Background In melanoma, cancer germline antigen (CGA)-directed vaccination has shown to induce objective clinical responses accompanied by strong anti-tumor immune responses. 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. Synthetic long peptide (SLP) vaccination has proven to elicit efficient anti-tumor CD4+ and CD8+ T cell responses and to have promising clinical effects. 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