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REVIEW

Using phosphoproteomics and next generation sequencing to discover novel therapeutic targets in patient antibodies

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ABSTRACT

Introduction: The goal of this review is to survey proteomics and next generation sequencing (NGS) approaches to discover phosphopeptide neoantigens that can be used as targets for individualized cancer therapy and diagnostics. Neoantigens are peptides or proteins that can be derived from tumor-specific or viral alterations containing mutated sequences including different post-translational modifications that will not be found in normal human cells.
These neoantigens can be highly specific for the individual affected tissue. Mass spectrometry combined with NGS allow identifying autoantibodies specific for neoantigens, including neoantigens that contain phosphorylated moieties.
Areas covered: We discuss recognition of neoantigens through the application of phosphoproteomics. We focus on phosphoprotein modifications that can be accurately identified with mass spectrometry and can serve as rarely described targets for immunotherapy and diagnosis in cancer and autoimmune diseases. Identification of these phospho-moiety containing neoantigens gives the possibility to search for corresponding autoantibodies, especially if NGS and mass spectrometry are combined.
Expert commentary: Identification of post-translational modifications with mass spectrometry gives possibilities to define modifications that are specific for cancer and autoimmune diseases. This technology develops rapidly, and gives opportunities to select neoantigens that are highly specific for cancer and immune diseases and to sequence autoantibodies involved.

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Neoantigens; autoantibodies; phosphopeptide; proteomics; NGS; next generation sequencing

1. Neoantigens

The goal of this review is to define cancer-associated phosphorylated neoantigens by phosphoproteomics and describe technology to sequence antibodies that might be specific for these neoantigens. Neoantigens are mostly proteins that can be derived from tumor-specific alterations with mutated sequences including post-translational modifications that will not be found in normal human cells. Neoantigens are ideal targets for immunotherapy because the host's immune system does not show central T-cell tolerance to these antigens and can recognize them as foreign [1]. In this review we only discuss neoantigens of protein origin with changed phosphorylated moieties, and we take these phospho-moieties as examples, although many more post-translational modifications exist. We exclude phosphoantigens that are not composed of amino-acid biopolymerisation, e.g. phosphometabolites.

A neoantigen with a coding alteration in an amino-acid sequence including specific post-translational modifications can be recognized as an immune determinant because it can participate in early tumor recognition and subsequent destruction by antigen-specific T cells. Binding affinity is not always correlated with the ability to trigger T-cell responses; only 1–2% of putative neoantigens can elicit T-cell reactivity [1,2]. Computerized forms of neoantigen prediction are scarce; the existing ones are hardly able to propose putative antigens that will be translated into peptides, cleaved by the proteasome, bound to human leukocyte antigen (HLA) molecules, and presented on the cell surface [1,3,4]. Neoantigens formed as a result of somatic mutations can be recognized by host immune cells, and are important for applying immunotherapy [3,5,6]. Although this phenomenon holds for phosphomodifications as well, it is rarely described in literature. Technological advance in the field of mass spectrometry (MS) makes it possible to discover neoantigens to investigate true in vivo peptide binding in the major histocompatibility complex (MHC) on the tumor cell surface [2]. However, the possibilities to include post-translational modifications are not largely included in recent research in general [7–11]. Incorporation of MS data into existing neoantigen identification methods, including post-translational modification methods, may lead to lower rates of false-positive predictions [2], and might allow finding specific antigens with modifications that are not directly recognized from genetic sequencing information [12–-12–14]. Nejo and coworkers [1] describe the results of a GAPVAC-101 clinical trial suggesting that it may be possible to combine both non-mutant antigen and neoantigen targets to elicit potential immune responses against heterogeneous glioma antigens [15]. The 'off-the-shelf' non-mutant peptide warehouse system is useful because it allows multiple personalized selection of antigen peptides that have been validated

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Article highlights

- Protein phosphorylation is a post-translational modification that is essential in the pathogenesis of tumor cells.
- MHC-restricted phosphopeptides are promising tumor-associated antigens for cancer immunotherapy
- Identification of neoantigens can be realized by combining high-throughput next generation sequencing and computational algorithms for MHC-peptide binding affinity.
- Autoantibodies against tumor-associated antigens are attractive targets with potential activity for tumors in vivo.

Mass spectrometry and NGS are highly amenable to study the possibilities to sequence antibodies specific for an antigen in serum.

for their immunogenicity and HLA presentation [16,17]. The non-mutant antigen ephrin type-A receptor 2 (EphA2), for instance, has been observed to be generally negative in normal glial cells, and be overexpressed in approximately 90% of glioblastoma (GBM) samples [18]. Many non-mutant antigens found in GBM are overexpressed in other cancers; HER2, for example, is overexpressed in approximately 30% of breast cancer patients, but it is also expressed at low levels in normal tissues, thus raising the risk of tumor-toxicity. Analysis of the cell surface expressions of HER2, IL-13Rα2 and EphA2 in 15 primary GBM samples showed that a novel treatment strategy co-targeting these three antigens was capable of capturing and eradicating nearly 100% of tumor cells in vitro [19]. HER2 and EphA2 are proteins that have been described to be involved in the pathogenesis of cancer. These proteins are all extensively phosphorylated (over 20 and 30 phosphosites, respectively https://www.phosphosite.org). The chance that phosphosites are involved in these immune responses is high.

2. Phosphopeptides and phosphoproteins as neoantigens

MHC-restricted phosphopeptides derived from phosphoproteins can be regarded as promising tumor-associated antigens (TAAs) for cancer immunotherapy. Phosphopeptide antigens are naturally processed and presented on human tumor cells by class I and class II MHC molecules [12,13,20]. Phosphopeptide-specific T cells can recognize intact human tumor cells, and phosphopeptide antigens may be closely linked to the malignant phenotype [20]. Dysregulated protein kinase activity is an indication of malignant transformation, and directly contributes to oncogenic signaling pathways leading to uncontrolled proliferation, cell survival, tissue invasion and metastasis [21].

2.1. Phosphorylation

Phosphorylation can influence both epitope conformation and T-cell receptor (TCR)/MHC binding, and makes it possible to target specific tumor-associated phosphopeptides originating from membrane bound proteins. Phosphorylation can have effects on the antigenic identity of MHC-bound peptides. The ability of T cells to recognize phosphopeptide-MHC molecules in both an epitope-specific and phospho-dependent manner can reside within the TCR. The phosphate-moiety can enable such modification-dependent and antigen-specific discrimination even for epitopes with few phosphorylation-induced conformational alterations [22]. In tumor cells deregulated phosphorylation may generate neoantigens by enhancing the binding of low-affinity peptides by MHC-class I [23,24]. Protein phosphorylation is a dominant mechanism in oncogenic signaling processes, and is preserved on peptides during antigen processing by MHC-I and MHC-II molecules [12]. For this reason, these peptides might not be degraded in the cell.

2.2. Phosphopeptides

Phosphopeptide antigens from cancer-related phosphoproteins could serve as immunological signatures of 'transformed self', while phosphorylation can enhance binding peptides to MHC-1 molecules, thereby creating neoantigens [25]. Phosphopeptides associated with human MHC II molecules have been revealed as a new cohort of tumor antigens that can be recognized by the immune system and be beneficial for cancer immune therapies [20]. Phosphopeptides are transported into the endoplasmic reticulum actively, but, when assembled with MHC, they seem to be protected from phosphatase activity, preserving the epitope for scrutiny by T cells at the cell surface [26]. MHC phosphopeptides can be used to elicit CD8 + T lymphocytes that only recognize the phosphorylated form of the peptides. Recognition of MHC phosphopeptides that are endogenously expressed on cancer cells by specific CD8 + T lymphocytes is a direct result of the level of expression and phosphorylation of the underlying source protein. The focus on phosphate modification makes it possible to select the complex mixture of MHC-associated peptides by mass spectrometry to identify a small number of peptides with high relevance to cellular growth control processes. This modification makes it possible to assess the expression of the epitope in a fast way, and not just the source protein in multiple tumors of the same or different histological types with CD8 + T lymphocytes and phosphopeptide-specific antibodies. This approach results in establishing a category of shared cancer antigens that are directly related to the cellular growth control processes. These shared antigens are likely targets for immunotherapy [12,13]. Phosphorylated peptides in MHC molecules represent a class of neoantigens expressed by cancer cells and recognized by CD8 T cells [27]. The phosphate-moieties on proteins create an antigenic form that distinguishes itself from unphosphorylated peptides [27]. Cancer cells display cancer-specific phosphopeptides in association with MHC class and Class II molecules. Insulin receptor substrate and the cell cycle regulator CDC25b are linked to cancer cell survival and expressed in a CD8 T cell memory response specific for phosphopeptides [13,14].

3. Autoantibodies

Autoantibodies are antibodies that have affinity for the presence of a patient's self-antigen(s). Antibodies can be generated against non-self antigens. Autoantibodies are generated against self-antigens that for some reason are seen as non-self

antigens, most probably due to coding changes in amino-acids sequence in a protein, conformational changes in the expressed epitope, and post-translational modifications (PTMs). The production of autoantibodies to self-antigens depends on the balance of immune tolerance. Biological activities of autoantibodies against self-antigens may induce paraneoplastic syndromes that reflect a cancer patient's body attempt to counteract tumor growth [28].

Autoantibodies are a hallmark of many autoimmune diseases; they can be present years before clinical symptoms arise, and be present even in healthy individuals (reviewed by Didier et al. [29]). The complexity of the human antibody repertoire is challenging in sequencing autoantibodies [30–-30–32]. The discovery that a person's memory, behavior, cognition and thought processes can be altered by autoantibodies led to changes in the approach for diagnosing neurological disorders. Identifying autoantibodies against neuronal cell surface antigens encourages immunotherapy [33]. Autoantibodies against TAAs are attractive targets to develop noninvasive serological tests for predicting a high risk of recurrence and/or treatment response [34]. Serum autoantibodies can be detected because tumor development is often accompanied with the expression of TAAs that may elicit immune responses that result in the generation of anti-TAA autoantibodies, even when the antigen expression is minimal [35].

An individual can make antibodies to an antigen, but the effectivity can vary. The success of the generation of a specific antibody or family of antibodies is almost an experiment in nature for every individual. Whether it works also depends on the generated titer of that antibody. For instance, patients with paraneoplastic syndrome can have high titers of antibodies that can be directed to neuro-antigens [36] and can, for that reason, be successful – but with severe neurological side effects. In these cases, cancer might develop, but these cancers have been observed to retract in sporadic cases. It is clear that these autoantibodies in paraneoplastic syndrome can recognize neoantigens on various tumors (e.g. melanoma, breast carcinoma).

3.1. Post-translational modifications

The ability of anti-cytokine autoantibodies (AAbs) to mediate disease manifestations may depend on the way how they neutralize or potentiate cytokine functions, which has been proposed to depend on the concentration of the AAb in circulation or in tissue, the avidity of the AAb or the epitopes recognized by the AAb [37,38]. Altered post-translational modifications or changes in the three-dimensional structure in these cytokine proteins have mostly not yet been investigated.

Vaccine approaches including post-translational modifications may have a unique advantage over genetically engineered T-cell approaches in that large numbers of antigens can be targeted simultaneously [1]. Using post-translational modifications to increase the specificity for erasing tumor cells could be an approach that deserves more attention in an experimental setting. Varley et al. [39] describe autoantibodies against a number of proteins that have been associated with well-defined diseases of the central nervous system. These antigens of protein origin are ion channels, including NMDAR and GABAAR and proteins including ligand-gated receptors and cell adhesion molecules. These autoantibodies target extracellular protein domains and have pathogenic potential because they can access their target at the neuroglial cell surface. The IgG1-predominant AQP4-antibodies can initiate complement-dependent cytotoxicity and antibody-dependent cellular cytotoxicity. Complement activation seems to be a prominent pathological mechanism, and the tendency of AQP4 to form densely-clustered orthogonal array particles on astrocytes may increase available divalent IgG-binding sites and hence complement binding potential [40]. AQP4, NMDAR, and GABAAR are all phosphorylated proteins with a series of phosphorylation sites [41–43] that potentially can be part of the specificity of these autoantibodies. Autoantibodies in NMDAR-antibody associated encephalitis target the extracellular amino-terminal domain of the NRI subunit of the NMDAR [44,45]. Extra-synaptic NMDARs are bound by autoantibodies, and subsequently the antibody binding to synaptic NMDAR can lead to lateral dispersion of the receptors and endocytosis. The synaptic effect may be due to disruption of the Ephrin-B2-NMDAR complex, since NMDAR synaptic content is regulated by the expression of Ephrin-B2 [46,47].

3.2. Epitopes

Sinmaz and coworkers [48] describe that research of autoantibody epitope specificity usually uses a cell-based assay followed by fluorescent microscopy or flow cytometry analysis [49]. Western blotting, radioimmunoassays and ELISAs have also been used to determine antigenic epitopes. Pathogenic autoantibodies might recognize cell surface antigens only in their native confirmation. The type of method utilized will determine whether the antigen epitope remains in its native three-dimensional conformation or is converted to a denatured or linear configuration. The type of status of cells used in cell-based assays – live, fixed or permeabilized – is important, as the type of status affects conformation and leads surprisingly to high levels of autoantibodies in healthy donors [39]. Ten percent overall seroprevalence of the antiNMDAR antibody with isotypes IgG, IgA and IgM was detected in both adult and healthy patient groups and could explained by the status of the cell [39,47]. Strone and coworkers [50] detected neoantigen-reactive T-cells in healthy donors, and showed that adoptive T-cell therapy focusing on neoantigens is feasible by transferring neoantigen-reactive T-cell receptor genes from healthy donors into patients' T-cells.

3.3. Methods and approaches

Moritz and Coworkers [51] describe the concept of autogenomics, and hypothesize that there may be systematic differences in sets of targeted antigens between patients. A gen-based approach, serological proteome analysis (SERPA), can be used for autoantigenomics (Figure 1) [52]. In this method, two-dimensional gel electrophoresis is coupled with immunoblotting using human sera in order to detect reactivity, followed by mass spectrometry or Edman sequencing to identify the

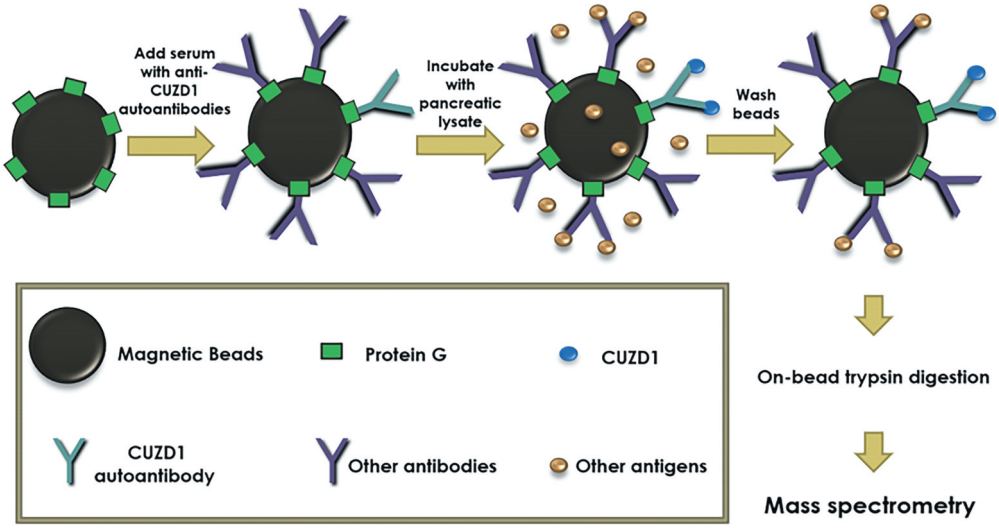

Figure 1. (a) array of different proteins bound by serum antibodies, (b) other techniques to select antigens directly via antibodies from serum, (c) result of the specific antigens bound to the disease specific antibodies, (d) analysis of autoantigens. (Copyright Moritz et al. [51]).

corresponding antigens. By analyzing the autoantigens, antigen clusters can be identified; for example, overrepresented antigens belonging to particular cellular components or signaling pathways [53].

Music and coworkers [54] developed an efficient immune-mass spectrometry approach to identify serum autoantibodies. All IgG antibodies, including autoantibodies, in serum are captured and purified on protein G beads, to be followed by addition of a protein mix containing thousands of human proteins. Then, the beads are washed extensively, followed by capture of antigen by antibodies that were digested by trypsin and subsequently identified by shotgun tandem MS analysis. This MS method can accurately quantify and identify proteins that may be difficult or even impossible to detect when other technology is used [54] (Figure 2).

Regarding transcriptomics, genome-wide microarray analyses and next-generation sequencing make it possible to comprehensively analyze gene sequences. This method even covers non-coding parts of RNA [55]. Transcriptomics methods have been used to support the identification of potential autoantigens [46,56,57]. High-throughput sequencing of the variable regions of the B and T cell receptors makes it possible to identify clonal receptor families [58]. Recombinant antibodies derived from clonal B cell families can be used to identify the corresponding antigen(s) (see Figure 1), although not yet in a high-throughput manner and large numbers of patients [51,57]. Using these techniques to focus on post-translational modifications specifically observed in tumors is an intriguing possibility to find antibodies that can be used for treatment.

4. Mass spectrometry and proteomics

Proteomics directly addresses proteins and gene products present in a particular cell state, reflecting the influence of

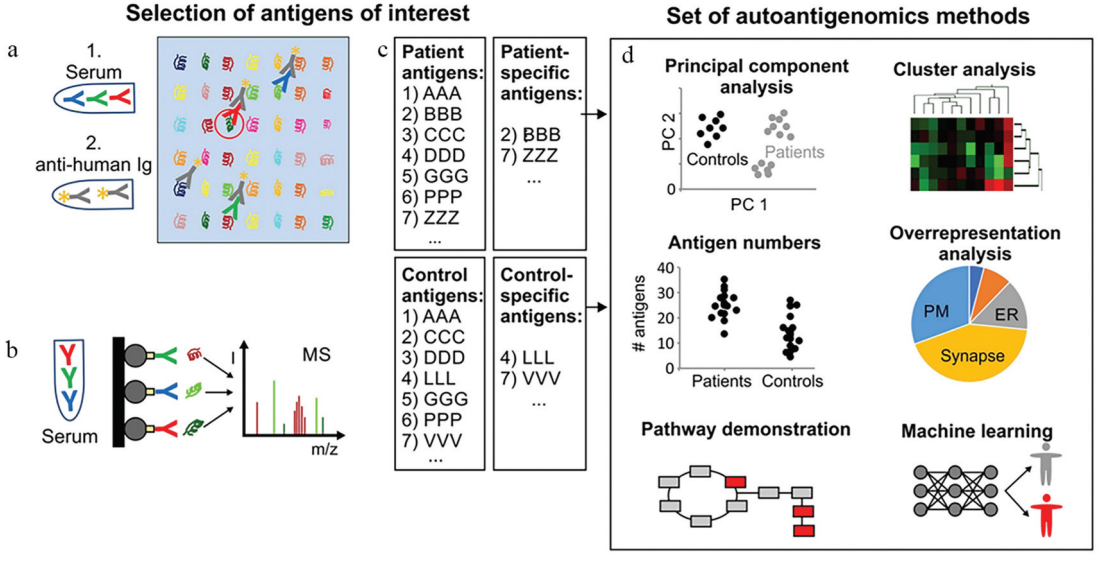

Figure 2. Methodology to identify antigens and autoantibodies from capturing with Protein G coated beads by mass spectrometry. (Copyright, Music et al. [54]).

genetic, environmental factors and post-translational modification. The proteome plays a substantial role in regulatory processes. MS is the standard approach to detect gene products, quantification and interaction of proteins [59–62].

Proteomics can be used to identify affinity-enriched autoantibodies, but with limited sequence length or sequence accuracy. The combination of proteomics and NGS (termed: proteogenomics) results in superior depth, read length, and sequence accuracy to detect specific antibodies and autoantibodies in serum [59]. The integration of proteomics with genomics and transcriptomics enables to identify specific post-translational modifications on antigens, including the antigens of autoantibodies and the sequence of these autoantibodies in serum [59]. Proteogenomic applications identify splice isoforms, single amino acid polymorphisms and other genetic variants [63].

4.1. Proteomic methods

In targeted proteomic methods, a limited set of predefined peptides with high reproducibility and specificity is analyzed (SRM, PRM) [62]. Proteomic methods are classified by pre-analytical Ig processing into top-down, middle-down and bottom-up. Top-down MS starts with intact Ig, and the fragmentation pattern elucidates information on the primary structure. Bottom-up MS is the process in which the Ig is enzymatically digested into peptides. The Ig primary structure is inferred from the peptide sequences. Either method can be refined by reducing the Ig into smaller fragments that can be analyzed intact (middle-down) or after further digestion into peptides (middle-up) [60,64].

Methods to get peptide data include data-dependent acquisition (DDA) modes, in which the mass spectrometer automatically fragments the top most abundant peptides peaks in each scan (Orbitrap-based MS), and data-independent acquisition (DIA) measurements such as sequential window acquisition of all theoretical mass spectra (SWATH) [62].

To identify antibodies in serum samples, two experimental pipelines need to be integrated: (1) high throughput sequencing of B lymphocyte cDNAs to generate a database of class-switched antibody variable domain sequence of a particular individual, and (2) a protein biochemistry and MS-based proteomics pipeline to identify peptides derived from the variable parts (CDR1, CDR2 and CDR3) of the antibodies. Boutz and coworkers [61] utilized high-throughput sequencing of the immunoglobulin variable domain (V gene) from a patient's B-cell population to construct a sample-specific antibody sequence database, enabling to apply shotgun-style MS proteomics to analyze serum antibodies. They developed a strategy to reduce false discovery and, at the same time, improve the accuracy of antibody identification by shotgun proteomics using high mass accuracy LC-MS/MS. High stringency filters were applied to groups of peptide-spectral matches rather than individual PSMs (peptide spectral matches). The use of filters on the mean precursor ion mass accuracy of peptide-spectral matches seems to be effective in distinguishing true and false identifications [61].

Shotgun proteomics, based on the DDA mode, is the most widely used MS approach for protein and PTM identification and quantification. PRM and SRM are specific, sensitive and accurate methods to quantify site-specific protein phosphorylations [65].

4.2. MS methods and techniques

High resolution mass spectrometric-based proteomics techniques are used for the detection of serum monoclonal antibodies [16]. High-end mass spectrometers with different fragmentation techniques, such as front-end and electron transfer dissociation and ultraviolet photodissociation (UVPD), can be used to increase the mass detection range and protein sequence coverage of antibodies [30] and open ways to identify specific antibodies present in serum or other biofluids.

When using MS to characterize and quantify MAbs, the variable region is the target; its uniqueness is compared to the endogenous polyclonal Ig repertoire obtained by NGS. The LC-MS miRAMM (monoclonal Ig rapid accurate mass measurement) technique allows simultaneous quantification of multiple MAbs. Clinical MS-based assays rely on immunoassays to quantify the specific MAbs. Still, MS-based clinical assays have distinct advantages over immunoassays because they do not necessarily rely on MAb-specific reagents, e.g. recombinant antigens and anti-idiotypic antibodies [66].

5. Next-generation sequencing

Next-generation sequencing (NGS) enables identifying neoantigen candidates as potential biomarkers or therapeutic targets for the individual patient [67]. Detection of somatic mutations by high-throughput NGS combined with computational algorithms for MHC-peptide binding affinity prediction can be used to identify candidate neoantigens which can be efficiently predicted [68]. The most common application of NGS is the identification of single nucleotide polymorphisms [69,70]. It is difficult to align smaller genomes to wild type reference sequences, and therefore it is hard to detect splicing events in the viral quasi-species [71]. Analysis of viral evolution in relation to T-cell responses shows that mutations are highly dynamic, with multiple mutations arising within targeted epitopes [72]. The combination of MS and NGS to find antibodies specific for an antigen (e.g. neutralizing activity) is intriguing. We describe below two examples mentioned in literature.

5.1. Ebola

A study by Park and Coworkers [73] showed that amino-acid variations in an Ebola epidemic were enriched in the mucin-like domain of the virus envelope glycoprotein and that the mutations occurred in the epitopes targeted by antibodies. B-cell receptor repertoire sequencing of sorted naïve and antigen-experienced B-cell populations can efficiently track B-cell clones among different tissues and blood. The study gives insight into central tolerance mechanisms in various diseases [74–76]. The advent of exome and whole-genome sequencing of patients with inherited disorders of B-cell development and function makes it possible to identify coding and noncoding variations that influence B-cell development [77,78]. Yadav and Coworkers [58] defined a strategy to identify neo-epitopes by combining whole-exome/transcriptome sequencing and MS to predict

MHC-I peptide immunogenicity. The identification of epitopes is essential to understand and manipulate CD8 T-cell immune response [79]. The possibility to detect changed phosphorylation moieties on proteins generates detection of antibodies specific for these modifications. Ivanov and coworkers [80] identified multiple phosphorylation sites in structural proteins of the Ebola virus (EBOV) and differences in phosphorylation profiles of EBOV particles, showing that phosphorylation of VP35 Thr-210 is necessary for viral polymerase activity and for interaction with nucleoprotein (NP). EBOV-neutralizing antibodies combining the IGHV3-15/IGLV1-40 immunoglobulin gene segments were identified in EBOV vaccines [81]. Park and coworkers [73] showed that genomes from three samples share a threonine-to-alanine mutation at amino-acid position 485, a position that is conserved among all members of the EBOV genus. Two of these samples have multiple mutations within a single B cell epitope in glycoprotein (GP). All EBOV structural proteins were detected with MS with high coverage, along phosphopeptides. A study by Khurana and coworkers [82] demonstrated that antibodies targeting antigenic sites had EBOV neutralizing activity in vitro and this indicated an important role for the C terminus of glycoprotein 1 (GP1) and a very conserved site in GP2. The combination of partly sequencing of specific parts in the complementary regions and the available data of antibodies obtained by NGS of B cells makes it a viable route for detecting autoantibodies in biofluids via antigen binding techniques.

5.2. HIV-1

A protruding heavy-chain third-complementarity-determining region (CDR H3 loop) in the antigen-combining surface is a feature of the monoclonal antibody 2909. This protrusion is also seen in the PG16 crystal and PG9 structure of strain-specific neutralizing antibodies. Although the structural motifs representing CDR H3 are found in other antibody structures, the highly solvent-exposed nature of this motif in these quaternary-specific CDR3 seems to be rare. Both monoclonal antibody 2909 and PG16 depend highly on the amino acid residue at position 160 in the V2 loop. A mutation at position 160 (N160K) in HIV-1 infectious molecular clone SF162 can result in pseudovirus switching and change monoclonal antibody 2909 and PG16 sensitivities. Therefore, the monoclonal antibodies 2909 and PG16 recognize different immunotypes of the same epitope, since monoclonal antibody 2909 does not neutralize broadly due to the N160K amino-acid substitution. The structural comparison of monoclonal antibody 2909 and PG16 shows that these antibodies use protruding CDR H3 structures, differentially shaped and sulfonated as could be observed by MS and possibly different heavy and light chain orientation is present to recognize a similar quaternary-specific epitope (mono-specificities in an oligo-clonal context) [83]. So not only chemical structures, but also how the shapes of these chemical structures are organized, generates the specific response of the immune system. Moreover, phosphorylation sites have been identified in all viral structural proteins [80] and could be part of such observations. Detecting these antibodies from serum combined with NGS still needs attention but is technically possible and opens ways to potentially produce neutralizing antibodies (Figure 3).

The identification of specific clonal expansions in immune sera for the production of monoclonal antibodies can be combined with NGS sequencing of B cells. By combining these two omics technologies, partial information of CDR regions including post-translational modifications can be obtained by MS and can subsequently be searched in NGS immunoglobulin data. In this way, antigen-specific antibodies from a specific clone can be identified [15].

6. Expert Opinion

Neoantigens normally are expected to arise from amino-acid mutation, but it has become more and more clear that also post-translational modification can trigger immune responses, including the production of antibodies. The analysis of autoantibodies might be not only a function of elevated concentrations of amino-acid mutated antigen, but also of the three-dimensional chemical structure. Moreover, post-translational modification can trigger autoantibody generation.

Mass spectrometry progresses rapidly in possibilities to screen for changes in post-translational modifications and their identification in cancer tissue and autoimmune diseases. Ideas are generated to isolate autoantibodies using these modified proteins and corresponding phosphopeptides as means to sequence these specific autoantibodies. The combination of NGS and MS is interesting because it provides opportunity to select relevant antibody sequences from the NGS reads using information of the variable part of the autoantibody that is enriched by the antigen. This approach is rather new, made possible by more and more advanced high resolution MS and sample preparation techniques including ion mobility and FAIMS separations in high-resolution mass spectrometers. These latter techniques improve the feasibilities to sequence in more and more depth autoantibodies.

6.1. Strengths

Combinations of orthogonal techniques such as MS and NGS give plenty of possibilities to select relevant antibodies in patient sera. The applications can be in the fields of cancer and autoimmune diseases. The knowledge to produce antibodies on an industrial scale is widely applied by biopharmaceutical companies that produce antibody–based biologicals. The process is described in literature, and therefore it is possible to use the knowledge in different research fields and in clinical settings.

6.2. Weaknesses

The sequencing of variable parts, especially regarding the CDR3 is not very well developed. Although more and more publications show the feasibility of full sequencing, examples of full sequencing of the CDR3 from patient sera are absent or extremely rare. One of the reasons is that the CDR3 contains less lysine and arginine moieties and most often starts with a sequence of ….CAR …. This results in the situation that the constant part is missing in tryptic peptides of CDR3 and alignment is difficult, therefore. The use of other enzymes might help to sequence CDR3 in higher coverage, but this has not yet been reported in literature.

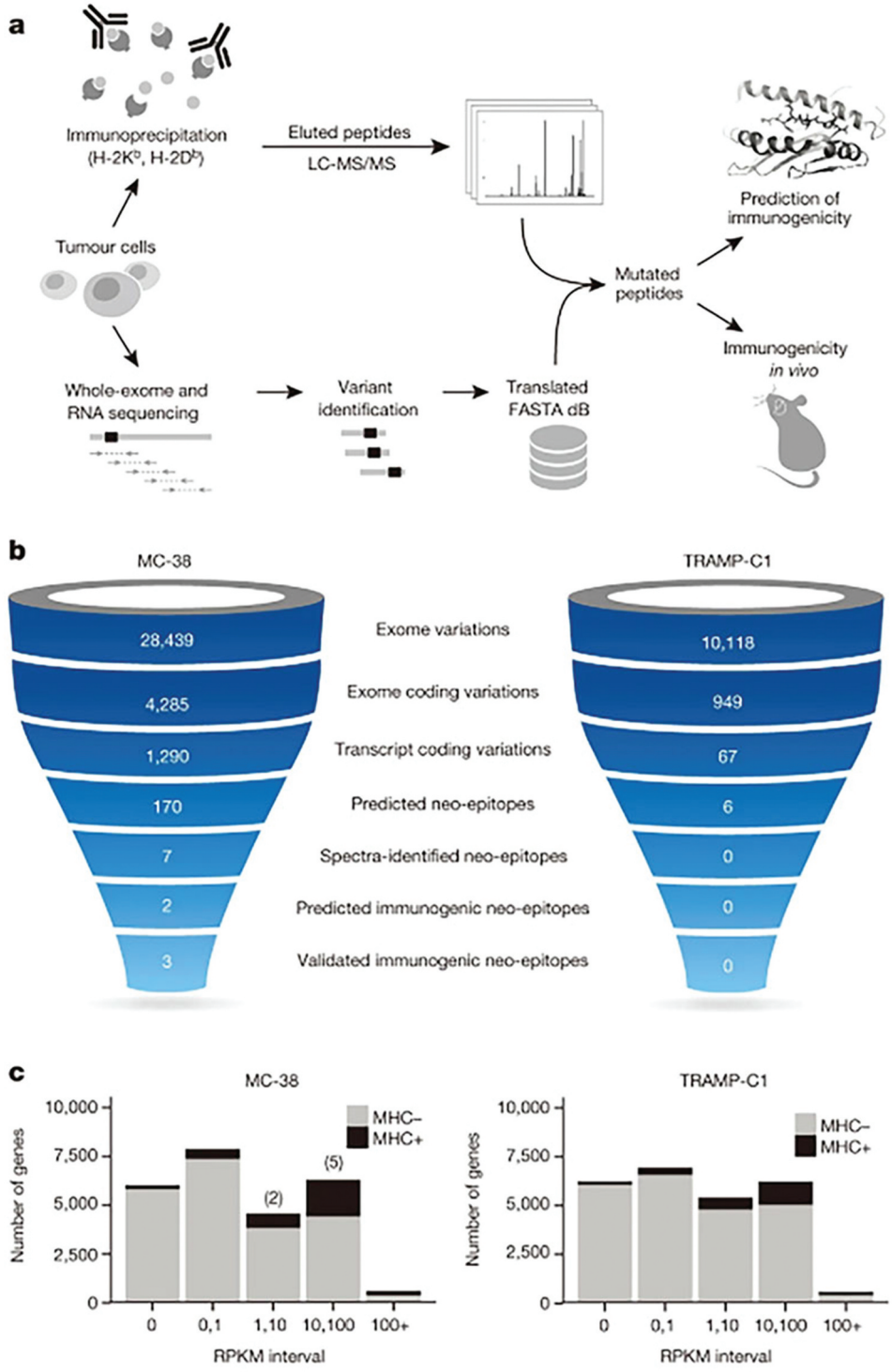

Figure 3. Schematic approach for identification of mutated MHC1-presented peptides using sequencing in combination with mass spectrometry. This approach can potentially be used to sequence the serum antibodies specific for these mutated peptides by sequencing and mass spectrometry (Copyright, Yadav et al. [78]).

6.3. Opportunities

The opportunities are enormous. If a simple pipeline for antibody selection from patient serum could be anticipated, this can open ways for diagnostics and development, and the production of new antibody medication.

6.4. Threats

The complexity of antibodies in serum is large, and tackling this complexity needs improvement in mass spectrometry and sample preparation. However, this is a pure technical challenge that can be overcome in the near future; and there are no theoretical reasons why this could not be realized.

6.5. Perspectives

Expensive antibody-based biologicals are developed in a relatively fast way. At the moment, roughly 25 antibody-based biologicals are used in the clinic. The possibility to find relevant antibodies in a relatively easy way opens ways to produce new medication cheaper and more focused on the individual patient. In addition, selecting antibodies of human origin would prevent specific side effects. Developing smart ways to produce these antibodies for cancer and infections will have a high social and economic impact.

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Glossary

AAbs	anti-cytokine autoantibodies
AQP4	aquaporin 4
CDR	complementary determining region
DDA	data-dependent acquisition
DIA	data-independent acquisition
EBOV	Ebola virus
FAIMs	field asymmetric waveform ion mobility spectrometry
FRET	fluorescence resonance energy transfer
GABAAR	y-aminobutyric acid receptor-A
GBM	glioblastoma
GP	glycoprotein
HLA	human leukocyte antigen
IP	immunoprecipitation
LC-MS	liquid chromatography-mass spectrometry
MAbs	monoclonal antibodies
miRAMM	monoclonal Ig rapid accurate mass measurement
MHC	major histocompatability complex
MRM	multiple reaction monitoring
MS	mass spectrometry
NGS	next-generation sequencing
NMDAR	N-methyl-D-aspartate receptor
NP	nucleoprotein
NR1	metabotropic glutamate receptor 1 (mGluR1)
PRM	parallel reaction monitoring
PSM	peptide spectral matches
PTM	post-translational modification
SERPA	serological proteome analysis
SRM	selected reaction monitoring
SWATH	sequential window acquisition of theoretical mass spectra
TAAs	tumor-associated antigens
TCR	T-cell receptor
UVPD	ultraviolet photodissociation

Declaration of interest

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

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