1181 THERAPEUTIC VACCINES CONSISTING OF CANCER GERMLINE ANTIGEN-BASED SYNTHETIC LONG PEPTIDES ARE IMMUNOGENIC IN HUMAN HEPATOCELLULAR CARCINOMA PATIENTS

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Background In melanoma, cancer germline antigen (CGA)-directed vaccination has shown to induce objective clinical responses accompanied by strong anti-tumor immune responses.[1] As CGAs are immunogenic and highly expressed by hepatocellular carcinoma (HCC) tumor cells, these have demonstrated to be attractive targets to be implemented in therapeutic anti-liver cancer vaccination as well.[2]Synthetic long peptide (SLP) vaccination has proven to elicit efficient anti-tumor $CD4^+$ and $CD8^+$ T cell responses and to have promising clinical effects.[3] We aimed to develop an SLP vaccine targeting HCC-restricted CGA-epitopes covering at least five different HLA super types that are highly prevalent globally.

Methods We applied an integrative pre-clinical approach of *in silico* epitope prediction, immunopeptidomics, and *in vitro* tools to select GSAs and validate CGA-SLPs in HCC patient-derived tumor infiltrating lymphocytes (TILs) and peripheral blood mononuclear cells (PBMCs).

Results Out of a set of 13 CGAs, previously shown to be expressed in primary human HCC tissues, two CGAs (i.e., CGA-A and -B) demonstrated no healthy tissue expression and covered >75% of HCC patients collectively (N = 55). Immunopeptidome analysis of human HCC-derived hepatocytes (N = 12), together with *in silico* CGA-related epitope predictions according to epitope immunogenicity, enabled identification of 196 and 220 potential epitopes for CGA-A and -B, respectively. HLA-A*02:01 binding of these epitopes was validated *in vitro* using a HLA-A2 stabilization assay and ranked accordingly. Six SLPs were designed incorporating 54 HLA-A*02:01, 25 HLA-A*01:01, 24 HLA-A*03:01, 27 HLA-A*24:01, and 15 HLA-B*07:02 predicted and/or validated CGA-A- and -B-related epitopes. Top three-ranked epitopes were selected to validate ex vivo intra-tumor immune reactivity using corresponding peptide-HLA-A*02:01 dextramers in human HCC-derived TILs. Tumors of 8/11 patients contained CGA-A- and CGA-B-specific TILs that were characterized by a tumor reactive phenotype. Upon *in vitro* enrichment, SLP immunogenicity was demonstrated through Interferon gamma ELISPOT in 2/3 of human HCC-derived PBMCs using an *in vitro* co-culture system with autologous antigen presenting cells.

Conclusions Here, we describe the intelligent design of a set of immunogenic SLPs comprising CGA-related epitopes for the global population that can be further exploited for the development of an off-the shelf anti-cancer vaccine to treat HCC.

REFERENCES

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Ethics Approval All study procedures were approved by the local ethics committee (Medische Ethische Toetsings Commissie Erasmus MC Rotterdam; NL58534,078.16). Patients had given informed consent for tissue and blood donation as well as usage of personal data.

Consent Patients had given informed consent for tissue and blood donation as well as usage of personal data.

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