Cancer Cell
Commentary

Targeting Immunosupportive Cancer Therapies: Accentuate the Positive, Eliminate the Negative

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In this Commentary we aim to provide an overview of some specific examples of cancer therapeutics, including targeted approaches using monoclonal antibodies and kinase inhibitors, as well as highlight novel approaches for enhancing immunological responses against tumors. We point out that a fundamental property of the cancer cell, genomic instability, confounds the targeted therapies that aim to induce cell death directly while simultaneously enhancing the potential for immunological attack by creating a large number of neoantigens. We argue for combinatorial strategies with agents that target tumor cells to release these antigens together with innovative therapies that enhance immunological responses by interfering with inhibitory checkpoints.

Introduction

The past decade has witnessed rapid advancements in a number of biomedical disciplines. Our understanding of the molecular basis of cancer and of signaling pathways that regulate immune responses to cancer has resulted in the development of exciting new therapies that have progressed or are progressing to the stage of clinical application. In this Commentary we aim to give an overview of some specific examples of these novel therapeutics, in particular "targeted" approaches, including monoclonal antibodies and small-molecule inhibitors (SMIs) of enzymatic function, and of approaches that aim primarily to enhance the host immune responses directed toward tumors. We hope to persuade the reader that the insights we have gained into some of the factors imposing limitations on the efficacy of recent approaches strongly argue for combinatorial strategies including both elements that target tumor cells directly and those that enhance immunological responses by interfering with inhibitory checkpoints within the immune system. In this way the sum of the total may greatly outweigh the sum of the parts.

Until recently, immunotherapeutic strategies have focused primarily on enhancing immune effector functions, founded on the premise that immune stimulation may enable the recognition of antigenic determinants that are expressed by the tumor but that remain only weakly immunogenic and incapable of eliciting protective responses in the unmanipulated host. Despite notable achievements in attempts to augment antitumor immunity by a variety of vaccination approaches, initial enthusiasm has been tempered by the fact that successes have been anecdotal and that the generation of measurable increases in antitumor effector responses have not often correlated with significant or durable objectively quantifiable clinical responses. This lack of clinical success has contributed to the marginalization of immunological approaches from the mainstream of anticancer therapies. We now recognize that at least part of this failure of immune-based strategies is attributable to the presence of numerous regulatory circuits that act to limit the magnitude and duration of immunological responses. In many cases such circuits may already be engaged in patients with cancer, limiting endogenous antitumor responses and thereby frustrating attempts to mobilize the immune system effectively to obtain therapeutic benefit. In this respect vaccination strategies may, at best, enhance effector responses that will subsequently be dampened by a shifting response in host homeostatic equilibrium, e.g., the upregulation of CTLA-4 on activated effector T cells that mediates a cell intrinsic inhibitory brake. At worst, they may directly induce the expansion of populations of regulatory T cells (e.g., $Foxp3^+CD4^+CD25^+$ cells) (Nishikawa et al., 2005) or suppressive antigen-presenting cells (e.g., plasmacytoid dendritic cells, $CD11b^+Gr1^+$ myeloid suppressor cells, or Th2-polarized tumor-associated macrophages), particularly as many of the currently identified targets are self or altered self antigens. Targeting these regulatory circuits would seem to be an imperative for optimizing cancer immunotherapies.

Conventional Chemotherapeutics

Together with radiotherapy and surgery, conventional cytotoxic drugs form a trinity that has provided the basis for mainstream anticancer therapies for the past millennium. Most of these agents have remained in use for many decades. Given the apparent dose-response relationship with tumor cell kill, they have often been used in protocols that push their dose to the limits imposed by toxic side effects, most commonly that of bone marrow suppression. Hematopoietic stem cell transplanta-

tion has even allowed escalation of chemoradiotherapy toward the limits imposed by organ toxicities other than the bone marrow. Rescue with autologous stem cells has demonstrated that dose escalation alone does not eradicate the malignancy in many cases, and the lower relapse rates following allogeneic transplantation for hematological malignancies has shown that an associated immune-mediated graft-versus-malignancy effect may be equally important in effecting cure. Intuitively, part of the difficulty inherent in combining conventional cytotoxics with immune-based therapies is that the damage caused to the lymphohematopoietic compartment will limit the ability of the host immune system to respond to further manipulations. Adoptive transfer of cellular populations that have not been exposed to chemotherapy in vivo provides one way to try to circumvent this problem (Dudley et al., 2002; Rapoport et al., 2005). The search for less globally toxic anticancer agents has been fuelled in recent years by an increased understanding of the aberrant intracellular signaling pathways involved in the pathogenesis of cancers, and an ability to manufacture potent monoclonal antibodies targeting a variety of cell surface molecules.

Small-Molecule Inhibitors

The development of "targeted" therapies has been a major advance in the management of patients with cancer. The term relates to increased target selectivity and encompasses a number of approaches that share the general aim of reducing the collateral toxicities caused by many cytotoxic chemotherapies or radiotherapy. In some cases target selectivity remains relatively broad (e.g., imatinib targets a number of tyrosine kinases such as ABL, PDGFR, and KIT), but clinical selectivity is enhanced by the supraphysiological target activity caused by activating mutations within malignant cells. In the case of imatinib, the lower selectivity is reflected by its efficacy in a number of different malignancies (reviewed in Tefferi and Pardanani, 2004; Lasota and Miettinen, 2006). For other targeted therapies, exemplified by monoclonal antibodies, selectivity is so exquisitely high as to endow true target specificity, modulated clinically by the level of target distribution on normal cell populations as opposed to their malignant counterparts.

Despite the revolutionary impact that targeted therapies have had on the management of some forms of cancer (Peggs and Mackinnon, 2003), experiential wisdom suggests that in many cases they offer improved survival but rarely the chance for cure. Thus treatment with SMIs of tyrosine kinases is often accompanied by the development of drug resistance (Rubin and Duensing, 2006; Krause and Van Etten, 2005). A variety of mechanisms contribute, including secondary mutations in the target gene that abolish binding of the inhibitor, "kinase switch" mutations, and gene amplifications. Even taking an optimal case scenario, where the mutated kinase activity is both necessary and apparently sufficient for disease pathogenesis (a primary case oncogene addiction), perhaps most closely approached in chronic myeloid leukemia (CML), the achievement of disease eradication at a molecular level (as measured by RT-PCR for *BCR-ABL* gene transcripts) has remained elusive (Press et al., 2006). Although a relatively low incidence of secondary resistance to imatinib has been reported since the initiation of large phase III studies, the number continues to slowly rise. In addition, mutations found at relapse may also be detectable prior to treatment, consistent with a model in which selective pressure favors outgrowth of pre-existent resistant clones (Roche-Lestienne et al., 2003). Thus, long-term use may be associated with a significant risk of treatment failure, and even in those few who achieve a molecular complete response, subsequent cessation of therapy has been associated with significant risk of relapse (Rousselot et al., 2007). While next-generation inhibitors may rescue failing patients, improve primary response rates still further, and delay the development of secondary drug resistance, it is likely that further cancer "pharmaco-editing" will drive new escape variants (Burgess et al., 2005; Sorel et al., 2006). It is also becoming clear that the majority of human cancers are unlikely to represent the optimal case scenario at the time of diagnosis. Cases of CML that have advanced beyond early chronic phase to accelerated phase or blast crisis are genetically more complex and exhibit lower response rates and response duration (O'Dwyer et al., 2002). The majority of advanced solid tumors are also genetically complex. In breast or colorectal cancers, it is estimated from sequence analyses that individual tumors accumulate an average of approximately 90 mutations, which lead to changes in the amino acid sequence of expressed proteins (Sjoblom et al., 2006). While only a subset may contribute to carcinogenesis, their number has been estimated to average over ten per tumor and perhaps reach up to almost 190 over a range of breast and colorectal cancers. They are predicted to affect a wide range of cellular functions, including transcription, adhesion, and invasion. It is unlikely that a given tumor will be critically dependent on any one abnormally activated kinase or signaling pathway for its malignant phenotype. For the majority of tumors, perhaps paradoxically to the original concept of targeted therapies, there may therefore be a requirement for the use either of combinations of multiple SMIs targeting different pathways or of SMIs with broader target selectivity for effective and durable activity. Rational implementation of combinatorial approaches may, in addition, require molecular profiling of individual tumor samples. Alternative combinatorial approaches include those using conventional chemotherapeutics or monoclonal antibodies.

Monoclonal Antibodies

Numerous monoclonal antibodies that directly target elements of the tumor cells' antigenic topiary are currently in clinical development (e.g., cetuximab and panitumumab targeting EGFR, trastuzumab and pertuzumab targeting ERBB2, and rituximab targeting CD20). In most cases

target specificity is not likely to be toward mutated self sequences or resultant conformational changes in proteins, but rather toward unaltered extracellular portions of mutated kinase receptors (e.g., EGFR) or molecules that are present on tumor cells but that are also present on their nonmalignant counterparts (e.g., CD20). Indeed, it remains unclear whether mutations of the intracellular domains of membrane receptor kinases affect the response to therapeutic monoclonals. Thus, toxicity may depend both on differential expression levels, and on the impact of damaging the nonmalignant antigen-expressing compartment. Some monoclonal antibodies may offer the potential for cure, or increased cure rates in combination with conventional chemotherapeutics (Feugier et al., 2005). This may be more likely when the nonmalignant counterparts are expendable and short-term toxicity to this compartment is unlikely to be a limiting factor, e.g., anti-CD20 therapy, where destruction of the $CD20^+$ B cell compartment induces a manageable immune deficit. By extension, efficacy may be related to the importance of the target to the continued survival of the malignant cell. If the tumor cell can avoid destruction by downregulation of the target molecule, a "therapeutically" induced form of immunoediting may result in the development of clinical resistance (Chu et al., 2002). Alternatively, if the target is not expressed by the putative cancer stem cell compartment, then relapse may be inevitable once therapy is discontinued. Inability to eradicate tumor stem cells may also be a mechanism for failure of SMIs. For example, imatinib may affect more differentiated leukemic cells in CML but not the leukemic stem cells (Michor et al., 2005).

Genomic Instability: The Interface of Targeted Therapies and the Immune System

The mechanisms of action of monoclonal antibodies are manifold but can be broadly categorized as direct or indirect. The former may include the blocking of function (e.g., hindering ligand binding, increasing internalization of receptors) and stimulating function (e.g., inducing apoptosis). The indirect mode of action is mediated by the immune system and includes the activation of complement-dependent cytotoxicity and both complement-dependent and antibody-dependent cellular cytotoxicity (Cartron et al., 2002). This is an important distinction to SMIs of cellular kinases which have not been generally reported to activate immune responses against tumor cells (Burchert et al., 2003). Indeed, SMIs such as imatinib may actually inhibit lymphocyte function (Dietz et al., 2004; Seggewiss et al., 2005). Given our increased understanding of the regulatory circuits controlling immune activation it is conceivable, if not probable, that the involvement of adaptive immune responses is constrained by regulatory mechanisms autonomous to the effector T cell compartment or mediated via other regulatory populations. Targeting these inhibitory pathways might allow engagement of therapeutically relevant immunological activities. Evidence that tumors may shape host immunity by selective deletion of high-avidity tumor-specific T cells (Molldrem et al., 2003) suggests that engagement of lower-avidity clones by interference with inhibitory peripheral tolerance mechanisms may be a prerequisite for effective immunotherapies.

The very genetic instability that may confound targeted SMI monotherapies may provide unique opportunities for the generation of antitumor immunity. Such mutational diversity can provide neoantigens that could be perceived by the immune system as nonself. Our analyses of the data set used by Sjoblom et al. (2006), which described 1307 mutations in 11 breast and 11 colorectal cancers based on analysis of cell lines or xenografts, provides unique insights into the potential impact of mutagenesis on the generation of novel antigens. Using in silico-based computer algorithms combined with high-throughput post hoc analyses, we found that a significant number of candidate tumor neoantigens arise as a consequence of the multiple gene mutations occurring in cancers (unpublished data). Individual breast and colorectal cancers accumulated an average of approximately 9.9 and 6.6 novel HLA-A*0201-binding epitopes, respectively, several within genes implicated in the neoplastic process. The increased frequency of predicted epitopes in breast cancer may reflect variability between tumor types that would be unaccounted for during conventional immune targeting against a limited number of know antigen targets. These findings predict that the ensuing immune responses would be patient specific and directed toward nonself. Since each tumor theoretically presents a number of targets for immunological attack, the possibility of immune evasion by target mutation is lessened. While not all of the predicted epitopes will be processed and presented on the cell surface in the context of HLA-A*0201, each tumor contains six distinct MHC class I molecules, including two loci each for HLA-A, -B, and -C, which can present additional mutant peptides dependent on the genotype of the individual. If we assume that other MHC I alleles would present neoepitopes at a similar frequency, then the total number per cell would be $6 \times 6.6–9.9$, or somewhere between 39 and 59 new antigens per tumor cell. Even if the algorithms are incorrect in 90% of cases, this nonetheless indicates a fairly large number (four to six) of neoepitopes per tumor generated by genomic instability.

Clearly, these arguments remain to some degree conjectural at present, since such potential neoepitopes have yet to be shown to be presented by tumors or antigen-presenting cells (APCs), and this remains an important proof of principle requiring confirmation. However, these unique tumor antigens have a number of potential advantages as targets for immunotherapy as compared to the shared, self antigens that have formed the nexus for clinical trials of vaccination and adoptive cellular therapy over the past decade (Parmiani et al., 2007). One perceived disadvantage has been the technical complexity of their identification and molecular characterization in the individual tumor/patient, which imposes limitations on the

rapidity of application of such sequence-specific therapies. Sequencing the entire genome of each individual tumor and subsequent selection of mutated peptides whose motifs are predicted to bind patient-specific HLA alleles remains at the limits of technical possibility. Even when feasible, the cost implications are sizeable, particularly given the need to generate patient-specific therapeutics targeting multiple peptides. In addition, the predictive algorithms for HLA binding and possible immunogenicity require further refinement to more reliably predict optimal targets. However, tumor cell destruction in situ can potentially provide a polyvalent tumor vaccine if appropriately presented by the host immune system, without an absolute requirement for knowledge of the targeted antigens. Amplification of these responses by interference with immune regulatory circuits may prove to be an obligate element of such strategies. The same approaches that have been applied to target cancer cell survival directly are now being applied to target elements of the immune system in order to enhance antitumor immunity. Most notable at present are the immunostimulatory monoclonal antibodies. These may interfere with the function of inhibitory receptors (antagonistic antibodies), promote the function of lymphocytes or professional APCs directly (agonistic antibodies), deplete regulatory populations, or interfere with inhibitory molecules expressed by the tumor or cells in the tumor microenvironment.

Accentuating the Positive

T cells specific for tumor antigens are now recognized to be present in significant numbers in patients with cancer, and many attempts to expand or activate these populations with conventional vaccination strategies have been attempted. Optimization of these approaches might be achieved by provision of costimulatory signals, either enhancing engagement of TCR molecules or promoting cell division, survival, or effector functions. The evolution of therapeutics aimed at directly enhancing the function of APCs or effector T cells has enabled the exploration of a number of novel combinatorial approaches. Stimulatory monoclonal antibodies targeting members of the TNF receptor family have shown promise in preclinical models (summarized in Melero et al., 2007). These include anti-CD40 (which induces IL-12 production by dendritic cells [DC], thus enhancing natural killer [NK] and NK T cell activation, T helper type I responses, and cytotoxic T lymphocyte [CTL] induction, as well as directly inhibiting tumor growth) (French et al., 1999; Tong and Stone, 2003), and anti-OX-40, anti-4-1BB, and anti-GITR (Sugamura et al., 2004; Melero et al., 1997; Ko et al., 2005), which enhance either the magnitude or duration of T cell responses. Oligodeoxynucleotide adjuvants containing unmethylated cytosine-guanine motifs (CpG-ODN) offer an alternate mechanism for activating DC (Krieg, 2007), and α-galactosylceramide for activating invariant NK T cells (Berkers and Ovaa, 2005), both promoting adaptive immunity. While detailing the complete spectrum of targets currently being evaluated is beyond the scope of this Commentary, consideration of specific approaches enlightens the likely course of clinical development of immunotherapeutics over the coming years. For example, a combination of antibodies directed toward the death-inducing TNF-related apoptosis-inducing ligand receptor (TRAIL-R), CD40, and CD137 (41-BB) has been shown to augment antitumor activity in TRAIL-sensitive murine tumor models (Uno et al., 2006). Induction of tumor apoptosis and antigen release from tumor cells and recruitment of innate immune cells into the tumor site by anti-DR5 (anti-TRAIL-R), coupled with augmentation of DC function induced by anti-CD40, and improved induction, activation, and survival of tumor-specific CTL facilitated by anti-CD137, are all likely to be important contributors to the favorable antitumor activity (Takeda et al., 2007; Tamada and Chen, 2006). One potential advantage of approaches relying on the synergy of multiple components is that they might reduce the toxicity induced by higher doses of each agent administered as monotherapy (e.g., immune responses may be constrained toward tumor-related antigens rather than ubiquitous self antigens). The severe toxicity experienced by normal volunteers receiving a "super-agonistic" costimulatory antibody directed toward CD28 (TGN1412) highlights the need for careful evaluation of these powerful new therapeutics (Suntharalingam et al., 2006; Sheridan, 2006), although other targets that do not obviate the requirement for TCR signaling in inducing T cell activation (a feature of super-agonists) will likely have more favorable toxicity profiles.

Drugs that have broad-ranging effects on cellular functions, such as those targeting elements of intracellular signaling pathways, offer intriguing new avenues to explore, although the pleiotropic nature of their effects makes prediction of outcomes and toxicities particularly challenging. For example, approaches that inhibit Stat3 either directly, or via inhibition of JAK2-induced phosphorylation of Stat3, offer a number of potentially additive benefits, including induction of increased apoptosis of tumor cells, reduced VEGF expression (and hence angiogenesis), and interference with inhibitory cytokine (e.g., IL-6) signaling (reviewed in Jing and Tweardy, 2005). Such agents are yet to reach clinical practice, but the importance of target selectivity is highlighted by the immunosuppressive activity of JAK3 inhibitors (Changelian et al., 2003).

Targeted therapies that augment costimulatory pathways therefore augur improved immunotherapeutic outcomes. However, all such approaches are potentially constrained by induction of regulatory inhibitory feedback mechanisms (Ko et al., 2005; Biagi et al., 2005). Eliminating the negative impact of these pathways on immune responses may ultimately confer optimal antitumor activity to these therapies.

Regulatory Circuit Blockade

Blockade of inhibitory immune checkpoints for therapeutic benefit offers considerable promise, particularly as combination with other treatment modalities that promote

crosspriming of antitumor immunity may yield additive or synergistic activity. The strategy that is the most advanced in clinical development involves antibodies that block CTLA-4, an inhibitory member of the immunoglobulin superfamily of receptors (Korman et al., 2006). Members of the immunoglobulin superfamily share features in both sequence and structure, and the majority bind members of the B7 ligand family. CTLA-4 shares the B7-1 and B7-2 ligands with CD28, a critical costimulatory molecule. Ligation of CD28 in concert with T cell receptor stimulation has been shown to enhance T cell proliferation by inducing production of IL-2 and antiapoptotic factors, and hence to decrease the number of ligated T cell receptors that are required for a given biological response (Viola and Lanzavecchia, 1996). CTLA-4 engagement selectively blocks augmentation of gene regulations by CD28-mediated costimulation but does not ablate gene regulation induced by TCR triggering alone (Riley et al., 2002). The function of CTLA-4 as a negative regulator of CD28-dependent T cell responses is most strikingly demonstrated by the phenotype of CTLA-4 knockout mice, which succumb to a rapidly lethal polyclonal CD4-dependent lymphoproliferation within 3–4 weeks of birth (Waterhouse et al., 1995; Tivol et al., 1995). Antibody-mediated blockade of CTLA-4 is particularly effective at enhancing secondary immune responses, more markedly in $CD4^+$ T cells, and has been shown in preclinical models to synergize with a number of other antitumor immunotherapies, while often having only modest effects as a monotherapy (reviewed in Korman et al., 2006). Furthermore, early clinical studies have shown that CTLA-4 blockade has activity as a monotherapy and, in keeping with murine models, enhanced activity in combination with some other therapies in the treatment of human malignancies including melanoma, renal, ovarian, and prostatic carcinomas (Peggs et al., 2006; Fong et al., 2006). Adverse immunological events have been a feature of some of the early studies but have generally proven manageable and the majority reversible. Other inhibitory members of the Ig superfamily offer further possible targets. For example, blockade of the PD-1:PD-L1 axis has shown considerable promise in its ability to rescue exhausted $CD8^+$ T cells in murine models of viral infection and to enhance antitumor activity (Barber et al., 2006; Iwai et al., 2005). Regulatory circuits involving non-cell-autonomous inhibition, most notably regulatory T cell populations, offer a further possible therapeutic target. The lack of unique cell surface markers for these populations has restricted the development of monoclonal antibodies as targeted therapies for depletion of these cells, particularly as many of their surface markers are shared with activated effector cells. Denileukin diftitox (ONTAK) is a fusion protein designed to direct the cytocidal action of diphtheria toxin to cells that overexpress the IL-2 receptor. Ex vivo studies indicate that it interacts with the high- and intermediate-affinity IL-2 receptor on the cell surface and undergoes internalization. Subsequent cleavage in the endosome releases the diphtheria toxin into the cytosol, which then inhibits cellular protein synthesis, resulting in rapid cell death. Preliminary studies in ovarian and renal cell carcinoma demonstrate an early reduction in circulating $CD4^+CD25^{high}$ regulatory T cells following denileukin diftitox therapy with preservation of the $CD4^+CD25^{int}$ memory T cell pool (Dannull et al., 2005), but possible depletion of $CD25^+$ effector cells with prolonged or repeated administration (Barnett et al., 2005). Administration prior to vaccination with DC transfected with tumor RNA enhanced tumor immunity as measured by subsequent in vitro analyses of cytokine production in recall responses to the DC vaccine (Dannull et al., 2005). The availability of SMIs selectively targeting regulatory T cell function would be a valuable addition to our existing therapeutic armamentarium. Many SMIs that are directed at pathways involved in peripheral tolerance are in development (reviewed in Muller and Scherle, 2006). These include inhibitors of indoleamine 2,3-dioxygenase, arginase 1, inducible nitric-oxide synthase, and the transforming growth factor receptor β receptor tyrosine kinase.

Combinatorial Approaches

Our current understanding of tumor immunology therefore suggests that conventional anticancer therapies might be viewed as immunosupportive therapies that have the potential to turn the tumor itself into a form of polyvalent in vivo cellular vaccine, and that immune checkpoint blockade with or without additional costimulatory receptor agonistic ligation might provide the immunological adjuvant necessary to realize a true therapeutic impact (Figure 1). Combination of regulatory circuit blockade with other targeted therapies is particularly attractive, as the more widespread toxicity to the immune system that may be a consequence of conventional chemotherapy or radiotherapy, and which intuitively might impact attempts to enhance immune responses, can be avoided. This requires that the targeted therapy be capable of inducing augmentation of tumor antigen presentation to the immune system. As previously discussed, monoclonal antibodies are certainly capable of initiating immune system activation that could be propagated by interference with regulatory circuits. Whether SMIs of intracellular kinases can effect such activity is less clear. In vitro these molecules often induce tumor cell apoptosis, a form of cell death commonly effected by many established chemotherapeutics both in vitro and in vivo. While apoptosis has long been considered as nonimmunogenic or even tolerizing, a gradual acceptance that not all forms of apoptosis are necessarily immunologically equivalent has developed. Massive apoptosis may change a normally tolerogenic crosspresentation of antigen (Sotomayor et al., 2001) into an effective crosspriming event (Rovere et al., 1999), and stressed apoptotic tumor cells have an enhanced capacity to activate dendritic cells and induce specific cytotoxic T cells, possibly via the intermediary of

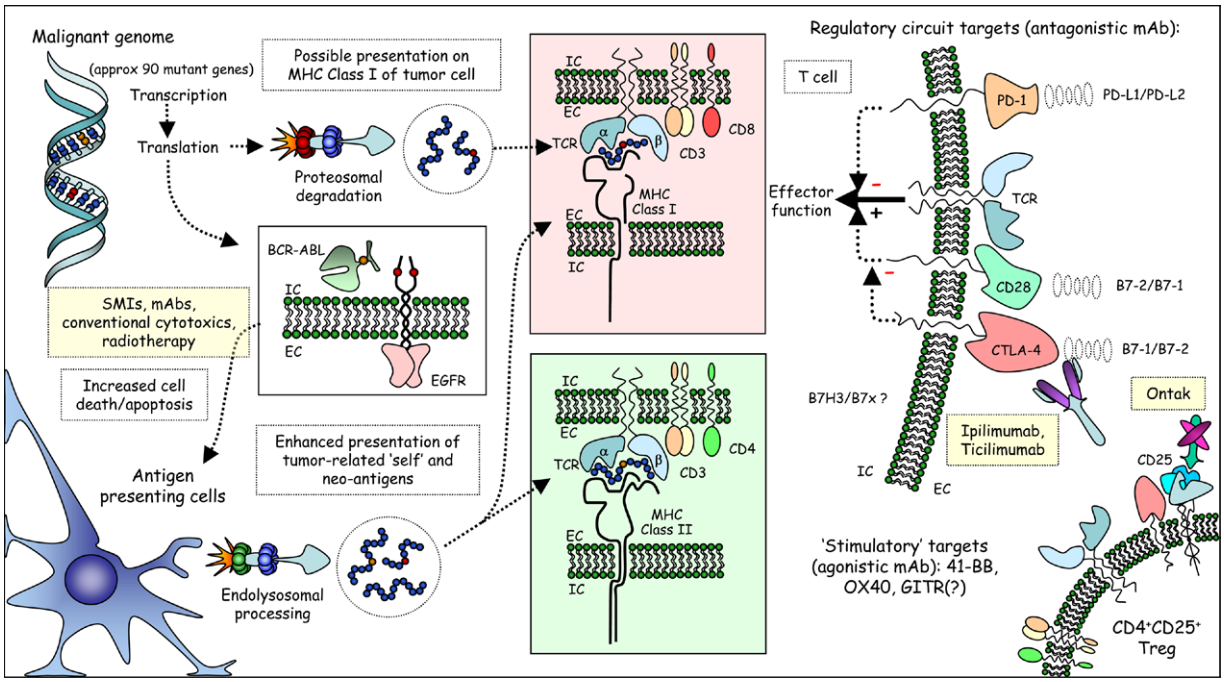

Figure 1. The Genomic Instability of Cancers Is Important Both in the Evolution of Their Malignant Phenotype and in Providing Potential Targets for Immune-Based Therapeutics

Mutant gene products, such as BCR-ABL or constitutively active epidermal growth factor receptor, provide potential targets for small-molecule inhibitors (SMIs) or monoclonal antibodies (mAbs). These, or other cytotoxic therapies, can indirectly enhance presentation of neoantigens via both the class II and class I MHC pathways of professional antigen-presenting cells. However, numerous regulatory circuits serve to limit the potential immune response directed toward these targets. Blockade of T cell-autonomous inhibitory pathways (e.g., CTLA-4) or inhibitory cellular populations (e.g., Foxp3$^+$ regulatory T cells) may sufficiently enhance endogenous immune responses to enable the unmasking of clinically meaningful antitumor activity. B7x and B7H3 are newer inhibitory members of the CD28:B7 immunoglobulin superfamily that may also be amenable to blockade.

heat shock protein induction (Feng et al., 2003). Uric acid release by injured cells is a key endogenous danger signal improving crosspriming (Shi et al., 2003), and calreticulin exposure on the cell surface may also be important in distinguishing between immunogenic and nonimmunogenic cell death (Obeid et al., 2007). Interestingly, imatinib has been reported both to directly enhance antigen presentation of mature dendritic cells and to inhibit dendritic cell differentiation from a number of different progenitors (Appel et al., 2005). In addition, it reversibly inhibits T cell proliferation by interfering with T cell receptor signal transduction (Seggewiss et al., 2005). Effects on antigen presentation and T cell responses induced by SMI therapies clearly warrant further study, and the context in which antigen is presented following their use (tolerogenic versus immunogenic) needs clarification. Murine models allowing these issues to be addressed are already well established. In light of this it is perhaps premature to exclude combinations of chemoradiotherapy and regulatory checkpoint blockade. These approaches have shown promise in preclinical models despite concerns that cytotoxic drugs might be detrimental to immunotherapies. Cytotoxic chemotherapies appear capable of inducing an appropriate milieu for presentation of tumor antigens (Nowak et al., 2002), and induction of lymphopenia may actually be a beneficial side effect providing an environment in which antitumor effectors can preferentially expand (Klebanoff et al., 2005). Contributory factors also likely include reduction in regulatory cell function or number (Ghiringhelli et al., 2004), increased antigen crosspresentation (Nowak et al., 2003a), partial activation of dendritic cells (Nowak et al., 2003b), and partial sensitization of tumor cells for cytotoxic T cell-mediated lysis (Bergmann-Leitner and Abrams, 2001). Appropriate timing of sequential therapies is likely to become an important factor in such combinatorial approaches. Given the difficulties inherent in planning trials that combine multiple new agents, particularly if this entails the involvement of a number of pharmaceutical companies, it is probable that these approaches will focus on those employing conventional chemotherapeutics in the short term.

Our hope is that the opportunities immune checkpoint blockade offers to enhance responses to antigens released by tumor cell death, perhaps augmented by additional manipulations directly promoting the function of lymphocyte receptors, will allow us to move into an era where immunotherapy emerges from the research domain to join the mainstream of oncological therapies, an exciting future that is fecund with opportunity to improve remission rates, prevent disease recurrence, and ultimately cure cancer.

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