Journal Pre-proof

Tumor antigenS heterogeneity and immune response-targeting neoantigens in breast cancer

Monica Benvenuto, Chiara Focaccetti, Valerio Izzi, Laura Masuelli, Andrea Modesti, Roberto Bei

PII: S1044-579X(19)30299-8

DOI: https://doi.org/10.1016/j.semcancer.2019.10.023

Reference: YSCBI 1711

To appear in: *Seminars in Cancer Biology*

Received Date: 30 September 2019

Accepted Date: 30 October 2019

Please cite this article as: Benvenuto M, Focaccetti C, Izzi V, Masuelli L, Modesti A, Bei R, Tumor antigenS heterogeneity and immune response-targeting neoantigens in breast cancer, *Seminars in Cancer Biology* (2019), doi: https://doi.org/10.1016/j.semcancer.2019.10.023

TUMOR ANTIGENS HETEROGENEITY AND IMMUNE RESPONSE-TARGETING NEOANTIGENS IN BREAST CANCER

Monica Benvenuto[a,b,1], Chiara Focaccetti[c,1], Valerio Izzi[d,1], Laura Masuelli[e], Andrea Modesti[a], Roberto Bei[a,*]

[a] Department of Clinical Sciences and Translational Medicine, University of Rome "Tor Vergata", Via Montpellier 1, 00133 Rome, Italy; modesti@med.uniroma2.it (A.M.); bei@med.uniroma2.it (R.B.)

[b] Saint Camillus International University of Health and Medical Sciences, via di Sant'Alessandro 8, 00131 Rome, Italy; monica.benvenuto@unicamillus.org

[c] Department of Human Science and Promotion of the Quality of Life, University San Raffaele Rome, Via di Val Cannuta 247, 00166 Rome, Italy; chiara.focaccetti@uniroma5.it

[d] Center for Cell-Matrix Research, Biocenter Oulu, Faculty of Biochemistry and Molecular Medicine, University of Oulu, Aapistie 7C, FI-90230, Oulu, Finland; valerio.izzi@oulu.fi

[e] Department of Experimental Medicine, University of Rome "Sapienza", Viale Regina Elena 324, 00161 Rome, Italy; laura.masuelli@uniroma1.it

[1] MB, CF and VI equally contributed to the work

*Corresponding author

E-mail address: bei@med.uniroma2.it

Full postal address: Department of Clinical Sciences and Translational Medicine, University of Rome "Tor Vergata", Via Montpellier 1, Rome, Italy

Tel: +39 0672596522

Abstract

Breast cancer is both the most common type of cancer and the most frequent cause of cancer mortality in women, mainly because of its heterogeneity and limited immunogenicity. The aim of specific active cancer immunotherapy is to stimulate the host's immune response against cancer cells directly using a vaccine platform carrying one or more tumor antigens. In particular, the ideal tumor antigen should be able to elicit T cell and B cell responses, be specific for the tumor and be expressed at high levels on cancer cells. Neoantigens are ideal targets for immunotherapy because they are exclusive to individual patient's tumors, are absent in healthy tissues and are not subject to immune tolerance mechanisms. Thus, neoantigens should generate a specific reaction towards tumors since they constitute the largest fraction of targets of tumor-infiltrating T cells.

In this review, we describe the technologies used for neoantigen discovery, the heterogeneity of neoantigens in breast cancer and recent studies of breast cancer immunotherapy targeting neoantigens.

Abbreviations

INDELs: insertions or deletions; **FS**: frameshift; **ORF**: open reading frame; **NGS**: next-generation sequencing; **WES**: whole exome sequencing; **TMG**: tandem minigene; **PPs**: peptide pools; **TILs**: Tumor Infiltrating Lymphocytes; **TNBCs**: Triple Negative Breast Cancer; **TCGA**: The Cancer Genome Atlas; **LNpos or LNneg**: lymph node metastasis positive or negative; **NSG**: NOD.SCID.gamma-c-null immunodeficient mice; **PDX**: patient-derived xenograft; **nsSNVs**: non-synonymous single nucleotide variants; **ASCs**: antibody-secreting cells; **SLP**: synthetic long peptide.

Keywords: Neoantigens, cancer vaccine, immune response, immunotherapy

Breast cancer tumorigenesis and classification

Breast cancer is both the most common type of cancer and the most frequent cause of cancer mortality in women [1], with a global burden of 2 088 849 new cases (11.6% of all new cancer cases) and 626 679 deaths (6.6% of all cancer deaths) in 2018 [2]. The most common presentation of breast cancer is invasive ductal carcinoma (50%-75% of patients), in which cancer cells spread from the milk duct into the periductal fibrous or fatty tissue, followed by invasive lobular carcinoma (5%-15% of patients), in which cancer cells originate from the milk-producing breast lobules and spread to the surrounding tissues and towards the duct. Mixed ductal/lobular carcinomas and other rarer forms make up the remainder of cases [3].

Various classification schemes exist for breast cancer; they are based on molecular findings and cytological subtypes, whole-transcriptome patterns, the presence or absence of inheritable cancer driver genes, etc. It is worth noting that the various classifications intersect the cell ontogeny of breast cancer at different levels and that no such scheme is unique or disconnected from the others; rather, they overlap to a variable extent to better describe the heterogeneity intrinsic to breast cancer and its relationship with immunogenicity and patient survival [4-6].

Typically, though rare mesenchymal forms exist [7] breast adenocarcinomas originate from the luminal or basal (myoepithelial) cell layers of either milk ducts or lobules and evolve through hyperplastic stages (typical and atypical hyperplasia) before turning into localized or invasive carcinomas [8-10]. The presence of different subtypes (luminal and basal) and further subdivisions within the luminal tumors (luminal A/B) are likely to reflect the effect of pre-existing predisposing mutations, the acquisition of *de novo* mutations on cell-of-origin patterns and the stepwise process of cell specification in the breast epithelium [11]. Thus, from the initial migratory stem cell, first, the basal cells emerge, which then further differentiate into luminal B types and, last, into luminal A cell types [12]. In parallel, tumors originating from the earliest undifferentiated cells form high-grade preinvasive neoplasms, which are likely the precursors to the more differentiated basal and luminal subtype tumors [3, 11, 12]. Additionally, tumors can also originate from dedifferentiated somatic cells and from committed precursors, giving rise to either basal, luminal and mixed or luminal A neoplasms [11].

Another major classification system and by far the most successful in predicting the clinical outcome of patients is based on the presence/absence of either a hormone receptor (HR, either the progesterone receptor or estrogen receptor – PR or ER, respectively) or the human epidermal growth factor receptor 2 (ERBB2, formerly HER2 or HER2/neu) on the surfaces of the neoplastic cells [3]. Accordingly, tumors with HR immunoreactivity (typically to ERα) but no ERBB2 reactivity are classified as HR^+ and account for approximately 70% of all breast cancer cases. Conversely, tumors with positive or negative HR immunoreactivity and strong ERBB2 reactivity are classified as $ERBB2^+$ and make up approximately 15% of all cases, with a third type of breast cancer showing no immunoreactivity at all (triple-negative breast cancers, TNBCs) forming the remaining 15% of cases [3]. Though different in their molecular underpinnings, the deleterious outcomes of HR and ERBB2 signaling in breast cancer are similar and, most important, clinically actionable. In both HR and ERBB2 cases, oncogenic growth is triggered by excessive receptor activity (due to the genetic and epigenetic overexpression of ER, PR and ERBB2, alterations in their posttranslational modifications and,

only for ERBB2, gene amplification); thus, inhibiting such signaling has a strong impact on the chances for survival [3, 11, 13]. The origins of TNBCs are more obscure, are likely heterogeneous, and include several inheritable or *de novo* mutations affecting signaling pathways (PI3K/AKT/mTOR, JAK/STAT or NOTCH), genome integrity controllers (BRCA1 or BRCA2), cell cycle-related proteins (TP53), etc. [14, 15]. Identifying a unique receptor and the signaling pathway on which it acts as the oncogenic driver of a neoplasm is, of course, an important factor for patient management. This finding will enable targeted therapeutic approaches that generally rely upon inhibiting intrinsic estrogen signaling with an ER antagonist (*e.g.*, tamoxifen) for HR^+ tumors and blocking ERBB2 signaling with a monoclonal antibody (*e.g.*, trastuzumab) for $ERBB2^+$ tumors [3]. The outlook for TNBCs is, unfortunately, far worse, although several therapies are currently in use, including antiangiogenic drugs, poly (ADP-ribose) polymerase (PARP) inhibitors and various chemotherapy combinations [3, 11, 12, 14].

In addition to the oncogenic mechanisms highlighted by the HR/ERBB2/TNBC classification, another significant element of risk for the neoplastic conversion of normal mammary cells is the presence of inheritable mutations in various genes (BRCA1, BRCA2, TP53, PTEN, ATM, CHEK2, and HRAS1), which is evidenced by family pedigrees and a history of the specific neoplasm in a first-degree relative with a history of breast cancer [1]; however, inheritable patterns only characterize 20%–25% of all affected families, and their etiological contribution to carcinogenesis remains the subject of investigation [1].

Tumor antigens and neoantigens in diagnosis and therapy

The aim of specific active cancer immunotherapy is to stimulate the host's immune response against cancer cells through direct immune system stimulation using a platform carrying one or more tumor antigens (TAs). The outcome of the cancer vaccine should be the recognition of the cancer cell-expressed TAs by specific immune effectors. Thus, the identification of TAs is crucial for the development of effective cancer vaccines [16]. MAGE-1 was the first TA recognized by the cytolytic T lymphocytes identified in human melanoma [17]. Indeed, the recognition of TAs by T cells in cancer patients is the first step for the identification of a putative TA. In particular, the ideal TA should possess several properties [18]. First, it should be immunogenic, i.e., it should be able to elicit T cell and B cell responses. Thus, effective antigen processing and presentation on the cell surface by the major histocompatibility complex (MHC; also known as the human leukocyte antigen, HLA, in humans) are crucial. In addition, a TA should be specific for the tumor and expressed at high levels on cancer cells [18, 19]. The current classification of TAs includes shared and unique TAs, which are further divided according to the HLA molecule into class I and class II HLA-restricted TAs [20, 21].

Angelova *et al.* [22], aware that understanding the dimension and the variability of the repertoire of tumor antigens is of utmost importance for the development of personalized immunotherapies, coined the term “cancer antigenome” in colorectal tumors. This definition can be easily translated to all tumor types. It applies to two categories of antigens generated during tumor onset and growth: nonmutated cancer germ line antigens and neoantigens arising from somatic mutations. In fact, an updated and detailed description of the entirety of the antigens comprising a tumor mass was given by Hollingsworth and Jansen [23] and

divides the molecules into two main categories: tumor-associated antigens (TAAs) and tumor-specific antigens (TSAs) (**Fig. 1**).

TAAs are *self*-proteins that are not cancer-specific but are shared between different types of tumors and are commonly expressed and "public" among patients. Induction of the T-cell response against these antigens is possible despite central tolerance, but it produces significant toxicity due to the attack on healthy tissues [24-27].

TAAs can be divided into several categories according to their pattern of expression in normal tissues. Indeed, shared TAAs include overexpressed antigens, tissue differentiation antigens and tumor germline antigens [16, 18, 21]. Overexpressed antigens are normal proteins with low expression in normal cells and high expression in cancer cells. Examples of antigens in this category are HER2/*neu* [28], human telomerase reverse transcriptase (hTERT) [29], TP53 [30], mesothelin [31], survivin [32], and livin [33]. Tissue differentiation antigens are found in tumors and in the normal tissue from which the tumor originates. These TAAs are normal proteins expressed in specific tissues or at specific differentiation stages or are oncofetal proteins with increased expression in adult cancer tissues. Examples include Gp100 [34], Melan-A/Mart-1 [35], tyrosinase [36], prostate-specific antigen (PSA) [37], carcinoembryonic antigen (CEA) [38], and mammaglobin-A [39]. Tumor germline antigens, or cancer testis antigens, are proteins normally expressed at high levels in the germinal tissues of the testis, ovaries, and placenta and at low levels in other normal tissues. They include melanoma-associated antigen (MAGE) [17], B melanoma antigen (BAGE) [40], G antigen (GAGE) [41] families, New York esophageal squamous cell carcinoma 1 (NY-ESO-1) [42], and synovial sarcoma, X breakpoint 2 (SSX-2) [43].

Tumor-specific antigens (TSAs) originating in cancer cells may be associated with cancer transformation and/or progression [16, 44] and are ideal targets for immunotherapy because they are expressed only by cancer cells and not by normal cells, enabling specific recognition by the immune system that leads to effective tumor eradication with minimal side effects [45]. TSAs include oncoviral antigens and neoantigens. Viral antigens are present only in cancer cells and are derived from oncogenic tumor viruses. Viral oncoproteins are integrated into the cellular genome of tumors, inducing cell transformation and cancerogenesis. Examples are the human papilloma virus (HPV) E6 and E7 proteins and the Epstein-Barr virus (EBV)-encoded nuclear antigen (EBNA-1) [46, 47]. Neoantigens are encoded in cancer cells by mutational events of various types: single base substitutions that generate missense or nonsense mutations, splice sites, stop codons or silent changes and insertions or deletions (INDELs) that do or do not create translational frameshifts (FSs) and generate novel open reading frames (ORFs). Both viral antigens and oncogene-derived mutations are expressed in specific tumor types but are shared among many patients ("shared" neoantigens). However, the majority of neoantigens are exclusive to the tumors of individual patients ("private" neoantigens), originate from somatic DNA mutations only in the tumor tissue, are absent in healthy tissues and are not subject to immune tolerance mechanisms. Neoantigens should generate a specific reaction towards tumors since they constitute the largest fraction of targets of tumor-infiltrating T cells [48].

The previous definition of tumor antigen was expanded by Bobisse *et al.* [49], who coined the term "Immunopeptidome": molecules that can be recognized by patients' autologous T cells when exposed on HLA. These molecules can be generated by epigenetic, transcriptional, translational or posttranslational alterations of tumor cells and could lead to the recognition

and elimination of cancer cells. In fact, the endogenous mutated cancer proteins can be processed into peptides, presented on the surface of tumor cells and identified as "nonself" antigens.

Indeed, during the tumorigenesis process, cancer cells may acquire driver mutations, which are responsible for cancer transformation, or passenger mutations, which are byproducts of the instability of the genome [50]. In a pancancer analysis that comprised thousands of patients carrying 19 different types of tumors, including breast cancer, Efremova *et al.* [51] found a correlation between the number of generated neoepitopes and the number of tumor-infiltrating lymphocytes (TILs). These neoepitopes were mainly derived from mutations in passenger genes, while a tiny fraction was derived from mutations in driver genes that comprised the neoepitopes shared among patients [52]. According to the driver-passenger model, driver mutations render a selective advantage to cancer cells, thereby promoting cancer occurrence, while passenger mutations play a negligible role in driving cancer [53].

The number of somatic mutations that arise during tumorigenesis depends on the mutational load of the cancer. Defects in DNA repair pathways and exposure to carcinogens contribute to the mutational load [54]. In particular, the mutational load is different between various types of cancer and ranges from less than 0.1 somatic mutation per megabase (Mb) for pilocytic astrocytoma to over 10 mutations per Mb for melanoma [55, 56]. Breast cancer has a low mutational rate of 1 mutation per Mb [55], while lung and skin cancers have high mutation rates [54]. Additionally, the number of neoantigens per mutation can vary among cancers, ranging from 0.93 in melanoma to 1.43 in kidney renal cell carcinoma [52].

Therefore, the peptides that form neoantigens are not present in the normal human genome, making them of particular interest for the control of tumor growth because reactive T cells against these neoantigens are not influenced by thymic central tolerance and thus recognize the TAs as foreign antigens [57]. However, not all mutated proteins are recognized by T cells. Indeed, neoantigens should be processed in short peptides of 9-15 amino acids and then presented on the cell surface by the MHC for recognition by T cells. Thus, only a small number of neoantigens are able to trigger immune responses [58].

Neoantigens could be used as biomarkers in cancer immunotherapy to predict patient response to therapies. Moreover, they could also be used as targets for the development of new therapeutic approaches, aiming to boost the reactivity of endogenous T cells against cancer cells [57]. Cancer vaccines targeting neoantigens are personalized cancer immunotherapies since the neoantigen present is unique to each tumor and thus for each patient [45].

The first two studies that highlighted the possible use of neoantigens as targets for cancer immunotherapies were from Lennerz and Zhou. The first work, which was based on expression-cloning strategies, demonstrated the presence of antitumor T cells that targeted neoantigens produced by somatic point mutations in five genes in a melanoma patient [59]. In the second work, the authors reported that a melanoma patient had complete tumor regression after treatment with adoptive immunotherapy with autologous TILs comprising T cells specific for two mutant antigens [60].

Later, a study based on exome sequencing data reported that many neoantigens are recognized by T cells in breast and colon cancer [61, 62], and by 2012, it was reported that genomics and bioinformatics methods could be employed for the identification of tumor-specific neoantigens. Studies based on these methods showed that the combination of next-generation

sequencing (NGS), computer-based prediction of epitopes, and immunological evaluation was able to identify and validate TSAs in highly immunogenic methylcholanthrene (MCA)-induced sarcoma cells and in murine B16-F10 melanoma tumor cells [63, 64]. Another study reported the use of whole exome sequencing (WES) for the identification of neoantigens, which were recognized by adoptively transferred tumor-reactive T cells, in human cancers [65]. Other studies confirmed the identification of neoantigens by epitope prediction algorithms [66-69].

New technologies for neoantigens discovery

Neoantigens represent "private" antigens, being specific for each patient, and generally not shared. Thus, this specificity might represent a core obstacle to overcome to discover patients common neoantigens, Indeed, immunogenic mutations must be identified individually for each subject and then experimentally validated. Technologies now enable the identification of nonsynonymous mutations that create neoepitopes identifiable by patient-derived T cells. However, the prediction of the possible immunogenic mutations is not easy, and a long and complex bioinformatics process, acting as a bottleneck, is needed to determine the number of epitopes to be experimentally validated (**Fig. 2**).

The first method for the identification of these neoantigens was the classical complementary DNA (cDNA) library screening approach, as employed by Lennerz [59]. This low-throughput approach was labor-intensive.

Genomic mutations that alter protein sequences and thus can produce neoantigens are nonsynonymous mutations [59, 70], gene fusions [71]), retained introns [72], FS INDELs [73, 74] and posttranslational modifications [75]. With the advent of NGS, all of these variants could be detected, with the exception of posttranslational modifications, which can be identified with mass spectrometry (MS) [76].

The first step in the discovery of neoantigens is the identification of any somatic mutations in the tumor by using NGS technology. WES or whole-genome sequencing (WGS) is performed on tumors and matched to normal DNA, and the somatic variants are detected using variant-calling algorithms [77, 78]. Two or more variant calling algorithms are employed to limit false positives [79, 80]. However, there is a need to improve the sensitivity of these algorithms because one problem with this approach is the presence of false negatives, somatic variants that remain undetected [81].

An alternative strategy employed for the identification of neoantigens is RNA sequencing (RNA-seq), which is used in combination with WES/WGS to select the predicted mutations that are expressed as RNA and thus considered for further analysis [82]. RNA-seq also provides additional information because it enables the identification of neoantigens derived from alternative splicing, gene fusion and posttranscriptional modification [83, 84].

Kahles *et al.* performed a pancancer analysis of the alternative splicing landscape in 8 705 cancer patients by reanalyzing RNA and WGS data. Indeed, alternative splicing in cancer may lead to cancer-specific RNA transcripts and proteins. This research group focused on five alternative splicing events: intron retention, exon skipping, mutually exclusive exons, and alternative 3' and alternative 5' splice site changes. In addition, the authors identified

neojunction (exon-exon junction)-derived peptides that formed potential neoantigens but were not found in normal samples [85].

Recently, Wang and colleagues developed a new computational pipeline (ScanNeo) for the analysis of INDELs in cancer genomes based on the analysis of RNA-seq data. Indeed, the presence of INDELs in the cancer genome leads to the formation of novel ORFs and thus to the possible production of novel tumor-specific neoantigens. DNA-seq analyses reveal INDELs based on DNA data, but the INDELs detected by DNA data could not be expressed as RNA. Thus, the authors suggested that the discovery of INDEL-derived neoantigens by RNA data is crucial. ScanNeo starts with an aligned RNA-seq BAM file, followed by three phases: 1) INDEL discovery; 2) INDEL annotation and filtering; and 3) neoantigen predictions, which are based on NetMHC and NetMHCpan algorithms. The authors also applied ScanNeo in a prostate cancer cell line (LNCaP) and validated their neoantigen predictions by matching them with data from MS. The authors concluded that ScanNeo enables neoantigen discovery from expressed INDEL mutations and could be a complementary tool to DNA-seq analyses [86].

NGS data were also used for the identification of HLA types with computational tools such as Polysolver [87] and Optitype [88]. Indeed, the predicted neoantigens set was selected using *in silico* peptide prediction algorithms or MS-based immunopeptidomics [89]. The *in silico* analysis selects for potential epitopes that can bind with high affinity to the predicted MHC/HLA molecules. In this way, mutated peptides that have a high likelihood of binding to HLA are selected. One approach is the use of NetMHC, an algorithm that is based on artificial neural network (ANN) training methods and position-specific scoring matrices (PSSM) to predict MHC-peptide binding. With this approach, more than 75% of the predicted proteins were confirmed to bind to MHC. NetMHCpan is another method for predicting MHC, but it is more speculative than the previous method [90-92]. A limitation of these *in silico* strategies is that they are not optimal for the identification of neoantigens presented by class II HLAs both because endosomal HLA class II peptide processing is relatively more complex and also because the HLA class II peptide binding groove is open at both ends, making the prediction results more complex. Thus, further studies are needed [93, 94].

Recently, different computational pipelines were developed that integrate all these described steps for the identification of neoantigens. These pipelines integrate WGS data with HLA allele typing, mRNA expression data, peptide processing prediction and HLA allele binding. Examples are pVAC-seq [95] , MuPeXi [96], Cloudneo [97] and TIminer [98] (**Fig. 2**).

MS-based immunopeptidomics can be used to identify neoantigens or to validate those predicted by *in silico* strategies. In particular, the tumor material is lysed and homogenized, and then, the peptide-HLA (pHLA) complexes are purified by immunoprecipitation. Finally, liquid chromatography-coupled tandem MS (LCMS/MS) is used to determine the amino acid sequences of the purified peptides [99-101]. This unique approach also enables the identification of neoantigens derived from posttranslational modifications [102]. However, MS-based immunopeptidomics has some limitations: this method is labor intensive, has low sensitivity and requires a large amount of tumor material for the analysis [89]. In addition, the efficient identification of the amino acid sequence of the peptides is dependent on the efficient ionization and fragmentation of the peptides, which is dependent on the biochemical properties of the amino acids. Therefore, some peptides may never be detected with immunopeptidomics [103].

One of the main limitations of neoantigen screening is that only a few selected neoantigens identified with NGS, *in silico* strategies or immunopeptidomics are immunogenic and thus recognized by T cells. Therefore, candidate mutated peptides are screened by immunological methods to test their immunogenicity [89].

Approaches based on tumor-reactive cytotoxic T lymphocytes (CTLs), peripheral blood mononuclear cells (PBMCs) or patient sera were developed in the 1990s, with the identification of a large number of aberrantly expressed antigens. Currently, these methods are referred to as "reverse identification" or "reverse immunology" [104].

As already mentioned, cDNA library screening was the first strategy employed [59]. Briefly, in this method, tumor cDNA and HLA molecules are overexpressed in cell lines and cocultured with T cells to identify the antigens that are able to elicit T cell activation. This approach led to the identification of many neoantigens in the past two decades [60, 105, 106].

New immunological methods have been developed to facilitate large-scale neoantigen discovery. One approach is the use of peptide-HLA multimer staining technologies. Briefly, *in silico* strategies are used to predict neoantigens by HLA binding, and a pHLA library is generated for the screening of TILs [107-109]. However, this approach is limited by the relatively low sensitivity of the *in silico* methods; thus, a tandem minigene (TMG) screening approach has been developed. This approach is used to evaluate T cell responses against neoantigens presented on the class I and II HLA molecules of the patient without the need for using *in silico* algorithms [110]. A minigene construct is developed for each mutation identified. It contains the mutated amino acid flanked by 12 amino acids of the wild-type sequence. A TMG construct that contains from 6 to 24 minigenes has also been generated. TMGs are synthesized and transfected into autologous antigen presenting cells (APCs). Alternatively, peptide pools (PPs), each containing as many as 24 mutated peptides, can be generated by synthesizing 25-residue peptides. Thus, neoantigens are presented through intracellular (transfected TMGs) and extracellular (pulsed peptides) pathways on autologous APCs to T cells. TMGs or PPs capable of inducing a specific T cell response are deconvoluted to identify the specific neoantigens. The use of these approaches has led to the identification of a great number of neoantigens in recent years [89, 110-113] (**Fig. 2**).

Recently, Joglekar *et al.* developed a new strategy for neoantigen discovery that is based on using signaling and antigen-presenting bifunctional receptor (SABR) libraries. SABRs are chimeric receptors composed of an extracellular complex with an antigen and MHC, and after the interaction with a TCR, these receptors are able to elicit an intracellular TCR-like signal. Thus, SABRs enable the identification of specific TCR-pMHC interactions [114].

Heterogeneity of antigens/neoantigens expression in the context of breast cancer cell-of-origin patterns and clinical subtypes

The heterogeneity intrinsic to the cell-of-origin patterns of breast cancer has a large impact on the load and type of antigens and neoantigens that the cancer cells exhibit on their surfaces, and although breast cancer has been historically considered to have relatively weak antigenicity [115, 116], more recent investigations depict large variations in the size and composition of the immune infiltrates in the different molecular subtypes of this neoplasm, which in turn indicate different antigenic loads and contexts [115-119].

The TIL burden is the most solid indicator of ongoing immune surveillance (and subsequent immunoediting) processes [120] within the breast tissue and has been reported as both an independent prognostic factor for survival and a predictor of systemic response to immunotherapy [115, 116]. Accordingly, TIL counts vary by breast cancer subtype, with TNBC and ERBB2$^+$ tumors showing consistently higher frequencies than HR$^+$ cases, findings consistent with the greater prognostic power of TIL counts in the former [115]. However, TIL counts include different lymphocyte subpopulations, the relative abundance of which might be linked to a better (B cells and CD8$^+$ T cells) or worse (T regulatory cells, Tregs) prognosis; thus, the combination of antigen load and immune subpopulations within the given subtype more accurately depicts the immunogenic context of breast cancer [115-119].

The majority of TAAs targeted in breast cancer are overexpressed proteins, and vaccines against ERBB2, MUC1 and hTERT have shown proven efficacy [116], though the overall mutational load varies significantly between HR$^+$ and HR$^-$ tumors and thus affects the number of TILs that are available to respond to the stimulus [116]. The growth of immunogenomic and precision medicine approaches has, however, led to the discovery of several new subtype-specific markers that are candidates for becoming therapeutic targets. For example, mutated BRCA1/2 and APOBEC3B show a potential as antigens to polarize antitumoral T-cell clones against HER2$^+$ breast cancer [116], and TP53 has a mutational burden that is consistently high in all breast cancer types (though it is particularly high in metastatic cases) [117], although its targeting as a neoadjuvant is the subject of phase I/II trials for grade III cancers only [115]. A large set of actual or potential TAAs adds to these more well-known antigens, although their origins are purely experimental and have yet to be validated in a clinical context. For example, in a recent multiplatform survey of 84 breast cancer cell lines [121], several proteins, mRNAs and miRNAs were associated with specific breast cancer subtypes and response to therapy. Considering only the proteins studied, luminal-type cell lines overexpressed ESR1 (that is, ER), various keratins (KRT8, KRT18 and KRT19) and the transcription factors GATA3 and FOXA1, while HER2$^+$ cells upregulated, at least, HER2, GRB7, PERLD1, STARD3 and C17ORF37, though several other proteins were also associated with subtype-specific survival [121]. In comparison, TNBC cells showed a wider span of potential markers, including the "pan-TNBC" markers EGFR, CAV1/2, MSN, and ETS1 and the specific keratins and integrins typical of the basal cell layer (KRT4, KRT5, KRT6A/6B, KRT13, KRT14, KRT15, KRT16, KRT17, ITGA6, ITGB4, and ITGB6), for the "basal A-like TNBCs"; mesenchymal cell markers, such as VIM, PLAT, TGFB1, TGFBR2, TIMP1, CTSC, SPARC, FN1, and FBN1 and cancer stemness markers, such as CD44, for the "basal B-like TNBCs"; and a large selection of extracellular matrix (ECM)-related proteins, which might also indicate that tumor subtypes have specific modes of interaction with the microenvironment [121], an interesting supposition for further research [122, 123]. Additional antigenic targets that are specific for molecular and clinical breast cancer subtypes include PD-L1, CD163 and FOXP3 for TNBCs [119] and MAPK10, BCL9, TRIM65, CD93, KITLG, CNPPD1, CPED1, CCDC146, TMEM185A, INO80D, and PSMD11 for lymph node-infiltrating tumors [117]. In addition to these possible markers, several others have been put forward in 2012 by The Cancer Genome Atlas (TCGA) milestone study [124], although none has been used in the clinic, and it should also be noted that recent reports suggest an increase in antigen and neoantigen burden in therapeutically targeted breast tumors [125], indicating a

dynamic evolution in the type of antigens present within a single patient during the course of chemotherapy and immunotherapy.
An equally large variability is also present in neoantigens, as introduced in the following paragraphs.

Neoantigen studies in breast cancer

The study of neoantigen generation in tumor models has focused mainly on tumors that show a high mutational load, such as melanoma and lung cancer; breast cancer shows "only" an intermediate mutational load [55]. Moreover, for many years, specific TAAs were the focus of breast cancer studies. The majority of these studies sought to define the roles of TAAs in the prognosis of and the cure for breast cancer. Neoantigens studies have only recently been widened to include investigation into other cancer types, analysis on the nature of the neoantigens generated, the onset of an immune response and the possibility of inhibiting or abolishing tumor growth in preclinical and clinical trials.
Zhang *et al.* [126] developed an analysis of the antibody response based on cancer neoantigen screening. Taking into consideration the anticancer potential of FS-derived neoantigens, they built a peptide array with all possible nonoverlapping FS peptides and used those that were reactive as vaccine antigens in a breast cancer mouse model (4T1). Two rounds of gene-gun genetic vaccination and a subcutaneous peptide boost were effective in inducing slower tumor growth compared to that induced by nonreactive FS peptides. The differences in final tumor volume were not significant, but the production of antibodies against the FS peptide vaccines was positively correlated with the diminished tumor volume. Splenocytes generated more IFN-γ spots, and a correlation was found between a small tumor and a high number of spots representing IFN-γ-release, indicating that the T cell immune response is related to tumor reduction [126].
The results regarding the preservation of these mutations between primary tumors and metastasis are contradictory. Ding *et al.* [127] reported that primary basal-like breast cancer and brain metastases shared mutations, while Shan *et al.* [128] reported a very small level of conservation between metastasized lobular breast cancer and the primary tumor resected years earlier.
Of more than 900 000 unique neoantigens identified through a wide examination of 20 tumor histotypes, only 24 were shared among a tiny fraction of the patients otherwise carrying different diseases, and those that were shared were derived from mutations in driver genes (BRAF, RAS, PIK3CA) [52].
Stephens *et al.* [129], with a systematic genome analysis, documented their observation of the landscapes of the driver mutations in breast cancer. They confirmed the following characteristics in 100 breast cancer patients: 79 HR^+ and 21 HR^-, the presence of somatic driver mutations (substitutions and small INDELs) that were already known (AKT1, BRCA1, CDH1, GATA3, PIK3CA, PTEN, RB1 and TP53) and the presence of mutations previously found in other cancer types (APC, ARID1A, ARID2, ASXL1, BAP1, KRAS, MAP2K4, MLL2, MLL3, NF1, SETD2, SF3B1, SMAD4 and STK11). This result was based on the analysis of 21 416 protein coding genes and 1 664 microRNAs and showed that multiple events led to changes. Cumulatively, they found driver point mutations in approximately 40 genes in the 100 patients studied, and seven of these were mutated in more than 10% of the

cases, which corresponded to 58% of the driver mutations. In more than one-fourth of the patients analyzed, there was only one driver mutation, and a total of 6 mutated cancer genes were found in a single subject. Despite the use of such a detailed analysis, the data were not helpful in completely clarifying the mutational processes that generate somatic mutations, leading to the conclusion that multiple distinct mutational processes contribute to the onset and development of disease. There was no correlation between the total number of somatic mutations and the age at diagnosis; neither was a correlation found between the number of driver mutations and the tumor grade score (calculated by the combination of the number of mitotic cells, the number of cancer cell nuclear pleomorphisms and the extent of tubule formation).

Wang *et al.* [117] compared the mutational patterns of lymph node metastasis positive (LNpos) and negative (LNneg) breast cancer subjects using the TCGA database. Nonsynonymous somatic mutations and the rate of mutations were higher in the LNneg group. Although the mutational patterns were different, some genes were conserved in the 10 genes with the most mutations (TP53, PIK3CA, TTN, CDH1, GATA3 and KMT2C). In the LNneg group, the majority of mutated genes were functionally related and linked to microtubule organization (BRCA1, PCNT, BIRC6, RP1, RB1, TRP, DNAH7, PAFAH1B1, DYNC1H1, DISC1, and AGTPBP1), while in the LNpos group, the neoantigens originated from proteins closely associated with the MAPK family, the BCL family, TRIM65, and CD98. In the primary tumor of the LNneg group, a higher infiltration of effector immune cells was reported (activated $CD4^+$/$CD8^+$ T cells, activated DCs and mast cells), suggesting that a higher mutational load enhances the stimulation of an immune attack of the tumor.

Zhang *et al.* [130] identified neoantigens specifically recognized by the T cells in advanced breast cancer patients: PALB2, ROBO3, PTPRS and ZDHHC16. From these patients, PDX mouse models were generated, and whole tumor exomes were compared to those of normal tissue to identify mutations. From this comparison, thousands of nonsynonymous single nucleotide variants (nsSNVs) were identified, and they were initially prioritized for their predicted binding affinity for HLA (as determined by the NetMHC software) and later functionally evaluated (with IFN-γ ELISPOT). Moreover, neoantigen-specific T cells were able to inhibit the growth of patient tumors implanted into NOD.SCID.gamma-c-null (NSG) immunodeficient mice by inducing TILs to produce IFN-γ.

Ding *et al.* [127] analyzed 47 luminal breast cancer samples and identified an average of 31 nsSNVs in each breast cancer genome investigated, demonstrating that nsSNVs are expressed and recognized by the immune system in many cases.

Young *et al.* [131] compared the individual antibody response in breast cancer patients in which antibody-secreting cells (ASCs) were generated from the tumor-draining lymph nodes of 20 subjects. Reflecting the large molecular heterogeneity of breast cancer, a unique antibody profile was defined for each patient through a one-dimensional western blot screening of MCF7 extracts, for which only a few bands were found in common with additional subjects. Identification of antibody-specific proteins was possible using a custom protein microarray. Cancer-testis antigen family (NY-ESO-1, and CTAG2), DEAD-box helicase 53 (DDX53), ferritin heavy polypeptide-like 17 (FTHL17), melanoma-associated antigen 4 (MAGE-4), and phosphoinositide-3-kinase regulatory subunit 1 (PIK3R1) were some of the proteins identified. When the profiles of plasma and ASC probes were compared, these proteins overlapped, but the plasma titer was greatly reduced.

Neoantigens have been shown to be the targets of tumor-specific $CD4^+$ and $CD8^+$ T cells, reinforcing their action through checkpoint inhibitor therapies [132]. Using data from the TCGA database, the results from an analysis of the FS INDEL contribution to the therapy response indicated that FS INDELs had an association with checkpoint inhibitors that was stronger than the association between FS INDELS and nsSNVs. The proportion of INDEL mutations in breast cancer is elevated, inferior only to that of renal cell carcinoma, as the authors specifically analyzed. The number of nsSNVs in breast cancer is low, as in TNBC. The number of FS neoantigens among all the tumor types analyzed was slightly higher, with those of TNBC still in a comparable range. In the analysis of a specific cohort of TNBC patients compared with patients with other invasive breast cancer subtypes, an increase in the fraction of patients achieving response was found. In fact, TNBC is characterized by a higher number of neoantigens originating from FSs and mutations, and the mutational load of BRCA-1-mutated TNBC is even higher, although no correlation was found between TNBC and the number of TILs [132].

Immune response targeting neoantigens

In the early days of anticancer vaccination strategy conception, the focus was on TAA choice. HER2, MART-1, MUC1, and NY-ESO-1 are some of the TAAs for which vaccines have been generated. Some level of response is conferred, although it cannot be generalized, and side effects are common [24, 27, 133]. Currently, the focus has moved to neoantigens. However, the identification of molecules that could be successfully exploited to generate specific vaccines is not a "walk in the park". The process is quite long, and each step reduces the possibility that a multitude of molecularly identified neoantigens will be useful, a situation that further reduces the possibility of generating specific cancer vaccines (**Fig. 2**).

The consensus is that hypermutated tumors generate a higher population of neoantigens that stimulate T cell activation and infiltration [48]. In contrast, the results obtained by McDonalds *et al.* [134] do not support the hypothesis that higher tumoral heterogeneity promotes greater immune infiltration targeting neoantigens. The mutant-allele tumor heterogeneity (MATH) bioinformatics analysis of a cohort of 1 000 breast cancer patients from TCGA (WES *vs* matched normal DNA) revealed that higher heterogeneity was associated with shorter overall survival (OS) because of the selective pressure of tumor-infiltrating cells. HR^- and TNBCs were found to be associated with higher heterogeneity, higher tumor grades, higher mutational burden and higher KI-67 expression but lower less infiltration of activated $CD8^+$ and $CD4^+$ T cells, B cells, DCs, and mast cells, higher infiltration of Tregs, lower expression of T-cell exhaustion markers (PD-L1, IDO2, ADORA2A, VISTA and CCR4) and lower levels of cytolytic enzyme (granzyme A, perforine) expression.

It is possible that the tipping point in the generation of neoantigen-based vaccines is not the number of activated infiltrating cells but the level of their specificity and their affinity against neoantigens. Aurisicchio *et al.* [135] recently confirmed this supposition in animal models, showing that high-affinity neoantigens (<50 nM) are better inducers of polyfunctional immune responses ($CD8^+IFN\gamma^+TNF\alpha^+$ and $CD8^+TNF\alpha^+IL2^+$) compared to low-affinity neoantigens (> 50 nM).

The number of studies reporting the successful design and application of neoantigen vaccines in breast cancer [130] and other cancer types is increasing daily. A specific response is

induced followed by the regression or inhibition of tumor growth. Tanyi *et al.* [136] injected autologous DCs pulsed with whole-tumor cell lysate into immunotherapy naïve patients with recurring ovarian cancer to generate polyfunctional T cells (IFN-γ, TNF-α, or IL-2) in response to tumor neoantigens. T cells were able to specifically kill tumor cells and suppress tumor growth in patient-derived xenograft (PDX) mice. Ott *et al.* [137] in melanoma patients demonstrated that a vaccine developed to target as many as 20 predicted personal tumor neoantigens is safe, feasible and creates a strong immunogenic response that had been absent before the vaccination, inducing polyfunctional $CD8^+$ and $CD4^+$ T cells able to discriminate between mutated and wild-type antigens. The results from Johanns *et al.* [138] support the hypothesis that targeting neoantigens is also feasible for tumors with lower mutational burden. In fact, in glioblastoma, on the basis of an immunogenomics pipeline to identify candidate neoantigens (WES and affinity calculation algorithms), a peptide vaccine was designed and administered to patients after treatment with an autologous tumor lysate DC vaccine. This combination of vaccines generated or increased the frequency of neoantigen-specific TILs ($CD8^+$ and $CD4^+$).

Li *et al.* [133] reported the targeting of neoantigens in the murine 4T1 and E0771 breast tumor models using synthetic long peptide (SLP) or polyepitope DNA vaccines and are currently enrolling neoadjuvant-treated TNBC patients in two clinical trials that will evaluate the safety of personalized neoantigen vaccines and the induction of specific T cell responses. The two clinical trials are designed to vaccinate patients with SLP administered with adjuvant (NCT02427581) or with polyepitope neoantigen DNA administered through muscular electroporation (NCT02348320).

Several trials, specific for or that include breast cancer patients, aim to investigate the contribution of neoantigen-derived formulations mainly against tumor recurrence (**Table 1**). However, the process of identifying better neoantigens has led to many problems; therefore, the trial developed by the Institut Curie in France (NCT02831634) seeks to study the preferable methodology for identifying tumor-mutated epitopes in patients with breast cancer. MHC I- and MHC II-restricted tumor neoepitopes were identified with either elution-HPLC performed on lymph node-derived tumor cell lines or with predictive algorithms on tumor sequencing data.

The primary outcomes of the ongoing trials are the safety and tolerability of the formulations or the survival or progression of disease in advanced stages. Immunogenicity and the induction of a specific T cell response against neoantigens included in the vaccine are secondary. The trial proposed by the Icahn School of Medicine at Mount Sinai (NCT02721043) seeks to evaluate the safety, tolerability and immunogenicity of peptide vaccines based on the patient's own tumor sequence (Personalized Genomic Vaccine 001, PGV001). A similar goal is stated in the mutanome engineered RNA immunotherapy (MERIT) study proposed by Biotech SE with TNBC patients. In this trial (NCT02316457), the efficacy of an RNA drug product, targeting TAAs commonly expressed in TNBC, and of a *de novo* synthesized RNA, targeting multiple neoantigens derived from the mutated epitopes of the patient, will be compared. The different vectors used to deliver neoantigens can also be investigated in a clinical trial, as in a study at NantBioScience in California; in this phase I trial (NCT03552718), previously treated breast cancer patients with HR^+, $HER2^{+/-}$, or TNBC are enrolled and will be evaluated using a yeast-based cancer vaccine (YE-NEO-001) composed of heat-killed yeast that was genetically modified to express patient-specific

neoantigens able to elicit $CD4^+$ and $CD8^+$ T cell responses against tumor cells expressing these specific epitopes.

A step forward approach is one that plans to use patients' blood cells engineered to express T cell receptors (TCRs) that are specific against neoantigens. Two clinical trials are actively recruiting patients with incurable or metastatic cancer, including breast cancer. The study proposed by Rosenberg at the National Cancer Institute (NCT03412877) aimed to administer autologous peripheral blood lymphocytes engineered to express a modified T cell receptor that recognizes unique mutated neoantigens expressed by the tumor. A second study (NCT03970382), proposed by PACT Pharma Inc., will administer an autologous adoptive T cell therapy in which cells have been engineered to express an autologous TCR targeting a neoepitope exclusively presented by a patient's tumor cells. In this study, cell therapy will be administered with or without nivolumab (an anti-PD-1 monoclonal antibody). In fact, the combination of neoantigen-based vaccines and checkpoint inhibitor drugs is the latest evolution of these studies. In the phase I clinical trial coordinated by W. Gillanders at the Washington University School of Medicine (NCT03199040), newly diagnosed clinical stage II-III TNBC patients who have completed the standard of care therapy (chemo/radiotherapy and surgery) will receive a neoantigen DNA vaccine alone or the vaccine plus durvalumab, a monoclonal antibody that blocks the interaction of PD-L1 with PD-1 and CD80. In the phase II trial (NCT03606967) conducted at the National Cancer Institute and coordinated by W. Gillanders, a personalized SLP neoantigen vaccine will be evaluated in combination with durvalumab and nab-paclitaxel (nanoparticle albumin-bound mitosis inhibitor paclitaxel) in metastatic TNBC. The induction of specific responses against neoantigens will be evaluated in a clinical trial, although the neoantigens are not direct components of the vaccine formulation (NCT02883062).

Conclusion and perspective

Despite social campaigns, global awareness and prevention protocols, breast cancer still remains the most common cause of death by neoplasia among women, with factors such as its cellular heterogeneity and limited immunogenicity contributing sensibly to its lethality..

Since the involvement of the immune system in distinguishing tumor growth was first understood, the effort spent in the investigation of the behaviors of both the tumor and immune system has increased recently. Several approaches have been developed and evaluated in clinical trials, and although many of them are promising and have been shown to be successful, a high population of patients still do not benefit from these therapies. Accordingly, the need for investigations into new lines of therapy or combinations is clear.

The investigation of neoantigens is one of the new approaches that has become feasible in recent years due to the evolution of bioinformatics, which has created new possibilities for curing cancer. The neoantigens that have already been employed in vaccine strategies have been demonstrated to be successful in reducing the volume of tumors not characterized by a high mutational load, such as those in breast cancer. The problems to overcome are the cost of the methodology and, more important, the time that the process requires from the analysis of the tumor tissue to the administration of a personalized vaccine formulation. Nevertheless, even when such a specific vaccine is administered to a patient, there is no guarantee that it will eradicate the disease.

Hopefully, with the improvement in computational strategies and assays for the identification, prioritization and immunogenicity prediction of neoantigens, this time-consuming process will be shortened and the cost will be reduced. Moreover, the use of a personalized vaccine in combination with other strategies, with the goal of unleashing a restrained immune system, could increase the fraction of patients who respond positively to therapy.

Acknowledgements

This study was supported by a grant from the University of Rome "Tor Vergata" (Mission Sustainability, Codice Unico di Progetto (CUP): E81I18000330005; R.B.) and by a grant from the Research Council for Health of the Academy of Finland (Grant 308867; V.I.). The authors thank Barbara Bulgarini for editorial assistance in the preparation of the manuscript.

References

[1] L. Semmler, C. Reiter-Brennan, A. Klein, BRCA1 and Breast Cancer: a Review of the Underlying Mechanisms Resulting in the Tissue-Specific Tumorigenesis in Mutation Carriers, J Breast Cancer 22(1) (2019) 1-14.
[2] F. Bray, J. Ferlay, I. Soerjomataram, R.L. Siegel, L.A. Torre, A. Jemal, Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries, CA Cancer J Clin 68(6) (2018) 394-424.
[3] A.G. Waks, E.P. Winer, Breast Cancer Treatment: A Review, JAMA 321(3) (2019) 288-300.
[4] C. Denkert, The immunogenicity of breast cancer--molecular subtypes matter, Ann Oncol 25(8) (2014) 1453-5.
[5] P. Poudel, G. Nyamundanda, Y. Patil, M.C.U. Cheang, A. Sadanandam, Heterocellular gene signatures reveal luminal-A breast cancer heterogeneity and differential therapeutic responses, NPJ Breast Cancer 5 (2019) 21.
[6] L.D. Miller, J.A. Chou, M.A. Black, C. Print, J. Chifman, A. Alistar, T. Putti, X. Zhou, D. Bedognetti, W. Hendrickx, A. Pullikuth, J. Rennhack, E.R. Andrechek, S. Demaria, E. Wang, F.M. Marincola, Immunogenic Subtypes of Breast Cancer Delineated by Gene Classifiers of Immune Responsiveness, Cancer Immunol Res 4(7) (2016) 600-10.
[7] M. Yin, H.B. Mackley, J.J. Drabick, H.A. Harvey, Primary female breast sarcoma: clinicopathological features, treatment and prognosis, Sci Rep 6 (2016) 31497.
[8] G. Tozbikian, E. Brogi, C.E. Vallejo, D. Giri, M. Murray, J. Catalano, C. Olcese, K.J. Van Zee, H.Y. Wen, Atypical Ductal Hyperplasia Bordering on Ductal Carcinoma In Situ, Int J Surg Pathol 25(2) (2017) 100-107.
[9] P. Clauser, M.A. Marino, P.A. Baltzer, M. Bazzocchi, C. Zuiani, Management of atypical lobular hyperplasia, atypical ductal hyperplasia, and lobular carcinoma in situ, Expert Rev Anticancer Ther 16(3) (2016) 335-46.
[10] F. Bertucci, P. Finetti, D. Birnbaum, Basal breast cancer: a complex and deadly molecular subtype, Curr Mol Med 12(1) (2012) 96-110.
[11] A.H. Sims, A. Howell, S.J. Howell, R.B. Clarke, Origins of breast cancer subtypes and therapeutic implications, Nat Clin Pract Oncol 4(9) (2007) 516-25.
[12] H. Olsson, A hypothesis about tumour development and the clinical features of hereditary breast cancers, Eur J Cancer 37(16) (2001) 2023-9.
[13] H. Hua, H. Zhang, Q. Kong, Y. Jiang, Mechanisms for estrogen receptor expression in human cancer, Exp Hematol Oncol 7 (2018) 24.

[14] A.C. Garrido-Castro, N.U. Lin, K. Polyak, Insights into Molecular Classifications of Triple-Negative Breast Cancer: Improving Patient Selection for Treatment, Cancer Discov 9(2) (2019) 176-198.
[15] C.K. Anders, V. Abramson, T. Tan, R. Dent, The Evolution of Triple-Negative Breast Cancer: From Biology to Novel Therapeutics, Am Soc Clin Oncol Educ Book 35 (2016) 34-42.
[16] L. Buonaguro, A. Petrizzo, M.L. Tornesello, F.M. Buonaguro, Translating tumor antigens into cancer vaccines, Clin Vaccine Immunol 18(1) (2011) 23-34.
[17] P. van der Bruggen, C. Traversari, P. Chomez, C. Lurquin, E. De Plaen, B. Van den Eynde, A. Knuth, T. Boon, A gene encoding an antigen recognized by cytolytic T lymphocytes on a human melanoma, Science 254(5038) (1991) 1643-7.
[18] S. Ilyas, J.C. Yang, Landscape of Tumor Antigens in T Cell Immunotherapy, J Immunol 195(11) (2015) 5117-22.
[19] G.A.A.K. Crain, J.J. Molldrem, Tumor-Associated Antigens, in: R.Z.a.B.R.B. Gérard Socié (Ed.), Immune Biology of Allogeneic Hematopoietic Stem Cell Transplantation, ScienceDirect2019, pp. 107-125.
[20] L. Novellino, C. Castelli, G. Parmiani, A listing of human tumor antigens recognized by T cells: March 2004 update, Cancer Immunol Immunother 54(3) (2005) 187-207.
[21] V.S. Farajzadeh, R. Nima, Tumor antigens, in: N.R.M. Keshavarz-Fathi (Ed.), Vaccines for Cancer ImmunotherapyAn Evidence-Based Review on Current Status and Future Perspectives, Elsevier2018, pp. 61-74.
[22] M. Angelova, P. Charoentong, H. Hackl, M.L. Fischer, R. Snajder, A.M. Krogsdam, M.J. Waldner, G. Bindea, B. Mlecnik, J. Galon, Z. Trajanoski, Characterization of the immunophenotypes and antigenomes of colorectal cancers reveals distinct tumor escape mechanisms and novel targets for immunotherapy, Genome Biol 16 (2015) 64.
[23] R.E. Hollingsworth, K. Jansen, Turning the corner on therapeutic cancer vaccines, NPJ Vaccines 4 (2019) 7.
[24] P. Menna, G. Minotti, E. Salvatorelli, Cardiotoxicity of Targeted Cancer Drugs: Concerns, "The Cart Before the Horse," and Lessons from Trastuzumab, Curr Cardiol Rep 21(5) (2019) 33.
[25] R. Bos, S. van Duikeren, H. Morreau, K. Franken, T.N. Schumacher, J.B. Haanen, S.H. van der Burg, C.J. Melief, R. Offringa, Balancing between antitumor efficacy and autoimmune pathology in T-cell-mediated targeting of carcinoembryonic antigen, Cancer Res 68(20) (2008) 8446-55.
[26] H. Uchi, R. Stan, M.J. Turk, M.E. Engelhorn, G.A. Rizzuto, S.M. Goldberg, J.D. Wolchok, A.N. Houghton, Unraveling the complex relationship between cancer immunity and autoimmunity: lessons from melanoma and vitiligo, Adv Immunol 90 (2006) 215-41.
[27] H.J. Stauss, E.C. Morris, Immunotherapy with gene-modified T cells: limiting side effects provides new challenges, Gene Ther 20(11) (2013) 1029-32.
[28] B. Fisk, T.L. Blevins, J.T. Wharton, C.G. Ioannides, Identification of an immunodominant peptide of HER-2/neu protooncogene recognized by ovarian tumor-specific cytotoxic T lymphocyte lines, J Exp Med 181(6) (1995) 2109-17.
[29] R.H. Vonderheide, W.C. Hahn, J.L. Schultze, L.M. Nadler, The telomerase catalytic subunit is a widely expressed tumor-associated antigen recognized by cytotoxic T lymphocytes, Immunity 10(6) (1999) 673-9.
[30] Y. Umano, T. Tsunoda, H. Tanaka, K. Matsuda, H. Yamaue, H. Tanimura, Generation of cytotoxic T cell responses to an HLA-A24 restricted epitope peptide derived from wild-type p53, Br J Cancer 84(8) (2001) 1052-7.
[31] K. Chang, I. Pastan, Molecular cloning of mesothelin, a differentiation antigen present on mesothelium, mesotheliomas, and ovarian cancers, Proc Natl Acad Sci U S A 93(1) (1996) 136-40.

[32] M.H. Andersen, L.O. Pedersen, J.C. Becker, P.T. Straten, Identification of a cytotoxic T lymphocyte response to the apoptosis inhibitor protein survivin in cancer patients, Cancer Res 61(3) (2001) 869-72.
[33] J.C. Schmollinger, R.H. Vonderheide, K.M. Hoar, B. Maecker, J.L. Schultze, F.S. Hodi, R.J. Soiffer, K. Jung, M.J. Kuroda, N.L. Letvin, E.A. Greenfield, M. Mihm, J.L. Kutok, G. Dranoff, Melanoma inhibitor of apoptosis protein (ML-IAP) is a target for immune-mediated tumor destruction, Proc Natl Acad Sci U S A 100(6) (2003) 3398-403.
[34] A.B. Bakker, M.W. Schreurs, A.J. de Boer, Y. Kawakami, S.A. Rosenberg, G.J. Adema, C.G. Figdor, Melanocyte lineage-specific antigen gp100 is recognized by melanoma-derived tumor-infiltrating lymphocytes, J Exp Med 179(3) (1994) 1005-9.
[35] Y. Kawakami, S. Eliyahu, K. Sakaguchi, P.F. Robbins, L. Rivoltini, J.R. Yannelli, E. Appella, S.A. Rosenberg, Identification of the immunodominant peptides of the MART-1 human melanoma antigen recognized by the majority of HLA-A2-restricted tumor infiltrating lymphocytes, J Exp Med 180(1) (1994) 347-52.
[36] R.F. Wang, E. Appella, Y. Kawakami, X. Kang, S.A. Rosenberg, Identification of TRP-2 as a human tumor antigen recognized by cytotoxic T lymphocytes, J Exp Med 184(6) (1996) 2207-16.
[37] J.M. Corman, E.E. Sercarz, N.K. Nanda, Recognition of prostate-specific antigenic peptide determinants by human CD4 and CD8 T cells, Clin Exp Immunol 114(2) (1998) 166-72.
[38] K.Y. Tsang, S. Zaremba, C.A. Nieroda, M.Z. Zhu, J.M. Hamilton, J. Schlom, Generation of human cytotoxic T cells specific for human carcinoembryonic antigen epitopes from patients immunized with recombinant vaccinia-CEA vaccine, J Natl Cancer Inst 87(13) (1995) 982-90.
[39] A. Jaramillo, K. Majumder, P.P. Manna, T.P. Fleming, G. Doherty, J.F. Dipersio, T. Mohanakumar, Identification of HLA-A3-restricted CD8+ T cell epitopes derived from mammaglobin-A, a tumor-associated antigen of human breast cancer, Int J Cancer 102(5) (2002) 499-506.
[40] P. Boël, C. Wildmann, M.L. Sensi, R. Brasseur, J.C. Renauld, P. Coulie, T. Boon, P. van der Bruggen, BAGE: a new gene encoding an antigen recognized on human melanomas by cytolytic T lymphocytes, Immunity 2(2) (1995) 167-75.
[41] B. Van den Eynde, O. Peeters, O. De Backer, B. Gaugler, S. Lucas, T. Boon, A new family of genes coding for an antigen recognized by autologous cytolytic T lymphocytes on a human melanoma, J Exp Med 182(3) (1995) 689-98.
[42] E. Jäger, Y.T. Chen, J.W. Drijfhout, J. Karbach, M. Ringhoffer, D. Jäger, M. Arand, H. Wada, Y. Noguchi, E. Stockert, L.J. Old, A. Knuth, Simultaneous humoral and cellular immune response against cancer-testis antigen NY-ESO-1: definition of human histocompatibility leukocyte antigen (HLA)-A2-binding peptide epitopes, J Exp Med 187(2) (1998) 265-70.
[43] M. Ayyoub, S. Stevanovic, U. Sahin, P. Guillaume, C. Servis, D. Rimoldi, D. Valmori, P. Romero, J.C. Cerottini, H.G. Rammensee, M. Pfreundschuh, D. Speiser, F. Lévy, Proteasome-assisted identification of a SSX-2-derived epitope recognized by tumor-reactive CTL infiltrating metastatic melanoma, J Immunol 168(4) (2002) 1717-22.
[44] K. Kakimi, T. Karasaki, H. Matsushita, T. Sugie, Advances in personalized cancer immunotherapy, Breast Cancer 24(1) (2017) 16-24.
[45] R.Y. Pan, W.H. Chung, M.T. Chu, S.J. Chen, H.C. Chen, L. Zheng, S.I. Hung, Recent Development and Clinical Application of Cancer Vaccine: Targeting Neoantigens, J Immunol Res 2018 (2018) 4325874.
[46] M.E. Ressing, A. Sette, R.M. Brandt, J. Ruppert, P.A. Wentworth, M. Hartman, C. Oseroff, H.M. Grey, C.J. Melief, W.M. Kast, Human CTL epitopes encoded by human

papillomavirus type 16 E6 and E7 identified through in vivo and in vitro immunogenicity studies of HLA-A*0201-binding peptides, J Immunol 154(11) (1995) 5934-43.
[47] W. Herr, E. Ranieri, A. Gambotto, L.S. Kierstead, A.A. Amoscato, L. Gesualdo, W.J. Storkus, Identification of naturally processed and HLA-presented Epstein-Barr virus peptides recognized by CD4(+) or CD8(+) T lymphocytes from human blood, Proc Natl Acad Sci U S A 96(21) (1999) 12033-8.
[48] B. Heemskerk, P. Kvistborg, T.N. Schumacher, The cancer antigenome, EMBO J 32(2) (2013) 194-203.
[49] S. Bobisse, P.G. Foukas, G. Coukos, A. Harari, Neoantigen-based cancer immunotherapy, Ann Transl Med 4(14) (2016) 262.
[50] M.M. Gubin, M.N. Artyomov, E.R. Mardis, R.D. Schreiber, Tumor neoantigens: building a framework for personalized cancer immunotherapy, J Clin Invest 125(9) (2015) 3413-21.
[51] M. Efremova, F. Finotello, D. Rieder, Z. Trajanoski, Neoantigens Generated by Individual Mutations and Their Role in Cancer Immunity and Immunotherapy, Front Immunol 8 (2017) 1679.
[52] P. Charoentong, F. Finotello, M. Angelova, C. Mayer, M. Efremova, D. Rieder, H. Hackl, Z. Trajanoski, Pan-cancer Immunogenomic Analyses Reveal Genotype-Immunophenotype Relationships and Predictors of Response to Checkpoint Blockade, Cell Rep 18(1) (2017) 248-262.
[53] Y. Hou, B. Gao, G. Li, Z. Su, MaxMIF: A New Method for Identifying Cancer Driver Genes through Effective Data Integration, Adv Sci (Weinh) 5(9) (2018) 1800640.
[54] I. Martincorena, P.J. Campbell, Somatic mutation in cancer and normal cells, Science 349(6255) (2015) 1483-9.
[55] L.B. Alexandrov, S. Nik-Zainal, D.C. Wedge, S.A. Aparicio, S. Behjati, A.V. Biankin, G.R. Bignell, N. Bolli, A. Borg, A.L. Børresen-Dale, S. Boyault, B. Burkhardt, A.P. Butler, C. Caldas, H.R. Davies, C. Desmedt, R. Eils, J.E. Eyfjörd, J.A. Foekens, M. Greaves, F. Hosoda, B. Hutter, T. Ilicic, S. Imbeaud, M. Imielinski, M. Imielinsk, N. Jäger, D.T. Jones, D. Jones, S. Knappskog, M. Kool, S.R. Lakhani, C. López-Otín, S. Martin, N.C. Munshi, H. Nakamura, P.A. Northcott, M. Pajic, E. Papaemmanuil, A. Paradiso, J.V. Pearson, X.S. Puente, K. Raine, M. Ramakrishna, A.L. Richardson, J. Richter, P. Rosenstiel, M. Schlesner, T.N. Schumacher, P.N. Span, J.W. Teague, Y. Totoki, A.N. Tutt, R. Valdés-Mas, M.M. van Buuren, L. van 't Veer, A. Vincent-Salomon, N. Waddell, L.R. Yates, J. Zucman-Rossi, P.A. Futreal, U. McDermott, P. Lichter, M. Meyerson, S.M. Grimmond, R. Siebert, E. Campo, T. Shibata, S.M. Pfister, P.J. Campbell, M.R. Stratton, A.P.C.G. Initiative, I.B.C. Consortium, I.M.-S. Consortium, I. PedBrain, Signatures of mutational processes in human cancer, Nature 500(7463) (2013) 415-21.
[56] M.S. Lawrence, P. Stojanov, P. Polak, G.V. Kryukov, K. Cibulskis, A. Sivachenko, S.L. Carter, C. Stewart, C.H. Mermel, S.A. Roberts, A. Kiezun, P.S. Hammerman, A. McKenna, Y. Drier, L. Zou, A.H. Ramos, T.J. Pugh, N. Stransky, E. Helman, J. Kim, C. Sougnez, L. Ambrogio, E. Nickerson, E. Shefler, M.L. Cortés, D. Auclair, G. Saksena, D. Voet, M. Noble, D. DiCara, P. Lin, L. Lichtenstein, D.I. Heiman, T. Fennell, M. Imielinski, B. Hernandez, E. Hodis, S. Baca, A.M. Dulak, J. Lohr, D.A. Landau, C.J. Wu, J. Melendez-Zajgla, A. Hidalgo-Miranda, A. Koren, S.A. McCarroll, J. Mora, B. Crompton, R. Onofrio, M. Parkin, W. Winckler, K. Ardlie, S.B. Gabriel, C.W.M. Roberts, J.A. Biegel, K. Stegmaier, A.J. Bass, L.A. Garraway, M. Meyerson, T.R. Golub, D.A. Gordenin, S. Sunyaev, E.S. Lander, G. Getz, Mutational heterogeneity in cancer and the search for new cancer-associated genes, Nature 499(7457) (2013) 214-218.
[57] T.N. Schumacher, R.D. Schreiber, Neoantigens in cancer immunotherapy, Science 348(6230) (2015) 69-74.

[58] R.F. Wang, H.Y. Wang, Immune targets and neoantigens for cancer immunotherapy and precision medicine, Cell Res 27(1) (2017) 11-37.
[59] V. Lennerz, M. Fatho, C. Gentilini, R.A. Frye, A. Lifke, D. Ferel, C. Wölfel, C. Huber, T. Wölfel, The response of autologous T cells to a human melanoma is dominated by mutated neoantigens, Proc Natl Acad Sci U S A 102(44) (2005) 16013-8.
[60] J. Zhou, M.E. Dudley, S.A. Rosenberg, P.F. Robbins, Persistence of multiple tumor-specific T-cell clones is associated with complete tumor regression in a melanoma patient receiving adoptive cell transfer therapy, J Immunother 28(1) (2005) 53-62.
[61] L.D. Wood, D.W. Parsons, S. Jones, J. Lin, T. Sjöblom, R.J. Leary, D. Shen, S.M. Boca, T. Barber, J. Ptak, N. Silliman, S. Szabo, Z. Dezso, V. Ustyanksky, T. Nikolskaya, Y. Nikolsky, R. Karchin, P.A. Wilson, J.S. Kaminker, Z. Zhang, R. Croshaw, J. Willis, D. Dawson, M. Shipitsin, J.K. Willson, S. Sukumar, K. Polyak, B.H. Park, C.L. Pethiyagoda, P.V. Pant, D.G. Ballinger, A.B. Sparks, J. Hartigan, D.R. Smith, E. Suh, N. Papadopoulos, P. Buckhaults, S.D. Markowitz, G. Parmigiani, K.W. Kinzler, V.E. Velculescu, B. Vogelstein, The genomic landscapes of human breast and colorectal cancers, Science 318(5853) (2007) 1108-13.
[62] N.H. Segal, D.W. Parsons, K.S. Peggs, V. Velculescu, K.W. Kinzler, B. Vogelstein, J.P. Allison, Epitope landscape in breast and colorectal cancer, Cancer Res 68(3) (2008) 889-92.
[63] J.C. Castle, S. Kreiter, J. Diekmann, M. Löwer, N. van de Roemer, J. de Graaf, A. Selmi, M. Diken, S. Boegel, C. Paret, M. Koslowski, A.N. Kuhn, C.M. Britten, C. Huber, O. Türeci, U. Sahin, Exploiting the mutanome for tumor vaccination, Cancer Res 72(5) (2012) 1081-91.
[64] H. Matsushita, M.D. Vesely, D.C. Koboldt, C.G. Rickert, R. Uppaluri, V.J. Magrini, C.D. Arthur, J.M. White, Y.S. Chen, L.K. Shea, J. Hundal, M.C. Wendl, R. Demeter, T. Wylie, J.P. Allison, M.J. Smyth, L.J. Old, E.R. Mardis, R.D. Schreiber, Cancer exome analysis reveals a T-cell-dependent mechanism of cancer immunoediting, Nature 482(7385) (2012) 400-4.
[65] P.F. Robbins, Y.C. Lu, M. El-Gamil, Y.F. Li, C. Gross, J. Gartner, J.C. Lin, J.K. Teer, P. Cliften, E. Tycksen, Y. Samuels, S.A. Rosenberg, Mining exomic sequencing data to identify mutated antigens recognized by adoptively transferred tumor-reactive T cells, Nat Med 19(6) (2013) 747-52.
[66] E.F. Fritsch, M. Rajasagi, P.A. Ott, V. Brusic, N. Hacohen, C.J. Wu, HLA-binding properties of tumor neoepitopes in humans, Cancer Immunol Res 2(6) (2014) 522-9.
[67] M. Rajasagi, S.A. Shukla, E.F. Fritsch, D.B. Keskin, D. DeLuca, E. Carmona, W. Zhang, C. Sougnez, K. Cibulskis, J. Sidney, K. Stevenson, J. Ritz, D. Neuberg, V. Brusic, S. Gabriel, E.S. Lander, G. Getz, N. Hacohen, C.J. Wu, Systematic identification of personal tumor-specific neoantigens in chronic lymphocytic leukemia, Blood 124(3) (2014) 453-62.
[68] M.M. van Buuren, J.J. Calis, T.N. Schumacher, High sensitivity of cancer exome-based CD8 T cell neo-antigen identification, Oncoimmunology 3 (2014) e28836.
[69] D.A. Wick, J.R. Webb, J.S. Nielsen, S.D. Martin, D.R. Kroeger, K. Milne, M. Castellarin, K. Twumasi-Boateng, P.H. Watson, R.A. Holt, B.H. Nelson, Surveillance of the tumor mutanome by T cells during progression from primary to recurrent ovarian cancer, Clin Cancer Res 20(5) (2014) 1125-34.
[70] A.L. Pritchard, J.G. Burel, M.A. Neller, N.K. Hayward, J.A. Lopez, M. Fatho, V. Lennerz, T. Wölfel, C.W. Schmidt, Exome Sequencing to Predict Neoantigens in Melanoma, Cancer Immunol Res 3(9) (2015) 992-8.
[71] T.C. Chang, R.A. Carter, Y. Li, H. Wang, M.N. Edmonson, X. Chen, P. Arnold, T.L. Geiger, G. Wu, J. Peng, M. Dyer, J.R. Downing, D.R. Green, P.G. Thomas, J. Zhang, The neoepitope landscape in pediatric cancers, Genome Med 9(1) (2017) 78.
[72] R. Lupetti, P. Pisarra, A. Verrecchia, C. Farina, G. Nicolini, A. Anichini, C. Bordignon, M. Sensi, G. Parmiani, C. Traversari, Translation of a retained intron in tyrosinase-related protein (TRP) 2 mRNA generates a new cytotoxic T lymphocyte (CTL)-defined and shared

human melanoma antigen not expressed in normal cells of the melanocytic lineage, J Exp Med 188(6) (1998) 1005-16.
[73] E.M. Inderberg, S. Wälchli, M.R. Myhre, S. Trachsel, H. Almåsbak, G. Kvalheim, G. Gaudernack, T cell therapy targeting a public neoantigen in microsatellite instable colon cancer reduces, Oncoimmunology 6(4) (2017) e1302631.
[74] M. Linnebacher, J. Gebert, W. Rudy, S. Woerner, Y.P. Yuan, P. Bork, M. von Knebel Doeberitz, Frameshift peptide-derived T-cell epitopes: a source of novel tumor-specific antigens, Int J Cancer 93(1) (2001) 6-11.
[75] J.C. Skipper, R.C. Hendrickson, P.H. Gulden, V. Brichard, A. Van Pel, Y. Chen, J. Shabanowitz, T. Wolfel, C.L. Slingluff, T. Boon, D.F. Hunt, V.H. Engelhard, An HLA-A2-restricted tyrosinase antigen on melanoma cells results from posttranslational modification and suggests a novel pathway for processing of membrane proteins, J Exp Med 183(2) (1996) 527-34.
[76] S. Hutchison, A.L. Pritchard, Identifying neoantigens for use in immunotherapy, Mamm Genome 29(11-12) (2018) 714-730.
[77] C. Xu, A review of somatic single nucleotide variant calling algorithms for next-generation sequencing data, Comput Struct Biotechnol J 16 (2018) 15-24.
[78] S. Hwang, E. Kim, I. Lee, E.M. Marcotte, Systematic comparison of variant calling pipelines using gold standard personal exome variants, Sci Rep 5 (2015) 17875.
[79] L. Ding, M.C. Wendl, J.F. McMichael, B.J. Raphael, Expanding the computational toolbox for mining cancer genomes, Nat Rev Genet 15(8) (2014) 556-70.
[80] Z.K. Liu, Y.K. Shang, Z.N. Chen, H. Bian, A three-caller pipeline for variant analysis of cancer whole-exome sequencing data, Mol Med Rep 15(5) (2017) 2489-2494.
[81] V.I. Jurtz, L.R. Olsen, Computational Methods for Identification of T Cell Neoepitopes in Tumors, Methods Mol Biol 1878 (2019) 157-172.
[82] T. Karasaki, K. Nagayama, H. Kuwano, J.I. Nitadori, M. Sato, M. Anraku, A. Hosoi, H. Matsushita, M. Takazawa, O. Ohara, J. Nakajima, K. Kakimi, Prediction and prioritization of neoantigens: integration of RNA sequencing data with whole-exome sequencing, Cancer Sci 108(2) (2017) 170-177.
[83] A.C. Smart, C.A. Margolis, H. Pimentel, M.X. He, D. Miao, D. Adeegbe, T. Fugmann, K.K. Wong, E.M. Van Allen, Intron retention is a source of neoepitopes in cancer, Nat Biotechnol 36(11) (2018) 1056-1058.
[84] S.K. Rathe, F.E. Popescu, J.E. Johnson, A.L. Watson, T.A. Marko, B.S. Moriarity, J.R. Ohlfest, D.A. Largaespada, Identification of candidate neoantigens produced by fusion transcripts in human osteosarcomas, Sci Rep 9(1) (2019) 358.
[85] A. Kahles, K.V. Lehmann, N.C. Toussaint, M. Hüser, S.G. Stark, T. Sachsenberg, O. Stegle, O. Kohlbacher, C. Sander, G. Rätsch, C.G.A.R. Network, Comprehensive Analysis of Alternative Splicing Across Tumors from 8,705 Patients, Cancer Cell 34(2) (2018) 211-224.e6.
[86] T.Y. Wang, L. Wang, S.K. Alam, L.H. Hoeppner, R. Yang, ScanNeo: identifying indel derived neoantigens using RNA-Seq data, Bioinformatics (2019).
[87] S.A. Shukla, M.S. Rooney, M. Rajasagi, G. Tiao, P.M. Dixon, M.S. Lawrence, J. Stevens, W.J. Lane, J.L. Dellagatta, S. Steelman, C. Sougnez, K. Cibulskis, A. Kiezun, N. Hacohen, V. Brusic, C.J. Wu, G. Getz, Comprehensive analysis of cancer-associated somatic mutations in class I HLA genes, Nat Biotechnol 33(11) (2015) 1152-8.
[88] A. Szolek, B. Schubert, C. Mohr, M. Sturm, M. Feldhahn, O. Kohlbacher, OptiType: precision HLA typing from next-generation sequencing data, Bioinformatics 30(23) (2014) 3310-6.
[89] A. Garcia-Garijo, C.A. Fajardo, A. Gros, Determinants for Neoantigen Identification, Front Immunol 10 (2019) 1392.

[90] A. Desrichard, A. Snyder, T.A. Chan, Cancer Neoantigens and Applications for Immunotherapy, Clin Cancer Res 22(4) (2016) 807-12.
[91] C. Lundegaard, K. Lamberth, M. Harndahl, S. Buus, O. Lund, M. Nielsen, NetMHC-3.0: accurate web accessible predictions of human, mouse and monkey MHC class I affinities for peptides of length 8-11, Nucleic Acids Res 36(Web Server issue) (2008) W509-12.
[92] V. Jurtz, S. Paul, M. Andreatta, P. Marcatili, B. Peters, M. Nielsen, NetMHCpan-4.0: Improved Peptide-MHC Class I Interaction Predictions Integrating Eluted Ligand and Peptide Binding Affinity Data, J Immunol 199(9) (2017) 3360-3368.
[93] M. Nielsen, O. Lund, S. Buus, C. Lundegaard, MHC class II epitope predictive algorithms, Immunology 130(3) (2010) 319-28.
[94] C. Lundegaard, O. Lund, C. Kesmir, S. Brunak, M. Nielsen, Modeling the adaptive immune system: predictions and simulations, Bioinformatics 23(24) (2007) 3265-75.
[95] J. Hundal, B.M. Carreno, A.A. Petti, G.P. Linette, O.L. Griffith, E.R. Mardis, M. Griffith, pVAC-Seq: A genome-guided in silico approach to identifying tumor neoantigens, Genome Med 8(1) (2016) 11.
[96] A.M. Bjerregaard, M. Nielsen, S.R. Hadrup, Z. Szallasi, A.C. Eklund, MuPeXI: prediction of neo-epitopes from tumor sequencing data, Cancer Immunol Immunother 66(9) (2017) 1123-1130.
[97] P. Bais, S. Namburi, D.M. Gatti, X. Zhang, J.H. Chuang, CloudNeo: a cloud pipeline for identifying patient-specific tumor neoantigens, Bioinformatics 33(19) (2017) 3110-3112.
[98] E. Tappeiner, F. Finotello, P. Charoentong, C. Mayer, D. Rieder, Z. Trajanoski, TIminer: NGS data mining pipeline for cancer immunology and immunotherapy, Bioinformatics 33(19) (2017) 3140-3141.
[99] M. Yadav, S. Jhunjhunwala, Q.T. Phung, P. Lupardus, J. Tanguay, S. Bumbaca, C. Franci, T.K. Cheung, J. Fritsche, T. Weinschenk, Z. Modrusan, I. Mellman, J.R. Lill, L. Delamarre, Predicting immunogenic tumour mutations by combining mass spectrometry and exome sequencing, Nature 515(7528) (2014) 572-6.
[100] M.M. Gubin, X. Zhang, H. Schuster, E. Caron, J.P. Ward, T. Noguchi, Y. Ivanova, J. Hundal, C.D. Arthur, W.J. Krebber, G.E. Mulder, M. Toebes, M.D. Vesely, S.S. Lam, A.J. Korman, J.P. Allison, G.J. Freeman, A.H. Sharpe, E.L. Pearce, T.N. Schumacher, R. Aebersold, H.G. Rammensee, C.J. Melief, E.R. Mardis, W.E. Gillanders, M.N. Artyomov, R.D. Schreiber, Checkpoint blockade cancer immunotherapy targets tumour-specific mutant antigens, Nature 515(7528) (2014) 577-81.
[101] M. Bassani-Sternberg, E. Bräunlein, R. Klar, T. Engleitner, P. Sinitcyn, S. Audehm, M. Straub, J. Weber, J. Slotta-Huspenina, K. Specht, M.E. Martignoni, A. Werner, R. Hein, D. H Busch, C. Peschel, R. Rad, J. Cox, M. Mann, A.M. Krackhardt, Direct identification of clinically relevant neoepitopes presented on native human melanoma tissue by mass spectrometry, Nat Commun 7 (2016) 13404.
[102] D.I. van der Lee, R.M. Reijmers, M.W. Honders, R.S. Hagedoorn, R.C. de Jong, M.G. Kester, D.M. van der Steen, A.H. de Ru, C. Kweekel, H.M. Bijen, I. Jedema, H. Veelken, P.A. van Veelen, M.H. Heemskerk, J.H.F. Falkenburg, M. Griffioen, Mutated nucleophosmin 1 as immunotherapy target in acute myeloid leukemia, J Clin Invest 129(2) (2019) 774-785.
[103] P. Faridi, A.W. Purcell, N.P. Croft, In Immunopeptidomics We Need a Sniper Instead of a Shotgun, Proteomics 18(12) (2018) e1700464.
[104] M. Paroli, F. Bellati, M. Videtta, C. Focaccetti, C. Mancone, T. Donato, M. Antonilli, G. Perniola, D. Accapezzato, C. Napoletano, M. Nuti, A. Bartolazzi, P.B. Panici, M. Tripodi, F. Palombo, V. Barnaba, Discovery of chemotherapy-associated ovarian cancer antigens by interrogating memory T cells, Int J Cancer 134(8) (2014) 1823-34.
[105] J. Huang, M. El-Gamil, M.E. Dudley, Y.F. Li, S.A. Rosenberg, P.F. Robbins, T cells associated with tumor regression recognize frameshifted products of the CDKN2A tumor

suppressor gene locus and a mutated HLA class I gene product, J Immunol 172(10) (2004) 6057-64.
[106] Y.C. Lu, X. Yao, Y.F. Li, M. El-Gamil, M.E. Dudley, J.C. Yang, J.R. Almeida, D.C. Douek, Y. Samuels, S.A. Rosenberg, P.F. Robbins, Mutated PPP1R3B is recognized by T cells used to treat a melanoma patient who experienced a durable complete tumor regression, J Immunol 190(12) (2013) 6034-42.
[107] N. McGranahan, A.J. Furness, R. Rosenthal, S. Ramskov, R. Lyngaa, S.K. Saini, M. Jamal-Hanjani, G.A. Wilson, N.J. Birkbak, C.T. Hiley, T.B. Watkins, S. Shafi, N. Murugaesu, R. Mitter, A.U. Akarca, J. Linares, T. Marafioti, J.Y. Henry, E.M. Van Allen, D. Miao, B. Schilling, D. Schadendorf, L.A. Garraway, V. Makarov, N.A. Rizvi, A. Snyder, M.D. Hellmann, T. Merghoub, J.D. Wolchok, S.A. Shukla, C.J. Wu, K.S. Peggs, T.A. Chan, S.R. Hadrup, S.A. Quezada, C. Swanton, Clonal neoantigens elicit T cell immunoreactivity and sensitivity to immune checkpoint blockade, Science 351(6280) (2016) 1463-9.
[108] C.J. Cohen, J.J. Gartner, M. Horovitz-Fried, K. Shamalov, K. Trebska-McGowan, V.V. Bliskovsky, M.R. Parkhurst, C. Ankri, T.D. Prickett, J.S. Crystal, Y.F. Li, M. El-Gamil, S.A. Rosenberg, P.F. Robbins, Isolation of neoantigen-specific T cells from tumor and peripheral lymphocytes, J Clin Invest 125(10) (2015) 3981-91.
[109] A.K. Bentzen, A.M. Marquard, R. Lyngaa, S.K. Saini, S. Ramskov, M. Donia, L. Such, A.J. Furness, N. McGranahan, R. Rosenthal, P.T. Straten, Z. Szallasi, I.M. Svane, C. Swanton, S.A. Quezada, S.N. Jakobsen, A.C. Eklund, S.R. Hadrup, Large-scale detection of antigen-specific T cells using peptide-MHC-I multimers labeled with DNA barcodes, Nat Biotechnol 34(10) (2016) 1037-1045.
[110] Y.C. Lu, X. Yao, J.S. Crystal, Y.F. Li, M. El-Gamil, C. Gross, L. Davis, M.E. Dudley, J.C. Yang, Y. Samuels, S.A. Rosenberg, P.F. Robbins, Efficient identification of mutated cancer antigens recognized by T cells associated with durable tumor regressions, Clin Cancer Res 20(13) (2014) 3401-10.
[111] E. Tran, M. Ahmadzadeh, Y.C. Lu, A. Gros, S. Turcotte, P.F. Robbins, J.J. Gartner, Z. Zheng, Y.F. Li, S. Ray, J.R. Wunderlich, R.P. Somerville, S.A. Rosenberg, Immunogenicity of somatic mutations in human gastrointestinal cancers, Science 350(6266) (2015) 1387-90.
[112] T.D. Prickett, J.S. Crystal, C.J. Cohen, A. Pasetto, M.R. Parkhurst, J.J. Gartner, X. Yao, R. Wang, A. Gros, Y.F. Li, M. El-Gamil, K. Trebska-McGowan, S.A. Rosenberg, P.F. Robbins, Durable Complete Response from Metastatic Melanoma after Transfer of Autologous T Cells Recognizing 10 Mutated Tumor Antigens, Cancer Immunol Res 4(8) (2016) 669-78.
[113] C. Linnemann, M.M. van Buuren, L. Bies, E.M. Verdegaal, R. Schotte, J.J. Calis, S. Behjati, A. Velds, H. Hilkmann, D.E. Atmioui, M. Visser, M.R. Stratton, J.B. Haanen, H. Spits, S.H. van der Burg, T.N. Schumacher, High-throughput epitope discovery reveals frequent recognition of neo-antigens by CD4+ T cells in human melanoma, Nat Med 21(1) (2015) 81-5.
[114] A.V. Joglekar, M.T. Leonard, J.D. Jeppson, M. Swift, G. Li, S. Wong, S. Peng, J.M. Zaretsky, J.R. Heath, A. Ribas, M.T. Bethune, D. Baltimore, T cell antigen discovery via signaling and antigen-presenting bifunctional receptors, Nat Methods 16(2) (2019) 191-198.
[115] C. Criscitiello, G. Viale, G. Curigliano, Peptide vaccines in early breast cancer, Breast 44 (2019) 128-134.
[116] D. Hammerl, M. Smid, A.M. Timmermans, S. Sleijfer, J.W.M. Martens, R. Debets, Breast cancer genomics and immuno-oncological markers to guide immune therapies, Semin Cancer Biol 52(Pt 2) (2018) 178-188.
[117] Z. Wang, W. Liu, C. Chen, X. Yang, Y. Luo, B. Zhang, Low mutation and neoantigen burden and fewer effector tumor infiltrating lymphocytes correlate with breast cancer metastasization to lymph nodes, Sci Rep 9(1) (2019) 253.

[118] P. Narang, M. Chen, A.A. Sharma, K.S. Anderson, M.A. Wilson, The neoepitope landscape of breast cancer: implications for immunotherapy, BMC Cancer 19(1) (2019) 200.
[119] T.A. Adams, P.J. Vail, A. Ruiz, M. Mollaee, P.A. McCue, E.S. Knudsen, A.K. Witkiewicz, Composite analysis of immunological and metabolic markers defines novel subtypes of triple negative breast cancer, Mod Pathol 31(2) (2018) 288-298.
[120] C. Focaccetti, V. Izzi, M. Benvenuto, S. Fazi, S. Ciuffa, M.G. Giganti, V. Potenza, V. Manzari, A. Modesti, R. Bei, Polyphenols as Immunomodulatory Compounds in the Tumor Microenvironment: Friends or Foes?, Int J Mol Sci 20(7) (2019).
[121] X. Dai, H. Cheng, Z. Bai, J. Li, Breast Cancer Cell Line Classification and Its Relevance with Breast Tumor Subtyping, J Cancer 8(16) (2017) 3131-3141.
[122] M. Moriggi, M. Giussani, E. Torretta, D. Capitanio, M. Sandri, R. Leone, S. De Palma, M. Vasso, G. Vozzi, E. Tagliabue, C. Gelfi, ECM Remodeling in Breast Cancer with Different Grade: Contribution of 2D-DIGE Proteomics, Proteomics 18(24) (2018) e1800278.
[123] A. Bergamaschi, E. Tagliabue, T. Sørlie, B. Naume, T. Triulzi, R. Orlandi, H.G. Russnes, J.M. Nesland, R. Tammi, P. Auvinen, V.M. Kosma, S. Ménard, A.L. Børresen-Dale, Extracellular matrix signature identifies breast cancer subgroups with different clinical outcome, J Pathol 214(3) (2008) 357-67.
[124] C.G.A. Network, Comprehensive molecular portraits of human breast tumours, Nature 490(7418) (2012) 61-70.
[125] C.A. Santa-Maria, T. Kato, J.H. Park, K. Kiyotani, A. Rademaker, A.N. Shah, L. Gross, L.Z. Blanco, S. Jain, L. Flaum, C. Tellez, R. Stein, R. Uthe, W.J. Gradishar, M. Cristofanilli, Y. Nakamura, F.J. Giles, A pilot study of durvalumab and tremelimumab and immunogenomic dynamics in metastatic breast cancer, Oncotarget 9(27) (2018) 18985-18996.
[126] J. Zhang, L. Shen, S.A. Johnston, Using Frameshift Peptide Arrays for Cancer Neo-Antigens Screening, Sci Rep 8(1) (2018) 17366.
[127] L. Ding, M.J. Ellis, S. Li, D.E. Larson, K. Chen, J.W. Wallis, C.C. Harris, M.D. McLellan, R.S. Fulton, L.L. Fulton, R.M. Abbott, J. Hoog, D.J. Dooling, D.C. Koboldt, H. Schmidt, J. Kalicki, Q. Zhang, L. Chen, L. Lin, M.C. Wendl, J.F. McMichael, V.J. Magrini, L. Cook, S.D. McGrath, T.L. Vickery, E. Appelbaum, K. Deschryver, S. Davies, T. Guintoli, R. Crowder, Y. Tao, J.E. Snider, S.M. Smith, A.F. Dukes, G.E. Sanderson, C.S. Pohl, K.D. Delehaunty, C.C. Fronick, K.A. Pape, J.S. Reed, J.S. Robinson, J.S. Hodges, W. Schierding, N.D. Dees, D. Shen, D.P. Locke, M.E. Wiechert, J.M. Eldred, J.B. Peck, B.J. Oberkfell, J.T. Lolofie, F. Du, A.E. Hawkins, M.D. O'Laughlin, K.E. Bernard, M. Cunningham, G. Elliott, M.D. Mason, D.M. Thompson, J.L. Ivanovich, P.J. Goodfellow, C.M. Perou, G.M. Weinstock, R. Aft, M. Watson, T.J. Ley, R.K. Wilson, E.R. Mardis, Genome remodelling in a basal-like breast cancer metastasis and xenograft, Nature 464(7291) (2010) 999-1005.
[128] S.P. Shah, R.D. Morin, J. Khattra, L. Prentice, T. Pugh, A. Burleigh, A. Delaney, K. Gelmon, R. Guliany, J. Senz, C. Steidl, R.A. Holt, S. Jones, M. Sun, G. Leung, R. Moore, T. Severson, G.A. Taylor, A.E. Teschendorff, K. Tse, G. Turashvili, R. Varhol, R.L. Warren, P. Watson, Y. Zhao, C. Caldas, D. Huntsman, M. Hirst, M.A. Marra, S. Aparicio, Mutational evolution in a lobular breast tumour profiled at single nucleotide resolution, Nature 461(7265) (2009) 809-13.
[129] P.J. Stephens, P.S. Tarpey, H. Davies, P. Van Loo, C. Greenman, D.C. Wedge, S. Nik-Zainal, S. Martin, I. Varela, G.R. Bignell, L.R. Yates, E. Papaemmanuil, D. Beare, A. Butler, A. Cheverton, J. Gamble, J. Hinton, M. Jia, A. Jayakumar, D. Jones, C. Latimer, K.W. Lau, S. McLaren, D.J. McBride, A. Menzies, L. Mudie, K. Raine, R. Rad, M.S. Chapman, J. Teague, D. Easton, A. Langerød, M.T. Lee, C.Y. Shen, B.T. Tee, B.W. Huimin, A. Broeks, A.C. Vargas, G. Turashvili, J. Martens, A. Fatima, P. Miron, S.F. Chin, G. Thomas, S. Boyault, O. Mariani, S.R. Lakhani, M. van de Vijver, L. van 't Veer, J. Foekens, C. Desmedt, C. Sotiriou, A. Tutt, C. Caldas, J.S. Reis-Filho, S.A. Aparicio, A.V. Salomon, A.L. Børresen-Dale, A.L. Richardson, P.J. Campbell, P.A. Futreal, M.R. Stratton, O.B.C.C. (OSBREAC), The

landscape of cancer genes and mutational processes in breast cancer, Nature 486(7403) (2012) 400-4.
[130] X. Zhang, S. Kim, J. Hundal, J.M. Herndon, S. Li, A.A. Petti, S.D. Soysal, L. Li, M.D. McLellan, J. Hoog, T. Primeau, N. Myers, T.L. Vickery, M. Sturmoski, I.S. Hagemann, C.A. Miller, M.J. Ellis, E.R. Mardis, T. Hansen, T.P. Fleming, S.P. Goedegebuure, W.E. Gillanders, Breast Cancer Neoantigens Can Induce CD8, Cancer Immunol Res 5(7) (2017) 516-523.
[131] A.R. Young, J.D.G. Duarte, R. Coulson, M. O'Brien, S. Deb, A. Lopata, A. Behren, S. Mathivanan, E. Lim, E. Meeusen, Immunoprofiling of Breast Cancer Antigens Using Antibodies Derived from Local Lymph Nodes, Cancers (Basel) 11(5) (2019).
[132] S. Turajlic, K. Litchfield, H. Xu, R. Rosenthal, N. McGranahan, J.L. Reading, Y.N.S. Wong, A. Rowan, N. Kanu, M. Al Bakir, T. Chambers, R. Salgado, P. Savas, S. Loi, N.J. Birkbak, L. Sansregret, M. Gore, J. Larkin, S.A. Quezada, C. Swanton, Insertion-and-deletion-derived tumour-specific neoantigens and the immunogenic phenotype: a pan-cancer analysis, Lancet Oncol 18(8) (2017) 1009-1021.
[133] L. Li, S.P. Goedegebuure, W.E. Gillanders, Preclinical and clinical development of neoantigen vaccines, Ann Oncol 28(suppl_12) (2017) xii11-xii17.
[134] K.A. McDonald, T. Kawaguchi, Q. Qi, X. Peng, M. Asaoka, J. Young, M. Opyrchal, L. Yan, S. Patnaik, E. Otsuji, K. Takabe, Tumor Heterogeneity Correlates with Less Immune Response and Worse Survival in Breast Cancer Patients, Ann Surg Oncol 26(7) (2019) 2191-2199.
[135] L. Aurisicchio, E. Salvatori, L. Lione, S. Bandini, M. Pallocca, R. Maggio, M. Fanciulli, F. De Nicola, F. Goeman, G. Ciliberto, A. Conforti, L. Luberto, F. Palombo, Poly-specific neoantigen-targeted cancer vaccines delay patient derived tumor growth, J Exp Clin Cancer Res 38(1) (2019) 78.
[136] J.L. Tanyi, S. Bobisse, E. Ophir, S. Tuyaerts, A. Roberti, R. Genolet, P. Baumgartner, B.J. Stevenson, C. Iseli, D. Dangaj, B. Czerniecki, A. Semilietof, J. Racle, A. Michel, I. Xenarios, C. Chiang, D.S. Monos, D.A. Torigian, H.L. Nisenbaum, O. Michielin, C.H. June, B.L. Levine, D.J. Powell, D. Gfeller, R. Mick, U. Dafni, V. Zoete, A. Harari, G. Coukos, L.E. Kandalaft, Personalized cancer vaccine effectively mobilizes antitumor T cell immunity in ovarian cancer, Sci Transl Med 10(436) (2018).
[137] P.A. Ott, Z. Hu, D.B. Keskin, S.A. Shukla, J. Sun, D.J. Bozym, W. Zhang, A. Luoma, A. Giobbie-Hurder, L. Peter, C. Chen, O. Olive, T.A. Carter, S. Li, D.J. Lieb, T. Eisenhaure, E. Gjini, J. Stevens, W.J. Lane, I. Javeri, K. Nellaiappan, A.M. Salazar, H. Daley, M. Seaman, E.I. Buchbinder, C.H. Yoon, M. Harden, N. Lennon, S. Gabriel, S.J. Rodig, D.H. Barouch, J.C. Aster, G. Getz, K. Wucherpfennig, D. Neuberg, J. Ritz, E.S. Lander, E.F. Fritsch, N. Hacohen, C.J. Wu, An immunogenic personal neoantigen vaccine for patients with melanoma, Nature 547(7662) (2017) 217-221.
[138] T.M. Johanns, C.A. Miller, C.J. Liu, R.J. Perrin, D. Bender, D.K. Kobayashi, J.L. Campian, M.R. Chicoine, R.G. Dacey, J. Huang, E.F. Fritsch, W.E. Gillanders, M.N. Artyomov, E.R. Mardis, R.D. Schreiber, G.P. Dunn, Detection of neoantigen-specific T cells following a personalized vaccine in a patient with glioblastoma, Oncoimmunology 8(4) (2019) e1561106.

Figure legends

Figure 1. Classification of tumor antigens. Tumor antigens can be divided in Tumor Associated Antigens (TAAs) and in Tumor Specific Antigens (TSAs). More common antigens are indicated in each section while type of events causing mutations are listed in the Neoantigens section. Distribution of each category of antigens (public, shared, private) is also indicated. Abbreviations: Nonsynonymous single nucleotide variants (nsSNVs), Insertion/deletions (INDELs), Open reading frame (ORF).

Figure 2. The bottleneck of neoantigens discovery. The discovery of neoantigens is a multistep process that comprises the sequencing of samples (tumor *vs* normal), the prediction of peptide processing and of HLA binding followed by immunological assays or selection of antigen specific cells. Main softwares and assays applied in each steps are listed next to the appropriate section. Moreover some software integrating all step for neoantigen identification are reported.

Table 1. Ongoing clinical trials on Breast Cancer neoantigens.

Identifier (study phase; location countries)	Title	Condition or disease	Date of Submission; Estimated Completion Date	• Estimated patients • Recruitment Status	• Primary Outcome; • Secondary Outcome
NCT02316457 (Phase 1; Belgium, Germany, Sweden)	RNA-Immunotherapy of IVAC_W_bre1_uID and IVAC_M_uID (TNBC-MERIT)	TNBC	Dec 15, 2014; Dec 2019	• 39 • Recruiting	• Safety; • Immunogenicity
NCT02348320 (Phase 1; USA)	Safety and Immunogenicity of a Personalized Polyepitope DNA Vaccine Strategy in Breast Cancer Patients With Persistent Triple-Negative Disease Following Neoadjuvant Chemotherapy	TNBC	Jan 21, 2015; Jun 30, 2020	• 30 • Recruiting	• Safety; • Immunogenicity
NCT02427581 (Phase 1; USA)	Safety and Immunogenicity of a Personalized Synthetic Long Peptide Breast Cancer Vaccine Strategy in Patients With Persistent Triple-Negative Breast Cancer Following Neoadjuvant Chemotherapy	TNBC	Apr 22, 2015; Aug 31, 2020	• 15 • Recruiting	• Safety; • Immunogenicity
NCT02721043 (Phase 1; USA)	Safety and Immunogenicity of Personalized Genomic Vaccine to Treat Malignancies	Solid Tumors	Feb 16, 2016; Jul 2020	• 20 • Active, not recruiting	• Dose-limiting toxicities
NCT02831634 (n.a.; France)	A Feasibility Study to Identify T-cell Responses to Neoepitopes in Tumor Invaded Lymph Nodes (NeoEpitope)	BRCA (and other)	Jul 11, 2016; Mar 1, 2018	• 25 • Completed	• Feasibility; • Immunogenicity
NCT02883062 (Phase 2; USA)	Carboplatin and Paclitaxel With or Without Atezolizumab Before Surgery in Treating Patients With Newly Diagnosed, Stage II-III Triple-Negative Breast Cancer	BRCA	Aug 30, 2016; Jul 1, 2020	• 72 • Recruiting	• Increase in TIL percentage; • Increase in pCR rate
NCT03199040 (Phase 1; USA)	Neoantigen DNA Vaccine Alone *vs* Neoantigen DNA Vaccine Plus Durvalumab in Triple Negative Breast Cancer Patients Following Standard of Care Therapy	TNBC	Jun 26, 2017; Sep 30, 2020	• 24 • Recruiting	• Safety; • Immunogenicity
NCT03412877 (Phase 2; USA)	Administration of Autologous T-Cells Genetically Engineered to Express T-Cell Receptors Reactive Against Mutated Neoantigens in People With Metastatic Cancer	BRCA (and other)	Jan 29, 2018; Mar 23, 2027	• 210 • Recruiting	• Response rate; • Immunogenicity, survival of engineered cells

NCT03552718 (Phase 1; USA)	QUILT-2.025 NANT Neoepitope Yeast Vaccine (YE-NEO-001): Adjuvant Immunotherapy Using a Personalized Neoepitope Yeast-Based Vaccine To Induce T-Cell Responses In Subjects with Previously Treated Cancers	BRCA (and other)	Jun 12, 2018; Dec 30, 2019	• 16 • Recruiting	• Safety; • Recurrence rate; DFS, OS, PFS
NCT03606967 (Phase 2; USA)	Nab-Paclitaxel and Durvalumab With or Without Neoantigen Vaccine in Treating Patients With Metastatic Triple Negative Breast Cancer	BRCA	Jul 30, 2018; Jul 31, 2019	• 70 • Not yet recruiting	• PFS; • Safety, Clinical RR, CBR, OS
NCT03970382 (Phase 1; USA)	A Study of Gene Edited Autologous Neoantigen Targeted TCR T-Cells With or Without Anti-PD-1 in Patients With Solid Tumors	BRCA (and other)	May 31, 2019; Dec 2020	• 148 • Recruiting	• Safety; • Cmax of NeoTCR-P1 in the PB; OS, PFS

Abbreviations: n.a. - not applicable; TNBC – triple negative breast cancer; BRCA –breast cancer ; DFS – disease free survival; OS – overall survival; PFS – progression free survival; pCR - pathologic complete response; RR – response rate; CBR – clinical benefit rate; TIL – tumor infiltrating lymphocytes; PB – peripheral blood; Cmax – maximum concentration.

Figure 1. Classification of tumor antigens.

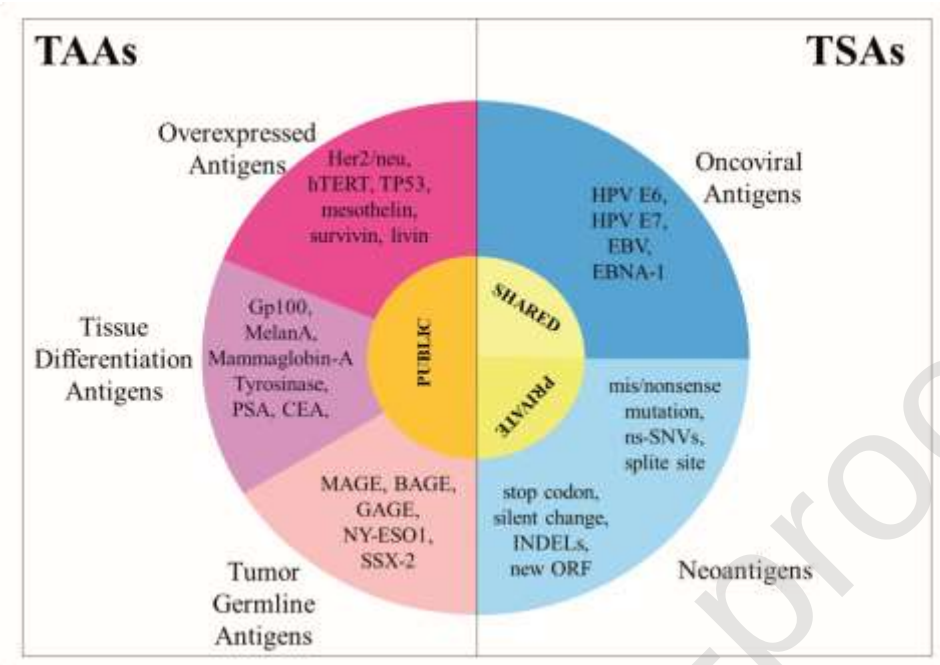

Figure 2. The bottleneck of neoantigens discovery.