Translating the Science of Cancer Dormancy to the Clinic

Julio A. Aguirre-Ghiso

ABSTRACT

The paradigm of metastasis has been significantly remodeled by the incorporation of cancer dormancy as a mechanism to explain long-term remission intervals followed by relapse. There is overall consensus on the potential impact of better understanding dormancy. Key cancer-cell autonomous and microenvironmental mechanisms might explain this biology and, in turn, the timing of metastasis. However, the approach and feasibility to apply this biology to clinical trials has been controversial. The discussion here provides insight into how these controversies are being resolved by the development of active clinical trials, thus bringing to reality opportunities to target cancer dormancy.

Consensus: Cancer Dormancy Is an Important Biological Process to Understand and Target

It is clear that the concept of cancer dormancy (1), and probably more so among those studying metastasis (2), is now common in discussions, articles, and research programs addressing the mechanisms of relapse. For some time the NCI and other funding and advisory agencies such as the Department of Defense and the National Breast Cancer Coalition have had the biology of cancer dormancy on their radar (3) and provocative questions, RFAs and topics in study sections include this theme. Further, as evidenced in the recent Cancer Grand Challenge initiative by the Cancer Research UK and the NCI, cancer dormancy is seen by the leadership in these organizations as the main problem in cancer that must be solved.

In the last 10 years publications on cancer dormancy have grown almost exponentially covering three main areas, angiogenic dormancy, cellular dormancy, and immune-mediated dormancy (1). Recently, the most prevalent topics focus on cancer dormancy at the cellular level, that is cancer cells entering a prolonged growth (G_0–G_1) arrest despite carrying promitogenic and prosurvival mutations, and how this process is controlled by stromal, vascular, and other cellular and extracellular matrix niches (4). Thus, the data points to an inextricable connection between cancer cells and the microenvironment in controlling cancer-cell dormancy. Further, a more detailed analysis is developing around the relationship between innate and adaptive immunity and cancer-cell dormancy (4).

Effort has been placed on using *in vivo* and/or *in vitro* 3D culture systems to discover ways to model cancer-cell dormancy, induce dormancy of cancer cells, or reveal vulnerabilities that would render them sensitive to new dormancy-specific therapies such as unfolded protein response inhibitors or when blocking adhesion signaling, to conventional antiproliferative therapies (5, 6). These include targeting processes such as autophagy, metabolism, stress signaling, nuclear receptors, adhesion signaling, and using drugs that reprogram the epigenome to induce and maintain dormancy (1). The available body of work and current active projects on dormancy clearly indicate that investigators see this stage of the metastatic cascade as a new opportunity to target the metastatic process. This is because the discovery that disseminated cancer cells (DCC) can originate very early in cancer evolution (7) and that early- or late-evolved DCCs can enter decades-long periods of dormancy, reshaped our notion of metastasis targeting. For the first time we gained insight into an unexpected biology, that is that cancer pauses its progression, revealing a window of opportunity for early intervention. Importantly, across several experimental and human studies it has become clear that even stage IV patients carry still-dormant cancer cells that coexist with growing lesions in the same or separate target organs (1). The implication is that even if a detectable lesion or lesions respond to therapy, undetectable DCCs evade therapy and persist only to later activate and complicate the patient's future prognosis. Thus, targeting dormant cancer may not only be applicable in the adjuvant setting but also in stage IV complementing antiproliferative, radiation, and/or surgical therapies. Metastasis has always been seen as the last battle for life and this notion has been focused mainly on the clinical reality that once lesions are overt they need to be removed by surgeons or targeted as best as possible by radiation, chemo, immune, or targeted therapies (7). While success stories always exist and there is continued progress, the hard truth is that metastatic disease once manifested as overt in a systemic fashion is very difficult to stop.

Controversy: Can We Ever Design a Cancer Dormancy-Targeting Clinical Trial to Prevent Relapse?

The question is how and when can we harness the biology of cancer dormancy to prevent and treat metastatic disease. Several questions emerge in these discussions: what biomarkers, systemic or cancer-cell specific can be used to find dormant cancer cells or niches that are in prodormancy versus proreactivation states? How do you design a dormancy-targeting clinical trial? How do we make sure we are "hitting" the target? How long does it take to read out a dormancy trial? The latter is the most troubling question for those in the clinical and pharmaceutical space because of the common paralyzing misconception that cancer dormancy trials must be only

Division of Hematology and Medical Oncology, Department of Medicine, Icahn School of Medicine at Mount Sinai, New York, New York. Department of Otolaryngology, Icahn School of Medicine at Mount Sinai, New York, New York. Department of Oncological Sciences, Icahn School of Medicine at Mount Sinai, New York, New York. Black Family Stem Cell Institute, Icahn School of Medicine at Mount Sinai, New York, New York. Tisch Cancer Institute, Icahn School of Medicine at Mount Sinai, New York, New York.

Corresponding Author: Julio A. Aguirre-Ghiso, Division of Hematology and Medical Oncology, Department of Medicine, Icahn School of Medicine at Mount Sinai, 1 Gustave L. Levy Place, New York, NY 10029-5674. Phone: 212–241–9582; Fax: 212–241–4096; E-mail: julio.aguirre-ghiso@mssm.edu

Cancer Res 2021;81:4673–5

doi: 10.1158/0008-5472.CAN-21-1407

adjuvant trials that take many years or a decade to complete and thus will be incompatible with industry timelines to support testing a new treatment.

The above questions have answers (or it is known where to look for them) and there are opportunities to start probing these concepts without running into cripplingly large budgets and long timelines of exclusively adjuvant trials. Nevertheless, more work is needed to check all the boxes to advance the approach of targeting cancer dormancy. While labs push the boundaries of research forward finding new targets in dormant cancer cells, critical effort will be needed to provide human sample correlate analysis (beyond the customary Kaplan–Meier curve) for these targets on DCCs or host compartments in patients with residual disease. The limited analysis of samples during residual disease seems to be more common in solid cancer analysis than in hematologic malignancies where probing the bone marrow to profile residual multiple myeloma or leukemia cells is a more common practice. Thus, as we discover targets and markers of dormancy or signals from the niches that induce or reverse dormancy, these should be tested in human specimens. Some of these examples have been reviewed recently (4). For example, a recent study showed (8) that detection of the nuclear receptor NR2F1, a dormancy marker and potential target identified in experimental head and neck, prostate, and breast cancer systems, can stratify DCCs into $NR2F1^{high}$ and $NR2F1^{low}$ profiles. This profiling of residual disease in patients with breast cancer revealed that patients that had $NR2F1^{high}$ DCCs had longer bone metastasis–free periods than patients that carried $NR2F1^{low}$ DCCs. This could be simply attributed to the growth arrest function of NR2F1, because its expression was inversely correlated with Ki67 expression. However, Ki67 detection in parallel samples could not inform on the rate of metastatic relapse. Thus, dormancy markers appear to provide information on not only proliferative capacity but also on additional transcriptional programs that maintain DCC phenotypes for long periods. Another approach to validate in patients' data from experimental dormancy signatures from a pancreatic cancer mouse models was recently reported (9). The authors performed single-cell RNA sequencing (RNA-seq) from primary tumors and from liver samples resected during primary lesion surgery that were apparently normal and that contained DCCs. This approach, although in a small number of patients, showed that dormancy signatures in the experimental models were traceable in human solitary DCCs in patients. These examples show how the biology of dormancy from research efforts can be probed and validated in human samples. Another approach is testing whether the host is able to produce the signals that promote dormancy and if present or absent, how do these inform on patient outcomes. In a recent study, it was demonstrated that $NG2^{+}$/$Nestin^{+}$ mesenschymal stem cells (MSC) that control hematopoietic stem cell (HSC) dormancy in mice control dormancy of breast cancer DCCs by producing produce BMP7 and TGFβ2. Analysis of patient samples from a clinical trial where bone marrow supernatants were collected through the course of treatment (10) showed that patients that had bone metastatic relapse, more commonly had no detectable levels of TGFβ2 and BMP7 in the bone marrow supernatant. Analysis of the metastasis-free proportions showed that patients with breast cancer positive for BMP7 in the bone marrow supernatant lived longer without metastasis than those that had undetectable BMP7. These data further support that not only DCCs may be a source of information but surrogate measures of functional prodormancy niches (producing TGFβ2 and BMP7 for example) may also inform on the state of dormant cancer cells in patients (10). Understanding how the niches that harbor DCCs change under specific circumstances (e.g., aging, immunosuppression, inflammation) is key to designing strategies to correct the niche function.

The above and other examples suggest that there are clear opportunities to probe the biology of dormancy in patient samples and obtain useful information. But have there been any clinical trials designed around the biology derived from cancer dormancy studies? The answer is yes. For example, a Phase II clinical trial was motivated by findings showing that that during dormancy, cancer cells can activate autophagy (1). This trial takes advantage of available FDA approved autophagy inhibitors [e.g., hydroxychloroquine (HCQ)] in combination with mTOR inhibitors to treat patients with breast cancer during remission phases and thus prevent locoregional or distant recurrences (clinical trial identifier: NCT03032406). Another study (clinical trial identifier: NCT04841148) will test safety and early efficacy of HCQ or a PD-L1 blocking antibody, with or without a CDK4/6 inhibitor, in patients with early-stage ER^{+} breast cancer. The patients to be treated are those who harbor DCCs in the bone marrow after definitive surgery and standard adjuvant therapy and thus are at risk of relapse. This trial appears to be designed following the notion that autophagy is turned on in dormant cancer cells fulfilling a survival function and that there are available drugs to inhibit this pathway. Further, the use of CDK4/6 inhibitors may allow creating a vulnerable population of DCCs to HCQ or PD-L1 targeting. There is ample evidence that mTOR inhibitors induce autophagy across various cancer types and thus, autophagy inhibitors may show improved combinatorial DCC killing effect, but only in cells that maintain this pathway active (as tested in NCT03032406). CDK4/6 inhibitors, although less studied as autophagy inducers, can also activate this catabolic pathway in some cancer types and thus, there may be promise in arresting still-proliferative DCCs and then sensitizing them to cell death with HCQ or other autophagy inhibitors. A similar trial design could be used in a short term clinical trial by testing dormancy markers as intermediate clinical endpoints for ER^{+} breast cancer to resolve the question of whether for example treatment with letrozole and/or CDK inhibitors induce dormancy of residual circulating tumor cells (CTC) and DCCs and whether dormancy cues that can be detected in circulation inform on potential prodormancy niches. These trials could be done by sampling patients under current standard of care in a year or less. Importantly, a separate clinical trial is attempting to screen for the presence of bone marrow DCCs in patients within 5 years after having being treated for breast cancer (clinical trial identifier: NCT02732171). One of the trial's goals is to have patients that have DCCs in their bone marrow and therefore at a higher risk of developing metastasis to be offered to participate in other trials targeting DCCs such as NCT03032406. Studies on dormancy and mobilization of DCCs from the bone marrow (1) led to a new Phase Ib trial (clinical trial identifier: NCT04197999) for GMI-1359, a small molecule, which concomitantly blocks E-selectin and CXCR4 function, both molecules implicated in DCC BM retention as well as metastatic progression. While safety is the main objective of this trial, other secondary objectives include pre and postdose CTC-enumeration to determine tumor-cell mobilization. Such trials could be adapted to understand the frequency of dormant DCCs in stage IV patients and to validate markers of dormancy discovered in the lab. Along the same lines this trial could also be used to test whether GMI-1359 affects dormancy pathways. Other trials such as NCT00072020 or NCT00172068 where zoledronic acid was found to reduce bone metastasis and to improve disease outcomes for women with established menopause or to eliminate DCCs from the bone marrow with a concomitant decrease in bone metastasis, respectively may be useful to test for dormancy markers in collected biospecimens that carry DCCs

or to probe for prodormancy cues produced by bone marrow (BM) niche cells as discussed above (10). This way questions on how dormancy is modulated by current therapies may also be answered. Using similar approaches for biomarker readouts as in (8–10) in a relatively short clinical trial samples could be collected before and after initiating the letrozole and/or CDK inhibitor treatment. These are a few examples on how clinical trials can be designed following the biology revealed from studying cancer dormancy. Additional information on these trials can be obtained following the provided identifiers.

It was shown that dormancy is defined by a specific epigenetic state and that malignant DCCs can be reprogrammed into this phenotype (ref. 8 and references within). These data led to a clinical trial where disseminated PCa residual disease that is not yet detectable by imaging (biochemical or PSA-detected recurrence) is reprogrammed into dormancy (clinical trial identifier: NCT03572387). This clinical trial repurposed 5-azacytidine and *all-trans* retinoic acid (both FDA-approved drugs) to treat patients with PCa after hormonal ablation. This study will also enumerate CTCs and detect NR2F1 (a gene induced by the 5-azacytidine and *all-trans* retinoic acid therapy; ref. 8 and references within) in these CTCs to determine the reprogramming success. In addition, plasma levels of cues that induce dormancy (1) such as LIF, GAS6, BMP4, and BMP7 are also measured to determine the systemic prodormancy or proreactivation state of the host upon treatment. This is proof that with careful thinking, the basic science on cancer dormancy can be developed into innovative trials that do not represent unsurmountable financial hurdles. These trials could be adapted to also use ctDNA or sequencing of DCC genomes to understand how dormancy and the used therapies shape the evolution of disseminated residual disease. Overall, identifying targets and how to read them out, developing markers and methods to detect the DCCs and dormancy- or reactivation-supportive niches during minimal residual disease in trials will help better understand how to target these residual cancer cells based on their unique biology. The ongoing work in this space will likely impulse a new era of clinical trials tailored to target dormant cancer cells in all their manifestations and put an end to the controversies associated with this path that is no longer theoretical and is being reduced to practice.

Authors' Disclosures

J.A. Aguirre-Ghiso reports grants and other support from HiberCell LLC outside the submitted work.

Acknowledgments

This work was supported by grants from NIH/NCI CA109182 and The V-Foundation. J.A. Aguirre-Ghiso is a Samuel Waxman Cancer Research Foundation Investigator.

Received May 4, 2021; revised June 16, 2021; accepted July 8, 2021; published first August 24, 2021.

References

1. Risson E, Nobre AR, Maguer-Satta V, Aguirre-Ghiso JA. The current paradigm and challenges ahead for the dormancy of disseminated tumor cells. Nature Cancer 2020;1:672–80.
2. Summers MA, McDonald MM, Croucher PI. Cancer cell dormancy in metastasis. Cold Spring Harb Perspect Med 2020;10:a037556.
3. Vessella RL, Pantel K, Mohla S. Tumor cell dormancy: An NCI workshop report. Cancer Biol Ther 2007;6:1496–504.
4. Phan TG, Croucher PI. The dormant cancer cell life cycle. Nat Rev Cancer 2020;20:398–411.
5. Aguirre-Ghiso JA. Methods of treating minimal residual cancer. USA2019 03.10.2019.
6. Carlson P, Dasgupta A, Grzelak CA, Kim J, Barrett A, Coleman IM, et al. Targeting the perivascular niche sensitizes disseminated tumour cells to chemotherapy. Nat Cell Biol 2019;21:238–50.
7. Klein CA. Cancer progression and the invisible phase of metastatic colonization. Nat Rev Cancer 2020;20:681–94.
8. Borgen E, Rypdal MC, Sosa MS, Renolen A, Schlichting E, Lonning PE, et al. NR2F1 stratifies dormant disseminated tumor cells in breast cancer patients. Breast Cancer Res 2018;20:120.
9. Dudgeon C, Harris CR, Chen Y, Ghaddar B, Sharma A, Shah MM, et al. A novel model of pancreatic cancer dormancy reveals mechanistic insights and a dormancy gene signature with human relevance. bioRxiv 2020:2020.04.13.037374. DOI: 10.1101/2020.04.13.037374.
10. Nobre AR, Risson E, Singh DK, Di Martino JS, Cheung JF, Wang J, et al. Bone marrow NG2+/Nestin+ mesenchymal stem cells drive DTC dormancy via TGF-β2. Nature Cancer 2021;2:327–39.