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Hypothesis: the generation of T cells directed against neoepitopes employing immune-mediating agents other than neoepitope vaccines

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ABSTRACT

The development of vaccines, especially RNA-based, directed against patient-specific tumor neoepitopes is an active and productive area of cancer immunotherapy. Promising clinical results in melanoma and other solid tumor types are emerging. As with all cancer therapy modalities, neoepitope vaccine development and delivery also has some drawbacks, including the level of effort to develop a patient-specific product, accuracy of algorithms to predict neoepitopes, and with the exception of melanoma and some other tumor types, biopsies of metastatic lesions of solid tumors are often not available. We hypothesize that in some circumstances the use of rationally designed combinations of "off-the-shelf" agents may prove an additional path to enable the patient to produce his/her own "neoepitope vaccine" in situ. These combination therapies may consist of agents to activate a tumor-associated T-cell response, potentiate that response, reduce or eliminate immunosuppressive entities in the tumor microenvironment, and/or alter the phenotype of tumor cells to render them more susceptible to immune-mediated lysis. Examples are provided in both preclinical and clinical studies in which combinations of "off-the-shelf" agents lead to the generation of T cells directed against tumor-derived neoepitopes with consequent antitumor activity.

The development of vaccines directed against neoepitopes of solid tumors has recently been and continues to be an active and potentially promising area of investigation. To date, ~150 clinical studies employing neoepitope vaccines have been reported. Of note, several trials employing RNA-directed vaccines are providing intriguing results. In one of the few randomized trials,[1] the messenger RNA-4157(V940) vaccine, based on individual patients' neo-antigens, plus anti-programmed cell death protein-1 (PD-1) monoclonal antibody (MAb), was randomized to anti-PD-1 alone in patients with completely resected stage IIIB-IV melanoma; the risk of recurrence was 44% lower in the combination arm. Employing another RNA-based vaccine (BNT122) custom-made for each patient, given in combination with anti-programmed death-ligand 1 (PD-L1) MAb followed by chemotherapy, neoepitope T-cell responses correlated with substantially longer relapse-free survival of eight patients with pancreatic cancer.[2] An adeno-based personalized neoepitope vaccine[3] has been administered to front-line patients with metastatic microsatellite-stable colorectal cancer and is showing trends in progression-free survival. Other promising clinical studies have been reported in smaller trials in patients with hepatocellular cancer, non-small cell lung cancer, and multiple tumor types[4]; all these single-arm studies also included anti-PD-1/L1 agents and/or chemotherapy or cytokine. Scores of preclinical studies of vaccines directed against neoepitopes have also been reported with promising results both as monotherapies and in combination with checkpoint antibodies and/or other immune-modulating agents. Dozens of review articles on neoepitope vaccines have been written in recent years describing the above.[5 6] The use of vaccines directed against neoepitopes that are shared among different tumors, such as the various mutations of RAS or p53, is an attractive approach, but each vaccine must be matched with the individual patient's class I/II alleles. Only 24 of 911,584 mutant neoantigens were discovered to be shared by at least 5% of patients.[7] Moreover only 0.42% of 3,760 anticipated neoantigens were detected in more than one tumor.[8] While the vast majority of T cells generated against neoepitopes have been $CD8^+$, $CD4^+$ T cells were generated with the use of a 13-mer mutated RAS peptide vaccine.[9]

While the use of patient individualized vaccines directed against neoepitopes is an extremely important and promising area of

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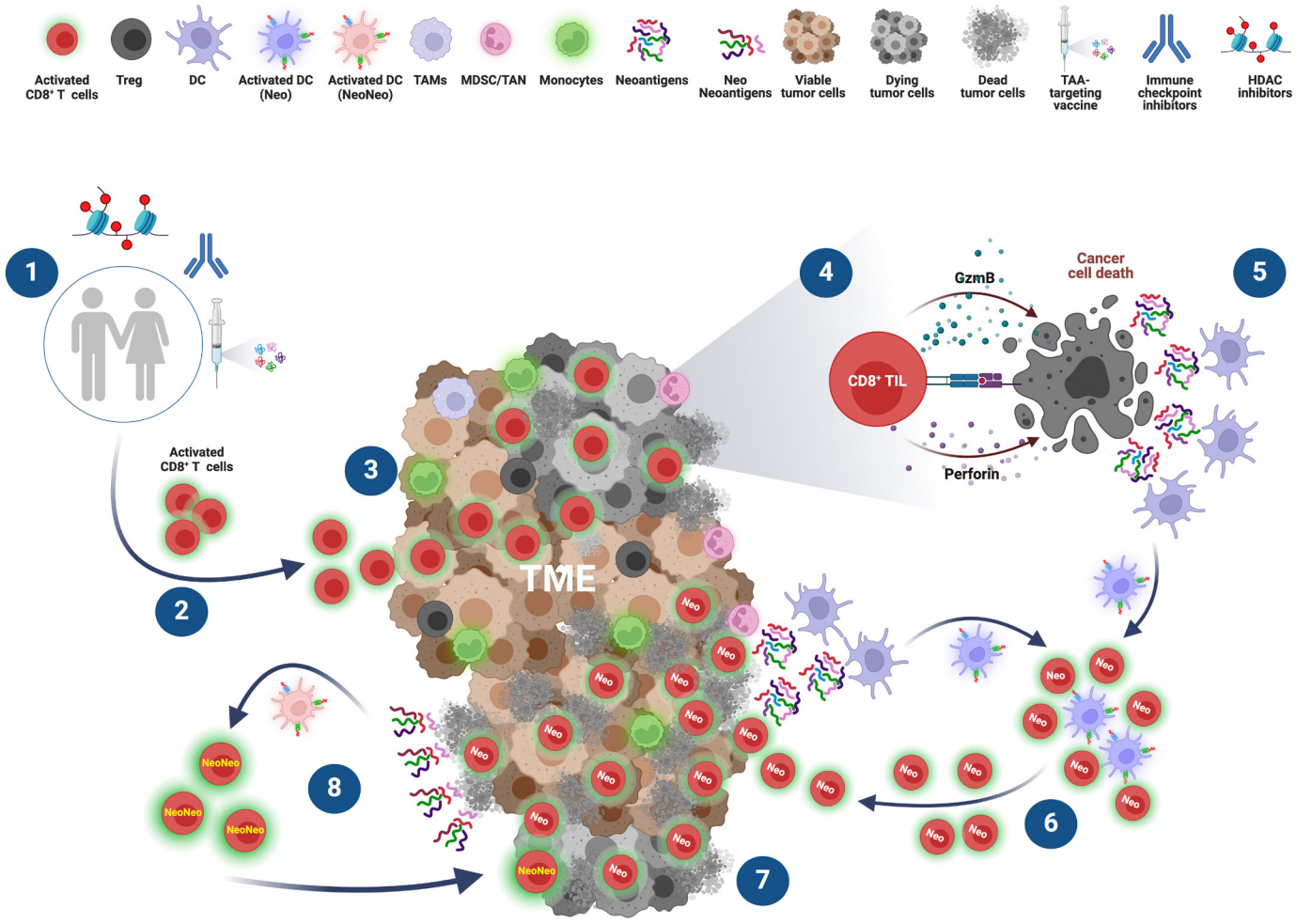

Figure 1 Generation of neoepitope-specific T-cell responses in the absence of neoepitope-targeted vaccination. (1) Multiple immune-mediating agents (2) activate CD8$^+$ T cells with lytic ability, (3) directing them to the tumor microenvironment. At the tumor site, (4) cytotoxic CD8$^+$ T cells mediate tumor destruction, with (5) consequent release of neoantigens. Dendritic cell (DC) uptake of released neoantigens triggers DC maturation, with consequent (6) priming and clonal expansion of CD8$^+$ T cells directed to neoantigens (Neo). Subsequent migration of Neo CD8$^+$ T cells to the tumor site (7) promotes tumor cell death, amplifying the expansion of Neo CD8$^+$ T cells. (8) Tumor cell destruction elicited by cytotoxic Neo CD8$^+$ T cells can promote the release and DC uptake of additional neoantigens, leading to the generation of new Neo CD8$^+$ T cells (NeoNeo). Created with BioRender.com. HDAC, histone deacetylase; MDSC, myeloid-derived suppressor cells; TAA, tumor-associated antigen; Treg, regulatory T cells; TAM, tumor-associated macrophage; TAN, tumor-associated neutrophil; TIL, tumor-infiltrating lymphocyte; TME, tumor microenvironment.

investigation, there are also some drawbacks associated with this technology: (1) they are labor and time intensive to produce for a given patient, (2) algorithms often do not predict the most immunogenic epitopes, and (3) in non-melanoma solid tumors and a few other exceptions, metastatic biopsies reflecting the progression of tumor phenotype are often not available; consequently, a neoepitope vaccine derived from neoepitopes identified from the analysis of mutations in a primary tumor does not necessarily reflect the immune editing in the tumor as a consequence of prior therapies or metastatic spread.

We hypothesize that in some studies the rational design of combinations of "off-the-shelf" immune-mediating agents can efficiently and effectively enable the host to generate T cells directed against neoepitopes capable of eliciting antitumor responses, and, in essence, enable the patient to produce his/her own "neoepitope vaccine" in situ (figure 1). We hypothesize for this to occur one must target multiple components of the immune system and immunosuppressive entities in the tumor microenvironment (TME)—all of which can be accomplished by employing "off-the-shelf" reagents. These regimens will most likely require combinations of agents to (1) activate an immune response, such as a vaccine to a tumor-associated antigen, or even certain chemotherapeutic agents or radiation, (2) potentiate that response, with the use of immunocytokines such as an interleukin (IL)-15 superagonist or a tumor-targeting IL-12, (3) epigenetically modify immune cells to induce greater proliferative and lytic capacity, and modify tumor cell phenotype with the use of an histone deacetylase (HDAC) inhibitor to render tumor cells more susceptible to immune-mediated

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attack, and/or (4) reduce or eliminate immunosuppressive entities such as regulatory T cells or myeloid-derived suppressor cells in the TME with agents directed against transforming growth factor (TGF)-β and/or IL-8. This approach is also not without its issues: excluding anti-checkpoint monoclonal antibodies, the majority of immune-mediating agents are not yet Food and Drug Administration approved, and thus clinical studies to test this hypothesis may require the use of multiple novel–novel combinations of agents, often developed by different Biotech/Pharma with reluctance to engage in such collaborative ventures. Moreover, review and regulatory committees may question the contribution of each agent in the combination therapy—a task requiring multiple clinical trials and years of effort. There is now strong evidence emerging that due to the multiple feedback mechanisms of the immune system, the contribution of a given agent as a single therapy can be quite different when used in combination with a second agent, and different again if a third agent is employed. We suggest that, after safety with multiple immune-mediated agents is established, such trials will be allowed to proceed to determine clinical efficacy, especially in patients with limited choices available. If successful, the components of the trial can then be evaluated in subsequent studies.

It is well known that the use of "off-the-shelf" vaccines directed against "self" tumor-associated antigens (TAAs) has had very little success, most certainly due to the relatively low levels and avidity of the T cells induced. Few trials have employed these "off-the-shelf" vaccines with checkpoint inhibitory antibodies in combination with other "off-the-shelf" immunocytokines to potentiate immune responses, agents to reduce or eliminate immunosuppressive entities in the TME, and/or agents to modulate tumor cells to render them more susceptible to immune-mediated cell lysis. Any level of tumor cell lysis would enable the host to generate neoepitope-specific T cells that recognize the tumor in "real time" and the potential cascade to additional neoepitopes ("neo-neo") for further tumor cell disruption. Evidence that latent recognition of neoepitopes is revealed by other "off-the-shelf" immunotherapies has been observed in clinical studies and correlated with clinical response (table 1). A recent neoadjuvant trial was conducted in patients with human papillomavirus-unrelated head and neck cancer employing the bifunctional anti-PD-L1/TGF-βR2 bintrafusp alfa. Pathologic tumor responses were observed in 5/14 (36%) patients, with the detection of a greater number of T cells to neoepitopes and responses to more unique neoepitopes in the TME correlating with

Table 1 Immune-mediating agents other than neoepitope vaccines show induction of neoepitope-specific T cells correlating with clinical responses

Agent	Disease	Reference
Bintrafusp alfa (α-PD-L1, TGF-βRII)	HPVneg SCCHN	Redman *et al*, Journal of Clinical Investigation, 132:e161400, 2022
α-PD-1+α-CTLA4	Advanced salivary gland cancer	Vos *et al*, Nature Medicine, 29:3077–89, 2023
α-PD-L1	Metastatic urothelial carcinoma	Holm *et al*, Nature Communications, 13:1935, 2022
α-CTLA4	Prostate cancer	Subudhi *et al*, Science Translational Medicine, 12:eaaz3577, 2020
Adoptive transfer vaccine primed autologous T cells+autologous DC tumor cell lysate vaccine+bevacizumab	Recurrent ovarian cancer	Bobisse *et al*, Nature Cancer, 4:1410–7, 2023
Allo-tumor cells (XRT)+GM-CSF+BCG	Melanoma	Podaza *et al*, Frontiers in Immunology, 11:1147, 2020
MAGE vaccine	Melanoma	Corbiere *et al*, Cancer Research, 71:1253–62, 2011
Autologous DC tumor cell lysate vaccine+bevacizumab±low-dose cyclophosphamide	Recurrent ovarian cancer	Tanyi *et al*, Science Translational Medicine, 10:eaao5931, 2018
α-PD-1+α-CTLA4	NSCLC, melanoma	McGranahan *et al*, Science 351:1463–9, 2016
α-PD-1	NSCLC	Rizvi *et al*, Science, 348:124–8, 2015

BCG, Bacille Calmette-Guérin; CTLA4, cytotoxic T-lymphocyte associated protein 4; DC, dendritic cells; GM-CSF, granulocyte-macrophage colony-stimulating factor; HPVneg SCCHN, human papillomavirus negative squamous cell carcinoma of the head and neck; NSCLC, non-small cell lung cancer; PD-1, programmed cell death protein-1; PD-L1, programmed death-ligand 1; TGF, transforming growth factor; XRT, external beam radiation therapy.

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the development of pathologic responses (table 1). In two studies, vaccination of patients with melanoma with a dendritic cell vaccine or a melanoma antigen gene (MAGE)-directed vaccine also led to clones of T cells in tumors directed against neoepitopes (table 1).

Evidence for this phenomenon is also seen in preclinical studies. For example, an adenovirus-based vaccine against the TAA carcinoembryonic antigen (CEA) was unsuccessful in generating antitumor responses; however, the combination of vaccine plus the IL-15 superagonist N-803 and the HDAC inhibitor entinostat generated T cells to neoepitopes and this combination was required for antitumor activity.[10] Similar results in the generation of neoepitopes were seen employing the CEA vaccine in CEA-transgenic mice in combination with bintrafusp alfa and an IL-8R inhibitor.[11]

The HDAC inhibitor entinostat plus anti-PD-1 was also shown to induce neoepitopes in the TME and antitumor activity in two murine bladder cancer models.[12] In another study,[13] a neoepitope vaccine induced some anti-neoepitope T-cell responses, but failed to induce antitumor activity. The addition of immunocytokines and anti-PD-1 to the regimen led to antitumor responses, increased induction of T cells to neoepitopes in the vaccine and, perhaps more importantly, to neoepitopes not in the vaccine. The deletion of the immunosuppressive CSF1 gene in tumors also induced the generation of neoepitopes and antitumor immunity.[14]

We hypothesize that in addition to the use of neoepitope vaccines, in some circumstances the use of rationally designed combinations of "off-the-shelf" immune-mediating agents may prove an additional path in the development of neoepitope-directed T cells capable of tumor cell destruction. As discussed above, this approach will require the understanding that unlike the combined use of chemotherapeutic agents, radiation, or small molecule targeted therapeutics, specific components of combinations of immune-mediating agents may not themselves have tumor-lytic properties, but may modulate other entities in the immune system and/or TME to enhance tumor control. This recognition will lead to the acceleration of clinical trials to validate, or not, this approach. As with all hypotheses, validation will require carefully designed, randomized, multicenter trials.

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