Cutting-Edge Developments in Oncology Research

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Abstract

The field of oncology research has made many successful advances, and new discoveries have started making headlines. As an example, the identification of immune checkpoint inhibition mechanisms in carcinogenic cells led to the development of immunoassays, which have helped many cancer convalescents recover. This article covers the most advanced cutting-edge areas of cancer research: exosomes, microbiomes, immunotherapy, nanocarriers, and organoids. Research on exosomes advances cancer detection and treatment modalities, as well as further understanding of mechanisms that regulate carcinogen cell division, proliferation, invasion, and metastasis. Microbiome consents the researchers to understand the disease cancer. Immunotherapy is the third method in the treatment of cancer. Organoid biology will be further expanded with the aim of translating research into customized therapeutic therapies. Nanocarriers enable cancer specific drug delivery by inherent unreceptive targeting phenomena and implemented active targeting strategies. These areas of research may also bring about the advent of the latest cancer treatments in the future. Malignant infections are one of the leading grounds for demise in the society. Patients are treated with surgery, radiation, and chemotherapy. In chemotherapy, the malignant cells are destroyed and the tumor burden is reduced. However, in most cases, resistance to chemotherapy develops. Therefore, there is a constant need for new additional treatment modalities and chemotherapeutic complex rules. Due to the rapid development in cancer research, I can only mention a few goals and treatment options that I have chosen; However, this review specializes in new and admirable significant strategies and compounds.

Keywords
► cancer treatment
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► medical oncology
► microbiome
► nanocarriers
► oncology
► organoid

Introduction

Considerable advancement has been done in the ground of cancer research through the use of state-of-the-art gear and technologies. Next-generation sequencing (NGS) is an example of cutting-edge technology. Besides specified to as high-throughput sequencing, NGS is the universal designation accustomed to explain numeral single leading nucleic acid sequencing technologies.¹ Liquid biopsy, or fluid phase biopsy, is the scrutiny of liquid body fluid tissue, frequently blood.² It provides a broad range of opportunities in the field of oncology and carcinogen treatment and is used as a

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singular manner to detect cancer. Hence, liquid biopsies can be accomplished more habitually and could be used to monitor carcinoma expansion, track a patient’s response to treatment, or as a “scrutiny” method for people who have completed the treatment but are at high risk of their disease recurring.\(^1\) This approach can be used to authenticate the efficacy of a malignancy treatment drug by analyzing a couple of liquid scintigraphy tissue sections in the duration of a limited number of weeks.\(^1\) These techniques permit for a great deal faster and less expensive sequencing of nucleic acids, that is, DNA and RNA in comparison with the formerly used Sanger sequencing (dideoxy chain termination sequencing), and thus have reformed the field of genetic makeup and cell biology. NGS additionally permits on behalf of less complicated exposure of transmutations in mutation sections, which leads to improvement in numerous novel proxies that can be used to treat the patients.\(^1\) Innovative maneuvers, medicines, and drugs have been devised and advanced for cancer treatment. Keyhole surgery using robotics has advanced and has made it feasible to envisage the motion of the tongs in three dimensions. This approach is at present utilized in esophageal, gastrocolic, and celiac cancer surgical procedures.\(^3\)–\(^5\)

Currently, immunotherapy have become a further approach for handling cancer patients. Honjo and Allison detected the insusceptible checkpoint, which brought the improvement in insusceptible checkpoint inhibitors.\(^6\) Notwithstanding these advances, gastrointestinal cancer malignancy is still a prime hassle in the way of latest treatment techniques. In this review article, the introduction and description describe five new regions of cancer research that could make contributions to cancer treatment in the upcoming times: exosomes, microbiome, immunotherapy, nanocarriers, and organoids. Despite its accepted application in medical oncology, clinicians and biomedical scientists are nevertheless struggling with an incredibly low degree of expertise of diverse cellular and subcellular techniques and understanding of treatment mechanisms of chemotherapeutics already utilized in medical oncology.\(^1\) There is no uncertainty that the certification of innovative chemotherapysensitive target molecules can optimize treatment success in clinical oncology.

**The Role of Exosome Investigation in Oncology**

An exosome is a minor molecule (micro vesicle) that is oozed out from cells. Its outer face has macromolecules derived from cellular membranes that measure between 15 and 150 nm in proportions. In addition, proteins and nucleic acids are determined in the matrix of exosomes.\(^7\) In recent times, several scholars have concluded that exosomes are concerned in numerous ailment mechanisms.\(^1\) Exosomes, which contain microRNAs, mRNAs, and proteins, have proven useful for retaining a wide range of practical amalgams.\(^8\)–\(^10\)

Countless cells practice the secretion of exosomes to express each other, and those exosomes also serve the goal of reaching remote cells. Malignant cancer cells can also release exosomes that incorporate particles that are conducive to cancer progression. Exosomes initiated by cancer cells can also destroy the blood-cerebral barricade, which subsidizes brain tumor.\(^10\)–\(^11\) Malignant cancer cells are additionally influenced via exosomes that surround normal cells.\(^12\)

Thus, in addition to the tumor microenvironment and pre-metastatic niche development, exosomes become deeply involved in cancer cell division, propagation, incursion, and metastasis.\(^13\)

Exosomes can also be applied to identify most cancers. Categories which are established in numerous body fluids, including blood, plasma, and urinal fluids. The identification and interpretation of exosomes from most malignant cancer cells are used to make detections on the occurrence of the disease.\(^14\) In the meantime, innumerable vesicles, such as many proteins (nucleotides), DNA, and microRNAs, are present in exosomes from ordinary cells; it is essential to separate them for most of them are associated with cancer.\(^1\) Currently, exosome exposure strategies are being developed for exosomes within the plasma of many periampullary and hepatopancreatic cancer patients, with exosomes constant in the bladder of most cancer patients. Therefore, the similarity of the mechanisms that control most cancer cell division, proliferation, invasion, and metastasis, along with improvements in most cancer detection and treatment techniques is strongly influenced by exosome research.\(^1\) While the discharge of exosomes from most malignant cancer cells can be restricted, the tumor microenvironment and signal transduction that facilitate the formation of the premetastatic region of interest cannot be achieved. Research is currently underway converging on the elimination of most cancerous exosomes.\(^15\)–\(^16\)

**Implementation of Microbiome in Cancer Research**

Different variety of pathogens live in the human anatomy out of which bacteria have the maximum essential association with the human anatomy. Bacteria can survive at any place inside the human anatomy, such as the digestive system, respiratory system, and oral cavities.\(^15\) In particular, the bacteria in the gut are abundant in types and amounts.\(^1\)–\(^10\)\(^,\)\(^18\)–\(^20\)

The average populace of different bacteria observed in the mortal gut is called the microbiome.

Modern improvements with NGS have produced even more specialization to the duodenal microbiome.\(^21\) Bacteria in human microbiome primarily belong to four files: *Firmicutes, Bacteroides, Proteobacteria*, and *Enterobacteriaceae*. Of these, the most prominent species are *Firmicutes* and *Bacteroidetes*.\(^22\) Dysbiosis is a circumstance where the multiplicity of the microbiome is abridged. Dysbiosis has been reported to be related with several ailments, comprising seditious bowel disease, colorectal cancer, diabetes, and allergic diseases.\(^23\) *Atoxopbium parvulum* and *Actinomyces odontolyticus* proliferate with size in the early stages of colorectal cancer (adenoma or intramuscular) over the course of the cancer advancement.\(^2\)–\(^24\) This suggests that a specific microbe may be related with the primary junctures
of colorectal cancer remission, which may be convenient for understanding premature exposure of cancer.

Countless researches have likewise been led to clarify the connection among the microbiome and the human immune system. Immunoglobulin A (IgA) antibodies, one of the utmost essential factors in the intestinal resistant system, are assumed to have a function in removal of pestilent microbes and restoring the intestinal environment. IgA antibodies detect, eradicate, and counteract infectious bacteria and toxins. It additionally preserves a mutual association by opening and capturing the host’s typical microbiome. This antibody called W27IgA apprehends to a portion of serine hydroxymethyl transferase, an enzyme intricated in bacterial growth. W27IgA antibodies bind to them and suppress the growth of Escherichia coli. The W27IgA antibody, however, does not attach to bacteria that overpower enterocolitis, including bifidobacteria and lactic acid bacteria. Thus, the microbe is intensely concerned with the resistance of the human gut. Lately, it has been installed that besides being involved in the intestinal immune system, microbiome also plays a broader role in the human immune system.

As the exploration of the microbiome progresses, its links to pathophysiology of numerous ailments such as cancer as well as its supervision of the human immune system become clear. It is, moreover, associated with lymph node metastasis, hepatocellular carcinoma, and remote metastasis. The research and study of the microbiome provide some evidence in improving and treating gastrointestinal cancer.

**The Growth of Immunotherapy in Cancer Treatment**

For the last few decades, surgery, chemotherapy, and radiation therapy have been the primary techniques of therapy for most cancers. Along with to these treatment options, immunotherapy has freshly fascinated universal interest. An individual’s immune system is stimulated by the cancer antigens to attack cancerous cells under typical conditions. Nevertheless, occasionally the immune system does not treat cancer cells as nonself or is unable to assault them. Even though therapies that prompt the immune system to counter against cancer cells have been analyzed for a long time, the usage of an affected person’s own immune system to treat cancer has not been recognized. Lately, the immunoassay center has demonstrated the effectiveness of both immunosuppressive measures and chimeric antigen receptor (CAR)-T cell therapy.

There are two main fundamental reasons why it may be problematic to prove the effectiveness of anticancer therapy for some time. Signal transduction by immune checkpoint compounds, including Programmed death 1 and CTLA4, overpowers cytotoxic T lymphocytes (CTLs). Suppression of immune checkpoint molecules that neutralize antibodies can initiate the subdual of cancer-specific CTLs which instantly activate the immune system and promote cancer eradication. Immunoassay has been shown to be effective and clinically applicable in many solid cancers, including melanoma, lung cancer, gastric cancer, and esophageal cancer. In addition to PD-1 and CTLA4, new immunoassay molecules containing LAG3 T cell Ig and ITIM domain and Signal regulatory protein α are also being actively studied. Although this treatment is favorable, cancer cases that answer to these treatments are narrow. This is due to the fact that the use of this treatment calls for the incidence of cancer-specific CTLs in the patient’s body.

A second problem with immunotherapy is that T cells do not apprehend the exact cancer cell antigens and the immune accelerators are very weak. By delivering CTLs to the victim’s body that recognize the exact cancer cell-specific antigen, CAR-T cells strengthen the immune accelerator. The CAR is made up of single chain Fv antigens (CD28, 4–1BB) and constitutive molecules (CD3ζ, 4–1). Next, CAR is instigated into T cells taken from cancer patients and CAR-T cells cells are generated. CAR-T cells secrete a specific antigen of cancer cells and are activated to damage these cells. The CAR-T cells link with high antibody specificity to cancer-specific antigens, as well as to cancer cells that are very proliferative and possess strong cytotoxic activity. The CAR-T treatment is operative in leukemia, including B-cell acute lymphoblastic leukemia and myeloma. While CAR-T cell therapy has a high beneficial effect, an obstinate and severe malignant singularity known as cytokine release syndrome has been acknowledged in some patients. The recent treatment for microsatellite instability-high colorectal cancer includes nivolumab and ipilimumab. The progression-free survival rates (9 months) and 12-month survival rates (71%) for the Nivolumab Plus Ipilimumab Cohort of Checkmate-142 were 87 and 85%, respectively.

Therefore, it is predicted that the further specialization of the cancer immune system and the improvement of different immunotherapies will subsidize to momentous improvements in cancer treatment. One hassle with immunotherapy is that there is no conclusive extrapolative biomarker. To find the new biomarker, we assess cytolytic activity (CYT) ratings. CYT rating is based on GZMA and PRF1 mRNA expression levels as a new measure of cancer immunity. Advances in biomarker novelty may assist many gastrointestinal cancer patients.

**Use of Organoids in Cancer Research**

The three-dimensional (3D) organoid arrangement is a biological culture-based, innovative, and physically applicable cellular stage. The organoid is a small and abridged model of an organ that is fashioned in vitro in 3D and represents the actual microscopic anatomy. With a few cells cultured from the tissue or cultivated cells as the preliminary substance, the organoids nurture and transmit into the vault membrane cellular pool, which subsidizes to their self-regenerative and distinction capabilities. A wide variety of cancer tissues and cells can also be studied to determine the traits of the stem embryonic stem cells or prompted pluripotent stem cells. The organoid structure is commonly referred to in 3D for the growth of stem cells or their innate cells. The
phylogeny and practical properties of diverse varieties of cancer tissue have been replicated in single-cellular suspensions or organoids generated from cell masses. In addition to culture-propagated cancer cells, these masses are quarantined from murine embryonic cells and humanoid tissues or cultured cells. The arrangements of organoids display the capability of cancer cells to self-regenerate, proliferate, and differentiate, and offer insight into important molecular pathways and biomolecular systems in many cancer treatments. Organoid systems have also been applied to the analysis of many genetic and biological processes, including locomotion, pressure reaction, cellular-cellular communication, and cell exchanges with a wide variety of cells, including fibroblasts, endothelial cells, and inflammatory cells.

Organoids, although not a complex and convenient technology, do require precise media, enhancements, and several intricate techniques, and their solicitation is mainly dedicated to the treatment of cancers (colorectal, prostate, breast, ovary, and esophageal cancer). The keratinocyte serum-free medium was modified to produce endoscopic esophageal biopsies, commemorated human esophageal epithelial cells, and 3D organoids from the murine esophagus. 3D organoid systems have materialized as a sturdy apparatus in basic fundamental research over the past few years that can be used for customized medication. In maximum circumstances, it may be beneficial to organize the patient’s organoid to investigate the susceptibility of new therapeutic agents to the treatment of cancer. Therefore, it seems that organoid biology is becoming more broadened with the purpose of interpolating research into customized medicine.

**Nanocarriers: Cutting-Edge Antineoplastic Drug Carriers in Cancer Treatment**

Biological nanocarriers are frictional nanomaterial systems that can deliver small molecular weight drugs or macrocellular anticancer agents, such as genes or proteins, to subcutaneous tissues during targeted treatment and accumulate in tumors in the same way as molecular carriers like antibodies and peptide-drug conjugates do. Furthermore, nanocarriers attenuate dilapidation, lessen renal absorption, and increase the solubility of insoluble anticancer drugs. In most cancers, angiogenesis produces new blood vessels for the tumor, but these new vessels have enlarged permeability or enhanced permeability and retention (EPR) effect, resulting in inactive nanocarriers as well as poor lymphatic drainage of the tumor tissue by delivering the release of chemotherapeutic agents into the tumor homeostasis. To take advantage of the abnormalities of tumor vascularization, nanocarriers must have a sufficiently diffuse half-life to target the tumor environment passively, inhibiting the movement of the mononuclear phagocyte system (MPS) and reticuloendothelial system by transporting anywhere in the bloodstream and releasing anticancer drugs into the tumor. For this resolution, the nanocarrier size to exit MPS should not exceed 400 nm and is more effective by the EPR effect in tumors less than 210 nm in diameter. Moreover, the surface of these nanoscale carriers must be hydrophilic and neutral or simply ionic to escape plasma proteins (opsonins) and stop macrophage attack. This is accomplished by coating the carrier exterior with hydrophilic polymers such as polyethylene glycol (PEG) or synthetic copolymers of polyethylene oxide (hydrophilic block) and propylene oxide (hydrophobic block). Further, blood vessels and cells contain negatively charged molecules that can repel nanocarriers with negatively charged exteriors. Therefore, one must use slightly negative or positive exteriors. On the surface of nanocarriers for containing active targeting, chemotherapeutic drugs are present by a combination of various components, such as monoclonal antibodies, antibody fractions, peptides, and growth factors.

In fact, nanocarriers permit the inclusion of multiple pursuing ligands due to the surface-to-area-to-volume ratio that comes with many binding options. The active targeting of new drug carriers is not increase the total tumor accretion of cytotoxic drugs at the site, but submissively allocates cytotoxic drugs in superior quantities than the consolidated systems to the tumor because the preliminary accretion of nanocarriers in the tumor affects the consequence of EPR before the target is formed. Nevertheless, the active cellular target enhances healing efficiency by reducing specificity and increasing the intake. Moreover, the use of peptides aims to defeat the multicellular resistance of nanocarriers and avoid the restrictions of sedentary targets, as in some hypovascular tumors. Active targeting nanocarriers can intensify antitumor capacity several times compared with nontargeting carriers. From tumor vasculature penetration, it can be accredited to this clinical failure because there are fundamentally certain restrictions on the procurement and entry of cancer cells. Furthermore, in budding tumors, cancer cells are close to the endothelial barrier and bind to receptors that initially penetrate the rest of the tumor, targeting nanocarriers. In this cutting-edge world, specific techniques have been defined to address these defects that reduce the transport of nutrients and oxygen to the tumor and increase the antitumor potential of nanocarriers by releasing less molecular anticancer drugs near the tumor vasculature. An extra drawback that contributes to the state-of-the-art clinical miscarriage of dynamic targeting nanocarriers is the inclusion of target in nanocarriers, which have enhanced immunogenicity and plasma protein absorption, reducing their blood flow time and their capability to passively target tumors. Among nanocarriers, there are polymer therapeutics (polymer-protein and polymer-drug conjugates) in which the drug is nonpolarly bonded or conjugated to the polymer, and particulate drug nanocarriers, in which the drug is trapped inside specific structures made from specific materials like polymers (polymeric micelles, dendrimers, and polymeric nanoparticles (NPs)) or organometallic compounds (chiral and zigzag carbon nanotubes).
Polymer Therapeutics: Explicit Biomarkers for Cancer Treatment

In polymer therapeutics, there are polymer–protein conjugates and polymer–drug conjugates, which are, among other things like nanosized linear water-soluble polymeric macromolecular structures that are joined to anticancer drugs and anticancer peptides by cleavable linkers proteins or small molecules that can be united with anticancer drugs and are constant for the transfer period of the cytotoxic component and discharges anticancer drug into the tumor.\(^6\)\(^3\) The synergistic combination of anticancer proteins with polymers diminishes its immunity and escalates its constancy and diffusion interval in the blood,\(^6\)\(^4\) but in the case of polymer-drug amalgamations, the polymers deliver improve the circulating time in blood for cytotoxic drugs, with improved aqueous solubility. Passive beaglued delivery to tumors and low toxicity increase the remedial value of the anticancer drug.\(^5\)\(^3\)\(^6\)\(^5\) In both cases, these constructions can be measured as “new chemical entities” with a penchant for drug carriers, with low drug loading and limited potential for active targeting due to the limited number of compound sites available in the polymer;\(^6\)\(^4\) in combination, PEG-L-asparagine (pegaspargase or Oncaspar), a polymer-protein conjugates, is administered intravenously.\(^6\)\(^6\)\(^6\)\(^7\) Pegaspargase is a primary polymer-protein conjugate authorized through the U.S. Food and Drug Administration (FDA) in 1990s for the treatment of acute lymphoblastic leukemia.\(^4\) Recent research does not have any new FDA approvals for cancer treatment, but there are enzymes (arginine deaminase) and bio biological response modifiers (interlukin 2, interferon-\(\gamma\), and antibody fragments).\(^6\)\(^8\)\(^6\)\(^9\) Research on polymer-drug compounds for the cure of age-related,\(^5\)\(^4\) cancer currently consists of at least 20 compounds (most of which are in closed state)\(^6\)\(^2\) and are mainly used instead of conventional cytotoxic drugs, for instance, platinate,\(^7\)\(^0\) doxorubicin, camptothecin, paclitaxel, methotrexate, and irinotecan. In phase III clinical trials, Xyotax (CT-2103 or OPAXIO), a polyglutamic acid (PGA)-paclitaxel conjugate, and NKTR-59, a polymeric conjugate of irinotecan, are close to commercialization.\(^5\)\(^3\)\(^7\)\(^1\)

In 1994, a manmade polymer-drug conjugate based on N-(2-hydroxypropylene) methacrylamide-doxorubicin became the key component in medical trials. Since then, no further polymer-drug conjugates based on artificial polymers such as HPMA, PGA or PEG have been submitted for medical trials. Additionally, numerous natural polymers can be classified as polymer-drug conjugates, though only some polysaccharides, hyaluronic acid, human serum albumin, and dextran have reached the stage of clinical trials.\(^5\)\(^3\) The most notable breakthrough in the direction of medical use of polymer-drug conjugates is the docosahexaenoic acid—paclitaxel conjugate (Taxol), which has recently entered the phase III clinical trials of cancer treatment.\(^2\)\(^7\)\(^2\) Although nearly all polymer-drug conjugates use passive targeting, active mechanisms with targets such as antibodies, peptides, and folate have evolved over the years.\(^5\)\(^3\) Polymer-drug conjugates\(^5\)\(^3\) are also being researched for their potential to inhibit specific kinases, accelerate apoptosis, or reduce angiogenesis (polycystic ovary syndrome).\(^7\)\(^3\)

Conclusion

As the exosomes enter the bloodstream or urinal tract, if the apprehending system is in place, it will become a much less intrusive test to make out cancer.\(^1\) Since exosomes contain not only DNA but also other genetic material and proteins, it is a novel instrument for cancer research that includes the early prognosis of cancer. Normal mobile homeostasis is predicted by the interchange of genetic biological substances across the membrane and enables such transport by vessels that transport the cargo to the suitable destination. They are immersed in the ubiquitous external environment and are featured by specific capabilities contingent on the secreted cell of the foundation.\(^1\) Exosomes contribute to the technique of chemo-organotropic metastasis and additionally involve extra essential chemooncogenic indicators in integrating the selection and function of the analogous exosome in chemo-organotropic metastasis. Ultimately, to detail the complex mechanisms of regulation and cross-communication that exist between cancer and stromal cells, further research is needed. Furthermore, the origin and biological impact of diversity in exosomes continue to be largely unknown due to the deficieny of analytical platforms and accessible equipment. The standard features of the human immune system such as accurate detection and removal of cancer cells, adaptation to the developing tumor, and memory of the immune system appear to be an excellent aggregate to develop an effective defense for long-term cancer regulation.

Microbiome may be an addition in advanced cancer prognosis and treatment. Exposure of a particular microorganism in the gastrointestinal tract can envisage particular cancer proliferation. Microbiome is extraordinarily essential for human health; at contemporary times its function in the context of cancer is clear. Microbial outcomes vary from improving cancer immunity and cancer treatment efficiency to promoting cancer advancement and preventing treatment effectiveness. These broad implications have prompted researchers to analyze these specific interfaces, along with how vicissitudes in the microbiome augment the survival and treatment potential of most cancers. For cancers such as gastric cancer, these interactions have been well established; however, they are rarely understood in other cases. Since nonsmall cell lung cancer is the bulk of lung cancer cases and one of the pinnacle causes of cancer deaths globally, the specificity of the mechanisms that affect microbiome evolution is compulsory for measures and treatment to prolong patient’s endurance and treatment reaction.

As the field of cancer immunotherapy has evolved, the focus of treatment has lifted from handling the disease site to treating specific tumor biological symptoms and its relations with the patient’s internal cancer autoimmunity set-point. Because the immune system has the ability to recollect and detect and destroy tumor forms, immunotherapy always has integral benefits compared with other therapies that do not have these two main indications. Finding out why
immunotherapy treatments work best in some cancers and in some patients is even more thought-provoking, while in others tumors that were once sensitive to treatment may become resistant. To be particularly effective, cancer immunotherapy must discover conducting to change the immune system in patients who show a low or no immune response to their tumors, even to the tumor microenvironment without tumor-infiltration T-cells. Despite the promise of immunotherapy for cancer treatment, only a minority of cancers respond to some of these treatments.

Nanotechnology is pragmatic in cancer treatment and has ushered a new era in cancer treatment. A variety of NPs, including organic and inorganic NPs, are already widely used in the medical treatment of a wide variety of cancers. Furthermore, nanocarrier delivery systems make available better platforms for combination therapy, which can help drug resistance to hypoxia, including flux transporter overexpression, defective apoptotic pathways, and hypoxia in the tumor microenvironment. The use of nanovaccine and synthetic antigen presenting cells has demonstrated greater efficacy of inoculations for immunotherapy.

Organoids can also help solve the problem of drug resistance and lead to the advancement of modified therapies. However, the preparation of organoids takes time and may take even longer to test for drug resistance. Present-day advances in in-vitro 3D culture technological expertise, comprising organoids, have opened new opportunities for the development of unique, more physical human cancer models. The genetic modification of organoids allows disease modeling in a setting that accesses the biological environment. In addition, organoids can be raised from patient-derived healthy and highly functional tumor tissue, undoubtedly allowing for patient-accurate drug testing and improving personalized treatment regimens. If we can overcome these problems, research on organoids can help overcome cancer. Therefore, these five new cancer research fields will make a significant difference to the diagnosis and treatment of most cancers.

Conflict of Interest
None declared.

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