# Immuno oncology, what have the last 3 decades brought?

## Abstract

A little over 30 years ago, the discovery and availability of recombinant growth factors for human lymphocytes, such as IL-2, prompted experimental use of IL-2 to both treat patients directly and for *ex vivo* expansion of lymphocytes for therapeutic reinfusion. In contrast to the standard practice of immunization, IL-2 therapy represented a pioneering step in developing a course of therapy that involved manipulation of the host immune system. Adoptive immunotherapy produced and still produces some stunning successes but not in all cases and not without the possibility of considerable and severe side effects. The recent approval of Mabs that are checkpoint inhibitors to treat such deadly cancers as melanoma, have opened the door for clinical testing of other Mabs to antigens related to immune surveillance. Adoptive cell immunotherapy has progressed to incorporate elements from adaptive therapy in so far as the cells may be engineered to specifically seek out antigens, as in the CAR-T cell therapy, or may be cultured with markers on specific cancers when appropriately specific antigens are known, such as prostate cancer antigens; MUC1, PAP, PSA, PSMA. Both the development of a range of immunotherapy agents and clinical experience with them continues with the promise to identify how to use this new armory effectively on the war on cancer.

**Keywords:** cancer immunotherapy, immuno oncology, adoptive immunotherapy, checkpoint inhibitor, cancer vaccines

Margaret A Baker
Independent Consultant, USA

**Correspondence:** Margaret A Baker, Independent Consultant, 641 Malvern Rd, Ardmore, PA 19003, USA, Tel 610-649-1840, Email meg.baker58@gmail.com

**Abbreviations:** APC, antigen presenting cell; BCG, bacille bcalmette-guerin; CTL, cytotoxic T lymphocyte; DC, dendritic cells; GM-CSF, granulocyte macrophage colony stimulating factor; Infa, interferon alpha; LAK, lyphokine activated killer cell; IL-2, interleukin 2; MUC1, mucin 1; NSCLC, non-small cell lung cancer; TREG, regulatory T-cell; Til, tumor infiltrating lymphocytes; NY-ESO, New York esophageal carcinoma antigen 1; MAB, monoclonal antibody; PAP, prostatic acid phosphatase; PSA, prostate specific antigen; PSMA, prostate specific membrane antigen; FDA, (U.S.) food and drug administration

## Introduction

The foot on the "gas pedal" driving towards new cancer therapies involving the host immune system is noticeably heavy. Meetings, the press and regulatory agencies give weekly or daily reports on the promise, prospect and products that represent new modalities in immuno oncology. These new developments represent advances in both the technical aspects – *in vitro* cell stimulation and expansion, vaccines and gene therapy, target identification and antibody therapeutics – as well as the understanding of when and how much to use the new tools in the tool kit.

## 30 years of modern immuno-oncology

To this foot soldier in the war on cancer, Steve Rosenberg planted a flag on the mountaintop when he began treating patients with melanoma in 1984 using a recombinant manufactured form of a cytokine, IL-2 and, subsequently, by also infusing IL-2 treated immune cells, LAK and TIL, to act as homing devices to wipe out cancer cells in the body from within.[1,2] Recombinant IL-2 represented possibly the first biologic drug put into clinical development and was called aldesleukin by its manufacturer, Cetus, and later became Proleukin®. Several other companies also devised and developed modified forms of human IL-2, such as teceleukin (Roche). These efforts and the discovery and cloning of other cytokines were largely enabled by

another new laboratory technique, PCR. Adoptive immunotherapy using IL-2 proved to have produced at least a few durable (29 years) responses.[3] So dramatic were the early media reports of the results and so unlike any other therapy, that many researchers – including those I worked with at Stanford University's Cancer Biology Research Laboratory in the mid '80s – that we believed that our research on novel cytoxic molecules might have just been made superfluous. The ability to use a synthetically produced cytokine to muster the immune system and to grow lymphocytes in the laboratory for use to clear cancer cells from the body appeared to be a viable and superior therapeutic method. Adoptive transfer of immune cells seemed a logical shortcut to promoting an immune response rather than by, for example, vaccination. Immunization, at the time, was generally prophylactic and effective in evoking the adaptive immune response to limit growth of foreign invaders, usually bacteria or viruses. However, immunization requires weeks or months before the system are properly primed using disease specific antigens to spur circulating lymphocytes with memory recognition. Interestingly, somewhat concurrently the hunt for tumor specific antigens was underway and it was demonstrated that injection of tumor tissue into mice yielded Mabs isolated by the technique of Kohler and Milstein described in 1975,[4] that recognized unique tumor markers.[5] What those antigens were was not entirely clear.

Debate as to whether immune surveillance could timely suppress or eliminate tumor cells was revived as new tools and the ability to manipulate and understand the genome became available. Using animal models, the question was finally addressed directly.[6] Evidence showed that the immune system could suppress or eliminate cancer cells providing renewed confidence in finding a way to encourage, co-opt, or reconstitute the system to produce cures. On the downside, clearly an out of control immune response – think anaphylactic shock or sepsis - has severe consequences and, in particular, invoking the uncontrolled release of immune stimulating signals, called the Cytokine Release Syndrome (CRS), can be life-threatening

or fatal. Thousands more careers in fields of immunology, cancer biology, and genomics and probably billions research dollars later, the ensuing 30 years has produced newly approved and about to be approved immunological based therapies for certain cancers. From the experience to date, there are several conditions to developing an effective immuno oncology therapy,

a. Removing or blocking immune suppression.

b. Enhancing tumor antigen recognition and/or a promoting homing or recruitment of cytotoxic lymphocytes to the cancer cells.

c. Maintaining control or carefully targeting the cytolytic processes.

d. Potentially combining immune mechanisms, in a timely manner, with each other or other modalities such as chemotherapy or radiation.

Thirty years ago, each of these steps were still giant black holes of complexity but dogged pursuit by many researchers and the courageous and hopeful participation of patients in experimental testing has led to reasonably safe therapies now in the early clinical use.[7,8]

## Nonspecific immune stimulation and adoptive therapy

T-cell stimulating cytokines, IL-2 which alone could produce responses in cancer patients[3] as could another newly identified cytokine, IFNalpha, approved for cancer therapy in 1986, and somewhat later GM-CSF, sargramostim or LEUKINE®, first approved for use after autologous bone marrow transplant in 1991, began to be used. Access to these factors for culture and expansion of lymphocytes was and still is key to the *ex vivo* manipulation and expansion of lymphocytes to treat cancer. Adoptive therapy may use either exogenously derived or autologous cells to the patient/host. Rosenberg et al. combined IL-2 treatment with exogenous IL-2 activation of autologous lymphocytes.[1–3,9] More recently, autologous cells used in adoptive immunotherapy are being re-engineered or "educated" to make them more selective homing devices for specific cancers cells. Autologous T-cells can be genetically engineered to express conventional or chimeric T-cell receptors for use in patients with leukemia, metastatic melanoma, synovial sarcoma, neuroblastoma and refractory lymphoma. The chimeric antigen receptor engineered T-cell (CAR-T) approach has succeeded in producing responses in leukemias expressing CD19[10] and the efforts are underway to expand the reperto ire of chimeric antigens.

In humans, in addition to specific T lymphocytes, NK cells play an important role in tumor immune surveillance. New therapeutic cell preparations comprising NK cells bioengineered to incorporate chimeric antigen receptors (CARs) and antibody receptors to further optimize targeting and potency in the therapeutic disease are being developed by a company headed by Patrick Soon-Shiong, NantKwest.[11] Adoptive immunotherapy of autologous cells bears the limitation of the complication of requiring the time and facilities to be able to harvest, educate or genetically engineer a cell line, and expand the cell population for later re-infusion to the patient.[7] A more universal approach using NK cells as proposed by Soon-Shiong may allow an "off the shelf" product which would shorten patient wait time.

## Mabs targeting the lymphocyte receptors and the checkpoint pathway

A large family of T cell receptors involved in immune surveillance, activation, or, alternatively, putting the system into check, has been discovered. The checkpoint pathway is engaged by tumors to effectively cloak themselves from host immune mechanisms or by T-cells themselves to prevent "exhaustion". The advent of the ability to select and develop highly potent, exquisitely selective, and nontoxic drugs in the form of monoclonal antibodies has also come to fore in the last two decades. The convergence of these two fields of research has led to a rapidly developing set of tools in the anticancer armamentarium, including the so-called "checkpoint inhibitors". The first therapeutic Mab, Muromonab-CD3 or OKT3, a murine antibody to the CD3 receptor on T-cells was approved by the US FDA in 1986 for use in acute graft vs host response not successfully controlled by steroid therapy. OKT3 blocks raging immune response by direct engagement of a receptor, CD3, on T lymphocytes first stimulating them, which can cause CRS and then causing T-cell apoptosis. Within the last decade, the ability to construct human antibody libraries, use transgenic mice with components of the human adaptive immune system, or use synthetic biology to formulate a human or human-like antibody has enabled development of Mabs with long *in vivo* half lives capable of high affinity and fuctional binding to a myriad of cellular and soluble targets. Mabs can be used as passive immunotherapy that is, directly targeting tumor surface antigens or receptors. Such Mab products have been approved for treating several forms of cancer. Specific Mabs may block tumor cell functions, such as growth receptors, as is the case for the anti-HER2/neu antibody trastuzumab (HERCEPTIN®), or block soluble factors that promote tumor growth, such as tumor vascular zing factor, VEGF, by bevacizumab (AVASTIN®). Alternatively, or in addition to the specific actions of the antibody therapeutic, the nonselective portion of the antibody, the constant region, can elicit effector functions and promote an cytolytic immune complex - usually involving NK cells - at the cites of multiple antibody binding, as with rituximab (RITUXAN®), a CD20 binding antibody used to treat lymphoma. This response is called antibody-dependent cell-mediated cytotoxicity (ADCC) and molecular engineering of the antibody constant regions to enhance ADCC functions of antibody therapeutics is another tool now in use in developing next generation biologic dugs.[12]

Immuno oncology has embraced antibodies with specificity allowing agonism of T-cell activating receptors or antagonism of checkpoint receptors. However, the practical use of the Mabs against T-cell antigens had an unfortunate initial misstep. The phase I trial of TGN1412 or CD28 Super MAB, a Mab with agonist activity specific to CD28, caused life-threatening immune inflammatory reactions in the small number of healthy volunteers to whom it was given.[13] That trial lead to an abundance of caution on the part of regulatory bodies including the US FDA in the design of subsequent trials of therapeutics targeting the T-cells. Nevertheless, the anti-CTLA-4 antibody, ipilimumab or YERVOY®, was approved in 2011 for the treatment of advanced melanoma. CTLA-4 is one of the "checks" in the balance of promotion and modulation of the immune system. Tremelimumab, CP-675,206 is another fully human anti-CTLA-4 antibody being developed by AstraZeneca also reached Phase III clinical trial stage for metastatic melanoma (See NCT00257205). The number of known T-cell modulating receptors and their cognate ligands has grown in complexity. A short list of target antigens on both the enhancement or "accelerator" side and the check or "braking "side of the system was summarized by Mellman et al.[14] For cancer immunotherapy, agonism of immune stimulating and antagonism of immune suppressing signals, it is hoped, will translate to the ability of TIL and/or NK cells to seek out and kill tumor cells . Targets for agonists that enhance T-cell stimulation and promote the immune system's potential to kill tumor cells – putting the foot on the accelerator - are, CD28, OX40,

GITR, CD137 (4-1BB), CD27, and HVEM. Antagonists should target antigens associated with down activation of T-cells and leniency towards tumor cell growth and killing inhibitory signals – those that put on the parking brake – and whose function should blocked are, CTLA-4, PD-1 on active CD4 positive T-cells, PD-L1 (B7-H1) on tumor cells, TIM3, BTLA, VISTA, and LAG-3. Several antibody therapeutics designed to relieve immunosuppressive signals – the "anti cloaking" or parking brake releasing activity and now called the checkpoint inhibitors – are approved or in late clinical development. KEYTRUDA®, pembrolizumab, formerly MK-3475, was the first PD-1 antibody to be approved in the US. Keytruda was approved by the US FDA in September 2014 for advanced melanoma.[15] Keytruda prescribing information specifies that it should be given only after ipilimumab and, if the patients melanoma expresses the V600E amino acid change in the BRAF cell signaling protein, a BRAF inhibitor such as vermurafenib (Zelboraf; Genentech/Daiichi Sankyo) or dabrafenib (Tafinla; GSK). Another PD-1 antibody, developed by BMS, MDX 1106, nivolumab or OPDIVO, has been approved in the US for unrespectable and chemotherapy resistant melanoma as well as advanced squamous NSCLC and Japan while PD-L1 antibodies under development are MDX=1105 (BMS), MPDL3280 (Genentech/Roche).

An OX40 (C134, TNFRSF4) antibody (MEDI 6383) completed Phase I clinical testing in 2013.[16] The phase I multi-center trial (NCT02221960) is designed to evaluate the safety and tolerability of MEDI6383 as a treatment for patients with solid tumors.[17] Urelumab , and anti-CD137 (an antigen also known as 4-1BB) antibody, formerly PF-05082566, PF-2566, being developed by Pfizer in partnership with Merck Serono, was recently retested in a small trial of patients whose lymphoma had progressed and showing a benefit in 40% of the 38 patients treated.[18,19] Another 4-1BB antibody is being developed by BMS.[18] An agonist anti-CD27 mAb, varlilumab or CDX-1127, is being developed by Celldex, Inc. (Hampton, NJ) and has shown ability to activate human T cells in the context of T cell receptor stimulation and activity in pre-clinical tumor models. In addition to the immune enhancing properties of CDX-1127, the mAb may also provide direct therapeutic effects against tumors with CD27 expression. Phase I clinical study results were report in 2014.[20] CDX-1127 is being developed for the treatment of B cell hematologic malignancies known to express CD27, including but not limited to chronic lymphocytic leukemia (CLL), Burkett's lymphoma, mantle cell lymphoma, primary lymphoma of the central nervous system and marginal zone B-cell lymphoma. CDX-1127 is also in Phase 1 development for the treatment of solid tumors, including metastatic melanoma and renal cell carcinoma. GITR, glucocorticoid-induced tumor necrosis factor receptor, is a member of the TNF receptor super family that is expressed on the surface of multiple types of immune cells, including regulatory T-cells, effector T-cells, B-cells, natural killer (NK) cells, and activated dendritic cells. Specifically, GITR activation increases the proliferation and function of effector T cells, as well as abrogating the suppression induced by inappropriately activated T regulatory cells.[12] GITR stimulation promotes anti-tumor immunity by increasing the activity of other immune cells such as NK cells, antigen presenting cells, and B cells. GITR, Inc. is developing TRX518, the first GITR-targeted therapeutic to enter human studies. TRX518 is currently in a Phase 1 a multi-site open label dose escalation study in adults with biopsy proven unrespectable Stage III or Stage IV melanoma or other solid tumor malignancies.[21]

LAG3, lymphocyte activation gene 3 proteins or CD223, is a receptor with high homology to CD4 expressed exclusively in activated T and NK lymphocytes. BMS-986016 is an agonist antibody that has entered Phase 1 safety testing. Tesaro also has a LAG3 antibody in preclinical testing Table 1. Preclinical studies have demonstrated a synergistic effect of LAG-3 and PD-1 in preventing autoimmunity in animal models.[22] A Phase I study of BMS-986016 alone and with nivolumab in patients with advanced solid tumors has been initiated at Memorial Sloan Kettering Hospital.[23] TIM3, T-cell immunoglobulin and mucin domain-containing protein 3 or CD366, is specifically expressed on Th1 cells and negatively regulates T lymphocyte response. No antibodies to TIM-3 have entered the clinic yet although Kyowa Hakko Kirin Co., Ltd filed for a patent on a possibly disease treating antibody composition in 2010 (WO2011/155607A1). VISTA is distinct from but bears homology to PD-L1 and is therefore part of the checkpoint system. No clinical candidates have been reported that antagonize VISTA. BTLA, B and T lymphocyte attenuator is an inhibitory receptor with structural and functional similarities to CTLA-4 and PD-1. HVEM, herpes virus entry mediator (CD270 or TR2), is a member of the TNF receptor super family (TNFSF14) and a ligand for BTLA. HVEM was found constitutively expressed by myeloid leukemia, B cell lymphoma, and multiple myeloma cells.[24]

## Cancer vaccines, tickling the adaptive response

In 1876, Coley demonstrated that injecting bacteria into tumors could help shrink them. In 1976, what was originally a tuberculosis vaccine, a bacterial preparation, BCG, was used experimentally and is still routinely used to treat bladder cancer. Instillation of a solution of BCG into the bladder stimulates the immune response. These are examples of early immuno oncology therapy using adjuvant, if not cancer vaccines. To be effective, a vaccine should engage the antigen presenting cells (APC) of the immune system, usually dendritic cells. In order to eradicate tumor cells, antigen-specific CD8+ cytolytic T cells (CTLs), which are responsible for tumor cell lysis, as well as antigen-specific CD4+ helper T cells that secrete cytokines that activate the CTL must be induced. In another aspect the APC educate and motivate B–cells to produce disease specific antibodies. CD4+ and CD8+ antigen epitopes are distinct and peptide based vaccines may limit or skew the response according to the epitope presentation. As discussed above, passive immunotherapy is use of cell directed specific antibodies that both identify malignant cells and recruit lymphocytes to the surface of the cells using the effector functions inherent to certain subclasses of immunoglobulin's, e.g. IgG1. Methods of delivering the antigens have included whole cells, proteins, peptides and DNA vaccines that provide coding sequences for the protein antigens (See Schlom for a review). Preventative HPV vaccines have been approved (GARDISIL®, CERVARIX®).[25]

The identity and source of the best antigens for the purpose of killing cancer cells is still an area of research. Antigens on breast, prostate, melanoma, and HPV induced cancer have been or still are actively being explored. More than 75 antigens have been clinically tested in various approaches of cancer vaccines and can be classified as onco-/fetal proteins such as CEA, p53 and MUC1; tissue specific antigens such as NY-ESO-1, Her2/neu (ERBB2), MAGE-3, PAP, PSA, PSMA and B-cell lineage specific antigens called anti-Id; Tumor or patient specific mutations, such as p53, EGFR; and a few specific glycoprotein antigens such as gp100 and STn.[26,27] Multiple forms of several antigens, particularly MUC1 and PSA, have been investigated in a number of vaccine platforms see Table 2. To date, only one product that is a therapeutic vaccine has been approved by the US FDA. Sipuleucel-T, PROVENGE®, manufactured and sold by Dendron, is an autologous cell cancer vaccine. It is a preparation of autologous CD54+ cells derived from the patient and activated

with PAP-GM-CSF for patient reinfusion. Sipuleucel-T is approved for the treatment of asymptomatic or minimally symptomatic metastatic castrate resistant (hormone refractory) prostate cancer. A dose of Sipuleucel-T contains a minimum of 50 million cells and a recombinant protein, PAP-GM-CSF. This construct links human prostatic acid phosphatase (PAP), an antigen expressed in prostate cancer tissue, directly to human GM-CSF. GM-CSF is cytokine that stimulates immune cell production.[28]

**Table 1** Summary of clinical stage Mab candidates to T-cell targets

| | Product, Antibody, Candidate Designation (Company Developing) | Relief Of Immune Suppression Targets/Checkpoints | Product, Antibody, Candidate Designation (Company Developing) |
|---|---|---|---|
| CD28 | TGEN 1412 | CTLA-4 | Yervoy, ipilimumab, MDX-010 (BMS) |
| OX40 (TNFRSF4) | MEDI 6383 (Astra Zeneca) | PD-1 | KEYTRUDA, pembrolizumab (Merck), Opidvo, nivolumab (BMS) |
| 4-1BB (CD137, TNFSF9) | Urelumab, PF-05082566 (Pfizer/ Merck Serono) | PD-L1/L2 | MDX-1105 (BMS), MPDL3280 (Genentech/ Roche) |
| GITR | TRX518 (GITR, Inc.) | LAG3 (CD223) | BMS-986016 |
| CD27 | Varlilumab, CDX-1127 (Celldex) | | |

BMS, Bristol myers squibb; Mab, monoclonal antibody

**Table 2** Cancer vaccines that have entered late stage clinical evaluation

| Type Antigen (A), Cells (C), or Vector (V) | Product (Developer/ Originator) | Product | Components/Adjuvant | Status |
|---|---|---|---|---|
| A | GARDISIL® (Merck) | Quadrivalent HPV vaccine (HPV4) | Virus-like particles (VLPs) prepared from recombinant L1 capsid protein of HPV types 16 and 18 and 6 and 11; contains amorphous aluminum hydroxyphosphate sulfate (AAHS) adjuvant | FDA approved for use in males and females |
| A | CERVARIX®(GSK), | Bivalent HPV vaccine, HPV2 | VLPs prepared from recombinant L1 capsid protein of HPV of HPV types 16 and 18; contains AS04, a proprietary adjuvant | FDA approved for use in females |
| A | NeuVax (Galena BioPharma) | nelipepimut-S or E75 | E75 is a peptide from HER2/neu (ERBB2) combined with GM-CSF | Ph III for prevention of breast cancer recurrence NCT01479244 |
| A | Stimuvax (Merck, Oncothyron) | BLP25 liposome vaccine (L-BLP25), emepepimut-S | MUC1 peptide (25 amino acids) encapsulated in liposome, tecemotide, plus MPL adjuvant | Ph III for NSCLC, Ph III for breast cancer. Merck discontinued development in 2014 |
| A | LYMPREVA (Biovest International, National Cancer Institute, USA) | Dasiprotimut-T | an autologous idiotype lymphoma cancer vaccine to be used in combination with GM-CSF (Id-KLH/GM-CSF), | April 2015, Received a negative opinion by EMA. Follicular non-Hodgkin's lymphoma; Waldenstrom's macroglobulinaemia; Mantle-cell lymphoma; |
| A | MAGE-A3 (GSK) | | recombinant MAGE-A3 protein and AS15 (a combination of QS-21 Stimulant adjuvant, monophosphoryl lipid A, and CpG7909, a TLR-9 agonist) | Phase III failures for NSCLC and also melanoma |
| A | Prophage Series G-100 (Agenus) | HSPPC-96 (heat shock protein, gp96) | heat shock protein, gp96, purified from patients' tumor tissue and QS-21 Stimulon, a saponin extracted from the bark of the Quillaja saponaria (soap bark) | Phase III trial in patients with newly diagnosed glioblastoma multiforme (GBM) |
| C | Provenge | Sipuleucel-T | autologous CD54+ cells derived from the patient and activated with PAP-GM-CSF for patient reinfusion | FDA Approved for |

Table Continued...

| Type | Product (Developer/ Originator) | Product | Components/Adjuvant | Status |
|---|---|---|---|---|
| **Antigen (A), Cells (C), or Vector (V)** | | | | |
| | (Dendron) | | | mCRPC |
| C | GVAX (Aduro) | | allogeneic pancreatic, prostate, or breast tumor cells engineered to express GM-CSF | Ph II for prevention of recurrence of advanced MDS or AML. |
| C | HyperAcute™ (New Link) | algenpantucel-L | human pancreatic cancer cells expressing a mouse gene | Phase III, in patients with borderline resectable or locally advanced unrespectable pancreatic cancer (NCT01836432). |
| V | TG4010 (Tansgene) | MVA-MUC1-IL2 | Targets MUC-1 using recombinant vaccinia (pox) virus expressing MUC1 and IL2 | Phase IIb/III study (NCT01383148) for patients with stage IV NSCLC |
| V | ProstVac (Bavarian Nordic) | PSA-TRICOM | vaccinia-fowl pox containing PSA gene and TRICOM (co stimulatory molecules) | Phase III asymptomatic or Minimally symptomatic metastatic castration-resistant prostate cancer (MCRPC). Phase II in patients with localized disease |
| V | Allovectin-7 (Vical) | velimogene aliplasmid | plasmid-based immunotherapeutic expressing HLA-B7 and β2 microglobulin genes, forming a major Histocompatibility class I complex | Phase III, failed to meet key endpoints in patients with stage III/IV Metastatic melanoma. |
| V | T-Vec (Amgen) | talimogene laherparepvec | Engineered HSV1 viral vector capable of replication and lysis of tumor cells and expressing GM-CSF. Injected directly into tumors. | Ph III metastatic melanoma (positive results reported in 2014) |

AML, acute myeloid leukemia; CEA, carcinoembryonic antigen; GBM, glioblastoma multiforme; GM-CSF, granulocyte macrophage colony-stimulating factor; HPV, human papilloma virus; HSV, herpes simplex virus 1; IL2, interleukin 2; MAGE-A3, melanoma-associated antigen 3; MCRPC , metastatic castration resistant prostate cancer; MDS , myelodysplastic syndrome; MPL, 3-O-deacyl-4'-monophosphoryl lipid A; MUC-1, mucin 1; NSCLC, non-small cell lung cancer; PSA, prostate-specific antigen; RF, recombinant fowl pox; RV , recombinant vaccinia; STn-KLH , Sialyl-Tn–Keyhole Limpet hemocyanin; EMA, European medicines agency

DNA vector vaccines, injecting the antigen coding sequence into the host in a system to allow *in vivo* expression of an immunogenic, have had a long development history. Several have reached advanced clinical testing. Two vaccinia vector based products, TG4010 and ProstVac, combine expression of antigen with a cytokine(s) to promote a tumor specific immune response have advanced to phase III testing. Another platform developed by Inovia called SynCon® that targets T-cell activation with tumor specific antigens and employs cytokines,[29] is currently in clinical testing with both a prostate cancer targeting vaccine, INO-5150, and one targeting cancers caused by the human papillomavirus, INO-3112, for cervical and head and neck cancer. The company reports that the prostate cancer vaccine employs engineered immunogens related to PSA and PSMA for prostate cancer.

Along the lines of a vaccine, but directed at and not containing the antigen, are molecules selective at promoting an immune attack that will destroy a target cell, such as a cancer cell. The first of a class of therapeutic called bispecific antigen-directed CD3 T-Cell engager (BiTE®), BLINCYTO® (Amgen), is being used to treat late stage acute lymphoblastic leukemia (ALL). In December of 2014, the USFDA approved Blincyto (blinatumomab) to treat patients with Philadelphia chromosome-negative precursor B-cell acute lymphoblastic leukemia

(B-cell ALL), an uncommon form of ALL.[30,31] Blinatumomab binds to CD19, an antigen on leukemia cells as well as the CD3 receptor on T-cells and therefore directly links the T-cell to the cancer cell. A trial of 185 patients with Philadelphia chromosome negative ALL produced complete remission in 32 percent of those treated for at least four weeks.[30]

## Combinations with immunotherapy

Exhaustion of tumor-associated antigen-specific T cells is a phenomenon that often occurs upon chronic antigen stimulation. T-cell exhaustion was demonstrated in a mouse model as a result of tumor recurrence but CD4+ T-cell functions may be restored by blockade of the T-cell inhibitory receptors PD-L1 and LAG-3 to produce an active cytolytic response.[22,32,33] Combining agents that produce PD-1 blockage with LAG-3 inhibition with added naive CD4+ T-cells may be a strategy for treating recurrent tumors in man.[32,34] IDO (indoleamine 2,3-Dioxygenase)is an enzyme that depletes tryptophan and initiates kyeueurine synthesis. Kyneurine promotes checkpoint blockade. Tumor cells express IDO and T-cells are known to have a high requirement for tryptophan. Therefore, an IDO inhibitor may work synergistically with the checkpoint antibodies, and specifically e.g. ipilimumab.[8] Like most cancer therapy, immune therapy does not produce responses in all patients. One of the next steps in the use of these agents will focus on identifying biomarkers that can point to the individual patients or, times in their course of treatment that would benefit optimally from its use.

For example, a Phase I/II study in the US with Kyowa's Poteligeo (mogamulizumab), an anti-CCR4 antibody with enhance ADCC and Opdivo (nivolumab) for PD-1 blockade in advanced or metastatic solid tumours was recently initiated.[35] CCR4, chemokine receptor 4 or CD194, is expressed by most adult T-cell leukemia-lymphoma tumors in patients and so represents a potential targeting antigen for immune therapy. There may be a strategy to prevent tumor responses that include the display of PD-1 ligand, PD-L1.[36] Thus, in a patient already ongoing antitumor response,[37] the use PD-1 blockade, could be used sequentially with another therapeutic or therapeutic regimen. Numerous combinations including the use of "armed" antibodies as antibody drug conjugates (ADC) or therapeutic vaccines or even untargeted chemotherapy regimens might conceivably benefit from a timely administration of PD-1 blockade or other checkpoint inhibitors and clinical investigations of such combinations are already underway.

## Conclusion

Immuno oncology therapy now enjoys almost daily press release, newly initiated studies, new drug approvals, and a wealth of publications. It is truly encouraging if not remarkable to see that cancer patients and their physicians have new options that can provide seemingly durable responses – though it is early times. The USFDA has already granted accelerated approval to several new immuno therapy agents. Accelerated approval is granted by the FDA for drugs targeting serious or life-threatening diseases where a surrogate marker of efficacy (e.g., progression-free survival) provides an indication that the drug is "reasonably likely" to benefit patients. Learning how to identify those who will respond and when to offer these products is the next step for applying these novel and potentially lifesaving therapies.[38]

## Acknowledgements

## Conflicts on interest

The author declares that there is no conflict of interest.

## Funding

## References

1. Cohen PJ, Lotze MT, Roberts JR, et al. The immunopathology of sequential tumor biopsies in patients treated with interleukin-2. Correlation of response with T-cell infiltration and HLA-DR expression. *Am J Pathol*. 1987;129(2):208–216.

2. Mule JJ, Rosenberg SA. Successful adoptive immunotherapy of established metastases with lymphokine activated killer cells and recombinant interleukin-2. *Immune Responses to Metastases*. 1987;11:69–94.

3. Rosenberg SA. IL-2: The first effective immunotherapy for human cancer. *J Immunol*. 2014;192(12):5451–5458.

4. Kohler G, Milstein, C. Continuous cultures of fused cells secreting antibody of predefined specificity. *Nature*. 1975;256(5517):495–497.

5. Kaprowski H, Croce C. Method of producing tumor antibodies. Wistar Intstitute. 1979.

6. Haynes N. Cancer microenvironment and immunity - lessons for immunotherapy. Walter and Eliza hall institute postgraduate lecture series. 2014.

7. Mark B, Faries MD. Adoptive T cell Therapy in Melanoma at the Angeles clinic Melanoma education symposium USA. 2015.

8. Michael Postow MD. New checkpoint inhibitors & immunotherapy in metastatic Melanoma the Angeles clinic Melanoma education symposium USA. 2015.

9. Muul LM, Nason-Burchenal K, Carter CS, et al. Development of an automated closed system for generation of human lymphokine-activated killer (LAK) cells for use in adoptive immunotherapy. *J Immunol Methods*. 1987;101(2):171–181.

10. Lanitis E, Poussin M, Klattenhoff AW, et al. Chimeric antigen receptor T cells with dissociated signaling domains exhibit focused antitumor activity with reduced potential for toxicity *in vivo*. *Cancer Immunol Res*. 2013;1(1):43–53.

11. NantKwest website. Natkwest.com

12. Rajasekaran N, Chester C, Yonezawa A, et al. Enhancement of antibody-dependent cell mediated cytotoxicity: a new era in cancer treatment. 2015;4:91–100.

13. Suntharalingam G, Perry MR, Ward S, et al. Cytokine storm in a phase 1 trial of the anti-CD28 monoclonal antibody TGN1412. *New Eng J Med*. 2006;355(10):1018–1028.

14. Mellman I, Coukos G, Dranoff G. Cancer immunotherapy comes of age. *Nature*. 2011;480(7378):480–489.

15. Keytruda prescribing information from the U.S. Food and drug administration.

16. Curti BD, Kovacsovics-Bankowski M, Morris N, et al. OX40 is a potent immune-stimulating target in late-stage cancer patients. *Cancer Res*. 2013;73(24):7189–7198.

17. Linch SN, McNamara MJ, Redmond WL. OX40 agonists and combination immunotherapy: putting the pedal to the metal. *Frontiers in Oncology*. 2015;5:34.

18. Berkot Bill. INSIGHT-Pfizer, Bristol revive cancer drugs that rev up immune system. Markets. 2015.

19. Bartkowiak T, Curran MA. 4-1BB agonists: multi-potent potentiators of tumor immunity. *Front Oncol*. 2015;5:117.

20. Bullock T, McClintik H, Jeong S, et al. Soc immunotherapy of cancer 2014 poster, immune correlates of varlilumab (CDX-1127) treated cancer patients are consistent with CD27 costimulatory activity. 2014.

21. http://www.gitrrx.com/therapeutic-program.php

22. Taku Okazaki, Il-mi Okazaki, Jian Wang, et al. PD-1 and LAG-3 inhibitory co-receptors act synergistically to prevent autoimmunity in mice. *J Exp Med*. 2011;208(2):395–407.

23. Safety study of anti-LAG-3 with and without anti-PD-1 in the treatment of solid tumors. *Clinical trials study*. 2015.

24. Derre L, Jean-Paul Rivals, Camilla Jandus, et al. BTLA mediates inhibition of human tumor-specific CD8+ T cells that can be partially reversed by vaccination. *J Clin Invest*. 2010;120(1):157–167.

25. Morbidity and mortality weekly report (MMWR). FDA licensure of bivalent human papilloma virus vaccine (HPV2, Cervarix) for use in females and updated HPV vaccination recommendations from the advisory committee on immunization practices (ACIP). 2010;59(20):626–629.

26. Cheever MA, Allison JP, Ferris AS, et al. The prioritization of cancer antigens: a national cancer institute pilot project for the acceleration of translational research. *Clin Cancer Res*. 2009;15(17):5323–5337.

27. Schlom J. Therapeutic cancer vaccines: current status and moving forward. *J Natl Cancer Inst*. 2012;104(8):599–613.

28. Dendron. Provenge prescribing information from the US. Food and drug administration.

29. Inovio pharmaceuticals company website: technology description and press release, 2015.

30. FDA News Release. FDA approves Blincyto to treat a rare form of acute lymphoblastic leukemia. First anti-CD19 drug to receive agency approval. 2014.

31. Blincyto prescribing information from the U.S. Food and drug administration.

32. Goding SA, Wilson KA, Xie Y, et al. Restoring immune function of tumor-specific CD4+ T cells during recurrence of Melanoma. *J Immunol*. 2013;190(9):4899–4909.

33. Taube JM, Anders RA, Young GD, et al. Co localization of inflammatory response with B7-h1 expression in human melanocytic lesions supports an adaptive resistance mechanism of immune escape. *Abstract Sci Transl Med*. 2012;4(127):127ra37.

34. Goding SA, Wilson KA, Antony PA. Combination of adoptive cell transfer, anti-PD-L1 and anti-LAG-3 antibodies for the treatment of recurrent tumors. Better with more *Onco Immunol*. 2013;2(8).

35. George Underwood G. BMS in new Opdivo combo collaboration. Daily News.2 015.

36. Ott PA, Hodi FS, Robert C. CTLA-4 and PD-1/PD-L1 blockade: new immunotherapeutic modalities with durable clinical benefit in melanoma patients. *Clin Cancer Res*. 2013;19(19):5300–5309.

37. Butterfield Lisa H. Clinical review: state of the art review: Cancer vaccines. *BMJ*. 2015;350:h988.

38. Pandha H, Pawelec G. Immune checkpoint targeting as anti-cancer immunotherapy: promises, questions, challenges and the need for predictive biomarkers at ASCO 2015. *Cancer Immunol Immunother*. 2015;64:1071–1074.