REVIEW

Emerging targets for anticancer vaccination: IDH

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The development of anticancer vaccines as a pillar of cancer immunotherapy has been hampered by the scarcity of suitable tumor-specific antigens. While response to immune checkpoint inhibitors is driven by T cells recognizing mutated antigens, the vast majority of these neoantigens are patient-specific, mandating personalized approaches. In addition, neoantigens are often subclonal present in only a fraction of tumor cells resulting in immune evasion of neoantigen-negative tumor cells. Isocitrate dehydrogenase (IDH)1 mutations, most frequently encoding for the neomorphic protein IDH1R132H, are frequent driver mutations found in the majority of diffuse World Health Organization grade 2 and 3 gliomas. In addition, IDH1R132H generates a shared clonal neoepitope that is recognized by mutation-specific T-helper cells. A recent phase 1 trial (NOA-16, NCT02454634) demonstrated safety and immunogenicity of IDH1-vac, a long IDH1R132H peptide vaccine in patients with newly diagnosed astrocytoma and provided evidence of biological efficacy based on imaging parameters. In addition, vaccine-induced IDH1R132H-reactive tumor-infiltrating T cells were identified. Here we discuss clinical and scientific implications and future developments of IDH-directed immunotherapies.

Key words: glioma, isocitrate dehydrogenase, neoantigen, vaccine, immunotherapy

NEOANTIGENS AS VACCINE TARGETS IN CANCER IMMUNOTHERAPY

The discovery that response to immune checkpoint inhibitors (ICIs) is correlated to tumor mutational burden (TMB) across cancer entities[1] has provided a mechanistic basis for targeting mutated antigens, so-called neoantigens, by vaccines. As most neoantigens generated by non-synonymous mutations are private,[2] personalized approaches using messenger RNA (mRNA) and peptide vaccines have been developed and translated into phase 1 clinical trials, also in gliomas.[3,4] Due to tumor-specificity of neoantigens, both side-effects and efficacy are favorable compared with vaccines targeting tumor-associated antigens (TAAs), which have previously been tested in patients with cancer including gliomas, most likely due to insufficient breach of endogenous tolerance to TAAs. Most personalized vaccine approaches have focused on the induction of cytotoxic CD8+ T-cell responses against neoepitopes presented on major histocompatibility complexes (MHC) class I. The majority of neoantigens, however, are presented on MHC class II molecules stimulating CD4+ T-helper cell responses.[5] The efficacy of ICIs critically depends on CD4+ T-helper cell responses in experimental glioma models[6] and mutation-specific CD4+ T-helper cells are capable of eradicating large tumors in patients.[7] Personalized neoepitope-specific vaccines induce CD4+ rather than CD8+ T-cell responses in cancer patients[8,9] and specific strategies to target MHCII-restricted patient-specific neoepitopes by vaccines may be developed with improving accuracy of currently available algorithms to predict presentation by MHCII allelotypes.[10,11] In addition to the absolute number of neoantigens, their clonality or clonal representation within the tumor has been shown to be a key determinant of response to ICIs.[12] Subclonal neoantigens are prone to immune evasion due to clonal selection.[13] In addition to a comparatively low TMB, gliomas are signified by a low neoantigen clonality, particularly in the recurrent situation, which is also a reason why trials with ICI as a monotherapy in this disease have been unsuccessful in unselected patient populations thus far.[14] Hence, recurrent driver mutations represent an attractive target for specific immunotherapies, as neoantigens derived from them are clonal and provide an opportunity for an off-the-shelf vaccine. Such off-the-shelf vaccines are also more cost-effective than personalized

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MUTANT IDH AS A NEOANTIGEN

Mutations in the gene for isocitrate dehydrogenase type 1 (IDH1) resulting in an exchange of histidine to arginine at position 132 of the amino acid sequence (IDH1R132H) are frequent events in diffuse gliomas. About 70% of World Health Organization (WHO) grade 2 and 3 gliomas harbor these disease-defining clonal driver mutations. Preclinical animal studies have provided evidence that neoepitopes are generated from IDH1R132H and presented on MHC to elicit a mutation-specific T-cell response effective in controlling tumor growth.[15,16] In an MHC-humanized mouse model transgenic for the human MHCII allele HLA-DR1, a long peptide vaccine covering the hotspot mutation R132H generated CD4+ T-helper cells which are capable of infiltrating syngeneic tumors and controlling their growth, most likely by releasing proinflammatory cytokines such as interferon-γ and tumor necrosis factor-α. In some patients with recurrent IDH1R132H-positive gliomas, spontaneous CD4+ T-cell responses can be detected.[15] Detection of T-cell responses depends on assay sensitivity,[17] and sequencing-based methodologies to improve this sensitivity are being developed.[18,19] In addition to mutation-specific T-cell responses, IDH1R132H-specific antibody responses can be elicited by IDH1-vac in MHC-humanized mice and can also be detected in some patients with recurrent IDH1R132H-mutant gliomas. Of note, there is currently no evidence, that any of the rarer IDH1 or IDH2 mutations elicit similar mutation-specific immune responses. Both in preclinical mouse models and in patients, there is evidence that IDH1R132H is naturally processed and presented on MHCII, albeit with similar affinity and avidity as the wild-type peptide.[15] In situ proximity ligation assays indicated that IDH1R132H is presented in human gliomas, both on MHCII+ tumor cells and on tumor-infiltrating myeloid cells.[20] As IDH1R132H is expressed only in tumor but not in normal cells, thus representing a true tumor-specific antigen, mutation-specific T-cell responses will only target tumor cells. Indeed, there is no evidence of off-target toxicity of an IDH1R132H-specific vaccine from preclinical or clinical studies. The antigenic function of IDH1R132H in principle applies to other types of tumors with this type of mutation, such as cholangiocarcinoma, osteosarcoma, and acute myeloid leukemia, although this has not been formally proven.

IMMUNOMODULATORY FUNCTION OF MUTANT IDH

Why do gliomas with IDH1R132H develop despite the evidence that this driver mutation generates an immunogenic neoantigen? One potential explanation is the location of gliomas in an immune sanctuary site. Another reason could be the specific enzymatic function of mutant IDH and its oncometabolite R-2-hydroxyglutarate (R-2-HG). Strikingly, when compared with IDH-wild-type gliomas, IDH-mutant gliomas display reduced and not enhanced CD4+ and CD8+ T-cell infiltration.[21-23] This is due to a dual immunomodulatory function of R-2-HG. This metabolite alters the intrinsic chemokine profile of tumor cells to repel rather than attract T cells.[24] In addition, R-2-HG secreted from tumor cells is taken up by tumor-infiltrating T cells and represses the activation of CD4+ and CD8+ T cells by blocking T-cell receptor (TCR) signaling and altering the cytokine profile.[23,25] In addition to its impact on T cells, there is profound skewing of the tumor-infiltrating myeloid cell phenotype in IDH-mutant compared with IDH-wild-type gliomas, resulting in an immunosuppressive myeloid phenotype.[26-29] Also in myeloid cells, this skewing is mediated by direct and indirect effects of R-2-HG. As a result, IDH-mutant gliomas may be more resistant to immunotherapeutic approaches beyond specific vaccines, including ICIs. In fact, inhibitors of the enzymatic function of IDHs revert the immunosuppressive microenvironment in gliomas and sensitize these tumors to ICIs in preclinical models. On a mechanistic level, IDH inhibitors reinvigorate TCR activation by enhancing nuclear factor of activated T cells (NFAT) signaling in (vaccine-induced) antigen-specific T cells and revert the immunosuppressive phenotype of tumor-infiltrating myeloid cells by normalizing tryptophan metabolism.[23,30] These data support clinical trials combining immunotherapies with IDH inhibitors in IDH-mutant gliomas.

DEVELOPMENT OF IMMUNOTHERAPIES TARGETING MUTANT IDH

Three different IDH1-directed mutation-specific peptide vaccines have been, or are currently being tested in four clinical trials (Tables 1 and 2). Available data from the multicenter first-in-man phase trial of the Neurooncology Working Group (NOA) of the German Cancer Society (NOA-16, NCT02454634), that met its primary endpoints, show that a long peptide vaccine covering the mutated region is safe and immunogenic,[31] with vaccine-induced immune responses in 93.3% of patients across multiple MHC alleles. This trial included 33 patients with newly diagnosed grade 3 and grade 4 IDH1R132H-positive astrocytomas. Patients with an oligodendroglial phenotype signified by allelic losses on chromosomes 1p and 19q and

Table 1. IDH1-directed vaccines in clinical trials

Name of the compound	Mechanism of action	Phase of clinical trial development	Company
IDH1-vac	Activation of IDH1R132H-specific T cells	I	German Cancer Research Center, Heidelberg University
PEPIDH1M	Activation of IDH1R132H-specific T cells	I	Duke University
IDH1R132H-DC vaccine	Activation of IDH1R132H-specific T cells	I	Beijing Tiantan Hospital

IDH, isocitrate dehydrogenase.

Table 2. Current on-going clinical trials using IDH1-directed vaccines

Clinicaltrial.gov identifier	Tumor type	Setting	Phase	Treatment arms	Target accrual
NCT02193347	Gliomas, WHO grade 2, IDH-mutant[a]	Recurrent	1	Single arm: PEPIDH1M vaccine + tetanus-diphtheria toxoid (Td) + temozolomide	24
NCT02454634	Astrocytoma, WHO grade 3 and 4, IDH-mutant[a]	Newly diagnosed	1	Single arm: IDH1-vac Radiotherapy and/or temozolomide	39
NCT03893903	Glioma, IDH-mutant[a]	Recurrent	1	Arm 1: IDH-vac Arm 2: IDH-vac + avelumab Arm 3: avelumab	60
NCT02771301	Glioma, IDH-mutant[a]	Newly diagnosed	1	Single arm: IDH1R132H-DC vaccine + radiotherapy + temozolomide	30

IDH, isocitrate dehydrogenase; WHO, World Health Organization.
[a] Only IDH1R132H-mutant tumors eligible.

persistence of nuclear ATRX expression were excluded. All patients in the trial were treated with radiotherapy and/or chemotherapy. To avoid the inclusion of patients with standard of care treatment-related pseudoprogression (PsPD), patients were screened and enrolled 4 weeks after completion of radiotherapy followed by an exclusion of patients with PsPD. Upon enrolment, patients received eight vaccines in total over a period of 6 months, integrated into adjuvant temozolomide chemotherapy. More than 90% of patients received all eight vaccines. More than 90% of patients had treatment-related adverse events, none of which was severe. Some 66% and 47% of the adverse events classified as possibly related to IDH1-vac were local administration site conditions (injection site induration or erythema, respectively), which is in the order of what was expected from subcutaneous peptide/protein vaccines administered with these adjuvants. Current follow-up data

in this single-arm trial show 3-year progression-free survival (PFS) of 63% [95% confidence interval (CI) 44% to 77%] and overall survival of 84% (95% CI 67% to 93%). Patients with immune responses showed a 2-year PFS of 82% (95% CI 62% to 92%). While these data do not prove clinical efficacy, several important findings recapitulate preclinical data and provide evidence of biological efficacy. (i) There was a strong positive correlation of intratumoral IDH1R132H peptide presentation in the tumor tissue, as assessed by proximity ligation assay at baseline, with the magnitude and sustainability of specific peripheral T-cell responses as quantified by a newly established mutation specificity score. These data support the concept that IDH1R132H is processed and presented in the tumor tissue and supports mutation-specific T-cell responses. (ii) There was a high frequency of PsPD, indicating intratumoral inflammatory reactions. In the safety dataset, PsPD occurred in 37.5% of
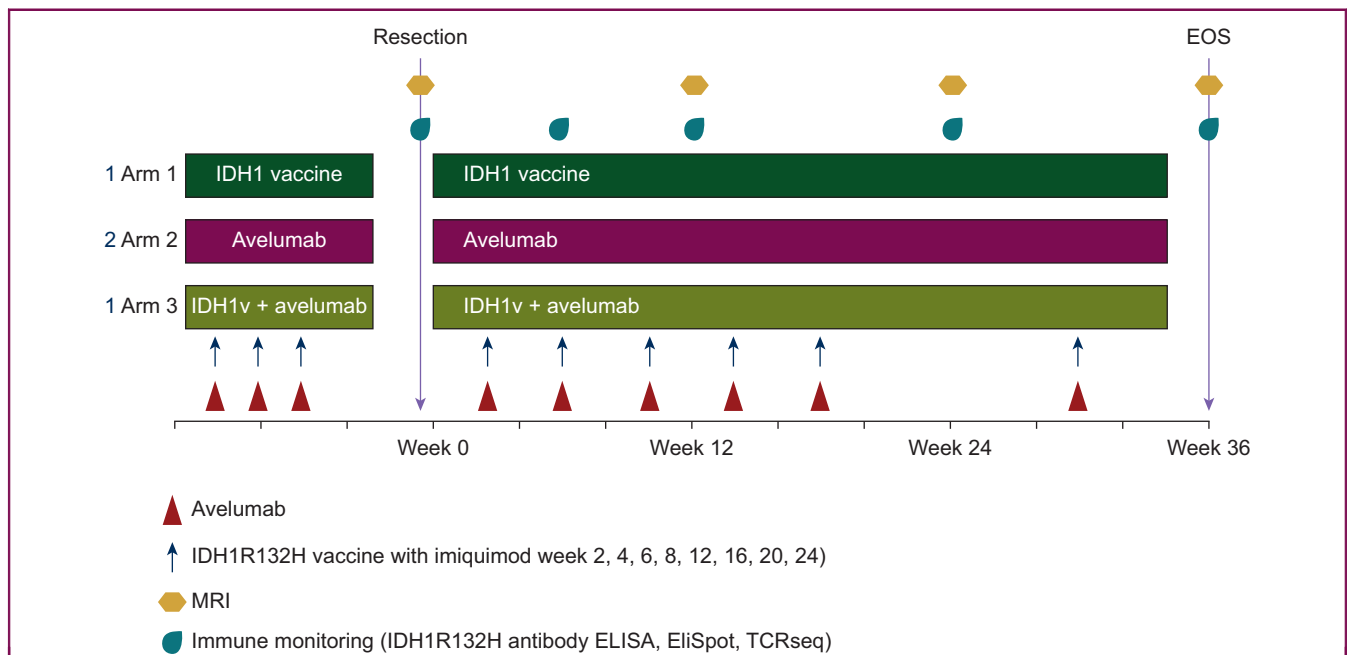

Figure 1. NOA21 window-of-opportunity trial enrolling patients with recurrent IDH1R132H-mutated gliomas eligible for resection.
Patients are randomized to receive neoadjuvant IDH vaccine, avelumab, or combined IDH vaccine and avelumab.
ELISA, enzyme-linked immunosorbent assay; EliSpot, enzyme-linked immunosorbent spot; EOS, end of study; IDH, isocitrate dehydrogenase; MRI, magnetic resonance imaging; TCRSeq, T-cell receptor-sequencing.

patients compared with 16.7% of patients in a molecularly matched control cohort. Importantly, PsPDs were associated with increased vaccine-induced peripheral T-cell responses and were not detected in patients without immune responses. (iii) Tumor-infiltrating IDH1R132H-reactive T cells were detected in a patient with PsPD. Combined single-cell TCR and RNA sequencing of these T cells revealed clonal expansion and an activated gene expression signature of cells bearing a TCR reactive to IDH1R132H. These data not only provide the rationale for a phase II clinical trial, but also form the basis for rational combination strategies to enhance the infiltration and intratumoral activity of IDH1R132H-reactive T cells induced by vaccines.

COMBINATION OF IDH1 VACCINE AND ICI

In addition to combinations with IDH inhibitors, ICIs may provide an opportunity to amplify vaccine-induced T-cell responses. Combining the IDH1 peptide vaccine with ICIs may enhance IDH1R132H-specific T-cell responses and may inhibit immune evasion of tumor cells due to counteracting inhibition of function of PD1-expressing T cells in the glioma microenvironment. Several clinical trials in patients with cancer including gliomas are currently underway to investigate the safety and efficacy of therapeutic combinations of cancer vaccines with immune checkpoint blockade. First results indicated clinical benefit of such combinations and demonstrated that their toxicity profile is acceptable and similar to safety profiles of monotherapy with ICIs. The ongoing NOA21 trial (Figure 1) evaluates, in a randomized phase 1 window-of-opportunity design, the safety and immunogenicity of IDH1-vac in combination with the programmed death-ligand 1 (PD-L1)-blocking ICI avelumab (NCT03893903).

CONCLUSION

IDH1R132H is a rare example of a shared driver mutation generating a clonal neoepitope. Preclinical data and the NOA16 trial provide evidence that active immunization using a long peptide is safe and induces mutation-specific proinflammatory CD4+ T-helper cell responses capable of infiltrating IDH1R132H-positive tumors. Single-cell sequencing analyses, TCR repertoire analyses, and TCR cloning not only provide a much more in-depth assessment of vaccine-induced T-cell responses, but also open the opportunity for adoptive T-cell therapy using TCR-transgenic T cells. Further developments include combination therapies with ICIs and IDH inhibitors.

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DISCLOSURE

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