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## Microfluidic models for adoptive cell-mediated cancer immunotherapies

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### Abstract

Current adoptive T cell therapies have shown promising results in clinical trials but need further development as an effective cancer treatment. Here, we discuss how 3D microfluidic tumour models mimicking the tumour microenvironment could help in testing T cell immunotherapies by assessing engineered T cells and identifying combinatorial therapy to improve therapeutic efficacy. We propose that 3D microfluidic systems can be used to screen different patient-specific treatments, thereby reducing the burden of *in vivo* testing and facilitating the rapid translation of successful T cell cancer immunotherapies to the clinic.

### Keywords

Microfluidics; adoptive cell therapy; T cell; cancer immunotherapy; 3D tumour model

### The era of cell-mediated cancer immunotherapy

In 1883, Elie Metchnikoff introduced the cellular theory of immunity, which states that host cells are the principal mediators of an immune response. This concept is the basis for the recent breakthrough of adoptive cell-mediated cancer immunotherapy (or adoptive cell therapy, ACT) currently in clinical trials. ACT relies on the isolation, genetic manipulation and reinfusion into patients of CD8<sup>+</sup> T cells expressing antigen receptors for tumours to

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mediate an effector function. However, the use of other lymphocytes as natural killer (NK) cells [1], dendritic cells, cytokine-induced killer (DC-CIK) [s1] cells [2] and CD4<sup>+</sup> helper T cells [3] could all be valid alternatives for cancer immunotherapy. We first review the present state of ACT for the personalised treatment of human cancers with its principal challenges to provide a context for potential microfluidics-based strategies for improvement at early stages of therapy design. We discuss *in vitro* 3D tumour models that recreate physiologically relevant tumour microenvironments (TMEs) in terms of cellular components and spatial organisation with obvious advantages over traditional 2D models. We focus our attention on microfluidic assays that hold promise for improving current strategies for cancer immunotherapy or helping to design new approaches. Finally, we highlight how microfluidic platforms can be used to test the efficacy of engineered T cells, and thereby accelerate the translation of T cell immunotherapy to the clinic.

### Strategies to engineer lymphocytes and approved clinical trials

ACT uses either endogenous tumour-reactive T cells called tumour-infiltrating lymphocytes (TILs) or host T cells engineered to express (i) highly specific T cell receptors (TCRs) for tumours or (ii) chimeric antigen receptors (CARs) derived from the single-chain variable fragments (scFv) of tumour-reactive monoclonal antibodies (mAbs). Additionally, an improved affinity and the inclusion of co-stimulatory molecules, such as those derived from inducible T cell co-stimulator [s2] (ICOS), 4-1BB and OX40 [s3][4,5] can help to improve treatment efficacy and prevent issues associated with immune tolerance. ON[s4]-switch CAR T cells enable, instead, precise control of the fate of the engineered T cells by small-molecule administration [6]. Techniques for engineering lymphocytes to express the desired receptor are classified as virus- or non-virus-based approaches. Virus-based methods include retrovirus [7], lentivirus [8] and lymphotropic herpes virus vectors [9]. Non-virus-based approaches rely, instead, on integration of plasmid DNA [4] or transgenes via electroporation or transposon-based systems [10], respectively. Other non-virus strategies include RNA-based electroporation and protein transduction of lymphocytes [5] for a transient expression of receptors of interest using mRNA or siRNA without genome alteration and with an expected reduced toxicity compared with DNA plasmid electroporation.

Despite the experimental nature of ACT, several strategies have recently been clinically approved for the treatment of liquid tumours. Clinical trials have yielded promising results for treating relapsed B cell acute lymphoblastic leukaemia (B-ALL) with T cells modified to express CD19 antibody as CAR infused into adult patients [11,12] or children [13,14]. Similarly, CD19-CAR T cells were infused into children and young adults with B-ALL or non-Hodgkin's lymphoma [15]. Clinical trials have also assessed ACT against solid tumours. For example, melanoma patients were treated with TIL ACT and high-dose interleukin (IL)-2 following non-myeloablative lympho-depleting conditioning [16] and the overall response rate was dramatically improved. A current Phase I clinical trial is administering HER2<sup>-</sup>[s5] CD28 CAR expressing T cells in advanced sarcoma patients and two other ongoing studies are using NY-ESO-1-specific T cells for patients with advanced synovial sarcoma/[s6] liposarcoma or a mixed population of sarcomas concurrently with palliative radiation therapy. A response was observed in four out of six synovial sarcoma

patients after lymphodepletion in a previous study with NY-ESO-1 genetically engineered lymphocytes [17].

Supporting methodologies to improve current outcomes focus on host conditioning regimens, IL-2 administration and immune checkpoint inhibition. The host conditioning regimen relies on chemotherapy aimed at depletion of T lymphocytes and could be administered before or after treatment with the purpose of (i) providing immunosuppression to prevent host inhibitory mechanisms and rejection of the infused T cells and (ii) supporting transferred T cell survival, proliferation and differentiation in a memory phenotype. Although the co-administration of IL-2 was used in current clinical trials [16–18], its role remains controversial.

Immune checkpoints have been found to play a crucial part in blocking T cells in the tumour microenvironment by activation of co-inhibitory pathways. Therefore, blocking antibodies have been approved by the FDA, such as nivolumab, lambrolizumab and pembrolizumab, targeting the programmed cell death protein 1 (PD-1); or ipilimumab against cytotoxic T-lymphocyte-associated protein 4 (CTLA-4). The therapeutic role of antibodies blocking CTLA-4 and PD-1/PD-L1 [s7] is discussed in a recent review [19] and additional immune checkpoints are under investigation. The strategy of combining immune checkpoint blockade with T cell therapy could lead to an enhancement of the efficacy of the transferred T cells in treating the cancer and improving clinical outcome. However, the side effects of blocking the natural immunosuppressive mechanisms must be taken into account and appropriately addressed.

## **Main limitations of current adoptive T cell therapies**

### **Poor TCR affinity, toxicity and host rejection**

Although various methodologies have been clinically approved, current therapies have met with limited success. These limitations include poor TCR affinity, humoral and cell-mediated immune responses, on- and off-target toxicity, poor T cell infiltration and impairment of T cell activity by the tumour microenvironment. Low TCR affinity can be overcome with improved design of the transducing vectors. Moreover, bispecific and trispecific T cells with increased affinity for tumours can be created by introduction of genes that encode TCRs and CARs while either retaining or not retaining the expression of endogenous TCR [20]. Toxicity, however, probably represents a major limitation of cell-mediated immunotherapy together with anaphylaxis and rejection of infused cells. Toxicity occurs when T cells attack normal tissue instead of tumours and it is mainly because of the targeting of normal tissues that express the same antigen or epitope of the tumour (on-target toxicity). This is especially apparent when foetal antigens that are typically highly expressed during development and merely downregulated (but not absent) in adulthood are targeted. In addition, low-level expression of tumour antigens in normal cells also makes it difficult to predict the potential on-target off-tumour toxicity. By contrast, when the normal tissues do not express the antigen or epitope of interest, off-target toxicities for TCR-expressing T cells can arise from an unexpected cross-reactivity with normal tissues or by mispairing of the TCR, although the latter has never occurred in clinical trials.

Additional toxicities are commonly manifested after CAR-T-cell administration as the cytokine release syndrome (CRS), the macrophage activation syndrome (MAS) and the tumour lysis syndrome, all mediated primarily by the excessive release of inflammatory cytokines. CRS has been observed, for example, in ACT targeting CD19 as a result of the great release of cytokines by CAR-expressing cells [21,22] but it can be effectively managed through the use of high-dose steroids or mAbs specific for inflammatory cytokines like IL-6 and tumour necrosis factor (TNF)- $\alpha$ .

### **Tumour microenvironment**

The use of T cell therapy for solid tumours is facing numerous challenges mainly because of the hostile tumour microenvironment. Once the engineered T cells reach the tumour, they need to (i) physically interact with cancer cells to engage the antigen and activate the cytotoxic pathway and (ii) proliferate to maintain a high number of active T cells intratumorally. The TME consists of supporting stromal cells, extracellular matrix (ECM), lymphatic vessels and blood vessels, and these contribute through different mechanisms to limit T cell interactions with the cancer cells (Figure 1a). In particular, T cell accumulation and proliferation at the tumour site is limited by the presence of stromal cells. Among stromal cells, cancer-associated fibroblasts (CAFs) often prevent T cell accumulation through secretion of a dense matrix (physical exclusion) and through CXCL12 production (chemical exclusion) [23]. The TME also contains transforming growth factor  $\beta$  (TGF- $\beta$ ) and IL-10, which are immunosuppressive, as well as regulatory T cells that act as a barrier preventing T cells from reaching the tumour [24].

Immune checkpoints and hypoxia also prevent T cells from approaching the cancer cells by suppressing their effector response. CTLA-4 accumulation on T cells after engagement inhibits T cell co-stimulation and activation. In addition, PD-L1 expressed by infiltrating monocytes, dendritic cells, macrophages and tumour cells [19,23] engages the PD-1 receptor on T cells to suppress the immune response and block cancer cell killing. Consequently, anti-CTLA-4 and anti-PD-L1 therapies have shown promise in overcoming the inhibitory signalling pathway [25]. Interestingly, T cell intratumoural migration is also negatively affected by the vasculature of the tumour microenvironment known to be leaky and highly irregular. Hence, normalisation of tumour vasculature by blocking vascular endothelial growth factor (VEGF) [26] or by targeting low-dose TNF- $\alpha$  [27] has been shown to improve T cell accumulation. Finally, regulatory T cells in the TME inhibit T cell function but modulation of the tumour stroma, by CAR T cells releasing IL-12, promoting a host immune response against the tumour [28] and resisting the regulatory-T-cell-mediated inhibition [29]. [s8]

### **Tumour models for T cell immunotherapy**

Owing to the vast range of possibilities to improve patient outcome through adaptive T cell cancer immunotherapy, and because of a widely variable patient response, it becomes crucial to perform preclinical tests of the developed strategies in a fast and reproducible way. One commonly used approach is to test engineered T cells for cancer immunotherapy using 2D standard tissue culture models and murine tumour models (Figure 1b) [4,6,7,9]. However,

despite advances in these methods, leading to therapies in clinical trials, substantial limitations remain. For example, murine models are complex, expensive and time-consuming and 2D *in vitro* tumour models, although simpler and faster, fail to mimic the native 3D tissue structure or the surrounding tumour microenvironment. To overcome some of these limitations, 3D *in vitro* models are being developed that offer the capability to investigate the role of the TME in a more physiologically realistic 3D condition compared with standard 2D *in vitro* assays [30]. TME architecture presents structural, mechanical and chemical cues that affect the expression of essential molecules such as integrins and matrix metalloproteinases that activate signalling pathways to regulate cell adhesion, EMT, migration and differentiation; and, although a degree of simplification is probably needed, it can be argued that standard 2D systems missing the features of 3D tissue organisation that play an essential part in cell growth, function, differentiation and interaction lack sufficient physiological relevance. Additionally, 3D *in vitro* models overcome the drawbacks of murine models in terms of scalability, cost and ease of use. Isolated cancer cells co-cultured in 3D hydrogels or scaffolds represent a way to study the interactions with lymphocytes [31] in a condition more comparable to the *in vivo* microenvironment. Thus, 3D *in vitro* tumour models allowing more physiologically relevant insights into tumour mass and immune system interactions in the TME could represent a more valuable assay for improved preclinical tests for immunotherapies against solid tumours in combination with or as an alternative to conventional methods. It has also been shown that lymphocytes capable of effectively targeting cancer cells cultured in 2D can fail to kill 3D tumours because of their different immune escape mechanisms [32].

A major approach to creating 3D *in vitro* tumour models for cancer research is to use spheroids or cell aggregates. Hanging-drop techniques are frequently used (Figure 1b), where gravity causes cells to aggregate inside a drop of media [33,34]. Low-adhesion microwells [35,36], encapsulation [37] and rotating-wall [39] vessel bioreactors are also commonly used for aggregate formation [38]. Aggregates can then be suspended in a hydrogel matrix to mimic the 3D microenvironment. This approach can also be used to produce a hypoxic environment, simulating the natural oxygen gradient that forms within tumours as a result of restricted diffusion into the spheroid core. Note that oxygen concentration gradients, although intrinsic to 3D assays, have not been reproduced in 2D culture systems. An alternative to multicellular aggregates from cell lines that preserves tissue heterogeneity is the use of patient-derived organotypic tumour explants as a 3D *in vitro* tumour model [39].

One example of 3D *in vitro* tumour models applied to study T cell immunotherapy was developed by Busse *et al.* to investigate the immunological properties in terms of tumour-associated antigen (TAA) and antigen-presenting molecules of tumour spheres of different cancer cell lines [40]. They found the 3D tumour spheres to be defective in antigen presentation because of a downregulated human leukocyte antigen [HLA] expression. As a result, 3D tumour spheres were not efficiently targeted by T cells in contrast to their corresponding 2D cultured cells. Similarly, Feder-Mangus *et al.* showed that 3D melanoma spheres more effectively inhibited TAA recognition by T cell compared with 2D cultures [41]. In a different approach, Dangles *et al.* showed that the different cell architecture between 2D and 3D cultures of two bladder cancer cell lines played a major part in TIL

activation by measuring their cytokine release [42]. 3D porous chitosan–alginate scaffolds were instead developed by Florczyk and colleagues to study tumour cell interaction with human peripheral blood lymphocytes [43]. Interestingly, Phan-Lai *et al.* showed that fibroblasts cultured in a 3D scaffold modulated the effect of specific T lymphocytes on breast cancer cells by co-seeding cancer cells, T lymphocytes and fibroblasts simultaneously [44]. Taken together, these findings confirm that 2D monolayers only partially mimic the *in vivo* condition and support the importance of testing T cell immunotherapeutic strategies in 3D tumour models.

## Potential for 3D microfluidic tumour models as preclinical assessment for cancer immunotherapy

In addition to the enhanced capabilities of *in vitro* 3D models to mimic the physical and spatial characteristics of a tumour microenvironment, microfluidic platforms offer other advantages [45]:

- Owing to their size, they provide savings through a reduction in the amount of biological materials and reagents needed.
- Glass and polydimethylsiloxane (PDMS), commonly used for microfluidic device fabrication, are transparent – representing optimal substrates to perform imaging with standard microscopy techniques.
- Microfluidic devices enable more-precise control of spatiotemporal dynamics and fluids in the TME providing the researchers with the possibility to impose molecular gradient profiles and different oxygen concentration levels.
- Microfluidic systems can draw upon many standardised quantitative assays to analyse biological phenomena, offer the potential for improved cell labelling and imaging techniques and the possibility to perform biochemical analysis on cells cultured in hydrogels without compromising the results from the matrix degradation procedure.

The contribution of microfluidics to cancer immunotherapy is limited thus far, focusing largely on techniques to prepare or isolate cells that could be potentially employed in a cancer immunotherapy strategy. Microfluidic devices show enormous promise, however, in studying underlining mechanisms that are difficult to observe and examine in animal models such as cell adhesion, cell migration and cell–cell interactions. For example, 2D chemotaxis of T cells to CCL21 and CCL19 [46] or chemotaxis of cancer cells and immune cells in 3D collagen gel [47] have been the focus of investigations in microfluidic devices. 2D cancer–immune-cell interaction has also been investigated by Mattei *et al.* [s11] who developed a microfluidic device to investigate the *in vivo* observation on the role of the immune system in melanoma growth further [48]. Specifically, the interactions between wild-type splenocytes and melanoma cells in 2D decreased tumour cell migration; their interaction was reduced when a transcription factor mediating immune responses [interferon regulatory factor (IRF)-8] was knocked-out resulting in higher tumour invasiveness. Another 2D

tumour model in a microfluidic device was developed to investigate the migration speed of lung cancer cells influenced by macrophages and myofibroblasts [49].

Existing microfluidic 3D tumour models, in addition to 2D microfluidic platforms, can replicate an organotypic TME as reported by Bersini *et al.* where the 3D model to study organ-specific extravasation included breast cancer cells, osteo-differentiated bone-marrow-derived mesenchymal stem cells and endothelial cells [50]. Similarly, a 3D bone microenvironment has been recreated to study prostate cancer cell metastasis [51] or the interactions between bone marrow cells and osteoblasts [52]. Moreover, supporting stroma cells in the TME have been considered to investigate the progression of pancreatic ductal adenocarcinoma [53] or ductal carcinoma *in situ* [54]. Most recently, Bischel *et al.* have co-cultured mammary epithelial cells, ductal carcinoma *in situ* (DCIS) cells and human mammary fibroblasts (HMFs) in a 3D hydrogel to study DCIS cell progression [55]. Primary adipose-derived stromal cells were instead co-cultured in a microfluidic system with a 3D breast cancer tissue model for testing nanoparticle-based photodynamic therapy [56]. Importantly, specific mechanisms at each level of the metastatic cascade, such as angiogenesis [57–61], EMT [62–65], intravasation [66–68] and extravasation [50,69–72] have been successfully modelled in 3D microfluidic platforms (Figure 2).

Here, we propose that novel or existing 3D pathophysiological models within microfluidic platforms could represent an immunogenic tumour microenvironment more relevant than 2D culture to help preclinical screening of T cell therapies (Figure 1). [s12]In these models, the possibility to modulate the 3D environment in terms of cell type, cellular architecture, ECM, administered cytokines and oxygen level offer the possibility to generate physiologically relevant conditions and to compare the antitumour activity of T cells engineered with different approaches. The combination of an *in vitro* microfluidic system with existing *in vivo* preclinical studies could, in fact, reduce the ethical and economical burden of animal models helping to speed up translation of only ‘optimal’ therapeutic strategies to patients, alleviating the unwanted clinical side effects. Specifically, 3D microfluidics models could provide benefits during the R&D step by aiding the design and optimisation of T cell therapeutic strategies and, then, during clinical trials they might offer a fast patient-specific preclinical test to fine-tune the therapy to be administered.

One example of a 3D microfluidic tumour model to explore the interaction between cancer and immune cells was reported by Bai *et al.* demonstrating the potential for a deeper investigation involving modelling, screening, evaluating and applying therapeutic approaches while reproducing the physiologically relevant microenvironment. Bai *et al.* investigated the role of macrophages on human lung adenocarcinoma (A549) aggregate dispersions. The microfluidic device integrated tumour cell aggregates in a 3D hydrogel co-cultured with different subtypes of human monocyte-derived macrophages and with an endothelial monolayer to recreate a 3D *in vitro* tumour microenvironment. The tumour-cell-macrophage interactions were tracked by time-lapse confocal microscopy. This microfluidic platform was key to identifying the role of each subtype of macrophages in tumour aggregate dispersion and demonstrated that M2a macrophages promote tumour dissemination by a contact-dependent mechanism through a  $\beta$ 2-integrin and ICAM-1 interaction [63].



These pre-existing 3D microfluidic tumour models could be applied to examine cancer immunotherapies offering the possibility to: (i) introduce multiple cell types in co-culture in a 3D ECM-like matrix for interaction studies; (ii) superimpose chemokine gradients for chemotaxis studies; (iii) visualise in real-time the cell interaction, migration and adhesion; and (iv) modulate the microenvironment with cytokine administration or hypoxic conditions to evaluate the part played by each[s13]. An easy manipulation of the tumour microenvironment to mimic the *in vivo* situation could enable rapidly screening multiple inflammatory conditions assessing the potential clinical outcome of different therapeutic strategies. As a result, 3D microfluidic tumour models will be useful in the screening of engineered T cell strategies in a more physiologically relevant TME and with control over the spatial cellular organisation.

To meet this need, our group recently developed a 3D multicellular microfluidic assay to analyse the function of TCR-engineered T cells [73,74]. In particular, T cells expressing hepatitis B virus (HBV)-specific TCR to target HBsAg-expressing HCC cells were produced [75,76] and the first proof-of-concept was demonstrated in a patient with end-stage HBV<sup>+</sup> HCC metastatic disease who had undergone a liver transplant [77]. TCR-engineered T cells are currently under investigation in a microfluidic platform. Importantly, this platform enables the testing of a variety of TCR-engineering strategies, supporting cytokine administration and/or changes in oxygen level similar to those typically found in the tumour microenvironment. Ultimately, microfluidic assays will provide insights into the mechanisms of T cell killing of tumour cells and their interactions with tumour cells or with other supporting cells present in the TME. The outcome from the microfluidics experiments will guide the improvement of T cell efficacy and/or the design of novel therapeutic strategies.

## Concluding remarks and future outlook

The key role of the tumour microenvironment in adoptive cell-mediated cancer immunotherapy is now widely recognised among immunologists and pathologists. Moreover, 3D microfluidic multicellular assays with real-time imaging and precise control over spatiotemporal parameters are poised to help elucidate the roles played by each cell type involved in this complex interaction. Although the utility of microfluidics is clear, there remain some barriers to overcome before we see widespread use in this application. PDMS, commonly used for microfluidic device fabrication, has limitations such as toxicity caused by the gradual release of non-cross-linked oligomers, or small, hydrophobic molecule absorption. The latter can be overcome by temperature-curing procedures and by coating the microchannel surfaces, or alternative biocompatible polymeric materials can be used for mass production. Other limitations of 3D microfluidics platforms, however, still exist; physiologically relevant matrix compositions and cell types need to be identified to mimic the native tumour microenvironment, and current microfluidic platforms need to be improved to better replicate the physiological complexity of the *in vivo* system. Despite these challenges, the potential value of microfluidics is evident. Because microfluidics systems can be seeded with patient-derived explants or cells, personalised immunotherapeutic strategies can be identified to fight the battle against cancer. This meets the rising demand for fast and reliable preclinical testing of engineered T cells to screen their

efficacy, compare different methodologies and to optimise therapeutic combinations of T cells and immunomodulatory agents that appear to be excellent candidates for therapy. Other synergistic combinations of T cells with radiotherapy or chemotherapy could be screened as well to identify the optimal response and to provide a rational and personalised therapy to cancer patients.

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## References

1. Childs RW, Carlsten M. Therapeutic approaches to enhance natural killer cell cytotoxicity against cancer: the force awakens. *Nat. Rev. Drug Discov.* 2015; 14:487–498. [PubMed: 26000725]
2. Lin T, et al. Clinical effects of autologous dendritic cells combined with cytokine-induced killer cells followed by chemotherapy in treating patients with advanced colorectal cancer: a prospective study. *Tumour Biol.* 2016; 37:4367–4372. [PubMed: 26499782]
3. Tran E, et al. Cancer immunotherapy based on mutation-specific CD4+ T cells in a patient with epithelial cancer. *Science.* 2014; 344:641–645. [PubMed: 24812403]
4. Finney HM, et al. Activation of resting human primary T cells with chimeric receptors: costimulation from CD28, inducible costimulator, CD134, and CD137 in series with signals from the TCR zeta chain. *J. Immunol.* 2004; 172:104–113. [PubMed: 14688315]
5. Beatty GL, et al. Mesothelin-specific chimeric antigen receptor mRNA-engineered T cells induce anti-tumor activity in solid malignancies. *Cancer Immunol. Res.* 2014; 2:112–120. [PubMed: 24579088]
6. Wu C-Y, et al. Remote control of therapeutic T cells through a small molecule-gated chimeric receptor. *Science.* 2015; 350:aab4077. [PubMed: 26405231]
7. Kochenderfer JN, et al. Eradication of B-lineage cells and regression of lymphoma in a patient treated with autologous T cells genetically engineered to recognize CD19. *Blood.* 2010; 116:4099–4102. [PubMed: 20668228]
8. Breckpot K, et al. Lentiviral vectors for cancer immunotherapy: transforming infectious particles into therapeutics. *Gene Therapy.* 2007; 14:847–862. [PubMed: 17361214]
9. Borenstein R, et al. Use of Amplicon-6 vectors derived from human herpesvirus 6 for efficient expression of membrane-associated and -secreted proteins in T Cells. *J. Virol.* 2004; 78:4730–4743. [PubMed: 15078955]
10. Singh H, et al. A new approach to gene therapy using Sleeping Beauty to genetically modify clinical-grade T cells to target CD19. *Immunol. Rev.* 2014; 257:181–190. [PubMed: 24329797]
11. Brentjens RJ, et al. CD19-targeted T cells rapidly induce molecular remissions in adults with chemotherapy-refractory acute lymphoblastic leukemia. *Sci. Transl. Med.* 2013; 5:177ra38.
12. Kochenderfer JN, et al. Chemotherapy-refractory diffuse large B-cell lymphoma and indolent B-cell malignancies can be effectively treated with autologous T cells expressing an anti-CD19 chimeric antigen receptor. *J. Clin. Oncol.* 2015; 33:540–549. [PubMed: 25154820]
13. Grupp SA, et al. Chimeric antigen receptor-modified T cells for acute lymphoid leukemia. *N. Eng. J. Med.* 2013; 368:1509–1518.
14. Maude SL, et al. Chimeric antigen receptor T cells for sustained remissions in leukemia. *N. Eng. J. Med.* 2014; 371:1507–1517.
15. Lee DW, et al. T cells expressing CD19 chimeric antigen receptors for acute lymphoblastic leukaemia in children and young adults: a phase 1 dose-escalation trial. *Lancet.* 2015; 385:517–528. [PubMed: 25319501]

16. Besser MJ, et al. Adoptive transfer of tumor-infiltrating lymphocytes in patients with metastatic melanoma: intent-to-treat analysis and efficacy after failure to prior immunotherapies. *Clin. Cancer Res.* 2013; 19:4792–4800. [PubMed: 23690483]
17. Burgess M, Tawbi H. Immunotherapeutic approaches to sarcoma. *Curr. Treat. Options Oncol.* 2015; 16:1–14.
18. Kochenderfer JN, et al. B-cell depletion and remissions of malignancy along with cytokine-associated toxicity in a clinical trial of anti-CD19 chimeric-antigen-receptor-transduced T cells. *Blood.* 2012; 119:2709–2720. [PubMed: 22160384]
19. Sharma P, Allison JP. The future of immune checkpoint therapy. *Science.* 2015; 348:56–61. [PubMed: 25838373]
20. Kalos M, June CH. Adoptive T cell transfer for cancer immunotherapy in the era of synthetic biology. *Immunity.* 2013; 39:49–60. [PubMed: 23890063]
21. Morrison C. Developers seek to finetune toxicity of T-cell therapies. *Nat. Biotechnol.* 2014; 32:1171–1172. [PubMed: 25489817]
22. Lee DW, et al. Current concepts in the diagnosis and management of cytokine release syndrome. *Blood.* 2014; 124:188–195. [PubMed: 24876563]
23. Joyce JA, Fearon DT. T cell exclusion, immune privilege, and the tumor microenvironment. *Science.* 2015; 348:74–80. [PubMed: 25838376]
24. Zhou G, Levitsky HI. Natural regulatory T cells and *de novo*-induced regulatory T cells contribute independently to tumor-specific tolerance. *J. Immunol.* 2007; 178:2155–2162. [PubMed: 17277120]
25. Smyth MJ, et al. Combination cancer immunotherapies tailored to the tumour microenvironment. *Nat. Rev. Clin. Oncol.* 2016; 13:143–158. [PubMed: 26598942]
26. Jain RK. Normalizing tumor microenvironment to treat cancer: bench to bedside to biomarkers. *J. Clin. Oncol.* 2013; 31:2205–2218. [PubMed: 23669226]
27. Johansson A, et al. Tumor-targeted TNF $\alpha$  stabilizes tumor vessels and enhances active immunotherapy. *Proc. Natl. Acad. Sci. U. S. A.* 2012; 109:7841–7846. [PubMed: 22547817]
28. Chmielewski M, et al. Of CARs and TRUCKs: chimeric antigen receptor (CAR) T cells engineered with an inducible cytokine to modulate the tumor stroma. *Immunol. Rev.* 2014; 257:83–90. [PubMed: 24329791]
29. Pegram HJ, et al. Tumor-targeted T cells modified to secrete IL-12 eradicate systemic tumors without need for prior conditioning. *Blood.* 2012; 119:4133–4141. [PubMed: 22354001]
30. Asghar W, et al. Engineering cancer microenvironments for *in vitro* 3-D tumor models. *Materials Today.* 2015; 18:539–553.
31. Augustine TN, et al. Establishment of a heterotypic 3D culture system to evaluate the interaction of TREG lymphocytes and NK cells with breast cancer. *J. Immunol. Methods.* 2015; 426:1–13. [PubMed: 26215372]
32. Feder-Mengus C, et al. New dimensions in tumor immunology: what does 3D culture reveal? *Trends Mol. Med.* 2008; 14:333–340. [PubMed: 18614399]
33. Kelm JM, et al. Method for generation of homogeneous multicellular tumor spheroids applicable to a wide variety of cell types. *Biotechnol. Bioeng.* 2003; 83:173–180. [PubMed: 12768623]
34. Raghavan S, et al. Formation of stable small cell number three-dimensional ovarian cancer spheroids using hanging drop arrays for preclinical drug sensitivity assays. *Gynecol. Oncol.* 2015; 138:181–189. [PubMed: 25913133]
35. Tu T-Y, et al. Rapid prototyping of concave microwells for the formation of 3D multicellular cancer aggregates for drug screening. *Adv. Healthc. Mater.* 2014; 3:609–616. [PubMed: 23983140]
36. Chambers KF, et al. 3D Cultures of prostate cancer cells cultured in a novel high-throughput culture platform are more resistant to chemotherapeutics compared to cells cultured in monolayer. *PLoS One.* 2014; 9:e111029. [PubMed: 25380249]
37. Alessandri K, et al. Cellular capsules as a tool for multicellular spheroid production and for investigating the mechanics of tumor progression *in vitro*. *Proc. Natl. Acad. Sci. U. S. A.* 2013; 110:14843–14848. [PubMed: 23980147]

38. Chen R, et al. Screening candidate metastasis-associated genes in three-dimensional HCC spheroids with different metastasis potential. *Int. J. Clin. Exp. Pathol.* 2014; 7:2527–2535. [PubMed: 24966965]
39. Fong ELS, et al. A 3D *in vitro* model of patient-derived prostate cancer xenograft for controlled interrogation of *in vivo* tumor-stromal interactions. *Biomaterials.* 2015; 77:164–172. [PubMed: 26599623]
40. Busse A, et al. Characterization of small spheres derived from various solid tumor cell lines: are they suitable targets for T cells? *Clin. Exp. Metastasis.* 2013; 30:781–791. [PubMed: 23519726]
41. Feder-Mengus C, et al. Multiple mechanisms underlie defective recognition of melanoma cells cultured in three-dimensional architectures by antigen-specific cytotoxic T lymphocytes. *Br. J. Cancer.* 2007; 96:1072–1082. [PubMed: 17342088]
42. Dangles V, et al. Impact of human bladder cancer cell architecture on autologous T-lymphocyte activation. *Int. J. Cancer.* 2002; 98:51–56. [PubMed: 11857385]
43. Florczyk SJ, et al. 3D porous chitosan–alginate scaffolds: a new matrix for studying prostate cancer cell–lymphocyte interactions *in vitro*. *Adv. Healthc. Mater.* 2012; 1:590–599. [PubMed: 23184794]
44. Phan-Lai V, et al. Three-dimensional scaffolds to evaluate tumor associated fibroblast-mediated suppression of breast tumor specific T cells. *Biomacromolecules.* 2013; 14:1330–1337. [PubMed: 23517456]
45. Sackmann EK, et al. The present and future role of microfluidics in biomedical research. *Nature.* 2014; 507:181–189. [s14]. [PubMed: 24622198]
46. Nandagopal S, et al. Combinatorial guidance by CCR7 ligands for T lymphocytes migration in co-existing chemokine fields. *PLoS One.* 2011; 6:e18183. [PubMed: 21464944]
47. Zhang Y, et al. High-throughput 3D cell invasion chip enables accurate cancer metastatic assays. *J. Am. Chem. Soc.* 2014; 136:15257–15262. [PubMed: 25285914]
48. Kim SK, et al. Inflammatory mimetic microfluidic chip by immobilization of cell adhesion molecules for T cell adhesion. *The Analyst.* 2012; 137:4062–4068. [PubMed: 22822477]
49. Hsu T-H, et al. The migration speed of cancer cells influenced by macrophages and myofibroblasts co-cultured in a microfluidic chip. *Integr. Biol.* 2012; 4:177–182.
50. Bersini S, et al. A microfluidic 3D *in vitro* model for specificity of breast cancer metastasis to bone. *Biomaterials.* 2014; 35:2454–2461. [PubMed: 24388382]
51. Hsiao AY, et al. Microfluidic system for formation of PC-3 prostate cancer co-culture spheroids. *Biomaterials.* 2009; 30:3020–3027. [PubMed: 19304321]
52. Zhang W, et al. Patient-specific 3D microfluidic tissue model for multiple myeloma. *Tissue Eng. Part C Methods.* 2014; 20:683–670. [s15].
53. Drifka CR, et al. A bioengineered heterotypic stroma-cancer microenvironment model to study pancreatic ductal adenocarcinoma. *Lab Chip.* 2013; 13:3965–3975. [PubMed: 23959166]
54. Sung KE, et al. Understanding the impact of 2D and 3D fibroblast cultures on *in vitro* breast cancer models. *PLoS One.* 2013; 8:e76373. [PubMed: 24124550]
55. Bischel LL, et al. Microfluidic model of ductal carcinoma *in situ* with 3D, organotypic structure. *BMC Cancer.* 2015; 15:12. [PubMed: 25605670]
56. Yang Y, et al. Evaluation of photodynamic therapy efficiency using an *in vitro* three-dimensional microfluidic breast cancer tissue model. *Lab Chip.* 2015; 15:735–744. [PubMed: 25428803]
57. Chan JM, et al. Engineering of *in vitro* 3D capillary beds by self-directed angiogenic sprouting. *PLoS One.* 2012; 7:e50582. [PubMed: 23226527]
58. Kim C, et al. A quantitative microfluidic angiogenesis screen for studying anti-angiogenic therapeutic drugs. *Lab Chip.* 2015; 15:301–310. [PubMed: 25370780]
59. Buchan DS, et al. The effects of time and intensity of exercise on novel and established markers of CVD in adolescent youth. *Am. J. Hum. Biol.* 2011; 23:517–526. [PubMed: 21465614]
60. Verbridge SS, et al. Physicochemical regulation of endothelial sprouting in a 3D microfluidic angiogenesis model. *J. Biomed. Mater. Res. A.* 2013; 101:2948–2956. [PubMed: 23559519]

61. Lim SH, et al. Complementary effects of ciclopirox olamine, a prolyl hydroxylase inhibitor and sphingosine 1-phosphate on fibroblasts and endothelial cells in driving capillary sprouting. *Integr. Biol.* 2013; 5:1474–1484.
62. Aref AR, et al. Screening therapeutic EMT blocking agents in a three-dimensional microenvironment. *Integr. Biol.* 2013; 5:381–389.
63. Bai J, et al. Contact-dependent carcinoma aggregate dispersion by M2a macrophages via ICAM-1 and  $\beta 2$  integrin interactions. *Oncotarget.* 2015; 6:25295–25307. [PubMed: 26231039]
64. Bai J, et al. Identification of drugs as single agents or in combination to prevent carcinoma dissemination in a microfluidic 3D environment. *Oncotarget.* 2015; 6:36603–36614. [PubMed: 26474384]
65. Kuo C-T, et al. Modeling of cancer metastasis and drug resistance via biomimetic nano-cilia and microfluidics. *Biomaterials.* 2014; 35:1562–1571. [PubMed: 24269156]
66. Zervantonakis IK, et al. Three-dimensional microfluidic model for tumor cell intravasation and endothelial barrier function. *Proc. Natl. Acad. Sci. U. S. A.* 2012; 109:13515–13520. [PubMed: 22869695]
67. Lee H, et al. A microfluidic platform for quantitative analysis of cancer angiogenesis and intravasation. *Biomicrofluidics.* 2014; 8:054102. [PubMed: 25332739]
68. Pisano M, et al. An *in vitro* model of the tumor–lymphatic microenvironment with simultaneous transendothelial and luminal flows reveals mechanisms of flow enhanced invasion. *Integr. Biol.* 2015; 7:525–533.
69. Riahi R, et al. A microfluidic model for organ-specific extravasation of circulating tumor cells. *Biomicrofluidics.* 2014; 8:024103. [PubMed: 24803959]
70. Chen MB, et al. Mechanisms of tumor cell extravasation in an *in vitro* microvascular network platform. *Integr. Biol.* 2013; 5:1262–1271.
71. Zhang Q, et al. A microfluidic-based device for study of transendothelial invasion of tumor aggregates in realtime. *Lab Chip.* 2012; 12:2837–2842. [PubMed: 22648473]
72. Jeon JS, et al. Human 3D vascularized organotypic microfluidic assays to study breast cancer cell extravasation. *Proc. Natl. Acad. Sci. U. S. A.* 2015; 112:214–219. [PubMed: 25524628]
73. Pavesi A, et al. Using microfluidics to investigate tumor cell extravasation and T-cell immunotherapies. *Conf. Proc. IEEE Eng. Med. Biol. Soc.* 2015; 2015:1853–1856. [PubMed: 26736642]
74. Koh S, et al. G04: engineered HBV-specific T cells: disentangling antiviral from killing capacity. *J. Hepatol.* 2015; 62:S188.
75. Gehring AJ, et al. Engineering virus-specific T cells that target HBV infected hepatocytes and hepatocellular carcinoma cell lines. *J. Hepatol.* 2011; 55:103–110. [PubMed: 21145860]
76. Koh S, et al. A practical approach to immunotherapy of hepatocellular carcinoma using T cells redirected against hepatitis B virus. *Mol. Ther. Nucl. Acids.* 2013; 2:e114.
77. Qasim W, et al. Immunotherapy of HCC metastases with autologous T cell receptor redirected T cells, targeting HBsAg in a liver transplant patient. *J. Hepatol.* 2015; 62:486–491. [PubMed: 25308176]

**Highlights**

- 3D microfluidic tumour models are effective in screening for T cell therapeutic strategies
- 3D microfluidics can decrease the cost and time for optimising T cell immunotherapy
- Microfluidic devices enable patient-specific preclinical tests of T cell therapies

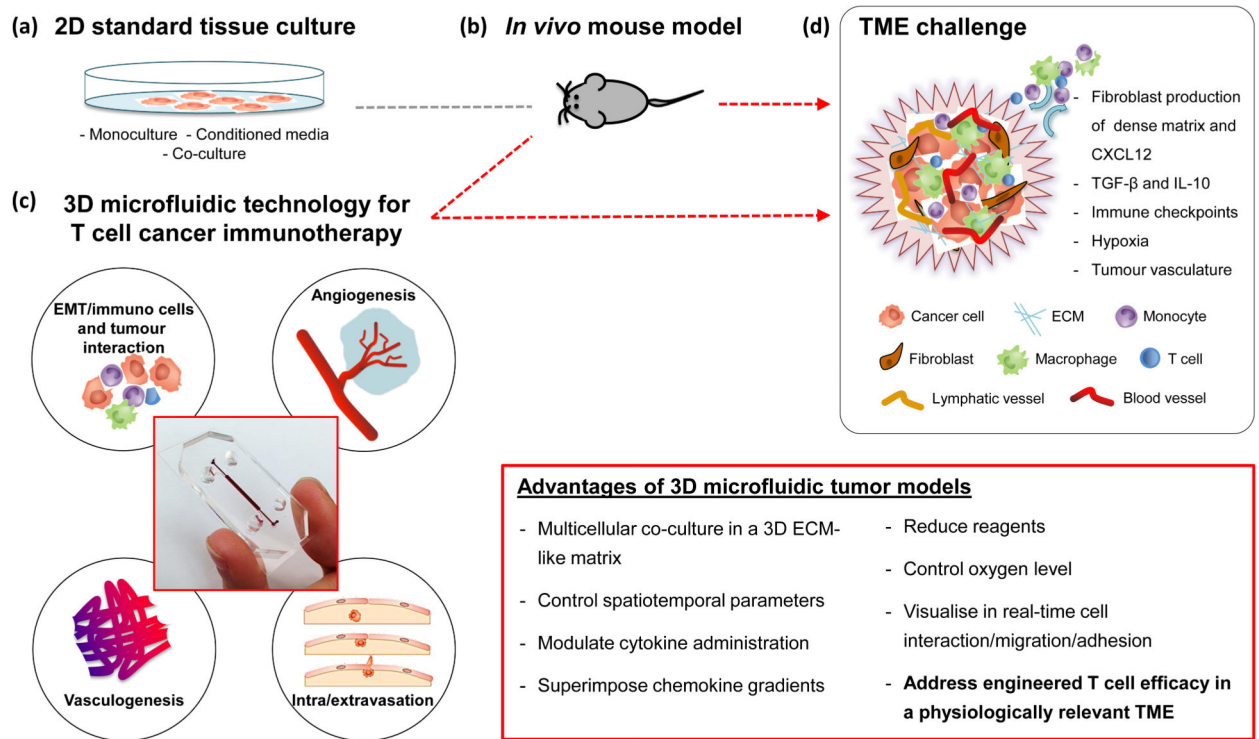
*Teaser:* Microfluidic platforms can be used for preclinical testing of engineered T cells to facilitate the translation of T cell immunotherapy to the clinic.

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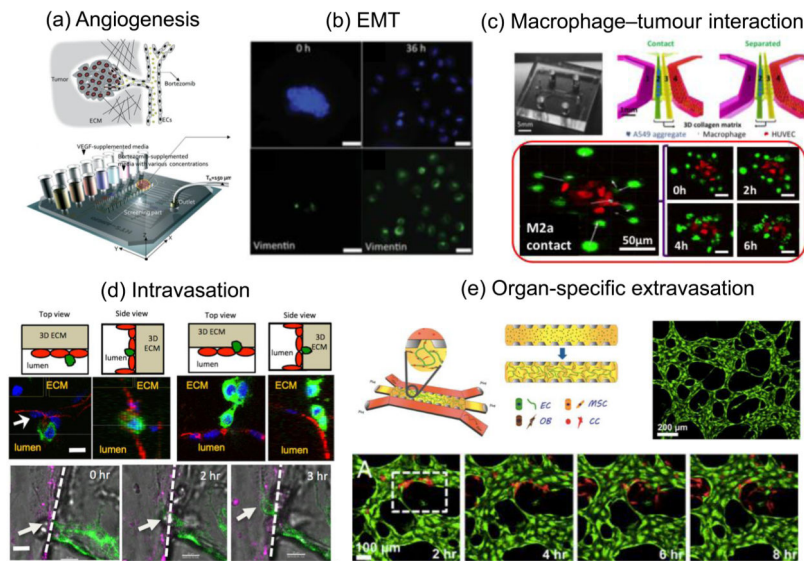
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**Figure 1.**

Pathways for preclinical screening of T cell cancer immunotherapies. Current preclinical models consist of **(a)** 2D standard tissue culture and **(b)** murine tumour models. **(c)** Existing or new 3D microfluidic platforms to study cell–cell interaction, angiogenesis, vasculogenesis, epithelial–mesenchymal transition (EMT), vasculogenesis, angiogenesis, intravasation and extravasation could be used as a substitute for 2D assays and even reduce the need for *in vivo* testing, providing an improved model for **(d)** the tumour microenvironment (TME). Abbreviations: CXCL12, stromal-cell-derived factor 1; TGF- $\beta$ , transforming growth factor  $\beta$ ; IL-10, interleukin 10.





**Figure 2.** Representative 3D microfluidic tumour models. **(a)** Angiogenesis: high-throughput microfluidic platform to screen antiangiogenic therapeutic drugs for cancer treatment [58]. **(b)** Epithelial–mesenchymal transition (EMT): immunostaining of vimentin, an EMT marker, on lung adenocarcinoma cell (A549) aggregates at 0 h and 36 h in co-culture with human umbilical vein endothelial cells (HUVECs). Blue: nuclei; green: vimentin [62] (scale bar: 30  $\mu\text{m}$ ). **(c)** Macrophage–tumour interaction: photograph and isometric view of the channel layout of the microfluidic device. Time-lapsed images of the M2a macrophage subtype [63]. **(d)** Intravasation: top and side views showing the device schematic with the endothelial monolayer, the tumour cells and the location of the 3D extracellular matrix (ECM). Confocal images, demonstrating intravasation of a single breast carcinoma cell (green) across the endothelium in red or magenta [66] (scale bar: 30  $\mu\text{m}$ ). **(e)** Extravasation: endothelial cells (ECs), mesenchymal stem cells (MSCs) and osteoblast-differentiated cells (OBs) seeded in the gel. ECs form vasculature, whereas MSCs and OBs create a bone-mimicking microenvironment for the extravasation of cancer cells (red) introduced into the vascular network (green) [72].