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POLYCLONAL IMMUNE RESPONSES TO ANTIGENS ASSOCIATED WITH CANCER SIGNALING PATHWAYS AND NEW STRATEGIES TO ENHANCE CANCER VACCINES

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Abstract

Aberrant signaling pathways are a hallmark of cancer. A variety of strategies for inhibiting signaling pathways have been developed, but monoclonal antibodies against receptor tyrosine kinases have been amongst the most successful. A challenge for these therapies is therapeutic unresponsiveness and acquired resistance due to mutations in the receptors, upregulation of alternate growth and survival pathways or inadequate function of the monoclonal antibodies. Vaccines are able to induce polyclonal responses which can have a multitude of affects against the target molecule. We began to explore therapeutic vaccine development to antigens associated with these signaling pathways. We provide an illustrative example in developing therapeutic cancer vaccines inducing polyclonal adaptive immune responses targeting the ErbB family member HER2. Further, we will discuss new strategies to augment the clinical efficacy of cancer vaccines by enhancing vaccine immunogenicity and reversing the immunosuppressive tumor microenvironment.

Keywords

Cancer immunotherapy; polyclonal; vaccines; antigens; active immunotherapy

INTRODUCTION

Manipulating the immune system to target malignancies through approaches ranging from autologous dendritic cell based vaccines to therapeutic immune-modulation [1-4] is now leading to clinical benefit, but a number of opportunities and challenges remain. Identifying novel tumor antigens continues to be a priority and insights from sequencing the cancer genome have revealed a number of genetic changes in cancer cells including somatic mutations, translocations, and amplifications which can lead to novel cancer specific antigens [5-7]. The role of post translational modification in the generation of novel cancer antigens is also an opportunity to be explored [7]. Characterizing the immunosuppressive environment of tumors and developing strategies to counter or evade these suppressive influences has been receiving considerable attention; and, an emphasis on the generation and regulation of antigen specific T cells has also been central to research in cancer

immunotherapy. However, we believe that the emerging recognition that cancer signaling pathways play pivotal roles in the biologic behavior of tumor cells creates an opportunity to rethink modern cancer immunotherapy strategies [8-10]. For example, there are clinically validated antigens found on cancer that have been targeted both by monoclonal antibodies and small molecules (Table 1). Interestingly, these targeted approaches are also generally associated with high rates of therapeutic resistance which does not typically involve loss of antigen expression, making them potential targets for alternative immunotherapeutic strategies [11, 12]. One such strategy is to develop polyclonal humoral immune responses by active immunotherapy.

THE THERAPEUTIC POTENTIAL OF POLYCLONAL IMMUNE RESPONSES TO HER2

An example of a credentialed tumor-associated antigen is the human epidermal growth factor receptor 2 (HER2), overexpressed in 20-30% of breast cancers and associated with more aggressive tumors and inferior overall survival [13]. Combinations of the anti-HER2 antibody trastuzumab and chemotherapy lengthen survival in metastatic HER2-overexpressing breast cancer [14]. However, progressive disease typically occurs within one year. Lapatinib, a potent reversible inhibitor of HER2 and EGFR tyrosine kinases [15], in conjunction with chemotherapy, enhances time to progression in these patients [16]. Unfortunately, responses to lapatinib are generally short-lived, and progression remains a significant clinical problem.

As mentioned, the overexpression of HER2 persists in trastuzumab and lapatinib-refractory tumors [11, 12, 17-19], and thus, targeting HER2 with a polyclonal adaptive immune response that includes both T cells and antibodies is a potential strategy. More than a dozen phase I and II studies of cancer vaccines have been conducted in breast cancer patients [20]. These vaccines have included proteins, peptides, modified tumor cells, and dendritic cells loaded with breast tumor antigens. In these studies, HER2 has been demonstrated to be immunogenic, with a suggestion that immunized patients had an improved clinical outcome [21-25].

We have developed an adenoviral vector vaccine expressing a kinase-inactive, full length human HER2 gene (Ad-HER2-ki), which we have demonstrated is non-oncogenic [26] to overcome safety the potential safety issues around using an oncogene as a vaccine. We have shown that this vector induces HER2-specific T cell responses and polyclonal antibody responses capable of mediating both antibody dependent cellular cytotoxicity (ADCC) and complement dependent cytotoxicity (CDC). This is distinct from the effects of trastuzumab, which mediates ADCC but not CDC. Similar results have recently been reported by another group using a similar adenoviral vector strategy [27].

In addition to these classical immune functions, the antibodies induced by Ad-HER2-ki had potent anti-proliferative effects on HER2-expressing tumor cells [28]. Similar results had previously been reported in animals and in human cancer patients (unpublished data) [29, 30]. We hypothesized that this anti-proliferative effect might be due to receptor downregulation and subsequently demonstrated that the HER2-vaccine induced antibodies within serum produced significant receptor internalization that did not occur when tumor cells were treated with trastuzumab, distinguishing the polyclonal serum antibodies from conventional monoclonal antibody approaches (Figure 1). We and others have observed that HER2 receptor internalization is not a function of trastuzumab [31-33]. Combining two monoclonal antibodies targeting different epitopes on HER2 has been observed to cause HER2 internalization [32, 34] and there is other evidence that supports the contention that multiple antibodies to different epitopes are more efficient at internalizing receptors [34-37]. We have identified 14 epitopes recognized by the HER2-vaccine induced antibodies [26]. The importance of the internalization lies in the possibility that internalized receptors may

meet one of two fates, either being recycled to the cell surface or degraded. Receptors recycled to the cell surface may continue to stimulate tumor growth, while receptor degradation would block growth factor signaling and clearly be the more desirable outcome for an anti-tumor strategy. There is also the possibility that receptor degradation may lead to more efficient antigen presentation to promote T cell responses against tumors cells expressing the receptor.

Finally, because the monoclonal HER2 targeting antibody trastuzumab synergizes with lapatinib, we tested whether vaccine induced antibodies induced by vaccinations against HER2 would synergize with lapatinib *in vitro* and whether combining lapatinib and Ad-HER2-ki immunization would lead to enhanced control of breast tumors *in vivo*. Our results establish that the combination was superior to either agent alone *in vitro* and *in vivo*. Although lapatinib and HER2-VIA target the same molecule their effects on signaling are different. Alone, the HER2-vaccine induced antibodies had their greatest effect on HER2 protein levels. As expected, lapatinib interrupted signaling through HER2 and thus the phosphorylation of downstream molecules. The combination of the two reagents resulted additionally in a reduction in levels of the anti-apoptotic protein survivin, which would result in enhanced tumor cell apoptosis. Thus, although combining lapatinib and trastuzumab has shown favorable clinical results [38], it is possible that the combination of lapatinib and a polyclonal anti-HER2 antibody response induced by vaccination will be superior because of the additional effects provided by polyclonal antibodies over a monoclonal antibody targeting a single epitope. It is also intriguing that lapatinib treatment can lead to stabilization and accumulation of HER2, enhancing trastuzumab-mediated cytotoxicity [39]. We expect similarly that it will potentiate the activity of vaccines targeting HER2.

The potential benefits of a vaccine strategy over a monoclonal antibody approach, with the induction of both T cell and polyclonal antibody responses, and multiple mechanisms of action resulting from polyclonal antibody induction, encourage the use of vaccine strategies. And there is increasing evidence that cancer vaccines can improve patient survival, renewing enthusiasm for cancer vaccine approaches. [40-43]. The synergy seen with the vaccine plus lapatinib suggests that their use in combination should also be evaluated clinically. And we will initiate this clinical study in the next 12 months. More broadly, we believe our results suggest that targeting receptor molecules using vaccines as a means to perturb signaling offers new opportunities to target cancer beyond the conventional lytic killing of cancer cells by the immune system.

THE THERAPEUTIC POTENTIAL OF POLYCLONAL IMMUNE RESPONSES TO OTHER CANCER SIGNALING ANTIGENS- HER3

Cancer immunotherapy has most often targeted antigens preferentially if not exclusively expressed by cancer cells. Due to the chronic nature of the progression of cells from pre-invasive to invasive and then metastatic disease, the long term expression of tumor antigens in the absence of appropriate co-stimulation can be associated with immunologic unresponsiveness [44]. Strategies that overcome this antigen specific unresponsiveness appear clinically promising including treatment with anti-CTLA4 antibodies [45-48]. An alternative strategy would be to target antigens that are not constitutively overexpressed by tumor cells, but rather, are rapidly induced in response to therapeutic interventions. Therapeutic resistance, either intrinsic or acquired following an initial period where a patients' cancer is responsive to the therapy, is a major obstacle to effective and sustained cancer treatment. It is almost universally true that despite sometimes impressive clinical results with kinase-targeted therapies, clinical experience dictates that most, if not all, treatment-responsive patients will eventually experience relapse as a result of acquired drug resistance [17, 18]. We hypothesize that these induced antigens would represent ideal

antigens to target by vaccination, as their acute expression would not be associated with immunologic unresponsiveness.

One such example is the overexpression of HER3 as a mechanism of induced resistance to EGFR and HER2 targeted therapies [49-51]. Although HER3 itself is not a tractable target for small molecule drug approaches because it lacks intrinsic tyrosine kinase activity, alternative approaches can be considered to ablate its signaling effects, either alone or as a potent heterodimer formed with EGFR or ERB2. One such alternative is the active immunization targeting HER3, which we have recently demonstrated generates HER3 specific immune responses that have anti-tumor effects *in vivo* and *in vitro* (manuscript in preparation). Thus, we believe that antigens that are upregulated by tumors in response to therapy represent a particularly good target for a cancer vaccine strategy.

RESISTANCE TO IMMUNE-MEDIATED KILLING BY T CELLS

Despite the utility demonstrated in experimental animal models, the application of this strategy must address shortcomings in current clinical cancer vaccine technologies. Although the benefits of therapeutic vaccination with autologous dendritic cells has been recently demonstrated, new technologies and insight into the requirements for inducing clinically relevant adaptive immune response provide an opportunity for use to improve the potency of cancer vaccines. For example, in tumor types which are refractory to conventional chemotherapy, immune effector cells remain highly capable to inducing killing when directed toward tumor cells. We demonstrated that metastatic human colorectal cancer (CRC) previously treated with conventional chemotherapy would be sensitive to T-cell killing mediated by carcinoembryonic antigen (CEA)/CD3-bispecific T-cell-engaging BiTE antibody (MEDI-565) [52]. We analyzed proliferation and lysis of CEA-positive (CEA+) CRC specimens that had survived previous systemic chemotherapy and biologic therapy to determine whether they could be killed by patient T cells engaged by MEDI-565 *in vitro*. At low concentrations (0.1-1 ng ml⁻¹), MEDI-565+ T cells caused reduced proliferation and enhanced apoptosis of CEA+ human CRC specimens. High levels of soluble CEA did not impair killing by redirected T cells and there was no increase in resistance to T-cell killing despite multiple rounds of exposure. This study shows for the first time that metastatic CRC specimens derived from patients previously treated with conventional chemotherapy can be lysed by patient T cells.

ANTIGEN DISCOVERY

In addition to well known tumor antigens, other antigens are being identified in subsets of common tumors, and there is increasing interest in their utility, particularly if they are in tumor subsets with a particularly poor prognosis. For example, cell surface proteoglycan, chondroitin sulfate proteoglycan 4 (CSPG4), is a potential target for monoclonal antibody-based immunotherapy for many types of cancer [53]. The lack of effective therapy for triple-negative breast cancer (TNBC) prompted us to examine whether CSPG4 is expressed in TNBC and can be targeted with CSPG4-specific mAb. CSPG4 protein expression was assessed in 44 primary TNBC lesions, in TNBC cell lines HS578T, MDA-MB-231, MDA-MB-435, and SUM149, and in tumor cells in pleural effusions from metastatic breast cancer patients. CSPG4 protein was preferentially expressed in 32 of the 44 (72.7%) primary TNBC lesions tested, in TNBC cell lines, and in tumor cells in pleural effusions from 12 metastatic breast cancer patients.

The effect of CSPG4-specific mAb 225.28 on growth, adhesion, and migration of TNBC cells was tested *in vitro*. CSPG4-specific mAb 225.28 statistically significantly inhibited growth, adhesion, and migration of TNBC cells *in vitro*. mAb 225.28 induced 73.1% regression of tumor metastasis in a TNBC cell-derived experimental lung metastasis model

(mAb 225.28 vs control, mean area of metastatic nodules = 44590.8 vs 165950.8 μm^2 ; difference of mean = 121360.0 μm^2 , 95% confidence interval = 91010.7 to 151709.4 μm^2 ; $P < .001$). Additionally, mAb 225.28 statistically significantly reduced spontaneous lung metastases and tumor recurrences in an orthotopic xenograft mouse model. The mechanisms responsible for antitumor effect included increased apoptosis and reduced mitotic activity in tumor cells, decreased blood vessel density in the tumor microenvironment, and reduced activation of signaling pathways involved in cell survival, proliferation and metastasis.

This study identified CSPG4 as a new target for TNBC. The antitumor activity of CSPG4-specific mAb was mediated by multiple mechanisms, including the inhibition of signaling pathways crucial for TNBC cell survival, proliferation, and metastasis.

NEW CANCER VACCINE STRATEGIES IN CLINICAL TRIALS AT DUKE

In addition to the recent activities in identifying important new antigens, improvement in antigen delivery for vaccination has occurred. For example, potent recombinant viral vectors have been clinically suboptimal due to the presence of neutralizing vector specific immune response. One alternative is the use of next generation vectors that can immunize in the setting of pre-existing vector immunity. A specific example involves the first generation recombinant serotype 5 adenovirus (Ad5) vectors which lack E1 expression and induce robust immune responses against encoded transgenes in pre-clinical models, but have muted responses in human trials because of widespread pre-existing anti-adenovirus immunity. Attempts to circumvent Ad5-specific immunity by using alternative serotypes or modifying capsid components have not yielded profound clinical improvement. To address this issue, we explored a novel alternative strategy, specifically reducing the expression of structural Ad5 genes by creating E1 and E2b deleted recombinant Ad5 vectors. We demonstrated that [E1-, E2b-]vectors retaining the Ad5 serotype are potent immunogens in pre-clinical models despite the presence of significant Ad5-specific immunity, in contrast to [E1-] vectors [54].

We extended these observations to pre-clinical models of cancer immunotherapy. A vector containing the carcinoembryonic antigen (CEA) gene insert was constructed to induce cell-mediated immunity (CMI) against the tumor-associated target and the CEA immunogenicity and in vivo anti-tumor effects of repeated immunizations with Ad5 [E1-, E2b-]-CEA compared with those observed with current generation Ad5 [E1-]-CEA were tested in Ad5 pre-immunized mice. We demonstrated that Ad5-immune mice immunized multiple times with Ad5 [E1-, E2b-]-CEA induced CEA-specific CMI responses that were significantly increased over those detected in Ad5-immune mice immunized multiple times with a current generation Ad5 [E1-]-CEA. Ad5 immune mice bearing CEA-expressing tumors that were treated with Ad5 [E1-, E2b-]-CEA had increased anti-tumor response as compared with Ad5 [E1-]-CEA treated mice. These results demonstrate that Ad5 [E1-, E2b-]-CEA can induce antigen specific immune responses which result in tumor growth inhibition despite the presence of pre-existing Ad5 immunity. These results have lead to an ongoing phase I clinical testing this viral construct in patients with advanced colorectal cancers which express CEA. An important endpoint of this study will be whether immune responses may be induced in patients with high titer anti-Ad antibodies [55].

Although adenoviral vectors have been a mainstay in gene transfer studies, even more novel vector systems can be generated with properties which exploit the role of dendritic cells to present antigen. One approach is to use a viral vector to deliver antigen to in situ DCs, which then activate tumor-specific T cell and antibody responses. However, vector-specific neutralizing antibodies and suppressive cell populations such as Tregs remain great challenges to the efficacy of this approach. We constructed a novel alphavirus vector that expressed CEA, and demonstrated that it, packaged in virus-like replicon particles (VRP),

was capable of efficiently infecting DCs, could be repeatedly administered to patients with metastatic cancer expressing the tumor antigen carcinoembryonic antigen (CEA) and that it overcame high titers of neutralizing antibodies and elevated Treg levels to induce clinically relevant CEA-specific T cell and antibody responses [56]. The CEA-specific antibodies mediated antibody-dependent cellular cytotoxicity against tumor cells from human colorectal cancer metastases. In addition, patients with CEA-specific T cell responses exhibited longer overall survival. These data suggest that VRP-based vectors can overcome the presence of neutralizing antibodies to break tolerance to self antigen and may be clinically useful for immunotherapy in the setting of tumor-induced immunosuppression.

NEW CANCER VACCINE STRATEGIES

We can also improve immunogenicity by enhancing the innate and adaptive responses by augmenting the activation signals to antigen presenting cells. For example, although critical for initiating and regulating immune responses, the therapeutic use of individual cytokines as anticancer immunotherapeutic agents has achieved only modest clinical success. Consequently, many current strategies have focused on the use of specific immunotherapeutic agonists that engage individual receptors of innate immune networks, such as the Toll-like receptor (TLR) system, each resulting in specific patterns of gene expression, cytokine production, and inflammatory outcome. However, these immunotherapeutics are constrained by variable cellular TLR expression and responsiveness to particular TLR agonists, as well as the specific cellular context of different tumors. We demonstrated that overexpression of MyD88, a pivotal regulator of multiple TLR signaling pathways, could circumvent these constraints and mimic coordinated TLR signaling across all cell types in a ligand-independent fashion [57, 58]. We generated an adenoviral vector expressing MyD88 and show that Ad-MyD88 infection elicits extensive Th1-specific transcriptional and secreted cytokine signatures in all murine and human cell types tested in vitro and in vivo. Importantly, in vivo intratumoral injection of Ad-MyD88 into established tumor masses enhanced adaptive immune responses and inhibited local tumor immunosuppression, resulting in significantly inhibited local and systemic growth of multiple tumor types. Finally, Ad-MyD88 infection of primary human dendritic cells, tumor-associated fibroblasts, and colorectal carcinoma cells elicited significant Th1-type cytokine responses, resulting in enhanced tumor cell lysis and expansion of human tumor antigen-specific T cells. Thus, Ad-MyD88 initiated robust antitumor activity in established murine tumor microenvironments and in human contexts, suggesting its potential effectiveness as a clinical immunotherapeutic strategy.

APPROACHES TO OVERCOMING THE IMMUNOSUPPRESSIVE TUMOR MICROENVIRONMENT

Finally, despite the advances in antigen identification, antigen delivery, and enhancement in immunogenicity, we need to explore strategies to overcome the profound suppressive tumor microenvironment (TME) and Treg population found in cancer patients that diminish anti-tumor immunity. Impairment of dendritic cells (DC), the most effective activators of anticancer immune responses, is one mechanism for defective antitumor immunity, but the causes of DC impairment are incompletely understood. We evaluated the association of impaired DC differentiation with angiogenesis-associated molecules D-dimer, vascular endothelial growth factor (VEGF), urokinase plasminogen activator (uPA), and plasminogen activator inhibitor (PAI-1) in peripheral blood from 41 patients with lung, breast, and colorectal carcinoma. Subsequently, we studied the effect of administration of the anti-VEGF antibody (bevacizumab) on DC maturation and function in vivo [59, 60]. Compared with healthy volunteers, cancer patients had a bias toward the immunoregulatory DC2, had deficits in DC maturation after overnight in vitro culture, and had a significant increase in immature myeloid cell progenitors of DC ($0.50 \pm 0.31\%$ vs. $0.32 \pm 0.16\%$ of peripheral

blood mononuclear cells, respectively, $P = 0.011$). A positive correlation was found between the percentage of DC2 and PAI-1 ($R = 0.50$) and between immature myeloid cells and VEGF ($R = 0.52$). Bevacizumab administration to cancer patients was associated with a decrease in the accumulation of immature progenitor cells ($0.39 \pm 0.30\%$ vs. $0.27 \pm 0.24\%$, $P = 0.012$) and induced a modest increase in the DC population in peripheral blood ($0.47 \pm 0.23\%$ vs. $0.53 \pm 0.30\%$). Moreover, anti-VEGF antibody treatment enhanced allo-stimulatory capacity of DC and T cell proliferation against recall antigens. These data suggest that DC differentiation is negatively associated with VEGF levels and may be one explanation for impaired anticancer immunity, especially in patients with advanced malignancies.

VEGF is increasingly emerging as a central player in the activity of immunosuppressive cell types that have been shown to be a major factor in the immunosuppressive TME, including specific subsets of TAMs (reviewed in: [61-63]) and MDSC (reviewed in: [64-66]). TAMs have diverse immunosuppressive functions including inhibition of CTL function and influencing the influx of Treg into the TME. MDSC are related to TAMs but represent a distinct and diverse population of myeloid cells based on phenotypic markers and gene expression studies. MDSC suppress innate and adaptive immune responses, affecting T, NK, and NKT cell function and blocking DC maturation. Both TAMs and MDSC are associated with the onset of resistance to anti-VEGF therapies.

Some therapeutic strategies are focused on enhancing maturation of MDSC precursors into mature myeloid cells and thus avoiding the suppressive effects of accumulating MDSC (reviewed in: [67]). Therapeutic strategies to bias macrophages to a more M1/anti-tumor macrophage population are also being studied, including blockade of the macrophage CSF1R [68, 69]. An alternate strategy is to block the development of MDSC and TAMs from their precursors by targeting VEGF. Using an anti-VEGF strategy is appealing for a number of reasons, including that it will starve the tumor blood supply as well as potentially reverse immunosuppression by MDSC and TAMs, and thereby enhance immune function. Multiple studies have shown that VEGF plays a role in DC dysfunction in cancer patients and have led to studies targeting VEGF [60]. For example, Manning et al. showed that an anti-VEGF MAb was able to trigger anti-tumor immunity in mice [70]. However, Gabrilovich and colleagues have reported that although an anti-VEGF therapy in cancer patients improved the differentiation of dendritic cells, it alone was not sufficient to induce antitumor immunity [71]. These findings suggest that an anti-VEGF strategy might enhance the response to a cancer vaccine in patients. To date there has been only a single clinical study combining a cancer vaccine with avastin and that study showed the vaccine could elicit immune responses and modulate PSA levels in patients with biochemically recurrent prostate cancer, despite the negative effects of avastin on DC function [72]. Further studies are warranted.

Another major factor that hampers the use of cancer vaccines to elicit clinically effective anti-tumor immunity are CD4(+)CD25(high)FoxP3(+) regulatory T (Treg) cells [73]. To directly address the role of regulatory T cells, we have performed clinical trials depleting human Tregs during cancer immunotherapy. In preclinical and clinical studies, we assessed the immune consequences of FoxP3(+) Treg-cell depletion in patients with advanced malignancies [74]. We demonstrated that a CD25(high) targeting immunotoxin (denileukin diftitox) depleted FoxP3(+) Treg cells, decreased Treg-cell function, and enhanced antigen-specific T-cell responses in vitro. We then attempted to enhance antitumor immune responses in patients with carcinoembryonic antigen (CEA)-expressing malignancies by Treg-cell depletion. In a pilot study ($n = 15$), denileukin diftitox, given as a single dose or repeated dosing, was followed by immunizations with dendritic cells modified with the fowlpox vector rF-CEA(6D)-TRICOM. By flow cytometric analysis, we report the first

direct evidence that circulating CD4(+)CD25(high)FoxP3(+) Treg cells are depleted after multiple doses of denileukin diftitox. Earlier induction of, and overall greater exposure to, the T-cell response to CEA was observed in the multiple-dose group, but not the single-dose group. These results indicate the potential for combining Treg-cell depletion with anticancer vaccines to enhance tumor antigen-specific immune responses and the need to explore dose and schedule of Treg depletion strategies in optimizing vaccine efforts. Alternative approaches to modulate Treg levels include cyclophosphamide (Cy) [75] and recent data also suggests that letrozole has effects on Treg [76]. One report has compared ONTAK and Cy and suggested that while Cy mediated double the reduction in Treg numbers when compared to ONTAK, only Cy had a negative impact on other T cell subsets and resulted in a lack of effective T cell priming [77] and we have observed similar effects using the predominant active Cy metabolite 4-hydroxycyclophosphamide (unpublished).

In addition to regulatory T cells, other “suppressor” cells have been described by us and others [78, 79]. We have identified a subset of CD4+ NKT cells in humans that diminish antigen specific CD8+ T cell expansion and are pursuing the biology of these cells in our cancer vaccine studies. And Matsushita et al., have recently reported on B10 cells, a regulatory B cell subset that play a role in preventing the onset of EAE in a mouse model but whose role in tumor immunity is yet to be defined. Thus, we face a myriad of different cell types whose suppressive function can impair the effectiveness of cancer vaccines and it is in the area of immunomodulation to counteract these cells that new approaches are needed to tip the balance in favor of anti-tumor immunity.

CONCLUSIONS

Although a variety of strategies have been employed to prioritize the development of therapeutic strategies directed toward these antigens, the emerging recognition of the role of aberrant signaling in cancer has lead to immunologic therapies, usually monoclonal antibodies targeting critical signaling pathways. Because adaptive immune responses are capable of inducing polyclonal immune effector responses which have different biologic effects than monoclonal antibodies or small molecule kinase inhibitors, we have begun to explore therapeutic vaccine development to antigens associated with these signaling pathways. We have shown that therapeutic cancer vaccines inducing polyclonal adaptive immune responses targeting the Erb family member HER2 have classical and non-classical anti-tumor effects, and these strategies should be explored to target other antigens associated with signaling pathways critical for survival of tumor cells or resistance mechanisms induced by therapy. More broadly, we are seeking to develop more potent cancer vaccines through the use of new adjuvant strategies with TLR adaptor agonists, new vector platforms that overcome anti-vector immunity and Treg, and we are developing strategies to overcome the immunosuppressive tumor microenvironment. Collectively, these studies will lead to increasingly better vaccines and we hope will build on the recent success of Provenge, which was the first cancer vaccine to be approved by the FDA and represents a new class of anti-cancer therapy.

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Figure 1. Binding of HER2-VIA results in HER2 receptor internalization. Human HER2+ breast cancer cells (SKBR3) were stained with DAPI (nuclear stain, blue) and an anti-HER2 MAb (red). Cells were then incubated with HER2-VIA from Ad-HER2 vaccinated mice for 5 minutes (Upper panel) or 2 hours (lower panel), and then visualized by fluorescence microscopy.

Table 1

Examples of antigens targeted by therapeutic monoclonal antibodies (MAb) and/or small molecule tyrosine kinase inhibitors (TKI).

Antigen	Therapeutic	Class	Reference
EGFR	Cetuximab	MAB	[80]
EGFR	Panitumumab	MAB	[81]
EGFR	Gefitinib	TKI	[82]
CD20	Rituximab	MAB	[83]
HER2	Trastuzumab	MAB	[14]
HER2	Lapatinib	TKI	[16]
VEGF	Avastin	MAB	[84]
VEGF	Sorafenib	TKI	[85]
VEGF	Sunitinib	TKI	[86]