecture requires two complementary approaches. First, establishing appropriate TEC subtypes and cell ratio ranges for different tumor types, originated from transcriptomic data. Second, develop *ad hoc* differentiation protocols necessary to sustain TEC phenotype heterogeneity within engineered tissues. Advanced spatial biology methods including nanoneedle-based spatiotemporal lipidomics offer non-destructive approaches to validate whether engineered tissues recapitulate native EC subpopulation architecture and to monitor phenotypic stability during culture and therapeutic intervention.[103]

Targeting tumor vasculature as therapeutic approach

Several strategies have been developed to target tumor vasculature to indirect inhibiting tumors growth and progression,[42,104] each with its own mechanisms and potential benefits. Many of these therapies, as summarized in Table 1 have reached clinics, opening a new avenue for cancer patients.

Anti-angiogenic therapies

The concept of anti-angiogenic therapy was first introduced in 1970s when Folkman discussed "starving tumors by eliminating the blood vessels".[53] Since then many angiogenic factors have been identified as indirect tumor targets, including VEGF and its receptor (VEGFR), FGF and its receptor (FGFR), DLL4 and its Notch receptors, and PDGF and its receptor (PDGFR).[54] Additionally, several anti-angiogenic therapies have received FDA approval for the treatment of advanced cancers. Bevacizumab (humanized anti-VEGF antibody), under the name AVASTIN®, was the first angiogenesis inhibitor approved by FDA in 2004.[105] As a single agent bevacizumab demonstrated anti-cancer activity in GBM[106,107] and metastatic RCC.[108] Following the bevacizumab patent expiration in 2017, FDA approved bevacizumab-awwb, a biosimilar to AVASTIN® for treatment of multiple cancers including metastatic CRC.[109] Furthermore, ramucirumab (human monoclonal VEGFR-2 antibody) also showed clinical benefits, including improved objective response rate (ORR) and overall survival (OS), leading to FDA approval for CRC,[110] HCC,[111] non-small cell lung cancer[112] and gastric cancer.[113] Lastly, lenvatinib (VEGF-2 inhibitor) was initially approved by FDA for treating radioactive iodine refractory, locally advanced, or metastatic differentiated thyroid carcinoma as it had significantly increased ORR compared to placebo.[114] This was followed by the additional FDA approval of the treatment for HCC[115] and RCC[116] based on its ability to improve ORR and OS in these cancers. These anti-angiogenic drugs have shown efficacy when used alone or in combination with other chemotherapeutic or immunotherapeutic treatments. However, although several of these therapies have received FDA approval for specific cancers, their efficacy in other tumor types remains largely unexplored. For example, ramucirumab is approved for the treatment of several cancers, but not for RCC. However, a vascularized RCC MPS was developed to test anti-VEGFR-2 therapy and demonstrated that ramucirumab could potentially be used for RCC treatment.[117] A more in-depth discussion of MPS applications for evaluating vascular-targeting agents will be presented in later sections. Some other rising anti-angiogenic therapies that are gaining recognition in preclinical environment include extracellular vesicles that exhibited the potential to suppress angiogenesis through the transfer of anti-angiogenic molecules,[118] nanoparticle-based delivery systems for anti-angiogenic agents[119] and targeting TAMs.[120] However, as previously described, some tumors rely on alternative ways of vascularization, such as

Table 1 Combination therapies and single agents targeting vasculature FDA approved. This table summarizes the therapies, types of cancer, and clinical trial numbers

Trial	Drug	Cancer	Date of the FDA approval
NCT02684006	Avelumab + axitinib	Renal cell carcinoma	14.05.2019
NCT03434379	Atezolizumab + bevacizumab	Hepatocellular carcinoma	29.05.2020
NCT02366143	Atezolizumab with chemotherapy and bevacizumab	Metastatic non-squamous, non-small cell lung cancer (NSq NSCLC)	06.12.2018
NCT03141177	Nivolumab and cabozantinib	Advanced renal cell carcinoma	22.01.2021
NCT02702388	Lenvatinib	Radioactive iodine refractory differentiated thyroid cancer	13.02.2015
NCT03517449	Pembrolizumab and lenvatinib	Endometrial carcinoma	21.07.2021
NCT02853331	Pembrolizumab and axitinib	Advanced renal cell carcinoma	19.04.2019
NCT00262847	Bevacizumab with chemotherapy	Epithelial ovarian, fallopian tube, or primary peritoneal cancer	13.06.2018
NCT02141295/E3200	Bevacizumab with FOLFOX4 (5-fluorouracil, leucovorin, and oxaliplatin)	Colorectal carcinoma	20.06.2006
BO17705E	Bevacizumab and INF alpha2a	Metastatic rencal cell carcinoma	31.07.2009
NCT04737187	Trifluridine and tipiracil with bevacizumab	Previously treated metastatic colorectal cancer	02.08.2023
NCT03737643	Olaparib with bevacizumab	Epithelial ovarian, fallopian tube, or primary peritoneal cancer	08.05.2020
Multiple	Bevacizumab-awwb (biosimilar) + 5-fluorouracil-based chemotherapy/ fluoropyrimidine-irinotecan- or fluoropyrimidine-oxaliplatin based chemotherapy/ carboplatin or paclitaxel/ interferon alpha/paclitaxel and cisplatin or paclitaxel and topotecan	Metastatic colorectal cancer, non-squamous non-small cell lung cancer, glioblastoma, renal cell carcinoma, cervical cancer	14.09.2017
NCT02435433	Ramucirumab	Hepatocellular carcinoma	20.05.2019
NCT00917384		Advanced gastric cancer or gastro-esophageal junction adenocarcinoma	21.04.2014
NCT02411448	Ramucirumab with erlotinib	Metastatic non-small cell lung cancer (NSCLC)	29.05.2020
NCT01183780	Ramucirumab with FOLFIRI (irinotecan, folinic acid, and 5-fluorouracil)	Metastatic colon cancer	24.04.2015
NCT01103323	Regorafenib	Advanced colorectal cancer	27.09.2012
NCT01271712		Advanced gastrointestinal stromal tumors	25.02.2013
NCT01774344		Hepatocellular carcinoma	27.04.2017
NCT00073307	Sorafenib	Advanced renal cell carcinoma	20.12.2005
NCT00105443		Unresectable hepatocellular carcinoma	19.11.2007
NCT00984282		Metastatic differentiated thyroid cancer	22.11.2013
NCT00410761	Vandetanib	Symptomatic or progressive medullary thyroid cancer	04.06.2011
NCT00561470	Ziv-Aflibercept with 5-fluorouracil, leucovorin, irinotecan-(FOLFIRI)	Metastatic colorectal cancer	04.08.2012
NCT00075218	Sunitinib malate	Gastrointestinal stromal tumors and advanced renal cell carcinoma	26.01.2006
NCT00428597		Rare type of pancreatic cancer	20.05.2011
NCT00375674		Recurrent renal cell carcinoma	16.11.2017
VEG113387	Pazopanib	Advanced renal cell carcinoma	19.10.2009
NCT00753688		Advanced soft tissue sarcoma	26.04.2012
NCT04322539/NCT02314819/NCT04322539	Fruquintinib	Metastatic colorectal cancer	08.11.2023
NCT02627963	Tivozanib	Advanced renal cell carcinoma	10.03.2021

vascular mimicry, making them unresponsive to anti-angiogenic treatments.[104] Screening patients for specific angiogenic markers before initiating therapy could help to stratify those who are most likely to benefit from treatment, ultimately enhancing therapeutic outcomes and informing the development of drugs targeting alternative methods of tumor vascularization.

Vascular disrupting agents (VDAs) and targeting vascular mimicry

Other ways to effectively target tumor vasculature, such as vascular disrupting agents (VDAs) or targeting vascular mimicry are currently being studied, either alone or in combination therapy. VDAs are small molecular agents such

as tubulin-binding agents (*e.g.*, combrestatins) or flavonoids which have shown anti-cancer effects by inducing selective vascular disruption in tumor vessels.[104] Several VDAs have reached clinical trials, the most recent one being crolibulin that showed decreased tumor perfusion 2–3 days post treatment in phase I.[121] While VDAs have demonstrated promising results in preclinical models, their effectiveness in clinical settings has been variable,[122] indicating a need for further research to refine these strategies and overcome potential resistance mechanisms. Furthermore, recent identification of the role of the transmembrane glycoprotein receptor CD44 in vascular mimicry has enabled the targeting of this non-angiogenic mechanism of tumor vascularization.[123] This led to a phase I clinical study using anti-CD44 monoclonal antibody RG7356 (NCT01358903) which demonstrated modest efficacy where 21% of the patients experienced disease stabilization over 6–35 weeks.[124] The variable clinical outcomes of VDAs highlight the need to better understand their effects. However, whether MPSs can serve as a suitable platform to study VDAs is uncertain, since most current models primarily recapitulate angiogenesis-driven mechanisms. In contrast, MPSs are particularly well-suited for investigating vascular normalization strategies, offering a controlled and physiologically relevant environment to evaluate interventions aimed at restoring vessel structure and function.

Vascular normalization

Proposed by Jain *et al.* vascular normalization aims to restore the structure and function of tumor blood vessels to improve the delivery of therapeutic agents and enhance treatment efficacy.[125] This approach works by restoring the balance between pro-angiogenic signals, like VEGF, and anti-angiogenic factors in the TME, helping to stabilize the existing blood vessels rather than completely depleting them. It is important to clarify that the normalized vasculature does not function completely as a normal vasculature, but the tumor-associated disrupted signaling are partially restored. A clinical study based on vascular normalization used a pan-VEGF receptor tyrosine kinase inhibitor in 30 GBM patients and reported increased tumor perfusion and patient survival.[126] Although all patients who responded to therapy had an increased survival, only seven patients responded to therapy.[126] In MPSs, vasculature is typically in the presence of tumor spheroids, making it responsive to tumor-derived signaling, leading to elevated VEGF levels that provide a physiologically relevant context for studying vascular normalization strategies. Additionally, these platforms allow the evaluation of combinatorial therapies and the investigation of potential synergistic effects on both the vasculature and tumor tissue.

Combination therapies

Currently, many clinical trials are assessing the efficacy of combinatorial approach, including anti-angiogenic therapies with either chemotherapy or immunotherapy. For example, combining bevacizumab with chemotherapy has improved ORR and OS in CRC patients compared to chemotherapy alone.[127] Similar observations were seen with bevacizumab in ovarian cancer.[128,129] However, combining bevacizumab with chemotherapy agent anthracycline and/or taxane in phase III for triple negative breast carcinoma showed no difference in OS (NCT00528567).[130,131] Ziv-aflibercept, a fusion protein used as a decoy for VEGFA and VEGFB has been used for patients with metastatic CRC in combination with FOLFIRI (5-fluorouracil, leucovorin, and irinotecan).[132] In a phase III clinical trial, it demonstrated significant improvements in ORR and OS for metastatic CRC (NCT00561470).[133] These results led to FDA approval for its use in patients with metastatic CRC resistant to oxaliplatin-containing regimen.[133] Furthermore, combination of anti-angiogenic drugs with immunotherapy has also reached clinical studies. A notable example is the combination of Pexa-vec, an oncolytic virus, and sorafenib, a multi-kinase inhibitor tested in a phase III clinical trial for HCC. However, this combination did not significantly improve the ORR (19.2%) or median OS (12.7 months) compared to sorafenib alone (ORR: 20.9%; OS: 14 months) (NCT02562755).[134] Dual blockade of VEGF and immune checkpoints has reached clinical trials for several solid tumors with bevacizumab being used as the anti-angiogenic agent in 50% of these studies.[54] A significant patient improvement in ORR and OS led to FDA approval of combination of anti-VEGF/VEGFR with anti-PD-1/PD-L1 for treating HCC and RCC.[135] Recently, promising clinical evidence in ORR and OS has been demonstrated in patients with advanced gastric cancer and gastroesophageal junction adenocarcinoma using anti-VEGF/VEGFR with anti-PD-1/PD-L1 combination therapy in phase I/II trials.[135] Phase III clinical trial (NCT02853331) use of anti-PD-1 pembrolizumab with receptor tyrosine kinase inhibitor axitinib showed enhanced ORR in RCC patients.[136] In the field of adaptive cell therapy, a phase II study combining sunitinib, a tyrosine kinase inhibitor, and autologous dendritic cell immunotherapy reported significant anti-cancer activity in 13 out of 21 patient with RCC.[137] However, a subsequent phase III clinical trial for the same combination was terminated due to lack of efficacy (NCT01582672). A phase II clinical trial combining bevacizumab and allogenic NK immunotherapy for recurrent solid tumors has been completed, but results are still pending (NCT02857920). Bevacizumab has also been combined with interferon alpha IFN-α or IFN-α-2a in two phase III clinical trials for RCC.[138] The study showed improvements in progression-free survival, and the combination with IFN-α also improved OS,[138] however, without statistical significance. Combinations of VDAs with anti-angiogenic or chemotherapy/immunotherapies have also shown potential in preclinical models,[139–141] but these effects have not been consistently translated to clinical

settings. The combination of bevacizumab and paclitaxel was shown in an MPS to produce better outcomes than either agent alone.[25]

Current therapeutic strategies, combining conventional and immune approaches with anti-angiogenic agents show promise but exhibit a high level of discrepancy between patients, highlighting the need for more refined models that accurately replicate the *in vivo* environment.[142] An important factor in these studies is the dosage of anti-angiogenic agents. High doses can lead to unintended consequences, including increased ECM deposition, enhanced infiltration of pro-tumorigenic immune cells (*e.g.* MDSCs, TAMs or Tregs), elevated hypoxia and metabolic stress, and increased immunosuppression.[142] Preclinical platforms such as MPSs could help address this challenge. By enabling the testing of multiple drug combinations and concentrations in a human-relevant, architecturally accurate *in vitro* model, MPSs could reduce the variability in clinical responses currently observed. Furthermore, using patient-derived cells within these systems may help tailor treatments to individual patients, paving the way for more personalized therapeutic approaches.

Additional clinical challenges persist, such as insufficient routine monitoring of the TME, inadequate assessment of vessel status and oxygenation, and the absence of predictive markers for vascular remodeling.[143] To address these issues, vascularized microfluidic tumor models mimicking human biology could bridge the gap in preclinical testing by recapitulating key TME features including perfused vessels, enabling real-time analysis of drug delivery, immune cell trafficking, and metastatic processes that cannot be adequately studied in conventional systems (Fig. 1).

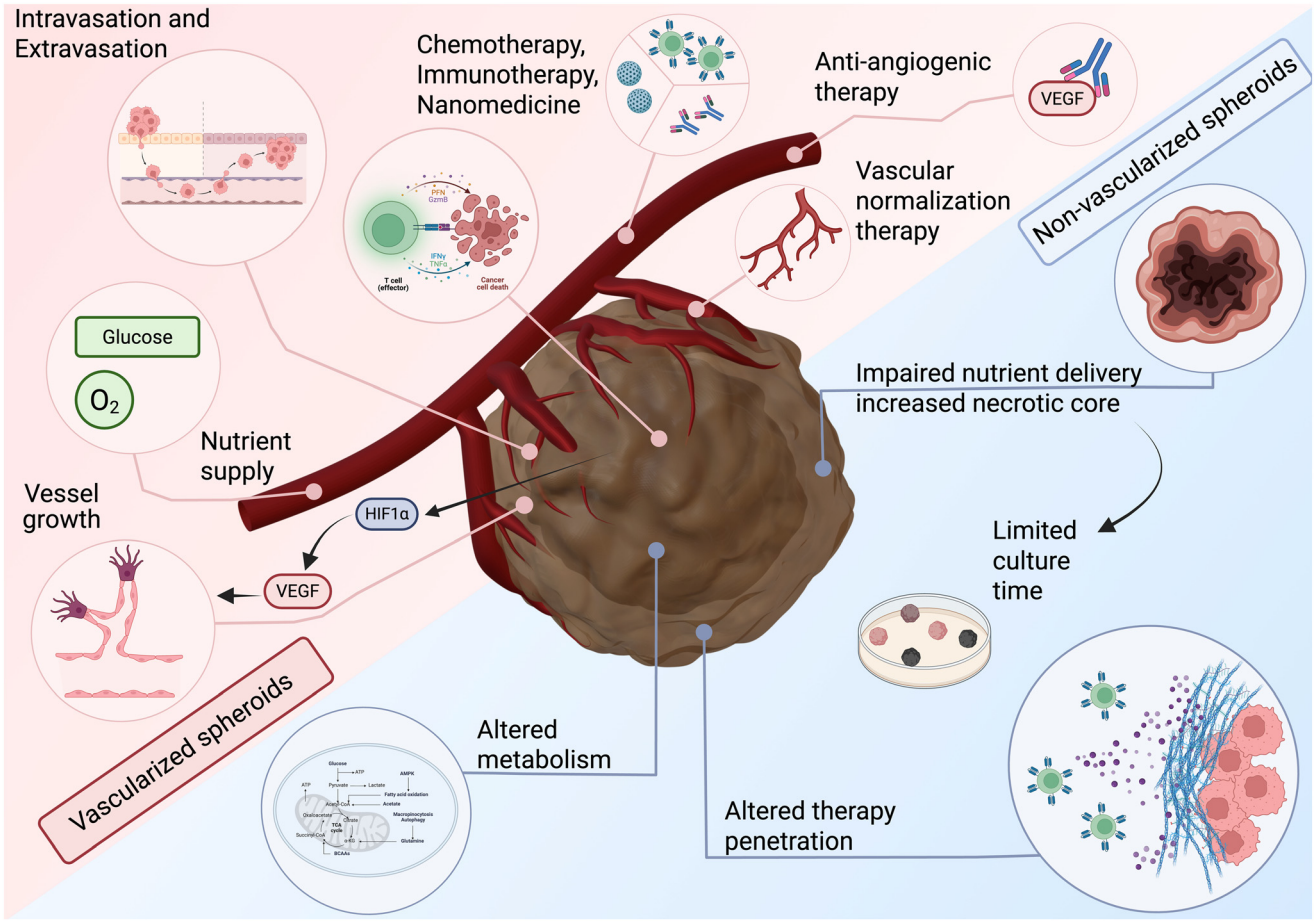

Fig. 1 Schematic overview of the advantages offered by vascularized 3D *in vitro* tumor models. These models closely recapitulate *in vivo* tumor physiology by supporting hypoxia-driven angiogenesis, which stimulates vessel growth through gradients of oxygen and pro-angiogenic factors. The presence of functional vasculature ensures direct delivery of nutrients and oxygen, enabling realistic modeling of tumor–stroma and tumor–immune interactions. Vascularized 3D models facilitate the study of key metastatic processes, including tumor cell intravasation and extravasation, and provide a robust platform for evaluating anti-angiogenic therapies, vascular normalization strategies, and the efficacy of immunotherapies and nanomedicine. Compared to non-vascularized spheroids, which suffer from limited nutrient diffusion, necrotic core formation, and altered drug penetration, vascularized models improve physiological relevance and predictive power for drug screening. The central 3D tumor image was created with NomadSculpt; surrounding annotations were created using BioRender. https://BioRender.com/wfw9oxb

Evolution of 3D *in vitro* tumor vascularization models

Understanding the biology of tumor vasculature, including its development, heterogeneity, and the influence of stromal and immune populations, is critical for evaluating therapeutic strategies. However, translating this knowledge into effective cancer treatments remains constrained by the inability of current preclinical systems to recapitulate the vascular barrier and microenvironmental molecular features that drive therapy resistance. 3D *in vitro* MPSs offer a controlled platform to recreate key aspects of tumor vascularization. By integrating engineered vascular networks with defined stromal and immune populations within controlled biochemical and biophysical microenvironments, these evolved platforms enable investigation of the tumor vasculature as a functional component of the TME rather than a static structural element. To achieve physiological relevance, vascularized MPS must integrate multiple components: an ECM that support the architecture of functional endothelial vessels, stromal cells that actively remodel vascular architecture, immune cells that modulate vascular function and spatially organized gradients of oxygen, nutrients, and molecular factors that reflect the interconnected therapeutic barriers discussed in the previous sections.

This section reviews the evolution of 3D *in vitro* tumor vascularization models, examining how engineering innovations progressively address the biological requirements established in our discussion of TME complexity. We focus on the selection and integration of cellular components, biomaterial strategies, and technological approaches that collectively enable the recapitulation of tumor vascular biology, bridging the gap between our understanding of vascular mechanisms and the engineered systems available for predictive preclinical testing.

Endothelial cell sources for *in vitro* vascularization

In vitro vascularization models have evolved significantly since 1988, when the first tube formation assay was established using human umbilical vein endothelial cells (HUVECs),[144] becoming one of the most widely utilized *in vitro* methods for studying angiogenesis due to its accessibility and ease of implementation. While HUVECs remain the predominant cell source of ECs in research, alternative options include endothelial progenitor cells (EPCs) and human pluripotent stem cell-derived endothelial cells (hPSC-ECs). HUVECs continue to be preferred for their availability, straightforward isolation procedures and well-established culture protocols. They have consistently demonstrated their ability to form vascular structures both *in vivo* and *in vitro*. While HUVECs are a reliable tool for vascularization studies, it is important to acknowledge that variation in genotype and phenotype may arise depending on the donor source and, being primary cells, they can exhibit senescence at higher passage numbers. HUVECs may also express inflammatory markers under specific environmental conditions, and they may not fully capture patient-specific or organ-specific endothelial characteristics.[145,146] EPCs are typically isolated from peripheral blood and bone marrow and have gained significant attention in vascularization research for their ability to rapidly proliferate and differentiate into ECs. Nevertheless, they face limitations as lack of unambiguous characterization, due to overlapping marker expression and challenges in optimizing culture conditions to promote robust microvessel formation.[147] Patient-derived induced pluripotent stem cells (iPSCs) offer a distinct advantage for generating personalized vascularized models: they can be differentiated into patient-matched endothelial cells, eliminating immune rejection and enabling personalized therapeutic testing.[49] Alongside ECs, hPSCs can differentiate into different cell populations, including perivascular cells[148] and smooth muscle cells,[149] they are renewable,[150] and offer the potential for precise genetic modification.[151] However, achieving high yields of hPSCs remains challenging, and there are concerns regarding the development of genetic and epigenetic changes during *in vitro* culture.[152] Indeed, the choice of EC source influences the physiological relevance and translational potential of vascularized MPS. While HUVECs offer practical advantages for standardized angiogenesis assays, their limited ability to capture patient-specific or organ-specific vascular phenotypes restricts their utility for personalized medicine applications. In contrast, patient-derived iPSC-ECs present significant promise for recapitulating individual vascular heterogeneity, yet their optimization and scalability remain ongoing challenges. Beyond the cellular components themselves, the success of *in vitro* vascularization critically depends on the biomaterial microenvironment in which endothelial cells reside. The ECM composition, mechanical properties, and biochemical cues provided by hydrogels directly determine whether endothelial cells can assemble into functional, perfusable vessel networks that recapitulate the structural and functional characteristics of native tumor vasculature.

Biomaterials for engineering vascular structures

Biomaterials for vascularization models include both natural and synthetic options, each with distinct advantages and limitations. Natural biomaterials like collagen, fibrin, gelatin, alginate, and hyaluronic acid (HA) closely mimic the native ECM, offering excellent biocompatibility and bioactivity. These materials offer ECM-like spatial guidance and structure by providing cell-adhesion sites, native signaling capabilities and allow for hydrogel remodeling and capillary-like network formation. However, they may exhibit batch-to-batch variation, which arise from differences in animal or tissue sources, variations in extraction and purification protocols, and inconsistencies in processing parameters such as temperature, pH, and enzymatic treatments. Synthetic biomaterials instead, such as polyethylene glycol (PEG),

polycaprolactone (PCL), and poly(lactic-*co*-glycolic acid) (PLGA) provide greater control over mechanical properties and degradation rates but may lack essential biological cues.[153] In vascularization-on-a-chip applications, fibrin-based hydrogels have demonstrated superior angiogenic properties compared to Matrigel, a tumor–derived basement membrane extract, or collagen type I alone, which can inhibit the development of perfusable vessel-like structures.[154,155] To address the limitations of individual materials, researchers are increasingly focusing on composite materials that combine the advantages of both natural and synthetic biomaterials, as well as developing bioactive materials that better mimic the complex native vascular environment.[156–158] Human platelet lysate has emerged as a bioactive matrix component to significantly enhance angiogenesis when combined with structural biomaterials such as fibrin or collagen.[159] Furthermore, techniques such as adjusting crosslinking density, protein and polymer concentrations have been developed to fine-tune the mechanical and structural properties of these hydrogels and better mimic the target tissue environment, influencing cell behavior and vascular morphogenesis.[160] Recently covalently crosslinked viscoelastic hydrogels with reversible physical interactions or dynamic covalent bonds, commonly known as dynamic hydrogels, have become of interest. They can undergo stress relaxation, allowing them to recover their structure after deformation, a property that closely resembles the mechanical properties of soft tissues and the natural ECM, making them ideal for mimicking native cellular microenvironments.[161] Interestingly, dynamic hydrogels have shown enhanced endothelial colony-forming cell-derived endothelial cells (ECFC-EC) contractility, integrin β1 clustering, and vinculin recruitment, activating FAK and metalloproteinase expression.[161] This resulted with support for vascular assembly and basement membrane deposition.[161] For a more comprehensive review of hydrogels used in 3D culture and vascularization, we refer the readers to the work in[162] and,[156] respectively. Altogether, selecting and tuning biomimetic hydrogels is central to engineering vascularized 3D spheroid-based MPS architectures, enabling to more faithfully recapitulate the tumor microenvironment and its therapy-resistant vascular niches.

3D spheroid architecture as a fundamental structural requirement for TME modeling

The predominant use of spheroids in vascularized MPS models is not arbitrary; rather, the 3D architecture is essential for recapitulating key TME features that drive therapy resistance. Unlike monolayer cultures, spheroids establish oxygen and nutrient gradients that simulate the microenvironmental heterogeneity of *in vivo* tumors. As spheroid grow (typically >400–500 μm in diameter), oxygen diffusion becomes limiting, creating a distinct hypoxic core that triggers HIF-1α signaling and pro-angiogenic factor secretion.[163] This diffusion-dependent hypoxia cannot be accurately modeled in 2D cultures. Furthermore, the 3D geometry enables cell–cell and cell–matrix interactions across multiple axes, rather than the single-plane interactions observed in 2D systems. In co-culture conditions, these 3D interactions govern mechanotransduction and signaling between tumor cells, fibroblasts, immune cells, and the developing vasculature. The necrotic core formation observed in larger spheroids also replicates the pathological architecture of poorly vascularized tumors and drives the selection of chemotherapy-resistant populations. Therefore, the use of spheroid is not merely a convenient culture format but a fundamental structural requirement for physiologically relevant modeling of tumor–vascular interactions and therapy resistance mechanisms.

Vascularization strategies: template-based and self-organized approaches

Replicating the complexity of tumor vasculature *in vitro* presents significant challenges, necessitating precise biological and mechanical conditions. Distinct methods have been developed to vascularize tumor organoids and spheroids, categorized broadly into template-based and self-organized approaches. Template-based methods rely on predefined structures or patterns to guide vascular network formation, offering precise spatial control over the organization of cells and materials. Key techniques include molding and bioprinting. Molding generates perfusable conduits by organizing cells and biomaterials within a thin molding plane, allowing for intricate vascular architectures.[152,156] Bioprinting provides precise deposition of cells and biomaterials, enabling the creation of organoids with pre-formed vascular channels and high experimental flexibility.[152,156] Other engineering-driven approaches, are microfabrication and laser degradation that allow for the creation of defined vascular structures through precise biomaterial manipulation.[164,165] While template-based methods provide precise spatial control over vascular network formation, they often fail to capture the dynamic heterogeneity, structural abnormality, and adaptive remodeling characteristic of tumor vasculature. In contrast, self-organized vessels allow endothelial and supporting cells to autonomously form networks that can dynamically rearrange in response to the tumor microenvironmental cues. These considerations are critical when designing MPSs, where replicating physiologically relevant vessel architecture, perfusion, and cell–cell interactions is essential to study tumor progression, drug delivery, and therapeutic responses *in vitro*. These self-organized approaches rely on the intrinsic ability of cells to interact, remodel, and self-organize, making the careful selection of cell types, supporting stromal populations, and extracellular cues critical to achieving functional vascular networks *in vitro*. A common method is the co-culture of endothelial cells with fibroblasts or mesenchymal stromal cells to support vessel formation, though other supporting populations such as pericytes,

smooth muscle cells, or immune cells can also be incorporated depending on the desired micronvironmental context.[152] Other techniques include co-differentiation of iPSCs into endothelial and supporting cells or assembly of organoid and vascular constructs, enabling the formation of physiologically relevant vascular networks.[166] For a comprehensive overview on vascularization strategies, we refer readers to another insightful review.[167] In contrast, our review narrows the focus on the application of vascularized models in enabling anti-tumor therapeutic development, highlighting their translational potential in advancing therapeutic strategies for cancer treatment. Therefore, we specifically discuss the distinct strategies used to build vascularized tumor spehroids and organoids *in vitro*, implemented both with and without microfluidic devices.

Non-microfluidic vascularization: morphogenesis and *in vivo* engraftment

Significant advancements have been achieved in *in vitro* vascularization techniques without microfluidic devices, primarily focused on mimicking structural morphogenesis and tissue integration for transplantation, rather than functional perfusion for tumor biology studies. Successful generation of vascular-like network has been achieved in hydrogels outside microfluidic devices.[168,169] Vascularization of organoids without microfluidic support is usually established *via* intra-organoid vascularization, as observed during the development of cerebral organoids following a co-differentiation methods, adding VEGF to human embryonic stem cells (hESCs) promoted vascularization without inhibiting neural differentiation.[170] In another study, overexpression of human E26 transformation-specific variant transcription factor 2 in hESC subpopulations contributed to the development of a complex vasculature in human cortical organoids.[171] Beyond morphogenesis, these models have demonstrated functional anastomosis upon transplantation. Co-differentiation method led to successful intravascular perfusion of *in vitro* generated vascularized iPSC-derived liver organoid following transplantation into an *in vivo* mouse model.[172] Assembly methods have also been used, where vascular organoids established independently were subsequently fused with specific tissue organoids to form vascularized constructs but where vasculature is not perfusable.[173,174] In the context of cancer, co-cultures of cancer cells with ECs have reported development of intra-tumoral vasculature that mimics the structural presence of vessels within the tumor mass.[175–177] Lastly, advances in bioprinting have enabled the introduction of perfusable channels, lined with ECs, into organ building blocks composed of patient-derived stem cell organoids.[178] While bioprinting approaches enable controlled perfusion through predefined channels lined with ECs, vascularization remains largely architecturally imposed rather than arising from biological self-assembly. In contrast, MPSs support the formation of biologically relevant self-assembled vascular networks that become perfusable under dynamic flow. Consequently, non-microfluidic vascularization strategies primarily achieve structural vascular morphogenesis, with perfusion confined to predefined engineered channels rather than dynamic microvascular networks. This structural limitation restricts their ability to capture dynamic vascular remodeling and adaptive structural changes in response to tumor microenvironmental cues, that influence drug delivery, nutrient exchange, and barrier function. By integrating perfusion into spatially organized, self-assembled vasculature within tumor spheroids, MPSs overcome these limitations, providing enhanced capabilities to model functional vascular–tumor interactions and more accurately predict therapeutic responses.

Microfluidic systems: enabling functional perfused vasculature and transport regulation

In the complex TME found *in vivo*, cells experience mechanical, biochemical and topographical cues that shape their behavior.[179,180] Microfluidic technology has enabled the development of tumor-on-a-chip models that mimic these microenvironmental conditions providing tunable matrix stiffness, controlled biochemical gradients, and spatial cell arrangement.

The evolution of microfluidic systems to generate tumor spheroid vascularization (Fig. 2) highlights a shift from simple 2D monolayers to complex, 3D tissue-mimetic models. The journey toward these systems began as early as 1948, when researchers successfully cultured single cells within glass capillaries to study adaptation to confined environments.[181] This concept evolved significantly with the generation of microelectromechanical systems (MEMS) in the 1980s, which integrated sensors, actuators, valves, and pumps, leveraging microscale fluid dynamics and advanced microfabrication techniques on silicon wafers. However, it was in the late 1990s that microfluidic devices become widely adopted in *in vitro* tissue culture with the introduction of soft lithography and polydimethylsiloxane (PDMS) replica molding to fabricate more accessible, biocompatible and transparent platforms for biomedical research.[182] Early microfluidic endothelial monolayer models progressed from demonstrating dynamic leukocyte–endothelial adhesion under flow in PMMA systems,[183] to developing computer-controlled PDMS platforms with integrated piezoelectric pumping that revealed shear stress-dependent endothelial alignment,[184] to creating two-layer vasculature systems to study metastatic cancer cell adhesion.[185] However, it was in 2010 that the term "organ-on-a-chip" was used for describing a model integrating endothelial monolayer and lung cells, demonstrating the potential of microfluidic platforms to model organ-specific microenvironments.[186] Building on these foundations, tumor models were developed using EC organization in monolayer,[187] marking the beginning of tumor-on-a-chip technology. These tumor models with an

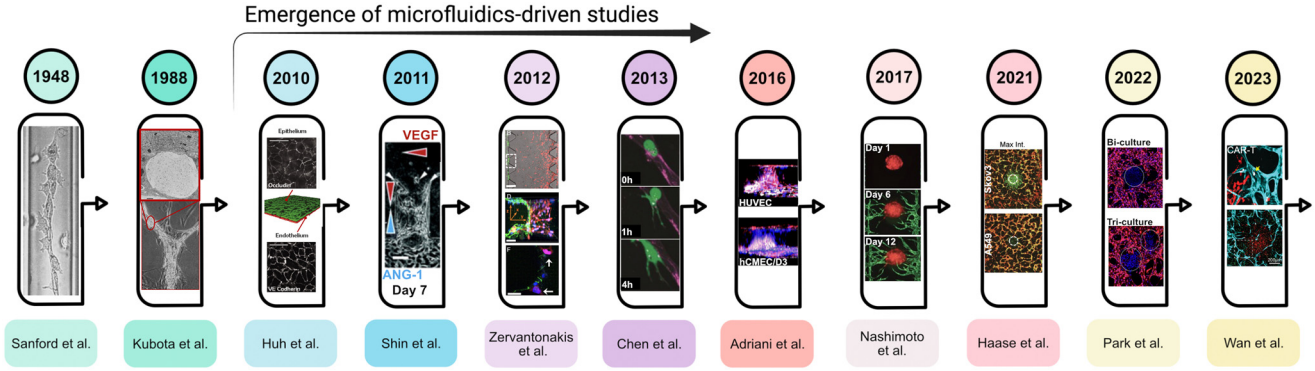

Fig. 2 Timeline of pivotal research advances for achieving physiologically relevant vascularized tumor-on-a-chip models. This timeline illustrates the evolution of experimental models from early cells grown in capillary tubes (1948),[181] through the development of capillary-like tube formation on matrices (1988),[144] to the introduction of organ-on-chip technologies such as lung- and tumor-on-a-chip with EC monolayers and 3D angiogenic sprouting (2010–2012).[186,187,198] Subsequent advances in organ-on-a-chip models include vascular networks with tumor cells, increased organ-specific cellular complexity and angiogenic sprouting toward tumor spheroids (2013–2017).[189,204,213] More recent milestones have seen the formation of self-assembled vascular networks for tumor spheroid vascularization, incorporation of stromal and immune cellular components within the spheroids (2021–2022),[205,206] and the application of immune cell interactions, such as CAR T-cells within vascularized tumor spheroids (2023),[207] reflecting the increasing cellular complexity and physiological relevance of vascularized tumor-on-a-chip models over time. The image was created with Affinity Designer 2. Image reproduced and adapted: from a public domain source,[181] under Creative Commons CC BY-NC-SA 4.0,[144] with permission through RightLink license,[189,204] with permission from Royal Society of Chemistry,[198,205] with permission through PNAS,[187] with permission through John Wiley and sons,[206] under Creative Commons CC BY-NC.[207]

endothelial monolayer were particularly valuable for studying key biological processes including intravasation[187,188] and extravasation,[189–191] epithelial–mesenchymal transition (EMT),[192,193] immune cell transendothelial migration,[194,195] and probe nanoparticle vascular permeability.[196,197] Organ-on-a-chip system enabled also mimicking angiogenic sprouting from an endothelial monolayer into an hydrogel,[198,199] and to test anti-angiogenic therapies.[200,201] Recent advances have leveraged microfluidic technology to promote the formation and maintenance of self-assembled, functional blood vessels around a tumor spheroid or organoid in an ECM-like hydrogel.[165,167,202] In this context, functional *in vitro* vasculature refers to vessels with an open, perfusable lumen, and physiologically relevant permeability, allowing for the study of transport-dependent processes. These vascularized tumor-on-a-chip models have opened new avenues for studying tumor vascular biology and therapy responses in more physiologically relevant *in vitro* settings[24,25,27,117,203–212] with remarkable implications for cancer research, therapy discovery, and personalized medicine.

The primary advantages of using microfluidic devices for the generation of perfused vasculature *in vitro* are: (i) possibility to establish molecular gradients to mimic physio/pathological conditions; (ii) real-time observation of functional responses and transport phenomena; 3) modeling the physiological barriers to drug/cell delivery with quantitative assessment of vessel permeability; (iii) mathematically predict flow patterns, (iv) possibility to integrate automated flow pumps to mimic shear stresses and vascular perfusion, (v) cost-efficiency with use of smaller amount of reagents and occupying much smaller footprint compared to 2D culture and animal models, (vi) standardized fabrication techniques or commercially-available channel layouts.[214] These devices support more physiologically relevant therapy testing by facilitating the recapitulation of cell–cell and cell–ECM interactions, the possibility of controlling multiple gradients and enabling the study of therapeutic agent effects on organ-specific cells after they have crossed the endothelial barrier.[213] Microfluidic devices are highly customizable and typically fabricated using soft lithography, where a pattern is first created on a silicon wafer *via* photolithography, and then replica molding is performed with a liquid polymer, commonly PDMS. PDMS is favored in microfluidic devices due to its ease of handling, low cost, gas permeability, and optical transparency for real-time imaging.[214] Microfluidic devices can be fabricated using other methods, such as micromolding, microetching, laser etching, injection molding, photopolymerization, and 3D printing.[215] Commercial options for microfluidic platforms for developing vascularized tumor models include devices from several companies, such as Ibidi, Mimetas, and AIM Biotech, which facilitate *in vitro* vascularization and support advanced therapeutic studies.

MPSs allow for the precise manipulation of the tumor microenvironment, enabling researchers to study tumor vascularization processes in controlled settings.[167,202] Similar to the progression observed in non-microfluidic vascularization strategies, the vasculature within microfluidic device was first developed on its own followed by the addition of single cancer cells[189] and subsequently cancer spheroids.[204,205] A key advantage of MPSs is their ability to recapitulate complex heterocellular interactions within the TME, integrating TECs, TAMs, CAFs, and tumor cells in controlled spatially defined architectures, allowing real-time analysis of angiocrine signaling, cell-type-specific contributions, and functional outcomes such

as vessel stabilization, immune cell trafficking, barrier integrity, and metastatic potential. By supporting diverse cell aggregates formats, from tumor-only spheroids to heterocellular assemblies, MPSs provide a translationally relevant framework to model tumor angiogenesis and vascular remodeling to evaluate therapeutic effects on both the tumor cells and the surrounding vasculature, including clinically relevant endpoints, such as tumor dormancy and metastatic risk.

Microfluidic strategies for vascularizing tumor spheroids generally fall into three design categories (Fig. 3): self-assembly (or vasculogenesis), angiogenic sprouting, and vascular bed assembly.[165] Three representative examples illustrate these distinct vascularization strategies. In the first approach (Fig. 3A), a tumor spheroid was co-embedded with ECs and stromal cells in a central hydrogel chamber of the microfluidic device. The ECs self-assembled into a network that surrounded

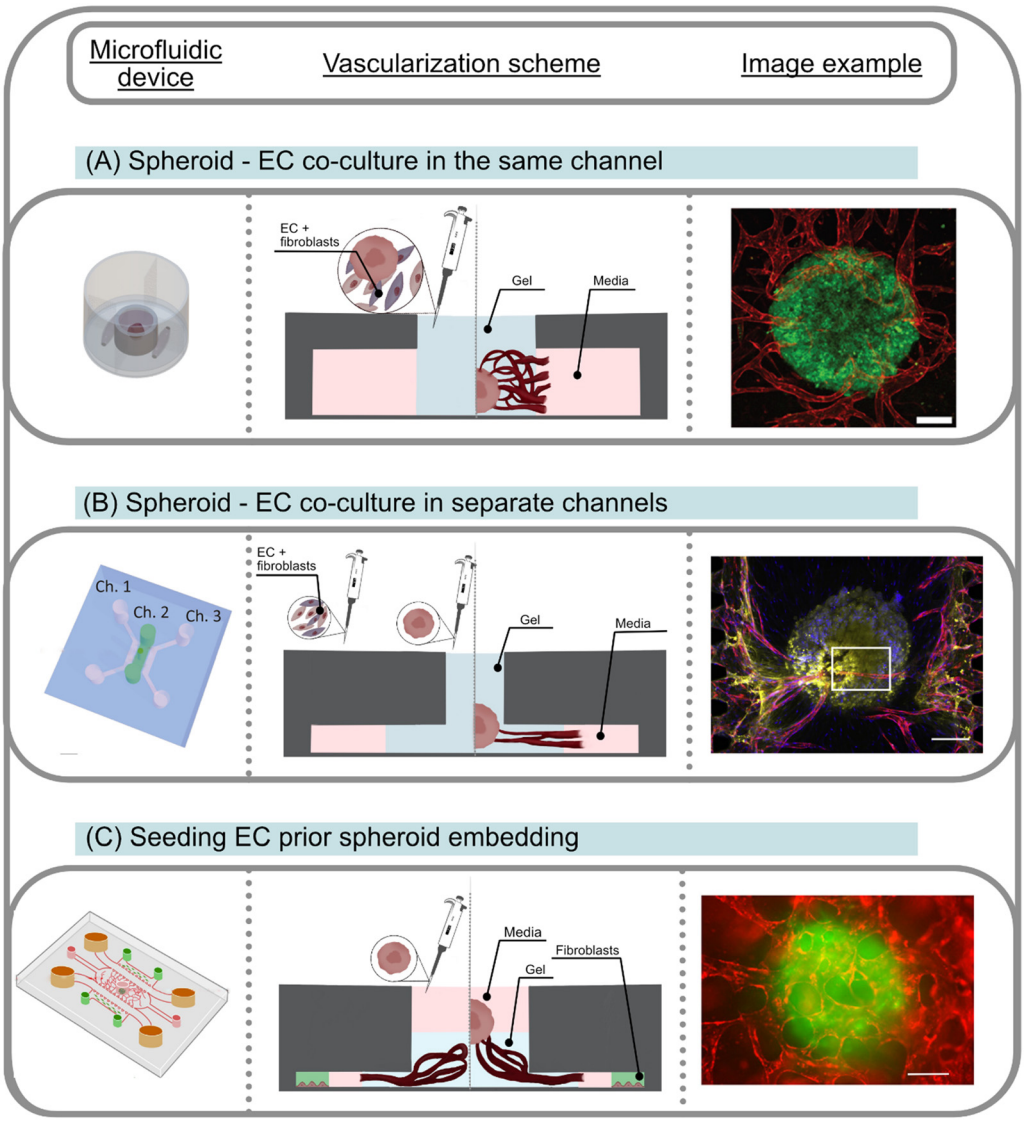

Fig. 3 Schematic representation of different vascularization approaches in 3D tumor microenvironment models. A) Co-culture system where a pre-formed tumor spheroid was embedded alongside endothelial cells and fibroblasts in a collagen–fibrin matrix within the central round chamber of the microfluidic system, allowing vascular networks to self-assemble around the tumor spheroid. Image adapted from Fig. 4 of,[26] showing an hepatocellular carcinoma spheroid co-cultured with HUVECs and fibroblasts in the OrganiX™ microfluidic insert, with vessels (red) forming around and penetrating tumor spheroid (green). Scale bar = 200 μm. (B) Co-culture system where a pre-formed spheroid was embedded in collagen-fibrin hydrogel and enabling vessel sprouting from both lateral fluidic channels containing endothelial cells. Image reproduced with permission from,[216] Copyright 2020, Elsevier, showing breast cancer spheroids embedded in collagen–fibrin hydrogel and vessels (red) sprouting and connecting with the tumor spheroid (yellow). Cell nuceli are in blue. Scale bar = 100 μm. (C) A five-channel microfluidic device was used to seed fibroblasts in two lateral channels, and endothelial cells in the central hydrogel channel to allow vasculature development before seeding the tumor spheroid on top of the "capillary bed". Image reproduced with permission from,[24] Copyright 2022, American Chemical Society, showing the esophageal tumor spheroids (green) reached by the underlying vasculature (red). Scale bar = 150 μm. The vascularization schematics were created using Procreate and Affinity Designer 2, with the microfluidic device in (A) modeled using NomadSculpt.

and penetrated the spheroid, modeling *de novo* vessel formation and structural integration with the tumor spheroid.[26] In the second approach (Fig. 3B), a tumor spheroid was inserted independently in the central channel while the ECs were seeded in the two adjacent side channels. ECs sprout into the hydrogel toward the tumor, mimicking the recruitment of host vessels by a growing tumor.[216] Lastly, in the third strategy (Fig. 3C), ECs were first inserted in the central channel to form a capillary bed, followed by a tumor spheroid seeded on top of the capillary bed[24] to allow the underlying vasculature to reach the spheroids, modeling the interaction of a tumor with an existing vascular network. All these methods led to a generation of a perfusable vasculature for monitoring tumor cells extravasation, migration or therapy delivery, and the effect that the tumor vasculature has on the therapy efficacy.

Applications of vascularized tumor-on-a-chip models

As described above, vascularized microphysiological tumor models have rapidly advanced as versatile platforms for investigating the complex interplay between tumors and their microenvironment.

Their major advantage lies in replicating the intricate interactions among multiple cellular constituents, such as TECs, TAMs, CAFs, and tumor cells within controlled, heterocellular configurations. These models enable researchers to explore a broad spectrum of applications, from elucidating the heterogeneity and modulation of the TME, to studying angiogenesis, vascular targeting, metastatic mechanisms, and the impact of microbiota and metabolites. Additionally, they provide valuable insights into biomarker discovery and the evaluation of therapeutic efficacy. By integrating diverse cellular and extracellular components, vascularized tumor-on-a-chip systems offer translational opportunities to recapitulate human-relevant conditions and address key questions in cancer biology and drug development. In this section, we detail key applications of vascularized tumor-on-a-chip systems, summarized in Fig. 4.

EMT, migration, intravasation and extravasation studies

Vascularized tumor-on-a-chip models have emerged as powerful tools for studying cancer migration and metastasis with distinct design architectures targeting specific

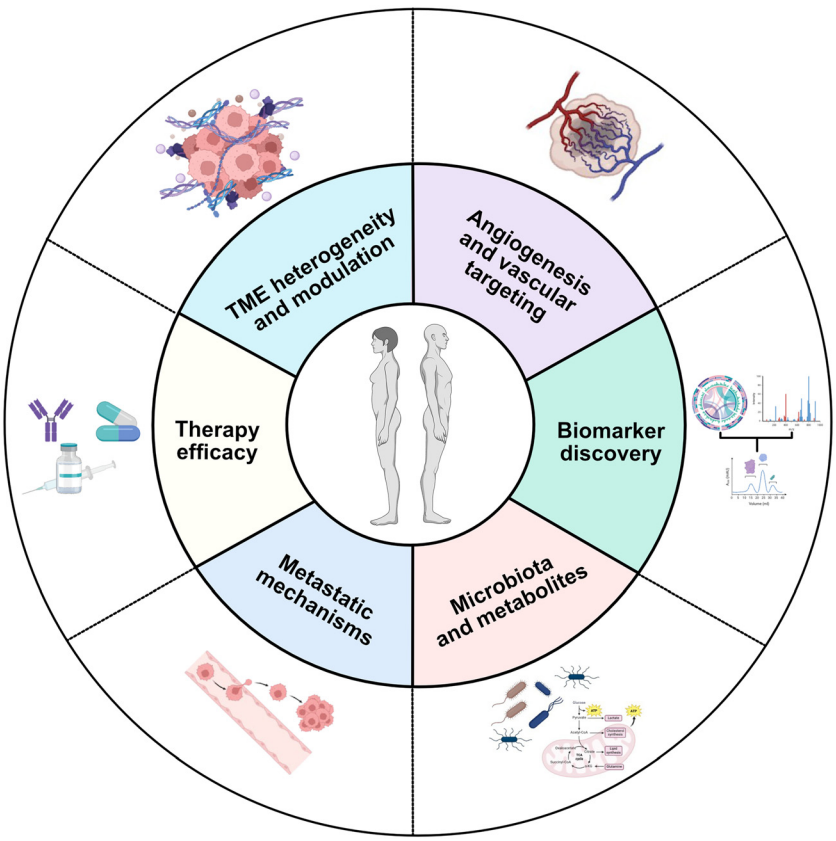

Fig. 4 Key research applications of vascularized tumor-on-a-chip models. Circular diagram illustrating the six major areas of investigation enabled by microphysiological systems (MPSs): tumor microenvironment (TME) heterogeneity and modulation, angiogenesis and vascular targeting, biomarker discovery, microbiota and metabolite interactions, metastatic mechanisms, and therapy efficacy assessment. The central human silhouettes emphasize the human-relevance and translational potential of these platforms in personalized medicine and drug development. The scheme for this image was created with Procreate and images in the outskirt of the scheme in BioRender. https://BioRender.com/i49z773.

mechanisms of vascular–tumor pathobiology driving each step of the metastatic cascade. While numerous organ-on-a-chip platforms have been developed to model various aspects of tumor biology, we highlight here representative vascularized systems that exemplify how design choices of vascular components enable mechanistic interrogation of specific metastatic processes. Vascularized tumor-on-a-chip models of CRC and pancreatic ductal adenocarcinoma were developed by using ECFC-derived ECs supported with normal human lung fibroblasts (NHLF), to investigate how TECs and stromal cells regulate tumor cell migration and invasion. The key vascular design feature was the generation of morphologically and functionally distinct EC clusters: P1, expressing genes related to cell cycle and immune pathways, and P2, expressing genes involved in tumor development and invasion, thereby recapitulating the known heterogeneity of tumor-associated vasculature observed *in vivo*.[217] The device layout featured multiple adjacent chambers that allowed sequential loading of 3D tissues at different time points, enabling control over the temporal dynamics of vasculature formation and its influence on tumor cell behaviors.[217] Complementing this focus on EMT and migration, a multi tissue-on-a-chip system was developed to specifically model tumor cell extravasation in breast and liver cancer models. The critical vascular design choice was the use of telomerase-immortalized microvascular ECs along tumor clusters, selecting an endothelial source that reflects the physiological properties of organ-specific microvasculature to study transendothelial migration. The platform demonstarted that inflammatory activation of TNF-α-stimulated endothelium in the model led to increased transendothelial tumor migration and increased vasculature porosity and leakiness, modeling the loss of endothelial barrier function that enables tumor cell extravasation during metastatic dissemination.[218] Instead, to address the contribution of mechanical cues to vascular–tumor interactions, two bioprinting-based vascularized tumor-on-a-chip systems were developed to investigate how mechanical forces and cell–matrix interactions regulate both tumor cell behavior and endothelial integrity during vascular–tumor interactions. One approach embedded patient-derived neuroblastoma tumor spheroids with ECs into bioprinted fluidic chips.[219] The key engineered design feature was the implementation of a programmable 3D-orbital shaker to standardize the endothelial cell coating within the channel, addressing a critical technical barrier to maintaining viable vasculature in tissue constructs. Mechanistically, the platform confirmed that the vessel formation requires the presence of supporting cells: fibroblasts, mesenchymal stem cells or adipose derived stem cells. Interestingly, they also reported that tumor spheroids attracted microvessels from the surrounding tissue, a vascular remodeling behavior reflecting the pro-angiogenic microenvironment during tumor progression.[219] An alternative bioprinting strategy directly bioprinted a ring-shaped endothelial compartment using a GelMA-fibrin bioink containing human cerebral microvascular endothelial cells (hCMEC/D3) and filled the core with a GelMA-alginate bioink loaded with GBM cells, with fibrin-based matrices selected for superior support for vasculogenesis. The critical discovery was that shear stress and cell–matrix mechanical interactions were key player for vasculogenesis and tumor–vascular interactions.[220] Furthermore, the model was exposed to simulated microgravity and showed a reduction in the spontaneous aggregation of cells and a significant reduction in cell junction proteins, pointing out that mechanotransduction affects not only the tumor but also vascular barrier function and integrity.[220] These studies highlight that vascularized tumor-on-a-chip platforms can recapitulate the multifaceted vascular and mechanical features underlying tumor cell migration, intravasation and extravasation. This capability is now enabling that is now translating into rational evaluation of therapeutic interventions designed to target vascular-mediated metastatic vulnerabilities.

Therapy efficacy studies

Therapeutic investigation in vascularized tumor-on-a-chip models comprises direct tumor targeting, indirect vascular-directed strategies, and combinatorial approaches. These studies typically embed tumor spheroid with the ECs and their supporting stromal cells within 3D hydrogel matrices made of fibrin[203] or collagen–fibrin combination[26,210] within microfluidic devices, as shown in Fig. 3. Critically, different MPS designs emphasize distinct vascular properties, some prioritize angiogenic morphogenesis and vessel maturation, others model functional barrier defects and leakiness, and others reconstruct oxygen gradients and metabolic heterogeneity, each capturing specific aspects of the tumor–vasculature interface relevant to drug delivery and resistance. Once the model is established, it is possible to proceed with the therapeutic intervention at different timepoints and assess how vascular barrier functions, and oxygen/nutrient gradients modulate drug penetration and efficacy.

Direct tumor targeting

Direct tumor targeting traditionally relies on chemotherapeutic compounds, however, other methods include using small-molecule therapies targeting specific molecular pathways (*e.g.*, tyrosine-kinase inhibitors), and immunotherapies, such as immune check-point inhibitors, TILs or CAR/TCR engineered T cells.[221] Therefore, implementing vasculature in *in vitro* tumor models is essential for studying direct targeting for a better understanding of therapy delivery dynamics, vascular interactions, and therapeutic efficacy in a more physiologically relevant context. Importance of vasculature for drug efficacy in spheroids has been described by several research groups. A CRC spheroid model in microfluidic device called "vascularized micro-tumor" (VMT) was developed to compare standard-of-care chemotherapy in VMT, 2D cell monolayer, 3D cell culture and a xenograft

model.[203] The study reported that vessels in the VMT mirror hallmarks of *in vivo* tumor-associated vasculature, with vessel leakiness and compression becoming more pronounced as tumor progresses over time. Importantly, these VMT better predicted tumor growth and response to chemotherapeutic drugs observed in preclinical *in vivo* murine xenograft models than 2D and 3D monocultures. However, this model exhibited upregulation only in hypoxia- and glycolysis-associated gene programs, with no significant enrichment in other pathways, suggesting a limited ability to fully recapitulate the complexity of *in vivo* tumor biology. Furthermore, Nashimoto *et al.* generated angiogenic sprouting from adjacent channels towards tumor spheroids, generating perfusable vasculature within several types of spheroids.[216] The platform demonstrated that vascular presence increased proliferation activities of tumor cells and decresed cell death in the spheroid. Moreover, by comparing the drug distribution under vascularized perfusion and in static conditions the drug had no dose-dependent response under perfusion conditions highlighting the impact of vascular transport dynamics on therapeutic response. A 3D vascularized lung cancer-on-a-chip (VLCC) was developed to model vessel maturation stages, featuring: (i) a lung decellularized ECM-based pro-angiogenic tissue-mimetic hydrogel, (ii) heterocellular tumor spheroids modeling solid lung cancer, and (iii) perfusable, large-diameter vessel-like structures recapitulating arterial, venous, and capillary functions.[222] In this model capillaries originated from the spheroid vasculature and demonstrated greater sensitivity to dose-dependent anti-cancer therapy compared with non-vascularized spheroids and 2D tumor models. Aside from chemotherapy, Dey *et al.* delivered both chemotherapy and anti-HER2 CAR T cells in a vascularized breast cancer model.[208] By using aspiration-assisted bioprinting, tumor spheroids were positioned at predefined distances from the perfusable vasculature that allowed assessment of the impact of spatial proximity on tumor angiogenesis and invasion.[208] Devices with tumors bioprinted proximal to the perfused vasculature exhibited higher diffusional permeability compared with distally bioprinted tumors and tumor-free controls.[208] They further conclude that tumors exhibited dose-dependent therapeutic responses and drug resistance, and that vascular delivery of HER2-targeted CAR-T cells induces endothelial recruitment, inflammatory cytokine and chemokine secretion, and effective antitumor activity, highlighting the platform utility for dissecting tumor-stroma crosstalk and evaluating targeted therapies.[208] Furthermore, the sequential addition of fibroblasts or patient-derived thyroid CAFs during tumor spheroid formation enhanced vascularization in several tumor cell lines and better CAR-T cell homing and killing efficiency under perfused conditions.[207] Interestingly, a vascularized human liver tumor spheroid model recapitulated hypoxic regions within the tumor and demonstrated how the presence of tumor vasculature limited the infiltration of engineered T-cells, without diminishing their cytotoxic capabilities.[26] Additionally, high-content multiplex imaging and spatial profiling of the TME allowed to characterize the immune context around the tumor and identify distinct vascular-mediated exhaustion patterns in the engineered T-cells. When it comes to brain, one of the significant obstacles in treating GBM is the BBB, which hinders the effective delivery of therapeutic agents to the tumor site. A BBB-tumor model was developed combining primary brain endothelial cells (BECs), astrocytes, and pericytes in a fibrin gel alongside a GBM spheroid from U87 cell line.[27] BBB presence reduced the U87 spheroid sensitivity to temozolomide in 3D microenvironment settings compared to 2D, potentially due to low pH supported by TME. This indicated that the 3D microenvironment and vasculature presence are sufficient to alter tumor behavior and drug sensitivity, allowing for a more accurate early-stage therapy validation. Proteomic analysis of the temozolomide-treated vascularized GBM model revealed several pathways involved in chemoresistance, with proteins overlapping with recurrent glioma patients, supporting the clinical relevance of this model. Furthermore, a vascularized lung spheroid model whith perfusable vessels sprouting towards the tumor[25] was developed to assess flow-directed sprouting and doxycycline treatment. By adjusting reservoir volumes, flow was directed from the human lung fibroblasts (hLF) channel to the hollow channel and sprouting consistently occurred opposite to flow, whereas without flow, HUVECs showed no directional preference, confirming that interstitial flow directs capillary sprouting orientation.[25] Notably, the degree of vascularization correlated with tumor aggressiveness. Another study, showed in 3D vascularized spheroid model that tumor spheroids from two cancer cell lines induced localized endothelial barrier disruption, resulting in leakier vessels within ≈1 mm of the spheroids,[206] while taxol treatment resulted in a reduced endothelial function and viability, alongside leakier and non-perfusable vessels at high concentrations of the drug (10×10^{-6} m), highlighting how chemotherapy also affect the vasculature functions. This vascular toxicity, often overlooked in efficacy studies, can be addressed in MPSs that uniquely enable this bidirectional interrogation, capturing both tumor killing and vascular disruption. This realization motivates investigation of vascular-directed strategies that intentionally modulate tumor angiogenesis and barrier function, an approach with a long clinical precedent, as discussed in the 'Targeting tumor vasculature as a therapeutic approach' section, but incompletely understood at the mechanistic level, now accessible through MPS interrogation.

Indirect tumor targeting *via* vasculature

The presence of vasculature within *in vitro* tumor models provides a key advantage for studying indirect tumor targeting using vascular disrupting or vasculature

normalizing agents. In MPSs, the self-assembly method allows targeting of vasculature at different stages of its development, enabling to elucidate the specific mechanisms by which these drugs influence vascular development, permeability, and tumor growth. Understanding these mechanisms will provide valuable insights into their efficacy and potential side effects. For example, an arterio-venous microfluidic platform was developed to model vascular permeability and maturation across two tumor types, consisting of two outer microfluidic channels, an arteriole (high pressure) and a venule (low pressure), connected by three tissue chambers filled with an ECM–cell mixture.[209] In this system, in 5–7 days, ECs self-assembled into a network that anastomosed with the channels, forming tight seals and establishing intraluminal flow while maintaining their leakiness, thereby providing a suitable platform for studying anti-angiogenic therapies. However, anti-VEGFR therapy (Apatinib and Vandetanib) was not effective in either breast or CRC whereas multi-targeted inhibitors (Linifanib, Cabozantinib targeting VEGFR/PDGFR/Tie2) induced vascular regression, demonstarting the possibility of testing indirect tumor targeting. This finding also demonstrates the platform capacity to predict clinical efficacy: as a similar combination of cabozantinib and nivolumab subsequently succeeded in RCC Phase III trials (NCT03141177),[223] validating the MPS-based predictions. The same model was used to study the metabolic changes in tumor post drug treatment with observation of a glycolytic hierarchy from tumor (highest) across ECs to stroma (lowest), highlighting the possibility of targeting the tumor metabolism with potentially low toxicity on vasculature. Furthermore, another study used primary RCC cells and normal-adjacent renal cortex cells to compare the effect of anti-angiogenic drugs. Microfluidic devices were fabricated by polymerizing collagen containing embedded tumor spheroids and sacrificial mandrel rods. After polymerisation, the rods were removed to create lumens within the matrix, which were subsequently seeded with HUVECs. Controlled, directional flow was then established through the lumens. This setup increased angiogenic factors, including ANGPTL4,[117] showing that tumor spheroid, but not healthy tissue, induced EC sprouting in a flow-directed microfluidic system, and VEGFR2-Fc blockade successfully suppressed sprouting.[117] A well known anti VEGFR2 therapy, ramucirumab, has been approved by FDA for treatment of several cancers, but not for RCC.[110–113] Vandetanib, also a VEGFR2 inhibitor[224] similarly lacks clinical validation in RCC, suggesting a potential new avenue to explore these drugs for RCC, evaluating their therapeutic efficacy within a vascularized tumor model. Interestingly, rather than using conventional inhibitors, in one study of vascularized HCC spheroids, VEGF or VEGFR RNAi-bound mesoporous silica nanoparticles (MSN) were delivered.[211] The device consisted of multiple channels: fibroblast, media 1, tumor, central, and EC channels. Angiogenic sprouting originated from the EC channel, traversing the central channel toward the tumor channel. Nanomedicine treatment was distributed through the central channel and did not affect EC viability or proliferation. A pronounced anti-angiogenic effect was observed when VEGFR RNAi MSN were applied, whereas VEGF RNAi MSN had no significant impact on angiogenic sprouting. Multiple control treatments, including MSN alone and free siRNA, showed no inhibitory effects, while sunitinib served as a positive control and significantly reduced sprouting. Overall, this platform effectively demonstrated VEGFR-targeted inhibition of angiogenesis, consistent with the central role of VEGF/VEGFR signaling in endothelial-driven sprouting, opening opportunities to translate this strategy into clinical applications.

While indirect targeting through the vasculature can modulate tumor growth, the success of vascular inhibition remains modest clinically, suggesting that isolated vascular targeting, like isolated tumor targeting, inadequately addresses the complexity of TME-mediated resistance and tumor–endothelial crosstalk, a limitation addressable through combinatorial strategies that simultaneously target both tumor cells and their vascular microenvironment.

Combinatorial strategies

Combinatorial strategies that target both tumor cells and vasculature have been successfully implemented in vascularized tumor-on-a-chip models. A vascularized GBM spheroid model in a microfluidic device achieved barrier-function mimicry incorporating human patient-derived xenograft GBM cells, embedded in a triculture BBB microvascular network formed by iPSC-derived ECs, pericytes, and astrocytes self-assembled in fibrin at defined ratios to generate perfusable vessels.[225] This platform demonstrated enhanced targeting and efficacy of AP2-functionalized, cisplatin-encapsulated nanoparticles that exploit low-density lipoprotein receptor-related protein 1 (LRP1) overexpression in GBM vessels to preferentially target tumor-proximal vasculature and improve drug delivery to the tumor. While liposomes, used in this study, are generally biocompatible and biodegradable, safety-related factors, such as particle size, surface charge, targeting ligand specificity to prevent off-target binding, and drug-payload neurotoxicity, require rigorous preclinical validation before clinical consideration for brain-targeted applications. A five-channel platform exemplifying vascular normalization, instead, demonstrated that reconstruction of perfusable esophageal tumor-on-a-chip model treated with prolyl hydroxylase (PHD) inhibitor increased vessel size while decreasing vessel permeability, leaving the normal vessels protected, enhancing cisplatin delivery and efficacy.[24] PHD inhibitors are primarily used as anemia-targeting drugs,[226] but these findings, pairing vascular normalization with direct chemotherapy, suggest a potential repurposing for tumor therapy. Additionally, a vascularized breast cancer platform capturing stromal remodeling employed multiple invasiveness-stratified tumor cell lines integrated into microfluidic devices containing ECs

and fibroblasts within a hydrogel, and investigated targeting IL-8 or stromal HA followed by trastuzumab or cetuximab delivery.[210] This platform demonstrated that more invasive tumor cell lines (SKBR3 and MDA-MB-468) induced stronger desmoplastic responses, including increased stromal HA deposition, vascular dysfunction, elevated interstitial fluid pressure, and impaired drug delivery, effects that were reversed by targeting IL-8 or stromal HA.[210] These findings highlight the need to incorporate therapeutic strategies that combine vascular targeting with anti-tumor treatments, extending beyond direct modulation of classical vascular regulators such as VEGFR2. Most of the vascularized tumor-on-chip methods consist of using two separate devices, one for generating tumor spheroids (either by low-attachment plates or hanging-drop method) and the other to integrate them with ECs (or EC spheroid) in a microfluidic device to promote vascularization and/or drug delivery, which leads to increased inter-exprimental variability. However, an integrated all-in-one platform consolidated spheroid generation and vascularization within a single device named "All-in-One-IMPACT".[212] The platform consists of self-assembled tumor spheroids directly on the chip using the hanging drop method in the cell culture channel. Tumor spheroids were assembled either with patient-derived cells or cell lines. Next, they injected a hydrogel containing ECs and fibroblasts, which integrates the spheroids into a 3D-patterned vascularized microenvironment along the culture channel. Treatment with bevacizumab, paclitaxel, and combination of both drugs reported highest tumor cell apoptosis with combinatorial approach. Bevacizumab with paclitaxel has already been approved as first line therapy for some cancers, including $HER2^+$ breast cancer,[227] highlighting potential translation for combinatorial vascular-cytotoxic strategies. For a summary of drug efficacy studies in vascularized tumor microfluidic models please refer to Table 2.

While both indirect (through vasculature) and combinatorial strategies (tumor + vasculature) have been explored in vascularized tumor-on-a-chip models, the field still has translational gaps to address. To bridge these gaps, validation efforts and comparative studies between different model strategies need to be designed following the efficacy results of therapies in the clinic. The diverse landscape of FDA-approved vascular-targeting therapies summarized in Table 1, offers a comprehensive benchmark set for such systematic evaluation. These approved regimens, spanning single-agent anti-angiogenic drugs to combinations with chemotherapy and immunotherapy across multiple cancer types, represent a clinically validated standard against which

Table 2 Therapy efficacy studies in vascularized tumoroid in microfluidic device

	Cancer model	Drug	Vascular cells	Outcome	Reference
Direct	Colorectal carcinoma	Leucovorin, oxaliplatin Anti-TGFβ (direct/indirect)	ECFC-EC + NHLF	More accurate	203
	Several types[a]	Paclitaxel	HUVEC + NHLF	No dose dependency under perfusion	216
	Lung cancer	Doxorubicin	HUVEC	Dose-dependency	222
	Glioblastoma	Temozolomide	BEC + AC + PC	Increased sensitivity Chemoresistance pathways increase	27
	Brain tumor	Taxol	HUVEC + NHLF	Impact on vasculature-leakiness	206
	Lung cancer	Doxorubicin	HUVEC + NHLF	Reduced tumor size	25
	Several types[b]	CAR-T cells	HUVEC + hLF	Increased T cell infiltration with higher dead cell density in tumor region	207
	Breast cancer	Anti-HER CAR-T cells	HUVEC + HDF	Decreased tumor growth	208
Indirect	Breast cancer, CRC	Anti-VEGFR + anti-PDGFR + anti-Tie2	ECFC-EC + NHLF	Multiple vascular-targeting regresses vasculature	209
	Clear cell renal cell carcinoma	Recombinant VEGFR2-Fc	HUVEC	Blocked tumor induced vascular sprouting	117
	Several types[c]	SIVEGFR/MSN miRNA	HUVEC + NHLF	Angiogenesis and tumor growth inhibition	211
Combinatorial	Breast cancer	Anti IL8/anti HA + trastuzumab/cetuximab	HUVEC + NHLF	Increased drug delivery and tumor cell death	210
	Esophageal carcinoma	PHD inhibitor + cisplatin	HUVEC + NHLF	Increased drug delivery and tumor cell death	24
	Several cell types and patient derived cells[d]	Taxol + bevacizumab	HUVEC + hLF	Highest apoptosis with combinatorial therapy	212

[a] Hepatocellular carcinoma, colorectal adenocarcinoma, mammary gland epithelial adenocarcinoma. [b] Renal cell carcinoma, small cell lung carcinoma, ovarian carcinoma. [c] Colorectal adenocarcinoma, adenocarcinomic alveolar basal epithelial cell, hepatocellular carcinoma, glioblastoma, renal cell epithelial adenocarcinoma, and mammary gland epithelial adenocarcinoma. [d] Colorectal adenocarcinoma, hepatocellular carcinoma, glioblastoma, alveolar basal epithelial cells adenocarcinoma.

current MPS technologies must be tested. Establishing whether vascularized MPSs can faithfully reproduce the differential responses observed across these diverse therapeutic regimens would not only validate these platforms for predictive preclinical testing but also enable mechanistic interrogation of resistance mechanisms.

Current limitations and technological bottlenecks in vascularized MPSs for therapy innovation

Although engineering methods have enabled the generation of increasingly complex vascularized tumor-on-chip platforms, several technical and translational bottlenecks still limit their routine use in therapy development. Reproducibility remains a major barrier to widespread adoption. Small variations in hydrogel composition or crosslinking can substantially alter angiogenic sprouting, vessel diameter, and permeability. Commonly used ECM-like hydrogels can produce variable outcomes, as vessel diameter and network structure are directly influenced by protein concentrations, which also affects gel stiffness. Outcomes in vascularization are also strongly influenced by cell type with endothelial and stromal cells from different donors, sources, or passages differing in their propensity to form stable, perfusable networks. Tissue specificity remains another unresolved challenge. ECs retain strong contextual identities, yet many vascularized MPS platforms rely on generic or non-matched endothelial sources that fail to reproduce organotypic phenotypes, permeability, or inflammatory responses. Achieving true tissue fidelity will require integrating patient-derived cells, specifically organ-specific ECs together with supporting stromal and immune components. This integration is key for enabling personalized modeling that captures patient-specific vascular phenotypes, tumor–endothelial interactions, and individualized therapeutic responses for functional precision medicine. Another limitation is the lack in most models of mural cells, such as smooth muscle cells and pericytes, which play a role in vessel stability, barrier integrity, and the ability to model vasomotion or pathological tone,[228] risking overlooking clinically relevant responses governed by mural–endothelial interactions. A material limitation, instead, is the widespread use of PDMS for device fabrication. PDMS is convenient to mold and optically clear, but it absorbs hydrophobic small molecules, including numerous chemotherapeutics and targeted agents, that may impact the actual dose experienced by cells.[229] In response, several groups and commercial platforms are moving towards thermoplastics or hybrid architectures that combine channels that minimize adsorption with gas-permeable materials for effective gas exchange. Furthermore, most vascularized MPSs operate as single devices or in small arrays, making them inherently low-throughput compared with conventional multi-well screening formats. This constrains their use to mechanistic studies and small drug panels rather than broad compound libraries or complex dosing schedules required for therapeutic screenings. However, perhaps the most pressing gap, is the lack of standardized metrics for assessing vascular generation and functionality. Ongoing efforts by experienced working groups are beginning to define metrics to characterize vessel functions (*e.g.*, permeability coefficients, perfusion rates, shear stress, and vessel maturation indices) and geometry (*e.g.* vascular diameter, vascular area coverage, branch lenght, and branch complexity) but these remain far from being widely implemented and standardized across vascularized tumor-on-chip studies. Standardization of experimental guidelines, detailing experimental workflows, quality-control checkpoints, and reporting criteria, will be essential for addressing the limitations in reproducibility and for accurately comparing vascularization strategies across platforms. These standardization efforts must balance the need for consistency with the flexibility required for continued innovation of these new technologies. In the context of clinical translation, there is a lack of systematic comparisons among MPSs and conventional models correlating with clinical outcomes. One study conducted a direct comparative validation showing that a vascularized microtumor model accurately recapitulated both tumor growth kinetics and chemotherapy response (FOLFOX regimen) observed in matched mouse xenografts for two CRC cell lines (HCT116 and SW480), whereas conventional 2D monolayer and 3D spheroid cultures showed significantly divergent responses.[203] However, without large-scale prospective correlation, it is difficult to quantify the true predictive value of these systems or to define when they provide added benefit over existing preclinical tools. To bridge this gap, future validation efforts must prioritize systematic co-trial studies where MPSs and standard preclinical platforms are directly compared with clinical outcomes across multiple therapeutic modalities to establish the predictive accuracy of these platforms. Furthermore, the establishment of open-access databases compiling MPS characterization data alongside clinical outcomes will be also useful to identify which techniques are best suited for specific therapeutic testing, enabling continuous model refinements and the development of predictive algorithms of therapy response.

Conclusion and forward-looking perspective

In this review we have presented vascularized tumor-on-chip platforms as human-relevant tissue models for therapeutic screening. We have detailed the fundamental role of tumor vasculature in driving therapeutic resistance through multiple interconnected mechanisms, and the need to accurately predict human-specific therapeutic responses. This has driven the development of MPSs that enable the generation of perfusable vascular networks for the functional

assessment of therapy efficacy, potentially shortening the timeline for discovering effective anti-cancer therapeutics. Advanced vascularized tumor platforms have been generated by leveraging bioprinting, microfluidic technologies, and dynamic perfusion systems. Patient-derived MPSs incorporating vascular, stromal, and immune components enable screening of treatment regimens based on biomarker feedback, advancing beyond purely genomic approaches toward functional precision medicine approaches, where MPSs may serve as a critical validation of therapeutic responses in the development pipeline. However, we have also discussed major constraints, including incomplete molecular heterogeneity representation compared to human tumors, and limited standardization and validation efforts. Several engineering approaches are beginning to address the listed technical limitations and bottlenecks, starting with parallelization efforts that include plate-integrated microfluidic systems, compatible with automated handling and imaging, that increase scalability and enable simultaneous testing of multiple dosages, demonstrating that higher-density layouts are technically feasible. However, further increasing the number of units per plate may comes at the expense of preserved physiological relevance, *e.g.*, too narrow gel channels cannot host cell aggregates >500 μm in diameter, which are representative of the diffusion-limited, gradient-rich tumor architecture observed *in vivo*. Emerging directions also include the integration of biomechanical and metabolic cues in the device. The use of dynamic hydrogels that can stiffen or degrade in response to cellular stimuli allows modeling of mechanotransduction-driven vascular remodeling, with implications for understanding hypoxia-mechanotransduction coupling.[180] Complementarily, vascularized MPSs integarted with metabolic and proteomic profiling could identify vascular-specific biomarkers to predict therapy response.

Another emerging direction regards the devlopemnt of multi-organ-on-chip platforms.[218] By modeling tumor tissues together with physiologically relevant distal organs, creating multi-organ tumor–vascular models, such as liver, kidney, or bone marrow, could provide a powerful framework for evaluating systemic responses and off-target toxicities of emerging anti-tumor agents.[8] Given that many vascular-targeting drugs exert unintended effects on healthy endothelium or non-tumor vascular beds, incorporating multi-organ architectures could substantially reduce the risk of advancing candidates that later fail due to unforeseen vascular or organ-specific toxicity, as the field moves toward regulatory acceptance and clinical translation. Indeed, the FDA's recent acceptance of organ-chip technologies into regulatory pathways[20] provides a clear encouragement for MPSs to become integral components of therapeutic development pipelines. Notably, on October 27, 2025, the FDA approved an investigational new drug (IND) application for a combination therapy comprising BAL0891, a dual TTK/PLK1 kinase inhibitor, and tislelizumab, an anti-PD-1 immune checkpoint inhibitor, based on efficacy data generated using a vascularized tumor immune microenvironment model (vTIME; Qureator Inc., USA).[230] This approval represents the first FDA IND decision in which efficacy data were derived exclusively from human vascularized organoid-based combination studies, without reliance on traditional animal proof-of-concept models. As regulatory bodies increasingly signal interest in MPS-based data, regulatory acceptance criteria, standardization protocols, and validation methodologies specific for MPSs are still under development since this process requires a coordinated approach, including scientists, industry partners, regulatory experts, and clinical investigators to develop and test standardized protocols.

Therefore, evaluating whether vascularized tumor-on-chip models are ready to drive therapeutic innovation reveals that we are approaching this goal. These platforms are transitioning from research tools to functionally human-relevant systems, demonstrating their emerging role in therapy development with potential to advance precision oncology through ongoing investment in technology development, standardization efforts and collaborative clinical validation studies.

Author contributions

Conceptualization: G. A. and I. P. conceptualized the review. Funding acquisition: G. A. and C. C. acquired the funding. Writing – original draft: G. A. and I. P. wrote the original draft. Writing – review & editing: G. A., I. P. and C. C. reviewed and edited the manuscript. All the authors approved the submitted manuscript.

Conflicts of interest

GA is inventor of the OrganiX™ plate, licensed to AIM Biotech Pte. Ltd.

Data availability

No primary research results, software or code have been included and no new data were generated or analyzed as part of this review.

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