Perspective

Challenging Roadblocks to Cancer Cure

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Abstract

The Pezcoller Symposium in Trento, Italy, June 2015, focused entirely on the question of why advanced cancer cure is so uncommon despite the extraordinarily rapid growth of invaluable therapeutic information. Participants were asked to define and to critically evaluate real and potential obstacles to permanent disease eradication. High-level concepts on potential road blocks to cures as well as opportunities for intervention in diverse areas of investigation ranging from genomic alterations to metabolism, microenvironment, immunity, and mechanotransduction were discussed. Provocative concepts and novel therapeutic avenues were proposed. What follows is a critical analysis of the highlights of this meeting. *Cancer Res; 76(17); 1–7. ©2016 AACR.*

Introduction

Although cures or long-term, disease-free survival can be achieved in certain cancers such as CML and testicular cancer, others, particularly solid tumors, have eluded them altogether. On the other hand, extended survival without definitive cure has been achieved in many malignancies, either as a result of prevention or chronic therapeutic approaches (e.g., prostate and breast cancer). Therapies range from traditional chemotherapeutic agents to targeted approaches with small molecules, to biological and, most recently, powerful immunotherapeutic approaches.

Tumors also range in pathogenetic mechanisms, which, in turn, may affect therapeutic responsiveness. Monogenic alterations, such as certain genomic translocation-driving leukemias and non–small cell lung cancers, are potentially treatable with targeted agents. In contrast, most solid tumors with their complex genetic alterations, their heterogeneity, clonal diversity, and intricate microenvironments have proven to be a much more arduous therapeutic problem.

Because of the difficulty in addressing cancers that are so diverse and resourceful, some have advocated a goal of turning lethal cancers into chronic diseases rather than curing them. Others would argue that this is a defeatist approach that sets a "low bar" and avoids decisive endpoints. For instance, improving drug potency through rigorous medicinal chemistry approaches [see recent successes with more powerful inhibitors of the androgen receptor (1)] or using more sensitive analyses (e.g., increasing sequencing depth or utilizing novel bioinformatics approaches), to identify low-frequency clonal events in primary tumors prior to their expansion (2), may avoid early-onset drug resistance and/or provide a rationale for the use of suitable combination therapy in a primary setting. As an example of the effect of heightened

efficacy, the potency of bcr-abl inhibition in chronic myelogenous leukemia (CML) has been increased by an order of magnitude by medicinal chemistry approaches that led to the synthesis of nilotinib, resulting in an approximately 30% increase in the molecular response rate over that observed with imatinib (3). That said, nilotinib resistance remains a clinical problem. Thus, Novartis scientists have developed an optimized, non-ATP binding site–targeted allosteric inhibitor of the bcr-abl kinase. It does not compete with ATP binding to the enzyme and has resulted in powerful responses in imatinib/nilotinib–resistant CML (4). Allosteric inhibitor–resistant cells have emerged in CML cells as well. Using an elegant bar-coding strategy, rare resistant clones, the vast majority of which were present prior to therapy, were identified as a series of low-frequency clonal events. The cells escaped detection even when sought by deep sequencing and required the above-noted biological selection/bar-coding strategy to be identified (5). The data also suggest that the use of a combination of ATP-binding site–directed and an allosteric inhibitor in the initial treatment phase of CML can eliminate rare clones that are resistant to both therapeutic modalities, perhaps improving clinical outcome.

Whatever the case, these findings reflect a need for methodologies that can detect rare, resistant clones in patients' cancers, both before therapy decisions are made and as therapy progresses. Although the clinical development of this practice will represent a technological challenge, its availability could significantly improve treatment outcome, much as in the case of bacterial infection therapy.

Aneuploidy

Because aneuploidy is a recognized hallmark of cancer, dissecting the mechanisms underlying its genesis may result in the discovery of novel therapeutic targets. Surprisingly, ample evidence suggests that aneuploidy does not provide a growth advantage to cells but instead may result in decreased fitness (6). One reason for this is that chromosome missegregation causes DNA damage and p53 activation, which in turn prevents cell-cycle progression. More importantly, aneuploidy is responsible for significant changes in protein composition, which, in turn, result in dysregulation of many cellular pathways.

In cancer, however, it is believed that continuously changing karyotypes, like those detected in certain primary tumor cells, has

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a negative impact on cellular fitness while stable aneuploid karyotypes are selected to support maximal proliferation (6). Thus, aneuploidy is not a uniform force supporting all aspects of tumor progression. In addition, there also appears to be a proliferation-targeted selection for the "right" disordered karyotype. Clearly, the ability to decode such a karyotype and thereby understand whether and, if so how, it favors heightened proliferation must be known, to optimize therapeutic targeting for this aspect of cancer cell behavior. Thus, the ability to deconvolute the aneuploidy versus proliferation (or any other cancer cell behavior) state of the cells of a given tumor will, very likely, becomes necessary practice prior to selecting mechanism-based therapy for a given cancer.

Decreasing the levels of the mitosis-specific, centromere-linked motor protein, CENP-E, results in aneuploidy and chromosomal instability and in an increase in spontaneous tumorigenesis in aged animals (7). In contrast, increasing aneuploidy also protects cells from chemically or genetically induced tumor formation. In this regard, the definition of aneuploidy can range from rare chromosomal arm events to more subtle genomic instability. Generally, low levels of genomic instability are tumor promoting, while high rates of genomic instability preferentially result in apoptosis. These findings support the notion that chromosomally unstable tumors exhibit enhanced sensitivity to DNA-damaging drugs. Thus, the connections between an aneuploid genome and tumor behavior are not uniformly productive of one predicted clinical outcome or another.

Novel bioinformatics tools are capable of predicting aneuploidy during cancer evolution as a function of the distribution and relative potency of three categories of genes, tumor suppressor genes, oncogenes, and essential genes (8). More recently, chromosome missegregation and DNA rearrangements have been mechanistically associated through the process of micronucleus formation, which supports chromothripsis (9). This mechanism appears to be responsible for significant alterations (such as copy number variations or rearrangements) that are associated with individual chromosomes. It is thereby capable of providing a potential survival advantage to tumor cells. How such micronucleus-trapped genes affect tumor cell fitness, proliferation, or drug sensitivity will also be of primary importance in planning ongoing therapy for a patient's cancer. Again, although technically challenging, especially in a clinical setting, obtaining clinically valuable insights that are tailored to these findings can lead to more accurate and effective therapeutic strategies. For example, understanding how aneuploidy affects *in vivo* tumor cell viability, proliferation, and, most importantly, the potential to induce patient lethality will be of paramount relevance.

Metabolism

Genomic alterations in cancer cells lead to metabolic reprogramming, in no small measure, to achieve independence from growth factors and other extracellular regulators. Moreover, expression of the same activating oncogenic mutation in different tissues can differentially influence the metabolic rewiring of tumor cells arising in these tissues (10). Not only can genetic alterations in tumor cells but also the interplay of neoplastic cells and its microenvironment, together, trigger metabolic reprogramming that supports tumor growth and survival. These changes, too, need to be understood and considered when selecting therapeutic strategies. From results that are now emerging, understanding and dealing with some cancer-associated metabolic changes may be a vital component of future, curative therapy.

Cell-cycle checkpoints

Simultaneous targeting of selected metabolic enzymes/pathways and driving oncogenes may represent an effective therapeutic approach (11). More specifically, targeting both cell cycle–proliferative checkpoints and the tumor viability–specific metabolic support system could be an effective therapeutic strategy in cancers with high proliferative rates. For example, the anaphase-promoting complex Cdc20 (APC^{Cdc20}) regulates mitotic progression. APC^{Cdc20} inhibition sensitizes otherwise resistant cancer cells to chemotherapy by inducing apoptosis (12). In addition, APC^{Cdc20} links cell proliferation to increased glycolysis and glutaminolysis, two processes that are highly active in proliferating cells (13, 14). Would combined chemotherapy or a specific attack on cell proliferation, *per se*, and glutaminase inhibition represent a form of synthetic lethality in chemotherapy-resistant cancers?

Similarly, prolonged mitotic arrest results in ATP depletion, and ATP normally accumulates in interphase and is consumed in mitosis. Interfering with these metabolic pathways can sensitize cells to taxanes, which affect microtubule function and arrest cells in mitosis (15). Furthermore, the inhibition of fatty acid synthesis results in cell-cycle arrest at G_2–M despite the presence of abundant fatty acids in the media. Hence, *de novo* lipogenesis is essential for cell-cycle completion (16). Conceivably, this "lipogenic checkpoint" at G_2–M can be therapeutically exploited for certain hyperproliferative cancers through the use of lipogenesis inhibitors (17–19). This strategy would combine the targeting of a key cell-cycle checkpoint by inhibiting the metabolic regulator(s) that links it to metabolic activity.

Krebs cycle protein mutation

On the other hand, metabolic reprogramming may be a mechanism utilized by tumor cells to sustain their transformed state. As an example, certain gain-of-function isocitrate dehydrogenase 1 and 2 (IDH1 and IDH2) mutations exist in human tumors, for example, acute myeloid leukemia and glioblastoma (20) but also in a variety of other tumors, including prostate cancer and cholangiocarcinoma (21, 22). These particular mutations also result in the accumulation and, eventually, the secretion by mutant enzyme-producing cells of an abnormal metabolite, 2-hydroxyglutarate (2HG). 2-HG is now used as a relevant tumor biomarker, and considerable evidence implies that it, alone, can contribute to aspects of a neoplastic phenotype (23–25). Interestingly, IDH2 mutations also result in both histone and genomic DNA methylation and in abnormal differentiation of hematopoietic precursor cells and leukemia. These abnormalities are reversible within days or weeks of IDH2 inhibition (26). Importantly, persistent IDH2 inhibition has been exploited, therapeutically, with continued, complete remission in multiple acute myelogenous leukemia (AML) patients.

This approach avoids AML chemotherapy-induced bone marrow suppression and is, therefore, more readily tolerated than standard treatment. The question of whether drug resistance will emerge is open, and searches for resistant hematopoietic precursor cell clones that display this property will be important in evaluating this possibility. In addition, the agent of choice does not appear to kill leukemogenic cells, thereby avoiding a basic feature of current leukemia treatment. Rather, it appears to induce their normal differentiation. A key to the future usefulness of this

novel therapeutic approach is whether or not resistant leukemia cells eventually emerge and if so, understanding the nature of their resistance. Similarly, it will be important to know why, beyond pharmacologic considerations, any IDH2-mutant leukemia fails to respond to IDH2 inhibition. For example, are such cells also defective in the function of a pathway(s) that connect(s) normal IDH2 function to normal marrow differentiation?

These results suggest that a deeper understanding of metabolic alterations in cancer, whether primary or resulting from reprogramming to support genetic events, contributes to certain major therapeutic roadblocks.

The Microenvironment and Immunity

The microenvironment and immune responses both play an important role in modulating cancer progression. Absence of the latter can even initiate cancer development (27). However, the tumor microenvironment is, in part, immunosuppressive, thereby limiting effective, naturally occurring antitumoral immunity (28). Finally, important and potentially targetable connections between metabolism and tumor immunity are beginning to be unraveled. For example, while glucose depletion in the extracellular space decreases T-cell function, PD-1 ligand (PD-L1) expressed by tumor cells stimulates glycolysis (29). Hence, it remains to be tested whether altering extracellular glucose concentration affects T-cell function with potential modulation of the immune response (30).

Mechanotransduction

One of the challenges to overcoming barriers to cancer cure is the effect of cellular mechanotransduction. Mechanical forces arising, at least in part, in the microenvironment affect the execution of essential cellular decisions, such as proliferation, differentiation, and maintenance of stemness. Importantly, these mechanical and cytoskeletal inputs represent a central mechanism to control the activity of the transcriptional regulators, YAP and TAZ. YAP/TAZ are well known as prime effectors of the Hippo signaling cascade. They play key roles in controlling the growth of whole organs, amplification of tissue-specific progenitor cells during tissue renewal and regeneration, and cell proliferation. In tumors, YAP/TAZ may play a key role in determining cell polarity and the appearance of an epithelial-to-mesenchymal transition. Indeed, deregulated/aberrant YAP/Hippo signaling can reprogram tumor cells into cancer stem cells, thus, potentially affecting tumor initiation, progression, and metastasis. As such, YAP/TAZ, as well as other players in the field of mechanotransduction, are appealing therapeutic targets in cancer (for a comprehensive review see ref. 31), and the preclinical effects of any new, YAP/TAZ–targeting drugs will be of major interest.

Macrophages

Tumor-infiltrating macrophages are thought to support tumor progression, prompting the development of drugs that impair their survival, block their infiltration, and generally impair their function (32). Multiple macrophage subtypes exist. Specifically, tumor-infiltrating macrophages can be protumoral (MRC1 or mannose receptor positive) or inhibitory (CD11c positive). Macrophage function can be mediated by a variety of intrinsic and extrinsic factors, including miRNA expression, chemokine secretion, or cross-talk with the tumor neovasculature. Blocking the interaction between a macrophage-secreted chemokine, such as

CCL2, and its receptor expressed on tumor cells can delay tumor progression in preclinical models. Interestingly, interruption of this blockade with neutralizing antibodies results in a rebound effect (33). Antimacrophage therapies, including CCL2-neutralizing antibodies and small-molecule inhibitors that block the activity of the colony-stimulating factor-1 receptor (CSF1R), are under investigation in patients with cancer. Klug and colleagues show that iNOS[+]/M1 macrophages, induced by the normalization of the vasculature by low-dose irradiation, are both required and sufficient to mediate effector T-cell recruitment into tumor tissue and successful tumor immune rejection through a nitric oxide–dependent mechanism. Thus, M1 macrophages might be utilized in immunotherapeutic strategies, for instance, by adoptive transfer of iNOS-expressing macrophages (34). In addition, mice lacking the RNAi and miRNA- processing enzyme Dicer exclusively in macrophages exhibited tumor-inhibiting macrophages that activate IFNγ and STAT1 in a cell-autonomous manner. This, in turn, mediates the recruitment of CD8^{+} T cells to tumors. Individual miRNAs expressed in tumor-associated macrophages appear to modulate the immunologic responsiveness of these cells when they are present in tumors.

In preclinical models of hepatocellular carcinoma, strategies are being developed to deliver either antitumoral or antiangiogenic molecules. These new approaches utilize antiplatelet drugs to reduce immune-mediated hepatic necrosis, inflammatory, extracellular matrix deposition, and, ultimately, the development of hepatocellular carcinoma. Using this approach, Tie2^{+} monocytes localized in the proximity of liver colorectal cancer metastases of mice and humans. In addition, Tie2-IFNα–expressing monocytes (TIM-IFN) colonized the liver and impaired the growth of colorectal carcinoma liver metastases. Here, it appears that intrahepatic IFNα released by these TIMs primarily acts on the hepatic microenvironment impairing tumor growth. Thus, interfering with the hepatic microenvironment may represent a novel approach to reduce incidence and progression of both primary and secondary liver tumors (35, 36).

Understanding the complex molecular interactions between tumor-promoting and -inhibiting macrophages may well be essential for the implementation of certain future therapeutic strategies.

Immune Checkpoint Control

The PD-1 pathway

At the heart of this cellular immunity–suppressing pathway are the PD-1 receptor and its ligands, PD-L1 and PD-L2. This pathway plays an important role in regulating the balance between T-cell activation and tolerance (37). In fact, PD-1 engagement can inhibit T-cell proliferation, cytokine production, cytolytic function, and survival. The PD-1 pathway is a key mediator of T-cell exhaustion in both cancer and chronic infection.

The remarkable therapeutic effects of PD-1 pathway blockade in certain cancers demonstrate the key role of this pathway in the inhibition of antitumor immunity. Advances in one's understanding of the role of this pathway in the control of T-cell activation, tolerance, and exhaustion have led to remarkable cancer therapeutic advances. Interestingly, highly mutated tumors appear to be more responsive to PD-1 blockade. Thus, understanding which mutations are immunogenic and the discovery of neoantigens that can be shown to activate cellular tumor immunity will lead to better refined therapeutic strategies, including prophylactic tumor

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vaccines (38, 39). Anti-PD-1 therapy is generally well tolerated, but autoimmune manifestations are known and have reasonably common side effects (40).

In the future, PD-1 blockade will likely be combined with other immunomodulatory blockade strategies, such as cancer vaccines, immunomodulators, or antiangiogenic agents among others to achieve enhancement and/or extinction of multiple concomitant tumor-immunomodulatory pathways.

Naturally Occurring and Engineered T-cell Transfer

Adoptive cell transfer (ACT)–based cancer therapy currently includes the use of tumor-infiltrating lymphocytes (TIL), chimeric antigen receptor (CAR-T), and T-cell receptor (TCR)–engineered T cells. ACT has emerged from principles of basic immunology and revolutionized clinical immunotherapy (for a comprehensive review see ref. 40).

Antigen-presenting cells

Antigen-presenting cells (APC) are essential for innate and adaptive immunity as well as self-immune tolerance. David Baltimore's group showed that, in genetically engineered murine models, Bach1 regulates the generation of APCs, specifically macrophages and dendritic cells. Impaired APC development gives rise to defects in downstream T-cell responses. In addition, APCs foster the emergence of an increase in hematopoietic stem/progenitor cells (41).

As large numbers of tumor-specific T cells for ACT can be manufactured by retroviral genetic engineering of autologous peripheral blood lymphocytes and can be expanded over the course of several weeks, double cell therapy was successfully attempted in patients with melanoma, using TCR-engineered T cells with a very short *ex vivo* manipulation, with dendritic cell vaccines. This resulted in antitumor activity albeit not durable (42).

CAR-T cells

CAR-T cells are T cells engineered to express artificial, synthetic antigen–recognizing, and binding receptors that consist of an extracellular single-chain variable fragment of a relevant mAb, a spacer domain, a transmembrane domain, and a signaling domain that activates T-cell effector function. They target cancer cells that abundantly express the tumor-specific antigen (t-sA) that is recognized by the CAR-T antigen-binding protein, ideally operating in a tumor-specific manner. In principle, they should only kill cells that produce the cognate t-sA.

Although clinical trials in patients with advanced B-cell leukemias and lymphomas treated with CD-19–specific CAR-T cells have induced dramatic, durable, and complete remissions (43, 44), solid tumors present a bigger challenge. Current problems include the fact that targets exclusively expressed by a given tumor species have yet to be identified in many different tumor types, requiring new and effective CAR-T species, each expressing a highly specific tumor antigen–binding polypeptide; off-target effects (e.g., targeting of normal cells); the identification of predictive biomarkers of success; and the emergence of tumor lysis syndrome when CAR-T cells perform their tumor cell work well. Nevertheless, some successes with treatment directed by other highly specific T-cell species have been obtained. For instance, *ex vivo* expansion of HER2-specific Th1 cells, after vaccine priming

and patient infusion, resulted in a 40% clinical response rate in patients with stage IV treatment-refractory breast cancer (45).

Successful engagement of this complex, technically demanding approaches by pharmaceutical and biotechnology companies, will require flawless and cost-effective manufacturing.

Tumor Heterogeneity

Tumor heterogeneity and acquired resistance to treatment via the emergence and growth of resistant tumor cell subpopulations is one of the major challenges facing the field of cancer therapeutics (46). Assessment of the state of heterogeneity within individual tumors and temporal dynamics of tumor evolution have become very important in one's understanding of tumor pathogenesis as well as in monitoring tumor evolution as a function of therapy (47).

For instance, some of the constraints posed by clonal evolution, favoring somatic events or pathway activation, may be exploited therapeutically. Having said this, the frequent presence of mutations acquired during tumor evolution and representing drivers of neoplastic subclones suggests the need for a combinatorial therapeutic strategy that takes into account the relative abundance of each tumor cells species in which a druggable driver is identified (48). In breast cancer, the microenvironment surrounding ductal carcinoma *in situ* (DCIS) plays a significant role in shaping the evolution of cells confined within ducts and acini, into subsequent invasive tumors (49). However, clonal evolution likely occurs in DCIS, suggesting that drivers can serve as prototypical "stem" mutations in breast cancer.

This can now be studied in patient-derived xenograft models (50) that reflect the 10 subtypes of breast cancer proposed by Curtis and colleagues (51). Progress in this effort could advance the cause of elegant clinical analysis of a breast cancer patient's tumor cells. One goal would be improved early detection of a tumor's ability to progress/invade from a stage that does not and, therefore, requires specific antiprogression treatment.

New Approaches to Therapeutic Discovery

One of the obstacles to therapy is parallel clonal evolution, which occurs under selective pressure, such as effective targeted treatment. Multiple metastases may coevolve, some showing response to therapy while others not. Strikingly, under the selective pressure of a PI3Ka inhibitor, numerous cells in some of the nonresponding metastatic sites exhibited a PTEN-loss phenotype that used alternative PI3K isoforms as a mechanism of resistance. This parallel evolution under selective pressure, elegantly demonstrated in a case of an index patient, supports the contention that PI3K pathway activity, achieved by diverse means in different sites, remains essential to tumor maintenance and progression in a tumor originally driven by this pathway (52).

Repeated access to metastatic tissue in a given patient represents a substantial technical difficulty. To overcome this problem, circulating tumor DNA (ctDNA) has been used to genotype colorectal tumors and track clonal evolution during treatment with cetuximab or panitumumab, which target the EGFR. Surprisingly, analysis of tumor cells that had acquired resistance to cetuximab revealed that, upon antibody withdrawal, *KRAS* clones decayed, and the majority of tumor cells regained drug sensitivity. ctDNA profiles of individuals who benefited from multiple challenges with anti-EGFR antibodies exhibited corresponding changes in the circulating level of mutant *KRAS*. This dynamic

adaptation to an intermittent drug schedule, if more commonly encountered, may constitute a significant challenge to targeted therapy and must be taken into account in determining response (53).

The concept of "basket" trials is also emerging to address actionable genomic alterations in a tumor-affected organ-independent manner. To this end, Baselga and colleagues designed an elegant, histology-independent, flexible, early phase II "basket" study of vemurafenib in patients with nonmelanoma cancers harboring *BRAF* V600 mutations. Interestingly, while some tumors responded, others, harboring the same mutation, did not. The important conclusion derived from these findings is that, predictably, the histologic context is an important determinant of response in *BRAF* V600–mutated cancers (54).

This is almost certainly a generalizable concept supported by elegant studies in genetically engineered mice. Utilizing either a germline or a conditional genetic approach, Barbacid and colleagues followed tumorigenesis in various organs in the absence of the target. Such studies revealed the absence of redundancy of related kinases, such as c-Raf versus a- and c-raf, where the former is essential for pancreatic and lung tumorigenesis, whereas the latter two are dispensable (55). Similarly, although Cdk2/6 are dispensable for lung cancer development, Cdk4 is essential (56). Finally, although EGFR appears to be essential for the initiation of pancreatic tumors, it has no therapeutic value in k-ras–driven lung adenocarcinoma, as is well known in the clinic (57). Thus, genetically engineered mice appear to be "cleaner" models to determine the role of effector kinases in tumorigenesis and reaffirm the concept that context needs to be taken into account when targeting genomic alterations.

It is widely accepted that the ability to modulate protein–protein interactions using small molecules will be a fundamental requirement for the development of chemical tools to explore biological mechanisms ("chemical biology") and to seed the discovery of new drugs with improved potency and therapeutic index. One important strategy, named diversity-oriented synthesis, aims to efficiently synthesize libraries of structurally complex and functionally diverse small molecules, using cheminformatic analysis. The 3D structures of the library are very diverse, allowing one to screen for target effects based upon 3D structure, a major step forward (58).

Conclusions

Major, even dramatic, progress in cancer therapeutics continues to emerge. Ever smarter small molecules and now immune perturbants and other biological therapeutics have appeared in recent years. Yet, a cure of advanced cancer remains largely unachieved.

The reasons for the limited success in curing cancer, articulated in this Pezcoller meeting, arise from a myriad of impediments. Thus, as now seems clear, overcoming multiple high hurdles will be needed to achieve desirable and enduring clinical outcomes.

For example, understanding the pathways to drug resistance in each affected patient's tumor will be critical to future success (59). This will require increasing attention to the multitude of biological processes that are important to tumor survival, not to speak of understanding and being able to monitor how effective agents actually eliminate tumor cells from patients. An ability to detect in real time the number, heterogeneity state, and state of drug responsiveness versus resistance of a patient's cancer cells will be needed. This will undoubtedly require novel and as yet unavailable technology (e.g., tumor cell bar coding that does not require a patient's tumor cells to be engineered genetically). For example, real time, clinically predictive biological monitoring of tumor cells, analogous to what happens during the therapy of certain cardiac, endocrine, gastroenterological, and infectious diseases will eventually be needed as a guide to clinical management.

Finally, knowing in detail what molecular capabilities manifested by a patient's tumor have the potential to promote future clinical lethality may, when suitably impeded, prove important to converting remissions or stable disease into long-term survival.

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Massimo Loda

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