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Strategies to eliminate cancer stem cells: Clinical implications

Carol Ann Huff[a,*], William H. Matsui[b], B. Douglas Smith[c], Richard J. Jones[d]

[a]Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, Bunting-Blaustein Cancer Research Building, Room 209, 1650 Orleans St., Baltimore, MD 21231, USA
[b]Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, Bunting-Blaustein Cancer Research Building, Room 245, 1650 Orleans St., Baltimore, MD 21231, USA
[c]Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, Bunting-Blaustein Cancer Research Building, Room 246, 1650 Orleans St., Baltimore, MD 21231 , USA
[d]Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, Bunting-Blaustein Cancer Research Building, Room 207, 1650 Orleans St., Baltimore, MD 21231, USA

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ABSTRACT

Over the past two decades, major advances in our understanding of cancer have translated into only modest increments in survival for the majority of cancer patients. Recent data suggesting cancers arise from rare self-renewing stem cells that are biologically distinct from their more numerous differentiated progeny may explain this paradox. Current anti-cancer therapies have been developed to decrease the bulk of the tumour mass (i.e. the differentiated cancer cells). Although treatments directed against the bulk of the cancer may produce dramatic responses, they are unlikely to result in long-term remissions if the rare cancer stem cells are also not targeted. Conversely, treatments that selectively attack cancer stem cells will not immediately eliminate the differentiated cancer cells, and might therefore be prematurely abandoned if clinical activity is judged solely by traditional response criteria that reflect changes in the bulk of the tumour. Re-examining both our pre-clinical and clinical drug development paradigms to include the cancer stem cell concept has the potential to revolutionize the treatment of many cancers.

1. Introduction

More than 30 new anti-cancer drugs have been approved over the past two decades. Although approval required all of these drugs to show a clinical benefit, survival rates for most cancer patients have improved only marginally.[1] A cardinal principle of cancer therapeutics has been that objective clinical responses will translate into improved survival. The clinical development of new anticancer therapies usually proceeds in a stepwise manner. Initial clinical trials (termed phase I) are designed to identify safe doses and to begin to look for evidence of clinical activity. Phase II trials focus on identifying efficacy in specific tumour types, while phase III trials are designed to compare emerging treatments with the current standard therapy. Most new therapies will not be optimally effective alone and will require further stepwise development in combination strategies. The major advantage of using clinical response as the primary endpoint is that it is measurable over weeks to months, allowing the stepwise process of drug development to occur more rapidly and efficiently. In contrast, demonstrating a survival benefit adds significant complexity to trial design, usually requiring the accrual of large patient numbers and long follow-up to provide statistical significance.

* *Corresponding author:* Tel.: +1 410 502 7600/7200; fax: +410 502 7213.
E-mail address: huffca@jhmi.edu (C.A. Huff).
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2. Response and survival: the dirty secret of cancer therapeutics

Although objective clinical response is the most commonly used measure of therapeutic efficacy, there is surprisingly little evidence that response is a good surrogate for improved survival. The major rationale for using clinical response as the basis for measuring therapeutic efficacy is the premise that a complete remission must precede a cure. Since cure can currently be established only with long-term clinical follow-up, a complete remission will always precede verification of cure. There are, however, numerous examples in which response does not predict an improvement in survival. It has been known for many years that patients with follicular lymphoma[2] who achieve a complete remission do not experience a survival advantage over those treated with a 'watch and wait' approach. In multiple myeloma, neither the magnitude nor the speed of a clinical response has an impact on survival. Similarly, significant clinical responses in prostate,[3,4] pancreatic,[5] breast[6] and lung cancer[7] have only minimally impacted survival. Even when responses are associated with a statistical improvement in survival, the benefits are often incrementally small, often only a few weeks to months, as seen in a recently published phase III trial in lung cancer[7] and similar trials in prostate[8] and pancreatic[9] cancer. Understanding the basis for this apparent paradox, that treatment response and survival may not be linked, is essential for progress in the development of effective therapeutics.

3. Imatinib in chronic myeloid leukaemia: the model for targeted drug development

The cytogenetic hallmark of chronic myeloid leukaemia (CML), the Philadelphia chromosome, has been an obvious candidate for selective targeting.[10] The Philadelphia chromosome abnormality in CML is characterised by a reciprocal translocation between chromosomes 9 and 22, resulting in the transfer of c-ABL gene sequences from chromosome 9 to a site adjacent to the BCR gene sequences on chromosome 22. This translocation produces a 210-kD BCR–ABL fusion protein ($p210^{Bcr–Abl}$) that is a constitutively active tyrosine kinase.[11] $p210^{Bcr–Abl}$ is not only specific for CML, but the genetic translocation also appears to be the initiating oncogenic event: mouse bone marrow transduced with a retrovirus encoding $p210^{Bcr–Abl}$ produces disease when transplanted into mice.[12,13] Thus, selective targeting of BCR–ABL should be the consummate therapeutic strategy for CML.

Imatinib was developed as part of a program to identify drugs that block the unregulated activity of protein kinases present in many cancers. Imatinib is a potent and relatively selective inhibitor of platelet-derived growth factor and the Abl tyrosine kinases,[14] including BCR–ABL.[15] Moreover, it demonstrated striking selective activity against CML cell lines and clinical progenitors in vitro, inhibiting >90% of CML progenitor growth at concentrations (1–10 μM) that had little activity against normal haematopoietic progenitors. Imatinib's clinical activity against CML has mirrored its in vitro activity,[16–18] and based on an interim analysis of a multicentre, randomised trial showing higher response rates for imatinib,[19] it has replaced interferon-α (IFN) as the standard-of-care for newly diagnosed CML patients.[20] Although follow-up is ongoing, to date this study has not shown a survival advantage for imatinib. Moreover, emerging data suggest that imatinib may not be able to completely eradicate CML. CML patients who achieve the best responses to imatinib (reverse-transcriptase polymerase chain reaction negativity for BCR–ABL transcripts) invariably relapse quickly when the drug is discontinued,[21] and many have evidence of progression despite remaining on the drug.[22]

4. Resistance to imatinib: molecular versus cellular mechanisms

Recent data are beginning to shed light on mechanisms responsible for clinical resistance to imatinib. Not only can BCR–ABL gene amplification or mutations prevent productive imatinib binding,[23] but secondary genetic mutations capable of driving BCR–ABL independent leukaemic growth may also be present, even at initial diagnosis.[24] However, these genetic mechanisms of resistance may be responsible for only a fraction of clinical imatinib failures. CML arises at the level of haematopoietic stem cells and, like their normal counterparts, CML stem cells undergo orderly differentiation. Differentiated cells constitute the bulk of the leukaemic mass in CML, whereas the stem cells responsible for disease maintenance are rare.[25] Several investigators have now provided evidence that imatinib has differential effects on CML cells depending on their state of differentiation: while imatinib is toxic to differentiated CML progenitors, CML stem cells may be relatively or even completely resistant to the drug.[26–28]

The basis for the differential activity of imatinib toward CML stem cells and their differentiated progeny is probably multifactorial.[27] CML stem cells share many biological properties with their normal counterparts,[25,29] that may limit the effectiveness of therapeutic strategies targeting BCR–ABL signalling. Haematopoietic stem cells are largely quiescent and normally express high levels of ATP-binding cassette (ABC) transporters, such as the multidrug resistance-1 gene[30] and ABCG2.[31] Both of these factors may limit the cellular uptake of imatinib, since it is a substrate for the ABC transporters.[32,33] Perhaps most importantly, BCR–ABL appears to have different effects on CML stem cells and their differentiated progeny.[27] The cellular expansion in CML occurs primarily in the differentiated progenitors, rather than the in stem cell pool.[25,34] Moreover, BCR–ABL expression appears to be required for the survival of CML progenitors, but the same does not appear to be true for CML stem cells where the BCR–ABL gene can be silent.[25,35–37] These data suggest that BCR–ABL may produce only subtle effects in CML stem cells, and thus its inhibition may similarly have only minor consequences for these cells.[27] Accordingly, primitive CML progenitors and their normal counterparts display similar resistance to imatinib.[27] Therefore, based on the longevity (possibly greater than 10 years) of their normal counterparts, CML stem cells probably survive for years even if BCR–ABL activity is completely inhibited[25,29]; eventually, because of intrinsic genomic instability, CML stem cells and their progeny may develop genetic resistance to imatinib.

5. Cancer stem cells and cancer therapeutics: the dandelion phenomenon

The rapid responses induced by imatinib in CML patients[19] are probably a consequence of its impressive activity against differentiated CML progenitors that make up the bulk of the leukaemia. Data suggesting that many of these early responses may not be durable[21,22] could be explained by CML stem cell resistance to imatinib. This pattern of activity is analogous to cutting a dandelion off at ground level; although this will eliminate the visible portion of the weed, the unseen root also needs to be eliminated to prevent regrowth of the weed.[10,27] Conversely, the slow, but often durable, response seen in IFN-treated patients[38] is consistent with activity directed principally at the rare CML stem cells.[27,39] This treatment effect mimics attacking only the root of the dandelion. Although this has no immediately discernible effect on the weed, over time the weed will eventually wither and die if its root has been eliminated.[10,27] Thus, treatments that selectively attack cancer stem cells will not immediately eliminate the differentiated tumour cells, and may be prematurely abandoned if clinical activity is judged solely by traditional response criteria that reflect changes in the bulk of the tumour.

The 'dandelion phenomenon' probably applies to other cancers too. The bulk of the tumour in multiple myeloma is comprised of neoplastic plasma cells.[40] These cells resemble their normal counterparts and lack replicative capacity. Within the tumour bulk, there is a rare population of myeloma stem cells, resembling post-germinal centre B cells, which have the ability to self-renew, differentiate, and maintain the disease. The novel anti-myeloma agents, bortezomib and lenalidomide, are quite active against the plasma cells in vivo, but appear to have little activity against myeloma stem cells in vitro.[41] This may explain why they have shown significant clinical activity, but cures are not seen. Conversely, rituximab (anti-CD20 monoclonal antibody) is very active against the myeloma stem cells in vitro,[42] but has little activity against the mature plasma cells, which lack expression of the target antigen. Although its activity in multiple myeloma has been disappointing,[43] parameters typically used to follow clinical response in myeloma (i.e. monoclonal immunoglobulin level and percentage plasma cells in the marrow) primarily measure the effect of therapies on the terminally differentiated plasma cells. The long survival of the myeloma plasma cells could have obscured activity against the myeloma stem cells responsible for maintaining the disease. Perhaps a longer duration of rituximab treatment could ultimately have demonstrated clinical responses using traditional criteria, by inhibiting new myeloma cell production for a sufficient period of time to allow terminally differentiated myeloma plasma cells to undergo spontaneous apoptosis.

Gemtuzumab, anti-CD33 antibody conjugated to calicheamycin, has been approved to treat acute myeloid leukaemia (AML). Although the leukaemic cells in AML express CD33, the stem cells more closely resemble normal haematopoietic progenitors[44] and may not express markers of more differentiated cells.[45,46] Clinical studies are also looking at antibodies directed at B cell markers, such as CD19 in acute lymphocytic leukaemia (ALL).[47,48] CD19 is a marker of differentiation and is not seen on most ALL stem cells.[49] Thus, targeting markers such as CD19 and CD33 in ALL and AML, respectively, is unlikely to effect a cure. Other cancers in which stem cells have been demonstrated include myelodysplastic syndrome,[50] breast cancer,[51] brain cancer[52,53] and lung cancer.[54]

Traditional response criteria measure tumour bulk and may not reflect changes in populations of rare cancer stem cells. Therapies that target mature cancer cells (i.e. the visible portion of the weed) may produce clinical improvement and dramatic responses. However, such therapies are unlikely to lead to cures unless the cancer stem cells (i.e. the roots) which are responsible for disease maintenance are also targeted.[10] Imatinib could even induce undetectable BCR–ABL expression by polymerase chain reaction without affecting the stem cells that represent less than 0.1% of the CML cell population.[25] Standard response criteria may not only overestimate the effect of therapy on the minute population of stem cells, but may also underestimate it, as with IFN in CML and rituximab in myeloma.

6. Targeting cancer stem cells

New drug development should focus not only on the drugs and their specific targets, but also on the pathogenesis and biology of the diseases being treated. For example, even when the initiating oncogenic event is definitively being targeted, as with imatinib in CML, inherent properties of cancer stem cells may make the target inaccessible or not necessary for cell survival. Cancer stem cells in CML,[25] AML,[44] ALL,[49] myeloma[40] and lung cancer[54] all biologically appear to be more like their normal counterparts than their differentiated progeny. Thus, therapy directed at stem cell-specific targets, rather than cancer-specific targets, may offer the best chance of eliminating cancer stem cells but at the expense of probably also eliminating their normal counterparts. Nevertheless, therapies with similar activity to the cancer stem cells and their normal counterparts should not necessarily be disregarded. For example, rituximab is very active against myeloma stem cells in vitro[40] and recent data suggest that inducing terminal differentiation of cancer stem cells may eliminate their self-renewal capacity,[27,40] but neither approach is specific for the cancer stem cells. However, myeloma and leukaemic stem cells originate from cells with self-renewal potential, but probably not from the most primitive haematopoietic stem cells.[25,29,55,56] If a treatment equally eliminated myeloma and leukaemic stem cells and their normal counterparts, the presence of more primitive normal stem cells could replenish the normal progenitor pool.

Because objective tumour response measures the differentiated cancer cells and not the rare cancer stem cells, survival remains the ultimate clinical endpoint of therapeutic efficacy. However, assessing survival requires long studies and large patient numbers, and is thus often impractical as the primary clinical endpoint. New clinical paradigms and methodologies are therefore needed. Consideration should be given to relying more heavily on pre-clinical modeling, to eliminating traditional measures of clinical response as trial endpoints, and to utilising novel statistical methods to evaluate activity on rare cancer stem cells.

A detailed knowledge of the effects of new treatments on cancer stem cells in the laboratory would greatly enhance

the development of clinical trials. However, healthy skepticism exists as to how well pre-clinical models reflect the actual clinical situation, and thus pre-clinical studies and clinical trials with new therapies often proceed somewhat in isolation. Pre-clinical models, such as immunodeficient mice and novel in vitro culture systems[40,44,51,52] for studying cancer stem cells, are being developed; emerging data suggest that many of these systems parallel the in vivo behaviour of cancer stem cells quite closely. Using the correct models, it may be possible to develop a detailed understanding of the mechanisms of action of new treatments, as well as strategies for optimising activity; this may allow a fully developed new approach to be taken directly from the 'bench to the bedside'.

Effective pre-clinical models for cancer stem cells may ease the task of clinical trial development, but will not eliminate the need for new clinical paradigms. For example, evaluating the efficacy of treatments against cancer stem cells should be possible by utilising them after debulking the differentiated cells that constitute the majority of the tumour. Such an approach could be considered in the many cancers where clinical debulking is successful, but transient. For example, IFN could be used in patients with CML after they have achieved remissions to imatinib, thus optimising the activity of both drugs against their respective targets: imatinib for the committed CML progenitors and IFN for the CML stem cells.[27] The primary endpoint of such a trial would be progression-free survival off imatinib with a secondary endpoint of serially measuring the CML stem cells in vitro. A similar trial design could be used with rituximab in myeloma, employing bortezomib for debulking. The fate of the cancer stem cells could also be assessed serially as a secondary endpoint using newly developed in vitro assays. A positive correlation between survival and in vitro cancer stem cell assays would validate the use of these assays for future clinical trials. Re-examining both our pre-clinical and clinical drug development paradigms to include the cancer stem cell concept has the potential to revolutionise the treatment of many cancers.

Conflict of interest statement

None declared.

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