Immunotherapy

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Harnessing the potential to fight from within – immunotherapy advances in cancer treatment

Strategies to treat cancer have largely involved surgical removal of the tumor, broad chemotherapies or radiotherapies that exterminate both tumor and non-tumor cells, and targeted therapies to specifically block functional pathways to eliminate tumor growth. However, despite the type of treatment, cancers often reach a refractory period after prolonged treatment, when the patients fail to respond, leading to disease recurrence. A likely cause for this recurrence is the escape of the tumor cells from immune surveillance, a process that allows the tumors to evade attack by the immune cells and trigger their constant growth. The positive and negative regulators of both innate and adaptive immune cells work in concert through multiple interactions to recognize and eradicate tumor cells. Thus, immunotherapy offers hope in cancer treatment. To this end, biotechnologists together with immunologists are working on challenging medical problems to innovate new therapeutic pathways for cancer therapy. In this editorial, we will discuss recent advances in immunotherapy that are mostly based on countering the pro-tumor properties of the negative regulators of the immune system, to enhance their anti-tumor response.

The role of T cells in immune response

The tumor microenvironment (TME) usually is infiltrated by numerous immune cells, of which the CD8+ cytotoxic T-lymphocytes (Tc) are the mainstay. The Tc cells recognize tumor specific antigens presented through antigen presenting cells (APC) and with the aid of CD4+ helper T-lymphocytes (Th) cells, kill malignant cells. However, a subgroup of the Th cells called regulatory T-lymphocytes (Treg) has anti-inflammatory and immune-suppressive properties that prevent the activation and cytotoxicity of Tc. Tumor specific T cells express certain checkpoint proteins to regulate their function in immune response. In a normal anti-cancer response, checkpoint proteins on the T cells bind to receptors on tumor cells and prevent T cell effector function, thereby preventing T cell exhaustion, a process which facilitates the retention of antigen-specific T cells in the

repertoire under chronic stimulation. This process in turn is used by the tumor cells in their own favor to suppress the immune response and trigger their continuous growth. These checkpoint proteins have thus evolved as targets for immunotherapy since blocking them using checkpoint inhibitors (CPI) can release the brake and activate T cell immune response.

Types of immunotherapies

Current immunotherapy strategies can be categorized into two major groups – active and passive, depending on how the method reengages the patients' immune system to enhance anti-tumor activities. The active arm primarily includes cancer vaccines, immunomodulatory antibodies against immune checkpoint proteins and immunostimulatory cytokines to induce the hosts' immune system.

The passive arm also known as adoptive cell transfer (ACT) is a type of cell-based therapy where tumor infiltrating lymphocytes (TIL) are isolated from the patient, engineered to express a tumor specific neoantigen to enhance their immune potential, followed by lympho depleting chemotherapy before reinfusion of the engineered immune cells back into the patient. Particularly, T cells are engineered to produce an anti- CD19 chimeric antigen T-cell receptor known as CAR-T cells (1).

Cancer vaccines

Vaccination was earlier considered to play a protective role only against infectious diseases, and was not considered as an effective strategy in cancer therapy, until sipuleucel-T (Provenge) was first approved by the FDA in 2010 to treat patients with metastatic prostate cancer. Since then, therapeutic cancer vaccines have been developed using antigens from cancer cells, nucleic acids that can generate tumor associated antigens or weakened cancer cells carrying a specific antigen. Oncolytic viruses can also be engineered to act as cancer vaccines and the first genetically modified virus that received FDA approval in 2015 to treat melanoma was Imlygic (talimogene laherparepvec). Imlygic is a genetically modified oncolytic virus designed to replicate within tumors and produce an immunostimulatory protein called granulocyte-macrophage colony-stimulating factor (GM-CSF). Two main reasons prevented Imlygic's success, first was its intra tumoral mode of administration that limited it to melanoma and failed to benefit visceral lesions, second was the competition that it faced from CPIs. Thus recent trials have explored combinations of this vaccine with CPIs in two melanoma trials. The objective tumor response rate was almost doubled in the combination arm compared to the CPI alone arm, in a Phase Ib/II trial where Imlygic was combined with the anti-CTLA-4 antibody ipilimumab. It will be interesting to see the results of the ongoing KEYNOTE-034 Phase III trial in patients with unresectable melanoma, which is evaluating the efficacy of Imlygic in combination with the PD-1 inhibitor pembrolizumab. Data from a recent Phase 1b trial where a neoantigen cancer vaccine candidate NEO-PV-01 was combined with the checkpoint PD-1 inhibitor nivolumab in advanced or metastatic melanoma, smoking-associated non-small cell lung cancer (NSCLC) and bladder cancer, showed

consistent prolongation of progression free survival (PFS) in patients from all three cancer types (2).

Checkpoint inhibitors

Although our immune system confers a surveillance mechanism to prevent invasion by external agents, maintaining the immune homeostasis is critical to prevent immune response towards self-proteins. Immune checkpoint proteins make sure this balance is maintained while keeping tumor antigens and infectious agents at bay. Cytotoxic T-lymphocyte antigen-4 (CTLA-4) and programmed death-1 (PD-1) are two such checkpoint proteins that bind to their receptors B7 (CD80/CD86) and PD-L1 respectively, expressed on tumor or APC cells, and prevent the T cells from killing cancer cells (*figure 1*).
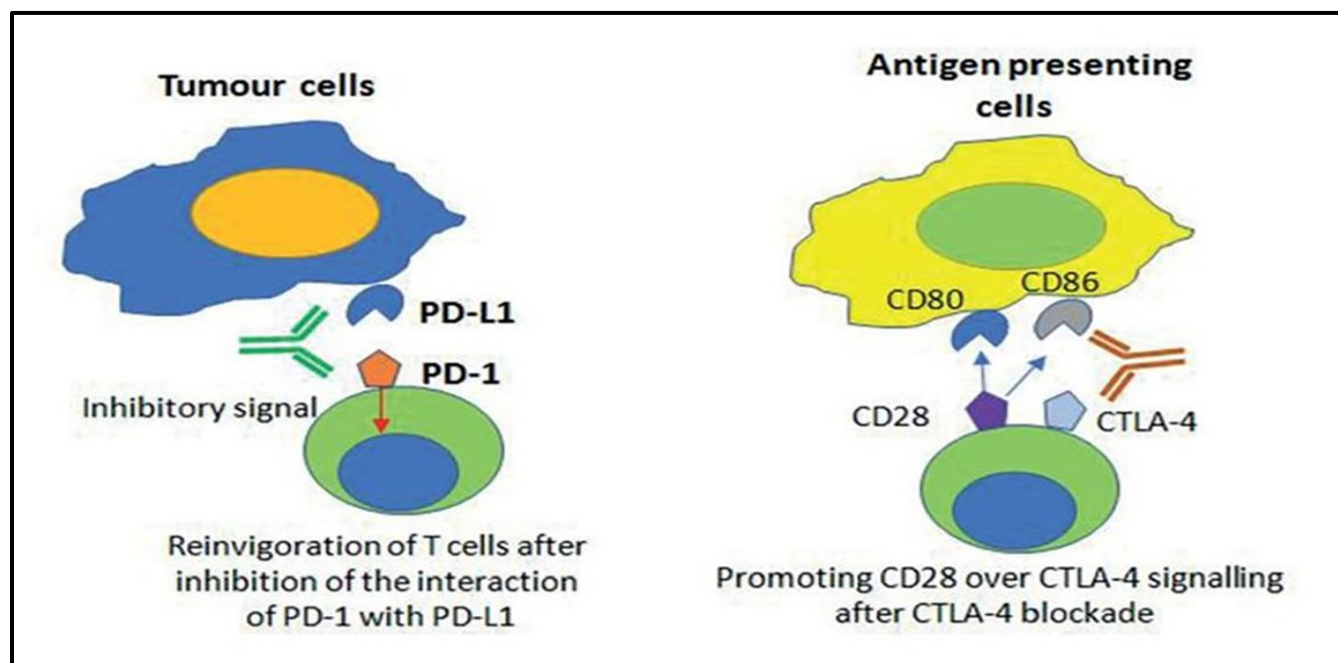

*Figure 1: Mechanisms of action of anti-PD-1 /PD-L1/CTLA-4 antibodies in the clinic using monoclonal antibodies as checkpoint inhibitors. [**Source:** ESMO Open. 2017]*

Multiple inhibitors which are mostly monoclonal antibodies against PD-1, its binding partner PD-L1 and CTLA-4 are already in the clinic showing great promise in certain cancer types. Ipilimumab (Yervoy), a monoclonal antibody against CTLA-4 was the first immune checkpoint inhibitor approved by the FDA in 2011, for treating unresectable metastatic melanoma. Despite increased response rates (overall survival and recurrence free survival) the use of ipilimumab was restricted, due to the serious immune related adverse events inflicted by this drug. The first anti-PD-1 inhibitor to receive FDA approval for the treatment of advanced melanoma was pembrolizumab (Keytruda) in September of 2014, shortly followed by nivolumab (Opvido) in December 2014. Both these drugs were later approved for other cancers including advanced non-small cell lung cancer (NSCLC), metastatic head and neck squamous cell carcinoma

(HNSCC), classic Hodgkin lymphoma, with metastatic gastric and cervical cancers where tumors express PD-L1, sorafenib resistant hepatocellular carcinoma, first line therapy for advanced renal cell carcinoma, metastatic squamous cell carcinoma of the esophagus with varying indications. Atezolizumab (Tecentriq) the first anti-PD-L1 inhibitor was approved by the FDA initially in 2016, to treat locally advanced or metastatic urothelial carcinoma following platinum based chemotherapy. Later atezolizumab has also been approved to treat patients with metastatic NSCLC and for the first-line treatment of patients with metastatic non-squamous NSCLC in combination with bevacizumab, paclitaxel, and carboplatin. A 26% overall response rate (ORR) was observed using pembrolizumab in a Phase I trial in 173 ipilimumab refractory melanoma patients. In another trial ipilimumab treatment resulted in 60% of patients showing severe grade 3 or 4 adverse immune reactions. In comparison, a study evaluating nivolumab across multiple cancer types in 296 patients, showed a 28% ORR in melanoma with only 14% grade 3 or 4 toxicities across all patients. These and other results have shown that anti-PD-1 based treatments are more favorable with regards to overall efficacy and reduced toxicity as compared to anti-CTLA-4 based treatments. This difference can be attributed to the varied distribution of these surface proteins, since PD-1 or PD-L1 are expressed on mature T cells and tumor or APC cells, whereas CTLA-4 is widely expressed on all T cells, leading to higher systemic toxicity beyond the TME (3).

With the development of these immune therapies, the fact that immunotherapy can augment the efficacy of chemotherapy, radiotherapy or even targeted therapies (eg: kinase inhibitors, hormone therapies, signal transduction inhibitors, gene expression modulators, apoptosis inducers, angiogenesis inhibitors) is now well recognized. Currently there are about 25 approved CPIs in the clinic and several 100 in preclinical and early phase clinical trials. Thus, it is not surprising that multiple combinatorial regimens using immune checkpoint inhibitors with other traditional therapies have been tested in the clinic, and multiple ongoing trials are based on these combinatorial approaches. Recently, several phase III trials have demonstrated the efficacy of combining PD-1 / PD-L1 inhibitors with chemotherapy in small cell lung cancer (SCLC), NSCLC, HNSCC and breast cancer. The synergistic potential demonstrated by combining CPIs with radiotherapy in preclinical studies have resulted in several trials evaluating these combinations in the clinic, however, with mixed results. A notable trial using this regime was a randomized phase III PACIFIC trial investigating the addition of durvalumab (anti-PD-L1) to platinum-based chemo radiotherapy in locally advanced (stage III) NSCLC, where the combination arm showed a remarkable increase in both progression-free survival (PFS) and overall survival (OS). In this particular trial the timely administration of durvalumab (14 days vs later) seemed to be critical for improving the OS in patients (1). In NSCLC, 6 trials have assessed the benefit of combining platinum based chemotherapy with anti-PD-1 therapy, and all of these showed variable, but significant, enhancement of PFS which also positively

correlated to the level of PD-L1 expression, and 4 of these trials showed improvement in OS. Based on several randomized trials, the frontline immunotherapy strategy is monotherapy in high-PD-L1, dual CPI in high-tumor mutational burden (TMB) or anti-PD-(L)1 in combination with chemotherapy in all-comers NSCLC in absence of ALK or EGFR addiction.

Despite the concern of associated immune toxicities, several preclinical studies have demonstrated the synergistic effects of combining two different checkpoint inhibitors, as manifested by increase in TIL, decrease in Treg and retraction of tumor, supporting the rationale for combining these inhibitors in the clinic. An early melanoma trial evaluating the combination of nivolumab with ipilimumab vs ipilimumab alone showed impressive ORR in the combination arm (40%) vs the monotherapy arm (20%), albeit with higher toxicities in the combination arm (53% vs 18%). Another dose-escalation study using the same drug combination in melanoma also showed prolonged progression free survival (PFS) with remarkably high ORR in the combination arm (63%vs 11%), although at the expense of higher toxicities. However, the immune related toxicities in both these trials were managed with drugs, and long term follow up revealed a longer OS in the combination arm. A subsequent well powered Phase III trial (Checkmate 067) in previously untreated melanoma also showed enhanced OS in the combination arm (58%) vs nivolumab alone (52%) and ipilimumab alone (34%); and enhanced complete response (CR) in the combination arm (11.5%) vs nivolumab alone (8.9%) and ipilimumab alone (2.2%). These and several other trials, some using pembrolizumab instead of nivolumab, all demonstrate the benefit of combining PD-1 and CTLA-4 inhibitors in melanoma treatment. The CPI combination Phase III trials in melanoma mostly showed benefit in patients with PD-L1 negative tumors (3). Early results from the CheckMate 142 Study, using the combination of nivolumab and ipilimumab in the treatment of patients with deficient DNA mismatch repair (dMMR)/high microsatellite instability (MSI-H) metastatic colorectal cancer (mCRC) showed 54.8% ORR and 78.6% disease control rate (DCR), with a manageable safety profile (1). A recent study that estimated the percentage of cancer patients who will respond to immunotherapy, reports the increase in response to checkpoint inhibitors from 0.14% in 2011 to 12.46% in 2018, showing 88% increase in response within 7 years which may be considered as a huge success. However, the efficacy of checkpoint inhibitors is still limited to certain types of cancer and better selection strategies by identifying appropriate response predictive biomarkers is necessary to expand the benefits to other cancer types.

CAR-T cell therapy

Currently approved CAR-T cells use autologous T-cells derived from the patient to construct a chimeric antigen receptor (CAR) consisting of a single-chain variable fragment (scFV) antigen-recognition domain, a CD3-derived T-cell activation domain, and a costimulatory domain (CD28, 4-1BB or both) (*figure 2*).
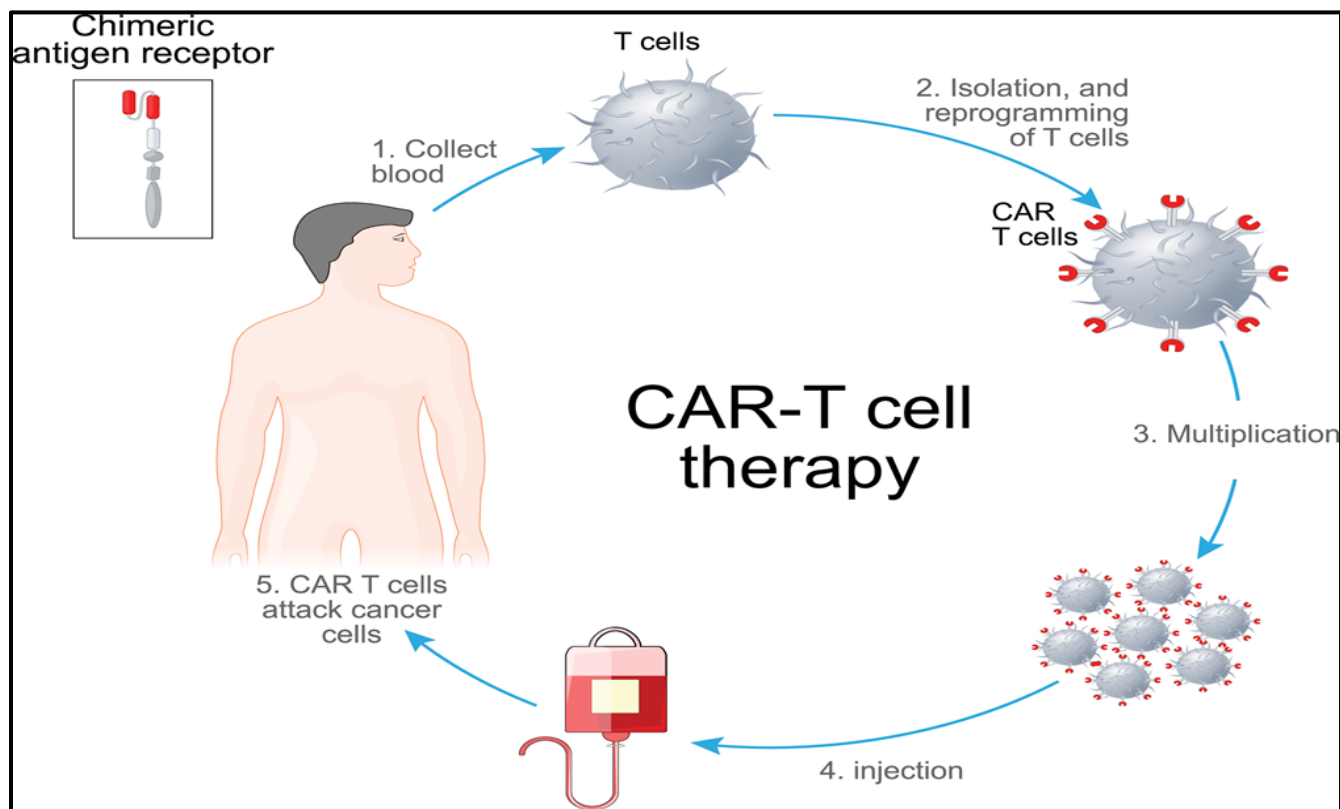
*Figure 2: CAR T-cell products with current FDA approval use a tumor-targeting domain (derived from a mouse antibody against the human B-cell tumor antigen CD19), and signaling domains derived from human immune activating receptors (CD28 or 4-1BB) [**Source:** iStock/Getty Images Plus].*

Tisagenlecleucel was the first CAR T-cell therapy to receive FDA approval in August 2017, for the treatment of children and young adults with B-cell acute lymphoblastic leukemia (B-ALL). This approval was based on the ELIANA trial conducted on 75 patients, that reported a strikingly high complete remission rate (CRR) of 60% and overall response rate of 81%. Most importantly, these responses were sustainable as evidenced by 80% relapse free survival (RFS) rate over 6-months, however, the treatment-related toxicity was very high, with 73% patients suffering from Grade 3-4 toxicities. Closely after this approval, in October of 2017, the second CAR-T therapy

axicabtagene ciloleucel was approved for refractory aggressive lymphoma, based on results from a Phase II multicenter ZUMA-1 trial. This trial again reported a very high ORR of 83% and a CR of 58%, but was associated with a high incidence of neurotoxicity (32%). Tisagenlecleucel was approved for a second time by the FDA in May 2018, for relapsed and refractory lymphoma based on the JULIET trial in diffuse large B-cell lymphoma (DLBCL) patients, which reported 40% CR with toxicity similar to the B-ALL trial. The next in line, CAR-T therapy based on similar principles which is showing very promising response in DLBCL, with fewer toxicities in lisocabtagene maraleucel. Despite the

approval and remarkably high efficacy of CAR-T therapies, the toxicities inflicted due to cytokine release syndrome (CRS) and neuro toxicities, usually ranging between 10-50% are extremely concerning. CRS is usually manifested in fever, hypoxia and ultimately may lead to organ failure. Neurotoxicity is characterized by various neurologic symptoms, including delirium and seizures. Concurrent trials with an effort to reduce these toxicities by using IL-6 blockers or monoclonal antibodies against IL-6 along with the CAR-T therapy are underway, but they are yet to show significant reduction of toxicity. The CAR-T cells recently entering clinical trials are designed to constitutively express both the costimulatory molecules CD28 and 4-1BB ligand to enhance T cell activation and cytotoxicity. Furthermore, like resistance to most drugs, the failure of CAR-T cell therapy is partly attributed to the loss of the CD-19 receptor. To counter this, CAR-T cells are also designed to express CD-22 or to express bispecific CD-19/CD-22. A study evaluating the efficacy of CD-22 CAR in patients who had previously relapsed on CD-19 CAR, reported an impressive 73% CR rate. Currently several trials are investigating the potential of bispecific CARs, and a few of these show encouraging response data, with reduced toxicities. Though still in its infancy, the efficacy of CAR-T cell therapy as demonstrated by data from multiple trials seems to hold promise for the future, particularly in blood cancers (4).

Need for predictive biomarkers

Despite the advances in immunotherapy in recent years, the number of patients who actually benefit from immunomodulatory treatments are still limited, due to lack of response predictive biomarkers that can tailor specific therapies aligned to the patients' needs. The identification of appropriate biomarkers can lead to optimization of benefits and help reduce unwanted toxicities, increase response rates and minimize costs.

Biomarkers explored in immunotherapy can be categorized into different groups such as serum proteins, tumor-specific receptor expression patterns, factors in the TME, circulating tumor cells, host genomic factors and tumor mutational burden.

High levels of interleukin-6 (IL-6) and C-reactive protein (CRP) in the serum have been reported to negatively correlate to interleukin-2 (IL-2) therapy response, in RCC and melanoma. Similarly, higher levels of soluble VEGF and CRP have been shown to correlate with lower clinical response (OS) in melanoma treated with ipilimumab. Elevated serum lactate dehydrogenase (LDH) level was also a negative predictor of benefit from ipilimumab, as analyzed from 3 separate trials. Cells in the peripheral blood, such as T cells, NK cells, dendritic cells, macrophages, and tumor cells, have been evaluated as predictive biomarkers in multiple clinical trials. Myeloid derived suppressor cells (MDSC), an immune cell population, was found to negatively correlate to the clinical benefit from ipilimumab. Some studies claimed that there is a positive correlation between increase in absolute lymphocyte count (ALC) and OR, but results from several studies put together show that irrespective of the treatment outcome there is always an increase in ALC.

The preponderance of certain cell types in the TME have also been evaluated as potential biomarkers. The reason of variations in the T cell populations residing within the TME are not clearly understood, but the types of cells present dictate whether the TME is inflammatory (increased effector T cells) or immunosuppressive (decreased effector T cells). Regardless of T cell type, the cells in an inflamed environment usually express high levels of T-cell checkpoints, such as PD-L1, B7H4, Tim-3, Lag-3 that can disable tumor-infiltrating effector cells. Furthermore, beta-catenin expression and intracellular hypoxia or release of soluble factors like IL-10 and transforming growth factor-beta (TGF-β) may result in inhibition of effector T cells. Melanomas with high Tc content, were found to be more likely to be associated with high PD-L1 expression, and resulted in improved prognosis. Similarly in NSCLC, increase in tumor infiltrating Tc and Th cells was a predictor of favorable response (5). PD-L1 expression on the tumor cells, has been the most sought after cell surface receptor that has been evaluated in multiple clinical trials as an independent predictive biomarker for favorable response. Though several studies reported a positive correlation between clinical response and PD-L1 expression on tumor cells, this is not always found to be true. This discrepancy potentially arises from the dynamic nature and heterogeneous expression of PD-L1 in patients. The FDA approved immunohistochemistry (IHC)-mediated detection of PD-L1 expression on tumors and mandated it as a prerequisite to treatment with anti-PD-1/PD-L1 inhibitors in several cancers. However, it has been found that PD-L1 tumor expression is not a reliable biomarker to determine patients who will respond. Moreover, one should not confuse between the tumors PD-L1 expression with soluble PD-L1 (sPD-L1) found in peripheral blood. Data from a study in pancreatic cancer patients suggest that sPD-1 and sPD-L1 are indicators of systemic inflammation and independent from tumoral PD-L1 expression. Another approved biomarker for predicting PD-1 inhibitor response is the presence of microsatellite instability (MSI) or deficient mismatch repair (dMMR), and several trials showed that the presence of MSI/dMMR increased tumor mutational burden (TMB) and subsequent response to PD-/PD-L1 inhibition. However, the presence of MSI/dMMR was not always associated with TMB and high TMB was itself used independently as a predictive biomarker. In the current scenario, further studies are necessary to ascertain these biomarkers as definite predictors of response (5).

Conclusions

The advancement of immunotherapy is inevitable with numerous preclinical studies and clinical trials geared to take cancer treatment to a new paradigm through immune modulation. With the discovery of better predictive biomarkers and implementation of the knowledge gained by consolidating data obtained from ongoing studies, immunotherapy will reach new heights in the near future. The CPIs will most likely expand to other cancer types and will end up gaining importance in other rational combinations. Whether the CAR-T cell therapies can be extended to solid malignancies is yet to be witnessed.

Author's Biography

Dr. Paromita Raha is a Biotechnologist who currently leads product development and food science research at a Food technology startup, Lecker Labs, manufacturer of the world's first automatic yogurt maker. She received her doctorate in Biotechnology from the Indian Institute of Chemical Biology in India by winning a competitive national fellowship. Later she pursued her postdoctoral research at the University of California at San Francisco in translational oncology. She has several years of research experience in preclinical oncology and she has worked as a Scientist in the drug discovery team in a pharmaceutical company. She has developed novel drug combinations in breast and lung cancer drug-resistant models, which have successfully translated to the clinic. Dr. Raha has authored multiple peer-reviewed scientific research papers in structural biology, preclinical oncology, and clinical research, and an invited book chapter in Medical Epigenetics. To remain abreast of current research, she freelances as an expert scientific reviewer and as a scientific editor. She also volunteers for outreach activities, particularly helping women and girls to pursue a career in STEM and has served as a Board of Director in a non-profit organization.

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