

A NEXT GENERATION DNA VACCINE CODING FOR THE IMMUNODOMINANT SEQUENCE OF ALPHA-ENOLASE WITH ENHANCED ABILITY TO INDUCE EFFECTOR T CELL RESPONSES TO CURE PANCREATIC CANCER

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Background Pancreatic ductal adenocarcinoma (PDA) is one of the most aggressive malignancies with a 5-year survival rate of 11%.¹ Only the 15% of patients have a resectable disease eligible for surgical resection followed by adjuvant chemotherapy to reduce the risk of relapse.² The glycolytic enzyme alpha-Enolase (ENO1) has been identified as PDA associated antigen.^{3,4} A non-integrating plasmid DNA vaccine encoding for full-length human ENO1 (FL-ENO1 vaccine) was able to slow tumor progression, inducing an integrated anti-tumor immune response, in mice engineered to spontaneously develop PDA (KPC).⁵ However, in FL-ENO1 vaccinated mice myeloid derived suppressor cells and regulatory T cells arose again, leading eventually to tumor recurrence. To optimize the ENO1 vaccine, we focused the research on the identification of the most immunogenic long epitopes widely presented by HLA molecules.

Methods A library of 14 peptides covering the sequence of ENO1 was synthesized to screen healthy donors and PDA patients for their capacity to recognize fractions of ENO1 through the stimulation of T cells with ENO1 peptides. According to the proliferative response and the cytokine release, the most immunogenic sequences of ENO1 were identified and cloned into the pVax plasmid (ENO3PEP vaccine). KPC mice were vaccinated at 8 weeks and every two weeks for a total of four rounds and sacrificed at 18 weeks of age either with empty, FL-ENO1 or ENO3PEP vaccine. The presence of anti-ENO1 specific antibodies and the number of specific T cells secreting IFN-gamma in response to ENO1 were assessed respectively by ELISA and ELISpot. Pancreas tumoral areas were analyzed on hematoxylin and eosin-stained sections, while the immune infiltrate was characterized through immunohistochemistry.

Results Three portions of ENO1 emerged as immunodominant as T cells from healthy donors and PDA patients stimulated with the related peptides showed the highest proliferation index and ratio of secreted IFN-gamma/IL-10 both compared to those stimulated with full-length ENO1. In KPC mice, the ENO3PEP vaccine i) significantly reduced the pancreatic tumor lesions, ii) increased the production of anti-ENO1 antibodies, iii) enhanced the secretion of IFN-gamma by ENO1-stimulated T cells, iv) recruited more T cells at tumor site compared to FL-ENO1 vaccine.

Conclusions The ENO3PEP vaccine, coding for the most immunogenic sequences of ENO1, was able to efficiently delayed tumor progression, inducing a strong integrated humoral and cellular response, emerging as potential next generation DNA vaccine suitable for immunotherapy in virtually all PDA patients.

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Ethics Approval The ENOAPA study for human samples was approved by A.O.U. Città della Salute e della Scienza di Torino Ethics Board; approval number 0011110, February 2nd, 2021. The study “Utilizzo della vaccinazione a DNA in combinazione con anticorpi monoclonali, inibitori chimici e chemioterapia per inibire la crescita e la metastatizzazione dell'adenocarcinoma pancreatico” for animal samples was approved by Ministero della Salute; approval number 597/2019-PR, July 30th, 2019.

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