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The End of an Era: The Cancer Stem Cell Hypothesis and Its Therapeutic Implications

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Preface

This paper was inspired by my investigations into the cancer stem cell hypothesis following a course in the molecular biology of cancer with Dr. Julia Kirshner. Cancer is one of the greatest health burdens in the world, and it has become clear in recent years that current therapies are not sufficient in reducing the worldwide cancer burden. Contemporary findings in cell and cancer biology illustrate the need for improvements of cancer treatments in order to reduce instances of drug resistance, relapse, and toxicity that result from chemotherapy, radiation, and surgery. The need to shift away from antediluvian cancer therapies is dire.

Abstract

Cancer, a leading cause of death in many developed countries, has become a global health burden. Much of the drain on healthcare systems has resulted from the high cost and ineffectiveness of conventional cancer treatments, which often lead to cancer relapse or metastasis. The failures of chemotherapy, radiation, and surgery have been elucidated by the cancer stem cell (CSC) hypothesis, which postulates that a small percentage of tumor cells drive tumorigenesis. These cells can sustain cancer through their capacity to self-renew and differentiate into specialized tumor cell types, similar to how healthy stem cells maintain healthy tissues. Conventional cancer treatments target the "bulk," non-CSC tumor cells, leaving behind drug-resistant cancer stem cells. Theoretically, treatments that eradicate entire populations of cancer stem cells prevent drug resistance, relapse, and metastasis of cancer, leading to more favorable outcomes for patients. Currently, therapies targeting CSCs are being explored both in the lab and in clinical trials, many of which show promise as effective drugs to be used in future widespread practice. Drugs that have the ability to destroy CSCs eliminate many of the side side effects and reduce the toxicity of current cancer therapies, if they are formulated to be CSC-specific. The CSC hypothesis has widespread global health implications, presenting a way to reduce the cost of cancer treatment and to prolong and improve lives of cancer patients.

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Introduction

Cancer is the second leading cause of mortality in the United States, accounting for one in every four deaths, and killing more people than car accidents, suicide, and communicable diseases combined. While cancer death rates have been declining in the recent years due to improved prevention, early detection, and pharmaceutical innovation, a vast number of mortalities occur because of treatment failures. Currently, chemotherapy, radiation, and surgery are the top three cancer treatment methods, which have been utilized in common practice for several decades. Cancer chemotherapy treatments have continued to evolve since the 1940s, after German use of chemical warfare agents prompted research on their therapeutic applications.[1] In 1965, combination chemotherapy was introduced into practice to combat cancer cell drug resistance; today, combining different chemotherapy drugs has become a common practice for oncologists.

Chemotherapy, radiation, and surgery are still the three most common cancer treatments. New chemotherapy drugs and surgical procedures continue to be discovered, increasing survival rates and quality of life for patients. However, drug resistance, relapse, and drug toxicity are common problems that chemotherapy either fails to address or causes. Prognosis for some types of cancer is generally very poor, despite improvements in treatments; cancer is the second leading cause of death in the United States, creating a financial burden on the healthcare system. Cancer research in the past decade has suggested that new

[1] Weisse, Allen B. (1991). Medical Odysseys: The Different and Sometimes Unexpected Pathways to Twentieth-Century Medical Discoveries. Rutgers University Press. p. 127.

treatment methods, other than chemotherapy, radiation, or surgery, are necessary to dramatically improve cancer patient outcomes.

The cancer stem cell hypothesis has dramatic therapeutic implications that have the potential to mitigate the failures of chemotherapy, radiation, and surgery. Formulated in the mid-1990s, the hypothesis postulates that cancer arises from a small population of tumorigenic cells. These cells, called cancer stem cells (CSCs) share many characteristics with normal stem cells found in every body tissue, such as the abilities to self-renew and differentiate into specific types of cells. Like normal differentiated and stem cells, CSCs divide infrequently, distinguishing them from differentiated tumor cells.

As chemotherapeutic agents target rapidly dividing cells, CSCs are often resistant to common cancer treatments, similar to normal differentiated and stem cells. While chemotherapy and surgery are able to remove or kill bulk tumor cells, they are not effective in destroying CSCs. For this reason, it is common for cancer patients to experience relapse or metastasis. The small numbers of CSCs that persist after traditional cancer treatments often have the ability to repopulate, sometimes at the site of the primary tumor and other times at a distant location. Because CSCs are such a small fraction of a tumor's total cells, they go undetected on routine cancer scans when they are by themselves, such as following chemotherapy, radiation, or surgery. For this reason, it is common in the clinical setting for patients to be in remission, then experience relapse years later.

It has become apparent in the recent years that cancer treatment must target cancer stem cells in order to prevent cancer relapse, drug resistance, and drug

toxicity. The objective of this research is to evaluate the question: how does the cancer stem cell hypothesis show promise in reducing the global cancer burden? This paper will describe the reasons for failures of traditional cancer treatments, elucidate the need for cancer treatments that target CSCs, evaluate the effectiveness of CSC therapies that have been studied in clinical trials, and assess the global health implications of basing future cancer therapies on the cancer stem cell hypothesis. It will take both a scientific and global health perspective, illustrating the intersections between both fields of study.

Literature Review

Cancer is one of the major global health issues, affecting millions annually across the globe. There are currently thousands of academic sources on different approaches that must be taken to combat the cancer crisis. Some sources describe how prevention must be improved and made more widespread; others focus on biological processes of cancer and how treatments must be modified. Sources collected for this research focus on the latter issue, and most reach the consensus that the cancer stem cell hypothesis has major therapeutic implications. This research is based mainly on primary research reports and somewhat on literature reviews from scientific journals.

Primary and secondary information from medical journals on the cancer stem cell hypothesis indicate that chemotherapy, radiation, and surgery, while reducing the bulk of tumor cells, present several problems in terms of long-term patient survival. Reviews of literature and meta-analytical studies indicate that chemotherapy often has unintended side effects, including toxicity. According to

both research reports and literature reviews, the cancer stem cell hypothesis has much potential in reducing the global cancer burden by being implicated in cancer treatments. Drugs that target CSCs mitigate many of the problems not accounted for or caused by chemotherapy, radiation, and surgery.

Primary reports from clinical trials of drugs that target CSCs indicate that in many cases, patient survival is improved when they are utilized alongside traditional cancer treatments. Research in laboratories has revealed that cancer stem cells are drug-resistant and have potential to cause relapse in patients who have undergone chemotherapy or surgery.

This study evaluates the implications of shifting cancer treatments away from traditional practices to utilizing cancer stem cell targeted drugs. It emphasizes the importance of treatments that destroy cancer stem cells, preventing the host of problems that arise when they are not eliminated. While clinical trials evaluate the effectiveness of single drugs, this research reviews literature and evaluates the effectiveness of different drugs that have been trialed.

Methodology

The majority of this research is a review of current literature. Most sources were found through PubMed, the major database for searches related to the biological sciences and medicine. From PubMed, primary scientific journal articles and secondary literature reviews were acquired. Several types of research papers were collected, including studies on how the current cancer treatments are ineffective and how the cancer stem cell hypothesis has therapeutic implications.

Interviews were also conducted with biologists and biochemists who specialize in cancer stem cell research. Four formal personal interviews were done with cancer biologists from various institutions. The individuals that were personally interviewed include Dr. Joerg Huelsken, Dr. Freddy Radtke, Dr. Cathrin Brisken, and Dr. Daniel Constam, all of whom conduct research at École Polytechnique Fédérale de Lausanne. One phone interview was conducted with Patrick Gaines, Executive Director of the Gates Center for Regenerative Medicine and Stem Cell Biology.

This methodological approach was utilized in order to gain a broad perspective on the cancer stem cell hypothesis, from research data, reviews of current literature, and dialogues with experts in the field. However, for this topic, choices in the Canton of Vaud, Switzerland were limited; thus in-person interviews were conducted only with experts from EPFL. However, each interviewee had a unique perspective on CSC research, providing a wide variety of expertise.

When evaluating the human subjects criteria, there were no ethical issues that arose. All interviewees gave full informed consent for the information disclosed in interviews to be utilized in this paper. Human subjects were not part of vulnerable populations. All sources, both primary and secondary, have been properly and consistently cited throughout this paper.

Results

1. Failures of traditional cancer treatments

Chemotherapy is the oldest, most well known and widespread cancer treatment utilized in medical practice. Radiation and surgery follow closely behind;

sometimes, two or three of the aforementioned treatments are used in combination. Chemotherapy drugs continue to be researched in clinical trials, as do radiation procedures and combination therapies. While much progress in improving overall cancer survival rates has been made, especially as a result of increased prescription of combination therapies, cancer patients often experience relapse, metastasis, or drug toxicity.

Most studies indicate that combination therapies are more effective in improving survival rates of certain types of cancer than single-drug treatments, though these results have not been replicated in all studies. Research conducted on combination therapy for head and neck cancer concluded that some chemotherapy combinations with radiotherapy more effectively treat patients with advanced stage head and neck cancer than radiation by itself.[2] Similarly, a study on outcomes and toxicity of radiotherapy combined with chemotherapy showed improvements in cancer survival, but a higher risk for toxicity.[3] Other studies have demonstrated increases in survival rates of combination therapy for advanced breast and lung cancers.[4][5] However, in a study of treatments for advanced non-small-cell lung cancer, it was found that certain chemotherapy drugs offered no significant

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[2] Bonner, J.A., Harari, P.M., Giralt, J. Azarnia, N. Shin, D.M. (2006). Radiotherapy plus cetuximab for squamous for squamous cell carcinoma of the head and neck. *New EngIand Journal of Medicine*, pp. 567–578

[3] Beijer, Y.J. Koopman, M., Terhaard, C.H.J., Braunius, W.W., van Es, R.J.J., de Graeff, A. (2013) Outcome and toxicity of radiotherapy combined with chemotherapy or cetuximab for head and neck cancer: our experience in one hundred and twenty-five patients. *Clinical Otolaryngology, 38*(1), 69-74. doi: 10.1111

[4] Romond, E.H., Perez, E.A., Bryant, J., Suman, V.J., Geyer, C.E., Davidson, N.E., et al. (2005). Trastuzumab plus adjuvant chemotherapy for operable HER2-positive breast cancer. *New England Journal of Medicine. 353*(16), 1673-84

[5] Arriagada, R., Bergman, B., Dunant, A., Pignon, J.P., Vansteenkiste J. (2004). Cisplatin-based adjuvant chemotherapy in patients with completely resected non-small-cell lung cancer. *New England Journal of Medicine,* 350(4): 351-60

advantage over other treatments in clinical trials.[6] From these studies, it can be concluded that in many cases, combination therapies offer significant advantages over chemotherapy alone. However, combination therapy has not been demonstrated to be effective against all types of cancer, and the severe toxicity of combined therapies makes it crucial that innovative treatment options be explored.[7] In addition, cancer tends to relapse and metastasize in later rounds of therapy, as it develops resistance to previous drugs. This section will examine the reason for the common failures of traditional cancer therapies using the cancer stem cell hypothesis as a framework for analysis. The problems of drug resistance, relapse, and toxicity will the focus of this section.

1a. Chemotherapy fails to eliminate all cancer cells, leading to drug resistance

Cancer stem cells, in recent years, have been implicated in cancer drug resistance. Currently, there is a low efficacy of chemotherapy, radiation, and surgical treatments on eradication of CSCs[8] Tumor drug resistance appears to be related to many characteristics of CSCs, both intrinsic and acquired.[9] For example, for many types of cancer, ionizing radiation is widely considered the best non-invasive therapy. However, in many cases, cancer stem cells are resistant, causing this

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[6] Schiller, J.H., Harrington, D., Belani, C.P., Langer, C.A., Sandler, Krook, J., Zhu J, Johnson D.H. (2002) Comparison of four chemotherary regiments for advanced non-small-cell lung cancer. *New EngIand Journal of Medicine*, *346* (2002), pp. 92–98

[7] Morrison R., Schleicher S.M., Sun Y., Niermann K.J., Kim S., Spratt D.E., Chung C.H., Lu B. (2011). Targeting the mechanisms of resistance to chemotherapy and radiotherapy with the cancer stem cell hypothesis. *Journal of Oncology, 2011:* 941876. doi: 10.1155/2011/941876

[8] Koch, U., Krause, M., Baumann, M. (2010). Cancer stem cells at the crossroads of current cancer therapy failures—radiation oncology perspective. *Seminars in Cancer Biology, 20* (2), pp. 116–124

[9] S. Vinogradov, X Wei. Cancer stem cells and drug resistance: the potential of nanomedicine . Nanomedicine (Lond) 7(4) 2013 597-618

method to fail.[10] CSCs utilize several biological mechanisms that contribute to their therapeutic resistance, described below.

First, cancer stem cells have a high level of relative dormancy and slow cell cycle kinetics; they are usually in a quiescent state.[11] Their low multiplication rate is a hallmark of normal somatic cells, with the exception of gut epithelial stem cells.[12] A generally accepted hypothesis is that CSC quiescence is a protection mechanism against cytotoxic therapy. Chemotherapy functions to stop tumor growth by inhibiting DNA replication or cell division of rapidly dividing cancer cells; thus, it fails to target CSCs.

Second, cancer stem cells have a high resistance to DNA damage and a high capacity for DNA repair.[13] Noncancerous stem cells have well-developed defense systems against mutation into carcinogenic CSCs; however, when rare genetic alterations do occur, these defense systems serve as barriers against chemotherapy and radiation that target cancerous DNA. In a study of cancer cell resistance against radiation, CSCs were able to repair DNA damage done by radiation much more quickly that all other types of cancer cells. [14] In addition, CSCs have downregulated telomerase function, conferring cellular immortality.[15] Telomerase an enzyme that

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[10] Morrison, et al., 2011

[11] Han, L., Shi, S., Gong, T., Zhang, Z., & Sun, X. (2013). Cancer stem cells: Therapeutic implications and perspectives in cancer therapy. *Acta Pharmaceutica Sinica B*, 3(2), 65-75. Retrieved October 20, 2014, from http://www.sciencedirect.com/science/article/pii/S2211383513000208

[12] Borst, P. (2012). Cancer drug pan-resistance: Pumps, cancer stem cells, quiescence, epithelial to mesenchymal transition, blocked cell death pathways, persisters or what? *Open Biology, 2*(5). Retrieved October 28, 2014, from NCBI.

[13] Morrison, et al., 2011

[14] Eyler, C. E. and Rich, J. N. (2008). Survival of the fittest: cancer stem cells in therapeutic resistance and angiogenesis, *Journal of Clinical Oncology*, 26(17), pp. 2839–2845.

[15] Morrison, et al., 2011

maintains repeated DNA sequences (telomeres) at the ends of chromosomal strands, which promote cell survival.[16]

Third, CSCs of several different cancer types have displayed a high expression of multiple drug resistant membrane transporters, namely ABC transporters.[17] These cell surface pumps function to preserve the genomes of normal stem cells against chemical mutagens, thereby precluding carcinogenesis. CSCs derive resistance to chemical mutagens, such as chemotherapy, from preexisting drug efflux pumps in normal stem cells from which CSCs originated, similar to DNA damage resistance.[18]

Fourth, cancer stem cells have a high resistance to apoptosis. Several mechanisms of CSC resistance to apoptosis have been identified, including the amplification of apoptosis inhibitor proteins. In a study of hepatocellular carcinoma CSCs, it was found that CSCs preferentially activate certain cell survival pathways.[19] Furthermore, CSCs have been shown to be resistant to the mitochondrial pathway of apoptosis[20] as well as a specific nuclear factor that is downregulated in CSCs.[21]

Fifth, the microenvironment of CSCs contributes to their resistance of common cancer therapies. Oxygen sensitizes cells to radiation due to its ability to

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[16] Dikmen, Z. G., Gellert, G. C., Jackson, S., et al. (2005) In vivo inhibition of lung cancer by GRN163L: a novel human telomerase inhibitor. *Cancer Research, 65*(17), pp. 7866–7873.
[17] Dean, M. (2009). ABC transporters, drug resistance, and cancer stem cells. *Journal of Mammary Gland Biology and Neoplasia, 14*(1), pp. 3–9.
[18] Dean, 2009
[19] Ma, S., Lee, T. K., Zheng, B.J., Chan, K.W., Guan, X.Y. (2008). CD133+ HCC cancer stem cells confer chemoresistance by preferential expression of the Akt/PKB survival pathway. *Oncogene, 27*(12), pp. 1749–1758.
[20] Vellanki, S.H.K., Grabrucker, A., Liebau, S., et al. (2009) Small-molecule XIAP inhibitors enhance γ-irradiation-induced apoptosis in glioblastoma. *Neoplasia, 11*(8). pp. 743–752.
[21] Sarkar, F.H., Li, Y., Wang, Z., Kong, D. (2008) NF-κB signaling pathway and its therapeutic implications in human diseases. *International Reviews of Immunology, 27*(5) pp. 293–319.

make DNA more vulnerable to damage.[22] Since radiotherapy is dependent on oxygen-free radicals, areas of low oxygen levels within tumors produce microenvironments that are conducive to radiation-induced destruction.[23] However, one study unexpectedly concluded that CSCs commonly reside near vascular areas.[24] Thus, antiangiogenic chemotherapy may increase CSC drug resistance to radiotherapy by decreasing oxygen availability.

Sixth, CSC heterogeneity contributes to their drug resistance. During therapy, treatment resistant cells within a heterogeneous tumor population can be preferentially selected for, resulting in a more aggressive and malignant outcome. Pharmacological eradication of entire tumors is made difficult by CSC heterogeneity, because they exhibit variable expression of drug-targeted genetic markers.[25] CSCs that have drug resistant properties flourish, later creating more drug-resistant tumors.

Several studies have validated the drug resistant properties of CSCs, indicating that alternate therapies and drug delivery methods are necessary to continue improving cancer patient outcomes. Continuing to utilize chemotherapy without therapies that target CSCs is inefficient from a public health perspective, as drug resistant cancer cells often metastasize or cause relapse. The importance of utilizing cancer therapies that target CSCs to combat drug resistance is discussed later.

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[22] Diehn, M., Cho, R.W., Lobo, N.A., et al. (2009) Association of reactive oxygen species levels and radioresistance in cancer stem cells. *Nature, 458*(7239), pp. 780–783.
[23] Diehn, et al., 2009
[24] Calabrese, C., Poppleton, H., Kocak, M., et al. (2007) A perivascular niche for brain tumor stem cells, *Cancer Cell, 11*(1) pp. 69–82.
[25] Morrison, et al., 2011

1b. Relapse

Cancer relapse is a common issue that affects a large percentage of cancer survivors. It begins with cancer cells that the first treatment didn't fully remove or destroy; there is growing evidence that these cells are usually drug-resistant CSCs. Furthermore, since CSCs are the only cancer cells that are tumorigenic, they are the only tumor cells that have potential to recolonize, either in the location of the primary tumor or at a distance. Thus, tumor recurrence due to CSCs is directly related to their drug-resistant properties. This section examines chemotherapy, radiation, and surgery failures in terms of cancer relapse.

Cancer stem cells consist a small fraction of all tumor cells in most cancers. Standard cancer treatment only shrinks tumors to their CSC progenitors, until the remaining cancer stem cells are below the detectable range.[26] The most common anti-cancer agents consist of paclitaxel, doxorubicin, and cisplatin. While they are capable of high cytotoxicity that kills the bulk of the tumor, they are non-targeting and often result in tumor relapse due to drug resistance.[27] For example, small lung cancer has been clinically characterized by early recurrence after complete response to combination chemotherapy initially.[28] One study conducted on this type of cancer found that after the initial cycles of treatment, CSCs persisted, frequently forming a chemoresistant tumor later.[29]

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[26] Han, et al., 2013
[27] Rich, J.N, Bao, S. (2007). Chemotherapy and cancer stem cells. *Cell Stem Cell, 1*(4), pp. 353–355.
[28] Hamilton G, Olszewski U (2013) Chemotherapy-induced Enrichment of Cancer Stem Cells in Lung Cancer. *Journal of Bioanalysis and Biomedicine, S9*(003), n.p. doi: 10.4172/1948-593X.S9-003
[29] Hamilton, et al., 2013

In a study of ovarian cancer, it was found that CSCs commonly lead to relapse from drug resistance after the initial rounds of treatment.[30] Ovarian cancer has been found to partially consist of heterogeneous CSCs, leading to drug resistance, thereby resulting in recurrence. Similar to small cell lung cancer, a regimen of chemotherapy following cytroreuctive surgery is initially effective. However, within a few months of this initial treatment, platinum-resistance of cancer stem cells leads to tumor relapse.[31] The secondary tumors are more drug resistant and malignant due to the selection for critical drug-resistant CSCs.

Growing evidence also suggests that chemotherapy may increase chances of acute myeloid leukemia relapse. Ley et al. found that while initial treatment with chemotherapy usually puts cancer into remission for 2-3 years, about 80 percent of AML patients die within five years of their diagnosis after chemotherapy fails to keep their cancer in remission and they experience relapse.[32] In all cases of relapse, chemotherapy failed to eradicate the "founding clone," or cancer stem cells. These findings indicate that eradicating CSCs and entire populations of their "subclones" is the key to achieving a cure for the disease.

Chemotherapy drug resistance of CSCs evidently leads to tumor relapse in many types of cancer. Chemotherapy drugs fail to eliminate tumorigenic CSCs,

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[30] Kakar, S., Ratajczak, M., Powell, K., Moghadamfalahi, M., Miller, D., Batra, S., & Singh, S. (2014). Withaferin A Alone and in Combination with Cisplatin Suppresses Growth and Metastasis of Ovarian Cancer by Targeting Putative Cancer Stem Cells. *Plos One, 9*(9). Retrieved October 10, 2014, from http://www.plosone.org/article/info:doi/10.1371/journal.pone.0107596

[31] Kakar, et al., 2014

[32] Ley, T., DiPersio, J., & Wilson, R. (n.d.). Chemotherapy May Influence Leukemia Relapse. *The Genome Institute*. Retrieved October 2, 2014, from http://genome.wustl.edu/articles/detail/chemotherapy-may-influence-leukemia-relapse/

causing a more malignant tumor to grow following initial treatments.[33] As chemotherapy preferentially selects for the most drug-resistant CSCs which are progenitors to more malignant bulk tumor cells, it is important that therapies targeting CSCs are utilized in conjunction with chemotherapy.

1c. Toxicity

Chemotherapy poses problems in addition to drug resistance and relapse, namely toxicity. Patients who undergo combination therapy are particularly at risk. However, combination therapy is frequently more effective than single chemotherapy drugs or radiation alone. Thus, it is often the treatment of choice, despite its unwanted side effects.

A retrospective analysis of combination radiotherapy and chemotherapy in patients with lung cancer associated high levels of toxicity with combined therapy. Compared to radiotherapy alone, simultaneous radiotherapy and cetuximab chemotherapy increase survival rates for locally advanced health and neck squamous cell carcinoma patients. However, of the 125 patients receiving treatment, five died due to heart failure from toxicity. Furthermore, dermatological toxicity prevented several patients from completing treatment and 59 percent of patients from receiving the full dose of cetuximab, leading to less successful outcomes from treatment. However, it was concluded that radiotherapy combined with cetuximab is justified by an increased two-year survival rate, despite an increased risk of toxicity.[34]

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[33] Constam, Daniel. Personal Interview. 12 Nov 2014.
[34] Beijer, et al, 2013

In addition to toxicity leading to cardiovascular failure, Bressler (1997) observed that neurotoxicity from chemotherapy has been commonly reported in patients taking antineoplastic drugs.[35] According to Bressler, there are frequently no treatments for these toxicities; thus, oncologists decide to discontinue chemotherapy treatment based on a number of criteria, including severity of symptoms and response to therapy. Among drugs that have caused neurotoxicity is 5-Fluoraouracil, which has led to cerebellar toxicity, ataxia of the trunk or extremities, and dizziness. Cerebellar toxicity appears to have an increased incidence with higher doses of the drug; though it has been observed at lower doses as well. Within weeks of stopping therapy, the syndrome is reversible; thus, physicians commonly decide to discontinue treatment to prevent further toxic effects.[36]

In a clinical trial of the efficacy of combined fluorouracil, cisplatin, and radiation therapy for treatment of localized carcinoma of the esophagus, Herskovic (1992) et al found that the combined therapy was much more effective than radiation alone, but with the cost of severe side effects. Compared with the control group who received radiation therapy alone, the experimental group receiving concurrent therapy with fluorouracil, cisplatin and radiation experienced increased toxic side effects. In the combined-therapy group, one patient died from renal and bone marrow failure. In 44 percent of patients receiving combined therapy, side effects were severe; in 20 percent they were life threatening, compared to 25

[35] Bressler, L. (1997). Neurotoxicity from Chemotherapy. *PMPR Rharmacotherapeutics, 1(*652). n.p. Retrieved November 2, 2014.
[36] Bressler, et al, 1997

percent and 3 percent, respectively, for those receiving only radiation. Despite the side effects, a significant survival advantage was observed for patients receiving combined chemotherapy and radiation as compared with radiation alone.[37]

It is clear that combined therapy is more successful than single chemotherapy drugs alone for treating many types of cancer; yet the toxicity of these combined therapies cannot be overlooked. To further improve survival rates of cancer patients, treatments other than combined chemotherapy and radiation must be utilized in order to eliminate these toxic side effects. Targeting CSCs reduces the need for combination radiotherapy and chemotherapy, or higher doses of chemotherapy. The destruction of CSCs with CSC-specific cancer drugs precludes relapse, because differentiated tumor cells cannot proliferate on their own.

2. The CSC hypothesis from a global health perspective

The cancer stem cell hypothesis is a key example of how cellular biology influences treatments at the population level, thus impacting public and global health. Theoretically, more effective cancer treatments than chemotherapy, radiation, and surgery, lead to lower rates of drug resistance, thus preventing cancer relapse and metastasis. Both of these complications place a burden on healthcare systems and their constituents, while pharmaceutical industries profit. Thus, the need for more effective and less toxic cancer therapies is evident, and the CSC hypothesis shows promise according to myriad experts around the globe.

[37] Herskovic, A., Martz, K., Al-Sarraf, M., Leichman, L., Brindle, J., Vaitkevicius, V., Emami, B. (1992). Combine Chemotherapy and Radiotherapy Compared with Radiotherapy Alone in Patients with Cancer of the Esophagus. *The New England Journal of Medicine, 326*(24), 1593-1598.

2a. The financial burdens of cancer

Cancer has a large financial impact on individuals and healthcare systems around the globe. According to a study of costs incurred by insured breast cancer patients, in 2004, cancer accounted for an average of 98 percent, 41 percent, and 26 percent of monthly income among breast cancer patients with income levels of <30,000, 30,000-60,000, and >60,000 US dollars, respectively.[38] In the same study, it was found that women lost a monthly average of 727 dollars after their breast cancer diagnosis.[39] Repeated chemotherapy treatments add to this burden; thus, metastasis increases the average percentage. According to Dr. Freddy Radke, developing drugs that successfully destroy CSCs reduces the risks of metastasis and relapse to near zero. As a result, CSC-targeted therapy hypothetically eliminates subsequent rounds of treatment, reducing the financial burden on families.

On a larger scale, the economy takes a hit from high costs of cancer. Currently, the NIH estimates that over 200 billion dollars is spent on cancer annually in the United States; this number continues to increase and is mainly due to indirect mortality costs.[40] Cancer mortality occurs when CSCs spread to distant locations, causing relapse and metastasis. Thus, eliminating this possibility with CSC-targeted therapies will greatly reduce cancer deaths, thereby diminishing the economic burden of cancer mortality.

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[38] Arozullah, A.M., Calhoun, E.A., Wolf, M., Finley, D., et al. (2004). Estimates from a study of insured women with breast cancer. *Supportive Oncology, 2*(3), pp. 271-278.
[39] Arozullah, et al., 2004
[40] American Cancer Society. Cancer Facts & Figures 2014. Atlanta, Ga. 2014.

2b. The psychosocial burden of cancer and chemotherapy

According to *Cancer Care for the Whole Patient: Meeting Psychsocial Health Needs,* people with cancer currently face risks such as physical impairment, disability, and inability to take part in daily activities, partially due to harsh treatment side effects. Many cancer patients have chronic cancer that requires intermittent or continuous treatment regimens, similar to heart disease. Cancer, in recent years, has increasingly met the definition of chronic; its permanence and resulting residual disability are caused by irreversible physiological changes, and its patients require continuous care. As a result of cancer's continuousness, patients and their loved ones must cope emotionally with the stresses created by the debilitating and often life-threatening treatments, and pain and fatigue even after they are declared in remission. Thus, psychological states categorized as depression are common in cancer patients, and fear of relapse is characteristic of those in remission.

As depression and stress in cancer patients are largely a result of the treatments they must undergo, shifting common therapies away from chemotherapy and radiation and towards targeted, CSC-specific drugs will prove effective in reducing the psychosocial burden of cancer. Diminishing the risk of relapse and metastasis with targeted drugs will also lessen fear and depressive feelings in patients. Furthermore, the milder to nonexistent side effects associated with CSC-targeted therapies will lessen the physical, therefore psychological, burden on cancer patients.

3. Necessary for cancer treatments to target CSCs to prevent relapse

While combined therapies have proven more effective in improving five and ten-year survival rates for most types of cancer, it has become clear in recent years that drug resistance of cancer stem cells to these treatments will continue to lead to relapse in certain patients. Thus, it is necessary for cancer stem cells to be eliminated in addition to bulk tumor cells; it is essential that novel treatments be utilized. According to much cancer research today, it is impossible to achieve a cure for cancer without eliminating all CSCs.[41] Currently, the most challenging barrier in cancer therapy lies in establishing a precise way to target and eliminate CSCs.[42] While this theory can be generalized across most types of cancer, certain studies suggest that the cancer stem cell theory may not explain initiation of all cancer types. For example, researchers have not yet isolated CSCs from B-cell precursor ALL.[43] Despite this example, the necessity for treatments to target CSCs has become evident. This section examines the potential implications of CSC-targeting therapies in terms of preventing drug resistance, relapse, and toxicity.

As CSCs are accepted as a chief source of cancer relapse after conventional therapy, failure to completely eliminate them with these therapies can lead to recurrence.[44] They constitute a discrete subpopulation of tumors, usually making up only five to 20 percent of all tumor cells.[45] However, they are the only tumor cells

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[41] Chen, L.S., Wang, A.X., Dong, B., Pu, K.F., Yuan, L.H., Zhu, Y.M. (2012). A new prospect in cancer therapy: targeting cancer stem cells to eradicate cancer. *Chinese Journal of Cancer, 31*(12), pp. 564–572.
[42] Chen, et al., 2012
[43] Bomken S, Fišer K, Heidenreich O, et al. (2010) Understanding the cancer stem cell. *British Journal of Cancer, 103*(4), pp. 439–445.
[44] Chen, et al., 2012
[45] Yu, et al., 2012

that are tumorigenic, indicating that they are the most important cancer cells to eliminate during therapy. They also have been found to promote angiogenesis during tumor development in certain types of cancer,[46] demonstrating that they lead to tumor cell proliferation in ways other that tumorigenicity. Several properties of CSCs are related to cancer relapse, indicating a need for CSC-targeted therapies.

Chiefly, the tumorigenic properties of CSCs contribute to cancer relapse months or years after the primary tumor was thought to have been eliminated. Similar to normal stem cells, CSCs are progenitors to differentiated tumor cells. CSCs maintain the ability to sustain the bulk tumor or, after undergoing specific genetic changes, give rise to new tumors in distant locations. CSCs are the only tumor cells that are able to support tumors and give rise to new, distinct tumors; thus, without them, tumor growth, metastasis, and relapse are impossible. The unique tumorigenic ability of CSCs makes them the ideal target for cancer drugs.

According to Yu et al (2012), in addition to tumorigenicity, cancer stem cells maintain the ability to self-renew. This characteristic, similar to tumorigenicity, was derived from normal, healthy stem cells. Asymmetric replication perpetuates stem cells in their undifferentiated state; during which one of the daughter cells remains an undifferentiated CSC while the other is genetically programmed for specialization. However, stem cells also retain the ability to divide symmetrically under stress to rapidly increase their population, resulting in two CSC daughter cells rather than two distinct cell types. These processes evolved to ensure both the

[46] Bao S.D., Wu Q.L., Sathornsumetee S., et al. (2006). Stem cell-like glioma cells promote tumor angiogenesis through vascular endothelial growth factor. *Cancer Research, 66*(16), pp. 7843–7848.

stability and versatility of a healthy, normal stem cell population.[47] Cancer stem cells derive both methods of division from normal stem cells, circumventing the latter mechanism that gives normal stem cells the ability to rapidly replicate.[48] This quality allows CSCs to quickly repopulate after chemotherapy, radiation, or surgery. Differentiated tumor cells do not maintain the ability to self-renew, indicating that targeting CSCs in cancer therapies has significantly more potential to reduce the risk of relapse.

An additional characteristic that contributes to the resilience of CSCs is their migratory ability. CSCs coopt the ability of normal stem cells to migrate to locations distant from the primary tumor, invade organs in these distant locations, and colonize by undergoing asymmetrical replication.[49] These metastatic colonies are largely responsible for tumor recurrence, and are often undetectable for months or years after initial therapies. Furthermore, metastatic tumor cells have several drug resistant properties not shared by primary tumor cells, making the prognosis for patients with relapse or metastasis highly unfavorable. Therapies that target CSC greatly reduce the risk for cancer relapse and metastasis, thereby eliminating a need for additional, toxic drug treatments following initial therapies.

Relapse remains a widespread fear among cancer survivors, and can be prevented with CSC-targeting therapies. Because of the biological differences that exist between CSCs and non-CSCs, similar to those between normal stem cells and differentiated cells, CSCs are the only cancer cells that maintain the ability to cause

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[47] Yu, et al., 2012
[48] Yu, et al, 2012
[49] Ratajczak M., Tarnowski M., Staniszewska M., Sroczynski T., Banach B. (2010) Mechanisms of cancer metastasis: involvement of cancer stem cells? *Minerva Medica, 101*(3), pp. 179-91.

recurrences.[50] Therapies that destroy differentiated cancer cells preferentially select for CSCs, increasing their drug resistant properties and resilience,[51] and kill differentiated cancer cells that lack potential to sustain cancer growth.[52] Thus, developing novel treatment strategies that are effective against CSCs[53] has become a top priority for many researchers in the pharmaceutical industry.

4. Barriers to overcoming CSC drug resistance with treatments that target CSCs

CSCs derive many of their characteristics from normal stem cells. They also appear to share more characteristics with healthy differentiated cells than differentiated tumor cells. Shared properties of CSCs and normal cells present a barrier for CSC-targeted treatment development, as many therapies that have the potential to destroy CSCs could also harm healthy cells.[54] However, it is imperative that signatures unique to CSCs be identified to develop CSC-specific treatments. Overcoming the problem of drug resistance precludes the ability of tumor cells to metastasize or relapse. Furthermore, first-round treatment cannot be repeated due to drug resistance of CSCs, making the prognosis less favorable as treatment options run out. However, targeting CSCs mitigates this problem, and thus, it is crucial that the several treatment resistance mechanisms discussed in the previous section on drug resistance of CSCs are overcome.

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[50] Chen, et al., 2012
[51] Morrison, et al., 2012
[52] Morrison, et al., 2012
[53] Chen, et al., 2012
[54] Gaines, Patrick. Personal interview. 11 Nov 2014.

The first of these mechanisms is the lower proliferation rate, or quiescence, of CSCs [55] than differentiated tumor cells. As tumors are histologically heterogeneous, different molecular features within a single tumor exhibit varying responses to cancer therapeutics.[56] Chemotherapy drugs target rapidly dividing tumor cells, while slowly proliferating cells, such as CSCs, escape.[57] Thus, the most drug resistant cells within a tumor, namely CSCs, proliferate, resulting in increased malignancy of cancer cells. Thus, it is important for novel therapies to target CSCs in order to eliminate the chance of relapse from drug resistance due to CSC quiescence. Several ways of overcoming this treatment barrier have been proposed, including blocking certain cell signaling pathways that cause cells to proliferate at a normal rate.[58]

Another treatment resistance characteristic of CSCs is their presentation of surface proteins that have the ability to efflux drugs across the plasma membrane.[59] These proteins are known as ATP-binding cassette transporters, and inhibiting them makes CSCs more sensitive to anti-cancer drugs. However, not all CSCs express these proteins. One study on tumorigenicity of different types of CSCs found that CSCs that expressed these transporters were more tumorigenic than ones that did

[55] Yu, Y., Ramena, G., Elble, R.C. (2012) The role of cancer stem cells in relapse of solid tumors. *Frontiers in Bioscience (Elite Ed)*4, pp. 1528–1541
[56] Singh A, Settleman J. (2010). EMT, cancer stem cells and drug resistance: an emerging axis of evil in the war on cancer. *Oncogene*, *29*(34), pp. 4741-51.
[57] Singh, et al., 2010
[58] Roesch A, Vultur A, Bogeski I, Wang H, Zimmermann KM, Speicher D, et al. (2013). Overcoming intrinsic multidrug resistance in melanoma by blocking the mitochondrial respiratory chain of slow-cycling JARID1Bhigh cells. *Cancer Cell, 23*(6), pp. 811–825.
[59] Yu, et al., 2012

not, though the CSCs without them were still tumorigenic but to a lesser degree.[60] Furthermore, the expression of ATP-binding cassette transporters is not limited to CSCs; normal kidney cells present high levels on their plasma membranes.[61] This similarity presents an additional problem for overcoming CSC drug resistance.

Finally, certain cell signaling pathways and transcription factors contribute to CSC therapy-resistance.[62] Among these is Wnt signaling, a group of signal transduction pathways that are important for cell proliferation and migration. They have been implicated in remaining cancer cells after radiation therapy[63] and are overexpressed in certain cancers. For example, Wnt is necessary for the radiation-resistance of mammary epithelial CSCs from mice.[64] Furthermore, early lung metastases overexpresses components of the Wnt signaling pathway, while the downregulation of these components inhibits metastasis.[65] However, signaling processes such as Wnt are necessary for certain healthy cell processes; thus, treatments inhibiting Wnt signaling must be delivered to the specific tumor site.

Clearly, cancer stem cells share many characteristics with healthy cells that make targeting them with drugs particularly difficult. However, as drug resistance results chiefly from these properties of CSCs, it is important that certain signatures

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[60] Patrawala, L, Calhoun, T, Schneider-Broussard, R, Zhou, J, Claypool, K, Tang, D.G. (2005). Side population is enriched in tumorigenic, stem-like cancer cells, whereas ABCG2+ and ABCG2- cancer cells are similarly tumorigenic. *Cancer Research, 65*(14):6207-6219.
[61] Dean, 2009
[62] Yu, et al., 2012
[63] Lindvall C., Bu W., Williams B.O., Li Y. (2007). Wnt signaling, stem cells, and the cellular origin of breast cancer. *Stem Cell Reviews, 3*(2), pp. 157-68.
[64] Woodward W.A., Chen M.S., Behbod F., Alfaro M.P., Buchholz T.A., Rosen J.M. (2007) WNT/beta-catenin mediates radiation resistance of mouse mammary progenitor cells. *Proceedings of the National Academy of Sciences, 104*(2), pp. 618-23.
[65] DiMeo T.A., Anderson K., Phadke P., Fan C., Perou C.M., Naber S., Kuperwasser C. (2009). A novel lung metastasis signature links Wnt signaling with cancer cell self-renewal and epithelial-mesenchymal transition in basal-like breast cancer. *Cancer Research, 69*(13), p.p. 5364-73.

of CSCs be targeted by novel therapies. Alternatively, drugs have been developed to chemically alter CSCs to make certain properties more similar to differentiated cancer cells. Thus, chemotherapy or other drugs targeting bulk tumor cells in combination with CSC-targeted drugs offers a promising solution to the problem of CSC drug resistance.

5. Potential therapies that target CSCs

Recent evidence has made the dire need for CSC-targeting therapies apparent. Cancer cannot be cured without eliminating CSCs in addition to bulk tumor cells; without CSCs, tumorigenesis becomes impossible.[66] While the problem of drug resistance has been somewhat mitigated by use of combination chemotherapy and radiation, the toxic effects of these combined therapies frequently make their outcomes unfavorable. Despite the success of combined therapies in improving cancer survival rates, for some types of cancer, these therapies are far from 100 percent effective. The cancer stem cell hypothesis presents the scientific community with a promising approach to finally discovering a cure for cancer, and several drugs that target CSCs have already undergone clinical trials.

Several facets of CSC-targeting therapies must be addressed in drug development,[67] including signatures of CSCs to be targeted and drug delivery methods. Certain cell signaling pathways that are implicated in cancer can be targeted in CSCs. However, these processes must either be specific to CSCs or drugs must be delivered to the exact location to the tumor, otherwise the risk of toxicity or

[66] Constam, Daniel. Personal Interview. 12 Nov 2014.
[67] Brisken, 2014

healthy cell damage exists. Thus, both the cellular mechanisms and delivery of drugs are of equal importance. This section examines bench research and clinical trials of CSC-targeting drugs, considering therapies that sensitize CSCs to conventional cancer drugs, drugs that inhibit certain CSC signaling pathways, and nanoparticle drug delivery.

5a. Drugs that target stem cell markers

Certain signatures of cancer stem cells play a vital role in their functioning, and thus make them logical targets for therapy. Often, cancer stem cells have higher expression of certain proteins or cell signaling pathways than bulk tumor cells, making them identifiable within a tumor. These markers include ABC transporters, cell-cell matrix receptors, and several cell signaling receptors.

Considering the similarities between normal, healthy stem cells and cancer stem cells is crucial for CSC-targeting drug development. This idea is particularly important for chemists creating drugs that target ABC transporters. Normal stem cells have a relatively high gene expression of drug efflux transporters from the ABC gene family,[68] allowing them to efflux drugs and toxins across their plasma membranes. While they allow healthy stem cells to effectively preserve their genomes against chemical mutagens that can lead to carcinogenesis, CSCs have coopted this resistance to DNA damage. Instead of protecting a healthy genome, however, they use the preexisting DNA repair systems of normal stem cells to prevent their DNA repair, thus preserving a genome that is conducive to

[68] Morrison, et al., 2011

carcinogenesis.[69] The high CSC expression of these transporters has been used to identify CSCs within a tumor, making them obvious drug targets within a cluster of cancer cells.[70] Drugs that block the function of efflux transporters or down-regulate their expression potentially have the ability to circumvent CSC chemoresistance mechanisms.[71]

5b. Drugs that target CSC signaling pathways

Signal transduction pathways that have well-established roles in the development and progression of tumors have recently found to be critical for CSC generation, differentiation, and drug resistance. Among these signaling pathways are Notch1 and Notch4, which have been implicated in breast cancer and leukemia; Wnt signaling, which is implicated in nearly every cancer type; and the hedgehog signaling pathways, which also is implicated in a broad range of cancers.

Notch: The Notch1 and Notch4 signaling pathways have been implicated in breast cancer and acute lymphoblastic leukemia; their unique levels of expression has aided chemists in the identification and isolation of breast CSCs.[72] Isolated CSCs from breast cancer are enriched for Notch4 and deficient in Notch1,[73] which presents potential therapeutic targets. Experimentally, inhibiting the Notch4 signaling pathway has prevented tumor formation.[74]

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[69] Morrison, et al., 2011
[70] Gatti L, Beretta GL, Cossa G, Zunino F, Perego P. (2009). ABC transporters as potential targets for modulation of drug resistance. *Mini-Reviews in Medicinal Chemistry,* 9(9), pp. 1102–1112.
[71] Gatti, et al., 2009
[72] Harrison H., Farnie G., Howell S.J., Rock R.E., Stylianou S., Brennan K.R., Bundred N.J., Clarke R.B. (2010). Regulation of breast cancer stem cell activity by signaling through the Notch4 receptor. *Cancer Research, 70*(2), pp. 709-718.
[73] Harrision, et al., 2010
[74] Real P.J., Ferrando A.A. (2009). NOTCH inhibition and glucocorticoid therapy in T-cell acute lymphoblastic leukemia. *Leukemia, 23*(8), 1374-1377.

Real et al's (2009) in vitro study of drugs that inhibit Notch1 signaling with gamma-secreatase inhibitors (GSIs) for treatment of t-cell acute lymphoblastic leukemia (T-ALL) demonstrates the effectiveness of anti-Notch1 therapies against T-ALL. T-ALL, while having a higher cure rate than the past with improved chemotherapy drugs, is highly associated with chemotherapy resistance and relapse. The chemotherapy drugs glucocorticoids play an integral role in lymphoid tumor treatment with their ability to induce apoptosis in lymphoid stem cells. However, patients with ALL in relapse have increased resistance to therapy with glucocorticoids. This resistance is chiefly due to immature T-cell resistance to glucocorticoid-induced cell death as a result of constitutive activation of Notch1 signaling. This clinical trial tested the effectiveness of GSIs in sensitizing the Notch1 pathway in ALL CSCs to glucocorticoids. It was found that inhibition of the Notch1 pathway by GSIs triggers a more robust response to glucocorticoid-induced apoptosis in T-ALL cells that would be otherwise resistant. However, while GSIs are effective inhibitors of the Notch1 pathway in cancer stem cells, making them effective anti-CSC agents, they often result in the inhibition of Notch signaling in the intestinal tract, leading to gastrointestinal toxicity. In the study, it was found that co-treatment with glucocorticoids inhibited this GSI-induced toxicity.[75] This issue also illustrates the importance of a drug delivery system that allows for specific targeting of cancer stem cells.

[75] Real, et al., 2009

Wnt/β-catenin pathway: The Wnt/β-catenin pathway has been implicated in the initiation and progression of many types of leukemia,[76] gliomas,[77] and colon cancer.[78] According to Dr. Joerg Huelsken, understanding the Wnt signaling pathway, in addition to other modes of cellular signal transduction, is critical to understanding the mechanisms of CSC tumorigenesis. Dr. Daniel Constam articulated the importance of the Wnt pathway in CSC differentiation and proliferation, indicating that its upregulation with mutations can lead to tumorigenesis. Mutations in genes that control the Wnt pathway have correlated to several types of cancers; the pathway, for example, leads to increased expression of anti-apoptotic genes.[79] This signaling pathway is critical for regulation of stem cell survival, and has been implicated in residual cancer following radiation therapy.[80] Thus, inhibition of the Wnt/β-catenin pathway shows promise in reducing the genomic instability and drug resistance that results from its upregulation. Several natural compounds have been found to be effective at inhibiting this signaling pathway, including piperine and curcumin.[81] Previously, both had been effectual in

[76] Barker, N. and Clevers, H. (2006). Mining the Wnt pathway for cancer therapeutics. *Nature Reviews Drug Discovery, 5*(12), pp. 997–1014.
[77] Shiras, A., Chettiar, S., Shepal, V., Rajendran, G., Prasad, G. R. , and Shastry, P. (2007). Spontaneous transformation of human adult nontumorigenic stem cells to cancer stem cells is driven by genomic instability in a human model of glioblastoma. *Stem Cells, 25*(6), pp. 1478–1489.
[78] Hadjihannas, M. V., Brückner, M., Jerchow, B., Birchmeier, W., Dietmaier, W., Behrens, Aberrant J. (2006). Wnt/β-catenin signaling can induce chromosomal instability in colon cancer. Proceedings of the National Academy of Sciences of the United States of America, 103(28), pp. 10747–10752.
[79] Huelsken, Joerg. Personal Interview. 25 Oct 2014.
[80] Yu, et al., 2012
[81] Kakarala M., Brenner D.E., Korkaya H., Cheng C., Tazi K., Ginestier C., Liu S., Dontu G., Wicha M.S. (2010) Targeting breast stem cells with the cancer preventive compounds curcumin and piperine, *Breast Cancer Research and Treatment, 122*(3), 777-785.

preventing a broad range of cancers.[82] [83] In addition, it was found that piperine increases the bioavailability of curcumin, giving the combination additional therapeutic efficacy.[84]

The Wnt/ β-catenin pathway has been seen to promote genetic instability and tolerance to DNA damage, which is part of a positive feedback loop in CSCs as mutations in DNA accumulate.[85] This instability continues to be promoted after radiation therapy, allowing cancer cells to develop adaptive mutations and survive treatments. Inhibitors of the Wnt signaling pathway have been developed in laboratories to prevent the tumorigenic ability of CSCs. Among these molecules are monoclonal antibodies against two specific Wnt pathways.[86] In a clinical trial of CSC drugs at the University of Colorado School of Medicine Gates Center for Regenerative Medicine and Stem Cell Biology, a patient who received a Wnt inhibitor drug has been four years in remission from a stage 4 diagnosis with no side effects.[87]

Hedgehog: According to a study by Merchanct, et al (2010), the Hedgehog (Hh) signaling pathway has additionally been implicated in a wide variety of

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[82] Goel A., Aggarwal B.B. (2010) Curcumin, the golden spice from Indian saffron, is a chemosensitizer and radiosensitizer for tumors and chemoprotector and radioprotector for normal organs. *Nutrition and Cancer, 62*(7), 919-930.
[83] Singh S., Khar A. (2006). Biological effects of curcumin and its role in cancer chemoprevention and therapy. *Anticancer Agents in Medicinal Chemistry, 6*(3), 259-70.
[84] Shoba G., Joy D., Joseph T., Majeed M., Rajendran R., Srinivas P.S. (1998). Influence of piperine on the pharmacokinetics of curcumin in animals and human volunteers. *Planta Medica, 64*(4), pp. 353-356.
[85] Eyler, et al., 2008
[86] Pang ,R.W.C. and Poon, R.T.P. (2007). From molecular biology to targeted therapies for hepatocellular carcinoma: the future is now. *Oncology, 72*(1) pp. 30–44.
[87] Gaines, 2014

cancers.[88] While early clinical trials with Hh inhibitors have validated the pathway as an anti-cancer target, several biological mechanisms of the Hh signaling sequence remain unclear. However, previously unexplained clinical phenomena can be partially accounted for by Hh signal transduction in the self-renewal of CSCs and development of metastatic disease. Additionally, inhibitors of the Hh signaling pathway are one among the few agents that are formally examining the CSC hypothesis in the clinical setting.[89]

5c. Nanoparticle drug delivery

Nanoparticle drug delivery offers significant advantages over small molecule pharmaceuticals used in clinical practice. Among its benefits is a reduced risk of off-target toxicities, which are common among drugs that target signaling pathways that are utilized in both CSCs and normal cells.[90] Furthermore, they address the issue of CSC drug resistance by sequestering drug agents at a high concentration and releasing them within CSCs following cellular uptake, overcoming their drug efflux resistance mechanisms.[91] Thus, nanoparticle drug delivery shows promise in reducing the financial burden of drug resistance and hospital admissions due to toxicity.

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[88] Merchant, A., & Matsui, W. (2010). Targeting Hedgehog - a Cancer Stem Cell Pathway. *Clinical Cancer Research: An Official Journal of the American Association for Cancer Research, 16*(12), 3130-3140. Retrieved November 10, 2014, from PubMed.
[89] Merchant, et al., 2010
[90] Shapira, A., Livney, Y.D., Broxterman, H.J., Assaraf, Y.G. (2011). Nanomedicine for targeted cancer therapy: towards the overcoming of drug resistance. Drug Resistance Updates, 14(3), pp 150-163.
[91] Burke, A., Singh, R., Carroll, D., Torti, F., & Torti, S. (2012). Targeting Cancer Stem Cells with Nanoparticle-Enabled Therapies. *Journal of Molecular Biomarkers and Diagnosis, (8),* n.p.

In one study done by Lim et al, the effectiveness of a sugar-encapsulated nanoparticle formulation for brain tumor treatment was investigated.[92] Curcumin, a natural anti-CSC agent, works by inhibiting the Wnt pathway. Its bioavailability was greatly increased when added to the nanoparticle formulation, increasing the rates of CSC cell cycle arrest and apoptosis.[93] A >50 percent decrease in the CSC populations were observed with this treatment, indicating that it has activity against CSC activity of some brain tumors.[94]

It is clear that a systematic approach is necessary for CSC-targeted drug delivery, taking into account the interactions of drugs with healthy cells. According to Dr. Patrick Gaines, general drug delivery is necessary to eliminate all metastatic sites of cancer. It is likely that a systemic but targeted approach to CSC destruction will prove most effective; nanoparticles accomplish both. However, several other biomedical technologies also show promise in achieving drug delivery targeted to CSCs; thus, further research is to be done in this area.

Conclusion

The burden of cancer on individuals, their families, healthcare systems, and the economy cannot be ignored. While chemotherapy, radiation, and surgical procedures are continually advancing and improving cancer survival rates, relapse and metastasis have not been addressed. The toxicity and other side effects of traditional cancer treatments cause physiological and psychological stress on

[92] Lim, K.J., Bisht, S., Bar, E.E., Maitra, A., Eberhart, C.G. (2011). A polymeric nanoparticle formulation of curcumin inhibits growth, clonogenicity and stem-like fraction in malignant brain tumors. *Cancer Biology and Therapy, 11*(5), p.p. 464–473.
[93] Lim, et al., 2011
[94] Lim, et al., 2011

patients, indicating a need for less harmful, more effective cancer treatments. The cancer stem cell hypothesis has shifted the focus of researchers from chemotherapeutic agents to drugs that target the only tumorigenic cells in cancer: cancer stem cells. Because chemotherapy fails to eliminate CSCs, more malignant, drug-resistant cancer cells often proliferate in metastatic sites or relapse years later, making patients' prognoses unfavorable. The cancer stem cell hypothesis is now widely accepted as a model for tumorigenicity, indicating a need for CSC-targeted therapies. In recent years, researchers have been working to identify CSC signatures that are targetable by drug agents. Several drugs are undergoing clinical trials, and have shown promise in eliminating CSCs and leading to remission without relapse. To reduce the global cancer burden, it is imperative that CSC-targeted therapies be trialed and developed by pharmaceutical companies.

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ISP Work Journal

20 October 2014

Preliminary Research, United Nations Library in Geneva, Switzerland

- Finalized topic, began outline of paper

21 October 2014

Preliminary research, UN Library

- Emailed doctors Keith Brennan (University of Manchester), Douglas Hanahan (EPFL), Peter ten Dijke (Leiden University Medical Center), and Patrick Gaines (University of Colorado Gates Center)
- Began collecting journal articles on failures of chemotherapy from Santa Clara University Databases

22 October 2014

Preliminary research, UN Library

- Received referral from Dr. Douglas Hanahan to contact Dr. Joerg Huelsken (EPFL)
- Emailed Dr. Joerg Huelsken, who researches Wnt and Notch signaling pathways in cancer stem cells, for permission to conduct formal interview
- Continued collecting scholarly articles on chemotherapy failures, primarily through the PubMed database

23 October 2014

Preliminary research, UN Library

- Patrick Gaines accepted interview request; formulated interview questions, schedule time for 13 November 2014 at 20.00
- Emailed Brennan, ten Dijke, and Huelsken for the second time, with requests to interview

24 October 2014

Preliminary research, SIT Office Nyon

- Completed initial outline of paper
- Collected journal articles on potential CSC-targeting therapies

27 October 2014

Research, UN Library

- Joerg Huelsken accepted interview request, proposes interview to be in Lausanne on 3 November 2014 at 16.00
- Formulated questions for interview with Dr. Huelsken

28 October 2014

Research, UN Library

- Incorporated scholarly articles and other sources into my initial outline
- Emailed Freddy Radtke (EPFL), asking for permission for formal interview

29 October 2014

Research, UN Library

- Received permission from Freddy Radke for interview, proposes interview to take place at EPFL on 5 November 2014 at 13.00

- Wrote introduction of paper, continue collecting scholarly articles and incorporating them into my outline

30 October 2014

Attend the seminar *Studying therapy escape mechanisms in a mouse model for BRCA1-mutated breast cancer,* conducted by Sven Rottenber, Institute of Animal Pathology, Bern, and hosted by Joerg Huelsken

- This seminar helped me gain background information on drug resistance mechanisms of CSCs, though some aspects of it were difficult to understand
- I had the opportunity to speak with Joerg Huelsken after the seminar about his work, which led me to revise my interview questions for him, making them more specific to his research

31 October 2014

Writing, UN Library

- Drafted an initial literature review for paper, detailing the scientific nature of the vast majority of my sources
- Drafted a rough methodology section of my paper

3 November 2014

Interview with Dr. Joerg Huelsken, École Polytechnique Fédérale de Lausanne, 16.00-16.20

- Gave me additional interview contacts, Dr. Daniel Constam (EPFL) and Dr. Cathrin Brisken (EPFL)

Emailed Dr. Constam and Dr. Brisken, asking for permission to interview them and mentioning referral from Dr. Huelsken

4 November 2014

Writing, UN Library

- Began writing the section of my paper that details potential CSC-targeting therapies, including information from my interview with Dr. Huelsken
- Received reply from Dr. Constam, proposing interview on 12 November 2014 at 14.00 in Lausanne

5 November 2014

Interview with Freddy Radtke, École Polytechnique Fédérale de Lausanne, 13.00-13.45

- Was asked to review section of paper where I paraphrase him to make sure information is accurate

Writing, Lausanne

- Continued writing CSC-targeted therapies section of paper, including interview with Dr. Radtke
- Sent this section to Dr. Radke

6 November 2014

Writing, UN Library

- Received approval from Dr. Radke to include section where I paraphrase him in my paper
- Completed draft of CSC-targeted therapies section

7 November 2014

Writing, UN Library

- Began writing the section of my paper on failures of chemotherapy
- Emailed Dr. Brisken again, following up about my previous email regarding an interview

10 November 2014

Writing, UN Library

- Continued writing the failures of chemotherapy section of my paper
- Received a reply from Dr. Brisken, proposing an interview on 14 November 2014 at the Starbucks near the Lausanne train station, at 17.00

11 November 2014

Writing, UN Library

- Completed draft of the failures of chemotherapy section of my paper
- Began drafting the cancer stem cell hypothesis from a global health perspective section of my paper

12 November 2014

Personal Interview with Dr. Daniel Constam, EPFL, 14.00-14.20

- Added information from this interview to my section on CSC-targeting therapies

13 November 2014

Phone interview with Dr. Patrick Gaines, 20.00-21.00

- Added information from this interview to my section on CSC-targeting therapies, as well as my section on the CSC hypothesis from a global health perspective

14 November 2014

Seminar: *Regulation of metabolism to allow tumor growth,* EPFL, 10.00, conducted by Matthew vander Heiden, MIT, The Koch Institute for Integrative Cancer Research, hosted by Etienne Meylan

- This seminar didn't directly address my topic, but it gave me valuable insight into mechanisms of tumor growth

Personal interview with Dr. Cathrin Brisken, Starbucks Lausanne, 17.15-17.45

- Added information from this interview to my section on CSC-targeting therapies

17 November 2014

Editing, UN Library

- Compiled all sections of paper
- Wrote conclusion, finalized bibliography

18 November 2014

Final editing of paper

Interviews

Dr. Joerg Huelsken
École Polytechnique Fédérale de Lausanne
3 November 2014

1. Which cell signaling pathways have you worked with as potential drug agent targets in CSCs?
I work with the Wnt gene family, which are involved in controlling cell growth and differentiation.

2. How does this cell signaling pathway in CSCs vary from that in normal cells?
We have found that genes that encode this pathway are mutated in cancer cells, indicating that mutations can cause healthy stem cells to become cancerous stem cells.

3. When developing drugs that could potentially destroy CSCs via the Wnt pathway, how do you avoid damaging healthy cells?
This is one of the biggest challenges in CSC research. CSCs share many characteristics with healthy cells, making systemic delivery of drugs challenging. Certain drug delivery methods, such as nanoparticle delivery, have shown promise in reducing toxicity. However, systemic delivery is possible if treatment is individualized and specific mutations in the Wnt pathway are identified.

4. How does the CSC hypothesis illustrate the interaction between public health and medicine?
CSC-targeted drugs show potential in eliminating toxic side effects and impact public health. Currently, many patients are hospitalized due to toxicity from chemo or radiotherapy, indicating a need for less harsh cancer pharmaceuticals.

5. How would such a dramatic shift as cancer treatments heavily reliant on chemotherapy to treatments that target CSCs happen in a reasonable amount of time?
Right now a lot of the research on CSCs is still being done in the lab. We have seen Phase I and II clinical trials happening, which have shown promise in reducing relapse rates. We need to see more of these types of trials and get these drugs on the market for use in common practice. One barrier faced is the incentive for pharma companies to continue developing chemotherapeutic agents, because they profit from the sale of such drugs.

Dr. Freddy Radtke
École Polytechnique Fédérale de Lausanne
5 November 2014

1. How does an understanding of cellular mechanisms of normal stem cell differentiation help us better understand CSCs?
Self-renewing cellular systems are continually differentiating from stem cell reservoirs, similar to how bulk tumor cells differentiate from CSCs.

2. How do the processes of self-renewal and differentiation in CSCs vary from those of normal stem cells?
In normal stem cells, these processes are tightly regulated. Differentiated cells are only produced from stem cells when there is a need for them. However, in CSCs, the cell signaling processes are less regulated because of mutations, disrupting homeostasis.

3. What cell signaling pathways in CSCs have you focused on?
We focus on the Notch signaling pathway in CSCs.

4. How could targeting CSCs with drugs impact the field of Global Health?
There are several ways. Once we develop drugs that successfully destroy CSCs, the risk of relapse is essentially zero. Same with metastasis. This would dramatically reduce healthcare costs, as subsequent rounds of chemotherapy after the first fails are costly and often unsuccessful. Also, when you reduce deaths, you reduce the burden cancer has on the economy; you don't lose the economic productivity of the person who has just passed away.

Dr. Daniel Constam
École Polytechnique Fédérale de Lausanne
12 November 2014

1. How does the Wnt signaling pathway impact CSC tumorigenesis?
Mutations in Wnt can create pro-differentiation signals, meaning that CSCs proliferate, while programmed cell death is downregulated

2. Why is it important to study such cell signaling pathways?
They have a lot of potential for drug therapy targets, and if they are effectively and specifically targeted, CSCs can be destroyed. Without CSCs, tumorigenesis is impossible.

3. What is the biggest problem of chemotherapy, in your opinion?
It doesn't target cancer stem cells. It's like removing the tip of the iceberg, while there is something much more dangerous beneath. Chemotherapy makes CSCs more dangerous by selecting for the most drug-resistant, tumorigenic ones.

Patrick Gaines
Phone Interview
13 November 2014

1. What are some of the clinical trials of cancer stem cell-targeting drugs that you have been involved with/overseen?
We have worked with hedgehog pathway inhibitors, Wnt inhibitors, and Notch inhibitors. These are pathways that are thought to be relied upon by CSCs.

2. Were the side effects of these drugs significant, and how do they compare to chemotherapy side effects?
Targeted therapies are generally better tolerated than chemotherapy. One of our first patients who received the Wnt inhibitor is now four years in remission from a stage 4 diagnosis without any side effects.

3. From what you have seen, which drug delivery methods that show promise for delivering CSC-targeting drugs to tumor sites (nanoparticle delivery, etc.)?
Systemic administration is required to get to all potential metastatic sites.

4. What are some of the challenges faced in developing therapies that target cancer stem cells?
Showing that the effect is cCSC-specific, and not just affecting generically all cells

5. Theoretically, what would the public health implications of widespread clinical use of CSC-targeting drugs be (given they are effective at destroying CSCs)? For example, how could lower rates of drug resistance among cancer patients positively impact populations?

Putative CSC therapies are being tested in combination with chemotherapy to decrease resistance. This would decrease toxicity and increase efficacy, thus prolonging life and avoiding admissions from toxicity.

Dr. Cathrin Brisken
Starbucks Lausanne
14 November 2014.

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1. What are some of the CSC-targeting therapies that have proven effective (in clinical trials) in destroying breast cancer stem cells?
This question is not in my field, as I work with female sex hormones and carcinogenesis, but these hormones impact cell signaling abilities of CSCs.

2. Why is it important to use living organisms in your research, rather than do experiments *in vitro?*
There are so many types of breast tissue, and they cannot all be replicated in the lab.

3. How have chemotherapy and radiotherapy been ineffective in the treatment of breast cancer?
They don't address the underlying problem of cancer; they just eliminate side effects of a deeper problem (CSCs, hormones, etc.). Radiotherapy for other types of cancer can actually cause mutations and lead to breast cancer later down the road.

4. Why should doctors switch to a more targeted approach to cancer?
Cancer is such an individual disease, and treatment varies so much from person to person. Signatures of different types of cancer have been identified that chemo and radiation do not address. They also have horrible side effects that could be eliminated with treatments that are directed to CSCs.