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Strategies for the Identification of T Cell–Recognized Tumor Antigens in Hematological Malignancies for Improved Graft-versus-Tumor Responses after Allogeneic Blood and Marrow Transplantation

Jenny Zilberberg[*], **Rena Feinman**, and **Robert Korngold**

Research Department and John Theurer Cancer Center, Hackensack University Medical Center, Hackensack, New Jersey

Abstract

Allogeneic blood and marrow transplantation (allo-BMT) is an effective immunotherapeutic treatment that can provide partial or complete remission for patients with hematological malignancies. Mature donor T cells in the donor inoculum play a central role in mediating graft-versus-tumor (GVT) responses by destroying residual tumor cells that persist after conditioning regimens. Alloreactivity towards minor histocompatibility antigens (miHA), which are varied tissue-related self-peptides presented in the context of major histocompatibility complex (MHC) molecules on recipient cells, some of which may be shared on tumor cells, is a dominant factor for the development of GVT. Potentially, GVT can also be directed to tumor-associated antigens or tumor-specific antigens that are more specific to the tumor cells themselves. The full exploitation of allo-BMT, however, is greatly limited by the development of graft-versus-host disease (GVHD), which is mediated by the donor T cell response against the miHA expressed in the recipient's cells of the intestine, skin, and liver. Because of the significance of GVT and GVHD responses in determining the clinical outcome of patients, miHA and tumor antigens have been intensively studied, and one active immunotherapeutic approach to separate these two responses has been cancer vaccination after allo-BMT. The combination of these two strategies has an advantage over vaccination of the patient without allo-BMT because his or her immune system has already been exposed and rendered unresponsive to the tumor antigens. The conditioning for allo-BMT eliminates the patient's existing immune system, including regulatory elements, and provides a more permissive environment for the newly developing donor immune compartment to selectively target the malignant cells. Utilizing recent technological advances, the identities of many human miHA and tumor antigenic peptides have been defined and are currently being evaluated in clinical and basic immunological studies for their ability to produce effective T cell responses. The first step towards this goal is the identification of targetable tumor antigens. In this review, we will highlight some of the technologies currently used to identify tumor antigens and anti-tumor T cell clones in hematological malignancies.

[*]Correspondence and reprint requests: Jenny Zilberberg, PhD, Research Department, Hackensack University Medical Center, 40 Prospect Avenue, Hackensack, NJ 07601, JZilberberg@HackensackUMC.org (J. Zilberberg).

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Keywords

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INTRODUCTION

Adoptive T cell therapy in the form of allogeneic blood and marrow transplantation (allo-BMT) has proven to be one of the few curative treatments for a number of drug-resistant hematological malignancies [1,2]. To date, the gold standard of immunotherapy used in the treatment of patients with acute myeloid leukemia (AML), lymphoma, chronic lymphocytic leukemia (CLL), chronic myeloid leukemia (CML), and multiple myeloma (MM) is the administration of donor lymphocytes along with, or at some time after, allo-BMT (in the form of donor lymphocyte infusions) [3]. The broad donor-derived $CD4^+$ and $CD8^+$ T cell repertoire targeting a diversity of undefined (allogeneic) tumor antigens is exploited in this setting [1,3,4].

Allo-BMT permits partial or complete remission in a significant percentage of patients. Although mature donor T cells in the donor inoculum facilitate patient immune reconstitution and mediate graft-versus-tumor (GVT) responses by destroying residual tumor cells that persist after conditioning regimens [5–7], the full exploitation of this clinical intervention is greatly limited by the development of graft-versus-host disease (GVHD). This primary complication of allo-BMT is due to the donor T cell allogeneic response against tissue antigens in the intestine, skin, and liver, which may or may not be shared by tumor cells [8–10]. Therefore, even in a fully major histocompatibility complex (MHC)-matched (in humans, HLA-matched) transplantation settings, the alloreactivity towards recipient cells is the key factor for the development of both GVT and GVHD effects. Unfortunately, the uncoupling of these two events to improve transplantation outcomes has yet to be achieved in a consistent and efficient manner. GVT can also be directed to tumor-specific target antigens that are not expressed by normal host tissues. Therefore, to reduce the development of GVHD and potentiate the GVT response, one active immune approach in the clinic has been the use of cancer vaccination after allo-BMT [11]. This combined strategy has an advantage over mere vaccination of the patient without allo-BMT, for the immune system of the patient has already been exposed and, hence, has become unresponsive to the tumor antigens, whereas the newly developing donor immune compartment can be educated to selectively target malignant cells. The first step towards this goal is the identification of targetable tumor antigens [12,13]. In this review, we will highlight some of the technologies currently used to identify tumor antigens and antitumor T cell clones in hematological malignancies.

CLASSIFICATION OF TUMOR ANTIGENS

Tumor antigens are classified according to their distribution as tumor-specific antigens (TSA), which are only expressed by the tumor, or as tumor-associated antigens (TAA), which can also be found in other normal cell types [11,13–16]. Unique tumor antigens, on the other hand, are those of patient-restricted expression whereas shared antigens are

commonly present across various samples of the same histologic subtype of malignancy and on different tumor types, but not in normal tissues, except for testis and placenta. Although shared antigens constitute an ideal group to develop broadly applicable cancer vaccines, the identification of unique TSA has the potential to develop into highly effective personalized immunotherapeutic interventions. Other classifications that stem from the combinations of these different types of antigens are as follows.

Unique TSA

These antigens result from somatic point mutations induced by carcinogens and, therefore, occur in a single tumor of one patient; thus, they represent a bone fide TSA not capable of being expressed by any normal tissue. Importantly, unique TSA have the potential to elicit more effective antitumor vaccine responses than shared antigens because of their resistance to immunoselection, particularly if the mutated protein is critical for the preservation of neoplastic cells. For a thorough review of identified unique tumor antigens, see Parmiani et al. [14].

Shared TSA

These antigens are expressed in different tumors but not in healthy tissues. The most prominent antigens among this group are the cancer-testis family of antigens including MAGE [17,18], BAGE, LAGE, GAGE, and NY-ESO-1 [19–22], which in normal tissues are restricted only to the testis and placenta but can be found in MM, breast, ovarian, and head and neck cancers [23,24].

Shared TAA

This category of antigens, although not tumor specific, is overexpressed in different types of tumors. Examples of these antigens are human telomerase reverse transcriptase [25], survivin [26,27], proteinase-3 [28,29], Wilms tumor gene-encoded transcription factor-1 (WT1) [30,31], mucin-1 [32], and preferentially expressed antigen of melanoma (PRAME) [33], which can be found only at very low levels in healthy tissues, such as the adrenal glands, ovaries, and endometrium. Shared TAA have been considered as potential targets for cancer immunotherapy. Arai et al. [34] demonstrated that $CD8^+$ cytotoxic T cell (CTL) clones specific for human telomerase reverse transcriptase peptides exerted cytotoxicity against leukemia cells in an HLA-A24–restricted manner, while sparing HLA-$A24^-$ leukemia cells or HLA-$A24^-$normal cells. PRAME is known to contain at least 4 different HLA-A*0201-restricted epitopes ($PRA_{100–108}$, $PRA_{142–151}$, $PRA_{300–309}$, and $PRA_{425–433}$) [35] recognized by CTLs [36]. Reports of PRAME antigen expression range from 47% to 70% of AML patients [37,38]. Proteinase-3 is also overexpressed in AML and CML [28,39,40] and WT1 is overtly present in several different types of leukemia [41]. In fact, WT1 ranked in the top 20 antigens with suggestive high therapeutic functionality according to the National Cancer Institute report on the prioritization of cancer antigens for acceleration of translational research [42]. WT1 is also among the most advanced targets for AML immunotherapy, as reflected by the relatively large number of WT1-targeted vaccine trials for AML patients [43]. In those patients, multiple WT1 CTL epitopes have been recognized as immunogenic (including $WT1_{37–45}$, $WT11_{26–134}$, $WT1_{187–195}$, and $WT1_{235–243}$) [43]. WT1 was also found to induce WT1-specific $CD4^+$ helper T cell

immunity in patients with AML through active immunization [44]. Mucin-1, an epithelial mucin present in a number of solid tumors, can also be found in MM cell lines and primary tumors [45–49]. For an in-depth review of tumor antigens recognized by T cells, and in particular those pertinent to hematological malignancies, see Novellino et al. [50], Borrello et al. [11], and Anguille et al. [41].

Minor Histocompatibility Antigens (miHA)

In the context of an MHC-matched allo-BMT, alloreactive $CD8^+$ and $CD4^+$ donor T cells can also be directed at non–MHC-encoded polymorphic peptides known as miHA, presented by both MHC class I and class II molecules [51,52] on allogeneic host cells. Many of these miHA are encoded by allelic genes that can differ between patient and donor because of single nucleotide polymorphisms (SNPs). The target molecules involved in the GVT response can be any of the TSA or TAA described above, as well as tissue- and tumor-specific miHA. Donor T cells have the advantage of recognizing all of these target antigens in an immunologically permissive environment, whereas in the patient, T cell responses are generated only against TSA and TAA and are subject to tolerizing mechanisms. Conceptually, in the allo-BMT setting, all tumor antigens presented in the context of MHC that are recognized by donor but not host T cells are miHA.

In 1978, Korngold and Sprent demonstrated that transfer of bone marrow cells containing T cells into lethally irradiated MHC-matched recipient mice caused GVHD, suggesting that miHAs were the main target for eliciting this disease [53]. The use of miHA as tumor targets after allo-BMT derives from the notion that some of these antigens are exclusively expressed on normal and malignant host hematopoietic cells permitting, hypothetically, the separation of GVT and GVHD pathological responses [54,55]. In some cases, miHA can also be considered as TAA in that they may be overexpressed in tumor cells in comparison to the rest of the hematopoietic compartment. The contribution of miHA in GVT has been evaluated mainly by isolating CTL with tumor lytic capability and studying their effect on normal host hematopoietic cells or nonhematopoietic fibroblast cells. Later on in this review, we will discuss work by the present authors using other technological approaches aimed at identifying and separating tumor versus tissue reactive T cell clones using spectratype analysis and T cell receptor (TCR) sequencing.

HA-1 and HA-2 constitute the first 2 miHA identified to be solely expressed on hematopoietic cells [54,56], including progenitor cells. Both of these antigens have been found to be expressed in all leukemia and MM cells [57,58]. Subsequently, a number of other hematopoietic miHA have been identified, including HB-1, an acute B-lymphoblastoid-leukemia-related antigen [59], and proliferation-associated nuclear element 1 gene [60,61], a B cell CLL–related antigen. Although ubiquitous, the ATP-dependent, interferon-responsive gene (ADIR) is also highly expressed in activated hematopoietic cells, including MM and various solid tumors [62]. LRH1, encoded by the P2X5 gene, is hematopoietic specific and expressed in leukemic cells and their $CD34^+$ progenitors [63]. A number of other miHA encoded by ubiquitously expressed genes appear to be preferentially expressed in activated hematopoietic cells and malignant cells (see Table 1 in references [13,64]). A retrospective analysis on the impact of a panel of 17 immunogenic miHAs

including HA-1, HA-2, HA-8, ATP-dependent, interferon-responsive, proliferation-associated nuclear element 1, LRH1, SP110, ECGF, and ACC2, in patients who received a partial T cell–depleted HLA-identical allo-BMT, revealed that in sibling transplantations, mismatches in one or more of the studied autosomal-encoded miHA resulted in an improved relapse-free survival rate, especially in MM patients [65].

Of note, mismatches in individual miHA, including HA-1, HA-2, and HA-8, have been associated with increased GVHD occurrence and lower relapse rates [66], although other studies could not confirm these results [67]. The adoptive transfer of miHA-specific CTLs selected on the basis of recognition of recipient hematopoietic cells but not skin fibroblasts has also unexpectedly been associated with GVHD. Likewise, in a murine model of BMT, infusion of tumor-specific CTLs identified by CDR3-size spectratype analysis was shown to induce a significant GVT response, but the same tumor-reactive Vβ family, which initially showed no hematopoietic alloreactivity was ultimately the causal entity of gut pathology in recipient mice, when administered at higher dosages [68]. Taken together, the results from these clinical trials and murine models suggest that responses to target tissue–related miHA are complex and may vary not only amongst different individuals but also between tissue types.

In 2009, The Translational Research Working Group of the National Cancer Institute specified a number of criteria for determining the suitability of a given tumor antigen for therapeutic application. The following characteristics were evaluated and prioritized in descending order to determine the “ideal” cancer antigen: (1) therapeutic function, (2) immunogenicity, (3) role of the antigen in oncogenicity, (4) specificity, (5) expression level and percent of antigen-positive cells, (6) stem cell expression, (7) number of patients with antigen-positive cancers, (8) number of antigenic epitopes, and (9) cellular location of antigen expression. Although there was no assessment of miHA using this prioritization analysis, it was concluded that none of the 75 tumor antigens studied fit all the criteria of the “ideal” cancer antigen. Nevertheless, 46 antigens were reported as immunogenic in clinical trials and 20 antigens had suggestive clinical efficacy in the “therapeutic function” category [42].

Approaches to Identify Tumor Antigens

The characterization of a tumor-specific CTL epitope from the human melanoma antigen MAGE-1 was reported in 1992 by Boon et al. [17,18]. Since this discovery, a number of technological advances have led to a great increase in the number of recognized TAA. The first strategy used to identify tumor antigens was peptide elution. This approach is based on high-liquid performance chromatography paired with mass spectrometric sequencing of the miHA peptide eluted from the cell surface of MHC molecules [62,69–72]. This technique, despite being very successful, has only yielded positive results in identifying HLA class I–presented miHA. In addition to peptide elution, forward or “T cell-to-antigen”–based strategy and reverse immunology are two major strategies that have been recently used in the identification of tumor antigens and $CD8^+$ and $CD4^+$ T cell epitopes contained in these tumor-specific proteins.

Forward Immunology Methods

Broadly speaking, these methods are characterized by the isolation of tumor-reactive T cells generated from an autologous peripheral blood mononuclear cell coculture with tumor cells or from individuals who underwent transplantation demonstrating a clinical response to donor lymphocyte infusions after allo-BMT, followed by the subsequent identification of the antigens that elicited a T cell response. For a review of immunogenic scenarios conducive for the identification of tumor antigens in this forward manner, see Kawakami et al. [73]. Forward immunological techniques and their variations can be applied to identify miHA presented in the context of either MHC-I or MHC-II.

After the isolation and expansion of CTLs, these lines can be used to isolate the tumor-specific cDNA that encodes the recognized CTL epitope by screening of cDNA expression libraries (Figure 1) derived from the tumor or Epstein-Barr virus (EBV)-transformed lymphoblastoid cell lines from members of CEPH families (Jean Dausset-Centre d'Etude du Polymorphisme Humain [CEPH]; http://www.cephb.fr/en/cephdb/), followed by genetic linkage analysis [74]. Genetic linkage analysis identifies the genomic locus of the miHA by pair-wise correlation of the miHA phenotype of large CEPH families with thousands of genetic markers identified in their genomes. Alternatively, as miHA are associated with common polymorphisms within the human population, they can also be identified using genetic association studies. This alternative genetic approach uses the extensive linkage disequilibrium found within the human genome to efficiently localize the target loci, based on recent advances of large-scale genotyping technologies and the assets of the International HapMap Project (www.hapmap.org). Using this approach, Spaapen et al. [75] identified a CD19-encoded miHA, presented by MHC class II molecules by correlating the miHA phenotypes of 23 CEPH individuals with the SNP genotypes derived from HapMap. Subsequently, the authors also identified the miHA recognized by the 1GF5 $CD4^+$ T cell clone, isolated from a patient with MM undergoing a strong GVT response associated with acute GVHD, using another derivate of forward techniques, a zygosity-genotype correlation analysis with embedded HapMap SNP genotypes from the "Utah residents with ancestry from northern and western Europe" (known as CEU population) [76].

Whole genome association scans is another forward approach that allows for high-throughput identification of miHA [77], where third-party EBV-B cell lines selected for their coexpression of pertinent HLA molecules, are genotyped for more than 1 million SNPs. The miHA are then identified by analysis of association between T cell recognition of these EBV-transformed lymphoblastoid cell lines and individual SNP genotypes measured in these lines [77,78], while recognition of nonhematopoietic fibroblasts and other stromal cells is ruled out to ensure that the T cell clone exclusively reacts against the hematopoietic (ie, of tumor origin) compartment [75].

All of these approaches, however, are limited by the low affinity interaction of the TCR with their specific MHC-peptide complex and the technical difficulties associated with library cloning platforms. Although yet to be applied to the identification of new miHA, Siewert et al. identified target antigens of $CD8^+$ T cells using combinatorial libraries coding for short peptides and a single-cell detection system with HLA-A*0201 MHC molecules presenting influenza matrix protein (flu58–66) peptides [79]. The MHC class I cDNA was

cotransfected along with a plasmid-coded combinatorial nonamer peptide library into COS-7 cells, which allowed antigen processing and presentation for T cell recognition. For screening, a reporter T cell hybridoma cell line was cotransfected with the specific CD8 TCR α and β chains and the super green fluorescence protein (sGFP) reporter gene under the transcriptional control of the nuclear factor of activated T cells enhancer. To identify and isolate an antigenic peptide, a single nuclear factor of activated T cells–activated TCR expressing T cell hybridoma cell that recognized the correct antigenic peptide expressing COS-7 cell during coculture was subsequently isolated and the antigenic peptide plasmid was cloned to determine the precise sequence of the peptide [79].

Reverse Immunology Methods

An alternative strategy used in the identification of tumor antigens is reverse immunology, in which the prediction of miHA on hematopoietic cells constitute the starting point and peptide candidates are subsequently screened for their capacity to induce a T cell–specific response [80,81] (Figure 2). In silico analysis uses prediction algorithms, such as SYFPEITHI [82] (http://www.syfpeithi.de/), SNP-derived epitope prediction program [83], which is based on SYFPEITHI, “BIMAS” (http://bimas.dcrt.nih.gov/molbio/hla_bind/), and TEPITOPEpan [84,85] (http://www.biokdd.-fudan.edu.cn/Service/TEPITOPEpan/) to determine peptides with putative strong binding to HLA-I and HLA-II molecules. However, because peptide-HLA binding affinity and proteolytic cleavage also play key roles in determining the biological feasibility of miHA, the vast majority of T cell responses detected using this original reverse manner approach were directed against epitopes that are not naturally processed and presented [86,87]. Recent technological advances from the HLA-associated peptidome of hematopoietic cells by mass spectrometric analysis (HLA peptidomics) SNP databases, MHC-tetramer technology, and multiparametric flow cytometric analysis were instrumental in the identification of eluted peptide candidates that can undergo HLA-restricted processing and presentation [80]. Likewise, proteasomal cleavage and transporter associated with antigen processing transport efficiency has been combined with reverse computational approaches to optimize the selection of candidate epitopes [88]. Feldhahn et al. described a different approach for large-scale detection of tissue-specific miHA [89] that uses netMHCpan, a high-throughput computational method for quantitative predictions of peptide binding to any HLA-A and -B locus protein of known sequence [90].

Innovative technologies and reduced costs of genomic sequencing have opened the door for the identification of tumor-associated genes through whole exome sequencing. Recently, Rosenberg et al. at the National Cancer Institute developed a screening method to identify mutated gene products from patients’ tumors and their potential T cell epitopes that may be recognized by isolated tumor-infiltrated lymphocytes (TILs). Although originally tested in melanoma, this approach could be extrapolated to other cancers, including hematological malignancies. Initially, whole exome sequencing of the tumor was compared to normal patient’s DNA to identify somatic mutations. Candidate mutated T cell epitopes identified in silico using netMHCpan were subsequently synthesized in COS-7 cell lines that were stably transduced to express the appropriate HLA (as described above), and assayed for their recognition by TILs. The adoptive transfer of TILs to patients that were generated after

exposure to these identified dominant target epitopes mediated significant and durable tumor regression [91].

PRODUCTION OF BULK T CELLS AS A TOOL FOR DISCOVERY OF TAA

The TCR is a heterodimeric protein, comprising an α and a β chain, that recognizes antigens presented by MHC molecules. Diversity of the TCR repertoire allows the adaptive immune system to protect the body against a vast array of potential pathogens, such as cancer cells and allo-antigens. These chains are somatically rearranged from individual gene segments to create millions of different surface receptors, with the majority of T cells expressing a single productively rearranged TCR α and β chain allele. The identification of tumor-reactive T cells and their TCR usage responsible for mounting a significant anti-tumor response is necessary for the discovery of novel tumor antigens. The generation of a sufficient number of tumor reactive cells is critical for determining their cognate tumor antigens by forward immunological approaches and for their adoptive transfer into patients as immunotherapy.

Unfortunately, the duration of the in vitro selection and expansion of tumor reactive T cells as shown in Figure 1(A-1) frequently leads to T cell exhaustion, reducing their capacity to produce cytokines and proliferate to an extent that makes them unsuitable for use as a tool for antigen discovery. A more feasible approach to generate sufficient number of functional T cells for therapeutic transfer has been the production of TCR-transgenic T cells restricted to a particular tumor epitope [92,93]. This approach uses viral transfer of the genes encoding the TCR α and β chains of identified tumor-specific clones into primary T cells. The advantage of viral transduction facilitates the generation of large amounts of antigen-specific CTLs in several days rather than several weeks, and thereby bypasses the development of proliferative senescence and its concomitant decrease in T cell killing activity.

Vβ CDR-3 size spectratyping and TCR deep sequencing, two techniques aimed at determining the TCR repertoire usage, have opened the possibility of dissociating GVT and GVHD responses by identifying allo-reactive and unique tumor-reactive T cell clones. Our group and others have extensively used spectratyping to predict Vβ families with in vivo allo-reactive potential in both murine models of BMT and clinical samples [94–97]. Using this technique, we identified those families capable of mounting a strong anti-tumor response [68,98] that overlapped only moderately with the induction of GVHD [68]. In particular, our approach has focused on in vitro mixed lymphocyte cultures, which expose donor T cells to hematopoietic allo-antigens from patient-derived peripheral blood mononuclear cells after the conditioning regimen to identify expanding T cell clones [94]. We are currently including mixed lymphocyte cultures of donor T cells stimulated with patient-derived tumor cells (obtained before conditioning) to distinguish unique donor-patient antitumor responses (work in progress). Although spectratype results can be further used to identify the particular TCR clone that is likely driving the expansion of a Vβ family [99], the feasibility for the rapid identification of tumor or GVHD effector T cells is low because of lengthy cloning techniques and the random selection of a limited number of colonies. The advent of next-generation high-throughput TCR sequencing can generate a sequence of tens of millions of TCRs from a single sample within a few days. This methodology uses a multiplex-based PCR method to identify TCRβ chains from genomic

DNA, which in turn can be used to pinpoint tumor-reactive T cells [100–102]. Although the conditions for the identification of the TCRα chain are currently being optimized, this technology has the potential to transform the recognition of relevant TCR clones that can then be engineered into primary T cells for downstream immunotherapeutic use [103].

SUMMARY AND FUTURE DIRECTIONS

The development of clinically efficacious cancer vaccines relies on the identification of targetable tumor antigens. In this review, we summarized some of the major and newest technological advances currently employed in the discovery of these antigens, as well as their limitations. We emphasized the use of these techniques for the recognition of miHA, TAA, and TSA as tumor targets of hematological malignancies in the context of allo-BMT. In particular we reviewed forward methods, which primarily start with identifying the T cell clone(s) responsible for mediating antitumor responses, as well as various modalities of reverse immunological approaches in which prediction of potential antigenic epitopes are screened for their capability of inducing a physiologically plausible GVT reaction. As de novo donor-derived immune cells can be much more readily educated to attack tumor cells, the use of the post–allo-BMT setting offers a unique environment to take advantage of cancer vaccines. We also discussed the techniques for recognition, selection, and expansion of those tumor-specific T cell clones that are necessary to guide the discovery process of tumor antigens. We believe that the recognition of potential tumor antigens, currently being used to generate dendritic cell peptide-loaded vaccines against a particularly relevant miHA, TAA, or TSA [13,24,104], will be streamlined by the capability to sequence the TCR of the responding T cell and to generate bulk quantities of tumor-targeting TCR-transduced T cells for adoptive immunotherapy. The combination of vaccination and TCR engineering will likely be used synergistically in upcoming years to enhance the antitumor response of donor T cells after transplantation and to provide a durable tumor remission, if not complete eradication.

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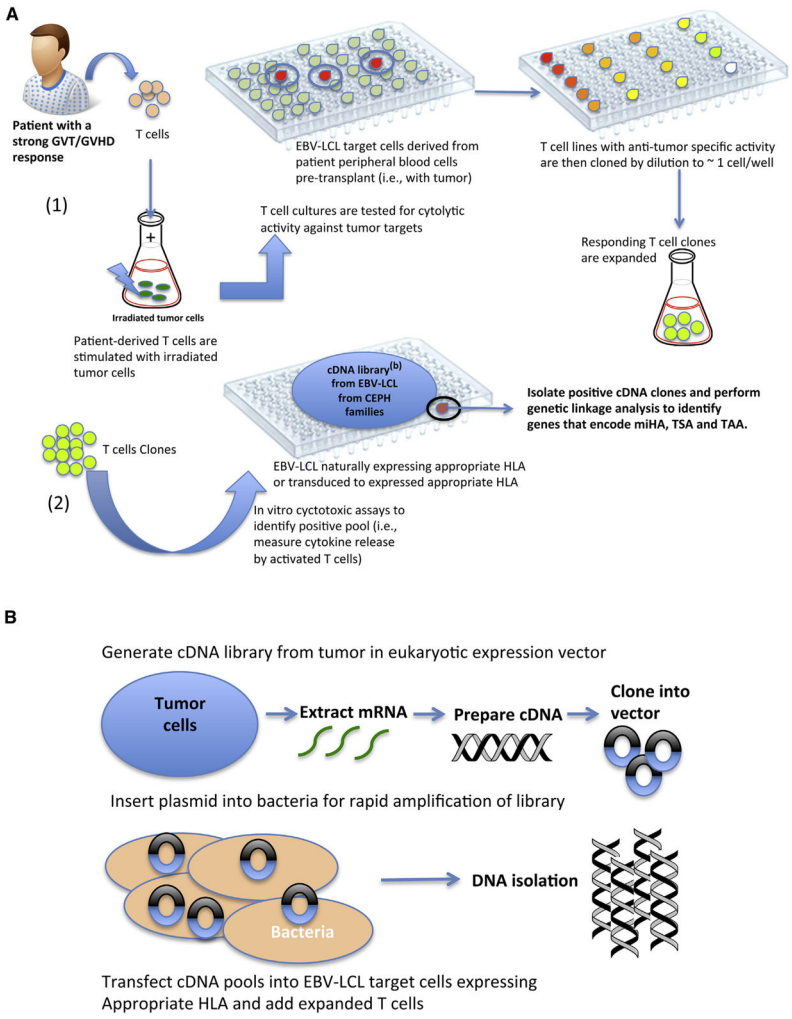

Figure 1.

Schematic representation of a forward immunology method for the identification of miHA as tumor targets. Panel (A-1): Identification and expansion of T cell clone with strong GVT/GVHD reactivity. Panel (A-2): The reactive T cell clone is then scanned against a cDNA library with EBV-LCL from CEPH families to identify the cDNA clone eliciting cytotoxic activity. Genetic linkage analysis is performed on this cDNA clone to identify genes encoding the potential tumor miHA. Panel (B): Preparation of cDNA library from patient-derived tumor cells or from EBV-LCL from CEPH families. Current methods for the production and expansion of T cell clones are discussed in more detail in the “Production of bulk T cells as a tool for discovery of TAA” section of this review.

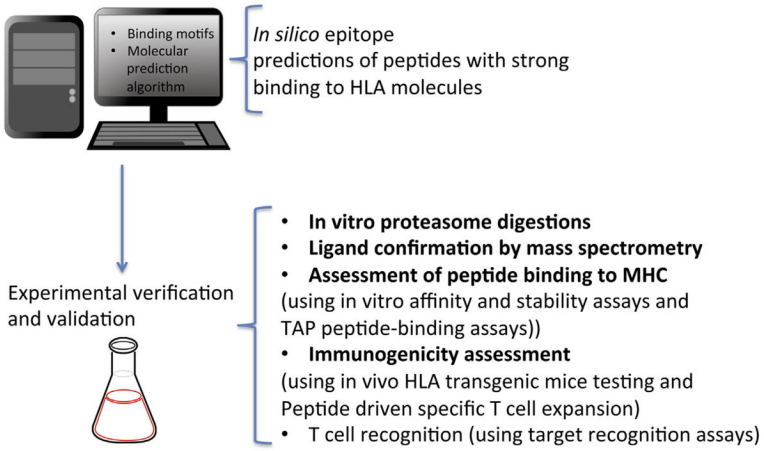

Figure 2.

Schematic representation of reversed immunological methods to determine miHA peptides. Steps for determining and validating miHA epitopes eliciting relevant antitumor T cell activity are depicted in this figure. A first phase of computational prediction determines a number of putative epitopes, which are then confirmed experimentally for their capacity to induce an in vivo T cell response.