Identification of Tumor Antigens Among the HLA Peptidomes of Glioblastoma Tumors and Plasma*

Bracha Shraibman‡, Eilon Barnea‡, Dganit Melamed Kadosh‡, Yael Haimovich‡, Gleb Slobodin§, Itzhak Rosner§, Carlos López-Larrea¶, Norbert Hilf¶, Sabrina Kuttruff¶, Colette Song¶, Cedrik Britten**, John Castle** ¶¶¶, Sebastian Kreiter**, Katrin Frenzel**, Marcos Tatagiba‡‡, Ghazaleh Tabatabai‡‡, Pierre-Yves Dietrich §§ §§§, Valérie Dutoit §§ §§§, Wolfgang Wick¶¶¶, Michael Platten¶¶¶, Frank Winkler¶¶¶, Andreas von Deimling¶¶¶, Judith Kroep¶¶¶, Juan Sahuquillo***, Francisco Martinez-Ricarte***, Jordi Rodon***, Ulrik Lassen‡‡‡, Christian Ottensmeier§§§, Sjoerd H. van der Burg

© 2018 Shraibman et al. Published under exclusive license by The American Society for Biochemistry and Molecular Biology, Inc.

The original version of this article was withdrawn by the authors. An error was discovered in the creation of the protein database file that was used for searching, which led to some incorrect associations between peptides and proteins. A corrected version of the manuscript has been supplied which contains the identical peptide identifications as the original, but the resulting number of proteins in various categories has now changed, and as a result some of the figures and supplementary files have changed also. The underlying conclusions of this study, however, remain unaltered. The corrected version can be accessed (Shraibman, B., Barnea, E., Kadosh, D. M., Haimovich, Y., Slobodin, G., Rosner, I., López-Larrea, C., Hilf, N., Kuttruff, S., Song, C., Britten, C., Castle, J., Kreiter, S., Frenzel, K., Tatagiba, M., Tabatabai, G., Dietrich, P.-Y., Dutoit, V., Wick, W., Platten, M., Winkler, F., von Deimling, A., Kroep, J., Sahuquillo, J., Martinez-Ricarte, F., Rodon, J., Lassen, U., Ottensmeier, C., van der Burg, S. H., Thor Straten, P., Poulsen, H. S., Ponsati, B., Okada, H., Rammensee, H. G., Sahin, U., Singh, H., and Admon, A. (2019) Identification of tumor antigens among the HLA peptidomes of glioblastoma tumors and plasma. Mol. Cell. Proteomics 18, 1255-1268).
ent cancers, including GBM, has drawn significant attention (3, 4), reviewed in (5–13). Much of the recent excitement about cancer immunotherapy stems from the success in treating patients with immune checkpoint modulators, which induce anti-cancer T cell immune reactions that can break tolerance and bring about complete responses in increasingly larger percentages of patients (14, 15). Identification of tumor antigens is needed for development of effective cancer immunotherapy, including GBM, and a good source for such antigens are the pools of HLA-bound peptides presented preferentially, or even exclusively, by the tumor cells (16), reviewed in (17–20). Tumor-specific antigens (TSA) and tumor-associated antigens (TAA) that can serve as candidates for cancer immunotherapeutics have been searched for extensively. Indeed, many such antigens were already identified, yet none has induced sufficiently strong anti-cancer immune reaction to eradicate the tumors in large cohorts of patients (18, 20, 21). One subset of preferred TAA are Cancer/Testis Antigens (CTA), which are aberrantly expressed in the malignant cells, and are normally expressed only in fetal tissues and in immune privileged sites, such as the male germ cells, placenta and ovary, but are absent from the normal somatic cells of any healthy tissue (22), reviewed in (23). Another special group of tumor antigens are neoantigens, which are attracting significant attention as potential targets for cancer immunotherapies (24). These are more likely common in tumors with higher mutational load and therefore less frequent.

HLA class I molecules are predominantly expressed as membrane anchored, cell-surface molecules. The HLA molecules are also present in the serum (27), and can be recovered from them in human plasma (sHLA) (28–30). Confounding factors for using sHLA peptides as a source for biomarkers of small amounts of sHLA molecules are absent in all healthy individuals (27, 31), and in addition, only one of the sHLA alleles are released to the circulation in larger amounts than others (32, 33).

It has been suggested that shedding of the sHLA molecules represents a mechanism for evasion of immune recognition of tumour cells, which release larger amounts of sHLA molecules relative to the healthy cells (33). Because the sHLA molecules carry with them their original load of bound peptides, the immunopeptidomes of the sHLA molecules of cancer patients include disproportionately large fractions of peptides originating from the tumour cells, even though the tumours constitute just small fraction of the body mass. It is therefore intuitive to assume that biomarkers of disease can be found among those sHLA peptides. Such plasma-sHLA molecules could then be a useful source of cancer biomarkers for early detection or recurrence during follow-up (28).

Analyses of the HLA peptidomes, based on immunooaffinity purification of the mHLA molecules from cell lines, tumor tissues or patients’ plasma, followed by analysis of the bound peptides by chromatography and tandem mass spectrometry (34) currently results in identification of thousands of HLA bound peptides (35–38). HLA peptides were previously isolated from GBM cell lines (39) and fresh tumors, including GBM (4, 16). Similarly, the HLA peptidomes of tumors, such as melanoma (40, 41), renal cell carcinoma (42, 43), leukemia (44) and multiple myeloma (45) have been characterized. Furthermore, several thousands of peptides have successfully been isolated from cancer patients’ plasma-sHLA molecules (28, 30).

In this research, more than 35,000 plasma-sHLA and tumor-mHLA peptidomes from patients were identified. These large peptidomes included several hundred peptides previously characterized and newly defined potential CTAs. We propose that these may serve as useful immunopectidomes for GBM, and may be a useful source of cancer biomarkers for early detection or recurrence during follow-up (28).

Analyses of the HLA peptidomes, based on immunooaffinity purification of the mHLA molecules from cell lines, tumor tissues or patients’ plasma, followed by analysis of the bound peptides by chromatography and tandem mass spectrometry (34) currently results in identification of thousands of HLA bound peptides (35–38). HLA peptides were previously isolated from GBM cell lines (39) and fresh tumors, including GBM (4, 16). Similarly, the HLA peptidomes of tumors, such as melanoma (40, 41), renal cell carcinoma (42, 43), leukemia (44) and multiple myeloma (45) have been characterized. Furthermore, several thousands of peptides have successfully been isolated from cancer patients’ plasma-sHLA molecules (28, 30).

In this research, more than 35,000 plasma-sHLA and tumor-mHLA peptidomes from patients were identified. These large peptidomes included several hundred peptides previously characterized and newly defined potential CTAs. We propose that these may serve as useful immunopectidomes for GBM, and may be a useful source of cancer biomarkers for early detection or recurrence during follow-up (28).

Analyses of the HLA peptidomes, based on immunooaffinity purification of the mHLA molecules from cell lines, tumor tissues or patients’ plasma, followed by analysis of the bound peptides by chromatography and tandem mass spectrometry (34) currently results in identification of thousands of HLA bound peptides (35–38). HLA peptides were previously isolated from GBM cell lines (39) and fresh tumors, including GBM (4, 16). Similarly, the HLA peptidomes of tumors, such as melanoma (40, 41), renal cell carcinoma (42, 43), leukemia (44) and multiple myeloma (45) have been characterized. Furthermore, several thousands of peptides have successfully been isolated from cancer patients’ plasma-sHLA molecules (28, 30).

In this research, more than 35,000 plasma-sHLA and tumor-mHLA peptidomes from patients were identified. These large peptidomes included several hundred peptides previously characterized and newly defined potential CTAs. We propose that these may serve as useful immunopectidomes for GBM, and may be a useful source of cancer biomarkers for early detection or recurrence during follow-up (28).

Analyses of the HLA peptidomes, based on immunooaffinity purification of the mHLA molecules from cell lines, tumor tissues or patients’ plasma, followed by analysis of the bound peptides by chromatography and tandem mass spectrometry (34) currently results in identification of thousands of HLA bound peptides (35–38). HLA peptides were previously isolated from GBM cell lines (39) and fresh tumors, including GBM (4, 16). Similarly, the HLA peptidomes of tumors, such as melanoma (40, 41), renal cell carcinoma (42, 43), leukemia (44) and multiple myeloma (45) have been characterized. Furthermore, several thousands of peptides have successfully been isolated from cancer patients’ plasma-sHLA molecules (28, 30).

In this research, more than 35,000 plasma-sHLA and tumor-mHLA peptidomes from patients were identified. These large peptidomes included several hundred peptides previously characterized and newly defined potential CTAs. We propose that these may serve as useful immunopectidomes for GBM, and may be a useful source of cancer biomarkers for early detection or recurrence during follow-up (28).

Analyses of the HLA peptidomes, based on immunooaffinity purification of the mHLA molecules from cell lines, tumor tissues or patients’ plasma, followed by analysis of the bound peptides by chromatography and tandem mass spectrometry (34) currently results in identification of thousands of HLA bound peptides (35–38). HLA peptides were previously isolated from GBM cell lines (39) and fresh tumors, including GBM (4, 16). Similarly, the HLA peptidomes of tumors, such as melanoma (40, 41), renal cell carcinoma (42, 43), leukemia (44) and multiple myeloma (45) have been characterized. Furthermore, several thousands of peptides have successfully been isolated from cancer patients’ plasma-sHLA molecules (28, 30).
centrifugation for 45 min at 18,000 rpm (40,000g, SS-34 rotor, Sorval, Thermo Fisher Scientific, Waltham, MA), at 4 °C. HLA class I molecules from the cleared lysate or from the fresh human plasma were immunoaffinity purified using the W6/32 mAb bound to Amino-Link beads (Thermo-Fisher Scientific) as in (28, 46). The HLA molecules with their bound peptides were eluted from the affinity column with five column volumes of 1% TFA. The eluted HLA class I proteins, and the released peptides were loaded on disposable C18 micro-columns (Harvard Apparatus, Holliston, MA), and the peptides fraction was recovered with 30% acetonitrile in 0.1% TFA, whereas the protein fraction was recovered with 80% acetonitrile in 0.1% TFA, as in (46). The peptide fractions were dried using vacuum centrifugation, reconstituted in 100 μl of 0.1% TFA, reloaded on C18 Stage-Tips (47), eluted with 80% acetonitrile, dried, and reconstituted with 0.1% formic acid for LC-MS/MS analysis.

Proteomics analysis was performed after trypsin digestion of the proteins in gel slices. Briefly, 40 μg protein sample from each tissue was run in 10% acrylamide gel, stained with Coomassie, and each lane of the gel was sliced into five slices. The proteins in the gel slices were reduced with 2.8 mM DTT at 60 °C for 30 min and carboxymethylated with 8.8 mM iodoacetamide in 100 mM ammonium bicarbonate at room temperature for 30 min and digested overnight at 37 °C in 10% acetonitrile and 10 mM ammonium bicarbonate with modified trypsin (Promega, Madison, WI) at a 1:10 (wt/wt) enzyme-to-substrate ratio. This was repeated for another 4 h with a similar amount of trypsin, followed by 150 min gradients of LC-MS/MS of the released peptides from each gel slice.

Identification of the HLA and Tryptic Peptides—Both the HLA and tryptic peptides were resolved by capillary chromatography/tandem mass spectrometry using a Q-Exactive-Plus mass spectrometer fitted with a capillary Ultimate 3000 RSLC nano-capillary UHPLC (Thermo-Fisher Scientific). The capillary LC systems were equipped with about 30 cm long, 75 micron inner diameter capillary columns, home-packed with 3.5 μm silica ReproSil-Pur C18-AQ resin (Dr. Maisch GmbH, Ammerbuch-Entringen, Germany) on a Micro Maser UHPLC (Thermo-Fisher Scientific). The reversed phase chromatography was performed on charge states between 2 to 7. The peptide match option was set to Preferred. Normalized collision energy was set to 25% and MS/MS resolution was 17,500 at 200 m/z. Fragmented m/z values were dynamically excluded from further selection for 20 s.

Data Analysis—The MS data was analyzed by the MaxQuant computational proteomics platform (49 version 1.5.3.8 and searched with the Andromeda search engine (50). Peptide identifications were based on the human section of the Uniprot database (http://www.uniprot.org) of July 2015 containing 69,693 entries. Proteins were declared positive identification with at least two identified tryptic peptides per protein. Mass tolerance of 4.5 ppm for the precursor masses and 20 ppm for the fragments were allowed. Methionine oxidation was accepted as variable modification for both tryptic and HLA peptides. Carbamidomethyl cysteine was accepted as a fixed modification for the proteomics data and as a variable modification for the HLA peptide data. Methionine sulfoxide and n-acetylation were set as variable modifications for both the proteomics and HLA peptidomics analyses. Minimal peptide length was set to seven amino acids and a maximum of two cleavage sites was allowed for tryptic peptides. The false discovery rate (FDR) was set to 0.01 for protein identifications, and 0.05 for the HLA peptides, because it resulted in identification of about twice as many true HLA ligands (see Discussion). The resulting identified protein tables were filtered to eliminate the identifications derived from the reverse database, as well as common contaminants.

Normalization of the HLA Peptidome and Proteome Data—The HLA peptidome of each of the LC-MS/MS runs was normalized according to its median. The medians were zeroed, and downregulated HLA peptides were defined as those changing by at least two folds in the plasma samples collected immediately before surgery, relative to samples collected at later clinic visits. The proteomics LC-MS/MS data was normalized using the LFQ and iBAQ tools of the MaxQuant software (49).

HLA Typing—DNA was extracted from blood of GBM patients using the DNeasy Blood & Tissue kit (Qiagen, Hilden, Germany) as per the kit instructions. The HLA typing was conducted with this DNA at the DKMS Life Science Lab (Dresden, Germany). The samples were processed within high-throughput workflow using next generation sequencing. HLA typing was based on the regions covering the peptide-binding domains and the identified HLA sequences were Exons 2 and 3 for HLA-A, -B, and -C, and Exon 1, 2, and 3 for HLA-DRB1 (51).

Definition of Tumor Antigen (TAA) Group—Selection of the peptide antigens was based on the cancer tumor database (CT gene database for human cancer genes; http://cancergene.mskcc.org) comprising of 277 different cancer-related genes (2005–2009) and the TAA database (http://cvc.dfci.harvard.edu/taa) (53).

RESULTS

Peptidomes are Identified From Tumors and Patients—Tumor samples were obtained from 10 different patients undergoing surgery. These freshly frozen tumor tissues were used for analysis of their membranal HLA peptidomes and their tumor proteomes. In addition, 106 plasma samples were obtained from 10 patients, and other 42 GBM patients (52 patients in total) were used for the analysis of their plasma-sHLA bound peptidomes. Additionally, control plasma of healthy donors (n = 6) and patients diagnosed with ankylosing spondylitis (n = 30), as an exemplar inflammatory process, were used for this study (Fig. 1, supplemental Table S1). The study was performed as part of the GAPVAC project (The European Gliona Actively Personalized Vaccine Consortium), which aimed to develop a personal immunotherapy treatment based on administration of synthetic copies of selected HLA peptides derived from tumor antigens to GBM patients. Here we describe the results of the plasma-soluble HLA (sHLA) peptidome analyses, the tumor membranal HLA (mHLA) peptidome and proteome analyses, performed in parallel with the GBM tumors. Overall, the tumor-mHLA peptidome analyses resulted in the identification of 24,650 different HLA peptides, derived from 7217 different source proteins, and the plasma-sHLA peptidome analyses resulted in the identification of 32,089 different HLA peptides, derived from 8281 different source proteins (supplemental Table S2). The proteomics analysis of these 10 GBM tumor samples resulted in identification of 7351 different proteins (supplemental Table S3).
The GBM HLA Peptidomes Include Many Peptides from Multiple Tumor Antigens—Among the patients’ plasma-sHLA and the tumor-mHLA peptidomes, 904 different HLA peptides derived from 143 known TAAs were identified. The reference set of TAAs was based on the cancer tumor database (CT gene database) (http://www.cta.lncc.br/) comprising 277 different TAAs (data accumulated between 2005 and 2009) (52) and the Tumor T cell Antigen database (TANTIGEN) (http://cvc.dfci.harvard.edu/tadb) (53) with 259 different TAAs, for a total set of 496 TAAs.

Derived from these 496 TAAs, a total of 763 plasma-sHLA and 611 tumor-membranal HLA peptides were identified. Importantly, up to 78.8% of the plasma-sHLA peptides, derived from this TAA group, were also detected among the tumor-mHLA peptidomes of the different patients. These results indicate that the plasma-sHLA peptidome may indeed provide a useful source of tumor antigens for diagnosis and immunotherapeutics (selected examples in Table I, and the entire list in supplemental Table S4). Four hundred five out of the 904 identified HLA peptides derived from TAAs were detected only in GBM plasma and tissue samples but not in any non-cancerous donors’ plasma samples, supporting their potential significance as authentic tumor antigens.

Among the TAAs expressed in the tumor cells, a subset of genes, normally expressed only in germline, embryonic and placenta cells, can be defined as CTAs, which are expressed at levels below a threshold of nine gcra units (expression units using background adjustment: GC content adjusted with Robust Multiarray Average, described in the BioGPS website) in all normal and essential tissues. Such CTAs were further defined as those derived from genes whose transcripts are expressed at significantly higher levels in the tumors tissues (according to BioGPS (55, 56), see Discussion). Using this filtration, a list of 145 CTAs was established (supplemental Table S5). In the HLA peptidome analysis described here, 19 different HLA peptides, belonging to 11 CTAs, were identified. An example for such antigen is the transcription factor SOX11 gene (SOX11) that is expressed only in fetal brain, according to BioGPS (supplemental Fig. S1B). In this analysis, two of its derived HLA peptides, AHSASEQQL and NFSDLVFTY, were observed in the plasma-sHLA and tumor-mHLA of the GBM patients and were detected in less than 5% of the non-cancerous blood donors’ plasma-sHLA peptidomes (Table I). Moreover, HLA peptides derived from male tissues such as testis or prostate, and identified in women’s plasma or GBM tissue, or HLA peptides derived from female tissues, such as placenta and ovaries, and identified in the men’s plasma or GBM tissue, can serve as potential immunotherapeutics candidates (Table II). For example, eight different HLA peptides originating from the ETV5 gene were identified in this analysis. This gene is expressed only in placenta but not at any other tissue in the body. Moreover, the eight ETV5 HLA peptides were identified mostly among the male GBM shLA and mHLA samples but not at any of the non-cancerous donors, suggesting their relevance to serve as proper biomarkers for the disease.

The Levels of Potential Biomarker Plasma-sHLA Peptides Are Reduced Following Surgical Removal of the Tumors—The presence of the plasma-sHLA peptides derived from tumor antigens may serve as surrogate biomarkers for different can-
| Gene name | Sequence | Count | GBM Peptidomes | sHLA Peptidomes | mHLA Peptidomes |
|-----------|----------|-------|----------------|----------------|----------------|
| ETV5      | FPDNQRPFL 0 | 0 | 1 | | |
|           | FSSSHEGFSY 0 | 1 | 0 | | |
|           | GPAPAPHL 0 | 1 | 1 | | |
|           | KVAGERYVY 0 | 3 | 3 | | |
|           | LPYAEGFAY 0 | 6 | 2 | | |
|           | MPGPPAHGF 0 | 2 | 2 | | |
|           | RPAMYDKL 0 | 2 | 1 | | |
| SOX4      | VPRPHOPLQM 0 | 0 | 0 | | |
|           | KIMEQSPDM 0 | 10 | 4 | | |
|           | SAASASAAL 0 | 19 | 0 | | |
|           | SISNLVFTY 0 | 4 | 1 | | |
| MDM2      | GEISEKAKL 0 | 9 | 2 | | |
|           | YTMKEVLFY 1 | 0 | 1 | | |
|           | GEISEKAKL 0 | 9 | 2 | | |
|           | SEQETLVRP 0 | 3 | 0 | | |
| DDR1      | AVGDGPPRV 0 | 8 | 6 | | |
|           | DSPQDLVSL 1 | 0 | 0 | | |
|           | FLAEDALNTV 0 | 6 | 6 | | |
|           | LPPPPONSV 0 | 0 | 0 | | |
|           | YLOVDLRL 0 | 0 | 0 | | |
| CSF1      | HSSGSVLPLGELE 2 | 3 | 0 | | |
|           | HTVDPGSAKQR 1 | 0 | 0 | | |
| TMEM108   | TVFSTSSL 0 | 2 | 2 | | |
| UBE2A     | REYKRVSA 2 | 0 | 0 | | |
| BST2      | DASAEVERL 0 | 0 | 0 | | |
|           | AAPQOLLIV 0 | 0 | 0 | | |
|           | VPLIIFTI 1 | 0 | 0 | | |
| NPM1      | DENEHOLSL 0 | 0 | 0 | | |
|           | EAIQDQW 0 | 0 | 0 | | |
|           | EGSPKVL 0 | 0 | 0 | | |
|           | EITPPA 0 | 0 | 0 | | |
|           | FPPA 0 | 0 | 0 | | |
| VHL       | VHL 1 | 13 | 1 | | |
|           | VE 1 | 6 | 1 | | |
|           | YEG 0 | 2 | 2 | | |
| BCAP31    | AESA 1 | 2 | 1 | | |
|           | KLDV 0 | 7 | 3 | | |
|           | KYMEELQVL 1 | 3 | 0 | | |
|           | LEKAENQVL 0 | 1 | 2 | | |
|           | THVLEGAGNKL 0 | 5 | 1 | | |
|           | TLSQQATL 0 | 0 | 0 | | |
| CDKN2A    | AAPGAPAAV 0 | 0 | 2 | | |
|           | LPVDLAEEL 1 | 7 | 2 | | |
| SART3     | AAFTRALEY 0 | 9 | 1 | | |
|           | AEAPRLAEY 2 | 2 | 2 | | |
|           | ARLEKDHISL 0 | 1 | 1 | | |
|           | AYIDFMK 2 | 3 | 0 | | |
|           | DHQVISVTF 0 | 5 | 1 | | |
|           | TVFVSNLPSYM 1 | 0 | 0 | | |
| PA2G4     | AEDLWTVKY 0 | 1 | 0 | | |
|           | DVAQGQTQTVGR 1 | 1 | 0 | | |
|           | IAPFTSISV 2 | 21 | 5 | | |
|           | KEGFVAQF 1 | 4 | 1 | | |
|           | TRIEGMIS 1 | 9 | 1 | | |
| COTL1     | FVISDRKEL 1 | 8 | 3 | | |
|           | VYVLESWHL 0 | 0 | 0 | | |
| ATIC      | KTLTPSAAY 1 | 2 | 0 | | |
|           | NLYPFVKT 0 | 0 | 0 | | |
|           | GPNMB 0 | 0 | 0 | | |

*The HLA Peptidome of Glioblastoma*

**Table I:** List of HLA peptides derived from the TAA group, identified among the GBM sHLA and mHLA peptidomes.
cancers, including GBM. Peptides detected among the plasma-sHLA peptidome, derived from tumor antigens that are not normally expressed at elevated levels in normal tissues, should be reduced in their levels after removal or reduction of the tumor load by treatments (57) such as the surgery performed for the GBM patients studied here. Most of the plasma-sHLA peptides of the individual patients remain relatively stable before and after surgery, and even a few months later, because these are self-peptides of the healthy tissues (example in Fig. 2A). This facilitated focusing on the minority of sHLA peptides that were reduced in their levels relative to the rest of the plasma-sHLA peptidome, to search among them for potential biomarkers. In this study, 94 different plasma samples were collected before and following surgery of the same 34 GBM patients as part of the GAPVAC project. The sHLA molecules of the individual patients were affinity purified and their peptidomes were analyzed separately by LC-MS/MS.

As many as 250 sHLA peptides, derived from 236 proteins were identified in any of the plasma samples of the noncancerous donors (supplemental Table S2, column B). Examples for such downregulated peptides are displayed in Fig. 2A. Some of the downregulated peptides were derived from known cancer related genes, such as the HLA-A*32:01 peptide RVNPLVKSF of FRMD3, which is not expressed in any of the normal tissues of the body, according to BioGPS. The disappearance of sHLA peptides from the plasma samples after surgery can be because of real reduction in their amounts, or because of chance misidentifications caused by the shotgun LC-MS/MS approach used here (examples in Fig. 2A).

The Plasma-sHLA and Tumor-mHLA Peptidomes of the Same Patients Are Highly Correlated—The large HLA peptidomes of the patients’ tumors-mHLA and plasma-sHLA enabled comparisons between these patients’ peptidomes. The peptidome analyses of the ten patients, from which both types of samples were available, indicated overlaps of up to 50% between the identified peptides of the individual patients (Fig. 2B). Multiple plasma samples of each patient were also considered as biological replica, and their LC-MS intensities were averaged. Multiple sHLA and the sHLA peptides of the individual patients shared between both types of samples (Fig. 2B) were averaged. In addition, the LC-MS signals of the plasma-sHLA peptides of different blood donors were correlated with Pearson correlations between 0 and 0.53 (supplemental Fig. S2B). As expected, both the tumor-mHLA and plasma-sHLA peptidomes of different blood donors were more similar when they shared HLA allotypes (examples in Fig. 3), for example, in patient “11–002”, as patient 11–002 shared between the plasma-sHLA and tumor-mHLA peptides of plasma A (taken before surgery) and tissue mHLA the HLA allotypes that belong to dissimilar HLA supertypes, are likely contaminating peptides, copurifying with the HLA molecules during the affinity purification. Furthermore, peptides that fit the sequence motifs of the HLA allotypes of the patients are more likely authentic HLA ligands of these allotypes (Fig. 3).

Similarity Between HLA Peptidomes of Individuals Sharing HLA Allotypes—As expected, both the tumor-mHLA and plasma-sHLA peptidomes of different blood donors were more similar when they shared HLA allotypes (examples in Fig. 3) and these patients shared more TAAa and CTAs peptides (supplemental Table S6) even though only small fractions of the peptides were shared among most patients (Fig. 4, supplemental Table S7). The shared peptides identified in the plasma-sHLA peptidomes of people harboring very different HLA allotypes that belong to dissimilar HLA supertypes, are likely contaminating peptides, copurifying with the HLA molecules during the affinity purification. Furthermore, peptides that fit the sequence motifs of the HLA allotypes of the patients are more likely authentic HLA ligands of these allotypes (Fig. 3).

The HLA Peptidomes Do Not Correlate with the Proteomes of the Tumors—The protein repertoires and their levels (measured in iBAQ values) of the different tumors, were much better correlated than the HLA peptidomes of the different tumors (Fig. 5, supplemental Fig. S3). As many as 4855 different proteins were identified in all ten tumors analyzed, out of the total number of 7351 proteins identified (supplemental Table S4).

Table I—continued

| Gene name | Sequence | Count # | sHLA non-cancerous | sHLA GBM | mHLA GBM tissue |
|-----------|----------|---------|-------------------|---------|-----------------|
| GPMB      | AYMRHNLQ | 2       | 0                 | 1       |
| IENSPGNV  | 0        | 3       | 0                 | 0       |
| IPTEVCTII | 0        | 0       | 0                 | 1       |
| KGGLVFLNR | 3        | 6       | 1                 | 1       |
| VFLNRAKAVF | 1     | 1       | 0                 | 0       |
| VLTSDDPAL | 0        | 1       | 0                 | 1       |
| CDK4      | ALTPVVVTL | 0       | 6                 | 1       |
| CDK4      | ALTPVVVTL | 0       | 6                 | 1       |
| CDK4      | ALTPVVVTL | 0       | 6                 | 1       |
| SOX11     | AHSASEQQL | 1       | 0                 | 1       |
| SOX11     | AHSASEQQL | 1       | 0                 | 1       |

May 14, 2019
Importantly, 1302 different HLA peptides, derived from 706 of the proteins identified in all of the tumor samples, were also detected in the tumor tissues mHLA and plasma sHLA peptidomes. None of these peptides was derived from the source genes of the sHLA peptides that were detected in the non-cancerous donors’ plasma (supplemental Table S2, column E). Among these 1302 different peptides, a subset of 32 HLA peptides belong to the TAA group (Table III). An example for such peptide is AHIKGVETI of Outer dense fiber protein 1 (ODF1). The mRNA of ODF1 is not expressed in any of the essential healthy tissues, except the testis in healthy individuals, according to the BioGPS (supplemental Fig. S1A) and its protein was observed by the proteome analyses in all the ten analyzed tumors. Furthermore, no peptides derived from this gene were observed in any of the plasma-sHLA peptidome of the noncancerous donors. HLA peptides derived from this gene were detected in the plasma-sHLA of two female GBM patients (Table II). Therefore, this gene and similar genes like it, whose proteins products are expressed in the tumor tissues and their derived sHLA peptides appear in the plasma-sHLA peptidomes, may serve as both, potential biomarkers and candidates for immunotherapeutics.

S3). Importantly, 1302 different HLA peptides, derived from 706 of the proteins identified in all of the tumor samples, were also detected in the tumor tissues mHLA and plasma sHLA peptidomes. None of these peptides was derived from the source genes of the sHLA peptides that were detected in the non-cancerous donors’ plasma (supplemental Table S2, column E). Among these 1302 different peptides, a subset of 32 HLA peptides belong to the TAA group (Table III). An example for such peptide is AHIKGVETI of Outer dense fiber protein 1 (ODF1). The mRNA of ODF1 is not expressed in any of the essential healthy tissues, except the testis in healthy individuals, according to the BioGPS (supplemental Fig. S1A) and its protein was observed by the proteome analyses in all the ten analyzed tumors. Furthermore, no peptides derived from this gene were observed in any of the plasma-sHLA peptidome of the noncancerous donors. HLA peptides derived from this gene were detected in the plasma-sHLA of two female GBM patients (Table II). Therefore, this gene and similar genes like it, whose proteins products are expressed in the tumor tissues and their derived sHLA peptides appear in the plasma-sHLA peptidomes, may serve as both, potential biomarkers and candidates for immunotherapeutics.

Some HLA Allomorphs Present Larger Diversity of Peptides In Both Tumors and Plasma—The 52 different GBM samples and 36 different noncancerous samples have undergone a complete HLA typing analysis based on DNA sequencing (51). This analysis facilitated the use of the NetMHC platform to fit the HLA peptides sequences to their likely presenting HLA allomorphs by selecting peptides with NetMHC rank equal or better than 2 (http://www.cbs.dtu.dk/services/NetMHC/). HLA

### Table II

| Gene name | Sequence | CTA description | # of identifications |
|-----------|----------|-----------------|----------------------|
| ETV5      | FPDNRQPFL| Expressed only in placenta | mHLA | mHLA | sHLA | sHLA | sHLA | sHLA |
|           | FSSSHEGFSY|                 | male | female | GBM male | female | non-cancerous male | non-cancerous female |
|           | GPAPAHPSL|                 | 0 | 1 | 0 | 0 | 0 | 0 |
|           | KVAGERYVY|                 | 0 | 1 | 0 | 1 | 0 | 0 |
|           | LPYAEGFAY|                 | 3 | 0 | 3 | 0 | 0 | 0 |
|           | MPQPPAHGF|                 | 1 | 1 | 5 | 1 | 0 | 0 |
|           | RPAMNKYQXL|                | 1 | 1 | 0 | 0 | 0 | 0 |
|           | VRPRPHQPLQM|              | 0 | 1 | 2 | 0 | 0 | 0 |
| ODF1      | AHIKGVETI| Expressed only in testis | mHLA | mHLA | sHLA | sHLA | sHLA | sHLA |
|           | MESPCSPCSPC|                | 1 | 0 | 0 | 2 | 0 | 0 |
|           | RIIDTPSEMGMFG|             | 2 | 0 | 0 | 0 | 0 | 0 |
| ACPP      | SAHDTTVSGLQ|            | 1 | 0 | 0 | 0 | 0 | 0 |
|           | AHIDTTVSGNL|              | 1 | 0 | 0 | 0 | 0 | 0 |
| TTK       | SPNSILKAA| Expressed only in testis and fetal organs | mHLA | mHLA | sHLA | sHLA | sHLA | sHLA |
|           |         |                 | 0 | 0 | 0 | 2 | 0 | 0 |
| SPA17     | DQKEKEKEKEK|            | 0 | 0 | 1 | 0 | 0 | 0 |
allomorphs with a consensus sequence motif such as HLA-B*35:01 and HLA-A*24:02 presented larger numbers of peptides relative to allomorphs such as HLA-B*27:05 or HLA-B*40:01 (Fig. 4 and supplemental Table S8). This phenomenon was observed in both GBM and in the non-cancerous donors’ plasma samples, as well as in the GBM tissues (Fig. 6, supplemental Fig. S4). The HLA-A*24:02 allele is common among glioma patients (58) and therefore HLA peptides presented by these alleles may become useful for treatment of larger groups of patients.

DISCUSSION

This study is an extensive immunopeptidome analysis of 106 GBM plasma samples, 10 GBM tumor tissues, and 36
A. 0.0302  B. 0.3459  C. 0.0043  D. 0.9614

Fig. 5  Comparison between the tumor proteomes and HLA peptidomes: Comparison between the tumor proteome and mHLA peptidome (A), plasma sHLA and tumor mHLA peptidome (B), between the tumor proteome and plasma shLA peptidome (C) of patient CPH-09, and comparison between the tumor peptidomes of two different patients, CPH-09 and BCN-018 (D), of patient CPH-09, and comparison between the tumor proteome and plasma sHLA peptidome (D), plasma sHLA and tumor mHLA peptidome (E), plasma sHLA and mHLA peptidome (F), and matched plasma-sHLA peptidomes of each of the patients, demonstrating that the plasma-sHLA peptidome contains many peptides derived from the tumors. These correlations, between the repertoires and LC-MS intensities of the mHLA and sHLA peptidomes of the individual patients, were much larger than the correlations between the HLA peptidomes and the proteomes of the patients’ tumors (supplemental Fig. S3). Low correlations in both repertoires and expression levels, between HLA peptidomes, proteomes, and transcriptomes, of the same cells, were suggested in previous publications (59, 60) but were found to be higher in others (61–64). Additionally, we demonstrate that the plasma-sHLA peptidomes are relatively stable, can be analyzed reproducibly, and represent the tumor-mHLA peptidomes. Comparative analysis of both mHLA and sHLA peptidomes may provide candidate peptides, potentially useful for immunotherapy and as biomarkers. Precision medicine based on large-scale body-fluid biomarkers may help to identify patients that are most likely to benefit from specific treatments, including, but not limited to immunotherapy (65–67). It is expected that different HLA peptides derived from the TAA and CTA genes will be presented among the patients’ sHLA allomorphs’ peptidomes. Thus, the search among the sHLA peptidomes of different people for disease biomarkers is not limited to specific peptides, but to the presence of different peptides derived from the selected TAA/CTA genes. A few of the 250 downregulated sHLA peptides observed in this study may serve as biomarkers for early detection of the disease or relapse. Indeed, potentially useful TAA can be selected based on different criteria. In our opinion, first and foremost are peptides that are derived from genes that not expressed in any healthy adult tissue, other than the immune privileged sites, such as the germline cells. Such HLA peptides are potentially useful for both immunotherapy and diagnosis. Plasma sHLA peptides that appear after treatment or remission are potentially useful for diagnosis, for early detection of relapse, or for both. sHLA and mHLA peptides that appear in large amounts in tumor proteome of the patient are potential targets for further studies. It is likely (but not necessary) that a few of the downregulated sHLA peptides are TAAs. Such selected biomarkers may serve as a tool to become useful for clinical exploitation. Furthermore, we consider that many peptides were not detected in this study in the plasma-sHLA peptidomes after surgery, also because of the nature of shotgun peptidomics methodology used here. Performing targeted LC-MS/MS analysis with the same or with different sets of samples is still required to exclude this possibility. The alternative use of data-independent analysis of HLA peptidome was already used for analyses of HLA peptidomes at higher reproducibility (68–71), and SRM were used to obtain more accurate presentation levels of the selected peptides in multiple analyses (72, 73). Smaller numbers of HLA peptides were identified with the LC-MS/MS data collected in this study when the FDR was set to 0.01 instead of 0.05 in the Andromeda search (50), performed within the MaxQuant analysis tool (49). This is expected, because the MS/MS fragmentations of many peptides are suboptimal in the data-dependent (shotgun) analysis performed in this study. The use of data-independent analysis for the HLA peptidome analysis may help to solve some of the data loss incurred (69, 71, 74). Alternatively, one can increase the FDR to 0.05 and facilitate this way the discovery of candidates for vaccine or biomarkers HLA peptides that would have been lost with 0.01 FDR (75). Here as well, a significant fraction of the peptides that were lost by use of 0.01 FDR, instead of 0.05, are true ligands of the HLA allomorphs of the patients (supplemental Table S9), as defined by the similarity of
sequence motifs to the peptides included in the 0.01 FDR (supplemental Fig. S5).

The blood-brain barrier (BBB) prevents entrance and exit of some cells and molecules (5, 6) to the brain and it is unknown if it allows passage of the circulating sHLA-peptide complexes. However, the BBB in GBM patients is partially broken by the local inflammatory conditions and differs in its tumor vessels morphology and in the hyper-permeability of its endothelial cells (5). The loss of functional integrity of the BBB allows passage of low molecular weight compounds, including different chemotherapeutics into the brain (76). Such changes in the BBB may also facilitate the release of sHLA molecules with their bound peptides into the circulation, thus allowing their detection in the plasma for further evaluations as tumor markers.

Ideally, HLA peptides useful to serve as immunotherapeutics or biomarkers should be derived from genes expressed at sufficient levels in the malignant tissues, but not at all in any of the other essential healthy tissues (18, 23). Fresh-frozen tumors can be used for exome, transcriptome, proteome and HLA peptidome analyses, allowing identification of neo-epitopes and tumor antigens (4, 16, 40–45, 63, 77). Selection of CTA candidates for immunotherapy can be based on high expression levels of their HLA peptides, mRNA and proteins in the tumors, and no expression in healthy tissues. Even though tumors and plasma are good sources of HLA peptides, healthy tissues are not normally available for analysis of their HLA peptidomes, whereas data about the gene expression levels is available in public databases. Very large and relatively accurate databases are publicly available, including data on gene and protein expression in many tissues, of numerous people. Examples include BioGPS (55, 56), TANTIGEN (53), and HPR (https://www.proteinatlas.org/) (78, 79). Although these databases are based on numerous studies, some discrepancies were observed between them in regard to different selected CTAs studied here. Using additional gene expression databases may alleviate some of these concerns and provide candidate immunotherapeutics with lower risk of inducing adverse effects.

The preferred CTAs, providing potentially useful HLA peptides discovered in this study, were defined as those derived from genes whose transcripts are expressed at levels below

### Table III

| Gene name | Protein name | Sequence |
|-----------|--------------|----------|
| ADAM17    | Disintegrin and metalloproteinase domain-containing protein 17 | THVETLLTF |
| CTNNA2    | Catenin alpha-2 | SFFKAMDFS |
| CTSN      | Pro-cathepsin H | ESAIAATGK |
| EGFR      | Epidermal growth factor receptor | TQDFMMYRT |
|          |              |          |
| KRAS      | GTPase Kras  |          |
| ODF1      | Outer dense fiber protein 1 |          |
| PAK2      | Serine/threonine-protein kinase PAK 2 |          |
| PPIB      | Peptidyl-prolyl cis-trans isomerase B |          |
| SCR1      | Secernin-1 |          |
| SNRP1     | Small nuclear ribonucleoprotein Sm D1 |          |
| WNK2      | GTPase-activating protein |          |

Withdrawn
nine gcroma units in all normal, essential tissues (according to BioGPS) and are expressed at significantly higher levels in the tumors tissues. Nine gcroma units of mRNA levels of expression was selected here as sufficiently low, because this is the highest measured mRNA expression level of many well-characterized CTAs in healthy (nontestis) adult tissues. Some of these CTAs, including CTAG1A (NY-ESO-1) and MAGE-A1 are well known TAAs, whose expression levels in healthy tissues are below 9 gcroma units. These CTAs were already used in multiple clinical studies without observable autoimmune reactions (19, 80–82) suggesting that genes expressed below these levels are possibly safe for clinical use.

The tumor-mHLA and plasma-sHLA peptidomes of different people can be compared while looking for HLA peptides shared between larger cohorts of individuals. HLAs with completely distinct binding motifs are not expected to bind and present shared peptide ligands. Importantly, significant similarities were observed in this study between the HLA peptidomes of different patients that share some of their HLA alleles (Fig. 3). In contrast, shared peptides, detected in the HLA peptidomes of different people who do not have any common HLA alleles or have HLA alleles belonging to different HLA supertypes (83) are more likely to be defined as contaminants, rather than true HLA ligands. For example, it is very unlikely that HLAs such as HLA-A*2, B*7 and B*27 will share any peptide ligands (83). On the other hand, shared tumor antigens that are detected in multiple patients that have similar HLA allotypes, are extremely important, because of their potential to become useful for treatment of multiple patients.

The HLA molecules of some HLA allomorphs, such as HLA A*24:02, are more abundant in the plasma of carriers of these alleles (32). In addition, some HLA allomorphs present more numerous peptides than others do (84). Indeed, here we observe similar phenomena at the HLA peptidome levels, some HLA allomorphs, such as HLA-A24, HLA-B35, and HLA-B51 present more diverse repertoires of peptides than others do, in both the tumors and the plasma. This implies that the use of plasma-sHLA peptidome analysis is probably more efficient for carriers of these alleles, because larger sHLA peptidomes can be recovered and identified from their plasma samples (Fig. 4 and Fig. 6). It may also mean that there is larger immune tolerance in the HLA-A*24 patients and, therefore, it may be more difficult to break the immune tolerance induced by circulating sHLA molecules, when attempting immunotherapy. Importantly, HLA-A*24 was claimed to be...
associated genetically with glioma (58). Therefore, the use of the sHLA peptidome analysis to search for disease biomarkers will need to be adjusted accordingly, to consider the HLA allotypes of the individual patients.

In conclusion, the data described here suggests a useful method for selection of biomarkers and cancer immune-therapeutics for GBM and provides large lists of such candidates. Such methodologies are likely useful for discovery of biomarkers and immune-therapeutics candidates for other cancers. Most importantly, the identification of numerous sHLA peptides derived from CTAs can represent a promising non-invasive strategy for the monitoring of patients’ response and progression during standard treatment modalities.

Acknowledgments—We thank Ilana Navon from the Smoler Proteomics Center at the Technion for performing the LC-MS/MS experiments. We acknowledge the advice and discussion with the members of the GAPVAC consortium, Stefan Stevanovic and Cécile Gouttefangeas.

DATA AVAILABILITY

The mass spectrometry proteomics data of the tissue proteomes, the mHLA and the sHLA peptidomes have been deposited to the ProteomeXchange Consortium (54) (http://proteomecentral.proteomexchange.org) via the PRIDE partner repository with the data set identifier PXD008127.

REFERENCES

1. Alifieris, C., and Trafalis, D. T. (2015) Glioblastoma multiforme: Pathogenesis and treatment. Pharmacol. Ther. 152, 63–82
2. Thakkar, J. P., Dolecek, T. A., Horbinski, C., Ostrom, Q. T., Lightner, D. D., Barnholtz-Sloan, J. S., and Villano, J. L. (2014) Epidemiologic and molecular prognostic review of glioblastoma. Cancer Epidemiol. Biomarkers Prev. 23, 1985–1996
3. Terasaki, M., Shibui, S., Narita, Y., Fujimaki, T., Aoki, T., Kajiwara, K., Yamada, A., Tomita, M., and Hirose, Y. (2011) The mass spectrometry proteomics data of the tissue proteomes, the mHLA and the sHLA peptidomes have been deposited to the ProteomeXchange Consortium (54) (http://proteomecentral.proteomexchange.org) via the PRIDE partner repository with the data set identifier PXD008127.
4. Neidert, M. C., Schoor, O., Trautwein, C., Trautwein, N., Christ, L., Melms, A., Honegger, J., Ramassese, H. G., Herold-Mende, C., Dittrich, P. Y., and Stevanovic, S. (2013) Natural HLA class I ligands from glioblastoma: Extending the options for immunotherapy. J. Neurooncol. 111, 285–294
5. Patel, M. A., and Pardoll, D. M. (2015) Concepts of immunotherapy for glioma. J. Neurooncol. 123, 323–330
6. Cohen-Inbar, O., and Zaaroor, M. (2016) Immunological aspects of malignant gliomas. Can. J. Neurol. Sci. / J. Can. des Sci. Neurol. 43, 494–502
7. Policova, J., Holubec, L., Kubikova, T., Priban, V., Hes, O., Pivovarova, K., and Treskova, I. (2017) Advances in experimental targeted therapy and immunotherapy for patients with glioblastoma multiforme. Anticancer Res. 37, 21–33
8. Swartz, A. M., Batich, K. A., Fecci, P. E., and Sampson, J. H. (2015) Peptide vaccines for the treatment of glioblastoma. J. Neurooncol. 123, 433–440
9. Ampie, L., Woof, E. C., and Dardis, C. (2015) Immunotherapeutic advances for glioblastoma. Front. Oncol. 5, 12
10. Oh, T., Sayegh, E. T., Fakunnejad, S., Oyon, D., Lamano, J. B., DiDomenico, J. D., Bloch, O., and Parsa, A. T. (2015) Vaccine therapies in malignant glioma. Curr. Neurol. Neurosci. Rep. 15, 508
11. Silvestri, J., S., Lee, S., Weinschenk, T., Singh-Jasuja, H., and Dietrich, P. R. (2016) Recent advances and future of immunotherapy for glioblastoma. Expert Opin. Biol. Ther. 16, 1233–1247
12. Ott, P. A., Hu, Z., Keskin, S., Li, S., Sun, J., Bozym, D. J., Zhang, W., Luoma, A., Giot, J.-P., Peter, L., Chen, C., Olive, O., Carter, T. A., Li, S., Li, J., Gjini, E., Stevens, J., Lane, W. J., Javert, I., Grothe, M., Daley, H., Seaman, M., Buchbinder, S., Shannon, N., Gabriel, S., Rodig, S., Blease, C., and Triebel, K. (2012) Exploiting the glioblastoma peptidome to discover novel tumour-associated antigens for immunotherapy. Brain 135, 1042–1054
13. Bassani-Sternberg, M., and Coukos, G. (2016) Mass spectrometry-based antigen discovery for cancer immunotherapy. Curr. Opin. Immunol. 41, 9–17
14. Rammensee, H.-G., and Jung, P. (2014) Exploiting the glioblastoma peptidome to discover novel tumour-associated antigens for immunotherapy. Brain 135, 1042–1054
15. Pol, J., Bloy, N., Buqué, A., Egermont, A., Sauvé-froidman, Galon, C. J., Tartour, E., Kromer, G., Galluzzi, L., Pol, J., Bloy, N., Buqué, A., Eger- mont, A., Cremer, I., Sauvé-froidman, Galon, C. J., Tartour, E., Zitvogel, L., Kromer, G., Galluzzi, L., Watch, T., Pol, J., Bloy, N., Buqué, A., Egermont, A., Cremer, I., Sauvé-froidman, Galon, C. J., Tartour, E., Zitvogel, L., Kromer, G., and Galluzzi, L. (2015) Trial Watch: Peptide-based anticancer vaccines Trial Watch: Peptide-based anticancer vac- cine. Oncoimmunology 4, e874417
16. Comber, J. D., and Philip, R. (2014) MHC class I antigen presentation and implications for developing a new generation of therapeutic vaccines. Ther Adv Vaccines 2, 77–89
17. Heemskerk, B., Kvitbogh, P., and Schumacher, T. N. M. (2012) The cancer antigen. EMBO J. 32, 194–203
18. Chen, Y. T., Scanlan, M. J., Sahin, U., Tureci, O., Gure, A. O., Tsang, S., Williamson, B., Stockert, E., Pfreundschuh, M., and Old, L. J. (1997) A testicular antigen aberrantly expressed in human cancers detected by autologous antibody screening. Proc. Natl. Acad. Sci. U.S.A. 94, 1914–1918
19. Whitehurst, A. W. (2014) Caus and consequence of cancer/testis antigen activation in cancer. Annu. Rev. Pharmacol. Toxicol. 54, 251–272
20. Schumacher, T. N., and Schreiber, R. D. (2015) Neonttagens in cancer immunotherapy. Science 348, 69–74
The HLA Peptidome of Glioblastoma

25. Charlton, R. K., and Zmijewski, C. M. (1970) Soluble HL-A7 antigen: localization in the beta-lipoprotein fraction of human serum. Science 170, 636–637
26. van Roold, J. J., van Leeuwen, A., and van Santen, M. C. (1970) Anti HL-A2 inhibitor in normal human serum. Nature 226, 366–367
27. Tabayoyong, W. B., and Zavazava, N. (2007) Soluble HLA revisited. Blood 110, 3121–3127
28. Bassani-Sternberg, M., Barnea, E., Beer, I., Avivi, I., Katz, T., and Admon, A. (2010) Soluble plasma HLA peptidome as a potential source for cancer biomarkers. Proc. Natl. Acad. Sci. U.S.A. 107, 18769–18776
29. Ritz, D., Gloger, A., Weide, B., Garbe, C., Neri, D., and Fugmann, T. (2016) High-sensitivity HLA class I peptidome analysis enables a precise definition of peptide motifs and the identification of peptides from cell lines and patients’ sera. Proteomics 16, 1570–1580
30. Ritz, D., Neri, D., and Fugmann, T. (2017) Purification of soluble HLA class I complexes from human serum or plasma deliver high quality immuno-peptidomes required for biomarker discovery. Proteomics 17, 1–6
31. Puppo, F., Scudeletti, M., Indiveri, F., and Ferrone, S. (1995) Serum HLA class I antigens: markers and modulators of an immune response? Immunol. Today 16, 124–127
32. Adamashvili, I. M., Fraser, P. A., and McDonald, J. C. (1996) Association of class I antigens: markers and modulators of an immune response? Immunol. Today 17, 321–334
33. Hunt, D. F., Henderson, R. A., Shabanowitz, J., Sakaguchi, K., Michel, H., Strychalski, N., Cox, J. A., and Appell, P. E. (1998) Characterization of peptides bound to the class I MHC molecule HLA-A2.1 by mass spectrometry. Science 255, 1261–1263
34. Granados, D. P., Laumont, C. M., Thibault, P., and Perreault, C. (2017) Human leukocyte antigen class I nature of self for T cells—a systems-level perspective. Cytokine nol. 34, 1–8
35. Schumacher, F.-R., Delamarre, L., Junhunjwala, P. T., Elias, J. E., and Lill, J. R. (2017) Predictive monitoring of HLA-peptide associations by high throughput analysis. Sci. Transl. Med. 9, 366–367
36. de Verteuil, D., Granados, D. P., Thibault, P., and Perreault, C. (2012) Origin of clinically relevant neoepitopes presented on native human melanoma melanocytes. J. Immunol. 188, 94–103
37. Fritsche, J., Rakitsch, B., Snipas, S., Kowalewski, D. J., Priemer, M., Stos-Zweifel, V., Hoerzer, H., Satelli, A., Sonntag, A., Goldfinger, V., Song, C., Mahr, A., Ott, M., Schoor, O., and Weinschenk, T. (2018) Translating Immunopeptidomics to Immunotherapy—decision-making for patient and personalized target selection. Proteomics 18, 200284
38. Shraibman, B., Kadosh, D. M., Barnea, E., and Admon, A. (2016) Human leukocyte antigen (HLA) peptides derived from tumor antigens induced by inhibition of DNA methylation for development of drug-facilitated immunotherapy. Mol. Cell. Proteomics 15, 3058–3070
39. Weinschenk, T., Gouttefangeas, C., Schirle, M., Gloger, A., Backert, L., Hoerzer, H., Satelli, A., Mahr, A., Ott, M., Schoor, O., and Weinschenk, T. (2016) Direct identification of peptide ligands in renal cell carcinoma cells. Mol. Cell. Proteomics 15, 3088–3097
40. Salti, R. H., Rammensee, H.-G., Stevanovic, S., and Stickel, J. S. (2015) HLA ligandome analysis identifies the underlying specificities of spontaneous autoimmune immune responses in chronic lymphocytic leukemia (CLL). Proc. Natl. Acad. Sci. U.S.A. 112, E166–E175
41. Tabayoyong, W. B., and Zavazava, N. (2007) Soluble HLA revisited. Blood 110, 3121–3127
42. Seliger, B., Dressler, S. P., Massa, C., Recktenwald, C. V., Altenberend, F., Bukur, J., Marincola, F. M., Wang, E., Stevanovic, S., and Lichtenthaler, F. (2011) Identification and characterization of human leukocyte antigen class I ligands in renal cell carcinoma cell lines. Proteomics 11, 2528–2541
43. Klett, M. G., Kowalewski, D. J., Schuster, H., Di Marco, M., Hennenlotter, J., Stenzl, A., Rammensee, H.-G., and Stevanovic, S. (2016) Carcinogenesis of renal cell carcinoma reflected in HLA ligands: A novel approach for synergistic peptide vaccination design. Oncoimmunology 5, e1204504
44. Kowalewski, D. J., Schuster, H., Backert, L., Berlin, C., Kahn, S., Kanz, L., Salti, R. H., Rammensee, H.-G., Stevanovic, S., and Stickel, J. S. (2015) HLA ligandome analysis identifies the underlying specificities of spontaneous autoimmune immune responses in chronic lymphocytic leukemia (CLL). Proc. Natl. Acad. Sci. U.S.A. 112, E166–E175
Khodadoust, M. S., Olsson, N., Wagar, L. E., Haabeth, O. A. W., Chen, B., Molecular & Cellular Proteomics 17.11, 2145.

Schuster, H., Peper, J. K., Bösmüller, H.-C., Röhle, K., Backert, L., Bilich, 67.

Tsiatas, M., Mountzios, G., and Curigliano, G. (2016) Future perspectives in cancer immunotherapy. Nature 543, 723–727.

Caron, E., Espona, L., Kowalewski, D. J., Schuster, H., Ternette, N., Alpízar, 68.

Croft, N. P., de Verteuil, D. A., Smith, S. A., Wong, Y. C., Schittenhelm, 72.

Tan, C. T., Croft, N. P., Dudek, N. L., Williamson, N. A., and Purcell, A. W. (2017) Antigen presentation profiling reveals recognition of lymphoma immunoglobulin neoantigens. Nature Immunology 18, 994–9951.

Sawyers, C. L. (2008) The cancer biomarker problem. Nature 452, 548–552.

Perz-Gracia, J. L., Sammenn, M. F., Bosch, A., Patiño-Garcia, A., Schalper, K. A., Segura, V., Bellmunt, J., Taberner, J., Sweeney, C. J., Choueiri, T. K., Martin, M., Fusco, J. P., Rodriguez-Ruz, M. E., Calvo, A., Prior, C., Paz-Ares, L., Pio, R., Gonzalez-Billabertiania, E., Gonzalez Hernandez, A., Práez, D., Piulats, J. M., Gurpide, A., Andueza, M., de la Puente, C., Paz-Ares, L., Hallin, I., Hamsten, C., Hansson, M., Hedhammer, M., Hercules, G., Kampf, C., Larsson, K., Lindskog, M., Lodewyckx, W., Lunde, J., Lundeberg, J., Magnusson, K., Malm, E., Nilsson, P., Olsing, D., Olsson, P., Ister, O., Ottosson, J., Paavilainen, L., Persson, A., Rimini, R., Rockberg, J., Runeson, M., Sivertsson, A., Skillerom, A., Steen, J., Stenvall, M., Sterky, F., Strömberg, S., Sundberg, M., Ternette, N., Tourie, S., Wahlund, E., Walén, A., Wang, J., Wernérus, H., Wester, K., Westergren, U., Xu, L. L., Hober, S., and Poole, D. (2016) Strategies to design clinical studies to identify predictive biomarkers in cancer research. Cancer Treat. Rev. 43, 57–67.

Tsitas, M., Mountzios, G., and Curigliano, G. (2016) Future perspectives in cancer immunotherapy. Annu. Trans. Med. 4, 273.

Croft, N. P., de Verteuil, D. A., Smith, S. A., Wong, Y. C., Schalper, K. A., Segura, V., Bellmunt, J., Taberner, J., Sweeney, C. J., Choueiri, T. K., Martin, M., Fusco, J. P., Rodriguez-Ruz, M. E., Calvo, A., Prior, C., Paz-Ares, L., Pio, R., Gonzalez-Billabertiania, E., Gonzalez Hernandez, A., Práez, D., Piulats, J. M., Gurpide, A., Andueza, M., de la Puente, C., Paz-Ares, L., Hallin, I., Hamsten, C., Hansson, M., Hedhammer, M., Hercules, G., Kampf, C., Larsson, K., Lindskog, M., Lodewyckx, W., Lunde, J., Lundeberg, J., Magnusson, K., Malm, E., Nilsson, P., Olsing, D., Olsson, P., Ister, O., Ottosson, J., Paavilainen, L., Persson, A., Rimini, R., Rockberg, J., Runeson, M., Sivertsson, A., Skillerom, A., Steen, J., Stenvall, M., Sterky, F., Strömberg, S., Sundberg, M., Ternette, N., Tourie, S., Wahlund, E., Walén, A., Wang, J., Wernérus, H., Wester, K., Westergren, U., Xu, L. L., Hober, S., and Poole, D. (2016) Strategies to design clinical studies to identify predictive biomarkers in cancer research. Cancer Treat. Rev. 43, 57–67.

Tsitas, M., Mountzios, G., and Curigliano, G. (2016) Future perspectives in cancer immunotherapy. Annu. Trans. Med. 4, 273.

Barnea, E., Melamed Kadosh, D., Haimovich, Y., Satumira, N., Dorris, M. L., Nguyen, M. T., Hamner, R. E., Tran, T. M., Coibert, R. A., Taurog, J. D., and Admon, A. (2017) The Human Leukocyte Antigen (HLA)-B27 Peptidome in Vivo, in Spondyloarthritis-susceptible HLA-B27 Transgenic Rats and the Effect of Erap1 Deletion. Mol. Cell. Proteomics 16, 642–662.

Agarwal, S., Sane, R., Oberoi, R., Olfest, J. R., and Elmqvist, W. F. (2011) Delivery of molecularly targeted therapy to malignant glioma, a disease of the whole brain. Expert Rev. Mol. Med. 13, e17.

Kalaora, S., Bamea, E., Merhavi-Shoham, E., Qutob, N., Teer, J. K., Shimony, N., Schachter, J., Rosenberg, S. A., Beisser, M. J., Admon, A., and Samuels, Y. (2016) Use of HLA peptidomopics and whole exome sequencing to identify human immunogenic neo-antigens. Oncotarget 7, 5100–5117.

Uhlén, M., Björling, E., Agaton, C., Szigyarto, C. A.-K., Amin, B., Andersen, E., Andersson, A.-C., Angelidou, P., Asplund, A., Asplund, C., Berglund, L., Bergström, K., Brumer, H., Cerján, D., Ekström, D., Elodei, A., Eriksson, C., Fagerberg, L., Falk, R., Fall, J., Forsberg, M., Björklund, M. G., Gumbel, K., Hallim, A., Hallin, I., Hamsten, C., Hansson, M., Hedhammer, M., Hercules, G., Campbell, C., Larsson, K., Lindskog, M., Lodewyckx, W., Lunde, J., Lundeberg, J., Magnusson, K., Malm, E., Nilsson, P., Odsing, D., Olsson, P., Liljedahl, M., Oster, O., Ottosson, J., Paavilainen, L., Persson, A., Rimini, R., Rockberg, J., Runeson, M., Sivertsson, A., Skillerom, A., Steen, J., Stenvall, M., Sterky, F., Strömberg, S., Sundberg, M., Ternette, N., Tourie, S., Wahlund, E., Walén, A., Wang, J., Wernérus, H., Wester, K., Westergren, U., Xu, L. L., Hober, S., and Poole, D. (2016) Strategic approaches as human protein atlas for normal and cancer tissue using HLA peptidomic digital maps of immune epitopes. Mol. Cell. Proteomics 4, 1920–1928.

Uhlén, M., Lindskog, M., Oksvold, P., CernCLUSIVE ATTEMPT WITHDRAWN FROM PUBLICATION. May 14, 2019.