

Targeting Heat-Shock Protein 90 (HSP90) as a Complementary Strategy to Immune Checkpoint Blockade for Cancer Therapy

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Abstract

The demonstration that immune checkpoint blockade can meaningfully improve outcomes for cancer patients has revolutionized the field of immuno-oncology. New biologic agents targeting specific checkpoints have shown remarkable durability in terms of patient response and, importantly, exhibit clinical activity across a range of human malignancies, including many that have traditionally proven refractory to other immunotherapies. In this rapidly evolving area, a key consideration relates to the identification of novel combinatorial strategies that exploit existing or investigational cancer therapies in order to optimize patient outcomes and the proportion of individuals able to derive benefit from this approach. In this regard, heat-shock protein 90 (HSP90) represents an important

emerging target for cancer therapy because its inactivation results in the simultaneous blockade of multiple signaling pathways and can sensitize tumor cells to other anticancer agents. Within the context of immunology, HSP90 plays a dual regulatory role, with its functional inhibition resulting in both immunosuppressive and immunostimulatory effects. In this Cancer Immunology at the Crossroads overview, the anticancer activity profile of targeted HSP90 inhibitors is discussed along with their paradoxical roles in immunology. Overall, we explore the rationale for combining the modalities of HSP90 inhibition and immune checkpoint blockade in order to augment the antitumor immune response in cancer. *Cancer Immunol Res*; 3(6); 583–9. ©2015 AACR.

Introduction

Harnessing the latent capacity of the immune system to control and/or eradicate human cancer has been a long-coveted, although elusive, frontier within the field of oncology. The primary goal of cancer immunotherapy is to trigger a self-sustaining cycle of immunity that is sufficient to amplify and propagate robust antitumor effects without inducing unrestrained autoimmune inflammatory responses (1). An increased understanding of the underlying mechanisms exploited by tumors in order to suppress adaptive immune responses and evade destruction has now translated into significant clinical advances. These successful strategies utilize new classes of immunotherapeutic agents—particularly those that modulate immune checkpoint proteins, including the immunoinhibitory receptors cytotoxic T-lymphocyte antigen-4 (CTLA-4) and programmed death 1 (PD-1; refs. 2, 3)—that serve to dampen tumor-directed T-cell activation and effector responses, respectively. Indeed, it is now established that pharmacologic blockade of such immune checkpoints, critical for the maintenance of self-tolerance, but whose dysregulation by tumors serves as a major mechanism of immune resistance, can promote immunogenic antitumor activity in a manner

showing enormous potential to revolutionize human cancer therapy (4).

A striking feature to emerge from the initial human trials evaluating monoclonal antibodies against CTLA-4 (e.g., ipilimumab) or PD-1 (e.g., nivolumab and pembrolizumab) was a remarkable durability of response, even after treatment discontinuation, which in turn predicts long-term patient survival (5). Moreover, antibody-mediated blockade of PD-L1, one of the ligands for PD-1, has also shown durable clinical benefits across multiple tumor types and appeared superior to those achieved using conventional chemotherapeutic or molecularly targeted approaches within the same indications (6). Conversely, however, the proportion of patients responding to these agents as monotherapy is typically low. This finding has thus prompted an intense exploration of novel combinatorial strategies using immune checkpoint modulators alongside other anticancer modalities, designed to augment antitumor immunity and improve overall response rates, and a large number of relevant trials are currently ongoing (reviewed in ref. 5). These include vaccines, chemotherapeutics, concomitant blockade of immunosuppressive pathways, targeting molecular pathways critical for tumor growth and maintenance, and angiogenesis inhibitors. In this Cancer Immunology at the Crossroads overview, we examine the rationale for pharmacologic inhibition of heat-shock protein 90 (HSP90) as a complementary strategy to immune checkpoint blockade for therapeutic intervention against human cancer.

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doi: 10.1158/2326-6066.CIR-15-0057

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Therapeutic Targeting of HSP90 in Cancer

HSP90 is a ubiquitously expressed molecular chaperone that plays an indispensable role in homeostasis by regulating the maturation and functional stability of an extensive array of

cellular substrates, referred to as "client" proteins (7). HSP90 also represents an essential component of the cellular heat-shock response and is transcriptionally induced in response to proteotoxic stress (8). As with other physiologic processes that may become co-opted by cancer cells, it has now become apparent that the chaperoning functions of HSP90 can be subverted during tumorigenesis to facilitate malignant progression and maintain a transformed cellular phenotype (9). In this regard, a large number of HSP90 client proteins have been implicated in the pathogenesis of human cancers, and have been shown to contribute to nearly every aspect of the oncogenic process, including immortality, aberrant survival, metabolism, genomic instability, and dissemination (10). Such oncoprotein clients are often expressed in labile states (i.e., mutant, amplified, and/or translocated) that are highly reliant on the HSP90 chaperone machinery acting as a biochemical buffer for their stability and function (11). Indeed, the buffering capacity of HSP90 against numerous environmental insults represents an important mechanism by which tumor cells may misappropriate HSP90 activity for selective advantage (12). Not surprisingly, then, elevated expression of HSP90 is commonly observed in tumor tissues, and this likely reflects a cytoprotective response to the stresses imposed by the hostile tumor microenvironment (13).

Since the initial demonstration two decades ago that natural product benzaquinone ansamycins (such as geldanamycin and its derivatives) were *bona fide* inhibitors of HSP90 with potent antitumor activity (14), pharmacologic blockade of HSP90 has emerged as an innovative approach for the development of novel antineoplastic agents, and small-molecule inhibitors of HSP90 now rank among the most actively pursued classes of agents in oncology (11). A number of key considerations arose during the characterization of selective HSP90 inhibitors that helped establish the feasibility of targeting this chaperone as a viable therapeutic strategy for cancer. First, and in contrast with traditional targeted therapies (e.g., kinase inhibitors) that selectively ablate only one or a few oncoproteins, functional inhibition of HSP90 results in the concurrent destabilization of hundreds of client proteins via ubiquitin ligase-proteasome degradation, thereby providing a means to simultaneously disrupt multiple oncogenic signaling cascades through a singular molecular target. Of note, the concomitant disruption of numerous signaling nodes in this manner serves to inhibit redundant pathways and feedback loops that can contribute to both intrinsic and acquired drug resistance mechanisms (15–17). Second, as a class, HSP90 inhibitors all display preferential and selective tumor retention characteristics (18). Although the underlying basis for this remains to be fully elucidated, evidence suggests that HSP90 exists in cancer cells as part of a highly active, multichaperone complex with greater affinity for targeted inhibitors than that observed in normal cells (19), and posttranslational modifications that occur as a consequence of cellular transformation have been described to account for tumor-selective HSP90 activation (20). Overall, these unique features of tumor selectivity and multimodal impacts on the malignant phenotype contribute significantly to an exploitable therapeutic index for this group of investigational agents.

At present, however, no HSP90 inhibitor has yet been approved for human therapeutic use. Although evaluation of the prototypical ansamycin-based compounds validated HSP90 as a druggable target for cancer treatment (11), their

clinical application was hampered due to a number of pharmacologic and safety limitations (21). Of a large number of second-generation, optimized small-molecule inhibitors of HSP90 that were subsequently developed, the resorcinol-based compound ganetespib (22) is currently the most clinically advanced (23). As single agents, HSP90 inhibitors, including ganetespib, have shown the strongest signals of efficacy in client protein-driven patient populations, most notably in anaplastic lymphoma kinase (ALK)-rearranged lung cancer and HER2-amplified breast tumors (24–26). Unfortunately, similar translational benefit has not been observed using HSP90 inhibitor monotherapy in other client protein-driven cancer populations. As a result, selective HSP90 inhibitors may ultimately show the most clinical utility as chemotherapeutic or molecularly targeted agent sensitizers (11), with HSP90 blockade offering a complementary strategy sufficient to confer superior therapeutic indices, overcome resistance mechanisms, and/or reduce treatment-related toxicities (9).

The HSP90 Paradox for Modulating Tumor Immune Responses

HSP90 is reported to play a number of important functional roles in processes that bridge innate and adaptive immune responses, including antigen presentation, lymphocyte activation and cross-priming, and the activation/maturation of dendritic cells (27, 28). Thus, targeted inhibition of HSP90 does not initially appear to be an intuitively attractive complementary approach for improving the T-cell-mediated antitumor functions induced by immune checkpoint blockade. Indeed, as part of the classical endogenous pathway of antigen presentation, data from *in vitro* studies suggested that HSP90 may be required for the binding and delivery of chaperoned, antigenic peptides with MHC I molecules to the cell surface (29). More recently, it has been shown that HSP90 can also facilitate direct tumor recognition by CD4⁺ T cells via a nonclassical, MHC II-mediated mechanism for presentation of intracellular tumor antigens (30). HSP90 also plays pivotal roles in efficient antigen cross-presentation and priming by antigen donor cells (31, 32), and in both the functional and phenotypic regulation of T lymphocytes (33). Abrogation of these processes due to loss of HSP90 activity would therefore predict for additional immunosuppression, and likely be counterproductive to the antitumor effects displayed by checkpoint modulators. Moreover, small-molecule inhibitors of HSP90 have been shown to block disease development and provide symptomatic control in a variety of inflammatory autoimmune disease models (34–37). Most of these effects arose following frequent drug administration schedules. Within the oncology setting, however, the tumor selectivity/retention properties of HSP90 inhibitor compounds permit the use of intermittent dosing regimens that lead to effective and sustained chaperone inhibitory activity within the tumor compartment and more restricted systemic immune system drug exposures.

Despite these considerations, several lines of experimental evidence now suggest that HSP90 inhibition may in fact overcome known barriers that can limit the effectiveness of immunotherapy. Loss of tumor antigen expression is one avenue that contributes to escape from immune recognition (38); thus, agents that either restore or enhance antigen expression are likely to be of considerable therapeutic relevance for promoting tumor

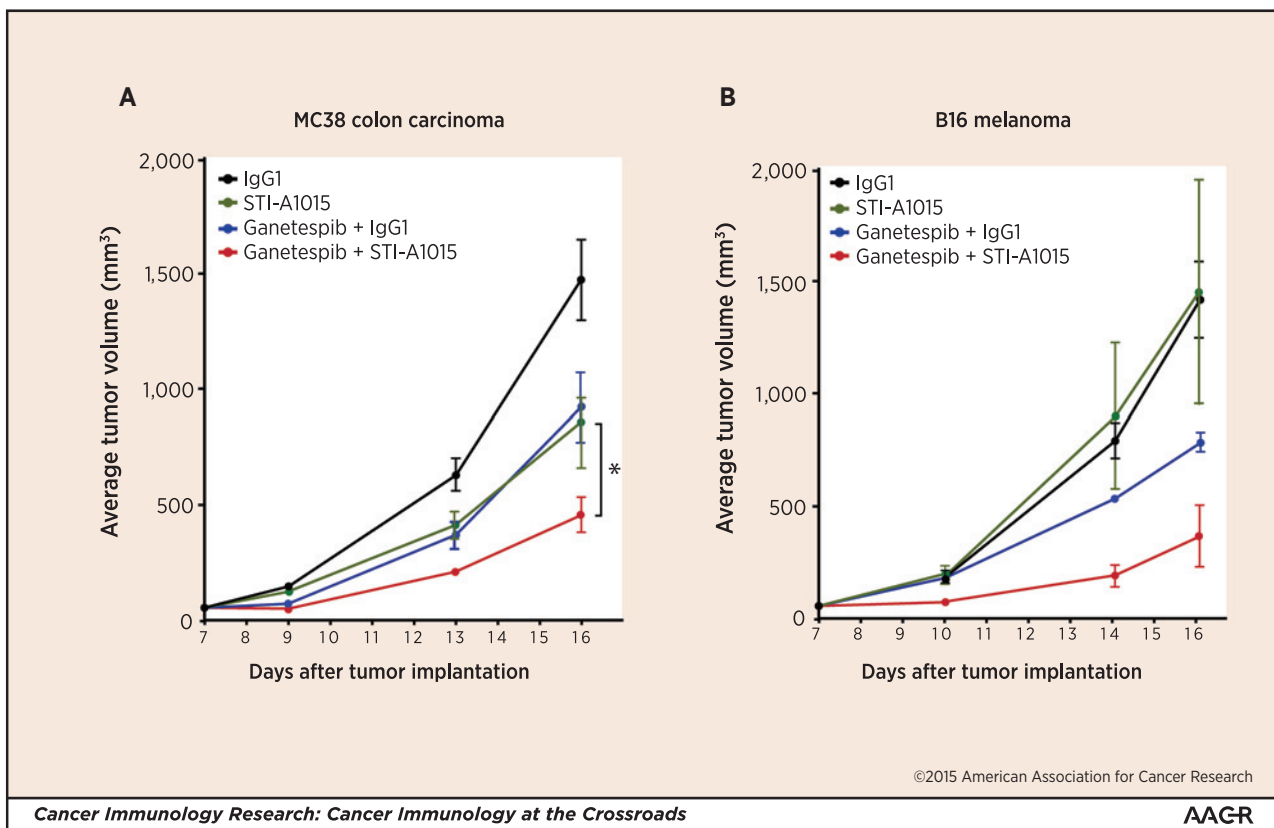


Figure 1.

The HSP90 inhibitor ganetespiB potentiates the antitumor efficacy of PD-L1 antibody treatment in syngeneic mouse tumor models. A, C57 BL/6 mice bearing established MC38 colon carcinoma tumors ($n = 7$ /group) were treated with 200 μ g of IgG1 control or the anti-PD-L1 antibody STI-A1015 (Sorrento Therapeutics, Inc.), either alone or in combination with 125 mg/kg of ganetespiB. GanetespiB was dosed on a weekly schedule (days 8 and 15), STI-A1015 was administered on days 8, 12, and 15. The combination of ganetespiB plus STI-A1015 displayed significantly greater antitumor activity than either individual agent (*, $P < 0.02$). B, C57 BL/6 mice bearing established B16 melanoma tumors ($n = 3$ /group) were dosed using a similar regimen as in A. Although administration of STI-A1015 alone had no effect on tumor growth, the efficacy of antibody treatment was potentiated by cotreatment with ganetespiB.

immunogenicity. In this regard, the ansamycin HSP90 inhibitor 17-allylamino-17-demethoxygeldanamycin (17-AAG) was identified as a potent stimulator of melanoma antigen expression as part of a screen for agents that could enhance T-cell recognition of melanoma tumors (39). This consequence of inhibiting HSP90 activity was subsequently validated in an expanded study evaluating 12 different and structurally diverse HSP90 inhibitor compounds, which confirmed that agents of this class all shared the capacity to increase the expression of tumor-specific differentiation antigens in a panel of melanoma and glioma cell lines (40). Moreover, pharmacologic blockade of HSP90 also resulted in elevated MHC I expression, a coordinate effect that combined with the enhanced antigen expression to promote increased T-cell recognition of treated tumors.

Further compelling evidence of "immune adjuvant" activity for HSP90 inhibitor therapy, mediated via augmented therapeutic T-cell recruitment and tumor-cell recognition, has been provided in a series of studies involving tumors that express the tyrosine kinase receptor EphA2, an established HSP90 client protein. *In vitro*, treatment of EphA2-positive cancer cell lines with another ansamycin inhibitor, 17-dimethylaminoethylamino-17-demethoxygeldanamycin (17-DMAG), improved tumor recognition by low-avidity EphA2 CD8⁺ T cells—an

effect that was related to proteasomal degradation of the client and subsequent MHC-dependent presentation of the derivative peptide antigens (41). Notably, tumor-cell recognition by these specific CD8⁺ T cells was further enhanced by combination of 17-DMAG with agonistic EphA2 antibody treatment. These observations were recapitulated *in vivo* using an EphA2-expressing sarcoma model, wherein 17-DMAG treatment inhibited tumor growth coincident with prolonged recognition by EphA2-specific CD8⁺ T cells and a reduction in suppressor cell populations, including regulatory T cells and myeloid-derived suppressor cells (42). Perhaps more significantly, coadministration of 17-DMAG in the context of either EphA2-centric immunization or adoptive transfer of anti-EphA2 CD8⁺ T cells resulted in superior therapeutic outcomes and the generation of long-term antitumor immunologic memory (42). Separately, a well-established consequence of HSP90 inhibition is the upregulation of the antiapoptotic protein HSP70 (11). Recent data have emerged suggesting a chemokine-like role for HSP70 in the recruitment of antitumor immune cells including CD3⁺/CD4⁺ and CD8⁺/GrB⁺ lymphocytes (43). Thus, what was considered a negative characteristic of HSP90 inhibitors could actually serve to facilitate antitumor immunity and support combination studies with various forms of immunotherapy.

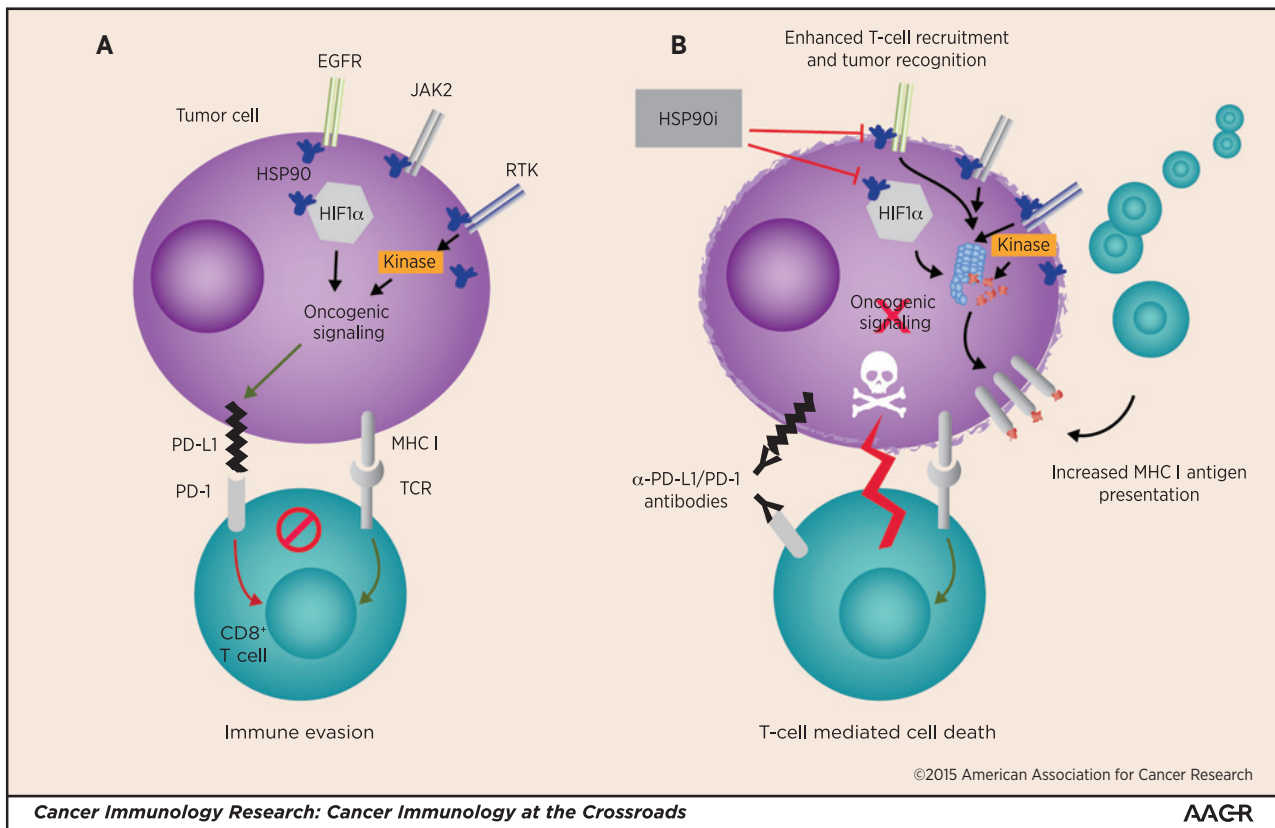


Figure 2.

Potential immunostimulatory activities conferred by HSP90 inhibition (HSP90i). A, within the context of immune checkpoint blockade, active engagement of the PD-1/PD-L1 axis dampens tumor-directed T-cell activation and effector responses, thereby contributing to immune evasion. B, targeted HSP90 blockade results in the proteasomal destruction (blue barrel) of multiple cellular client proteins [e.g., receptor tyrosine kinases (RTK)], resulting in increased expression of MHC I-complexed antigens (rouge-red peptide fragments) at the tumor-cell surface. This in turn promotes susceptibility to immune-mediated attack via enhanced effector T-cell recruitment (aqua blue cells) and tumor-cell recognition. Among the client proteins, oncogenes that may drive PD-L1 expression (e.g., mutant EGFR, JAK2, HIF1 α) would be subjected to destabilization. Consequent loss of PD-L1 expression in the tumor-cell compartment would limit the effectiveness of the immune checkpoint, restore T-cell-mediated cytotoxic activity, and complement the activity of selective PD-1 or PD-L1 antibody therapies. In addition, HSP90 inhibition itself may exert direct cytotoxic effects on tumor cells.

With specific respect to immune checkpoint blockade, a correlation between EGFR activation and a signature of immunosuppression that included upregulation of the PD-1/PD-L1 signaling axis has recently been reported in lung cancer (44). Of particular relevance, the authors showed that PD-L1 expression was induced in mutant EGFR-driven lung tumors and that pharmacologic blockade of the PD-1/PD-L1 pathway in genetically engineered mouse models resulted in reductions in tumor burden and improved overall survival. Interestingly, it was found that while both EGFR- and KRAS-mutant mouse tumors expressed PD-L1, only EGFR-driven models responded to PD-1 blockade. This description of tumor microenvironment remodeling and immune evasion facilitated by oncogenic EGFR signaling strongly implicates an important mechanistic role for PD-L1 expression as an adaptive response in this process. Of note, mutant EGFR oncoproteins are acutely reliant on the chaperone activity of HSP90 for their conformational stability and function (45, 46) and are accordingly sensitive to destabilization following HSP90 inhibitor treatment (47). A similar link between PD-L1 expression and HSP90 client protein activity, specifically due to the presence of oncogenic rearrangements of ALK, has also been described in lymphoma pathogenesis (48). ALK fusion proteins rank among

the most sensitive of all HSP90 client proteins (49, 50) and, at least within the context of ALK-driven lung cancer, have provided a compelling example of the relationship between client protein–driver dependence and clinical efficacy of HSP90 inhibitors (11). PD-L1 expression is also regulated by the HSP90 client proteins HIF1 α and JAK2 (51, 52), and it is likely that this list will continue to expand. In light of these considerations, it is not unreasonable to suggest that potent inhibition of HSP90 alongside immune checkpoint–targeted therapy may be therapeutically beneficial for cancers that aberrantly express PD-L1 induced via HSP90 client-mediated mechanisms, and this rational approach warrants further investigation.

In terms of combining the modalities of HSP90 inhibition and immune-checkpoint release, we have obtained preclinical evidence of superior therapeutic indices achieved with a combination of ganetespib plus anti-PD-L1 antibody treatment in two PD-L1–expressing, syngeneic mouse tumor models (Fig. 1; D.A. Proia; unpublished data). In allograft tumors derived from MC38 colon carcinoma cells, ganetespib monotherapy induced a comparable degree of tumor growth suppression to that seen following treatment with STI-A1015, a fully humanized IgG1 antibody designed with effector function to specifically bind

PD-L1 (Sorrento Therapeutics, Inc.); combining both agents resulted in a significant improvement in antitumor efficacy. Consistent with previously published data (53), anti-PD-L1 antibody administration as monotherapy was largely ineffective at inhibiting B16 melanoma tumor growth; however, ganetespib cotherapy strongly potentiated the tumor response in this highly aggressive cancer model. Although the mechanistic basis for this combinatorial benefit remains to be determined, such findings support the premise that targeting HSP90 may represent a complementary and therapeutically advantageous approach together with immune checkpoint blockade for augmenting antitumor immune responses.

Concluding Remarks

The immuno-oncology landscape is presently undergoing a major transformation due to advancements in immunotherapeutic drug development, best exemplified by the clinical introduction of targeted biologics against the CTLA-4 and PD-1/PD-L1 immune checkpoints. Validation of immune checkpoint blockade as a meaningful therapeutic approach was initially provided by the anti-CTLA-4 antibody ipilimumab in melanoma patients, with an impressive durability of response (yet relatively low proportion of responders) stood in stark contrast to remarkable response rates but limited duration of benefit seen with other molecularly targeted strategies, such as selective BRAF kinase inhibition (54). Significant and durable patient responses following PD-1/PD-L1 blockade have now been demonstrated in other cancer types that, unlike melanoma, have traditionally not been considered susceptible to immunotherapy. For example, immunotherapy has historically proven ineffective in non-small cell lung cancer (NSCLC); however, clinical trials of nivolumab have revealed not only statistically significant response and disease stabilization rates in advanced, chemorefractory NSCLC patients but also an unprecedented durability of response (55). Moreover, the phase III study CheckMate 017 (NCT01642004), comparing nivolumab with docetaxel in patients with second-line squamous cell NSCLC, has been terminated early after demonstrating a clear survival advantage for the PD-1 inhibitor over the chemotherapeutic agent. In addition, pembrolizumab has recently been granted Breakthrough Therapy designation by the FDA for the treatment of NSCLC patients lacking EGFR mutations and ALK rearrangements whose disease has progressed on or following platinum-based chemotherapy. Clearly, both anti-PD-1 drugs are imminently poised to extend their previous regulatory approval in melanoma into NSCLC, and

anti-PD-L1 agents likely to soon follow based on their recent, encouraging signs of activity in the same indication (56, 57). In this rapidly evolving field, expanding the scope of potential cancer indications and identifying strategies that increase the frequency of response in individuals able to derive benefit from this type of therapeutic intervention remains an important consideration.

The development of selective inhibitors of HSP90 over the past two decades paved the way for an exponential increase in our understanding of HSP90 biology and its intimate relationship with malignancy. HSP90 also represents a critical modulator of multiple innate and adaptive immunologic processes, and it now appears that inhibition of this molecular chaperone within this context may elicit a duality of responses, both immunostimulatory and immunosuppressive. As outlined above, accumulating evidence suggests that tumor-based HSP90 inhibition can directly influence, and is compatible with, T-cell-mediated antitumor immunity and may promote enhanced tumor surveillance and recognition through exploitation of client protein-dependent and -independent mechanisms. Figure 2 is a schematic diagram showing how tumor-based HSP90 inhibition may enhance antitumor T-cell immunity. Furthermore, the discovery that oncoprotein-directed dysregulation of PD-1/PD-L1 signaling can occur as an adaptive response for endogenous antitumor immunity raises the possibility for upstream intervention through HSP90 blockade, and it is tempting to speculate that additional, relevant pathways sensitive to HSP90 inhibition with the capacity to restore and/or amplify immune function are yet to be uncovered. Continued exploration of targeting HSP90 function as a modality for galvanizing an immune-based attack on tumors, particularly with respect to cooperative activity alongside immune checkpoint agents, is therefore warranted.

Disclosure of Potential Conflicts of Interest

D.A. Proia is the Director/Cancer Biology and has ownership interest (including patents) in Synta Pharmaceuticals. G.F. Kaufmann is VP Research, Early Development, Global Partnerships, and has ownership interest (including patents) in Sorrento Therapeutics, Inc.

Acknowledgments

The authors wish to dedicate this article to their dear colleague and friend Amar Singh, whose vision and tireless efforts brought us together to review and explore the concept laid out here. They also thank Dr. Richard Bates for help in preparation of the article and Walter Storkus for critical review.

Received February 27, 2015; accepted March 16, 2015; published OnlineFirst May 6, 2015.

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