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Recent Progress in Antitumoral Synthetic Vaccines

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ABSTRACT: Antitumoral synthetic vaccines offer a promising alternative to overcome problems associated with traditional treatments. Numerous vaccine prototypes have been described in the last two decades; however, none of them have been revealed satisfactory in clinical trials due to side effects, low bioavailability, uncertain molecular composition, and/or reproducibility. Here we highlight major advances in carbohydrate-based vaccines, which open new perspectives in cancer immunotherapy.

Cancer is a major cause of mortality worldwide. Around 8.2 million people died from cancers in 2012, and the World Health Organization now estimates that mortality could increase up to 80% by 2030. However, despite the recent progress in tumor treatment, current therapeutic regimens are still deficient due to intolerable side effects and often incomplete tumor clearance. For these reasons, extensive research programs are under investigation to develop innovative medical strategies aiming at protecting and/or treating the human population from cancers. Among them, immunotherapy-based approaches belong to the most promising alternatives. In particular, synthetic or semisynthetic vaccines have afforded impressive results in animal models, alone or in synergy with other treatments, although major issues and hypotheses remain to be addressed.

Briefly, a large majority of antitumoral vaccines are composed of tumor-associated carbohydrates antigens (TACAs) displayed at the surface of malignant cells and immunogenic protein or (lipo)peptide carriers providing stimulating epitopes for T-cells.[1] It is indeed now accepted that the combination of these structural elements within a single molecule is required to induce the T-cell dependent immune response, which leads to the secretion of high-affinity IgG antibodies (Abs) against tumors expressing the same TACAs, and to induce the activation of long-lasting memory B-cells and cytotoxic T-cells. However, until now most of the reported vaccines have been proved unsatisfactory in advanced clinical trials due to important hurdles,[2] including their low molecular definition, the low reproducibility and selectivity of immune response, and the lack of tailored immunization and adjuvant strategies. In this viewpoint, our objective is to highlight major advances in the design of carbohydrate-based synthetic vaccines, which, in our opinion, open new perspectives in cancer immunotherapy.

A landmark achievement in this field has been described by the group of Danishefsky.[3] Because of the heterogeneity of the tumor glycocalix and the evolution of the TACAs expression during the disease progression, they hypothesized that the insertion of multiple TACAs within the same vaccine construction might induce a multifaced response against tumors and thus ensure the complete destruction of cancer cells at different stages of the disease. With this aim, this group has designed a synthetic unimolecular pentavalent peptide combining five TACAs expressed by prostate and breast cancers, i.e., Globo-H, GM2, STn, TF, and T_N, which was conjugated to the keyhole limpet hemocyanin (KLH) carrier in a high epitope ratio (i.e., 505/1). Subsequent immunological studies using QS-21 as an adjuvant revealed the production of both IgG and IgM directed against each TACA and in similar titers than using a pool of monoantigenic vaccines. Moreover, these antibodies were proved by flow cytometry analysis to react with breast cancer cell lines expressing these carbohydrate antigens. In long-term investigations on glycopeptide antigens from the tandem repeat sequence of mucin MUC1 of epithelial tumor cells, Kunz's and Li's groups have synthesized vaccine candidates composed of a MUC1 glycopeptide displaying both the T_N and ST_N antigens, which was further conjugated to the bovine serum albumin (BSA) carrier.[4] Here again, immunization of Balb/c mice was found to promote a remarkable Ab response with predominant IgG1 isotype antibodies and a strong binding to breast MCF-7 tumor cell line. The studies from both groups demonstrate that future vaccine candidates might capitalize on several TACAs instead of only one to improve immunological responses.

While conjugate vaccines represent a promise in the development of tumor therapeutic vaccines, the inherent immunogenicity of the carrier protein is known to be responsible for the suppression of the immune response toward the desired TACA or glycoconjugate epitope. To avoid anticarrier immune response, self-adjuvating, multicomponent vaccines have been proposed by Boons and co-workers.[5] These multicomponent vaccines incorporate Pam3CysSerK4, a Toll-like receptor 2 (TLR2) ligand, and a dodecapeptide bearing a T_N antigen residue and was found to elicit high titers of IgG Abs. Reasoning that TLR2 ligands are able to enhance local inflammation, leading to the activation of the adaptive component of the immune system, Payne and co-workers recently synthesized vaccines incorporating a per-glycosylated MUC1 peptide as the B-cell epitope covalently linked to Pam3CysSer as TLR2 ligand, with or without the tetanus toxin peptide as helper T-cell epitope.[6] The tricomponent vaccines

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incorporating the T-cell epitope proved to be significantly more immunogenic than the dicomponent vaccines. Moreover, the per-glycosylated MUC1 vaccines were able to elicit a high level of IgG Abs in murine models in the absence of an external adjuvant, liposomal preparation or carrier protein.

The first example of self-adjuvanting four-component vaccines has been described by Renaudet et al.[7] This new generation of vaccine prototype displays within a cyclopeptide scaffold (i) a cluster of T_N antigen as B-cell epitope; (ii) a helper T-cell peptide epitope (PADRE); (iii) a cytotoxic T-cell peptide epitope ($HER_{420-429}$); and (iv) a TLR-2 agonist (palmitic acid). Immunological studies on mice have revealed both tumoral regression and a significant increase of survival without administration of external adjuvant. Further investigations on cellular and molecular mechanisms have indicated that the position of the lipid moiety in the peptide sequence significantly affects the magnitude of B-cell and cytotoxic T lymphocytes (CTLs) responses, mainly because of different cross-presentation pathways.

To elicit CTLs and IgG Abs against cancer-expressed MUC1, the level of glycosylation is also an important issue. As a matter of fact, nonglycosylated as well as densely glycosylated MUC1 sequences were ineffective. The minimum requirements to stimulate CTLs and to induce specific Abs were identified in the tripartite vaccine synthesized by Boons and co-workers, which contains a tumor-associated glycopeptide derived from MUC1, a poliovirus T-helper epitope, and Pam3CysSerK4.[8] Multivalent vaccines were also obtained by solid-phase synthesis coupling MUC1 glycopeptide antigens to the Pam3CysSerK4 lipopeptide through click reactions.[9] The vaccines raised immune responses in mice without the use of any external adjuvant. Among them, the vaccine containing four copies of a MUC1 sialyl T_N antigen also showed a remarkable cluster effect.

In another attempt to simplify MUC1 vaccine structure but retain the stimulating effect, the MUC1 tandem repeat glycopeptide HGVTSAPDTRPAPGSTAPPA was coupled to three known T-helper epitope peptides derived from tetanus toxoid. These new two-component antitumor vaccines induced a much stronger immune response when administered in plain buffer instead of with Freund's adjuvant.[10]

Capitalizing on the exceptionally strong immune reactions induced by a synthetic vaccine displaying a sialyl T_N saccharide coupled to tetanus toxoid as the carrier protein, Kunz and co-workers synthesized new vaccines consisting of tetanus toxoid and MUC1 glycopeptide with a T-antigen or a more stable T-antigen mimetic. The T-antigen mimetic was characterized by two fluorine substituents in 6- and 6′-positions of the pyranose rings.[11] Of note, strong and selective immune reactions were elicited by both the vaccines showing that the primary OH groups of the T-antigen carbohydrate part of the glycopeptide antigen can be replaced by fluorine without reducing the immunogenity and selectivity of the construct.

With respect to vaccines having the natural antigen structures, those displaying mimetic antigen structures should present the important advantage to be less sensitive to enzymatic degradation. Therefore, to improve the stability toward glycosidases and enhance the *in vivo* bioavailability, structural modifications to native antigens have been proposed. Very recently, a fully synthetic vaccine composed of four clustered T_N-antigen mimetics and an immunostimulant peptide (OvaPADRE), conjugated to the a cyclopeptide scaffold, has been reported.[12] This vaccine prototype elicited a robust and long lasting IgG/IgM Abs response and induced protection in mice through a mechanism mediated by B cells. Of note these Abs bind to MCF-7 human breast cancer cell lines expressing the native carbohydrate antigens. This clearly suggests that biologically relevant antibody specificities were induced.

In conclusion, since pioneering hypotheses on immunotherapy of tumors, synthetic vaccines represent milestones in the field of nontraditional cancer therapies. In particular, herein we highlighted (i) the use of multiple TACAs within the same vaccine; (ii) the replacement of external adjuvant with self-adjuvanting, multicomponent vaccines; and (iii) the employment of mimetic antigen structures, as some pivotal issues to take into account in the tortuous way of the development of safe and efficient therapeutic vaccines against cancer.

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Author Contributions

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Notes

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REFERENCES

(1) Wilson, R. M.; Danishefsky, S. J. A vision for vaccines built from fully synthetic tumor-associated antigens: from the laboratory to the clinic. *J. Am. Chem. Soc.* **2013**, *135*, 14462−14472.

(2) Miles, D.; Roché, H.; Martin, M.; Perren, T. J.; Cameron, D. A.; Glaspy, J.; Dodwell, D.; Parker, J.; Mayordomo, J.; Tres, A.; Murray, J. L.; Ibrahim, N. K. The Theratope Study Group. Phase III multicenter clinical trial of the sialyl-Tn (STn)-keyhole limpet hemocyanin (KLH) vaccine for metastatic breast cancer. *Oncologist* **2011**, *16*, 1092−1100.

(3) Zhu, J.; Wan, Q.; Lee, D.; Yang, G.; Spassova, M. K.; Ouerfelli, O.; Ragupathi, G.; Damani, P.; Livingston, P. O.; Danishefsky, S. J. From synthesis to biologics: preclinical data on a chemistry derived anticancer vaccine. *J. Am. Chem. Soc.* **2009**, *131*, 9298−9303.

(4) Cai, H.; Huang, Z.-H.; Shi, L.; Sun, Z.-Y.; Zhao, Y.-F.; Kunz, H.; Li, Y.-M. Variation of the glycosylation pattern in MUC1 glycopeptide BSA vaccines and its influence on the immune response. *Angew. Chem., Int. Ed.* **2012**, *51*, 1719−1723.

(5) Ingale, S.; Wolfert, M. A.; Buskas, T.; Boons, G. J. Increasing the antigenicity of synthetic tumor-associated carbohydrate antigens by targeting toll-like receptors. *ChemBioChem* **2009**, *10*, 455−463.

(6) Wilkinson, B. L.; Day, S.; Malins, L. R.; Apostolopoulos, V.; Payne, R. J. Self-adjuvating multicomponent cancer vaccine candidates combining per-glycosylated MUC1 glycopeptides and the Toll-like receptor 2 agonist Pam3CysSer. *Angew. Chem., Int. Ed.* **2011**, *50*, 1635−1639.

(7) Renaudet, O.; Dasgupta, G.; Bettahi, I.; Shi, A.; Nesburn, A. B.; Dumy, P.; BenMohamed, L. Linear and branched glyco-lipopeptides vaccines follow distinct cross-presentation pathways and generate different magnitudes of antitumor immunity. *PLoS One* **2010**, *5*, e11216.

(8) Lakshminarayanan, V.; Thompson, P.; Wolfert, M. A.; Buskas, T.; Bradley, J. M.; Pathangey, L. B.; Madsen, C. S.; Cohen, P. A.; Gendler, S. J.; Boons, G.-J. Immune recognition of tumor-associated mucin MUC1 is achieved by a fully synthetic aberrantly glycosylated MUC1 tripartite vaccine. *Proc. Natl. Acad. Sci. U.S.A.* **2012**, *109*, 261−266.

(9) Cai, H.; Sun, Z.-Y.; Chen, M.-S.; Zhao, Y.-F.; Kunz, H.; Li, Y.-M. Synthetic multivalent glycopeptide-lipopeptide antitumor vaccines: Impact of the cluster effect on the killing of tumor cells. *Angew. Chem., Int. Ed.* **2014**, *53*, 1699−1703.
(10) Cai, H.; Chen, M.-S.; Sun, Z.-Y.; Zhao, Y.-F.; Kunz, H.; Li, Y.-M. Self-adjuvating synthetic antitumor vaccines from MUC1 glycopeptides conjugated to T-cell epitopes from tetanus toxoid. *Angew. Chem., Int. Ed.* **2013**, *52*, 6106−6110.
(11) Hoffmann-Röder, A.; Kaiser, A.; Wagner, S.; Gaidzik, N.; Kowalczyk, D.; Westerlind, U.; Gerlitzki, B.; Schitt, E.; Kunz, H. Synthetic antitumor vaccines from tetanus toxoid conjugates of MUC1 glycopeptides with the Thomsen-Friedenreich antigen and a fluorine-substituted analogue. *Angew. Chem., Int. Ed.* **2010**, *49*, 8498−8503.
(12) Richichi, B.; Thomas, B.; Fiore, M.; Bosco, R.; Qureshi, H.; Nativi, C.; Renaudet, O.; BenMohamed, L. A cancer therapeutic vaccine based on clustered Tn-antigen mimetics induces strong antibody-mediated protective immunity. *Angew. Chem., Int. Ed.* **2014**, DOI: 10.1002/anie.201406897.