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The premise of personalized immunotherapy for cancer dormancy:

Cancer dormancy vaccines

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Abstract

Progress in cancer therapies has resulted in improved survival of patients with early stage breast cancer. However, mortality remains high in patients with distant recurrence of the disease after initially successful treatment of early stage breast cancer. To this end, tumor recurrences have been attributed to the presence of dormant tumor cells in breast cancer patients and cancer survivors. Current clinical practice guidelines recommend a “wait and watch” approach for tumor recurrence. This is because of our limited understanding of tumor dormancy. Dormant tumor cells are quiescent, and thus, do not respond to chemotherapies or radiation therapies, and they are not operable. Therefore, immunotherapy is the only option for the treatment of tumor dormancy. However, gaps in our knowledge as to dormancy-specific antigens prevent a relapse preventing vaccine design. Here, we provide a critical review of cancer immunotherapy, and discuss empirical evidence related to naturally-occurring tumor dormancy and treatment-induced tumor dormancy at the site of primary tumor and in distant organs before and after cancer therapies. Finally, we suggest that personalized vaccines targeting dormancy-associated neoantigens, which can be given to patients with early stage disease after the completion of neoadjuvant therapies and tumor resection as well as to cancer survivors could eliminate relapse causing dormant cells and offer a cure for cancer.

Naturally-occurring and treatment-induced tumor dormancy

Tumor cell dormancy is an early stage of cancer development or tumor recurrence during which malignant cells are present without detectable solid tumors. Tumor dormancy exists in two forms as naturally-occurring tumor dormancy prior to the establishment of primary cancer, and treatment-induced tumor dormancy after the completion of cancer therapies. Naturally-occurring or primary tumor dormancy is supported by two empirical evidence. First line of evident comes from the detection of malignant cells in post-mortem autopsies of individuals who had no clinical sign of cancer (1–5). Second line of evidence is obtained from non-metastatic cancer patients by the detection of local dormancy at the site of primary

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tumor and disseminated tumor dormancy in the bone marrow or in the circulation (6–10). These dormant cells could outgrow following surgical removal of primary cancer because of the surgery-induced inflammation and wound healing mechanisms (11,12). Cancer therapies, while eliminating residual tumor cells, could also induce secondary tumor dormancy, which is termed treatment-induced dormancy. This type of tumor dormancy is evident from the detection of chemotherapy-induced dormant cells being in a state of senescence engulfing nondormant tumor cells to enhance their survival during chemotherapies at the site of primary breast cancer (13). In colorectal cancer, presence of dormant cells at the tumor site is reported to promote chemoresistance (14). Distant tumor dormancy following successful completion of cancer therapies has been reported in cancer survivors and animal models (15–18). In fact, neoadjuvant chemotherapies select for Ki67⁻ naturally-occurring dormant cells, and at the same time, induce both apoptosis and tumor dormancy in Ki67⁺ tumor cells resulting in an increased number of Ki67⁻ and Ki67^{low} dormant cells (treatment-induced dormancy) (13,19,20). In the in vitro studies, we have reported that Ki67^{low} indolent dormant cells show a sluggish proliferation rate which was counterbalanced by spontaneous cell death so as to remain in a dormant state without being able to establish solid tumor until proliferation exceeds spontaneous cell death and results in tumor relapse (19). In fact, single cell dormancy is not merely regulated by cell cycle arrest (21) as it could exist as Ki67⁻ quiescent cells being in non-proliferative state, and Ki67^{low} indolent dormant cells with sluggish cell division counterbalanced by apoptosis which prevent tumor mass formation (Figure 1).

Distant recurrence of breast cancer occurs at different rates with ER⁺ tumor cells showing very long dormancy, and TNBC showing a high recurrence rate. This is likely due to a prolonged use of hormonal therapy in the former but termination of cancer therapies upon initial responses in the latter. In fact, ER⁺ tumor cells remain under pressure by hormone deprivation therapies until they develop resistance and escape from hormonal therapies. On the other hand, lack of a prolonged targeted therapy for TNBC rescues dormant cells from any therapeutic pressure which could in turn result in a quick recurrence. In addition, TNBC usually express PD-L1 to evade the immune surveillance. To this end, current advances in targeted therapies for TNBC by means of the immune check point blockades, showing promising results in clinical trials (22), are expected to inhibit or delay recurrence of TNBC.

Therapeutic targeting of cancer dormancy

Tumor dormancy can be regulated by cell-intrinsic factors such as tumor suppressor genes as well as cell-extrinsic factors such as angiogenesis, hypoxia, immune cell infiltrates and tissue structure. These factors could induce different mechanisms of dormancy including cell cycle arrest, autophagy or senescence. These microenvironmental cues of tumor dormancy have been reviewed previously (23–28). Accordingly, several factors such as VEGF, autophagy inhibitors or senolytic compounds have been used to overcome tumor dormancy. Nevertheless, the purpose of immunotherapy is to target dormant tumor cells directly through the recognition of dormancy-associated neoantigens.

While early stage cancers originate from naturally-occurring dormant tumor cells, metastatic cancers originate from regrowth of naturally-occurring or treatment-induced dormant cells

that were disseminated into distant organs. Distant recurrences could occur early or very late after initially successful treatment of primary cancers. In the Netherlands, HER2 positive and triple negative breast cancers had the highest recurrence rates within two years (29). Within 10 years, the highest rates of distant recurrence and regional recurrence were detected in patients with HER2 positive and triple negative breast cancers, respectively (29). The rates of breast cancer recurrences vary in distant organs including the bone (38.9% in HER2 positive and 45.1% in triple negative), liver (40.1% in HER2 positive and 27.5% in triple negative), lungs (35.2% in HER2 positive and 41.4% in triple negative) and brain (25.3% in HER2 positive and 23.1% in triple negative) (30). Tumor recurrence is common in all types of human cancers. According to the American Cancer Society (31), a 5-year survival rate for different types of cancer in the United States is less than 50% (Table 1). Therefore, a cancer cure requires an effective therapeutic targeting of tumor dormancy in order to prevent cancer recurrences.

Two major goals have been pursued for targeting tumor dormancy including the elimination of dormant cells or keeping them in a dormant state and prevent their regrowth (Figure 2). To achieve these goals, three strategies have been envisioned (Figure 2). First strategy involves the inhibition of pathways that support survival of dormant tumor cells and induce apoptosis. For instance, Kurppa et al reported that treatment of non-small cell lung cancer establishes senescence-like dormant cells characterized by upregulation of YAP/TEAD activity; thus, they proposed that inhibition of YAP and TEAD could result in the elimination of dormant tumor cells (18). Zhang et al reported that inhibition of mitochondrial respiration by means of small molecule VLX600 induced tumor cell death in colon cancer cells (32). However, a complete elimination of dormant cells is a challenging task. The toxicity and efficacy of these treatment approaches and the risk of activating redundant mechanisms of tumor dormancy by such targeted therapies remain to be determined. Second strategy involves inducing cell proliferation in quiescent dormant cells in order to render them susceptible to chemotherapies (33). This strategy is highly risky because of rescuing tumor cells from dormancy, which may facilitate recurrence of chemoresistant tumor clones or induction of secondary tumor dormancy. Third strategy is to use immunotherapy, which can induce apoptosis in dormant cells or prolong tumor dormancy and prevent distant recurrence of the disease. T cells can induce apoptosis in dormant tumor cells that have become resistant to additional doses of these conventional therapies (19,20). Tumor clones that survive T cell-mediated apoptosis can still remain in check by the immune system without resuming cell division. This strategy seems to be more feasible by inducing dormancy-specific memory T cells that produce IFN- γ upon recognition of dormant cells, thereby keeping quiescent cells in a dormant state and preventing distant recurrence of the disease (34). In fact, IFN- γ has been shown to induce long-term G1 cell cycle arrest in cervical cancer (35). This effect has been attributed in part to caspase 3 activation which also results in tumor immunoediting by downregulation of HER2 tumor associated antigen (36). The IFN- γ -induced STAT1 activation has also been shown to result in the downregulation of cyclin E and cyclin A or the upregulation of miRNA-28 family and CDK6 for the induction of tumor dormancy (37,38). Activated STAT1 can also induce cyclins D1, D2, D3 and CDK4 for the induction of cell cycle arrest in tumor cells (39). IFN- γ can also induce tumor cell dormancy via STAT1-independent

mechanisms by upregulating the cell cycle inhibitor p27 (40). IFN- γ can be released by innate immune cells such as NK cells and M1 macrophages, and induced by radiation therapies such that neutralization of IFN- γ could reverse radiation-induced tumor dormancy resulting in tumor relapse (41). In addition, evidence from the presence of naturally-occurring tumor dormancy without causing cancer support the feasibility of this strategy. For instance, post-mortem examination of women in their forties showed 39% of histologic breast malignancy in a dormant state (2), though only 1% of women in this age range get breast cancer. Interestingly, all autopsied individuals aged 50 to 70 had in situ carcinomas in the thyroid gland (42), whereas the incidence of thyroid cancer in this age group is only 0.1% (1). The equilibrium phase of tumor immune surveillance (43), which has been demonstrated in an animal model of sarcoma (44), is also an indicative of the ability of the immune response to prevent the recurrence of dormant tumor cells. After all, somatic cells in any biological system have developed survival mechanisms, which make the elimination of tumor cells arising from normal cells difficult. However, retention of tumor cell dormancy in a quiescent state by means of immunotherapy or dormancy vaccines would be more feasible. Once tumor cells become dormant and quiescent, they do not respond to chemotherapy, radiation therapy, or hormone deprivation therapy. However, we have reported that dormant cells that were established by chemotherapy or radiation therapy would remain highly susceptible to tumor-sensitized T cells (20).

Immunotherapeutic targeting of tumor dormancy for the prevention of distance recurrence of cancers

Mortality remains high in patients with metastatic recurrence of cancers. Recent advances in immunotherapy of cancer by means of antibody therapies (45,46) and immune checkpoint blockade (47–50) have prolonged survival of patients, but many patients do not respond to these therapies (49,51,52), and those who respond would remain at risk of tumor recurrence (50,53,54). This is because proliferating tumor cells could escape from cancer therapies by i) changing themselves through tumor antigen loss, MHC class I downregulation, mutations in antigen presentation machinery, and upregulation of the immune checkpoint molecules such as PD-L1, as well as by ii) changing their microenvironment through the secretion of immune suppressive cytokines/chemokines that induce Tregs and/or MDSCs for the suppression of anti-tumor immune responses. In addition, short-term and long-term side effects of these treatments limit their efficacy and impact quality of life of cancer patients. Therefore, a relapse-free cancer cure remains a major challenge for cancer therapeutics. To this end, tumor recurrence has been attributed to the presence of dormant tumor cells particularly in patients with breast cancer (8,17,55), and in patients with prostate cancer (56,57), ovarian cancer (58), melanoma (59–61), and hepatocellular carcinoma (62). Dormant tumor cells are quiescent, and thus, do not respond to chemotherapy or radiation therapies, and they are not operable. In addition, being in a quiescent state reduces risk of tumor escape because major changes take place during cell division. Therefore, immunotherapy is the only option for the treatment of tumor dormancy. In fact, we have demonstrated that dormant tumor cells remain highly susceptible to immunotherapy if targeted before clinical recurrence (63).

A critical review of published literature on human vaccines suggests that administration of immunotherapy in a preventive setting, i.e., during dormancy/latency and as a relapse prophylaxis, but not during active disease, can provide a cure for the disease. In fact, all human vaccines have been effective in a prophylactic setting before clinical symptom of the disease is evident. The rabies vaccine is an exception and can be used as post-exposure because the incubation period or dormancy for rabies is 1–3 months, which provides a window for vaccination as disease prophylaxis. Lessons learned from the application of the rabies vaccine during clinical latency suggest that cancer immunotherapy can be successful during tumor dormancy. However, two major gaps in our knowledge prevent the development of an effective relapse prophylactic cancer vaccine. First, dormancy-associated antigens are unknown to be used in a vaccine formulation, and second, the molecular mechanism of the establishment or maintenance of tumor dormancy is not completely understood. Although dormant tumor cells might contain cancer stem cells that did not respond to chemotherapies, tumor dormancy is mainly induced by chemotherapies and does not always show markers of cancer stem cells (64–66). A similar correlation between tumor dormancy and senescence or autophagy exists (19). In fact, tumor dormancy and senescence or autophagy or stemness are not mutually exclusive. Several reports have shown that the upregulation of various stress-induced UPR-associated genes like heat shock proteins and cyclophilin B regulate metastatic tumor dormancy (67–69). Given that chemotherapy could induce mutations in surviving tumor clones (70,71), it is expected to detect chemotherapy-induced mutant peptides in residual dormant tumor cells as antigenic targets following neoadjuvant chemotherapy. In addition, tumor cells can survive cancer therapeutics through the expression of survival receptors that transmit signals for the induction of anti-apoptotic genes in tumor cells. For instance, in humans, endothelin receptor A (ET_A) acts as a survival receptor by inducing the expression of anti-apoptotic genes in prostate cancer (72). Its ligand, ET-1 is produced by the prostate epithelia (72). The presence of the ET_A/ET-1 pathway at the tumor site could make tumor infiltrating T cells less effective in patients with prostate cancer. A higher responsiveness of melanoma patients to immunotherapy compared with patients with prostate cancer or ovarian cancer could be because ET_A is upregulated in prostate and ovarian cancers but not in melanoma (73,74). Human DCs also produce ET-1 upon activation (74), which in turn support survival of T cells during activation as well as tumor cells that express ET_A. Therefore, use of dormancy-associated neoantigens as personalized vaccine is expected to induce immune responses for the retention of tumor dormancy and prevention of tumor relapse even if dormant cells manage to survive apoptosis under immune pressure.

Future of cancer immunotherapy: personalized vaccines for cancer dormancy

Although cancer vaccine approach has been abandoned for a while, emerging evidence about neoantigens has renewed interest in cancer vaccines. Neoantigens are derived from random somatic mutations in tumor cells which are not present in normal cells (75,76). Thus, neoantigens can be recognized as tumor-specific targets by the immune system. Several clinical trials have shown that tumor neoantigens are effectively recognized by CD8⁺ and CD4⁺ T cells, thereby triggering anti-tumor immune responses (77). A

comprehensive review summarizing the empirical evidence on naturally occurring neoantigens and treatment-induced neoantigens suggest that dormant cells could express mutant neoantigens that can be used as target for immunotherapy (78). In fact, the neoantigen-specific T cells represent the most potent tumor-rejection T cell populations (79–81). However, naturally-occurring neoantigen-specific T cells in patients are typically rare because of a low clonal frequency and inefficient cross presentation of tumor-specific neoantigens (82,83). Therefore, neoantigen tumor vaccine has been developed to potentiate tumor-specific T cell responses. The safety and efficacy of these vaccines have been shown against late stage melanoma (84), as well as glioblastoma which has a low mutation rate (85). Currently, neoantigen-based vaccines are in clinical trials for cancer patients (86–90). Nevertheless, the efficacy of cancer vaccines, in general, is limited because of the immunosuppressive tumor microenvironment, tumor immunoediting and evasion from the immune response. Heterogeneity of proliferating tumor cells and their constant change during cell division creates these problems and inhibit the efficacy of cancer vaccines.

It is important to note that the lack of a solid tumor during dormancy excludes the immune suppressive tumor microenvironment, and a quiescent state of dormant cells dismantles tumor evasion mechanisms taking place during cell division. After all, human vaccines against infectious diseases are effective in a preventative setting, which could be the case when targeting tumor dormancy for the prevention of metastatic recurrences. In fact, we have reported that Ki67⁻ dormant cells do not undergo immunoediting and are highly susceptible to the immune response (19,20). Therefore, it is expected that immunization during tumor dormancy will prevent tumor recurrences. Nevertheless, tumor neoantigens have been identified during active stage disease rather than during tumor dormancy. This is because of the lack of a specific and clinically feasible strategy for the detection of dormant tumor cells in patients, and the right timing for vaccination prevent the development of immunotherapies for tumor dormancy. To identify breast cancer dormancy, CellSearch technology has been developed (91). This technology relies on epithelial markers for the detection of dormant tumor cells residing in the bone marrow or circulating in the blood. However, not all dormant cells express epithelial markers. Addition of mesenchymal markers to the detection panel (92) would reduce but not eliminate false negative data. After all, not all circulating tumor cells result in tumor recurrence as they have been detected in breast cancer survivors even two decades after a successful treatment of early stage diseases. Nevertheless, patients with circulating tumor cells are at a greater risk of tumor recurrence (93). Very recently, we have addressed these barriers by proposing a less invasive approach for the detection of Ki67⁻ dormant tumor cells in tumor biopsies or surgical excisions of cancer patients before or after neoadjuvant therapies (unpublished data). The Ki67⁻ dormant cells can be isolated by a laser capture microdissection for the extraction of DNA/RNA. Ultra low input whole genome sequencing (WGS) or whole exome sequencing (WES) technology (94) allows detecting heterogeneity of neoantigens in each individual in order to select those that are more commonly expressed by dormant cells but not by normal mammary tissue for the design of synthetic long peptide (SLPs) containing MHC class I and II neopeptides (95). Whether mutations can form tumor neoantigens depends on several factors: 1) translation of the mutated sequence into protein; 2) processing and presentation of neoantigens by MHC; 3) affinity of mutant peptide-MHC complex with T cell receptor

(TCR) (96). Therefore, the prediction of neoantigens requires not only identification of genome-expressed mutations, but also using RNAseq data and patients' MHC types. Frame shift mutations resulting from insertions/deletions (InDels) create alternative open reading frames (ORFs) with novel tumor-specific antigens. A pan-cancer analysis of 19 cancer types from The Cancer Genome Atlas demonstrated that frame shift-derived neoantigens were present in every cancer type (97). The discovered mutations can be screened using predictive algorithms for MHC peptide binding affinity (patient's HLA-A, B, C, DPA, DPB, DQA, DQB, DRA, DRB alleles) to identify the most immunogenic peptide candidates for ex vivo evaluation, and then manufacturing personalized cancer vaccines. The HLA typing algorithms such as OptiType, Polysolver, and PHLAT show up to 99% accuracy (98). The accuracy of predicting MHC class II epitopes has been problematic. However, using multiple MHC class II binding prediction algorithms such as TEPITOPE (58), netMHCII (59), and SMM-align (60), PHLAT (99), HLA-VBSeq (100), and seq2HLA (101) increase the chance of an accurate neoantigen prediction. Alternatively, a hidden Markov model (HMM)-based MHC binding predictor accommodating peptide sequences of variable length which is trained on recent Immune Epitope Database (IEDB) content can be used (102).

The objective of this manuscript is to highlight the need for developing a personalized dormancy vaccine for the prevention of cancer recurrences. This article addressed different types of tumor dormancy without getting into more detailed mechanistic studies which have been addressed in other publications. We propose that a personalized vaccine targeting dormancy-specific neoantigens, can be given to patients with early stage disease after the completion of neoadjuvant therapies and tumor resection as well as to cancer survivors after an initially successful completion of conventional therapies for the elimination of relapse causing dormant cells. This novel vaccine is nontoxic and will shift the current "wait and watch" approach to an active immunotherapy of tumor dormancy.

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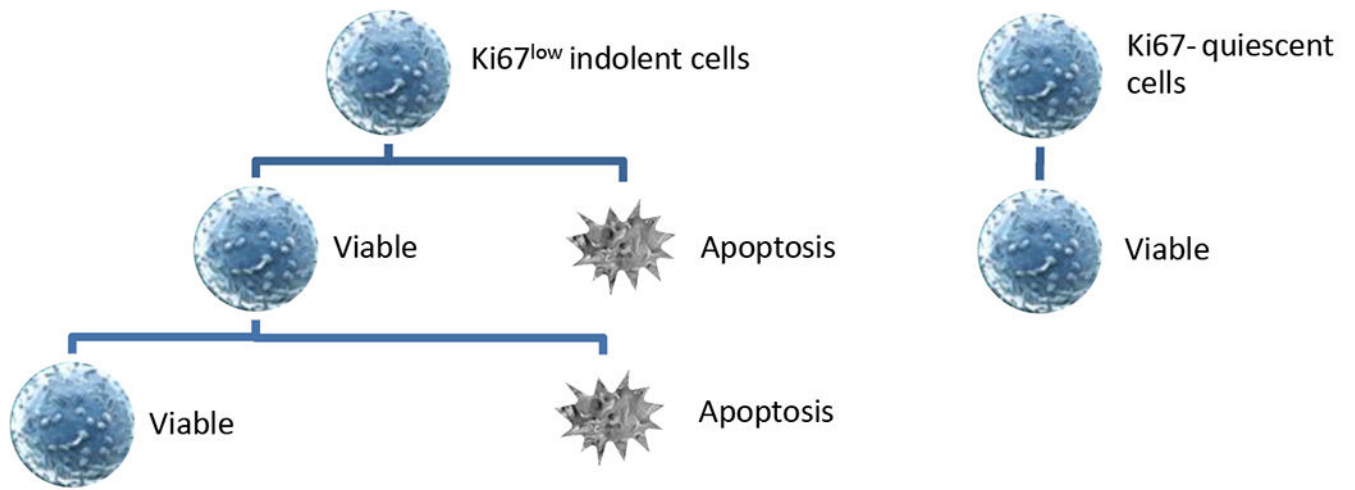


Figure 1. Two types of tumor dormancy.

Dormant tumor cells contain Ki67^{low} indolent cells and Ki67⁻ quiescent cells. Indolent dormant cells are characterized by sluggish proliferation rates counterbalanced by spontaneous cell death, keeping total number of cells unchanged. Quiescent dormant cells are arrested in G0 and incapable of cell division during dormancy.

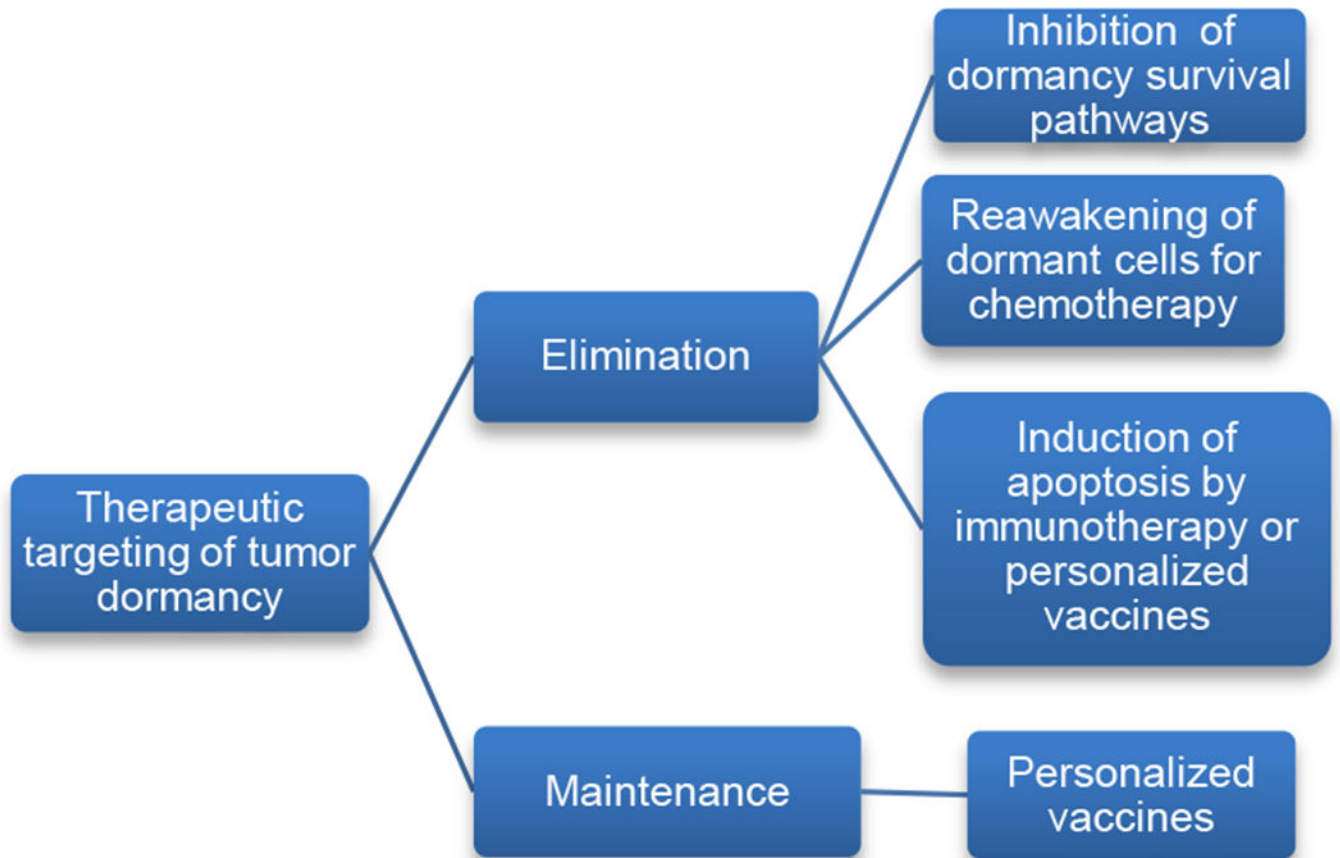


Figure 2. Immunotherapies could eliminate dormant cells or arrest them in a dormant state and prevent tumor relapse.

To eliminate dormant tumor cells, three strategies are being tested which include inhibition of cell survival pathways, reawakening of dormant cells in order to render them susceptible to chemotherapy, or induction of apoptosis by immunotherapy or personalized vaccines. Dormancy-specific personalized vaccines can establish memory, thereby preventing the recurrence of dormant cells that escape from apoptosis.

Table 1.

Relapse-free survival

Cancers	% Survival (5y)
Breast	29
Colon & Rectum	35
Kidney & renal pelvis	37
Leukemia	37
Lung & bronchus	60
Melanoma	27
Non-Hodgkin lymphoma	37
Oral cavity & pharynx	37
Ovary	31
Prostate	35
Testis	16
Thyroid	24
Urinary bladder	39
Uterine cervix	17
Uterine corpus	29

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