

Chapter 1

Primer on Cancer Immunotherapy and the Targeting of Native Proteins

Valentin Barsan and Paul C. Tumeh

Abstract Immunotherapy has notable potential for achieving durable clinical responses in many cancer types. The ability to readily measure the genomic landscape and infiltrating immune spectra of individual patient tumors offers mechanistic insights for combination therapy selection. Immunotherapeutic approaches through immune checkpoint blockade or stimulation, immune cell therapies, as well as tumor vaccination are being studied as mono and combination therapy in multiple cancer types. Uniquely, many immunotherapies target “native” self-proteins and thus herald a paradigm shift in cancer management in which the drug target is no longer an oncogenic protein but rather a normal signal that impacts the interactions of myriad immune cell types with both cancerous and normal cells. Native proteins in immunology are found in multiple isoforms with distinct interaction partners and at heterotypic transient cellular interfaces. Methods for evaluating the presence and function of native proteins for therapeutic targeting necessitates resolving for tumor–immune cellular interactions to understand which cell type is expressing which native protein isoform in the contextual (variably inflamed) tumor microenvironment. Just as tumor genomics has facilitated the selection of targeted therapies, precision immuno-oncology necessitates a comprehensive understanding of the immune system and the native proteins that govern its coordinated behavior. This primer on the relevant immunobiology, its clinical assessment, and therapeutic implications establishes a framework for conceptualizing the clinical advances in cancer immunotherapy that are the focus of this volume.

Keywords Immuno-oncology • Immunogenomics • Cancer immunotherapy • Checkpoint blockade • Immunobiology • Tumor biology • Adaptive immunity • Native proteins

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1.1 An Intersection of Oncology and Immunology

The tissues that form human organs are composed primarily of two symbiotic cellular components: the parenchyma and the stroma. The parenchyma establishes unique tissue function whereas the stroma comprises an admixture of resident tissue cells (fibroblasts, dendritic and mast cells), vascular and lymphatic endothelial cells, inflammatory cells (lymphocytes, macrophages, myeloid cells), regenerative mesenchymal stem cells, as well as structural matrix proteins and proteoglycans [1]. Healthy tissues maintain a dynamic balance of these composite cellular and structural components across time and despite environmental stressors to achieve resilient “youthful” organ function. However, genomic instability (germline and somatic variants) in cells results in the development of cancer hallmarks [2] and the accompanying loss and compromise of normal tissue function at which time patients present for clinical consultation. Beyond “driver” mutations [3] that establish key mechanisms for neoplastic progression, nonsynonymous somatic mutations (that alter the amino acid sequences of the proteins encoded by the altered genes) can encode the aberrant translation of a diverse set of peptide “neoantigens” that, when recognized as foreign, triggers tumor immunogenicity [4]. Rudolph Virchow first proposed a link between inflammation and cancer in the 1860s when he observed leukocytes infiltrating neoplastic tissues [5]. A century later, it was postulated that lymphocytes can recognize and eliminate aberrant cells [6, 7]. More recently, “immunoeediting” has been proposed as an active process in which immune cells both eliminate cancerous cells through immuno-recognition yet simultaneously promote neoplastic progression secondary to collateral inflammation [8]. Each patient’s cancer is therefore wholly unique – an evolutionary outcome of successive neoplastic cellular divisions within distinct tumor microenvironments shaped through time as much by the patient’s immune system as by successive therapeutic interventions.

The presence, subtype, location, and density of infiltrating immune cells in the tumor microenvironment characterize the degree of tumor inflammation. Diverse immune cell subtypes of varying immune “fitness” within each tissue stroma [9] and in the lymphatic system facilitate the intricate intercellular processes of discriminating self from nonself. Feedback control through suppression of inflammation is equally important in tuning the nature of the immune response to counter neoplastic cellular behaviors with sufficient, yet limited, on-target responses. Cancer immunotherapy and autoimmunity are thus finely related and likely coexist along a clinical spectrum in which the discriminate recognition of self from nonself determines the efficacy and toxicity profile of immunotherapeutics. Cancer immunotherapy therefore entails harnessing the power of the immune system to eliminate cancerous cells while preserving the integrity and function of otherwise healthy tissue. Historically speaking, the cancer drug development paradigm has entailed designing one drug to target one protein which is usually mutated and specific to a tumor type. The paradigm shift in cancer immunotherapy extends beyond targeting immune cells instead of cancerous cells. Rather, coordinating tumor immunity

entails targeting native nonmutated proteins instead of oncogenes. These native proteins are expressed by different immune cell types of varying fitness, in multiple isoforms (with distinct interaction partners), across discrete tissue compartments, and at heterotypic and transient cellular interfaces. Therapies that target the immune system are thus fundamentally different in biologic mechanism, pharmacokinetics, and clinical application than therapies that target key cancer pathways. Conversely, therapies that target driver mutations in oncogene pathways of cancer cells can inadvertently dampen critical intracellular pathways in immune cell activation.

Targeting native proteins introduces a level of biologic and clinical complexity with which we have limited experience in oncology. Methods for evaluating the presence and function of native proteins necessitate resolving for tumor-immune cellular interactions to understand which cell type is expressing which native protein isoform in what contextual (inflamed or noninflamed) tumor microenvironment. Just as each cancer has a distinct mutational landscape so too each patient presents with a unique immune system whose fitness is shaped by genetics, age, vaccination and pathogen exposure history, as well as the environment. For example, epidemiologic studies associate the development of mumps in childhood with protection against ovarian cancer ostensibly due to primed immune surveillance [10]. Important environmental influences on the immune system and cancer progression are intuitive yet complexly interrelated. These include diet and exercise that can modulate gut/airway/skin microbiomes, UV/airborne/ingested carcinogens, and infectious exposures. In health, the immune cells can recognize both pathogens (i.e., viruses, bacteria, fungi, and parasites) as well as mutated cells to effectuate a targeted cytotoxic response with limited collateral inflammatory damage to surrounding tissues. When the immune system cannot effectively discriminate between self and nonself, autoimmune diseases (such as rheumatoid arthritis, diabetes, lupus) develop. The balance between self-tolerance and autoimmunity thus underpins the mechanisms by which immunotherapies have been applied to treat cancer. Our deepening understanding of the immune system at a molecular level has led to broad therapeutic advances in immunomodulatory monoclonal antibodies, cellular therapies, and vaccination strategies that are now being studied in all cancer types alongside conventional approaches of surgery, chemotherapy, and radiation. Understanding when, where, and how the diverse cells of the immune system interact to mount a coordinated cytotoxic immune response against cancer establishes the foundation for implementing these insights in clinical settings.

1.2 Innate and Adaptive Immunity

An effective and specific cytotoxic immune response against a tumor is coordinated by multiple cross-priming agonist and antagonist signals coordinated between varied cells of the innate and adaptive immune systems [11, 12]. These systems are comprised of more than 200 immune cells types and more than 300 immune cell state

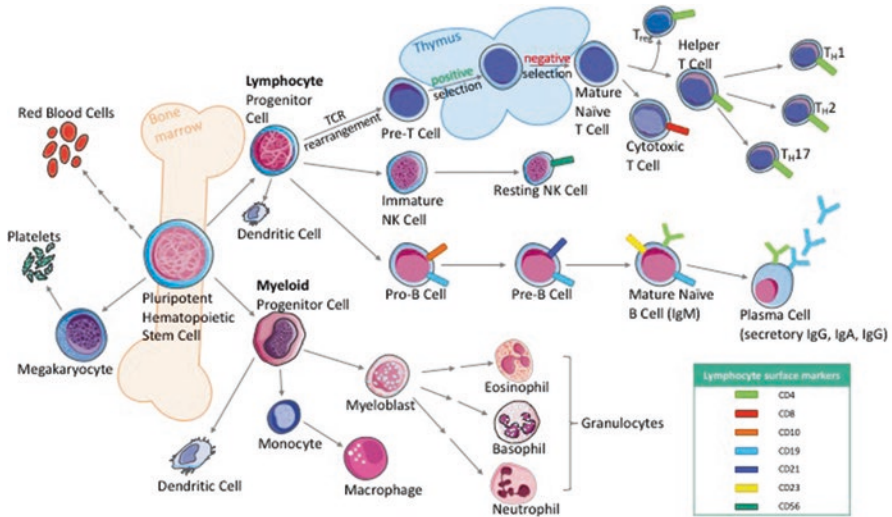


Fig. 1.1 Immune cell growth and differentiation. Cells of the immune system differentiate across myeloid or lymphoid lineages from hematopoietic stem cell precursors in the bone marrow. Hundreds of additional cell types and intermediate states exist. Partial maturation of T cells in the thymus and B cells in the bone marrow is followed by further differentiation in peripheral lymphoid tissues throughout development. Lymphocytes are commonly characterized by the surface expression of cluster of differentiation (CD) markers as well as the types of cytokines or antibodies produced

transitions [13]. All cells of the immune system differentiate (that is, increasingly functionalize) across myeloid or lymphoid lineages from hematopoietic stem cell precursors in the bone marrow (Fig. 1.1). Cells of the myeloid lineage include red blood cells, platelets, granulocytes (eosinophils, neutrophils, basophils), mast cells, and macrophages. Cells of the lymphoid lineage include natural killer (NK) cells, T cells ($\gamma\delta$, NK, $CD4^+$ and $CD8^+$ subsets), and B cells. Antigen-presenting dendritic cells may derive from either myeloid or lymphoid lineages. The myeloid and lymphoid lineages are functionally characterized by innate or adaptive cellular behaviors. The *innate* component includes most immune cells of the myeloid compartment as well as NK cells whereas the *adaptive* component consists solely of lymphoid (B and T) cells and their myriad subtypes. Partial maturation of T cells in the thymus and B cells in the bone marrow in utero is followed by further differentiation in peripheral lymphoid tissues after birth and attainment of immunocompetency under antigenic stimulus. Immune cells and their degree of differentiation are commonly characterized by expression of surface clusters of differentiation (CD) or the types of cytokines they secrete. Adaptive immunity is defined by the ability to discern and remember immunologic threats based on foreign, mutated, or atypically expressed antigens. At baseline, both components of the immune system are “on alert” until a threat has been identified at which time, rapid innate immune activation occurs and

primes an adaptive response. Because each major immune cell type has an active and a regulatory form, the balance between these states characterizes the quality of an immune response.

Cells of the *innate* immune system use generic methods to recognize foreign pathogens based on nonspecific and nonhuman molecular patterns such as single-stranded RNA or lipopolysaccharide. Germline-encoded non-self-reactive receptors on neutrophils, macrophages, natural killers, and mast cells respond to generalized pathogen-associated molecular patterns (PAMPs) such as mannose receptors or toll-like receptors shared by many classes of microbes [14]. Innate cells such as macrophages and neutrophils migrate into tissues through expression of high-affinity integrin, kill microbes through phagocytosis and reactive oxygen species (triggered by interferon- γ), induce inflammation (through tumor necrosis factor, IL-1 and IL-6), activate T cells and NK cells (through IL-12), and initiate tissue repair (through secretion of immunosuppressive interleukins, TGF- β , and fibroblast growth factors). Innate immunity defense mechanisms further include the complement cascade and inflammation. The complement system is comprised of nine major factors (C1 to C9), most of which are pro-enzymes present in normal serum and not increased by antigenic stimulation. The complement cascade facilitates inflammation, leukocyte recruitment, anaphylatoxin production, mast cell degranulation, opsonization for phagocytosis, secondary signals for B-cell activation, and the formation of membrane attack complexes against pathogenic cells. Tissue inflammation stimulates the adaptive immune response, enables the elimination of invasive foreign pathogens through controlled passage of immune cells, and initiates tissue repair.

Tissue inflammation also influences the resident cells within a tumor microenvironment. In an environment of chronic inflammation, myeloid cell differentiation can be skewed [15] toward the expansion of myeloid-derived suppressor cells (MDSCs). MDSCs are a heterogeneous subpopulation of immune cells (including macrophages, neutrophils, and dendritic cells) with potent immunosuppressive functions. Whereas M1 macrophages release interferon- γ and are responsible for phagocytosis, M2 macrophages release cytokines (IL-4, IL-10, TGF- β) that curtail inflammatory responses and foster immune tolerance [16]. Macrophages also serve as important regulators of tumor angiogenesis by producing various pro-angiogenic molecules such as erythrocyte growth factor (EGF) and vascular endothelial growth factor (VEGF). Tumors can foster immuno-tolerance in the microenvironment through the manipulation of cytokines (increased secretion of IL-6, IL-10, and TGF- β ; consumption of IL-2) that encourage infiltration of inhibitory immune cells such as MDSCs and regulatory T cells (Tregs). Several therapeutic approaches (PDE5 inhibitors, COX-2 inhibitors, ARG1 inhibitors, bisphosphonates, gemcitabine, and paclitaxel, among others) play a complementary role in promoting antitumor immune responses by inhibiting the function or proliferation of MDSCs [17]. Immune cells also acquire distinct metabolic characteristics [18] that influence the plasticity of their immunological phenotypes and functions.

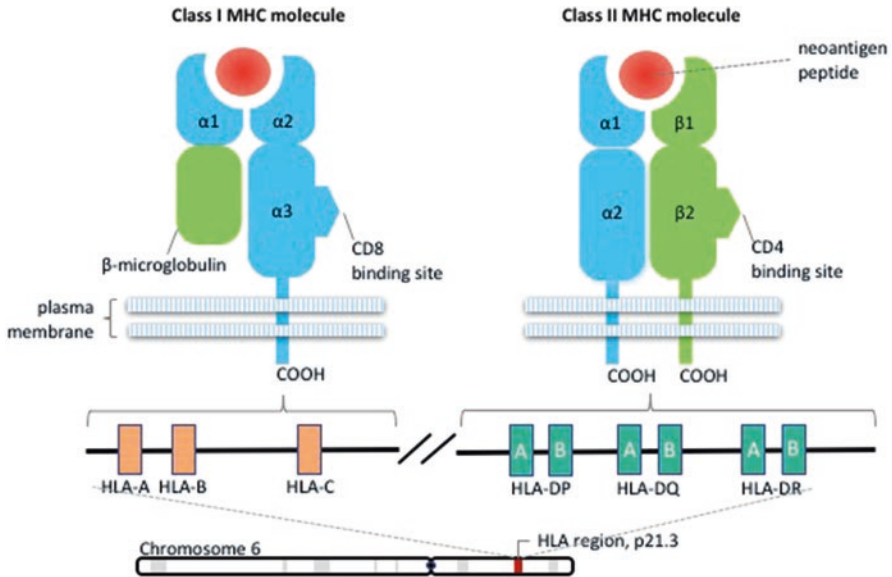


Fig. 1.2 Class I and Class II MHC Molecules. The maternal and paternal HLA haplotypes are located at chromosome 6, on the short arm at position p21.3, and encode the genes for MHC. HLA haplotypes are codominantly expressed. Both MHC Class I and MHC Class 2 consist of an alpha (heavy) and a beta (light) chain. The class I HLA molecule contains an alpha chain anchored to the cell membrane. The peptide antigen of 8 to 11-mer amino acids (red) is presented in a groove formed from a pair of alpha-helices on a floor of antiparallel beta strands. The class I alpha chains are coded for by genes within the MHC (e.g., HLA-A, HLA-B), whereas the beta chain, beta-2 microglobulin, is encoded on chromosome 15, not in the MHC. The class II HLA molecule is MHC-encoded by both alpha and beta chains each anchored to the cell membrane without beta-2-microglobulin. The peptide antigen of ~15-mer amino acids is presented in a groove formed from a pair of alpha helices on a floor of antiparallel beta strands. Class II antigens are constitutively expressed on B cells, dendritic cells, and monocytes and can be induced during inflammation on many other cell types that normally have little or no expression. Genes within the MHC (e.g., HLA-DP/Q/R) code for both chains

1.3 Orchestrating Adaptive Immunity

All human cells express a cell-surface major histocompatibility complex (MHC) that is genetically encoded by the human leukocyte antigen (HLA) locus. HLAs are inherited as haplotypes from both parents and expressed co-dominantly as MHC on all cells (Fig. 1.2). The MHC thus functions as an authenticating cell surface complex that physically presents peptides to adaptive immune cells [19] and enables the immune system to distinguish between self and nonself. HLA typing has thus enabled the matching of transplanted organs [20] and cells to minimize rejection. The HLA locus contains more than half of the four to five million single nucleotide polymorphisms (SNPs) in each individual genome [21]. This genomic variability implies enormous diversity in any given patient's relative immune fitness and

susceptibility to immunologic disorders or infectious agents. MHC diversity explains why tissue transplantation remains so challenging and perhaps as well why autoimmune and infectious susceptibilities cluster by subtype. Proteins encoded by the three key MHC class I genes (HLA-A, HLA-B, and HLA-C) are present on the surface of most cells to present peptides that are internally processed and exported from inside the cell. MHC class I thus facilitates immune surveillance of intracellular pathogens or aberrant proteins. Cells that do not express MHC are indiscriminately attacked by NK cells of the innate immune system. Downregulation of MHC by cancer cells suggests a therapeutic utility of NK cell therapy [22]. The six main MHC class II genes (HLA-DPA, HLA-DPB, HLA-DQA, HLA-DQB, HLA-DRA, and HLA-DRB) encode cell-surface proteins that display peptides derived from circulating, extracellular proteins to the immune system. MHC class II molecules are expressed only on antigen-presenting cells (APCs), such as dendritic cells.

APCs are activated by recognition of antigens that bind surface MHC which induces downregulation of cell-adhesion molecules to facilitate migration from the tissue of residence to a lymph node for antigen presentation to residing adaptive immune T and B lymphocytes. APCs serve as the critical link for priming the adaptive immune cells. Dendritic cells and macrophages are “professional” APCs and critically link the innate and adaptive immune systems. Since their discovery in 1973 [23], dendritic cells have been shown to develop from either myeloid or lymphoid hematopoietic lineages which thereby creates distinctive subsets of dendritic cells that have discreet functions tuned by their tissue of residence and microenvironment (these nuances are especially relevant in vaccine development). The main dendritic subtypes include plasmacytoid DCs (pDCs) and conventional DCs (cDCs). Both pDCs and cDCs are comprised of additional subtypes that have discrete morphology, tissue distribution, surface marker expression, and cytokine production which consequently lead to distinct pathways to T-cell activation. Also, tumor-associated macrophages are ontogenetically diverse [24] and specially tuned to the function of their host tissue. APCs such as dendritic cells or macrophages phagocytose (engulf) and process antigens released from tumor cells to present them to T and B cells. Engagement of the T- or B-cell receptor with MHC peptide is a necessary first step in lymphocyte cell activation. The complementarity determining region (CDR) determines the specificity of a lymphocyte receptor to its cognate antigen. T lymphocytes express clonal T-cell receptors (TCRs) on their surface that recognize antigenic peptides presented by host cells whereas B-cell receptors (BCRs) are secreted as soluble antibodies (immunoglobulins) upon antigen recognition. Lymphocyte receptors also exhibit tremendous genetic diversity to enable the recognition of so many potential antigens presented by MHC. The generation of diverse TCRs and BCRs begins with immature T and B lymphocytes through VDJ recombination, a process in which *germline* DNA is spliced to recombine noncontiguous variable (V), diversity (D), and joining (J) region gene segments and collectively encode the complementarity determining region 3 (CDR3) [25] of a given naïve (antigen inexperienced) lymphocyte. Diversity of the CDR3 region is increased by the deletion and template-independent insertion of nucleotides at the V-D and D-J junctions and further through somatic hypermutation in the BCR.

These receptors recognize residues of peptide antigens in MHC as well as polymorphic residues of the MHC molecule itself. An estimated 100,000–750,000 peptide-MHC class I complexes are expressed for each allelic product (HLA-A and HLA-B loci) [26], and each individual carries $\sim 10^7$ different TCRs [27] each detecting up to 10^6 variations of a given peptide sequence [28].

Immature T and B cells must subsequently demonstrate the ability to discern between harmful and innocuous antigens through a tolerance process prior to their release into circulation. B or T cells optimally recognize only one antigen. Developing T cells undergo tolerance and maturation in the thymus whereas B cells do so in the bone marrow. To establish immunologic tolerance in these organs, immature T and B cells undergo positive selection (weak receptor interaction with self-antigen allows for cell survival) and negative selection (lymphocytes that bind too strongly to self-antigens are signaled to die). Randomly generated TCRs and BCRs recognizing endogenously expressed self-epitopes (peptide/MHC “ligandomes”) are variably pruned in the thymus [29] and marrow [30], respectively, during their development to limit immunological self-destruction. Mature lymphocytes continuously recirculate between blood and peripheral lymphoid tissues, localizing and extravasating into tissues when guided by chemokine gradients from tissue-resident sentinel innate immune cells. The patient’s adaptive immune cells are thus finely tuned within a discrete range of binding affinities – a process that when disrupted can result in autoimmunity or when engineered *ex vivo* enables potent cellular therapies. Paradoxically, self-reactive adaptive immune cells theoretically comprise an autogenous source of potential anticancer activity. Autoimmunity eliminates cancerous cells.

Through interaction with APCs, the lymphoid cells of the *adaptive* immune system evolve with exquisite specificity to surface and soluble antigens through selective clonal expansion of T and B lymphocytes. The tumor draining lymph node is a more immuno-active microenvironment in which high throughput antigen exposure by APCs to standby lymphocytes occurs. In lymph nodes, naïve T and B cells recognize tumor antigens and can become activated. The mode of cancer cell death (apoptosis versus necrosis) influences the degree and quality of antigen spreading [31], in which previously intracellular immunogenic antigens are released because of cell lysis [32] thereby broadening antitumor responses to additional antigens. T cells exert immune effects through cellular interactions whereas B cells become activated upon antigen recognition to differentiate into antibody-producing plasma cells. Secreted antibody subtypes (immunoglobulins) are frequently measured in infectious diseases as titers and clinically indicate primary versus repeat/historical antigen exposures. B-cell homing areas enable rapid antibody secretion and are found primarily in the splenic follicles, marrow pulp, lymph nodes, and mucosal-associated tissues. Mature B cells are educated (antigen-specific) APCs that present to effector CD4 T cells via MHC-II, who will in turn activate B cells to undergo “class switching” and “affinity maturation” to produce clonal circulating antibodies of varying kinetics and increasing potency. A rapid adaptive immune response is initiated by T and B cells if the presented antigen has been recognized previously.

Both tumor and transplant rejection are mediated mainly by cytotoxic T lymphocytes. T-cell activity is controlled by a combination of antigen-specific signals from the TCR as well as antigen-independent signals from myriad co-receptors [33]. The TCR binds specific short stretches of amino acids (i.e., peptides) presented by MHC molecules located on all host cells, and notably APCs (Fig. 1.3). VDJ recombination produces a TCR that is composed of two different proteins chains (α and β whose ratios change throughout cellular maturation as well as in diseased states) and CD3 which encodes an invariant transmembrane protein complex that relays surface signals for secretion of pro-inflammatory cytokines such as IL-12 and interferon gamma. The MHC molecules expressed in the thymus restrict a mature T cell to a predetermined spectrum of antigens. Each T cell expresses monoclonal membrane-bound TCRs that all recognize the same specific peptide/MHC complex during physical contact between the T cell and an APC (MHC class II) or any host cell (MHC class I). T-cell subtypes are characterized broadly by their co-receptors: CD4 on helper and regulatory T cells is specific for MHC class II whereas CD8 on cytotoxic T cells is specific for MHC class I. The subtypes of effector CD4⁺ and CD8⁺ T cells are often characterized by the specific cytokines (interleukins) produced upon their activation. Activated CD8⁺ (killer) T cells engage in direct cytotoxic activity whereas activated CD4⁺ (helper) T cells support other lymphocytes, for example, by promoting the maturation of B cells into plasma cells and memory B cells and activating cytotoxic T cells and macrophages. To mount an effective immune response beyond activation through MHC-peptide and TCR binding, T cells require additional costimulatory signals. A critical priming costimulatory signal in naïve T cells is CD28, which binds to B7-1 and B7-2 (CD80/86) on the APC [34]. Without CD28:B7 interaction, the naïve T cell remains anergic (refractory to activation or unresponsive). The most differentiated effector and memory (antigen-experienced) T cells [35] are least dependent on costimulatory signals due to avidity maturation that reduces the activation threshold of these subtypes.

Once activated, T cells reduce expression of CD28 and upregulate surface immune checkpoint molecules which are native proteins that facilitate feedback inhibition and limit cytotoxic activity. Unrestrained T-cell activation would otherwise lead to malignant proliferation or autoimmune disease. CTLA-4 is one such *inducible* surface checkpoint molecule that is upregulated on T cells after activation, has higher affinity for the ligands CD80 and CD86, and is also constitutively expressed on a variety of Tregs [36]. CTLA acts as an “off switch” when bound to CD80 or CD86 on the surface of APCs. CTLA-4 blockade hence produces both a direct enhancement of T-cell effector function and a concomitant inhibition of regulatory T-cell activity [37]. Programmed cell death-1 (PD-1) is expressed later and functions as an inhibitory homologue of CD28 following T-cell activation. A key mechanism by which cancer cells diffuse the host immune response is the upregulation of PD-1 that bind to PD-1 on tumor-specific CD8⁺ T cells [38] as well as NK T cells and B cells. PD-1 is a member of the extended CD28/CTLA-4 family of T-cell regulators that is highly expressed on *activated* T cells whose two ligands PD-L1 and PD-L2 have been found to be expressed as immuno-escape behaviors of several cancers. PD-L1 and PD-L2 are also expressed on cells of the immune system

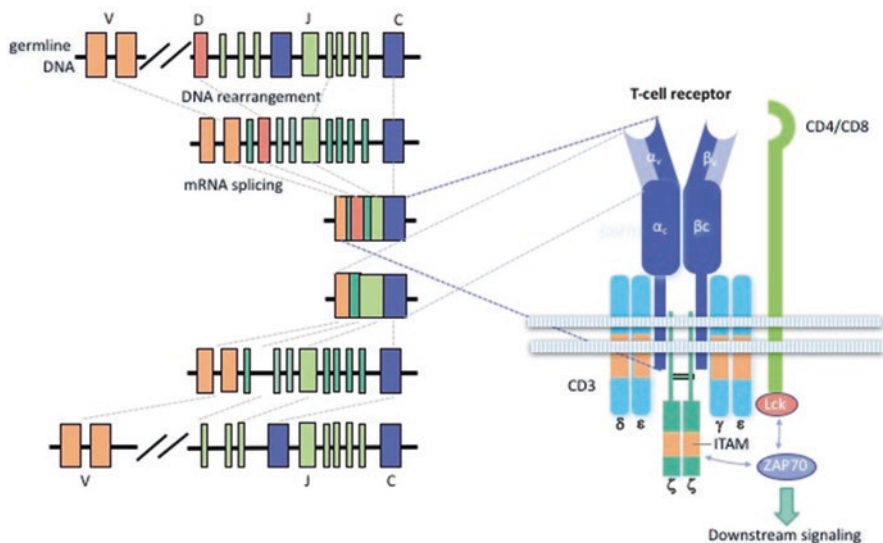


Fig. 1.3 The T cell receptor. The mature T cell heterodimer consists of alpha- and beta-subunit chains that are formed by rearranged germline DNA of variable (V), diversity (D), joining (J), and constant (C) regions. The TCR alpha chain is generated by VJ recombination, whereas the beta chain is generated by VDJ recombination. Signalling is initiated by aggregation of TCR by MHC-peptide complexes on APC. Costimulation is required from CD4 on helper-T cells or CD8 on cytotoxic T cells. The intracytoplasmic region of the TCR is too short to transduce a signal from the cell surface so CD3 facilitates signalling through the TCR. Once MHC-peptide binds the TCR, lymphocyte cell-specific protein tyrosine kinase (Lck) is activated and phosphorylates tyrosine residues within the immunoreceptor tyrosine-based activation motifs (ITAMs) of the CD3 and zeta chains, enabling zeta chain-associated protein kinase 70 (ZAP-70) recruitment to the TCR which triggers downstream signaling events required for T cell activation

(upregulated on macrophages and DCs in response to bacterial lipopolysaccharide as well as activated T, B, and NK cells). PD-L1 can also interact (like CTLA-4) with the CD80 receptor on T cells, sending a further immunosuppressive signal. In addition, PD-L1 is also expressed constitutively on nonlymphoid tissues such as the heart, lung, placenta, and skeletal muscle where it may serve to downregulate TCR signaling in PD-1+ cytotoxic T-lymphocytes and therefore protect against autoimmune-mediated tissue damage. Multiple additional co-receptors that modulate T-cell activation and inhibition have become the central focus of checkpoint blockade or stimulation (Fig. 1.4). The activation of T-cell subtypes is dependent on the balance of antagonist (e.g., CTLA-4, PD-1, LAG3, TIM3) and agonist native proteins (e.g., GITR, OX40, ICOS) on *both* the APC and T cells [33]. The therapeutic targeting these native proteins implies modulating complex cellular interactions both within the tumor bed and in lymphoid organs where APCs and T cells interact to amplify immune responses. Antibodies that mimic or block the effect of these checkpoint or agonist receptors or ligands aim to enhance the immune response against tumor cells. Chronic recognition of an antigen (such as that present in a malignant clone or

in a chronic viral infection) may lead to feedback inhibition on effector T-cell function, resulting in a phenotype termed “exhaustion” [39]. Several therapeutic strategies aim to revitalize exhausted T cells. The plurality of inducible signaling molecules that exist as native proteins across diverse immune cell subtypes underscores the challenge in developing a comprehensive understanding of tumor-immune cell interactions.

1.4 Immunobiology of Cancer Immunotherapy

The cancer immunity cycle [40] and the cancer-immune set point [41] have established the conceptual frameworks through which tumor-specific T cells amplify a highly specific, cytotoxic, and clonal response to tumor neoantigens after priming by APCs. In a dynamic multidirectional process, immune cells interact in three distinct tissue compartments: the tumor microenvironment (TME), the draining lymph nodes, and the bloodstream. Each environment can be theorized to exhibit unique biology of variable diagnostic and therapeutic utility. In the tumor bed, resident APCs such as dendritic cells initiate an immune response through maturation to present antigens from the TME, in the form of peptide–MHC molecule complexes, to naïve (antigen inexperienced) T cells in secondary lymphoid tissues [42] such as the draining lymph node. There, T cells are activated when their surface TCRs recognize the cognate peptide–MHC-I on the APC. Now-primed effector and regulatory T cells traffic into the tumor by extravasation from blood vessels as triggered by chemokine gradients of tissue inflammation and TME endothelial upregulation of selectins. Cancer cells evade immune regulation by disrupting this cycle through multiple mechanisms including downregulation of MHC/antigen presentation, expression of inhibitory cell surface molecules that inhibit cytotoxic T cells (PD-L1, FasL), and upregulation of inhibitors of apoptosis (Bcl-XL, FLIP). Checkpoint inhibitors have been developed and approved for various tumor types with the objective of reducing the effector T-cell threshold for activation and thereby facilitating more sensitive immunogenicity (i.e., autoimmunity) preferably towards neoplastic cells that appear more foreign [43]. PD-1 and CTLA-4 antibody-based therapies appear to have broad spectrum antineoplastic activity and may well serve as a backbone therapy [44, 45] in immuno-oncology. However, the spatiotemporal differences between PD-1 and CTLA-4 checkpoints in the tumor microenvironment and germinal lymph tissues imply variable downstream immunomodulatory consequences that depend on the composition of differential immune cell infiltrates as well as a plurality of local factors [46]. Because of their nuanced effector functions, the same immune cell types and cytokines may promote or prevent tumor formation depending on the biological (i.e., tissue and TME) context in which they act.

Inflamed tumors are characterized by the presence of tumor-infiltrating lymphocytes (TILs) whose differential and dynamic representations, relative densities, and spatial distributions form the antitumor immune response [47, 48], and these factors have predictive clinical value [49, 50]. Tumor infiltration with CD8⁺ T cells and an

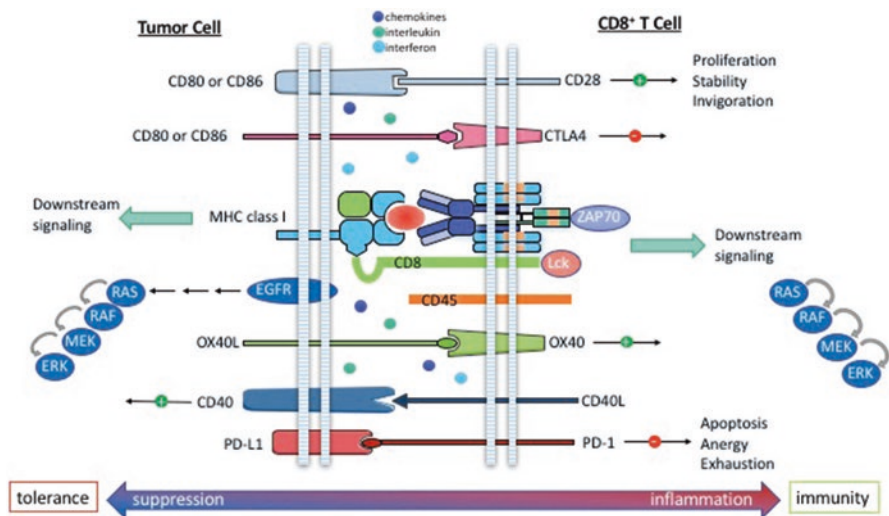


Fig. 1.4 The summative effects of co-stimulatory and inhibitory interactions regulate T cell responses. T cells activate downstream signaling to these ligand-receptor interactions only if they first recognize the cognate MHC-antigen through the TCR. Ligands can bind multiple receptors (with distinct kinetics of expression) giving rise to costimulatory or inhibitory signals amidst a milieu of circulating chemokines, interferons, and interleukins. Costimulatory receptors are often expressed on naïve and resting T cells whereas inhibitory receptors are upregulated only after T-cell activation to facilitate feedback inhibition. There are common pathways within both tumor and immune cells that may have variably suppressive or inflammatory effects depending on summative signals and tissue contexts

associated chemokine/interferon gene signature has been correlated with favorable clinical outcomes to checkpoint blockade, and this phenotype can be influenced by specific oncogene pathways activated within the TME as well as by host commensal microbiota that synergistically activate the immune system. The tissue-specific architecture of different cancer types reveals a range of somatic mutations [51, 52] as well as inflammatory landscapes [53, 54] from very focal expansion of TILs within tumor islets to diffuse inflammation throughout the tumor stroma. Spatial relationships are key to understanding cellular interactions within unique TMEs. Pleiotropic stromal determinants further nuance tumor-immune cellular interactions and intercommunications in the tumor microenvironment [41, 55, 56], and multiple immunosuppressive factors may mediate intrinsic resistance to immunotherapy [57, 58]. T cells are inhibited in the TME by surface membrane native proteins (checkpoints: PD1, CTLA4, LAG3, TIM3, BTLA, Adenosine A2AR), soluble factors, and metabolic alterations (IL10, TGFbeta, adenosine, IDO, arginase) [59], as well as inhibitory cells (cancer-associated fibroblasts, Tregs and Bregs, MDSCs, tumor-associated macrophages). The milieu required for optimal functioning of the immune system is thus defined by summative effects of antigen-presenting cells, immuno-modulating cytokines, and optimal surface molecule expression by both tumor cells and infiltrating lymphocytes.

Cancer progression (immune escape) relies on the exhaustion or suppression of antitumor immune activity which can be attributed to both sustained immune cell presence as well as the presence of regulatory lymphocyte subtypes [60]. Certain mutations in cancer cells are more strongly immunogenic (a subject of concerted research), and some cancers attempt to discard those mutations, which renders them resistant to therapies that boost the immune system [61]. Additional mechanisms of evading immune recognition include loss or somatic mutations of HLA haplotypes or JAK1/JAK2 genes [62, 63] that disrupt antigen presentation as well as upregulation of alternate immune checkpoints [64]. Clonal evolution of a primary cancer and the epithelial-to-mesenchymal transition that coordinates metastasis [65] can be promoted by immune cells [66] in inflammatory TMEs. Tumors can evolve to physically exclude immune cells as commonly seen in melanoma liver metastases and colorectal cancers [67, 68]. Inclusion of some immune cells can even promote tumor growth. For example, recruitment of macrophages via cytokines promotes epidermal growth factor (EGF) production, which nurtures tumor growth while macrophage digestion of the surrounding stroma enables tumor metastasis. Targeting macrophages and/or other protumorigenic immune cells may thus alter the TME and extend the cytotoxic properties of CD8⁺ T cells. Epigenetic regulation of cellular transcription is an additional mechanism for immune escape within the TME. Histone deacetylation and methylation can inhibit both the expression of T-cell stimulating ICAM1, CD80, CD86 as well as dampen neoantigen peptide-MHC loading [69].

Fundamentally, a functional antitumor T-cell response results in the production of IFN γ which facilitates effector functions as well as induces PD-L1 that mediates adaptive resistance [70]. Interferon and interleukin signaling convergence [71–73] most proximately explains the summative functional orientation of lymphocyte cytotoxicity within the TME. The summative quality of peptide–MHC–TCR interactions and TCR signaling in multiple anticancer T-cell clones directed against cancer antigens is subject to dynamic regulation by native co-stimulatory and co-inhibitory proteins. Cytokines dynamically regulate checkpoint expression [74] at nodes of the cancer-immunity cycle and therefore further modulate T-cell responses. Immunotherapies targeting native proteins such as molecular checkpoints thus have variable effects within a patient’s primary tumor, across any metastatic sites, throughout the lymphoid structures in which immune responses are organized, and subsumed into the patient’s systemic immunity. The relationship between tumor-specific antigens, the ratios of immune cell types targeting those epitopes, and the native proteins that orchestrate their interfaces may best capture the dynamisms of tumor evolution and immune response.

The intrinsic nature of many tumor antigens limits host antitumor responses since such “neoantigens” are often aberrantly expressed self-proteins that are merely subtle alterations of the normal protein. Mutations generate a range of immunogenic neoantigens. For example, insertions and deletions (indels) may make more foreign-looking peptides than SNVs whereas silent mutations may go unnoticed by the immune system since the encoded amino acid is not changed. Moreover, binding affinity of neoantigens to MHC may not correlate as strongly to immunogenicity as

binding stability [75] of peptide-MHC with the TCR. The net mutational load of a tumor theoretically increases the probability that an antigenic peptide is presented, recognized, and targeted by the adaptive immune system. Mutations are acquired through either exogenous exposure to mutagens (i.e., radiation, carcinogens, or oncogenic viruses) or endogenous mechanisms that abrogate the integrity of DNA replication (i.e., mismatch repair deficiency) [76, 77]. Accordingly, different tumor types have characteristic mutational frequencies and patterns [78, 79] whose antigenic load arises from viral or genomic variants that encode ostensibly immunogenic peptides for MHC presentation and variable T-cell binding avidity [80–82]. The mutational load across tumor types is highly variable. However, the quantity of neoantigens may not directly correlate with their quality (i.e., immunogenicity) or specificity. For example, pediatric and hematological cancers have the lowest mutation rates (approximately one mutation per megabase for chronic lymphocytic leukemia) compared with cancers exposed to environmental mutagens that randomly increase the mutation burden, such as melanoma and lung cancer (~15 mutations per megabase for melanoma). In more mutated tumors, immunosurveillance may be especially prevalent. For example, a 200-fold increase in risk of nonmelanoma skin cancers was demonstrated in renal transplant patients, highlighting a curious role for cancer immunosurveillance at sites exposed to highly mutagenic UV radiation [83]. Most tumor antigens are presumably weakly immunogenic as they often appear related to host antigens and lack “danger signals” such as PAMPs. Virally mediated cancers (up to 25% of human cancers) contain foreign pathogen antigens, and therefore, have distinct mechanisms for inducing immune responses beyond mutational load. Patients exhibit a spectrum of susceptibilities to infectious, autoimmune, and cancer-immune processes based on their immune fitness. Clinical measurements of immune function inform disease pathogenesis and treatment optimization.

1.5 Biomarkers and Diagnostics in Immuno-oncology

Biomarker methods help explain cellular functions and relationships in tumor immunology and immuno-genomics [84, 85]. Immune monitoring is needed for comprehensive assessment of the immune status in patients and to establish biomarkers of diagnosis, prognosis, and response to therapy. The standard of care diagnostic workup in cancer has progressed from stage (radiographic imaging) and grade (histology) to encompass myriad molecular features that are either pathognomonic for diagnosis or prognosticating for disease risk. Consensus guidelines (such as those set forth by NCCN, CAP, ASCO, ESMO, etc.) are continuously updated to broaden clinical utility of companion and complementary diagnostic biomarkers. The pathologist’s assessment of a tumor specimen can involve a range of methods that assay the biologic continuum from DNA to RNA to protein alongside complementary immunoassays that measure patient’s immune responses. Proteomic considerations are uniquely important in immuno-oncology diagnostics since the

targeting of native proteins implies understanding their cellular localization, splice and isoform variations, signaling pathways, post-translational modifications, and interaction networks and complexes. Tumor samples can be evaluated for clinical biomarkers through multiparametric immunohistochemistry, fluorescence in situ hybridization (FISH), gene expression profiling, polymerase chain reaction (PCR), and next generation sequencing (NGS). The resident immune cells of tumor specimens and lymph tissues may be studied through multiparameter flow cytometry to ascertain the functional orientation of lymphocytes through surface CD markers. Increased numbers of T-cell infiltrates within the tumor microenvironment have been associated with improved survival in several tumor types, including colorectal cancer and ovarian cancer [86, 87].

Multiple levels of post-transcriptional regulation imply that the relationship between RNA expression and protein abundance are often not linear. The primary (DNA), secondary (RNA), tertiary (peptide), and quaternary (cellular) levels of structure imply emergent properties at each scale. Hence, the co-evaluation of biomarkers in a sample helps elucidate functional relationships of gene and regulatory networks. Proteomic data are generally obtained using “shotgun proteomics” in which the combination of liquid chromatography (LC) and tandem mass spectrometry (MS/MS) identifies peptides by matching MS/MS spectra against theoretical spectra of all candidate peptides represented in a reference protein sequence database. This approach is limited since all protein-coding sequences in the genome are not known nor accurately annotated. Thus, distinct protein products of gene models are uncharacterized in protein reference databases (such as Ensembl, RefSeq, or UniProtKB) and, consequently, mutated native proteins and alternative splice forms are not being routinely measured.

The large majority of mutations in human tumors are unique to the individual tumor, necessitating the identification of mutations that can form neoantigens within clinically actionable timeframes using NGS and bioinformatics [88]. The clinically relevant types of genomic alterations (single nucleotide variants, insertions and deletions, amplifications, and gene fusions) each pose different diagnostic challenges beyond variable tumor purity in most clinical specimens [89]. PCR amplification and counting methods are limited by cost and throughput necessitating a compromise between a limited number of genes assayed in a large cohort or all coding genes sequenced in a small number of samples. Over the past decade, NGS has dramatically scaled our ability to sensitively and comprehensively identify mutations. The sensitivity of sequencing-based approaches is determined by the sequencing methods (amplicon vs enrichment library preparation) and read depth required to detect variants at clinically significant variant allele frequencies. Owing to the high sensitivity of NGS, rigorous analytical and clinical validations have been necessary to establish the accuracy and reproducibility of NGS panels that precedes their routine clinical use. Whole exome and whole transcriptome sequencing methods have broadened the measurable content beyond gene panels to enable identification of specific tumor neoantigens [90]. Detected neoantigens, when qualified by host HLA allotype (i.e., are presented by host MHC) may reveal not only the tumor “mutanome” but more specifically which mutated peptides are most immunogenic

for focusing the immune response through various therapeutic strategies including cellular therapies and tumor- or patient-specific cancer vaccines. Concordance across neoantigen profiling methods has been limited by variations in exome capture methods, DNA and RNA target enrichment, sequencing depth, bioinformatics for variant calling, intracellular neoantigen processing and MHC binding predictors, filtering for expression, and ranking criteria of vaccine peptides based on myriad criteria [85]. Large series of epitopes are being evaluated for their recognition by T cells from multiple independent T-cell repertoires to methodically examine the mechanisms of neoantigen presentation and T-cell recognition. Strong neoantigen-MHC binding, however, does not necessarily equate to strong immunogenicity since binding affinity and binding stability have different consequences on immune cell responses. Validation of immunogenic neoantigens through functional assays remains a limiting step for translating *in silico* predictions into the clinic. Functional assays of immunogenicity include ELISPOT, flow cytometry, capillary chromatography, tandem mass spectrometry workflows, and proteomics [91, 92].

Common methods for studying TME heterogeneity (IHC and flow cytometry) rely on a limited repertoire of phenotypic markers. Deconvolution algorithms applied to bulk tumor RNA-seq or methylation data can resolve aggregate transcripts into their relative TIL constituents [93] to identify cell types involved in immunoediting. These methods are beginning to demonstrate clinical relevance since *a priori* knowledge of the densities and types of TILs [94] helps characterize the TME per the degree of immune cell polarization towards inflammatory or immunosuppressive cell types. RNA-seq deconvolution algorithms aim to enumerate (immune and tissue) cell subsets from tissue expression profiles and are predicated on applications of machine learning such as classification and regression, position-specific scoring matrices, support vector machines, hidden Markov models, and artificial neural networks. These methods still require extensive further validation in mixed tissues and clinical samples. Myriad additional computational genomics tools have been developed to mine tumor immunologic and genomic data effectively and provide novel mechanistic insights, predictive biomarkers, and therapeutic targets [95]. The clinical utility of gene signature-based methods, however, strongly depends on the fidelity of reference profiles that deviate in cells undergoing heterotypic interactions, phenotypic plasticity, or disease-induced dysregulation. Transcriptional signatures reflective of T-cell orientation, exhaustion, and memory differentiate activation states and proliferation of key subsets of exhausted T cells defined by co-expression patterns such as T-bet or eomesodermin (eomes) in combination with PD-1 [96–98]. Intra- and inter-patient sample variability in immune cell subset composition, inter-patient genetic variability, as well as prior and concurrent treatment medications are all clinical parameters that confound our ability to establish validated assays to generate biomarker data sets.

Longitudinal monitoring of response to immunotherapies presents additional opportunities for understanding mechanisms. Immunotherapies such as checkpoint blockade enable the mounting of a measurable antitumor immune response over variable clinical timeframes. The conventional Response Evaluation Criteria In Solid Tumors (RECIST) using radiographic methods (e.g., CT and MRI) must

account for pseudoprogression in which tumors appear to grow radiographically secondary to inflammation [99, 100]. Owing to the inter and intra-tumoral heterogeneity [101] in metastatic disease, serial or functional imaging [102] such as anti-CD8 immuno-PET of multiple discrete lesions may be necessary to represent immunodynamics across multiple sites. Radiographic assessment thus contextualizes the relationships between circulating biomarkers and tumor inflammation through the course of therapy. Pharmacodynamic biomarkers for dose selection and toxicity anticipation are the basis of ongoing investigation. Unlike small-molecule-targeted agents such as Raf/MEK/PI3K inhibitors that block detectable signals in tumor cells, the targeting of native proteins does not infer an obvious signal for measurement of response. Considerable research is underway to understand and optimize proximal biomarkers unequivocally reflecting dose-dependent native protein target inhibition for immunotherapies. Immunodynamics measurement through complementary and mechanistic approaches [103, 104] will enable the implementation of novel endpoints in efficacy, dose selection, treatment sequencing, and toxicity.

Peripheral monitoring of tumor and immune biomarkers through blood draw is desirable because of the relative ease and standardization of sample collection as well as the ability to trend circulating tumor variants, immune cell subtypes, and pharmacodynamics over time. Existing peripheral monitoring approaches include flow cytometry for minimal residual disease, circulating tumor and exosomal DNA and RNA sequencing to monitor variant allele frequencies, circulating tumor cell isolation for expression analysis, tetramer assays for trending clonal evolution of antigen-specific T cells, and measurement of plasma cytokines and chemokines through ELISPOT assays. Circulating tumor DNA tests have been shown to provide complementary information to that found in primary tissue biopsies [105]. Peripheral evaluation of checkpoint blockade has revealed temporal variance in the differential leukocyte composition and markers of T-cell functionality such as clonality and expression of ICOS on sorted peripheral immune cell subtypes [106, 107]. Analysis of the T-cell receptor repertoire (such as by deep sequencing of the V β CDR3 region) has proven increasingly cost-effective and useful for clonality assessment and minimal residual disease detection. T-cell clonality in immuno-oncology enables the tracking of clonal expansion or contraction through serial sampling. Blood sampling also helps establish biomarker baselines for trending key organ function (kidney, liver, etc.) with vigilance towards inflammatory toxicity of immunotherapy such as cytokine release syndrome [108]. The differential immune measurements through blood draw are further confounded by any systemic immune response to opportunistic infections (viral, bacterial, fungal, parasitic) which are more common and can go undetected in immunosuppressed patients. Evaluation of the circulating microbiome through 16sRNA and metagenomic sequencing can measure aggregate effects of therapies on commensal and infectious flora [109–111]. Ongoing efforts are underway to establish the sampling timeframes and mechanistic relationships between circulating biomarkers as a proxy of dynamic antitumor immune activity within the tumor stroma [112, 113].

1.6 Targeting Native Proteins Within Cellular Networks

Treatment complexity is increasing as durable clinical responses require the use of numerous therapies in combination and in sequence [114]. Checkpoint blockade may appear to be the opposite of precision medicine as it can be applied broadly because of the targeting of native proteins. However, we still need biomarkers to understand when and why most patients do not respond while those who do may do so profoundly. Clinical outcomes are influenced by differences in patients' immune repertoires, their capacity to process and present antigens, the "quantity and quality" of tumor antigens generated, as well as the ability of cancer to suppress antitumor immunity. T cells are key effectors [115] and it is hypothesized that T cells can be genetically targeted to any antigen and enhanced to overcome immune escape mechanisms to achieve tumor eradication. As reviewed in this primer, T cells operate within a dynamic network of immune cell subtypes who are systemically under tumor micro-environmental influences and paracrine signals that continuously modulate their functional orientation. Beyond standard approaches of surgery, radiation, and chemotherapy, a variety of approaches to eliciting an antitumor immune response have been developed. These include anti-tumor antibodies (mono-, bi-, and multispecific), immune checkpoint blockade, T-cell costimulatory agonists, immunomodulators (cytokines, cyclic dinucleotides, IDO inhibitors), toll-like receptor agonists, cancer vaccines and oncolytic viruses, as well as adoptive T and NK cell therapies [116]. Conventional cytotoxic approaches modulate the composition and functional bioactivity of TILs beyond their intended effects on neoplastic cells [117].

The degree of nonlinear thinking required to first perceive and then influence network interactions [118] between immune cells and cancerous ones is central to advances in immuno-oncology. Combination immunotherapies that influence the immune set point may prevent immune escape by targeting complementary mechanisms through which tumor cells avoid elimination by the immune system. Mechanistic synergy can be achieved in: (1) priming – via tumor antigen-expressing dendritic cells or tumor cells transfected with genes that render them immunogenic; (2) amplification – checkpoint blockade or agonistic monoclonal antibodies against costimulatory molecules or immune-potentiating cytokines; and (3) removal of the inhibitions – eliminating mechanisms that self-regulate the strength of the immune response, such as inhibitory receptors or regulatory T cells.

Rational combination strategies must incorporate an understanding of pathway implications in both tumor and immune cell subtypes. The number of therapeutic combinations is exciting because the amount of antitumor activity is unprecedented. Most combinations are currently two drugs, but when adding sequence and dose (which are currently nearly guess work) the combinations become much more complex. Since the receptor occupancy of pembrolizumab (anti-PD-1 monoclonal antibody) is 3–6 months, the concept of monotherapy and sequencing of combinations becomes ever less well defined. Patients may therefore ultimately stratify into checkpoint inhibitor experienced vs checkpoint inhibitor naïve cohorts from a

line-of-therapy standpoint. Because of pharmacokinetics and principles of immune memory and regulation, the regimens that will best combine chemotherapy and radiotherapy with immunotherapy are likely not conventional regimens. Improving upon therapy doublets thus requires a comprehensive biomarker strategy. Combination therapies across therapeutic modalities may mitigate toxicity while adding mechanistic synergy. For example, the radiation “abscopal” (off-target) effect is an immunologic-based phenomenon [119] where local treatment leads to a systemic immune response through, presumably, massive neoantigen release, production of IFN-gamma, upregulation of T cells, and immune sensitivity to radiated/injured tissue. Chemotherapy reforms the immune profile since lymphocyte counts decline, then revives through homeostatic proliferation [120], and this leads to a restoration of T-cell responses.

Biomarker strategies must reconcile shared pathways across tumor and immune cells. For example, MEK inhibition of tumor cells also blocks naïve T-cell priming (through interference of the RAS MAP kinase pathway) and causes an increase in incompletely exhausted PD-1^{lo} CD8⁺ T cells in tumors and eventually depletes TILs as tumors relapse. Alternatively, the MAP kinase pathway is only needed for naïve T-cell expansion and differentiation into memory cells, and thus MEK and PD-1L1 may act by preventing rather than reversing T-cell exhaustion [121]. It may therefore be necessary, in some cases, to withhold a MEK inhibitor as the patient responds as this may inhibit immune priming while instead focusing on checkpoint blockade plus monoclonal combination therapies in the BRAF pathway, β -catenin pathway, or PI3K pathway. The tumor microenvironment (tissue type, proximity to the external world and associated microbiome, vascularity, etc.) will necessitate tissue-specific understanding of how immunotherapies act within certain cancer types beyond the likelihood for generation of immunogenic peptides (TMB) and expression of (inducible) checkpoint antigens. The immunoglobulin isotype of checkpoint blockade monoclonal agents further influences therapeutic antibody function by dictating their structural characteristics and specificity [122]. The type of immune response towards monoclonal antibodies targeted against native proteins can therefore be fine-tuned. Incorporating relevant biologic correlative data will enhance our ability to interpret complex and nuanced clinical responses to drug combinations and can help reduce the likelihood of taking a neutral or antagonistic combination through to phase III. Immune host–microbiota interactions also influence tissue-specific immune fitness (i.e., through gastrointestinal and respiratory flora) and thus systemic immunity. The composition of the microbiome is molded by environmental influences such as diet, exercise, hygiene, and sleep. As cancer patients are often immunosuppressed through chemotherapy which alters the host microbiome [123], antibiotics indiscriminately and broadly eradicate both pathogenic bacteria and commensal flora that may act as an adjuvant to cancer immunotherapy. For example, commensal bacteria have been shown to enhance PD-L1 blockade in murine models [111, 124].

Many have foreseen that checkpoint modulation will supplement chemotherapy as the cornerstone of cancer therapy either directly or after interventions targeting inflammation, by vaccination to boost T-cell repertoires or by adoptive T-cell

transfer [125]. Anticipating adaptive resistance followed by immune escape [126, 127], successive therapies must incorporate multiparametric biomarkers that capture the dynamic immunobiology within the tumor microenvironment. Linking adaptive and innate immunity has become a key area of focus in drug development [128]. Through leveraging complementary mechanisms of action, strategies may be devised for rational therapeutic combinations that promote durable immune responses [129]. For example, approaches that promote inflammation of an otherwise noninflamed tumor present as scientifically rational options to be studied as combination partners for checkpoint inhibitors that facilitate adaptive immunoresistance. Mechanistic-based approaches thereby increase the likelihood that effective combinations with acceptable toxicity profiles will progress into phase II/III trials that are prolonged and costly. For example, the recognition that PD-1 and PDL-1 have primary action in the tumor microenvironment whereas CTLA-4 acts in the lymphoid system provides a strong mechanistic basis for testing the combination of nivolumab and ipilimumab for therapeutic synergy [130, 131]. Anti-CTLA4 blockade expands T cells in all compartments of the body (not just those relevant to the cancer), and such global considerations for targeting native proteins are fundamental to understanding clinical responses. With so many therapeutic combinations possible [132], methods to assess the additive benefits of each intervention are needed.

Immunotherapy has catalyzed a change in the delivery of oncology care since the increasing complexity of cancer treatment increases costs. Therefore, optimizing personalized approaches to therapy will be critical to establishing and differentiating clinical value of both diagnostics and therapeutics. It remains to be determined if immunotherapies can advance to even earlier stages of disease such as in the neoadjuvant setting when tumor burden may facilitate enhanced immune system “education.” Co-development programs are needed to build upon the historic one drug one protein drug development paradigm. These considerations and their rational implementation into clinical practice are will foster the next wave of advances in cancer immunotherapy.

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