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Review

Shared neoantigens for cancer immunotherapy

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Exploration of neoantigens holds the potential to be productive in immuno-oncotherapy. Among tumor-specific antigens, neoantigens result from genetic instability that gives rise to non-synonymous somatic mutations, highly specific to tumor cells. In addition to point mutations, gene rearrangements, indels leading to frameshifts, chromosomal translocations or inversions that may lead to fusion proteins, alternative mRNA splicing, and integration of genetic material of oncogenic viruses into the host genome provide consistent sources of neoantigens that are absent in healthy tissues. Out of these alterations, 2%–3% may generate T cell neoepitopes, possibly detectable by TCRs. Neoantigens are absent in healthy tissues and are thus at low risk of triggering autoimmunity. In addition, the host lymphocytes have not been rendered tolerant toward them and it is possible to induce immune responses against them. Here, we overview the two categories of neoantigens, i.e., private and shared, and their use in immuno-oncotherapy in selected pre-clinical and clinical studies. The vast majority of commonly occurring tumor-specific mutations are cancer causing and are permanently expressed by all malignant tumor cells, preventing the latter from escaping vaccine-induced anti-neoantigen immunity. The use of public neoantigens combined with efficient vaccine platforms can provide non-personalized "off-the-shelf" therapeutic vaccine candidates for broad-spectrum immunotherapy purposes.

INTRODUCTION

Responsible for ~10 million deaths a year, cancer is the second cause of death in the world. In 2020, the most currently detected cancers were those of breast, lung, colorectal, and prostate.[1] Key immuno-oncotherapy developments currently providing new therapeutic options against solid and hematologic malignancies involve advances in cytokine treatment, immune checkpoint inhibitors, monoclonal antibodies, bispecific T cell engagers, adoptive transfer of chimeric antigen receptor (CAR)-T and T cell receptor (TCR)-T cells and therapeutic cancer vaccines. (1) Cytokines are major regulators of innate and adaptive immunity and allow cell crosstalk and activation of the immune system of cancer patients.[2] (2) Immune checkpoints prevent excessive immune responses. By promoting an immunosuppressive microenvironment, tumor cells can induce expression of immune checkpoint elements on T cells, thereby counteracting their effector functions. Immune checkpoint inhibitors can thus unleash T cells at given stages of their continuous differentiation and rejuvenate the T cells that have been primarily initiated by specific antigen presentation and appropriate co-stimulation.[3] Immune checkpoint inhibitors can also help initiation of T cell triggering in the secondary lymphoid organs, before T cells migrate to the tumors.[4] (3) Monoclonal antibodies can directly target tumor cells by recognizing the surface tumor antigens and kill them via antibody-dependent cell cytotoxicity.[5] (4) Bispecific T cell engagers recruit T cells to tumor cells, usually by targeting simultaneously CD3 and a tumor surface antigen.[6] (5) TCR-T or CAR-T cells are engineered to harbor selected TCR or surface chimeric antibodies built to specifically target tumor antigens, respectively detecting tumor-specific epitopes in the context of major histocompatibility complex (MHC) molecules or membrane-associated antigens.[7] (6) Therapeutic cancer vaccines activate the patient's adaptive immune cells by delivering relevant tumor antigens to the immune system through the use of appropriate immunization strategies. Among these approaches, antigen-specific immuno-oncotherapies comprising TCR-T or CAR-T cell therapies and cancer vaccines are based on two distinct categories of tumor antigens: tumor-associated antigens (TAAs) and tumor-specific antigens (TSAs).[8,9]

TAAs are self-proteins, resulting from transcription of germline DNA. Over-expressed by tumor cells, TAAs are usually absent or rarely expressed in healthy tissues, at least after the perinatal life.[10] Mature T cells can be unresponsive to TAAs as a result of the thymic or peripheral immune tolerance against self-components. However, TAAs may be detected by adaptive immune cells because of their unusually high, atypical, or mis-localized expression by malignant cells. Active induction of adaptive immunity against TAAs counteracts immune tolerance, and therefore may entail risks of off-tumor (multi)organ autoimmunity. Typical TAA categories are (1) antigens that are over-expressed and required for tumor survival, like human epidermal growth factor receptor (EGFR), human epidermal growth factor receptor-2 (HER2), and carcino-embryonic antigens (CEACAMs); (2) antigens with cell type- or tissue-specific expression profile, like tyrosinase in melanoma cells; and (3) cancer-testis antigens, which are normally only expressed by gametes and trophoblasts, epigenetically silenced at adulthood, but aberrantly re-expressed in several cancer types, like the strongly immunogenic 5T4 oncofetal antigen and New York esophageal squamous cell carcinoma 1 (NY-ESO-1).

In contrast to TAA, TSAs result most frequently from somatic mutations, are highly specific to tumor cells, and notably include

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neoantigens.[11] During carcinogenesis, genetic instability gives rise to somatic mutations both in non-coding and coding chromosome regions. Non-synonymous mutations in the coding DNA regions generate aberrant mutated neoantigens. In addition to point mutations accumulated during oncogenesis, gene rearrangements, indels leading to frameshifts, chromosomal translocations or inversions leading to fusion proteins, and alternative mRNA splicing can also provide consistent sources of neoantigens.[12] Likewise, oncogenic viruses, comprising papillomaviruses, Epstein-Barr virus, Kaposi's sarcoma-associated herpesvirus, human T cell lymphotropic virus, and hepatitis B and C viruses can also generate neoantigens due to the integration of their genetic material into the host genome.[13] The rise of new technologies driven by new-generation sequencing, bioinformatics, cancer atlas, epitopes prediction tools, and mass spectroscopy of MHC-bound peptides continuously improves identification of potential neoantigens as targets for immuno-oncotherapy.

Although the use of neoantigens in immuno-oncotherapy is promising, their clinical application still faces major limitations. Indeed, among the large number of tumor mutations, only a very small percentage generate neoepitopes capable of being presented by MHC molecules and eventually detected by TCRs. Moreover, as neoantigens closely resemble the self-proteome, they can only generate low-affinity TCRs, since high-affinity TCRs capable of efficiently recognizing self-antigens have been eliminated by central and/or peripheral T cell tolerance mechanisms. Immunotherapy against personal neoantigens (see below) presents an additional challenge; their mosaic intratumoral expression profile requires the incorporation of an appropriate assortment of several neoantigens into the immunotherapy strategy to achieve an acceptable coverage of neoantigen diversity. Considering the anarchic expression profiles of personal neoantigens, it is uncertain whether induced T cells could achieve a significant level of tumor growth control. Moreover, substantial HLA polymorphism also makes neoantigen design a difficult task, which in some cases could mean taking into account only the HLA alleles most frequently represented in human populations.

Here, we overview the two categories of neoantigens, i.e., personal and public neoantigens, and their use in immuno-oncotherapy in selected pre-clinical and clinical studies.

Neoantigens

In the last decade, genome/exome sequencing demonstrated that, during tumorigenesis, cells are able to acquire a mutanome of tens to thousands of somatic mutations.[14] Among them, only a few are driver mutations or immune-escape mutations that confer growth and/or fitness advantages to the cancer cells. Malignancies with large mutanomes leading to high neoantigen burden, like melanoma and lung cancers, or those with microsatellite instability, like colorectal, gastrointestinal, and endometrial cancers, are more prone to induce efficient anti-tumor T cell immunity. However, of the large number of mutations that occur in tumor cells, only ~3% give rise to neoepitopes. Those are properly processed by antigen presentation machineries and able to bind with good affinity to relevant MHC molecules to ultimately allow for their possible detection by appropriate TCRs.[15] Emergence of neoepitopes is based on the generation of anchor amino-acid residues, required for neopeptides to fit into the groove of the MHC molecules to be presented at the surface of tumor cells.[16,17] Alternatively, neoepitopes can also be generated or enhanced by point mutations leading to non-anchor amino-acid substitutions, usually localized at the central positions of the neoepitopes that point toward the TCR contact sites.[18] Whether anchor or non-anchor residues generate the more strongly immunogenic neoepitopes remains debatable.

Neoantigen-based immuno-oncotherapy relies either on triggering naive T cells or on amplifying pre-existing endogenous T cells. Since neoantigens are not germline and not expressed in healthy tissues, the specific T cells able to recognize them should theoretically neither be affected by central or peripheral tolerance mechanisms nor expected to trigger autoimmunity.[19–21] The increased relative affinity of the mutated neopeptide compared to the wild-type self-peptide can be determinant in the strength of neoantigen T cell immunogenicity.[22] Based on these properties, neoantigens are currently thought to be able to trigger specific T cells with potent on-target and without off-tumor effector functions, which amply justifies their exploration in cancer immunotherapies.

PERSONAL NEOANTIGENS

Tumor genomes are immune edited as a result of an active immune surveillance involving malignant cell interaction with the immune system. In fact, malignant cells able to activate the adaptive immunity are progressively eliminated until the tumor immunoediting leads to generation of cells expressing neoantigens that are not, or are only barely, recognized by the immune cells. These personal or private neoantigens, which are non-annotated in databases, are totally specific to a given tumor or, more often, to emerging tumor sub-clones. This results in clonal evolution and a mosaic expression profile of personal neoantigens, usually generating an intra-tumor heterogeneity. Consequently, vaccine candidates with the potential to target multiple neoantigens selected from an individual neoantigen repertoire appear to be best suited to induce immunity, for they can reach the greatest possible number of tumor cell sub-populations. However, identifying relevant neoantigens is challenging. In fact, it has to be considered that, under the immune selective pressure, the tumor clones that lack neoantigens targeted by the initial immune response may evade or resist to the T cell responses. Therefore, in the context of an incessantly evolving mutational landscape, many questions challenge the current paradigm, notably whether personal neoantigens, not expressed by all cells of a tumor, are appropriate targets for an effective and sustainable immunotherapy.[23] It is difficult to estimate the proportion of cells that must express the targeted neoantigens within a tumor for the expected immunotherapy effect to become significant.

Even though personalized cancer vaccines have shown some encouraging clinical results, their development is costly and logistically laborious. In fact, such vaccines necessitate identifying suitable

personal neoantigens by (1) tumor DNA/RNA sequencing to establish the unique mutanome of individual patients, (2) epitope prediction for HLA binding, (3) identification of epitope-flanking regions that possibly facilitate antigen processing, and (4) verification of non-identity with self-proteome.[18] Importantly, it is not guaranteed that all neoepitopes identified by genome/exome sequencing and prediction algorithms are indeed presented by MHC molecules. In fact, this approach alone leads to high frequencies of false-positive predicted neoepitopes. Consequently, the DNA/RNA sequencing methods can be advantageously complemented by immunopeptidomic analyses, based on immunoprecipitation of tumor cell MHC molecules and sequencing of co-immunoprecipitated peptides that are actually presented by the tumor cell presentation machineries.[24] An additional degree of difficulty arises from the fact that this strategy requires personalized Good Manufacturing Practices (GMP) vaccine production, during the short 3- to 4-week post-biopsy/surgery period.

Selected preclinical immunotherapies targeting personal neoantigens

One of the pioneer preclinical studies in the domain of personal neoantigens concerned identification of a unique tumor mutated antigen, i.e., an L47H substitution in the L9 ribosomal protein that was only expressed by the UV-induced 6132A murine tumor. This neoantigen was characterized by its largely exacerbated $CD4^+$ T cell-stimulatory property compared to the wild-type peptide.[25] Subsequently, an MHC-II (I-E^d)-restricted epitope derived from the L11 ribosomal protein was identified in a chemically induced murine fibrosarcoma that harbored a single N-to-H point substitution. Immunization of BALB/c mice with only the mutated epitope protected against a challenge with the same fibrosarcoma.[26] Later, exome next-generation sequencing of the B16F10 murine melanoma cells identified 962 somatic point mutations, with 58% of the mutanome occurring in the expressed genes.[27] One-third of 50 mutated peptides tested *in vivo* were immunogenic in mice, with 60% of them inducing T cell responses, preferentially against the mutated rather than the wild-type epitopes. Further immuno-oncotherapy experiments established the extent of the immunogenicity of nonsynonymous mutations and qualified mutated epitopes, based on single-amino-acid substitutions, as potential immuno-oncotherapy candidates.[27] In another study, immunization with identified tumor-specific mutant proteins along with anti-programmed cell death-1 (anti-PD-1) and/or anti-cytotoxic T-lymphocyte associated protein-4 (anti-CTLA-4) therapy showed the anti-tumor efficacy of therapeutic vaccines based on synthetic peptides in murine tumor models.[16,28]

Other authors also sought to make the identification of immunogenic neoepitopes more efficient. To this end, they combined the full-exome sequencing and transcriptome studies to mass spectrometric analysis of MHC-I-presented peptides in the MC38 colorectal and TRAMP-C1 prostate murine tumors. Interestingly, of ~1,300 amino-acid point mutations identified by sequencing, only 13% had anchor residues to bind to MHC-I molecules, a small fraction of which were confirmed by mass spectrometry as actually presented by MHC-I on the surface of MC38 or TRAMP-C1 cells. Vaccination of mice with peptides identified with this approach induced T cell responses associated with anti-tumor activity *in vivo*.[29]

Selected immunotherapy clinical trials based on personal neoantigens

To date, >150 neoantigen-based immuno-oncotherapy clinical trials have been initiated.[30] So far, personalized neoantigen therapies, mainly based on adjuvanted peptides, showed weak performances despite their administration together with chemo- and/or immune checkpoint inhibitor therapies. A phase Ib clinical trial of a personalized neoantigen vaccine, called NEO-PV-01, in combination with an antagonist anti-PD-1, showed good safety but only partial responses.[31] In patients with advanced melanoma, non-small cell lung cancer (NSCLC), or bladder cancer, examples of personalized neoantigen-based vaccines contained up to 20 peptides, typically 14–25 mers, selected for their predicted affinity to bind HLA-A or -B. During the design and production of the vaccine, patients were treated with anti-PD-1 (nivolumab) for 12 weeks. From week 12 on, the peptide vaccine formulated with the TLR3 agonist polyinosinic-polycytidylic acid stabilized with poly-L-lysine (poly-ICLC) adjuvant[32] was administered subcutaneously over 3 months, with five prime injections followed by two boost shots with selected neoantigen combinations. Neoantigen-specific T cells have been shown to migrate to the tumors and display cytotoxic anti-tumor effects. Among the vaccinated patients, the objective response rate (ORR) at 12 months was 59%, 39%, and 27%, respectively in melanoma, NSCLC, and bladder cancer patients. The overall survival (OS) at 12 months was 93%, 83%, and 67%, respectively, in these cohorts. Although patients continued to receive anti-PD-1 treatment concurrently with vaccination, the PD-L1 status did not correlate with ORR in any cohort. The 1-year OS rate in all cohorts was comparable to that of anti-PD-1 monotherapy. Therefore, it was difficult to decipher the specific effect of the neoantigen vaccine in this study. Post-treatment analysis of peripheral blood mononuclear cells (PBMCs) of melanoma patients with >9-month progression-free survival (PFS) showed an increased frequency of effector memory T cells and fewer naive T cells. Accordingly, after the treatment, the TCR repertoire evolved toward more clonality in the cohort with >9-month PFS. Higher frequencies of transcription factor 7 $(TCF7)^+$ $CD8^+$ tumor-infiltrating lymphocytes (TILs), which are the best correlate of response to anti-PD-1 treatments, were also observed in these patients. Of the 193 neopeptides tested, 58% induced $CD4^+$ or $CD8^+$ T cell activation, suggesting that the vaccine induced a specific and durable response mediated by both MHC-I and -II antigen presentation.[31]

In another phase Ib clinical trial, patients with non-squamous NSCLC were treated with NEO-PV-01 in combination with pemetrexed and carboplatin chemotherapy and anti-PD-1 pembrolizumab immunotherapy.[33] The treatment was well tolerated in a vast majority of patients. Up to 20 unique peptides were used for vaccination and 55% of them generated T cell responses. Both $CD4^+$

and CD8$^+$ T cell responses were respectively detected against 39% and 34% of epitopes. In addition, the treatment induced "epitope spreading," i.e., induction of T cell immunity against non-vaccinating neoantigens, notably specific to the Kirsten rat sarcoma virus (KRAS) shared neoantigen (see below), positively associated with extended PFS.[33]

A therapeutic vaccination using BioNTech's mRNA technology called autogene cevumeran has also been performed in a phase I clinical trial in patients with pancreatic ductal adenocarcinoma, in which mutation-derived neoantigens are suitable for immunotherapy.[34] From surgically resected tumors, up to 20 MHC-I or -II restricted neoantigens per patient were identified to generate individualized mRNA-based vaccines. Following surgery, patients were first administered with anti-PD-L1 (atezolizumab), followed by an eight-injection cycle of autogene cevumeran prime, and then followed by four-drug chemotherapy, before a booster injection. This vaccination regimen induced *de novo* neoantigen-specific T cells in eight of 16 patients, 50% of whom targeted more than one neoantigen included in the personalized vaccine. The T cell responses were amplified after the boost and the expanded T cells included neoantigen-specific polyfunctional effector CD8$^+$—but not CD4$^+$—T cells. The vaccine-responder patients had a longer mean recurrence-free survival than non-responders. However, these results should be considered with caution, as responders and non-responders had different overall clinical profiles. One can find among them different mean primary tumor sizes, varying statuses regarding pancreato-duodenectomy vs. distal pancreatectomy associated with splenectomy, and perhaps most confoundingly different pancreatic cancer stages.[34] In addition, with personalized neoantigens, which may not be expressed by all cells in a tumor, the tumor immune escape is inevitable. Extensive large-scale trials are required to confirm long-term efficacy of such mRNA-based immunotherapy approaches.[35]

A recent phase IIb clinical trial compared anti-PD-1 (pembrolizumab) monotherapy to combination of pembrolizumab and personal neoantigen mRNA-4157 vaccine from Moderna in patients with resected high-risk, stages IIIB-IV cutaneous melanoma.[36] Patients received pembrolizumab followed by vaccination with nine doses of mRNA-4157 encoding up to 34 personal neoantigens. With very few adverse effects, combinational treatment showed an improvement in both recurrence-free survival and distant metastasis-free survival.[36]

SHARED NEOANTIGENS

Shared neoantigens result from mutated antigens that are common in cancer patients and thus can be available as "off-the-shelf" vaccines and potentially used as broad-spectrum immuno-therapies. The vast majority of the commonly occurring tumor-specific mutations are cancer causing and essential to malignancy. In fact, global genome analyses of human cancers identified ~300 cancer-initiating genes and indicated that ~57% of the analyzed tumors possess potentially actionable oncogenic event.[37] Therefore, in contrast to personal neoantigens characterized by their mosaic expression within tumors, the shared neoantigens offer the advantage of avoiding mosaicism, loss of targeted antigen, and immune evasion. Like personal neoantigens, these public mutations may generate T cell epitopes that are absent in normal and healthy tissues.[38] The adaptive immune system has not been rendered tolerant to these antigens via central or peripheral tolerance mechanisms and therefore they are at low risk of inducing autoimmunity.[29,39] Among the most attractive public neoantigens are those generated by somatic mutations or indels in p53 transcriptional factor, KRAS, V-Raf murine sarcoma viral oncogene homolog B (BRAF), epidermal growth factor receptor (EGFR), human epidermal growth factor receptor 2 (HER2), phosphoinositide 3-kinases (PI3Ks), and TCRγ chain alternate reading frame protein (TARP), which we review below with a view to designing off-the-shelf immuno-oncotherapies (Figures 1 and 2; Table 1). Obviously, like personal neoantigens, the good T cell immunogenicity of these shared mutated neoantigens is determined by the potential of their common neoepitopes to be presented by the restricting elements of the patients, depending on their HLA haplotype.

Mutated p53 neoepitopes

Activation of the p53 transcriptional factor drastically halts the development of premalignant or malignant cells by responding to outside stress factors including hypoxia, DNA damage, oxidative stress, hyperproliferative signals, nutrient deficiency, or action of oncogenes.[40,41] p53 operates via induction of growth arrest, senescence and apoptosis, modulation of tumor stroma, angiogenesis, metabolism, and blockade of immuno-surveillance, resulting in invasion and metastasis.[42] Mutations in p53 are the most prevalent in various tumor types, as demonstrated by the unbiased sequencing of thousands of cancer genomes.[43,44] From 4,928 tumor samples tested in 48 independent projects, the National Cancer Institute data portal (https://portal.gdc.cancer.gov/genes/ENSG00000141510) identified 1,342 mutations spread across several p53 domains. Importantly, ~75% of all p53 mutations were common, missense, and localized in its DNA binding domain (DBD).[45] Among them, 30% belong to hotspot mutations, including R175, G245, R248, R249, R273, and R282, which occur at ~50% across all human cancer types.[45] These DBD mutations reduce the ability of p53 to bind DNA and abolish the transactivation of its target genes. A number of studies have shown that p53 "hotspot" mutations such as R175H, Y220C, and R248W are immunogenic and can be recognized by human T cells, as shared neoepitopes.[46,47]

Adoptive cellular therapy targeting p53 mutations achieved durable clinical responses in patients with advanced solid cancers.[48] A simple expansion of TILs, naturally reactive against p53 mutations, resulted in partial response in only two patients among 12. In addition, transfusion of patients with peripheral blood lymphocytes transduced with an allogenic TCR specific for p53 R175H neoepitope resulted in objective tumor regression in a patient with chemo-refractory breast cancer.[48]

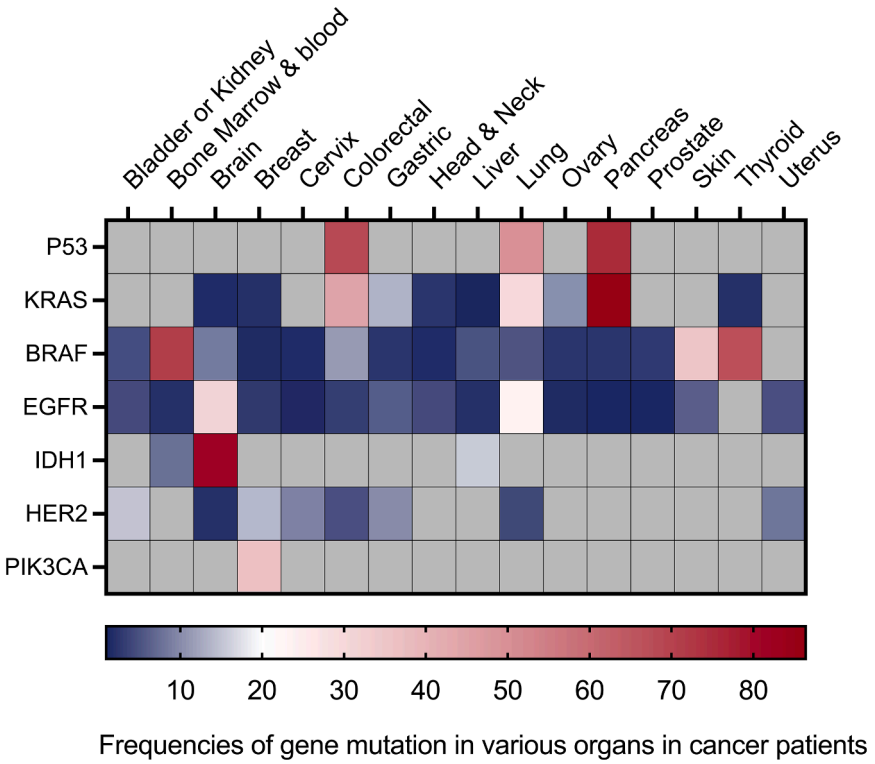


Figure 1. Frequencies of gene alterations in the relevant oncoproteins that can be potential shared targets of immuno-oncotherapy in diverse cancer types

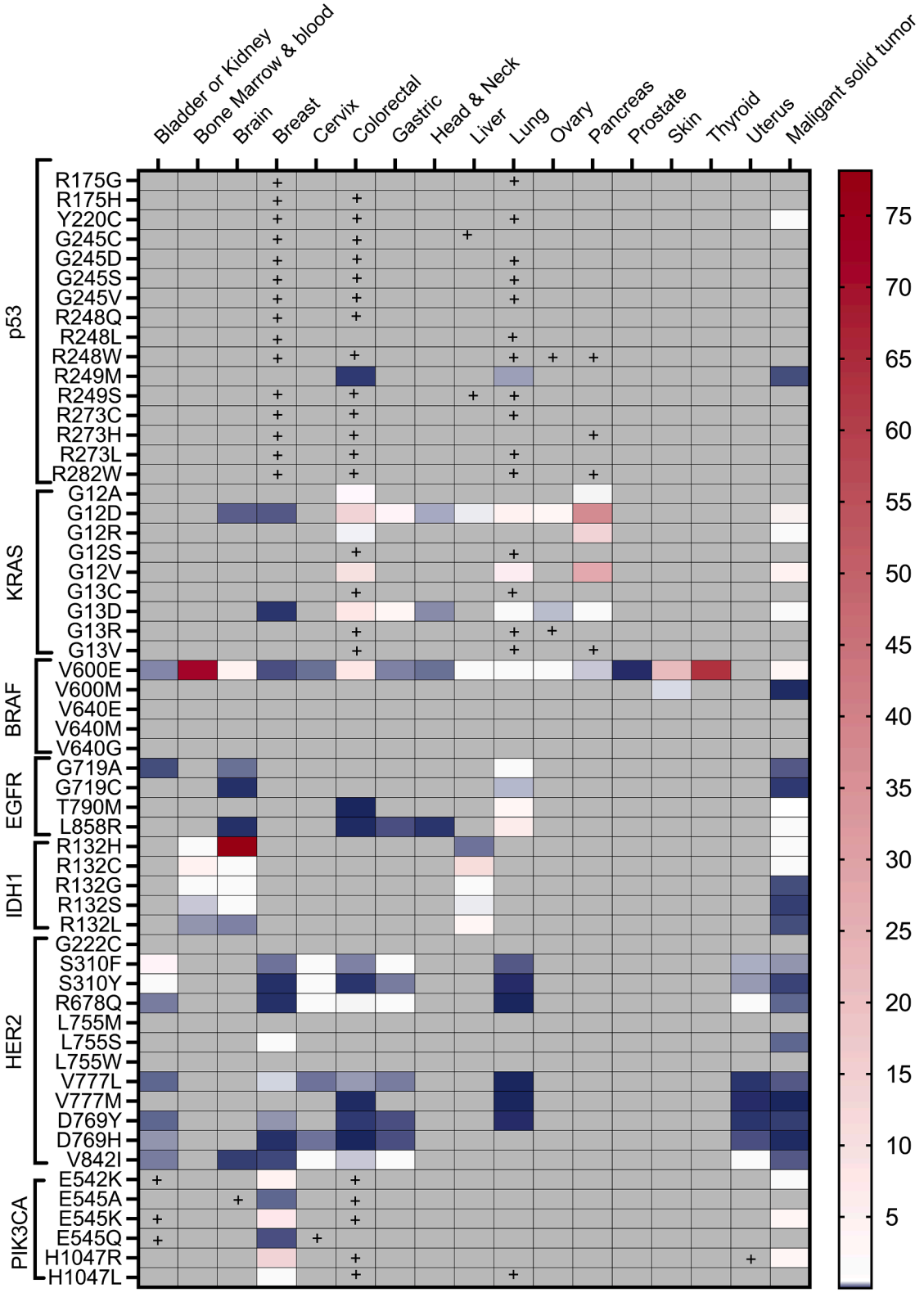
The data are extracted from GDC NIH data portal (https://portal.gdc.cancer.gov/).

Mutated KRAS neoepitopes

KRAS belongs to the *RAS* gene family and plays a crucial role in the regulation of cell division and growth. KRAS is a small guanosine triphosphatase (GTPase) that switches between its inactive GDP-bound and active GTP-bound forms.[49] The KRAS active form triggers the mitogen-activated protein kinase (MAPK) which activates cell survival, proliferation, and differentiation.[50] Missense somatic mutations in KRAS, including G12D, G12C, G12V, and G13D, result in accumulation of the active GTP-bound form and GTPase hyperactivity, leading to dysregulation of cellular processes, uncontrolled cell proliferation, and tumor formation. Mutations in KRAS are frequently found in various human cancers, including pancreatic, colorectal, and lung cancers, making it one of the most studied oncogenes. KRAS mutations are detected in 27% of human cancers, 85% of pancreatic cancers, 44% of colorectal cancers, 19% biliary cancers, and 19% of endometrial cancers. KRAS G12D and G12V mutations account respectively for >40% and ∼22%–33% of KRAS mutations in cancers. The KRAS G13D mutation represent 16% of KRAS mutations detected in colorectal cancers.[51,52] The large distribution of these mutations and their ability to induce immune response in humans[53] make KRAS mutations promising candidates for immuno-oncotherapy. The spontaneous T cell responses specific to mutated KRAS neoepitopes in cancer patients provided the rationale for the adoptive T cell therapy and TCR-T cell therapy.[54,55]

Several pre-clinical studies in mice showed the anti-tumor effect of mutated KRAS-targeting vaccines.[56–58] Notably, delivery of KRAS G12D neoantigen peptide formulated in CpG in liposomes (lipoplex) reduced the growth of syngeneic tumors expressing KRAS mutations in C57BL/6 and BALB/c mice.[56] However, despite some promising pre-clinical and clinical studies, the mutated KRAS-based vaccines have shown contradictory results regarding their immunogenicity in animals. While peptide vaccines have only induced immune responses in a small proportion of pancreatic cancer patients,[59] several reports indicate that immune responses to mutated KRAS epitopes can be induced by personalized neoantigen vaccines.[33] These vaccines would induce the "epitope spreading" mechanism indicative of an expansion of the immune response in which the immune system, having recognized one epitope, begins to recognize other epitopes that have become available after tumor cytolysis by T cells.

KRAS neoepitopes can be presented by both MHC-I and -II molecules. The reported affinity of KRAS G12 mutant epitopes for human HLA-I molecules varies from 16 nM to 28 μM.[60] Neoepitopes generated by KRAS G12V and G12D mutations can be efficiently presented by HLA-A*11:01.[61] Successful attempts have been made to improve the affinity of HLA-A*11:01-restricted TCRs to recognize KRAS G12D and KRAS G12V.[62,63] The affinity of the neoepitope generated by the KRAS G12D for HLA-C*08:02 is also notable. Importantly, adoptive transfer of *in vivo* expanded TILs specific to KRAS G12D and restricted to HLA-C*08:02 has been performed in a colorectal cancer patient with lung metastases and led to objective tumor regression of the vast majority of metastatic lesions.[55] More recently, a single infusion of autologous T cells engineered to express two allogeneic HLA-C*08:02-restricted TCRs specific to KRAS G12D resulted in the regression of visceral metastases with an overall partial response of 72%.[54] Subsequent studies of lymphocytes from successful adoptive cell therapy cases have shown that the mutant KRAS G12D peptide, but not the wild type, could stabilize HLA-C*08:02. This stabilization results from an aspartame residue present in this mutant epitope, which is essential for the formation of the ionic bridge with an arginine located at the position 156 on the HLA-C α2 helix.[64] In a clinical trial (NCT03953235), a therapeutic off-the-shelf vaccine encoding 20 shared neoantigens, including 5 KRAS neoepitopes resulting from G12D, G12R, G12V, G13D, and Q61H mutations, and 2 TP53 neoepitopes with R213L and S127Y



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mutations, was used in combination with immune checkpoint blockade.[65] Despite only short-term clinical improvements, probably due to the advanced cancer stages of the participants, T cell responses to HLA-matched KRAS neoepitopes were observed in five out of 16 patients. The strongest responses were detected against KRAS G12D in one HLA-A*02:01-positive patient and two HLA-A*11:01-positive patients. Notably, the addition of the strongly immunodominant yet tumor-irrelevant p53-derived neoepitopes to this vaccine resulted in a significantly reduced response to the KRAS neoepitopes. The authors suggested that, to avoid such epitope competition, the combination of various neoantigens in one vaccine should be avoided and advantageously replaced by parallel vaccination with multiple vaccines.[65]

There are several reports that KRAS neoepitopes can bind MHC-II molecules. Analyzing the TILs from colorectal cancer patients, TCRs specific for KRAS G12V and restricted by HLA-DPB1*03:01 or HLA-DPB1*14:01 could be isolated.[66] In a follow-up to the clinical trial CTN-98010,[67] in which patients with resected pancreatic cancer were given a vaccine composed of seven KRAS neoepitopes, several TCRs isolated from a surviving patient were able to potently recognize KRAS neoepitopes presented by HLA-DR*0404/0401 or HLA-DQ*0302.[68]

Given the low immunogenicity of KRAS G12-mutated neoepitopes, the weak level of vaccine development, mainly based on adjuvanted peptides, and the difficulty of keeping the active cytotoxic T cells "on site," the use of mutated KRAS-based vaccines seems for the moment challenging for complete solid tumor eradication. However, they can be effective in the elimination of metastases and residual cancer cells after surgery. In the "ELI-002 7P" phase I/II ongoing clinical trial, the immunotherapy targets seven KRAS/NRAS (neuroblastoma RAS viral oncogene homolog) G12 and G13 mutations in pancreatic and colorectal cancer patients who remained cancer positive despite previous treatment.[69] In that study, the presence of residual cancer cells was detected either on a circulating tumor DNA test or by progressively increasing levels of the tumor protein markers. Following a 6-month vaccine course, 77% of patients reduced their levels of tumor markers, with six achieving complete responses. Importantly, this therapy stimulated both helper and cytotoxic T cells in over half of the patients. Long-term monitoring showed sustained memory T cells in 75% of recipients after booster immunizations, with higher T cell responses correlating strongly with reduced tumor relapse rates.[70]

Mutated BRAF neoantigens

BRAF is a serine-threonine protein kinase, involved in the transduction of mitogenic signals via activation of the downstream RAS proteins. Several dozens of BRAF mutations have been identified in ∼7% of human cancers. The substitutions V600E, V600K, and V600A, D, G, L, M, Q, or R represent respectively 98%, 5%–10%, and 5% of BRAF mutations.[71,72] These mutations are detected in 67% of thyroid papillary cancers, 41% of cutaneous melanoma, and 11% of colorectal cancers.[51,52]

Of particular interest is the BRAF V600E mutation, an oncogenic driver that contributes to constitutive activation of the MAPK pathway, leading to uncontrolled cell proliferation and oncogenicity.[71,73] BRAF V600E is detected in ∼3% of diagnosed cancers, and constitutes 95%, 71%, and 62% of BRAF mutations, respectively, in thyroid papillary cancers, colorectal cancers, and cutaneous melanoma.[51,52] BRAF mutations are target of chemotherapies inhibiting BRAF V600E-mediated gain -of functions.[74] Interestingly, the shared neoantigen generated by BRAF V600E substitution is presentable by HLA-A2.[75] BRAF V600E^{+} HLA-A2 melanoma patients showed presence of T cell responses specific to this neoepitope.[76] The high prevalence (∼50%) of HLA-A2 among melanoma patients makes BRAF V600E neoepitope attractive for melanoma immunotherapy. BRAF V600E is also considered as a potentially pertinent neoantigenic target to be used in thyroid immuno-oncotherapy.[77]

Mutated EGFR neoepitopes

The tyrosine kinase EGFR is a transmembrane receptor that initiates signaling cascades through ligand-induced dimerization and consequently activates multiple downstream effectors involved in cell proliferation.[78] Overexpression and/or increased activity of EGFR is observed in many cancers. The most common EGFR genomic alterations are deletions in the exons 2–7, 19, and 21 and the L858R, T790M, and G719X mutations. EGFR is overexpressed in 50%–78% of triple-negative breast cancers,[79,80] 60%–80% of colon cancers,[81] 80% of renal cancers,[82] up to 60% of ovarian cancers,[83] 40%–89% of NSCLC,[84] 41% of adenocarcinomas, and 40% of malignant glioma.[85] EGFR mutations are detected in 23% of NSCLC, from which half are EGFR ΔE746-A750. The EGFR L858R-activating mutation occurs in 28% of EGFR mutations in NSCLC.[51] EGFR T790M is the secondary acquired mutation that confers resistance to the EGFR tyrosine kinase inhibitors.[52,86] The most common mutated form of EGFR, i.e., variant III (EGFRvIII), is a truncated form caused by an in-frame deletion of 801 bp from exon 2–7 leading to the deletion of 267 amino acids and generation of the LEEKKGNYVVTDH junction peptide.[87] The latter harbors an epitope that is immunogenic both in mice and humans and is the most tested candidate in EGFR-targeting vaccines. The EGFRvIII variant is present mainly in glioblastomas but also in NSCLC, breast, prostate, colon, and head and neck cancers and enables cancer cells to proliferate faster and decrease apoptosis after DNA damage.[88–91]

Pre-clinical studies in mice demonstrated that vaccination against various parts of EGFR controls tumor growth.[92–94] Notably, a preclinical study in subcutaneous or orthotopic glioblastoma in

Figure 2. Frequencies of the public point mutations with the potential to generate neoantigens as targets of immuno-oncotherapy in diverse cancer types
The data are extracted from My Cancer Genome (https://www.mycancergenome.org). "+" indicates that the mutation has been observed among case studies but it is not thus far possible to quantify its frequency.

Table 1. Shared neoepitopes on relevant human oncoproteins for which at least a presentation by an HLA-restricting element has been demonstrated

Shared mutations generating neoepitopes	Identified HLA-restricting Element	AA epitope sequence	Reference
p53 R175H p53 Y220C p53 R248W	HLA-A*02:01 HLA-A*02:01 HLA-A*68:01	HMTEVVRHC VVPCEPPEV SSCMGGMNWR	Lo et al. and Malekzadeh et al.[46,47]
KRAS G12D KRAS G12C KRAS G12V KRAS G13D	HLA-A*02:01 HLA-C*08:02 HLA-A*11:01 HLA-A*11:01 HLA-A*03:01 HLA-DPB1*03:01 HLA-DPB1*14:01 HLA-A*11:01	VVVGADGVGK VVVGACGVGK VVVGAVGVGK VVVGAGDVGK	Wang et al.; Poole et al.; Zhang et al.; Sim et al.; Rappaport et al.; and Ai et al.[61–66]
BRAF V600E	HLA-DQA1*03 HLA-DQB1*03 HLA-A*02:01 HLA-A*03:01 HLA-A*11:01	KIGDFGLATEK	Holt et al. and Tate et al.[51,52]
EGFR L858R EGFR T790M EGFR ΔE746-A750 EGFRvIII	HLA-A*31:01 HLA-A*11:01 HLA-A*02:01 HLA-C*15:02 HLA-A*03:01 HLA-A*11:01 HLA-B*08:01 HLA-A*02:01	HVKITDFGR KITDFGRAK MQLMPFGCLL LTSTVQLIM LEEKKGNYVVH	Masuda et al.; Park et al.; Pabla et al.; Minner et al.; Siwak et al.; Prabhakar et al.; Libermann et al.; Dimou et al.; Lin et al.; and Li et al.[79–85,99,133,134]
PIK3CA H1047R *PIK3CA* H1047L *PIK3CA* E542K	HLA A*03:01 HLA A*11:01 HLA A*11:01 HLA-DPB1*04:01 HLA-DPA1*01:03	ALHGGWTTK AISTRDPLSK	Holt et al.; Tate et al.; Chandran et al.; Shen et al.; and Iiizumi et al.[51,52,107,108,135]
IDH1 R132H	HLA A2.DR1	–	Yan et al. and Schumacher et al.[110,111]
TARP	HLA-A*02:01 HLA-DR53 HLA-DR1 HLA-DR9 HLA-DR13 HLA-DR15	FVFLRNFSL FLRNFSLML MQMFPPSPLFFFLQ QLLKQSSRRLEHTF	Kobayashi et al. and Wolfgang et al.[117,119]

C57BL/6 murine model showed that therapeutic vaccination with LEEKKGNYVVTDH controlled tumor growth and that the anti-tumor effect was largely enhanced by the G-6-Y amino-acid substitution, which maximizes proteasome cleavage.[17] However, possible autoimmune complications should be considered when transferring this technology to patients. In another preclinical study in A/J mice, a multi-epitope vaccine targeting EGFR L858R, T790M, and ΔE746-A750 epitopes prevented EGFR-driven lung tumorigenesis in (1) a syngeneic subcutaneous model under therapeutic conditions, and (2) mice with inducible lung-specific expression of EGFR L858R/T790M, where vaccination was performed in a prophylactic framework.[94]

In clinic, the CDX-110/rindopepimut vaccine targeting an EGFRvIII-derived 13-mer peptide, in combination with the alkylating agent temozolomide, has been used to treat brain tumors, along with radiotherapy. This immunotherapy was effective in post-surgery treatment of EGFRvIII-harboring glioblastoma multiforme (GBM) patients in multiple phase I and II clinical trials.[95–97] The treatment was well tolerated, resulted in improved PFS and OS, and elicited a robust immune response. However, a subsequently initiated phase III clinical trial, where GBM patients received rindopepimut and temozolomide (n = 371) or temozolomide alone (n = 374), did not reveal any significant improvement in OS.[98] Nevertheless, numerous studies reported the good immunogenicity of EGFR-derived neoantigens in humans, and this shared neoantigen is still considered an attractive target for immuno-oncotherapy.[74] The ability of EGFR L858R and EGFR ΔE746-A750 to bind HLA-I was predicted *in silico* and confirmed *in vitro* in binding assays.[99] EGFR L858R is mainly presented by HLA-A*31:01, HLA-A*33:01, HLA-A*68:01, HLA-B*08:01, and HLA-B*27:05, while EGFR ΔE746-A750 is mainly presented by HLA-A*03:01, HLA-A*11:01, and HLA-B*08:01. Further analysis of patients from the TCGA lung adenocarcinoma cohort with EGFR L858R or ΔE746-A750

alterations showed that the presence of these HLA alleles correlated with a better prognosis both in disease-free survival and OS.

In a phase I clinical trial, 24 stage III/IV NSCLC patients were treated with a neoantigen-based vaccine composed of a selection of driver mutations from various shared neoantigens, matched for their HLA.[74] Each patient received the mixture of 5–12 peptides for 2–6 targets. Within 3–4 months following the vaccination, seven of 24 patients showed partial to complete objective clinical responses, all of them harboring EGFR-mutated tumors. Two commonly shared EGFR mutations, i.e., T790M and L858R, were immunogenic in four of the responding patients. The study suggested that the multi-neoantigen vaccines had more therapeutic potential than the vaccination against one non-synonymous single-nucleotide variant.[74]

Targeting EGFR vIII with CAR-T cells has shown conflicting results. In a first-in-human phase I clinical trial (NCT02209376),[100] anti-EGFR-vIII CAR-T cells were infused into 10 glioblastoma patients and successfully infiltrated the tumors. Three patients had severe neurological complications, and no EGFR-directed toxicity or systemic cytokine release syndrome was observed. Nine patients had stable disease for 1 month after infusion. Although no tumor regression was detected in any of the patients, one patient did not require further treatment for more than 18 months after CAR-T infusion. In another phase I clinical trial (NCT01454596), patients with recurrent glioblastoma were treated with 6.3×10^6 to 2.6×10^{10} anti-EGFRvIII CAR-T cells, but the trial failed to demonstrate any clinically meaningful effect.[101] There was also severe toxicity and one treatment-related death.[101]

Mutated *HER2* neoepitopes

Among the most extensively studied oncogenes in solid tumors is HER2.[102] In addition to HER2 protein overexpression in certain types of cancer, especially breast cancer, HER2 somatic mutations may have major impacts in cancer progression and can be therapeutic targets.[102] Common activating somatic mutations of this receptor have been described in various types of cancer.[37] HER2 presents an overall incidence of 3.13% in cancer patients. HER2 mutations are mainly located in its extracellular, transmembrane, and catalytic domains and are thought to promote oncogenic signals. Among HER2 mutations, S310F/Y in the extracellular domain, insertions of exon 20, and the L755S and V777L mutations located in the catalytic domain are predominant. HER2 mutations are not associated with its expression level but are concurrent with microsatellite instability, tumor mutanome size, neoantigen burdens, and tumor-infiltrating immune cells. In cohorts of cancer patients treated with checkpoint inhibitors, those with improved objective response rates had mutated HER2 compared with those with wild-type HER2.[103]

Analysis of blood from NSCLC patients revealed neoepitope-specific $CD4^+$ T cells specific to KRAS G12V and the internal tandem duplication (ITD) of *ERBB2* gene encoding HER2. TCRs specific to these common neoantigens were isolated to generate TCR-T cells. HER2-ITD-specific TCR clonotypes were found in the patient's tumor but not in the non-adjacent lung.[104]

Mutated *PIK3CA* neoepitopes

PI3Ks are a family of kinases involved in many vital cellular functions such as proliferation, differentiation, and intracellular trafficking.[105] PI3Ks phosphorylate the 3′ position of the inositol ring of phosphatidylinositol, generating second messengers that are important in signal transduction pathways. Mutations in the PIK3CA catalytic subunit α are the most frequent oncogenic mutations detected in human cancers and are of primary importance in cell-cycle progression and malignant transformations.[106] PIK3CA mutations are present in 14% of malignant solid tumors, 36% of breast cancers, and 21% of bladder cancers. The PIK3CA H1047R, E545K, and E542K mutations constitute respectively 38%, 20%, and 12% of all PIK3CA mutations in breast cancers and there is increasing evidence that PIK3CA neoantigens can induce both MHC-I- and -II-mediated immune responses.[51,52]

The most common PIK3CA hotspot mutation, i.e., H1047 L/R, triggered tumor necrosis factor (TNF)α production by $CD8^+$ TCR transduced T cells, exclusively in the context of HLA A*03:01 allele.[107] In NOD SCIDγ mice engrafted with HLA $A*03:01^+$ HCC70 human breast cancer cell line, adoptive transfer of $CD8^+$ T cells transduced with a PIK3CA H1047L/R-specific TCR mediated the regression of established tumors. Analysis of PBMCs from HLA $A*03:01^+$ cancer patients also showed that PIK3CA was spontaneously immunogenic and could drive T cell clonal expansion *in vivo*. Another study showed that T cells isolated from healthy HLA-A*11:01-positive donors were able to recognize the PIK3CA H1047L mutation after *in vitro* stimulation.[108] Specific TCRs from $CD8^+$ T cells were also isolated and shown to be able to control the growth of tumor cells expressing PIK3CA H1047L-resulted neoepitope and HLA-A*11:01 *in vitro*. In a similar study, TILs were isolated from a colorectal cancer patient and tested against autologous antigen-presenting cells primed with PIK3CA neoepitopes *ex vivo*. As a result, a TCR specific for PIK3CA N345K and restricted by the HLA-II pair HLA-DPB1*04:01/HLA-DPA1*01:03 was identified.[109]

Mutated IDH1 neoepitopes

Gliomas are the most frequent primary brain tumors in adults and commonly contain mutations in the gene encoding the metabolic enzyme isocitrate dehydrogenase (IDH)1.[110] The gain-of-function R132H point mutation in the catalytic site of IDH1 is recurrent and present in >70% of diffuse grade II and grade III glioblastomas.[110] The product of the mutated IDH1, the oncometabolite 2-hydroxyglutarate, notably inhibits the function of histone and DNA demethylases, resulting in DNA hypermethylation and malignant transformation. The IDH1 R132H mutation generates a shared neoantigen, detected almost in all tumor cells, which contains an HLA-II-restricted $CD4^+$ T cell neoepitope. Vaccination of A2.DR1 MHC-humanized mice with adjuvanted IDH1 R132H:123-142 peptide triggered neoepitope-specific $CD4^+$ T cells, able to control the growth of a syngeneic tumor expressing IDH1 R132H.[111]

Based on these preclinical results, a phase I clinical trial has been performed in 33 patients with newly diagnosed grade III and IV IDH1

R132H-expressing gliomas. The intra-tumoral IDH1(R132H) neoepitope was presented in pre-treatment tumor tissue. The peptidic vaccine-based immunotherapy showed *de novo* induction of T cell immune responses in >90% of patients across multiple HLA alleles,[112] suggesting a promiscuity of epitope presentation by MHC-II molecules.[111] $CD4^+$ T cells of the responders were able to produce TNF-α, IFN-γ, and IL-17 after *in vitro* stimulation with the relevant peptide. Two patients without immune response to the vaccine showed progression within 2 years, while patients with immune responses showed a 2-year progression-free rate of 95%. Treated patients showed high rates of "pseudo-progression," defined as development of mass lesions corresponding to intra-tumoral infiltration and inflammatory reactions. Pseudo-progression was associated with increased vaccine-induced peripheral T cell responses, concomitant with longer survival. In a patient with pseudo-progression, $CD4^+$ TILs were enriched in a single clonotype with TCR specific to IDH1 R132H neoantigen.[112] This clinical trial provided proof of concept of the feasibility of this immunotherapy in human neuro-oncology.

Shared frameshift neoepitopes

Tumors with a defective DNA mismatch repair system leading to microsatellite instability, prone to DNA polymerase slippage during DNA replication, possess particularly high mutational burdens. In these tumors, indels can result in nucleotide bases in numbers that are not multiples of three in microsatellite regions of coding genes. These indels generate frameshift peptides that are sources of tumor-specific neoantigens, which notably can be shared across the patients with microsatellite instability.[113] Compared to single-amino-acid substitutions, DNA frameshifts generate more disruptive aberrations and can generate more immunogenic T cell epitopes.

In a recent study, 209 frameshift peptides shared across human colorectal, gastric, and endometrial tumors with microsatellite instability have been selected and verified for their absence in healthy tissues. There was also evidence that, from a single frameshift DNA mutation, multiple frameshift peptides could be generated as a result of alternative mRNA splicing. More than 80% of the frameshift peptides selected were shared across the three cancer types. Frameshift peptides with an average length of 17 amino acids and predicted to harbor HLA-I and/or -II epitopes were selected to design poly-antigens of ∼6,000 amino acids to be encoded by adenoviral or MVA-based vaccination vectors. The strong immunogenicity for both $CD4^+$ and $CD8^+$ T cells generated by these vectors was first shown in the murine model. The vaccination vector was able to activate human $CD8^+$ T cells by inducing *in vitro* presentation of encoded frameshift neoepitopes by human antigen-presenting cells.

There is an extremely high risk of developing hereditary non-polyposis colorectal cancer (HNPCC) in the carriers of the autosomal dominant Lynch syndrome mutation, resulting in DNA mismatch repair deficiency.[114] Frameshift peptide-specific T cells were detected not only in HNPCC patients but also in asymptomatic carriers of this mutation. It therefore appears that frameshift neoepitopes are expressed prior to the onset of HNPCC and that T cells specific to them exist in these individuals and may contribute to the control of malignant cell growth. Based on such widely shared frameshift neoantigens, a vaccine for the prevention and interception of HNPCC with DNA mismatch repair deficiency has been designed. This vaccine was shown to induce a broad immune response and successfully completed a phase I/IIa clinical trial.[115,116]

In addition to frameshift mutations in DNA, alternative mRNA splicing comprising skipped exons, retained intron, alternative 5′ or 3′ splice sites, and mutually exclusive exon usage is also a source of novel tumor antigens.[22] An interesting example is the TARP antigen, which has not been considered by its authors as a shared neoantigen.[117] In fact, from the unrearranged constant *TCRγ* segment located on chromosome 7p14.1, a subset of transcripts shorter than *TCRγ* transcripts can be expressed.[118] The 58-amino-acid-long protein encoded by an alternate ORF, i.e., TCRγ chain alternate reading frame protein (TARP), has no similarity with any protein sequences in databases.[119] The *TARP* transcript(s) were first unexpectedly discovered in healthy human prostate epithelial cells.[120] It is now well established that TARP is highly expressed in various cancers, including breast, endometrial, human androgen-sensitive prostate cancer, salivary gland tumors,[121] and acute myeloid leukemia.[118,122,123] The expression of TARP transcripts is correlated with cell proliferation and metastases.[121] Helper T cells are able to recognize $TARP^+$ prostate and breast tumor cells through $TARP_{1\text{-}14}$ neopeptide, restricted by HLA-DR53, or $TARP_{14\text{-}27}$ neoepitope, restricted by HLA-DR1, HLA-DR9, HLA-DR13, and HLA-DR15.[117]

In addition to HLA-II-restricted TARP immunogenic regions,[117] the TARP:27–35 region contains an immunodominant T cell epitope restricted by HLA-A*0201 and shown to be immunogenic for $CD8^+$ T cells in HLA-A2/K^b MHC-humanized transgenic mice.[124,125] The native TARP:27–35 and an anchor-modified TARP:29–37 segment harboring the L9V substitution[125] have been used for immunotherapy in prostate cancer patients either as adjuvanted peptides or loaded on autologous dendritic cells. These trials resulted in significant reduction in tumor growth rate in a majority of patients.[125]

Limitations and prospects of shared neoantigen applications

Although the clinical use of shared neoantigens in immuno-oncotherapy has shown encouraging results, clinical trials based on this approach are sometimes considered disappointing overall.[126] The relatively low efficacy of immunotherapy based on shared neoantigens could be attributed to the assumption that the only malignant cells with a proliferation advantage are those that are weakly immunogenic and therefore lacking suitable tumor antigens, including shared neoantigens.[126] According to this hypothesis, induction of T cells against such shared neoantigens will not properly target proliferating and metastatic tumor cells. However, the most commonly used shared neoantigens, such as those mentioned in this review (Table 1), result from driver mutations. These shared neoantigens arise mainly in the very early stages of carcinogenesis

and are also necessary for the maintenance of the malignant phenotype. It is therefore difficult to imagine a negative selection of tumor cells that, under the pressure of an immune response, could lose the expression of driver neoantigens. However, the loss of HLA molecules capable of presenting the corresponding neoepitopes by tumor cells is entirely possible and has even been clinically proved.[54] In this case, the combination of immunotherapy based on shared neoantigens with adjuvants capable of generating an inflammatory tumor microenvironment could restore HLA expression and overcome this difficulty.

One of the main challenges of using (shared) neoantigens in immuno-oncotherapy is the low probability that derived neopeptides can be presented by a significant number of HLA alleles distributed in human populations.[14] However, if a particular shared neoepitope and a matched HLA allele can be found during patient screening, the potential benefits of this approach may be considerable and deserve to be explored through clinical trials.[55,127,128]

CONCLUSIONS

Antigen-specific immuno-oncotherapy shows real promise if appropriate T cell neoepitopes from tumor-specific mutations can be identified, used with optimal vaccination platforms and, when necessary, combined with immune checkpoint blockers to ensure a strong and sustained anti-tumor T cell immunity. Neoantigens can trigger specific T cells with potent on-target effects, without off-tumor toxicity. However, of the large number of tumor-occurring mutations, only a very small percentage give rise to neoepitopes able to bind with good affinity to relevant MHC molecules, possibly detectable by TCRs. It is expected that most of the neoepitopes generated by single-amino-acid substitutions are less immunogenic than foreign exogenous pathogen-derived epitopes. In fact, the oligoclonal TCR repertoire capable of (cross)recognizing a wild-type self-peptide with good affinity has usually been rendered tolerant by negative selection, leaving only a narrow residual TCR repertoire capable of neoepitope recognition. Therefore, neoantigens, by virtue of being close to self-proteome, may often be of low affinity. Neoantigen-specific TCRs are capable of finely recognizing neoepitopes bearing a single non-anchor substitution with relatively low affinity. Hence, it is admitted that TCR affinity for the MHC-epitope complex impacts TCR signaling strength and thus the magnitude of the T cell response. In this case, it is understandable why neoantigens derived from somatic mutations are key targets of T cells unleashable by immune checkpoint inhibitors.[129,130] Thus, the action of anti-checkpoint inhibitors may have a greater rejuvenating impact on T cells carrying low-affinity TCRs.[131,132] Personalized cancer vaccines can activate and expand tumor-specific T cells, while the checkpoint inhibitors have the potential to help unleash these specific T cells, providing strong rationale for exploring the combined potential of these immunotherapies.

Another limitation of immunotherapy against neoantigens, particularly personal neoantigens, is the mosaicism in the profile of the neoantigen repertoire expressed within a tumor. If immunotherapy is to be based on private neoantigens, multiple neoantigens will need to be incorporated in a single vaccine to compensate for neoantigen expression mosaicism and to cover as many tumor cells expressing the targeted neoantigens as possible. It is also why shared neoantigens, which are cancer drivers and most likely to be expressed in tumor precursor cells and consequently by all tumor cells, are more relevant. In addition, therapies based on shared neoantigens do not require personalized design and individual GMP production of the vaccine.

The HLA haplotype of cancer patients obviously plays an instrumental role in (neo)antigen T cell-based vaccine efficacy. The substantial polymorphism of this locus makes antigenic design a difficult task, since it has to account for this great variability, while considering the most frequently represented HLA alleles capable of presenting given epitopes in targeted populations.

Despite the induction of T cell responses against relevant (neo)antigens, whether private or common, tumor-intrinsic or -extrinsic coordinated mechanisms can lead to tumor escape from T cell immunity. Tumor-intrinsic mechanisms comprise epigenetic modifications leading to downregulation or loss of expression of MHC or cognate (neo) antigen and defective antigen processing and/or presentation by tumor cells. Tumor extrinsic mechanisms, like hypoxic and immunosuppressive local tumor microenvironment, presence of regulatory T cells, myeloid-derived suppressor cells, M2-polarised tumor-associated macrophages, and T helper 2 $CD4^+$ T cells, as well as anti-inflammatory cytokines, may obstruct T cell functions. Therefore, the use of powerful vaccination strategies, like viral vectors, can be an asset to induce specific T cells of the highest quality as well as long-standing memory. Such vectors can be advantageously combined with immune checkpoint blockers and/or adjuvants that can reshape the tumor ecosystem toward a niche more permissive to TILs and to promote a “cold-to-hot” intratumor inflammatory switch.

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AUTHOR CONTRIBUTIONS

A.G. and L.M. wrote most of the parts on personal and shared neoantigens. F.L.C. compiled the bibliography and wrote the paragraphs on BRAF and shared frameshift neoepitopes and the research behind the figures. P.A. compiled the bibliography and wrote the paragraph on HER2-mutated neoepitopes. P.C. had a critical scientific contribution to the review as a whole. L.M. wrote most of the introduction and conclusion.

DECLARATION OF INTERESTS

P.C. is the founder and CSO of TheraVectys. A.G., F.L.C., and P.A. are employees of TheraVectys. L.M. has a consultancy activity for TheraVectys. A.G., F.L.C., P.C., and L.M. are inventors of a pending patent directed to the potential of lentiviral vectors encoding KRAS mutations in immuno-oncotherapy.

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