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Engineered Niches to Analyze Mechanisms of Metastasis and Guide Precision Medicine

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Abstract

Cancer metastasis poses a challenging problem both clinically and scientifically, as the stochastic nature of metastatic lesion formation introduces complexity for both early detection and the study of metastasis in preclinical models. Engineered metastatic niches represent an emerging approach to address this stochasticity by creating bioengineered sites where cancer can preferentially metastasize. As the engineered niche captures the earliest metastatic cells at a non-vital location, both non-invasive and biopsy-based monitoring of these sites can be performed routinely to detect metastasis early and monitor alterations in the forming metastatic niche. The engineered metastatic niche also provides a new platform technology that serves as a tunable site to molecularly dissect metastatic disease mechanisms. Ultimately, linking the engineered niches with advances in sensor development and synthetic biology can provide enabling tools for preclinical cancer models and fosters the potential to impact the future of clinical cancer care.

Introduction

Currently, cancer often goes undetected until identified by radiological studies, or the patient becomes symptomatic. While improvements in cancer screening, diagnostics, and treatments have decreased overall cancer mortality by approximately 30% since 1991 (1), over 90% of cancer deaths are due to the development of metastatic disease (2). Consequently, a critical need exists to improve metastasis-specific diagnostics and therapeutics. However, such research is limited by the stochastic nature of the initially microscopic metastatic lesions. A

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gap exists in current technologies for the early identification and analysis of metastasis in both preclinical models and clinical practice.

Synthetic metastatic niches represent an emerging technology for the mechanistic interrogation of the metastatic microenvironment. This tool can also have implications for the development of cancer diagnostics. The synthetic niches provide a pre-defined location for metastatic events, enabling mechanistic studies of dormancy and disease progression and, when implanted subcutaneously, act as readily accessible sites for clinical monitoring. Bioengineered metastatic niches support a platform for non-invasive imaging, cell collection, histological analysis, and biomarker identification that could be a rich source of information in clinical and preclinical settings (Figure 1). Importantly, synthetic metastatic niches reflect many aspects of metastatic disease observed in the native metastatic niche, e.g. the recruitment of an aggressive population of tumor cells similar to those in lung metastases (3).

In this review, we provide an analysis of engineered niches for mechanistic investigations of metastasis. Furthermore, we describe the potential of these niches to integrate within current clinical practice. The intersection between cancer biology and engineering is becoming ever more intertwined, and new engineered technologies will enable transformative insights and provide a means to screen novel therapeutics.

Bioengineered Niches for Metastatic Insights

Engineered metastatic niches represent a technological opportunity to study extravasation and colonization of metastatic sites (4). A century ago, Paget postulated the “Seed and Soil” hypothesis, in which he recognized that the distribution of metastatic cells to specific secondary organs was initiated by a favorable environment in those organs (5). Decades of work have helped to elucidate that the “soil” of these environments was altered by signaling from the primary tumor to create a pre-metastatic niche (PMN), and that mobilization and extravasation of bone-marrow derived cells played a pivotal role in this process (6). Secreted factors and exosomes from primary tumors enabled this mobilization and facilitated the formation of the PMN (7–11). The stochastic nature of pre-metastatic and metastatic lesions challenges the ability to analyze the dynamics and molecular mechanisms of localized sites at early time points in disease progression. Most studies isolate entire organs, or sections of organs with metastatic lesions, resulting in confounding data associated with the adjacent healthy tissues. Synthetic niches, to which tumor cells metastasize, provide tunability to enable enrichment or depletion of particular factors within a PMN, to examine the role of specific cell types in the microenvironment, tumor cell recruitment, and phenotypes of invading tumor, immune, and stromal cell populations. *In vitro* models have provided insights into the initial alterations at metastatic sites, with mechanisms subsequently validated *in vivo* (12–16). However, *in vitro* models cannot fully recapitulate the *in vivo* environment, and thus the synthetic niche may provide an alternative or complementary approach for studying mechanisms of metastasis.

The biological component of the engineered niche is comprised of infiltrating cells that contribute to the formation of connective tissue, a vascular network, and immune cells that

are recruited as part of the foreign body response to an implanted material (17). The presence of disease alters the immune system, and these alterations are reflected within the niche. The niche is formed by conditioning of immune cells from the vasculature, which ultimately attracts aggressive metastatic tumor cells (3,18–20). In a murine breast cancer model, tumor inoculation induced an influx of $Gr1^{hi}CD11b^{+}Ly6C^{-}$ myeloid derived suppressor cells, accompanied by decreases in $CD11c^{+}$ dendritic cells (DCs) and $F4/80^{+}CD11b^{+}$ macrophages (21). Single-cell RNA-seq performed on the immune populations in synthetic niches showed gene expression by multiple cell types that were associated with immunosuppressive, pro-tumor phenotypes. This pro-tumor environment was illustrated through the up-regulation of *S100a8* and *S100a9,* facilitating T cell suppression and colonization of metastatic cells in vital organs (22–24). In this context, the niche was formed by the progression of immune cell recruitment to a synthetic implant. This non-specific attraction of immune cells from the circulation to the engineered niche led to a suppressive environment formed within the niche when disease was present, which reflects the systemic disease dynamics (22). Furthermore, although engineered niches enable studies of metastasis, these engineered sites may not identically recapitulate metastatic sites. The synthetic niches contain cells and other factors not found in native metastatic lesions (e.g. connective tissue fibroblasts vs lung fibroblasts) that interact with cells recruited from the vasculature. Simultaneously, the tunability of these systems and ease of access positions the engineered metastatic niche as a provocative tool for studying cancer metastasis.

Analysis of metastatic tumor cells captured at a synthetic niche indicated that these cells possessed a migratory and metastatic phenotype, and a transcriptomic profile similar to the metastatic cells that homed to the lung (3). Furthermore, engineered metastatic niches redirect systemic immune cells, altering the primary tumor microenvironment (25). This attraction of tumor-conditioned immune cells and metastatic cells indicated that the engineered niche largely phenocopies a natural metastatic niche (natural MN). As described below, engineered metastatic niches have taken many forms based on the specific question being addressed. These engineered niches range from entirely synthetic implants, to cell-laden sites that require *ex vivo* culture prior to implantation. The relatively nascent stage of these systems implies that the possible design criteria for specific applications has significant potential, and this potential for plasticity is the focus of a recent review (26). Collectively, the engineered niche thus provides a unique platform to study the interactions between disseminated tumor cells, stroma, and immune cells (27).

Niche-driven insights into factors driving the metastatic cascade

Exosomes shed from the primary tumor are known to modify distal sites including lung tissue and bone marrow, to promote an environment conducive to metastasis (8). Building on this knowledge, incorporating exosomes into engineered MNs increased accumulation of metastatic cells, demonstrating that exosome-derived signals can influence MN function (28).

Extracellular matrix (ECM) proteins also contribute to the capture of metastatic cells, which has been confirmed using synthetic MNs. Polycaprolactone (PCL) scaffolds coated in collagen IV and fibronectin recruit more metastatic cells than uncoated scaffolds (29).

Alternatively, cancer-associated fibroblasts encapsulated in retrievable alginate beads produce ECM that enables capture of metastasizing peritoneal cells (30). These platforms identified the functional role of specific ECM components in cancer metastasis, with the potential for use as an engineered cell-capture therapy.

Secreted factors play complex roles in MN formation and are involved in tumor cell attraction, immunomodulation, and ECM deposition. Delivery of factors from the synthetic niches enabled investigation of the role of these proteins in metastatic progression. For example, erythropoietin (EPO) and CXCL12 loaded scaffolds demonstrated that the release of EPO enhanced melanoma cell recruitment, while CXCL12 release had no impact on cell recruitment (31). A separate report showed that CXCL12 delivery increased the metastasis of CXCR4+ melanoma cells to hyaluronan-based engineered niches. These disparate findings suggest that engineered niches can be harnessed to examine unique aspects of MN formation across cancer models (32). Another study implementing synthetic MNs reported that the presence of haptoglobin increased metastatic cell colonization (33). Localized lentiviral gene delivery also induced over-expression of specific cytokines at the implant. Lentiviral overexpression of CCL22, CCL2, CXCL12, and IL10 was performed from the engineered MNs. Gene delivery has been performed both prior to and following tumor cell inoculation. In both cases, delivered factors altered the immune cell composition of the implant. CCL22 increased metastatic tumor cell recruitment, while IL-10 reduced tumor cell recruitment (20,34). Importantly, these studies highlight the mechanistic insights associated with manipulating the synthetic niche. Changing one component, such as CCL2 for IL10, can lead to alterations within the environment that can obscure the contribution that a factor makes to a specific cellular response. For example, lentiviral expression of CXCL12 and CCL2 had no effect on tumor cell recruitment, but CXCL12 delivery increased immune cell recruitment (34). The ability to bias the abundance and phenotype of localized immune cells to screen tumor cell attractors illustrated the diverse capabilities of the engineered niche to dissect the metastatic cascade *in vivo*.

Hypoxia plays a large role in both primary tumors and metastatic sites as this environment promotes immune evasion, epithelial-mesenchymal transition, and the formation of the MN (35). Hypoxia stabilizes hypoxia inducible transcription factors (HIFs) that modulate many downstream pathways. Importantly, HIF signaling increases expression of lysyl oxidase (LOX) and similar proteins, which lead to alterations in ECM crosslinking and assembly. Elevated LOX expression and the concomitant changes in ECM are important steps in the formation of the PMN (35–38). While the role of hypoxia in cancer has largely focused on primary tumors, the impact of hypoxia on MN function has been analyzed. This study was performed by loading $CoCl_2$ into synthetic engineered niches, which served to stabilize HIF-1α, and to simulate some aspects of hypoxia. Scaffolds loaded with $CoCl_2$ mimicked many hypoxic responses for HUVECs *in vitro*. *In vivo*, the hypoxic niches exhibited increased vascularity compared to controls, yet did not influence tumor cell recruitment (39). The extent of HIF and LOX induced remodeling of ECM in the synthetic niche has yet to be determined *in vivo*, but engineered 3D microenvironments have shown cancer cell-directed ECM remodeling *in vitro* (40). Biomaterials have been developed with aligned paths for metastatic cell migration, indicating niche architecture and ECM-mediated alterations may significantly impact metastasis (41). Collectively, engineering the synthetic niches with

specific factors or architectures allows for the function of novel factors to be identified or investigated using several disease models.

Organ-Specific Synthetic Niches

Conditioning of the PMN has been proposed to account for specific cancer types metastasizing to specific organs (e.g. lung, brain, liver, and bone for breast cancer) (42). MN development in the lung is associated with distinctive tumor-conditioned stromal and immune cells (38,43). The ability to engineer the MN allows for the localized presentation of organ-specific ECM proteins, chemokines, and organ-derived cells to analyze their impact on metastatic events and cancer cell recruitment.

In the late 1990s, human bone was implanted into humanized SCID mice to study the metastasis of prostate cancer, identifying that human prostate cancer cells metastasized to human bone but not human lung and intestine fragments (44). This study motivated multiple biomaterial-based models to study metastasis and the leukemic niche within bone marrow (45–52). Biomaterial scaffolds have been developed to mimic bone microstructure, introduce osteogenic cues, and encapsulate osteoblastic cells (48,53–55). These approaches established humanized and tunable bone metastasis models (56). Furthermore, expression of human cytokines reduced tumor burden in hematochimeric mice compared to non-humanized mice (57). A more comprehensive review of tissue engineered bone for modeling malignancies can be found in Mcgovern et al (58).

Lung and liver-mimetic synthetic niches were developed by harnessing ECM derived from metastases of the respective tissues to coat microporous scaffolds (29). ECM from the organs of tumor bearing animals (i.e., diseased ECM) enhanced tumor cell colonization to the synthetic niche, which was further modified to verify myeloperoxidase as a key regulator of tumor cell colonization. Interestingly, the ECM from tumor-free animals did not promote tumor cell recruitment, consistent with the role of conditioning by the primary tumor in the function of natural and engineered niches. Lung tropic tumor cells proliferated at a higher rate on diseased lung ECM, demonstrating the organ and disease-specific influence of the MN. In particular, with further tissue engineering advances, the ability to create an array of organ-specific synthetic niches may be possible. The ability to create a bone, brain, lung, and liver mimic that could all be easily accessed will facilitate greater understanding of the homing of cells to these distinct microenvironments. We expect with improved understanding of the processes driving organotropism, therapies could be designed to interfere with these interactions.

Engineered mimics of metastatic sites are novel tools to enable the cellular and molecular mechanisms of metastatic cancer to be investigated. Studies examining factors driving the metastatic cascade generally have been conducted in breast, melanoma, ovarian, and colon cancer models. Studies of engineered niches and organ-specific metastasis have not typically spanned disease models, and thus the robustness of a scaffold in multiple models of metastatic disease is an area of active study. Furthermore, animal models of cancer, while widely used, are not fully representative of all aspects of human disease. Human cancer cells or patient derived xenografts may better recapitulate some aspects of human disease, yet are typically performed in immunocompromised mice, removing or minimizing the role of

adaptive immunity, an important facet of cancer metastasis. Several of the studies reported in this review have used both immunocompetent and immunocompromised mice, supporting that concept that the engineered niches are capturing key components of the metastatic cascade. Nevertheless, pairing of the engineered niches and metastatic models with humanized mice may help to accelerate discoveries most relevant to human metastasis.

Clinical Potential of Engineered Diagnostic Sites

Current Clinical Paradigm

The diagnosis of cancer involves a combination of analyses, including imaging, blood tests, and biopsies to identify tumor presence, blood-borne biomarkers, and tumor phenotype (Table 1). Imaging often provides the first indication of a cancer diagnosis and, once the diagnosis is confirmed, imaging is routinely used to measure changes in tumor size as the disease progresses. However, most imaging technologies can only detect tumors that are approximately 1 cm in size (59). The diagnostic and prognostic utility of such scans is limited, as tumor size is not a direct correlate for aggressiveness, heterogeneity, or response to certain therapies (59). Additionally, while small tumor size is often correlated with Stage I disease, a tumor of 1 cm may have up to 100 million cells (60). By this point, cancer cell heterogeneity can be extensive, with new mutations driving aggressive disease biology and treatment resistance. To this end, modalities that provide an earlier window of opportunity to detect and diagnose disease would provide a means to treat a patient when cancer cell heterogeneity is low, potentially optimizing treatment response. Blood tests and biomarker quantification allow for relatively easy and repeated analyses, yet many blood tests are not malignancy-specific and cannot be used in isolation (61). As an example, CA125 is used for monitoring ovarian cancer and has been effective for determining recurrence, though monitoring has not conclusively led to improved outcomes (61,62) . Markers for breast cancer recurrence (CA-27.29) are not widely applied, as their sensitivity and specificity have not been well established (63). Biopsy of primary tumors and subsequent histological analysis can be valuable for prognosis, defining stage and grade of disease (64). Histopathology of a tumor can identify disease subtype (e.g., receptor status in breast cancer) and aid in initial treatment selection, but this tissue resource cannot be monitored after tumor resection. Biopsy of distal sites can be used to confirm the presence of metastatic disease, and the corresponding disease subtype, which can then be compared to the primary tumor to track disease evolution. Thus, analysis of the MN could be used to inform and guide treatment decisions.

As next-generation sequencing and computational power increase, the reach of precision medicine into the field of oncology is expanding. Clinical decisions made with current tools are based on population-level results from large clinical trials, providing benefit for many. However, embedded in this paradigm is an empiric philosophy where a treatment is applied, the treatment response measured, and, if indicated, the patient is transitioned to next line therapy. This course can be long and arduous, and individual patient care can suffer at the expense of this treatment process. A critical need exists to move beyond this paradigm, as patients could benefit from a personalized medicine approach capable of detecting early systemic events, improving prognostic stratification, and monitoring response to therapy in

real-time. Furthermore, therapy selection is typically guided by information obtained from the primary tumor, which provides perspective on the initial disease biology, yet may not reflect metastatic sites. We propose that engineered niches provide the ideal platform to expand upon current diagnostic capability.

Biopsy for metastatic diagnostics

Metastatic tumor cell detection and analysis: Research into early metastatic diagnostics has focused largely on liquid biopsy as an alternative to clinical diagnostics and therapeutics based on the primary tumor. Most of this effort stems from the analysis of circulating tumor DNA (ctDNA), exosomes, or circulating tumor cells (CTCs) (65). Such technologies have been reviewed extensively (66–68). Elevated concentrations of tumor markers such as ctDNA and exosomes are found in the blood of cancer patients (68,69). ctDNA concentrations have been studied as surrogates for tumor burden in patients with metastatic disease, although some reports have found a lack of correlation (70,71). While, in theory, detecting ctDNA is an attractive target for diagnostic purposes, concerns associated with sampling have been identified. For example, breast cancers with a 1 cm tumor diameter are detectable by mammography (93%), but do not typically release enough ctDNA to be detected in a blood sample (72). The presence of ctDNA is even lower for a difficult to detect 0.5 cm diameter tumor. Cancer-specific exosome markers have shown prognostic value, including transforming growth factor beta 1 (TGF-β1) as a marker for ovarian cancer and epidermal growth factor receptor (EGFR) for glioblastoma (73). These technologies have also shown promise for monitoring the response to immunotherapies, as the primary tumor may initially gain volume due to the immune infiltrate (74). Of note, exosomes and mutated ctDNA can also be found in healthy individuals, decreasing the sensitivity and specificity in interpreting these findings (68,75). Conceivably an engineered diagnostic site could be leveraged for these diagnostic tests, and may also provide a sample enriched in factors of interest.

The direct analysis of metastatic cells has the potential to examine active disease and its evolution, along with the capacity to identify targets based on the biology of the cancer cell. At present, only one CTC device, CellSearch, is approved by the FDA (76). CellSearch implements antibodies for EpCAM and cytokeratin to isolate and identify CTCs, which are generally associated with poor prognosis (77). However, CellSearch and CTC isolation cannot distinguish cells with metastatic potential (typically 0.01% of CTCs), from those cells that will ultimately be biologically inert, thereby limiting the clinical relevance of CTC identification (78). Engineered diagnostic sites represent an alternative to bulk analysis of CTCs, because these sites have the capacity to accumulate an aggressive population of cancer cells and also decrease the systemic metastatic burden (20,21,28,79). Engineered niches capture cells prior to colonization of solid organs (20). Importantly, early detection with scaffold implantation, combined with surgical resection of the primary tumor, provided a survival advantage in pre-clinical models (22). Information collected from these niche-derived metastatic cells, which are phenotypically dissimilar from circulating CTCs, and cancer cells that make up the primary tumor (32), may be analyzed to direct specific therapies most relevant to the treatment of metastasis.

Immune cell detection and analysis: Elevations in specific immune cell populations in the blood have shown prognostic value in cancer management. Accordingly, the platelet-to-lymphocyte ratio (PLR) and neutrophil-to-lymphocyte ratio (NLR) were associated with poor prognosis, but little consensus on clinically meaningful values is available (80,81). In addition, high concentrations of regulatory T cells (Tregs) in the blood were identified as biomarkers for cancer, but the prognostic value of these T cells was dependent on the type of cancer analyzed. Tregs indicated poor prognosis for hepatocellular carcinoma and breast cancer (82,83), yet suggested a favorable prognosis for colorectal cancer and specific lymphomas (84,85). Such variability suggests that identifying changes in blood cell ratios and correlating these findings to cellular events in the MN may augment the function of the MN as a diagnostic.

Peripheral blood gene expression signatures have been developed to monitor cancer progression, particularly in lung cancer and breast cancer models. For lung cancer, studies with varying gene signatures have reported relatively high diagnostic accuracy (AUC = 0.81–0.98) (86–88). In the context of breast cancer, two separate studies developed gene signatures that distinguished breast cancer patients from healthy controls with accuracies of 80% and 77% (89,90). More recently, such signatures have been applied to predict disease relapse. One study established a signature that predicted post-surgical recurrence with an AUC of 0.88, where clinical parameters predict recurrence with an inferior AUC of 0.66 (91). Although these methods are promising for the early detection and management of cancer, they are limited by many of the same challenges as CTC analysis, namely that many of the changes observed do not necessarily correlate to metastatic burden.

Gene signatures derived from engineered metastatic sites monitor the immune dynamics of the local microenvironment to reflect disease progression and response to therapy. Profiling gene expression at the engineered niche, primarily in immune cells, was employed to generate a multivariate gene signature as a method to predict the likelihood of metastatic progression (22). Implementation of the signature separated healthy and early stage cancerous mice from animals with moderate or late stage disease with an accuracy of 92.3%, using supervised and unsupervised algorithms. As metastatic sites are conditioned by the immune system, measuring immune alterations at the engineered niche identified metastatic progression prior to the arrival of tumor cells. This strategy also demonstrated the capacity to monitor responsiveness to tumor resection based on analyzing immune cells at the niche (22), which may suggest the potential to monitor response to immunotherapies. Engineered diagnostic sites could be harnessed to molecularly stage metastatic disease, as an alternative to traditional staging methods based on imaging and histopathology of primary tumors. In a translational setting, a patient treated with the standard of therapy, yet at high-risk for distant recurrence, would undergo implantation of the scaffold at an easily accessible site (abdominal wall). This site would then be sampled and the tissue molecularly profiled and results stratified akin to OncotypeDX or MammaPrint, which could then inform clinician decisions regarding treatments at early time points in the metastatic setting. Molecular staging may impact the understanding of disease subtypes and serve as a powerful tool for precision medicine that can be harnessed prior to substantial distant disease burden (92).

Biomarker measurement and sensors

The utility of an engineered niche as a diagnostic will be enhanced with the development of non-invasive analytical technologies that identify disease-relevant features. Relative to native metastatic sites, the engineered niche represents a pre-defined location for analysis, which supports the use of advanced imaging technologies and the integration of sensors. Importantly, non-invasive monitoring supports longitudinal analysis of the niche, which can be coupled with discrete analysis by biopsy for more detailed molecular information. Finally, sensors can be designed to analyze the microenvironment of the niche, including cancer cells, immune cells, and other tissue-associated factors, reflective of the MN, which cannot be assessed by liquid biopsy.

Multiple imaging approaches have demonstrated the feasibility of detecting distinct structural features of the niche that reflect disease progression. Inverse spectroscopic optical coherence tomography (ISOCT) measures the micro and nanostructure of tissues, and when applied to engineered diagnostic sites detected structural changes sufficient to distinguish tumor bearing from tumor free animals at a pre-metastatic stage of disease (20,79). More recently, spectral ultrasound imaging (SUSI) was able to detect microenvironmental alterations in a synthetic niche during metastasis (18). The changes detected by ultrasound were determined to be cellular in nature, and may represent immune cell changes at the scaffold or the increased presence of endothelial cells in tumor bearing animals (18). Collectively, these studies highlight the opportunity for non-invasive disease detection that may be eventually translated to simple clinical or at-home monitoring.

Engineered diagnostic sites can also be designed to measure local concentrations of cancer-associated biomarkers that may not be detectable through liquid biopsy. In a recent study, a polymeric chamber with a permeable membrane was implanted in the resection bed of ovarian and testicular tumors (93). The chamber contained two populations of superparamagnetic nanoparticles tagged with antibodies for different components of the beta subunit of human chorionic gonadotrophin (hCG-β). The nanoparticles aggregated as hCG-β diffused into the device, which could be monitored with MRI through changes in the transverse relaxation time. These sensors are designed to serve as integrators of biomarker concentration. Measurement of integrated local expression of biomarkers over time may be useful for early detection of recurrence by MRI (93). This approach demonstrates the capacity for non-invasive *in vivo* monitoring of soluble cancer biomarkers and may enable common clinical imaging techniques to measure recurrence long before a detectable tumor forms.

Sensors that have been employed for monitoring primary tumors may ultimately be adapted to the engineered MN. An implantable NMR sensor and wireless reader were employed to monitor pH continuously over several days (94). Changes in relaxation time associated with decreased intratumoral and peritumoral pH were identified when compared to the pH from the contralateral control side of the study animals. Additionally, ongoing work on the Implantable Microsystems for Personalized Anti-Cancer Therapy (IMPACT) project at University of Edinburgh seeks to measure signals, such as pH and oxygen, using tumor implanted wireless sensors (95). As these technologies continue to develop, they may

provide unique insights into MN formation by providing measurements of dynamic changes in the microenvironment during niche development.

Responsive implants

Synthetic biology offers the opportunity to create diagnostic systems that respond to dynamic molecular signals *in vivo* (96,97). Macrophage cell lines have been engineered to express luciferase, for non-invasive visualization under the arginase-1 promoter, which was activated in the tumor microenvironment (96). Another approach harnessed a synthetic circuit in human embryonic kidney cells (98). Cells were encapsulated in alginate-poly(L-Lysine)-alginate beads to protect against xenogeneic rejection and were injected subcutaneously in mice to create a melanin biomedical tattoo that darkened upon the development of hypercalcemia. This innovative strategy utilized endogenous human proteins to create a persistent, *in vivo* synthetic circuit enabling the early detection of cancer progression. However, this detection strategy is nonspecific as many cancers do not induce hypercalcemia, while at the same time, other benign medical conditions are associated with this anomaly. Note that cell-based approaches may be challenging to translate, due to the regulatory hurdles, technical challenges, and expense associated with a cellular engineering and maintaining cell survival.

A number of technologies are emerging that may ultimately be translated toward the non-invasive or minimally-invasive monitoring of engineered niches. Analyte-responsive smart tattoos (99) for glucose sensing could be modified for tumor monitoring. Traditional sensors for glucose, pH, or other analytes could be adapted to the niches, and in the circumstance where a long-term implant is undesirable, degradable electronics could be used (100–102). Microneedle sampling is being developed for vaccine delivery, which penetrates only superficial layers of skin to sample the skin microenvironment and may prove to be a useful tool for minimally invasive monitoring of engineered MNs (103–105). Although these technologies have yet to be harnessed for oncology, future work will likely couple engineered MNs with these minimally invasive technologies to enable deeper analysis of alterations associated with metastasis and ultimately serve as precision tools in the diagnosis and prognosis of metastatic disease.

The pre-clinical data combined with non-invasive detection suggests that engineered niches have promise for use as a diagnostic for early detection, and for monitoring responses to therapy in the clinic. Multiple platforms have been able to capture metastatic cells with direct utility for monitoring disease progression, for which clinical studies have only recently been initiated (e.g., MTrap, NCT03085238). Engineered niches are fabricated from FDA-approved materials, and studies have been designed to establish that the synthetic niches do not pose risk to patients as they capture and retain tumor cells. Similar technologies have begun testing as a component of cancer vaccines in human clinical trials, and will be the first to examine the safety of these platforms (NCT01753089). The synthetic metastatic niches have been effective for monitoring disease recurrence and the response to therapy in pre-clinical models, yet the course of cancer progression in humans will require rigorous scrutiny.

Outlook

Although synthetic MNs are nascent in their development, this technology holds promise for preclinical studies of cancer metastasis and clinical translation for early detection, metastatic staging, and monitoring response to therapy (Figure 1). Synthetic niches reflect many aspects of native MNs, and these implants can be engineered to tune the metastatic microenvironment. Tunability enables controlled presentation of ECM or soluble proteins to molecularly dissect niche composition and function *in vivo*. Clinically, engineered diagnostic sites can provide unique, dynamic information about a metastatic niche that is not captured by the current clinical paradigm. Clinical technologies for the early detection and monitoring of metastatic cancer are a major area of interest, but many emerging technologies are limited by sampling issues. Synthetic niches enable an alternative and potentially enriched source that can feed into many existing analysis pipelines. For example, gene expression signatures can be used to monitor changes in synthetic niches similarly to their use in examining primary tumors for prognosis, and repeated analysis of the synthetic niche provides the opportunity for dynamic monitoring (22).

Development of engineered niches is occurring at a critical time in cancer therapy, with a recent expansion of treatment options for patients with metastatic disease. Implementation of these niches enables diagnosis when disease and mutational burden are low, presenting unprecedented opportunities for therapeutic intervention. Using engineered diagnostic sites as devices for the identification of early metastatic events, may improve therapeutic ability to forestall distant disease progression. Additionally, engineered niches also exploit the possibility for identification of novel markers, and the discovery of new targeted treatments. For example, vaccines could be directed towards neoantigens identified from metastatic cells found in the niche, or the molecular analysis of immunologic markers could help to direct the appropriate immunotherapy. Cells from the niche can also be converted to an experimental platform, akin to cell lines or organoids, to evaluate the effectiveness of emerging interventions. This system could be particularly useful for examining the metastatic microenvironment and factors associated with dormancy, stemness, and disease progression.

While additional work remains to fully realize the potential of these synthetic niches, tissue engineering and precision medicine are converging, and the resulting tools may have transformational benefits for science, medicine, and patient care. These niches are, in part, capturing systemic immunological changes, which are present in cancer, yet are also observed in autoimmune diseases, transplant rejection, and other conditions with prolonged, asymptomatic prodromes. In addition to enabling diagnosis and implementation of precision therapies, translation of this technology to humans could enable mechanistic insights into the early stages of human disease that have previously been elusive.

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References

1. Siegel RL, Miller KD, Jemal A. Cancer Statistics , 2019. CA Cancer J Clin. 2019;69:7–34. [PubMed: 30620402]
2. Seyfried TN, Huysentruyt LC. On the origin of cancer metastasis. Crit Rev Oncog. 2013;18:43–73. [PubMed: 23237552]
3. Bushnell GG, Hardas TP, Hart RM, Zhang Y, Oakes RS, Ronquist S, et al. Biomaterial Scaffolds Recruit an Aggressive Population of Metastatic Tumor Cells In Vivo. 2019;1–13.
4. Aguado BA, Bushnell GG, Rao SS, Jeruss JS, Shea LD. Engineering the pre-metastatic niche. Nat Biomed Eng. 2017;1.
5. Paget S The Distribution of Secondary Growths in Cancer of the Breast. Lancet. 1889;133:571–3.
6. Kaplan RN, Psaila B, Lyden D. Bone marrow cells in the “pre-metastatic niche”: Within bone and beyond. Cancer Metastasis Rev. 2006;25:521–9. [PubMed: 17186383]
7. Hiratsuka S, Watanabe A, Aburatani H, Maru Y. Tumour-mediated upregulation of chemoattractants and recruitment of myeloid cells predetermines lung metastasis. Nat Cell Biol. 2006;8:1369–75. [PubMed: 17128264]
8. Hoshino A, Costa-Silva B, Shen T- L, Rodrigues G, Hashimoto A, Tesic Mark M, et al. Tumour exosome integrins determine organotropic metastasis. Nature. 2015;527:329–35. [PubMed: 26524530]
9. Liu Y, Gu Y, Han Y, Zhang Q, Jiang Z, Zhang X, et al. Tumor Exosomal RNAs Promote Lung Pre-metastatic Niche Formation by Activating Alveolar Epithelial TLR3 to Recruit Neutrophils Article Tumor Exosomal RNAs Promote Lung Pre-metastatic Niche Formation by Activating Alveolar Epithelial TLR3 to Recruit Neutrophils. Cancer Cell; 2016;30:243–56. [PubMed: 27505671]
10. Kowanetz M, Wu X, Lee J, Tan M, Hagenbeek T, Qu X, et al. Granulocyte-colony stimulating factor promotes lung metastasis through mobilization of Ly6G+ Ly6C+ granulocytes. Proc Natl Acad Sci. 2010;107.
11. Chafe SC, Lou Y, Sceneay J, Vallejo M, Hamilton MJ, Dedhar S. Carbonic Anhydrase IX Promotes Myeloid- Derived Suppressor Cell Mobilization and Establishment of a Metastatic Niche by Stimulating G-CSF Production. Cancer Res. 2015;75:996–1009. [PubMed: 25623234]
12. Kusuma N, Anderson RL, Pouliot N. Laminin α5-derived peptides modulate the properties of metastatic breast tumour cells. Clin Exp Metastasis. 2011;28:909–21. [PubMed: 21938437]
13. Park CC, Zhang H, Pallavicini M, Gray JW, Baehner F, Park CJ, et al. B1 Integrin Inhibitory Antibody Induces Apoptosis of Breast Cancer Cells, Inhibits Growth, and Distinguishes Malignant From Normal Phenotype in Three Dimensional Cultures and in Vivo. Cancer Res. 2006;66:1526–35. [PubMed: 16452209]
14. Skardal A, Devarasetty M, Forsythe S, Atala A, Soker S. A Reductionist Metastasis-on-a-Chip Platform for In Vitro Tumor Progression Modeling and Drug Screening. Biotechnol Bioeng. 2016;113:2020–32. [PubMed: 26888480]
15. Kusuma N, Denoyer D, Eble JA, Redvers RP, Parker BS, Pelzer R, et al. Integrin-dependent response to laminin-511 regulates breast tumor cell invasion and metastasis. Int J Cancer. 2012;130:555–66. [PubMed: 21387294]
16. Hapach LA, Mosier JA, Wang W, Reinhart-king CA. Engineered models to parse apart the metastatic cascade. npj Precis Oncol; 2019.
17. Anderson JM, Rodriguez A, Chang DT. Foreign body reaction to biomaterials. Semin Immunol [Internet]. 2008;20:86–100.
18. Bushnell GG, Hong X, Hartfield RM, Zhang Y, Oakes RS, Rao SS, et al. High Frequency Spectral Ultrasound Imaging to Detect Metastasis in Implanted Biomaterial Scaffolds. Ann Biomed Eng. 2019.
19. Lee J, Li M, Milwid J, Dunham J, Vinegoni C, Gorbatov R, et al. Implantable microenvironments to attract hematopoietic stem/cancer cells. Proc Natl Acad Sci U S A. 2012;109:19638–43. [PubMed: 23150542]
20. Azarin SM, Yi J, Gower RM, Aguado BA, Sullivan ME, Goodman AG, et al. In vivo capture and label-free detection of early metastatic cells. Nat Commun. 2015;6.

21. Rao SS, Bushnell GG, Azarin SM, Spicer G, Aguado BA, Stoehr JR, et al. Enhanced survival with implantable scaffolds that capture metastatic breast cancer cells in vivo. Cancer Res. 2016;76:5209–18. [PubMed: 27635043]
22. Oakes RS, Bushnell GG, Orbach SM, Kandagatla P, Zhang Y, Morris AH, et al. Metastatic conditioning of myeloid cells at a subcutaneous synthetic niche reflects disease progression and predicts therapeutic outcomes. Cancer Res. 2019.
23. Lim SY, Yuzhalin AE, Gordon-Weeks AN, Muschel RJ. Tumor-infiltrating monocytes/macrophages promote tumor invasion and migration by upregulating S100A8 and S100A9 expression in cancer cells. Oncogene. 2016.
24. Hiratsuka S, Watanabe A, Sakurai Y, Akashi-Takamura S, Ishibashi S, Miyake K, et al. The S100A8-serum amyloid A3-TLR4 paracrine cascade establishes a pre-metastatic phase. Nat Cell Biol. 2008;10:1349–55. [PubMed: 18820689]
25. Aguado BA, Hartfield RM, Bushnell GG, Decker JT, Azarin SM, Nanavati D, et al. Biomaterial Scaffolds as Pre-metastatic Niche Mimics Systemically Alter the Primary Tumor and Tumor Microenvironment. Adv Healthc Mater. 2018;1700903:1–11.
26. Aguado BA, Bushnell GG, Rao SS, Jeruss JS, Shea LD. Engineering the pre-metastatic niche. Nat Biomed Eng. 2017;1:77.
27. Carpenter RA, Kwak JG, Peyton SR, Lee J. Implantable pre-metastatic niches for the study of the microenvironmental regulation of disseminated human tumour cells. Nat Biomed Eng. 2018;2:915–29. [PubMed: 30906645]
28. de la Fuente A, Alonso-Alconada L, Costa C, Cueva J, Garcia-Caballero T, Lopez-Lopez R, et al. M-Trap: Exosome-Based Capture of Tumor Cells as a New Technology in Peritoneal Metastasis. J Natl Cancer Inst. 2015;107.
29. Aguado BA, Caffe JR, Nanavati D, Rao SS, Bushnell GG, Azarin SM, et al. Extracellular matrix mediators of metastatic cell colonization characterized using scaffold mimics of the pre-metastatic niche. Acta Biomater. 2016;33:13–24. [PubMed: 26844426]
30. Vlieghere E De, Gremonprez F, Verset L, Mari L, Jones CJ, Craene B De, et al. Tumor-environment biomimetics delay peritoneal metastasis formation by deceiving and redirecting disseminated cancer cells. Biomaterials. 2015;54:148–57. [PubMed: 25907048]
31. Ko C, Wu L, Nair AM, Tsai Y, Lin VK, Tang L. The use of chemokine-releasing tissue engineering scaffolds in a model of inflammatory response-mediated melanoma cancer metastasis. Biomaterials. 2012;33:876–85. [PubMed: 22019117]
32. Ieranò C, Alterio CD, Giarra S, Napolitano M, Rea G, Portella L, et al. CXCL12 loaded-dermal filler captures CXCR4 expressing melanoma circulating tumor cells. Cell Death Dis. 2019.
33. Aguado BA, Wu JJ, Azarin SM, Nanavati D, Rao SS, Bushnell GG, et al. Secretome identification of immune cell factors mediating metastatic cell homing. Sci Rep. Nature Publishing Group; 2015;5:17566.
34. Bushnell GG, Rao SS, Hartfield RM, Zhang Y, Oakes RS, Jeruss JS, et al. Microporous scaffolds loaded with immunomodulatory lentivirus to study the contribution of immune cell populations to tumor cell recruitment in vivo. Biotechnol Bioeng. 2019.
35. Rankin EB, Giaccia AJ. Hypoxic control of metastasis. Science. 2016;352:57–63.
36. Erler JT, Bennewith KL, Nicolau M, Dornhöfer N, Kong C, Le Q- T, et al. Lysyl oxidase is essential for hypoxia-induced metastasis. Nature. 2006;440:1222–6. [PubMed: 16642001]
37. Cox TR, Rumney RMH, Schoof EM, Perryman L, Høye AM, Agrawal A, et al. The hypoxic cancer secretome induces pre-metastatic bone lesions through lysyl oxidase. Nature. 2015;522:106–10. [PubMed: 26017313]
38. Erler JT, Bennewith KL, Cox TR, Lang G, Bird D, Koong A, et al. Hypoxia-induced lysyl oxidase is a critical mediator of bone marrow cell recruitment to form the premetastatic niche. Cancer Cell. 2009;15:35–44. [PubMed: 19111879]
39. Lee HR, Pelaez F, Silbaugh AM, Leslie F, Racila E, Azarin SM. Biomaterial Platform To Establish a Hypoxic Metastatic Niche in Vivo. ACS Appl Bio Mater. American Chemical Society; 2019;2:1549–60.

40. Devarasetty M, Skardal A, Cowdrick K, Marini F, Soker S. Bioengineered Submucosal Organoids for In Vitro Modeling of Colorectal Cancer. Tissue Eng Part A. 2017;23:1026–41. [PubMed: 28922975]
41. Jain A, Betancur M, Patel GD, Valmikinathan CM, Mukhatyar VJ, Vakharia A, et al. Guiding intracortical brain tumour cells to an extracortical cytotoxic hydrogel using aligned polymeric nanofibres. Nat Mater. 2014;13:308–16. [PubMed: 24531400]
42. Minn AJ, Kang Y, Serganova I, Gupta GP, Giri DD, Doubrovin M, et al. Distinct organ-specific metastatic potential of individual breast cancer cells and primary tumors. J Clin Invest. 2005;115:44–55. [PubMed: 15630443]
43. Hiratsuka S, Goel S, Kamoun WS, Maru Y, Fukumura D, Duda DG, et al. Endothelial focal adhesion kinase mediates cancer cell homing to discrete regions of the lungs via E-selectin up-regulation. Proc Natl Acad Sci U S A. 2011;108:3725–30. [PubMed: 21321210]
44. Nemeth JA, Harb JF, Barroso U, He Z, Grignon DJ, Cher ML. Severe Combined Immunodeficient-hu Model of Human Prostate Cancer Metastasis to Human Bone. Cancer Res. 1999;1987–93. [PubMed: 10213511]
45. Xia T-S, Wang G-Z, Ding Q, Liu X-A, Zhou W-B, Zhang Y- F, et al. Bone metastasis in a novel breast cancer mouse model containing human breast and human bone. Breast Cancer Res Treat. 2012;471–86.
46. Seib FP, Berry JE, Shiozawa Y, Taichman RS, Kaplan DL. Tissue engineering a surrogate niche for metastatic cancer cells. Biomaterials. 2015;51:313–9. [PubMed: 25771021]
47. Vaiselbuh SR, Edelman M, Lipton JM, Ph D, Liu JM. Ectopic Human Mesenchymal Stem Cell-Coated Scaffolds in NOD / SCID Mice : An In Vivo Model of the Leukemia Niche. Tissue Eng Part C. 2010;16.
48. Lee J, Li M, Milwid J, Dunham J, Vinegoni C, Gorbatov R, et al. Implantable microenvironments to attract hematopoietic stem / cancer cells. Proc Natl Acad Sci. 2012;
49. Bersani F, Lee J, Yu M, Morris R, Desai R, Ramaswamy S. Bioengineered Implantable Scaffolds as a Tool to Study Stromal-Derived Factors in Metastatic Cancer Models. Cancer Res. 2014;7229–39. [PubMed: 25339351]
50. Holzapfel BM, Wagner F, Loessner D, Holzapfel NP, Thibaudeau L, Crawford R, et al. Species-specific homing mechanisms of human prostate cancer metastasis in tissue engineered bone. Biomaterials. 2014;35:4108–15. [PubMed: 24534484]
51. Moreau JE, Anderson K, Mauney JR, Nguyen T, Kaplan DL, Rosenblatt M. Tissue-Engineered Bone Serves as a Target for Metastasis of Human Breast Cancer in a Mouse Model. Cancer Res. 2007;10304–9. [PubMed: 17974972]
52. Qiao H, Tang T. Engineering 3D approaches to model the dynamic microenvironments of cancer bone metastasis. Bone Res. 2018.
53. Martine LC, Holzapfel BM, McGovern JA, Wagner F, Quent VM, Hesami P, et al. Engineering a humanized bone organ model in mice to study bone metastases. Nat Protoc. 2017;12:639–63. [PubMed: 28253234]
54. Moreau JE, Anderson K, Mauney JR, Nguyen T, Kaplan DL, Rosenblatt M. Tissue-engineered bone serves as a target for metastasis of human breast cancer in a mouse model. Cancer Res. 2007;67:10304–8. [PubMed: 17974972]
55. Thibaudeau L, Taubenberger A V, Holzapfel BM, Quent VM, Fuehrmann T, Hesami P, et al. A tissue-engineered humanized xenograft model of human breast cancer metastasis to bone. Dis Model Mech. 2014;7:299–309. [PubMed: 24713276]
56. Quent V, Taubenberger A, Reichert J, Martine L, Clements J, Hutmacher D, et al. A humanised tissue - engineered bone model allows species - specific breast cancer - related bone metastasis in vivo. J Tissue Eng Regen Med. 2018;494–504. [PubMed: 28714574]
57. Shafiee A, Mcgovern JA, Lahr CA, Meinert C, Moi D, Wagner F, et al. Immune system augmentation via humanization using stem / progenitor cells and bioengineering in a breast cancer model study. Int J Cancer. 2018;1482:1470–82.
58. Mcgovern JA, Griffin M, Hutmacher DW. Animal models for bone tissue engineering and modelling disease. Dis Model Mech. 2018;11.

59. Hodi FS, Ballinger M, Lyons B, Soria J, Nishino M, Tabernero J, et al. Immune-Modified Response Evaluation Criteria In Solid Tumors (imRECIST): Refining Guidelines to Assess the Clinical Benefit of Cancer Immunotherapy. J Clin Oncol. 2019;36.
60. Narod SA. Disappearing breast cancers. Curr Oncol. 2012;19:59–60. [PubMed: 22514490]
61. Egan TK. Monitoring Patients Undergoing Cancer Therapy 6 6 6. Lab Med. 2000;31:666–71.
62. Scholler N, Urban N. CA125 in ovarian cancer. Biomark Med. 2007;1:513–23. [PubMed: 20477371]
63. Rack B, Schindlbeck C, Juckstock J, Genss E- M, Hepp P, Lorenz R, et al. Prevalence of CA 27.29 in Primary Breast Cancer Patients before the Start of Systemic Treatment. Anticancer Res. 2010;30:1837–41. [PubMed: 20592389]
64. Rakha EA, Reis-filho JS, Baehner F, Dabbs DJ, Decker T, Eusebi V, et al. Breast cancer prognostic classification in the molecular era : the role of histological grade. Breast Cancer Res. 2010;
65. Pantel K, Alix-Panabieres C. Bone marrow as a reservoir for disseminated tumor cells: a special source for liquid biopsy in cancer patients. Bonekey Rep. 2014;3:584. [PubMed: 25419458]
66. Ma M, Zhu H, Zhang C, Sun X, Gao X, Chen G. "Liquid biopsy"-ctDNA detection with great potential and challenges. Ann Transl Med. 2015;3:235. [PubMed: 26539452]
67. Poudineh M, Sargent EH, Pantel K, Kelley SO. Profiling circulating tumour cells and other biomarkers of invasive cancers. Nat Biomed Eng. 2018;2:72–84. [PubMed: 31015625]
68. Soung YH, Ford S, Zhang V, Chung J. Exosomes in Cancer Diagnostics. Cancers (Basel). 2017;9.
69. Anker P, Mulcahy H, Chen XQ, Stroun M. Detection of circulating tumour DNA in the blood (plasma/serum) of cancer patients. Cancer Metastasis Rev. 1999;18:65–73. [PubMed: 10505546]
70. Xu RH, Wei W, Krawczyk M, Wang W, Luo H, Flagg K, et al. Circulating tumour DNA methylation markers for diagnosis and prognosis of hepatocellular carcinoma. Nat Mater. 2017;16:1155–61. [PubMed: 29035356]
71. Newman AM, Bratman S V, To J, Wynne JF, Eclov NC, Modlin LA, et al. An ultrasensitive method for quantitating circulating tumor DNA with broad patient coverage. Nat Med. 2014;20:548–54. [PubMed: 24705333]
72. Fiala C, Diamandis EP. Utility of circulating tumor DNA in cancer diagnostics with emphasis on early detection. BMC Med. BMC Medicine; 2018;16:1–10.
73. Szajnik M, Derbis M, Lach M, Patalas P, Michalak M, Drzewiecka H, et al. Exosomes in Plasma of Patients with Ovarian Carcinoma: Potential Biomarkers of Tumor Progression and Response to Therapy. Gynecol Obs. 2013.
74. Kurra V, Sullivan RJ, Gainor JF, Hodi FS, Gandhi L, Sadow CA, et al. Pseudoprogression in cancer immunotherapy: Rates, time course and patient outcomes. American Society of Clinical Oncology; 2016.
75. Chen AY, Braunstein GD, Anselmo MS, Jaboni JA, Viloria FT, Neidich JA, et al. Mutation detection with a liquid biopsy 96 mutation assay in cancer patients and healthy donors. Cancer Transl Med. 2017;3:39.
76. Riethdorf S, Fritsche H, Muller V, Rau T, Schindlbeck C, Rack B, et al. Detection of circulating tumor cells in peripheral blood of patients with metastatic breast cancer: a validation study of the CellSearch system. Clin Cancer Res. 2007;13:920–8. [PubMed: 17289886]
77. Miller MC, Doyle G V, Terstappen LW. Significance of Circulating Tumor Cells Detected by the CellSearch System in Patients with Metastatic Breast Colorectal and Prostate Cancer. J Oncol. 2010;2010:617421. [PubMed: 20016752]
78. Raimondi C, Gradilone A, Naso G, Cortesi E, Gazzaniga P. Clinical utility of circulating tumor cell counting through CellSearch((R)): the dilemma of a concept suspended in Limbo. Onco Targets Ther. 2014;7:619–25. [PubMed: 24790460]
79. Spicer GLC, Azarin SM, Yi J, Shea LD, Backman V. Detection of extracellular matrix modification in cancer models with inverse spectroscopic optical coherence tomography Detection of extracellular matrix modification in cancer models with inverse spectroscopic optical coherence tomography. Phys Med Biol. 2019;
80. Sylman JL, Mitrugno A, Atallah M, Tormoen GW, Shatzel JJ, Tassi Yunga S, et al. The Predictive Value of Inflammation-Related Peripheral Blood Measurements in Cancer Staging and Prognosis. Front Oncol. 2018;8:78. [PubMed: 29619344]

81. Mei Z, Shi L, Wang B, Yang J, Xiao Z, Du P, et al. Prognostic role of pretreatment blood neutrophil-to-lymphocyte ratio in advanced cancer survivors: A systematic review and meta-analysis of 66 cohort studies. Cancer Treat Rev. 2017;58:1–13. [PubMed: 28602879]
82. Zhou Y, Shao N, Aierken N, Xie C, Ye R, Qian X, et al. Prognostic value of tumor-infiltrating Foxp3+ regulatory T cells in patients with breast cancer: a meta-analysis. J Cancer. 2017;8:4098–105. [PubMed: 29187886]
83. Wang Y, Liu T, Tang W, Deng B, Chen Y, Zhu J, et al. Hepatocellular Carcinoma Cells Induce Regulatory T Cells and Lead to Poor Prognosis via Production of Transforming Growth Factor-beta1. Cell Physiol Biochem. 2016;38:306–18. [PubMed: 26799063]
84. Hu G, Li Z, Wang S. Tumor-infiltrating FoxP3(+) Tregs predict favorable outcome in colorectal cancer patients: A meta-analysis. Oncotarget. 2017;8:75361–71. [PubMed: 29088871]
85. Curiel TJ. Regulatory T cells and treatment of cancer. Curr Opin Immunol. 2008;20:241–6. [PubMed: 18508251]
86. Showe MK, Vachani A, Kossenkov AV, Yousef M, Nichols C, Nikonova E V, et al. Gene expression profiles in peripheral blood mononuclear cells can distinguish patients with non-small cell lung cancer from patients with nonmalignant lung disease. Cancer Res. 2009;69:9202–10. [PubMed: 19951989]
87. Zander T, Hofmann A, Staratschek-Jox A, Classen S, Debey-Pascher S, Maisel D, et al. Blood-based gene expression signatures in non-small cell lung cancer. Clin Cancer Res. 2011;17:3360–7. [PubMed: 21558400]
88. Rotunno M, Hu N, Su H, Wang C, Goldstein AM, Bergen AW, et al. A gene expression signature from peripheral whole blood for stage I lung adenocarcinoma. Cancer Prev Res. 2011;4:1599–608.
89. Aaroe J, Lindahl T, Dumeaux V, Saebo S, Tobin D, Hagen N, et al. Gene expression profiling of peripheral blood cells for early detection of breast cancer. Breast Cancer Res. 2010;12:R7. [PubMed: 20078854]
90. Sharma P, Sahni NS, Tibshirani R, Skaane P, Urdal P, Berghagen H, et al. Early detection of breast cancer based on gene-expression patterns in peripheral blood cells. Breast Cancer Res. 2005;7:R634–44. [PubMed: 16168108]
91. Carlsson A, Wingren C, Kristensson M, Rose C, Ferno M, Olsson H, et al. Molecular serum portraits in patients with primary breast cancer predict the development of distant metastases. Proc Natl Acad Sci U S A. 2011;108:14252–7. [PubMed: 21844363]
92. Bushnell GG, Wicha MS, Jeruss JS, Shea LD. Precision health for breast cancer metastasis: biomaterial scaffolds as an engineered metastatic niche to define, study, and monitor metastatic progression. Oncoscience. 2019;1.
93. Daniel KD, Kim GY, Vassiliou CC, Galindo M, Guimaraes AR, Weissleder R, et al. Implantable diagnostic device for cancer monitoring. Biosens Bioelectron. 2009;24:3252–7. [PubMed: 19442510]
94. Vassiliou CC, Liu VH, Cima MJ. Miniaturized, biopsy-implantable chemical sensor with wireless, magnetic resonance readout. Lab Chip. Royal Society of Chemistry; 2015;15.
95. Marland JRK, Blair EO, Flynn BW, González-Fernández E, Huang L, Kunkler IH, et al. Implantable Microsystems for Personalised Anticancer Therapy. In: Mitra S, Cumming DRS, editors. C Circuits Biol Sens Process Cham: Springer International Publishing; 2018 page 259–86.
96. Aalipour A, Chuang HY, Murty S, D'Souza AL, Park S min, Gulati GS, et al. Engineered immune cells as highly sensitive cancer diagnostics. Nat Biotechnol. 2019;37:531–9. [PubMed: 30886438]
97. Slomovic S, Pardee K, Collins JJ. Synthetic biology devices for in vitro and in vivo diagnostics. Proc Natl Acad Sci U S A. 2015;112:14429–35. [PubMed: 26598662]
98. Tastanova A, Folcher M, Müller M, Camenisch G, Ponti A, Horn T, et al. Synthetic biology-based cellular biomedical tattoo for detection of hypercalcemia associated with cancer. Sci Transl Med. 2018;10:1–12.
99. Heo YJ, Takeuchi S. Towards Smart Tattoos : Implantable Biosensors for Continuous Glucose Monitoring. Adv Healthc Mater. 2013;43–56. [PubMed: 23184404]
100. Koo J, MacEwan MR, Kang SK, Won SM, Stephen M, Gamble P, et al. Wireless bioresorbable electronic system enables sustained nonpharmacological neuroregenerative therapy. Nat Med. Springer US; 2018;24:1830–6.

101. Shin J, Yan Y, Bai W, Xue Y, Gamble P, Tian L, et al. Bioresorbable pressure sensors protected with thermally grown silicon dioxide for the monitoring of chronic diseases and healing processes. Nat Biomed Eng. Springer US; 2019;3:37–46.

102. Li C, Guo C, Fitzpatrick V, Ibrahim A, Zwierstra MJ, Hanna P, et al. Design of biodegradable, implantable devices towards clinical translation Nat Rev Mater. Springer US; 2019;

103. Al Sulaiman D, Chang JYH, Bennett NR, Topouzi H, Higgins CA, Irvine DJ, et al. Hydrogel-Coated Microneedle Arrays for Minimally Invasive Sampling and Sensing of Specific Circulating Nucleic Acids from Skin Interstitial Fluid. ACS Nano. American Chemical Society; 2019;13:9620–8.

104. Mandal A, Boopathy AV., Lam LKW, Moynihan KD, Welch ME, Bennett NR, et al. Cell and fluid sampling microneedle patches for monitoring skin-resident immunity. Sci Transl Med. 2018;10.

105. Samant PP, Prausnitz MR. Mechanisms of sampling interstitial fluid from skin using a microneedle patch. Proc Natl Acad Sci U S A. 2018;115:4583–8. [PubMed: 29666252]

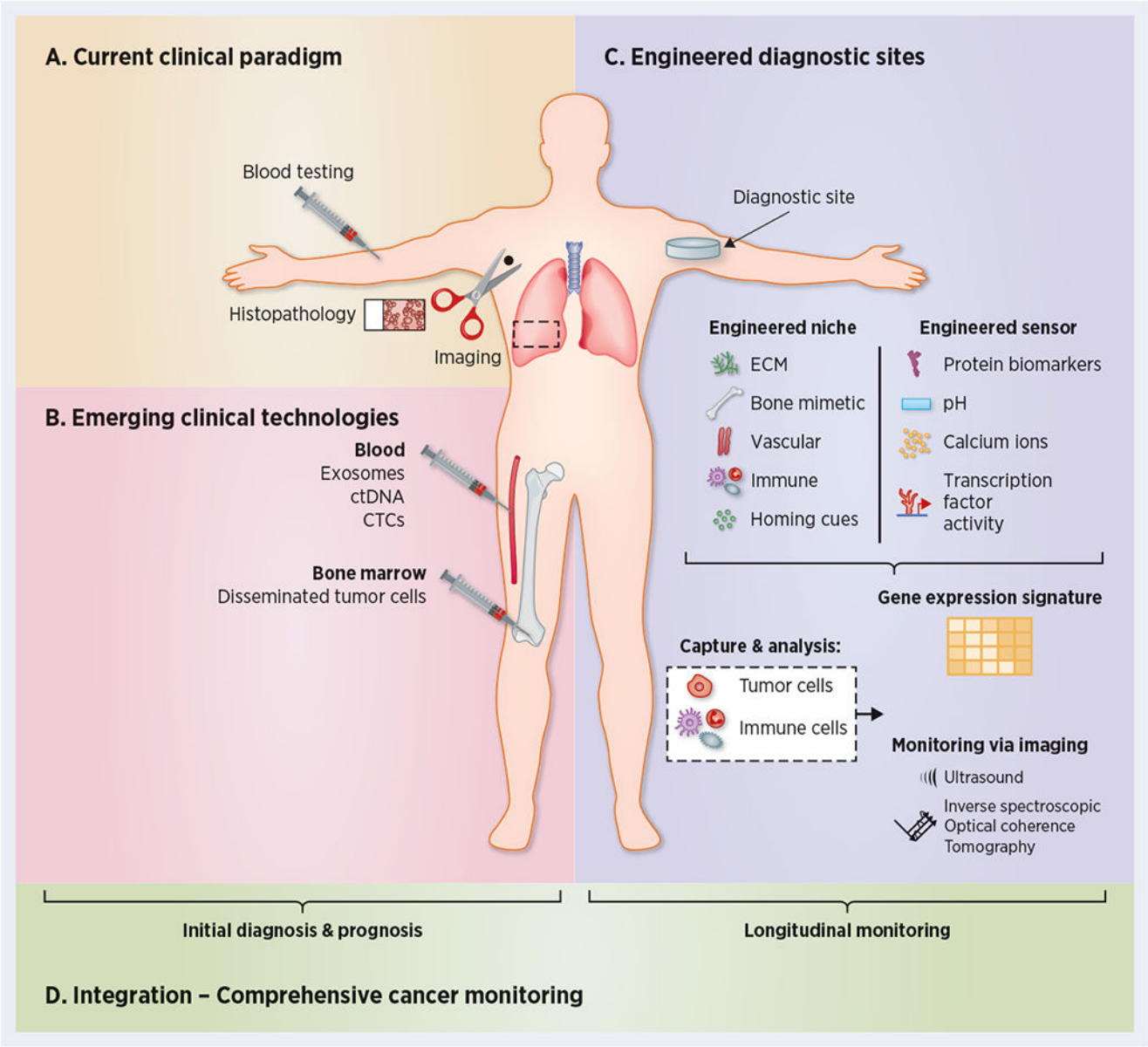

Figure 1:

Engineered diagnostic sites contribute unique information to current clinical paradigm and emerging technologies.

Table 1:

Limitations of the technologies used in the current clinical paradigm

Method	Examples	Limitation	Clinical Need
Imaging	MRI, CT, SPECT, PET and ultrasound	Cannot reliably detect tumors < $1cm^3$	-Earlier detection -Move beyond volumetric assessments
Blood tests	CEA (colon cancer) CA-125 (ovarian cancer) CA-27.29 (breast cancer) Complete blood count	Not always cancer specific	-Multivariate measurements more specific to malignancy and progression
Histopathology	Cytokeratin (carcinomas) Ki67 (proliferation) ER, PR, HER2 (breast cancer)	Snapshot measurement	-Location to biopsy after resection -Longitudinal, real-time measurements