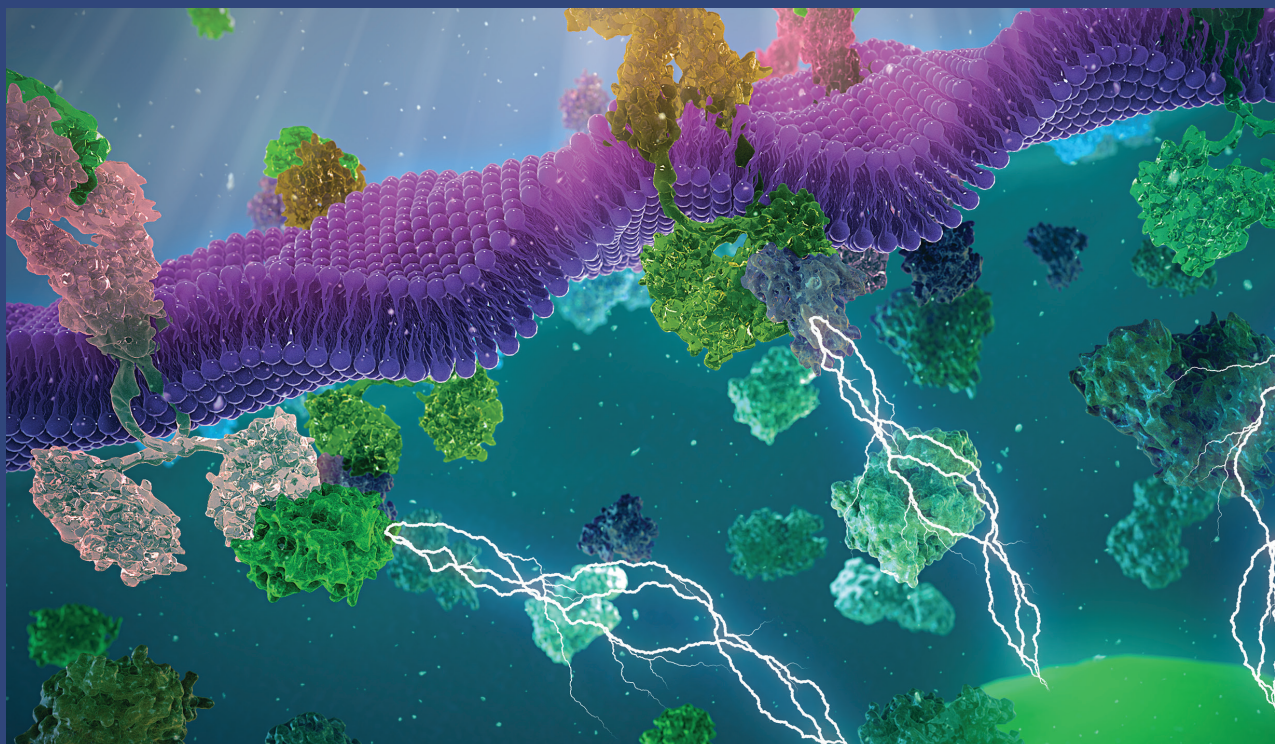


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the November 10, 2015 Issue of *Oncology Times*

Cancer Stem Cells: Current Perspectives, Future Directions



Cancer stem cells (CSCs) are becoming an increasingly greater focus of cancer research, as evidence suggests that they may be integral to tumor formation. Understanding the properties and characteristics of CSCs may lead to improvements in cancer diagnosis, therapy, and outcomes. *Cancer Stem Cells: Current Perspectives, Future Directions*, distributed by *Oncology Times*, offers insight into the role of CSCs in tumor initiation, progression, and metastasis, as well as the signaling pathways implicated in cancer, with a focus on gastric and gastroesophageal cancers. The potential for inhibition of these signaling pathways is also reviewed.

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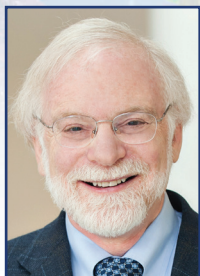
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Do Cancer Stem Cells Hold the Key to Controlling Cancer Growth and Spread?



Interview with Max S. Wicha, MD

Max S. Wicha, MD

Distinguished Professor of Medical Oncology and Director Emeritus

University of Michigan Comprehensive Cancer Center

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Despite advances in chemotherapy, targeted agents, and radiation therapy, the prognosis for patients with advanced cancer has remained poor.¹ Drug resistance, metastasis, and recurrence—even after extended periods of remission—pose persistent challenges to cancer management.² A growing body of evidence indicates, however, that a subset of cancer cells, called cancer stem cells (CSCs), may hold a key to controlling cancer and potentially achieving durable clinical responses.¹ “Ultimately, patient survival depends on getting rid of these cancer stem cells—the seeds we see in the cancerous tumor after treatment,” said Max S. Wicha, MD, Distinguished Professor of Medical Oncology and Director Emeritus at the University of Michigan Comprehensive Cancer Center in Ann Arbor.

CSCs Drive Tumorigenesis

Normal stem cells are undifferentiated cells in the body that can self-renew, propagate differentiated cells, and proliferate extensively.³ Laboratory studies have shown that entire organs can be generated from a single stem cell.³ These discoveries have fueled interest in stem cell therapy for a wide variety of diseases, including neurological, inflammatory, and endocrine disorders.³

CSCs are malignant cancer cells that share the capacity of normal stem cells for self-renewal and proliferation and can differentiate into the heterogeneous population of cancer cells that comprise a malignant tumor,⁴ Dr Wicha explained. A common misconception is that all CSCs arise from mutated normal stem cells, but some CSCs may arise from progenitor cells when a mutation endows these cells with the capacity for self-renewal, normally reserved to stem cells (Figure 1).^{5,6} The CSC model of cancer formation is hierarchical, in contrast with the traditional stochastic model (see *Models of Carcinogenesis* on page 3).^{5,7} A growing body of evidence suggests that CSCs are the drivers not only of tumor initiation and heterogeneity, but of treatment resistance, cancer recurrence, and metastasis, as well.^{8,9}

While the idea that cancers can arise from stem cells goes back about 150 years,⁵ it was not supported by experimental evidence until the late 1990s. Bonnet and Dick showed that a small subset of acute myelogenous leukemia (AML) cells were capable of transferring AML into immunosuppressed mice. The proteins expressed on these cells were similar to those expressed on normal hematopoietic stem cells.¹⁰ A few years later, the role of CSCs in tumorigenesis in solid tumors was supported

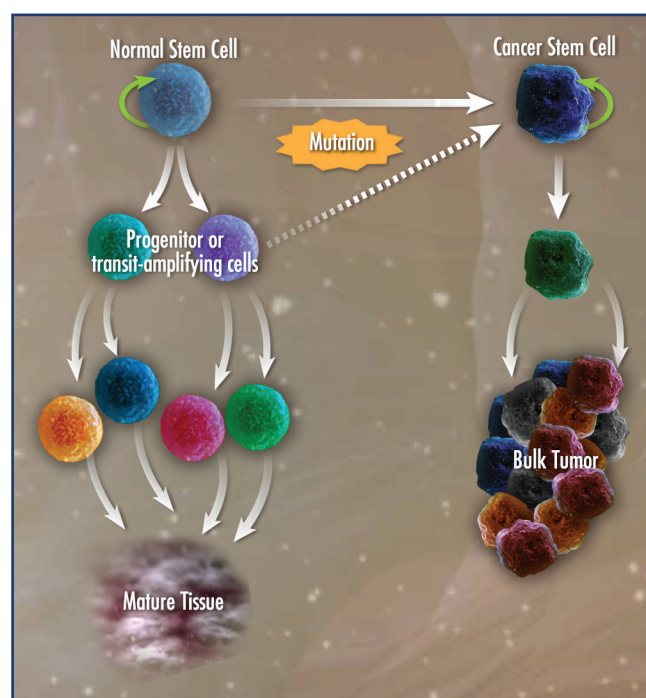
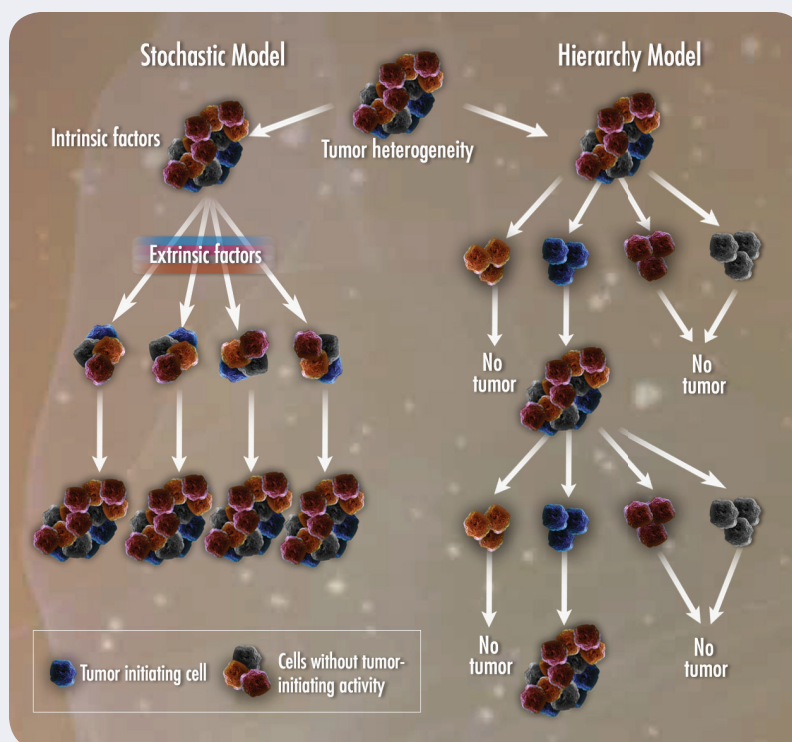


Figure 1. Stem cell development: normal and cancer stem cells (CSCs). Normal tissues develop from a central stem cell that grows and then differentiates to create progenitor and mature cell populations. Normal stem cells have the capacity to self-renew (shown by a curved green arrow), develop into mature tissue (shown by a variety of different color cells), and proliferate. CSCs develop via mutation of normal stem cells or progenitor cells. They go on to grow and differentiate to create primary tumors (the dashed line shows that it is unknown which specific types of progenitors are involved in the generation of CSCs). CSCs can self-renew, generate heterogeneous populations of daughter cells, and proliferate, just like normal stem cells.⁶

Models of Carcinogenesis

The classical model of cancer formation, termed the stochastic model, defines tumor cells as biologically equivalent. Intrinsic factors, such as signaling pathways and levels of transcription factors, and extrinsic factors, such as the microenvironment, host-specific factors, and immune response, result in varied and unpredictable behavior of the tumor cells. Therefore, tumor-initiating activity cannot be attributed to any specific type of cells. Conversely, the hierarchy model proposes that tumors are made up of biologically distinct types of cells with varying functions and behaviors. Tumor growth can only be initiated by a subset of cells known as cancer stem cells (CSCs), which can self-renew and differentiate to nontumorigenic progeny that comprise the tumor mass (Figure).¹

According to Max S. Wicha, MD, research suggests that both models are correct.^{2,3} *“What we know now is that the CSCs themselves can mutate. As cancers develop, the CSC that started the tumor can then mutate and produce a new clone, and at the top of the clone is a CSC. There can be more than one CSC in an individual cancer. Therefore, in a way, the stochastic model and the CSC model are both correct: stem cells mutate and get selected out and each stem cell then generates a clone. Thus, within the tumor are multiple stem cells and multiple clones that come from these tumors.”*



The stochastic and hierarchy models of tumor heterogeneity.

The stochastic model holds that cancer arises through random mutation and clonal selection. Other clones can be selected out with treatment, but the cancers continually mutate. In this model, any cell can become cancerous.⁴ The CSC model, termed the hierarchy model, is at the other end of the spectrum. It holds that cancers originate only in cells that can self-renew and then produce the differentiated cells that make up the bulk of the tumor.⁴

1. Dick JE. Stem cell concepts renew cancer research. *Blood*. 2008;112:4793-4807.
2. Chen K, Huang YH, Chen JL. Understanding and targeting cancer stem cells: therapeutic implications and challenges. *Acta Pharmacol Sin*. 2013;34:732-740.
3. Chen S, Huang EH. The colon cancer stem cell microenvironment holds keys to future cancer therapy. *J Gastrointest Surg*. 2014;18:1040-1048.
4. Wicha MS, Liu S, Dontu G. Cancer stem cells: an old idea—a paradigm shift. *Cancer Res*. 2006;66:1883-1890.

by the finding that human breast cancers also could be transferred to immunosuppressed mice by a small tumorigenic subset constituting only about 1% to 5% of the cancer cells.¹¹

“We need different clinical endpoints in assessing clinical trials that are designed to target CSCs.” – Dr Wicha

The finding that most cells in cancer tumors are non-tumorigenic has important therapeutic implications, Dr Wicha noted. Cancer treatments that target the non-tumorigenic cells will cause tumor regression; however, if they do not affect the CSCs or their signaling pathways, these cells will persist and potentially regenerate the

tumor, resulting in relapse.¹¹ One implication of this finding is that “current evaluation of treatment may be inadequate,” he pointed out.

Treatment efficacy is based on tumor shrinkage, which is usually defined in the clinic as tumor shrinkage of at least 50%.⁵ “The problem with that traditional endpoint is that for the vast majority of cancers, tumor shrinkage does not translate to patients living longer,” Dr Wicha said. An explanation for this may be that tumor regression is a mark of the effect of a treatment on the bulk tumor cell population, rather than the CSCs.¹²

CSCs tend to be resistant to conventional cancer therapies, similar to the resistance to apoptotic therapies observed in normal stem cells.⁵ “An issue critical

to understanding why CSCs are treatment resistant is whether CSCs are discrete populations of cells in cancer or whether non-CSCs can revert to stem cells,” Dr Wicha said. Recently, breast cancer stem cells were shown to exist in two states: the epithelial-mesenchymal transition (EMT) state and the mesenchymal-epithelial transition state (MET). In the EMT state, the cells are relatively quiescent but localized at the invasive tumor front, from where they can disseminate via the bloodstream to distant sites and seed micrometastases. In the MET state, the cells are capable of extensive proliferation, growing new tumors.¹³ Both cell states are needed to form metastases, and evidence suggests that plasticity of breast CSCs allows them to transition from one state to the other.¹³

Stem Cell Divisions Predict Cancer Risk

The incidence of cancer across different tissues varies widely, but the reason for these differences is unclear.¹⁴ For example, the lifetime risk for cancers in the alimentary tract varies by a factor of 24 (0.20% in the small intestine but 4.82% in the large intestine). Such differences cannot be explained fully by environmental or genetic factors, which account for only one-third of the risk variation.¹⁴ Recent evidence points to stem cells as the key to the differences seen among the incidences of cancer in different organs. Most cells in tissues are differentiated and short-lived; however, stem cells, with their capacity for self-renewal, are essentially “immortal.” Tomasetti and Vogelstein analyzed stem cells in different organs and the number of times these stem cells divide, because mutations can happen any time a cell divides; they found a close linear relationship between the number of stem cell divisions in, and the incidence of, cancer in that organ.¹⁴

“The findings of Tomasetti and Vogelstein have been misunderstood as attributing cancer to ‘bad luck,’” Dr Wicha said, “undercutting the role of stem cells in tumorigenesis.” However, the data are highly supportive of the origin of most cancers in stem cells. What the investigators state is that the majority of the differences in cancer risk can be attributed to “‘bad luck,’ that is, random mutations arising during DNA replication in normal, noncancerous stem cells.”¹⁴ In other words, mutations occur at a constant rate based on how often the cell divides. Based on the linear correlation of 0.804, 65% of the differences in cancer risk among different tissues can be explained by the total number of stem cell divisions in the tissues.¹⁴ However, mutations can be caused by other factors as well. “The best example of this is carcinogenic tobacco smoke,” he said. “In this case, the lung cancer incidence would be

higher than expected from just the number of stem cell divisions in lung tissue because of the influence of the environmental carcinogen.”

Target Selection May Be the Key to Effective Therapy

During the self-renewal process, normal stem cells interact with their microenvironment (termed the stem cell niche) via tightly regulated signaling pathways. In the early stage of cancer formation, after the stem cell or progenitor cell receives its first mutation, these pathways become dysregulated, allowing the CSCs to expand in an abnormal fashion.⁵ “We are finding that CSCs are driven by a limited number of key pathways,” Dr Wicha said (Figure 2).⁸ CSCs also interact with other components of the cellular microenvironment, such as cytokines, growth factors, and stromal cells (Figure 3).¹

“We are finding that CSCs are driven by a limited number of key pathways.”
– Dr Wicha

An expanded understanding of the key signaling pathways of CSCs and of the interactions of these cells with the tumor microenvironment is providing new insights into the mechanisms responsible for the resistance of CSCs to conventional cancer therapies and potential targets for new treatment approaches.⁸ Evidence has shown that not only are CSCs resistant to chemotherapy and radiation therapy, but that their number can actually be increased by these treatments.¹⁵

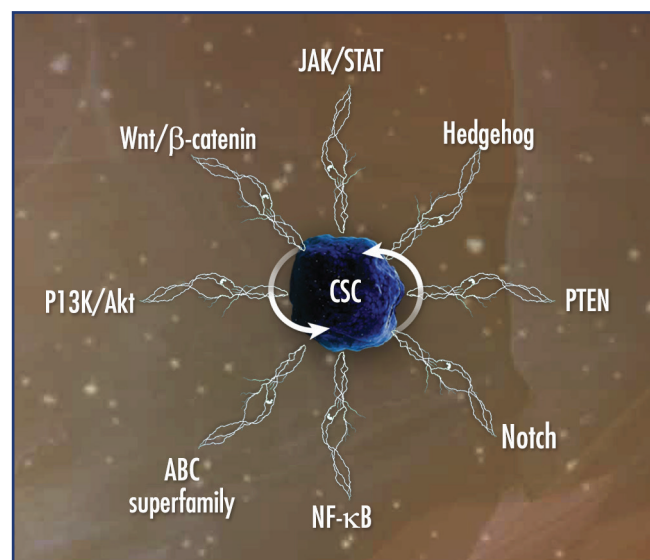


Figure 2. Key signaling pathways in cancer stem cells (CSC) development. Dysregulation of signaling pathways plays a crucial role in the ability of CSCs to self-renew and differentiate. Depending on the signaling pathway involved, CSCs gain the ability to initiate cancer formation or cause tumor recurrence.⁸

As Dr Wicha explained, evidence shows that cells being killed by chemotherapy secrete inflammatory mediators such as cytokines; some of these are interleukin (IL)-6 and IL-8, which then act to stimulate CSCs. Damage to normal tissue induces a similar response. Injured cells release the same cytokines, signaling the normal stem cells in the tissue to reproduce.¹⁶ “This is healthy when it occurs during normal tissue regeneration, but during treatment of cancer with chemotherapy CSC stimulation leads to their increase,” he added.

Dr Wicha noted that new approaches to cancer treatment include targeting CSCs and their signaling pathways, such as Notch, Wnt, Hedgehog, and JAK/STAT, which regulate the CSC internal circuitry. Cancer treatments may also target inflammatory cytokines, such as IL-6 and IL-8, which mediate the interaction between CSCs and the tumor microenvironment, he added. The blockade of IL-8 receptors as a potential treatment approach has been studied in breast cancer by Dr Wicha’s group and others.¹⁷ “Targeting some of the pathways involved in CSC self-renewal may not only stop CSCs from reproducing, but also lead to their differentiation into non-stem cells, thereby making them chemotherapy-sensitive,” Dr Wicha said. Evidence suggests that when IL-8 receptor blockers are used in combination with chemotherapy, the CSC population decreases to a greater degree than with chemotherapy alone, perhaps

because the CSCs are being prompted to differentiate and become sensitive to chemotherapy.¹⁷

Regarding the development of new cancer treatment strategies, it is important to remember that CSCs represent only a small fraction of the tumor cell population, Dr Wicha noted. If these cells are not killed, the tumor will regenerate. Even if the CSCs are destroyed, the non-CSCs that form the bulk of the tumor can, however, still undergo several rounds of cell division leading to spreading of the cancer. Therefore, for the treatment of advanced cancers, the optimal approach is thought to be a combination of a stem-cell targeting agent with a debulking agent that can destroy the large mass of the tumor cells. That debulking agent can be chemotherapy, for instance, because chemotherapy is effective at targeting the bulk cells, while CSCs are resistant to chemotherapy and radiation therapy.¹⁶ According to Dr Wicha, “When treating micrometastatic disease in the adjuvant setting, CSC-targeted therapy alone may be potentially curative. If we knock out the micrometastases, the cancer may not grow back.” For this reason, treatment strategies that target CSCs and the mechanisms responsible for the interaction between CSCs and their microenvironment may represent an important approach to improving patient outcomes.¹⁶

CSC Immunotherapy May Improve Outcomes

New insights into the biology of CSCs and nontumorigenic cancer cells are providing the rationale for immunologic approaches to targeting CSCs. The gene expression profiles and expressed antigens displayed by CSCs and non-stem cancer cells differ. Immunotherapies that target the differentiated cancer cells that form the tumor bulk may not effectively target the antigens expressed by CSCs. In addition, CSCs themselves exhibit heterogeneity.¹ Thus, molecular profiling of CSCs and cancer tumors and targeting of immunotherapy at heterogeneous CSC populations “represent one of the most exciting areas in cancer research. Specifically, targeted immunotherapy offers the potential for durable responses in patients with cancers who previously had few therapeutic options,” noted Dr Wicha.

CSCs can evade the immune system even more efficiently than the differentiated cells forming the tumor bulk. For example, CSCs express high amounts of programmed death (PD)–ligand 1 (PD-L1). The PD1/PD-L1 pathway (an immune checkpoint) is one of two recognized immunoinhibitory pathways that contribute to an immunosuppressive microenvironment that protects cancer cells from immune destruction.¹ Thus, a current approach to the treatment of a variety

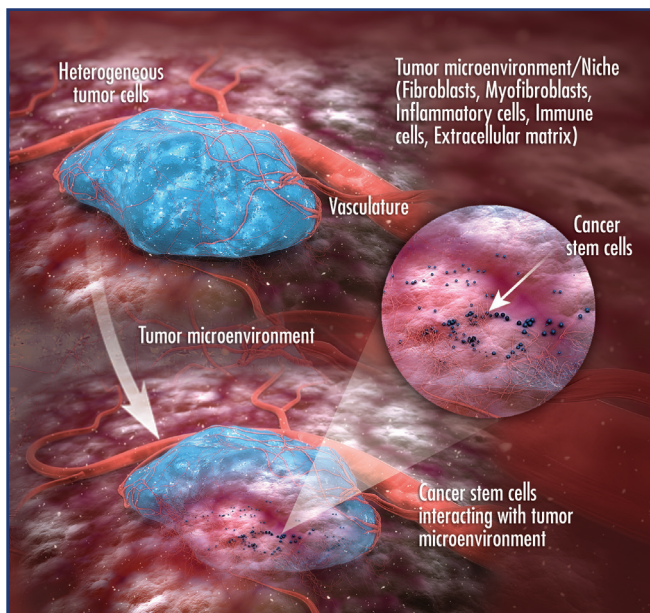


Figure 3. The interaction of CSCs with their microenvironment. CSCs exist within a microenvironment of stromal cells, immune cells, neighboring vasculature, and secreted factors that are produced by these cells. These elements create a niche where CSCs survive and propagate into the cells that define the tumor mass.^{1,5}

of cancers focuses on combinations of such immune checkpoint blocking therapies, other immunotherapies (such as IL-6 or IL-8 inhibitors), and vaccines that target CSCs.¹

“The vision for the future of cancer therapy is to base treatment selection on a complete molecular diagnosis of the tumor, including an evaluation of the stem-cell profile of that particular tumor,” noted Dr Wicha. From an analysis of the immune infiltrates of the tumor, it also will be possible to know whether the patient is mounting an immune response against the tumor. Dr Wicha believes that cancer treatment will move toward a combination approach—targeting the tumor bulk, CSC populations, and immune components. This will lead to “substantial, rather than merely incremental, gains in treating cancer. Most importantly, this future approach may offer a more durable patient response as opposed to an increase in survival of only 1 or 2 months.”

New Therapies, New Challenges

With new treatments come new challenges. As Dr Wicha explained, “The first challenge is to determine whether an agent that targets CSC pathways will be cytotoxic to normal stem cells, because they use the same pathways. Early trials must test low doses of these agents and escalate them carefully,” he said. “Several phase 1 trials have already shown that most agents targeting CSC pathways can be given effectively with relatively low toxicities and that, based on biopsies performed before and after treatment, the targets are being hit.” Going forward, researchers will analyze whether combining these agents with chemotherapy or immunologic agents will have the potential for increased side effects, such as inducing autoimmunity against the normal stem cells. “These investigations must proceed carefully,” he cautioned.

“The second challenge is the need to rethink the use of traditional endpoints of tumor regression in clinical trials,” Dr Wicha noted. Because CSC-targeting agents do not cause tumor regression, he explained, investigators must work with the United States Food and Drug Administration to determine how to demonstrate conclusively that these agents provide a benefit. “What are the acceptable endpoints?” he asked. “What should we be measuring?” Obviously, phase 1 trials are designed to

study potential agents that target CSC signaling alone, with careful monitoring of potential toxicity risks. Phase 2 studies will then assess chemotherapy alone compared with chemotherapy plus an agent targeting a CSC pathway. Potential endpoints in these studies will likely be time to develop new metastases, time to tumor progression, and progression-free or overall survival, rather than tumor regression.

A neoadjuvant trial design that assesses the level of complete pathological response has great appeal for CSC research in a number of tumor types, Dr Wicha said. Complete pathologic response with neoadjuvant therapies is associated with a favorable outcome and has already led to the approval of a new agent to be used as dual anti-HER2 therapy in patients with HER2-positive breast cancer.¹⁸ Another possible endpoint in the neoadjuvant setting is the measurement of residual CSCs after treatment, as the presence of these cells after neoadjuvant therapy has been associated with a poor prognosis.¹⁵

The other technology receiving increased attention is the isolation of circulating tumor cells. As Dr Wicha explained, circulating tumor cells are highly enriched in stem cell markers in patients with breast cancer. Whereas 1% to 5% of cells are CSCs in primary cancers, studies have shown that closer to 30% to 50% of circulating tumor cells express stem cell markers.^{11,19,20} Circulating tumor cells may prove useful as biomarkers for patients in clinical trials; isolating and measuring circulating tumor cells may be a way to monitor patients and determine the efficacy of potential treatments.²⁰ “The utility of these assays as predictive of outcomes must be proven in rigorous clinical trials,” Dr Wicha noted, “but this is the kind of research now being explored, as agents that target CSC pathways are increasingly used in the clinical research setting.”

Conclusion

CSCs as potential therapeutic targets may be instrumental in developing therapies that control cancer and allow for the achievement of durable clinical responses in patients. Expanded understanding of the biology of CSCs, their key signaling pathways, molecular diagnosis of tumors, and appropriate clinical trial endpoints will help in the development of agents targeting key signaling pathways.

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