

Opinion

# Archetypes of checkpoint-responsive immunity

Kwok Im,<sup>1,2</sup> Alexis J. Combes,<sup>1,2</sup> Matthew H. Spitzer,<sup>3</sup> Ansuman T. Satpathy,<sup>4</sup> and Matthew F. Krummel<sup>1,\*</sup>

**Responsiveness to immune checkpoint blockade (ICB) therapy in cancer is currently predicted by disparate individual measures – with varying degrees of accuracy – including tumor mutation burden, tumor-infiltrating T cell densities, dendritic cell frequencies, and the expression of checkpoint ligands. We propose that many of these individual parameters are linked, forming two distinct 'reactive' immune archetypes – collections of cells and gene expression – in ICB-responsive patients. We hypothesize that these are 'seeds' of antitumor immunity and are supported by specific elements of the tumor microenvironment (TME) and by actions of the microbiome. Although removing 'immunosuppressive' factors in the TME is important, understanding and parsing reactive immunity is crucial for optimal prognosis and for engaging this biology with candidate therapies to increase tumor cure rates.**

## Evaluating ICB therapy and targets

**Immune checkpoint blockade (ICB;** see [Glossary](#)) therapy mainly targets two T cell-associated inhibitory signaling pathways in human cancers. Anti-**CTLA-4** ICB is understood to work by blocking CTLA-4-derived inhibitory signals which would otherwise be delivered to naïve or **T effector (T<sub>eff</sub>) cells** by antigen-presenting cells (APCs) or other cells bearing the ligands B7-1 (CD80) or B7-2 (CD86) [1,2]. In mouse models, and possibly in humans, anti-CTLA-4 may also function by depleting **regulatory T (T<sub>reg</sub>) cells** [3] which constitutively express large amounts of surface CTLA-4 and would otherwise attenuate the immune response [4,5]. Anti-PD-1 or anti-PD-L1 ICB is commonly understood to work by blocking **PD-L1/2** from engaging **PD-1** on T cells alongside the T cell receptor (TCR), which would otherwise result in the recruitment of phosphatases and attenuate T cell activation.

The potential for anti-CTLA-4 antibodies (Abs) to cure cancers was first shown in mouse models of melanoma [6,7]. The first clinical approval for an anti-CTLA-4 drug, **ipilimumab** (NCT00094653)<sup>†</sup>, was thus sought in stage III and IV melanomas in which patient prognosis was extremely poor (median survival <12 months). In this Phase III study, highly durable **response rates** were observed in 6–21% of patients [8] thus leading to US FDA approval in 2011 for the treatment of metastatic melanoma, and shortly after in 2012 by the Canadian (Health Canada) and European (EMA) medicines agencies. Although adverse side effects are observed, most cases are reversible with appropriate treatment.

The anti-PD-1 Ab **nivolumab** was also tested in a clinical trial of melanoma and demonstrated higher response rates (52%) compared to ipilimumab alone (34%) [9]. In a second Phase III clinical trial involving patients with advanced melanoma (NCT01844505)<sup>‡</sup>, nivolumab combined with ipilimumab resulted in longer progression-free survival (PFS) and a higher overall response (OR) than either treatment alone [9]. However, higher adverse events were observed in combinatorial ICB (59%) compared to nivolumab (21%) or ipilimumab alone (28%), leading to the preferential use of nivolumab as the standard of care for advanced stage melanoma.

## Highlights

ICB therapies function for some cancers because they oppose suppressive immunity and engage antitumor 'reactive' immunity.

We introduce the concept of reactive immunity archetypes: these are networks of cells and gene expression profiles that align with specific immune functions – in this case involving tumor rejection. We focus on the nature of these reactive archetypes given that these can be engaged by ICB.

Integrating a network of studies, we argue that at least two reactive immunity archetypes are responsive to ICB.

The licensing of immune-reactive archetypes in ICB may be dependent on the availability of the former and the interactions both outside and within the TME.

Thorough understanding of these reactive archetypes, their composition, spatial localization, and origin may be required for identifying ICB responsiveness and for designing orthogonal immunotherapies that can enhance reactive immunity.

<sup>†</sup>Department of Pathology and Immunology Initiative, University of California at San Francisco, San Francisco, CA 94143, USA

<sup>‡</sup>UCSF CoLabs, University of California at San Francisco, San Francisco, CA 94143, USA

<sup>§</sup>Department of Otolaryngology, School of Medicine, University of California at San Francisco, San Francisco, CA 94143, USA

<sup>¶</sup>Department of Pathology, Stanford University, Stanford, CA 94305, USA

\*Correspondence: [Matthew.Krummel@ucsf.edu](mailto:Matthew.Krummel@ucsf.edu) (M.F. Krummel).



A second anti-PD-1 drug, **pembrolizumab**, has also been FDA-approved for treatment of non-small cell lung cancer (NSCLC) as a result of 41% response rates [10]. In a Phase I clinical study (NCT01295827)<sup>iii</sup>, NSCLC patients were selected based on their tumors expressing high amounts of PD-L1 on many cells and were given pembrolizumab every 2–3 weeks. Adverse events relating to the immune system occurred in 13% of patients. Recently, pembrolizumab was also FDA- and EMA-approved for the treatment of adult and pediatric patients with **mutational microsatellite instability-high (MSI<sup>hi</sup>)** in multiple tumor types [11]. Patients with MSI<sup>hi</sup> cancers were pulled from five different clinical studies collectively and the results revealed a 39.6% OR with approximately 78% of responses lasting >6 months, with similar adverse events to the previously mentioned clinical trials. To our knowledge, this marks the first time a cancer treatment for an indication is based on a common biomarker rather than on the primary site of origin.

We postulate here that the biology of the immune response, rather than the tissue of origin of the tumor, gives rise to the variability of responses to ICB therapy. For this, we advance the concept of an **archetype** – a recurrent motif in the immune system that spans multiple tumors and comprises a common core set of cells and/or gene expression profiles [12].

### Immune archetypes: a balancing act of multiple, linked states for functional goals

In viable tissues and immune systems, cell states support one another. For example, **type 1 conventional dendritic cells (cDC1s)** support CD8<sup>+</sup> T cell effector functions, whereas **type 2 conventional dendritic cells (cDC2s)** support CD4<sup>+</sup> T cells [5,13,14]. The functional linkage of these cell types may represent an evolution-selected pairing of functions to achieve a specific type of response. As opposed to focusing on the state of a single cell type, we argue that an immune system should be abstracted and conceived of by considering the prevalence of a collection of cells with linked states. The prototypical collections of these cells that define the system and its functional goals are then considered to be an archetype of that response.

The involvement of the immune system in wound-healing, cellular metabolism, and remodeling of tissues during development provides us with diverse blueprints of how a collection of cells can be differentially assembled for tissue homeostasis [12]. The resulting immune archetypes, as constructs, help to elucidate how different implementations of the immune system can program immunity to promote defense against pathogens in some cases, while separately and actively supporting host viability when faced with other types of biological changes [12]. We propose that different tumors grow because they have programmed these archetypes, and some will resemble 'the wound that never heals' [15], whereas other cancer types might activate archetypes that are normally used for tissue remodeling during development [16]. We postulate that each tumor effectively has such a **dominant tumor archetype**, and, as such, one that has 'suppressed' immunity. The concept of tumor immunosuppression is well established and can include, for example, the recruitment of tumor-associated macrophages or neutrophils; this is beautifully reviewed by others [17].

Conversely, according to our model, **reactive immune archetypes** are the collections of cell types that can mediate tumor regression. Although the cells comprising reactive archetypes are necessarily rare in growing tumors, we postulate that they are the seeds of a productive immune response, which ICB ultimately enhances, and their initial presence is therefore deemed to be crucial.

In **Figure 1A** (Key figure) we outline the selection of distinct cells and cellular states that comprise an archetype. We focus especially on a few of the reactive archetypes that are outlined in this

### Glossary

**Archetype:** a collection of cells and gene expression profiles defined by a functional goal for the immune system; a recurring motif of immunobiology, defined at the multicellular level.

**Assay for transposase-accessible chromatin with deep sequencing (ATAC-seq):** a technique to assess genome-wide chromatin accessibility, for example in cancer settings.

**CD8<sup>+</sup>PD-1<sup>+</sup>LAG3<sup>+</sup> phenotype:** associated with exhausted CD8<sup>+</sup> T cells.

**CTLA-4:** an immune checkpoint receptor in T lymphocytes; serves to downregulate T cell activation and adaptive immunity – an outcome that does not antagonize tumor growth.

**Dominant tumor archetypes:** the collection of cells and their linked phenotypic states that support tumor growth.

**Exhausted T (T<sub>EX</sub>) cells:** a subset of T cells defined by the progressive loss of effector function and sustained expression of the inhibitory receptors PD-1, TIM-3, LAG-3, and CTLA-4.

**ICB-resistant:** a tumor that is nonresponsive to ICB drugs.

**Immune checkpoint blockade (ICB):** antibodies/agents used to derepress antitumor immunity.

**Ipilimumab:** anti-CTLA-4 antibody; an ICB agent used to treat some cancers.

**Mutational microsatellite instability high (MSI<sup>hi</sup>):** a condition of genetic hypermutability that results from impaired DNA mismatch repair.

**Neoantigens:** the repertoire of peptides that have been newly formed but not previously recognized by the immune system.

**Nivolumab:** anti-PD-1 antibody; an ICB agent that is used to treat some cancers.

**PD-1:** an immune checkpoint inhibitory receptor found on T and B lymphocytes; serves to downregulate T cell activation and adaptive immunity by inducing T cell death – an outcome that does not antagonize tumor growth.

**PD-1<sup>hi</sup>ICOS<sup>+</sup> phenotype:** CD4<sup>+</sup> conventional T cells that can enhance the efficacy of anti-CTLA-4 antibodies.

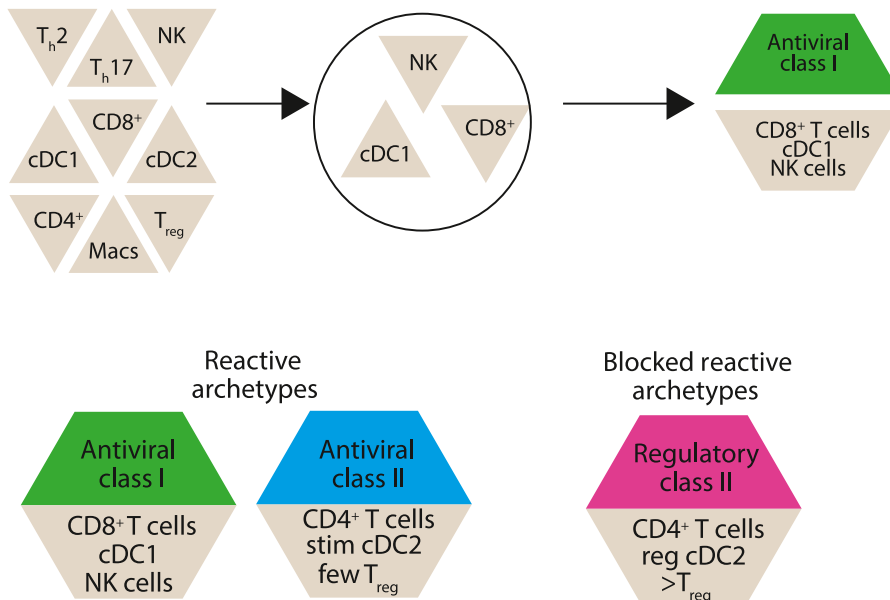
**PD-L1/2:** an immune checkpoint ligand expressed by myeloid and tumor cells (and other cells) in the tumor microenvironment; serves to downregulate adaptive immunity – an outcome that does not antagonize tumor growth.

**Pembrolizumab:** anti-PD-1 antibody; and ICB agent that is used to treat some cancers.

## Key figure

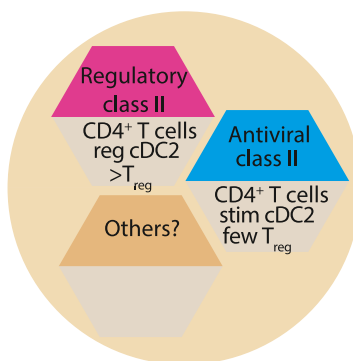
### Proposed reactive archetypes of immune checkpoint blockade (ICB) responsiveness

#### (A) Constructing a reactive archetype:

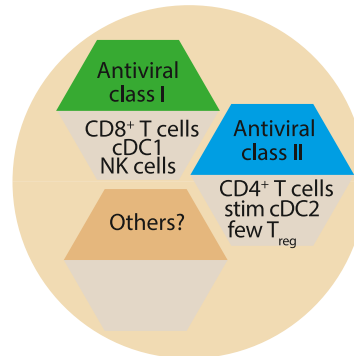


#### (B) Immune checkpoint response by cancer type

##### Head and neck carcinoma



##### Melanoma



**Reactive immune archetype:** the collection of cells and their linked phenotypic states (archetypes); we deem these to be necessary for ICB therapies to work effectively.

**Regulatory T (T<sub>reg</sub>) cells:** a subset of T cells that repress antitumor immunity.

**Response rate:** median survival of cancer patients measured in years or tenths of years.

**T effector (T<sub>eff</sub>) cells:** a subset of T cells that have differentiated to mediate target cell killing, here antitumor immunity.

**Tertiary lymphoid structures:** ectopic lymphoid formations found for example in tumoral tissues and exhibit similar characteristics to the architecture of lymph nodes.

**Tissue-resident memory (T<sub>RM</sub>) cells:** a subset of T cells that express CD69 and CD103, and occupy tissues without recirculating in the periphery.

**Tumor mutational burden (TMB):** a genomic biomarker that can predict responsiveness to immunotherapy.

**Type 1 conventional dendritic cells (cDC1s):** known for their efficient processing and crosspresentation of exogenous antigens by MHC class I molecules to activate CD8<sup>+</sup> T cells.

**Type 2 conventional dendritic cells (cDC2s):** induce CD4<sup>+</sup> T cell polarization towards the type 1 T helper (Th1), Th2, and Th17 phenotypes.

**Unfolded protein response (UPR):** an intracellular signaling pathway induced by the accumulation of unfolded proteins in the endoplasmic reticulum (ER), a condition referred to as ER stress.

**Figure 1.** (A) An immune archetype emerges as the selection of individual immune cell types (top, left) that have been shown to interact cohesively (black circle) and work together to ultimately create an effective immune response. In one example, this might potentiate a specific response against a viral pathogen, named here as an antiviral class I response (top, right, green hexagon) [21,25]. Among the reactive archetypes, we consider class I (green hexagon) and class II (blue hexagon), each of which has shown different collections of cells for predicting ICB responsiveness in melanoma patients [5,91]. Immune infiltrates resembling the class II archetype but with substantial T<sub>reg</sub> infiltrates are considered to constitute a 'blocked' response.

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review. We believe that sparse numbers of cells comprising a reactive immune archetype can coexist in a tumor that is predominantly populated with the dominant archetype, in the same way as type 1 T helper ( $T_H1$ ) cells are found in every  $T_H2$  cell-dominated *Leishmania* sp. lesion [18]. Presaging the subsequent discussion, Figure 1B outlines a rough understanding of how specific cancer types seem to have different propensities to support specific reactive archetypes. For example, ~40% of melanoma patients present a class I 'responsive' archetype, accounting for the majority of patients responding to anti-PD-1 ICB [13], but this is not observed in head and neck squamous cell carcinoma (HNSCC) [5]. However, a less common reactive archetype in some melanoma patients (a class II archetype) is nucleated by cDC2<sup>+</sup>/CD4<sup>+</sup> infiltration, and this archetype is relatively common in HNSCC [5]. Because these reactive archetypes are different, identifying those that are most abundant and malleable will be advantageous for predetermining what ICB treatment regimen might be most efficacious for a given patient.

### Class I: a CD8-based reactive archetype for ICB responsiveness

CD8<sup>+</sup> T cells are characterized by their cytolytic function (cytotoxic T cells, CTLs), which is mediated by the interaction between the TCR and the peptide–MHC class I complex (pMHC). CD8<sup>+</sup> 'tumor-infiltrating lymphocytes' (TILs) preferentially recognize pMHC containing self-proteins that are produced more by tumors than by healthy tissues [19], mutated self-proteins that are produced uniquely in the tumor [20], or seemingly irrelevant antigens such as influenza virus epitopes [21]. In mouse tumor models, tumor-specific CD8<sup>+</sup> T cells are notably capable of lysing target cells when taken from the tumor-draining lymph node (dLN) but are incapable of doing so when taken from the nearby TME [22]. This insufficiency may be cell-intrinsic; many tumor-resident CD8<sup>+</sup> T cells exhibit high expression of inhibitory checkpoint receptors PD-1 or CTLA-4 and harbor transcriptional states that are distinct from those of *bona fide* effector or tissue-resident memory T cells [23]. These so-called **exhausted T ( $T_{EX}$ ) cells** lack the ability to effectively perform cytotoxicity [24]. How then can  $T_{EX}$  cells become reactive?

Evidence from mouse tumor and chronic viral infection models (e.g., lymphocytic choriomeningitis virus clone 13 infections, among others) suggests that some CD8<sup>+</sup> T cell subsets differ in their state of exhaustion and have the potential to reinvigorated effector functions following PD-1 ICB [25]. In human studies, the frequency of a subset of CD8<sup>+</sup> T cells expressing CD103<sup>+</sup>CD69<sup>+</sup>, called **tissue-resident memory ( $T_{RM}$ ) cells**, correlated with better patient overall survival (OS) in a multitude of cancer types [26–29]. In contrast to the notion that T cell dysfunction is marked by high expression of PD-1, augmented numbers of PD-1<sup>hi</sup>  $T_{RM}$  cells have been demonstrated to predict a positive response to ICB in NSCLC patients [30]. Correspondingly, several research teams have recently shown that CD8<sup>+</sup>PD-1<sup>hi</sup>  $T_{RM}$  cells from OT-1 mice infected with vesicular stomatitis virus or Epstein–Barr virus can reduce tumor growth of **ICB-resistant** and poorly immunogenic tumors following intratumoral injection of these viral peptides [31,32]. Other studies in melanoma patients have reported that the detection of CD8<sup>+</sup> T cells with dual upregulation of PD-1 and CTLA-4 in melanoma tumors is also associated with increased responses to anti-PD-1 Ab therapies and OS [33,34]. These features suggest that some CD8<sup>+</sup>PD-1<sup>hi</sup>  $T_{RM}$  populations, rather than being consistently exhausted, may be poised for malleability and might represent a key cell type within the class I-reactive archetype.

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reactive' archetype; all these are separate from the dominant immune infiltrate which appears to 'accommodate' tumors [92]. (B) In malignancies such as melanoma, both class I- and class II-based responders are observed and appear to be mutually exclusive [5]. Head and neck squamous cell carcinoma patients (left circle) were demonstrated to have little class I reactive immunity, whereas melanomas may comprise class I or class II reactive infiltrates (left and right circles) [5]. Abbreviations: cDC1, conventional type 1 dendritic cell; cDC2, conventional type 2 dendritic cell; Macs, macrophages; NK, natural killer cell;  $T_H1$ , type 1 T helper cell;  $T_H17$ , type 17 T helper cell;  $T_{reg}$ , regulatory T cell.

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How do CD8<sup>+</sup> TILs become activated? We and others revealed that TME cDC1s in mouse tumors (characterized by the expression of the CD103 integrin) are unique in expressing high amounts of the stimulatory cytokine IL-12 [35–37], as well as in their capacity to cross-present tumor antigens on MHC I by maintaining ingested proteins in a neutral pH environment [35]. They are also essential for repriming incoming T cells, as evidenced from adoptive transfer experiments in EL4 tumor-bearing mice [35]. Before this, a tumor-clearance role for members of the cDC1 lineage was hinted at when assessing the phenotype of *Batf3*<sup>−/−</sup> mice (lacking the key transcription factor for the entire cDC1 lineage). In that background, investigators observed the notable absence of antigen cross-presentation to CD8<sup>+</sup> CTLs, as well as associated defects in the clearance of H31m1 fibrosarcoma cells, relative to wild-type mice [38]. In humans, immunoprofiling of intratumoral cDC1 (expressing BDCA3) taken from melanoma, renal, and NSCLC tumors demonstrated high frequencies of cDC1s when patients received anti-PD-L1 or anti-PD-1 Abs, and these treatments and profiles were associated with higher OS in the patients relative to controls [13,39]. In addition, loss of cDC1 in *Batf3*<sup>−/−</sup> mice results in failed CTL responses to anti-PD1 Ab, as evidenced from the reported increased tumor sizes and failure to prime an endogenous CTL-mediated response, compared to controls [40,41]. In addition, upon gene signature analysis of total RNAs taken from The Cancer Genome Atlas (TCGA) the prevalence of cDC1 cells in tumors was deemed to be prognostic for OS in 12 different tumor types from cancer patients, including breast, HNSCC, and lung [35]. Moreover, similar results of RNA gene signature analysis were observed when separate melanoma patient cohorts were assessed [13,42].

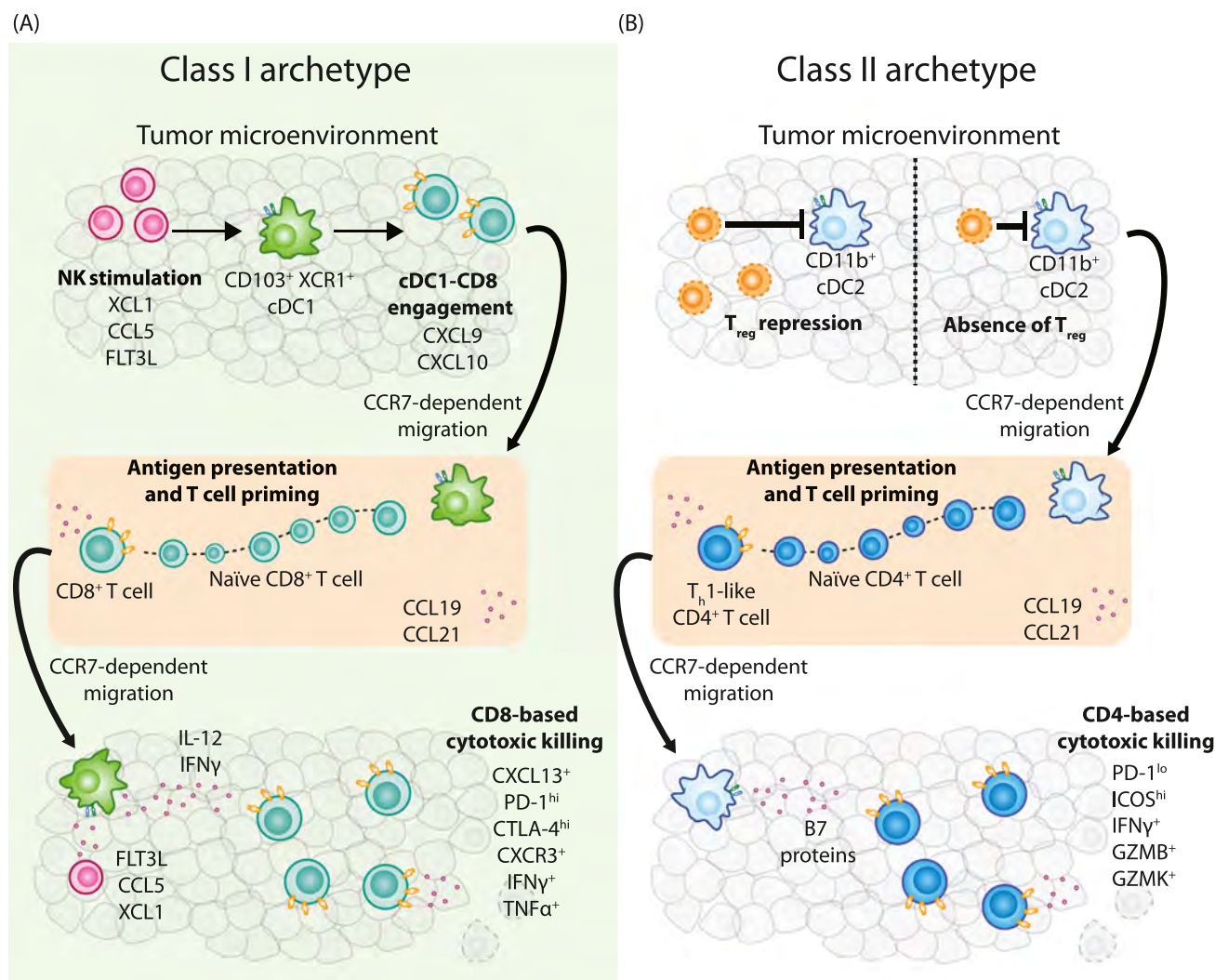
Subsequent work demonstrated that cDC1s are essential for carrying antigen from B16 tumor-bearing mice to the dLN and for priming new CD8<sup>+</sup> T cell clones [41,43]. In these examples, lineage tracing and immunoprofiling of CD103<sup>+</sup> expressing cDC1s revealed that these cells are the predominant mechanism by which intact fluorescent tumor protein antigens made their way to the dLN. Mechanistically, this required upregulation of chemokine receptor CCR7 by cDC1 [35,41], after which cDC1s migrated to the lymph node (LN) [43]. Imaging and functional experiments in mice have also shown that cDC1 can not only prime new T cells directly in the LN but also hand off the antigen via a unique dendritic cell (DC)–DC synapse (antigen resides within discrete vesicles inside DCs); the antigen is then transferred to CD8<sup>+</sup> T cells as well as to other DC subsets [44]. This in turn allows substantial activation and proliferation of new CTLs [44]. Taken together, the presence and trafficking of cDC1s into the TME should be considered to be a major requisite component of a reactive class I archetype.

Lastly, two separate lines of evidence have shown that a third cell type, the natural killer (NK) cell, is a crucial component of the class I reactive archetype. NK cells express high amounts of chemokines CCL5 and XCL1, which bind to receptors on cDC1s and are found in close association with cDC1s in tumors [13,42]. Meanwhile, NK cells are producers of the pseudo-cytokine Flt3L (a growth factor for cDC1), and, in a mouse model of NK cell depletion, the overall frequencies of cDC1s were reduced and this was accompanied with increased tumor growth relative to controls [13]. NK frequencies were highly associated with cDC1 numbers in human melanoma [13] and the presence of these cells was associated with a predicted improvement in OS and ICB responsiveness in two separate melanoma studies [13,42]. We postulate that local expression of specific NK ligands, often produced by cellular stress, might activate/support these cell types in some tumors, leading to the enrichment of NK cells, then cDC1s and a class I reactive archetype responsive to ICB (Figure 2A).

### Class II: a CD4-based reactive archetype for ICB responsiveness

A direct role for CD4<sup>+</sup> T cells in tumor immunity has been demonstrated in multiple mouse models either by adoptive transfer of tumor-reactive CD4<sup>+</sup> T cells or by selectively depleting CD4<sup>+</sup> T cells [45,46]. Many human and murine tumor cells upregulate MHC class II, particularly upon exposure





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**Figure 2. Mechanisms of action of class I and class II archetypes in immune checkpoint blockade (ICB) responsiveness.** (A) We propose that class I responsiveness begins with either the availability of CD8<sup>+</sup> T cells and the delivery of FLT3L, CCL5, and XCL1 from natural killer (NK) cells, or CD8<sup>+</sup> T cell priming from migratory CD103<sup>+</sup>XCR1<sup>+</sup> type 1 conventional dendritic cells (cDC1s) that have egressed (top right black arrow) to the tumor-draining lymph node (TDLN) via chemokines such as CCL19 and CCL21, thus supporting tumor antigen-specific CD8<sup>+</sup> T cell maturation. CD8<sup>+</sup> T cells then migrate back (left bottom black arrow) into the tumor microenvironment to perform cytotoxic killing on tumor cells. Studies have shown that CD8<sup>+</sup>PD-1<sup>hi</sup> T cells can be reinvigorated via ICB strategies in melanoma patients, improving overall survival [33,34]. (B) Class II responsiveness (right) begins with the limited presence of regulatory T (T<sub>reg</sub>) cells in the tumor microenvironment, licensing CD11b<sup>+</sup>CCR7<sup>+</sup> type 1 conventional dendritic cells (cDC2s) to egress (top right black arrow) into the TDLN via CCL19 and CCL21 for CD4<sup>+</sup> T cell priming. After interacting with CD11b<sup>+</sup>CCR7<sup>+</sup> cDC2s, *de novo* produced tumor antigen-specific CD4<sup>+</sup> T cells could then migrate (bottom left black arrow) back into the tumor microenvironment to elicit CD4<sup>+</sup>ICOS<sup>+</sup>PD-1<sup>lo</sup> T cell-dependent tumor rejection. Recent studies suggest that this 'blocked' reactive archetype might be harnessed for better ICB strategies [5,91]. Abbreviation: T<sub>h</sub>1, type 1 T helper cell.

to IFN- $\gamma$ , making CD4<sup>+</sup> T cells capable of direct cytotoxicity via a 'class II' axis [47,48]. This direct cytotoxic role for CD4<sup>+</sup> T cells is less well appreciated, but is evident from their production of granzymes, specifically granzyme K [49]. CD4<sup>+</sup> T cells in mice and humans also act by collaborating with phagocytes or B cells [50] and by coordinating immune activity that drives responsiveness [48,51–54].

We and others investigated potential partners of CD4<sup>+</sup> T cells in propagating a class II-reactive tumor immune setting [5,49,55–59]. Dominant APCs presenting antigens on MHC class II and

triggering CD4<sup>+</sup> T cells in tumors were typically cDC2s, as shown by mouse genetic and *in vitro* experiments [5], although in some cases cDC1 cells could also apparently substitute for cDC2 [55]. In one study, CD11b<sup>+</sup> cDC2s purified from the TME of tumor-bearing mice induced CD4<sup>+</sup> T cell expansion and proliferation *ex vivo* compared to other myeloid cell phenotypes; moreover, depletion of these myeloid populations using *Irfa*<sup>-/-</sup> mice resulted in a decreased ability to control tumor growth in B16 tumor-bearing T<sub>reg</sub>-depleted mice relative to controls [5]. In this study, T<sub>reg</sub> cells restricted the ability of cDC2s to profoundly stimulate CD4<sup>+</sup> T cells to adopt a **PD-1<sup>hi</sup>ICOS<sup>+</sup> phenotype** and become T<sub>eff</sub> cells [5]. One probable role for T<sub>reg</sub> cells in modulating cDC2 function may involve regulating the transcription and surface quantities of costimulatory molecules [5,60]. In humans, the same relationship between cDC2 and CD4<sup>+</sup> T cell numbers and phenotypes was found in head and neck tumor biopsies; in addition, these frequencies and phenotypes were inversely correlated with T<sub>reg</sub> frequencies [5]. Together, these results led us to hypothesize that some archetypes might be 'blocked' instead of simply being absent (Figure 1A,B); in other words, most archetypal components may be present, but additional immune cells (here T<sub>reg</sub> cells) could prevent the archetypal collection of cells from mediating antitumor immunity. We postulate that unblocking the pathways related to the recruitment, proliferation, and survival of T<sub>reg</sub> cells in 'blocked' class II archetypes might contribute to re-establishing ICB responsiveness.

### The role of tumor mutational burden in dictating ICB responsiveness?

Several studies have suggested that the **tumor mutational burden (TMB)** correlates with response to immunotherapy in cancer [61–63]. These studies posit that a fraction of these nonsynonymous somatic mutations become exposed as neoepitopes, thus providing the specific TCR–pMHC complexes necessary to enable an antitumor response. Although there have been loose associations between TMB in colon cancer and features of MSI<sup>hi</sup> (high TMB checkpoint-responsive) versus MSI<sup>lo</sup> (low TMB, non-responsive) [64], a recent study reported that no TMB cut-off could distinguish between the groups of lung and melanoma cancer patients that exhibit significantly different survival rates in response to ICB [65]. If indeed TMB is predictive of responsiveness in some patient cohorts, it is reasonable to speculate that the predictive value might reflect either (i) a larger availability of **neoantigens** for T cell activation, or (ii) map to higher tissue variability in a highly mutated tumor, and whose variability might result in danger signals that enrich components of the reactive immune archetype (see the stress response discussion in the following section). Noteworthy, in one recent study, TMB was associated with ICB responsiveness in mouse MC38 colorectal carcinoma tumor models; the second most correlated gene signature was intratumoral DC-derived *Cxcl9* (encoding CXCL9) – a chemokine described in mice as being produced by intratumoral cDC1s that functions to attract CD8<sup>+</sup> T cells into tumors via its ligand, CXCR3 [66,67].

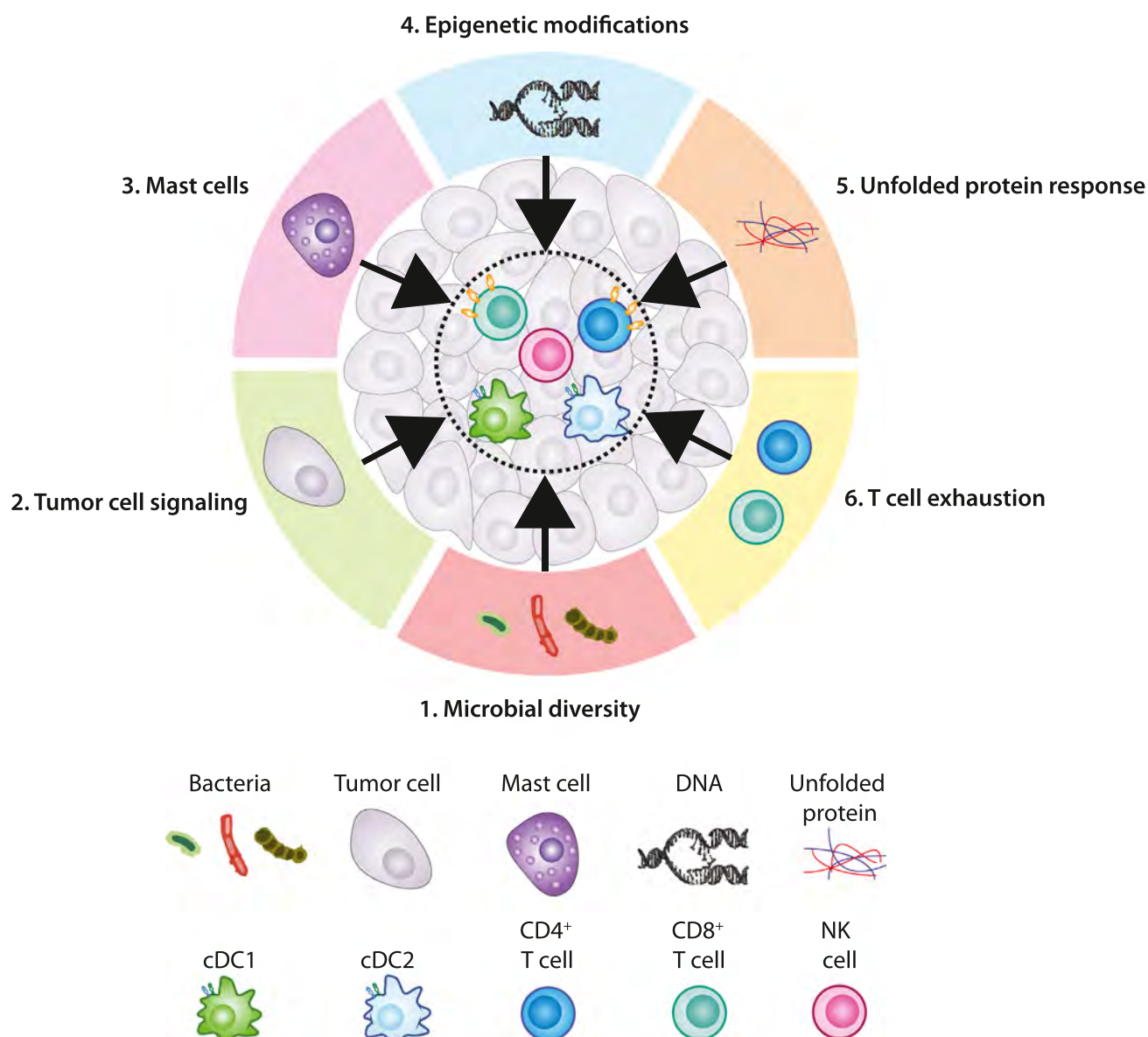
### What is the role of PD-L1 expression in predicting responsiveness?

A biomarker for potential ICB responsiveness in the past has been the intratumoral expression of costimulatory ligands, notably PD-L1 in response to anti-PD-1 ICB. However, although PD-L1 levels were used to stratify patients via immunohistochemistry in the pembrolizumab trials listed previously, such PD-L1 status alone was deemed to be a poor predictive marker for OS in melanoma patients receiving nivolumab and/or ipilimumab [68]. We posit that assessing PD-L1 expression in tumors likely neglects accounting for the availability of reactive immunity (the archetype alluded to here) and fails to enumerate all the cell type(s) that express PD-L1 ligands.

### Pathways towards upregulating reactive archetypes

What additional features of these archetypes remain to be revealed, and what are the components? We argue that various individual factors must clearly influence individual cell frequencies, such when assessing cDC1s. Likely factors may include Flt3L, prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) [42], and β-catenin

signaling [66,69]; indeed, these have modulated cDC1 frequencies in various tumor-bearing mouse models. We discuss in the following text additional intrinsic and extrinsic factors that likely facilitate class I and/or class II reactive archetypes (Figure 3).



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**Figure 3. Stressors modulating responsive immunity.** Some of the main aspects of determining immune checkpoint blockade (ICB) responsiveness include relying on which stressors (black arrows) impair which types of immune cells in the tumor microenvironment (TME) (hashed inner circle). Some key stressors of archetypes can include (1) microbial diversity affecting ICB responsiveness in mice and humans [82,83,93–95]. Another example is cotreatment with anti-PD-1 ICB and *Akkermansia muciniphila* in MCA-205 tumor-bearing mice which increased CD4<sup>+</sup>/Foxp3<sup>+</sup> regulatory T (T<sub>reg</sub>) cell ratios in tumor tissues and reduced tumor growth relative to controls [83]. (2) Tumor cells can also promote WNT/β-catenin signaling, as well as prostaglandins, to disrupt the recruitment of type 1 conventional dendritic cells (cDC1s) in a class I archetype setting [42,66,69]. (3) Mast cell TNF<sup>+</sup> gene signatures correlated with better overall survival in nasopharyngeal carcinoma patients and may lead to increasing cytolytic potential in CD8<sup>+</sup> T cells [79–81]. (4) Epigenetic modifications in chromatin accessibility affect CD8<sup>+</sup> T exhausted phenotypes and could be an attractive target for reinvigorating antitumor immunity [73,96–99]. (5) Unfolded protein responses can influence the regulation of dendritic cell (DC) development and survival in addition to CD4<sup>+</sup> and CD8<sup>+</sup> T cell activation [74,76,100]. (6) Finally, different states of exhausted T cell phenotypes in the TME or periphery can yield different outcomes in terms of potential anti-PD-1 and anti-PD-L1 ICB responsiveness [70,73,92]. Defining unknown stressors and providing further details of current stressor pathways might help to better inform ICB responsiveness. Abbreviation: NK, natural killer.



### Reversing moderate T cell exhaustion

As described previously, tumor-specific  $T_{RM}$  cells and putatively 'exhausted' T cells can overcome ICB resistance [25,30–32]. In one study of humanized mouse models of bladder cancer, CD40-expressing cDC1s induced antitumor immunity by reversing **CD8<sup>+</sup>PD-1<sup>+</sup>LAG3<sup>+</sup> phenotype**  $T_{EX}$  signatures and could reduce tumor growth in anti-CD40 Ab-treated mice compared to controls [70]. In another example, five distinct putative  $T_{EX}$  phenotypes were enriched in melanoma patient tumors and demonstrated antitumor activity against melanoma-associated antigens or neoantigens [32]. Regarding these subsets of apparent  $T_{EX}$  cells, we argue that it is likely that several previous studies aimed at reinvigorating  $T_{EX}$  cells [23,25,30,33,34] may have either (i) reactivated these subsets – that were not 'fully' exhausted but which retained cytolytic capabilities, (ii) activated a subset of sparse  $T_{eff}$  memory-related cells (*TCF7*- and *IL7R*-expressing) [26,30,31,71,72], or (iii) activated new T cells generated in LNs [73].

### Epigenetic modifications

Epigenetics may stabilize  $T_{EX}$  cells against reinvigoration, and may thus be a determining factor in ICB responsiveness (Figure 3). **Assay for transposase-accessible chromatin with deep sequencing (ATAC-seq)** analysis revealed that the epigenetic profiles of  $T_{EX}$  cells were distinct from those of  $CD8^{+} T_{eff}$  and  $CD8^{+}$  memory T cells in lung cancer patients [72]. Additional studies on human tumor biopsies and tumor-bearing mouse models have shown that gene regulatory elements in *cis* (e.g., enhancer/promoter) and *trans* (e.g., transcription factors) in the  $CD8^{+}$  T cell differentiation program can alter the ability of  $T_{EX}$  cells to be reprogrammed for ICB responsiveness [23,25,32,70,71]. For example, in basal cell carcinoma patients treated with anti-PD-1 Ab blockade, ICB responsiveness was correlated with the chromatin accessibility of *cis* elements at loci relevant for the regulation of terminal T cell differentiation and exhaustion [e.g., *TOX*, *PDCD1* (PD-1)], as well as with *trans* motifs downstream of TCR signaling and T cell exhaustion (e.g., *NFKB1*, *NFKB2*); this suggested that chromatin regulators might be used to identify ICB-responsive T cell subsets [32]. This idea is further supported by studies of *cis* elements in  $T_{EX}$  cells (e.g., *TCF7*) from melanoma and NSCLC patients [23], reporting that these key transcription factors, together with epigenetic programming at such loci, underlie mechanisms of T cell dysfunction and might be useful in predicting therapeutic reprogrammability. Future studies focusing on how these  $T_{EX}$  phenotypes shape the TME and interact with other immune cell types will be key to developing better strategies for achieving ICB responsiveness.

### Modulating endoplasmic reticulum (ER) stress

ER stress and activation of the **unfolded protein response (UPR)** contribute to the development and progression of many cancers. For example, XBP1 and C/EBP homologous protein (Chop) on DCs can dictate the development, survival, and activation of T cells in tumors, as depicted in Figure 3. For example, DC-specific *Xbp1* depletion reduced tumor growth and increased IFN- $\gamma^{+}$  expression on  $CD4^{+}$  and  $CD8^{+}$  TILs in a tumor-bearing mouse model of ovarian cancer [74]. In addition, the presence of intratumoral DCs expressing Chop negatively correlated with the number of  $CD45^{+}CD3^{+}$  T cells from ovarian cancer patients, suggesting that intrinsic ER stress mechanisms in DCs could modulate antitumor immunity [74]. However, it is unclear whether these or other ER-stress markers influence ICB responsiveness in mice or humans. Given the relationship between ER stress and UPR activation [75,76], blockade of UPR might be considered as a strategy to upregulate ICB reactive immune archetypes, presumably the archetypes that are relevant to essential DC subsets.

### Mast cells (MCs)

MCs are equipped with a broad range of receptors and costimulatory molecules to rapidly respond to incoming signals and secrete a variety of stored and newly synthesized mediators

[77]. For example, MCs can produce Flt3L in humans and mice [78] and may hypothetically substitute for NK cells in a class I reactive immune archetype. Indeed, microlocalized MCs seen in tumors from NSCLC patients were found to express a variety of cytokines under stress, such as TNF- $\alpha$ , IFN- $\gamma$ , IL-6, and chymase, that correlated with tumor growth inhibition and improved OS in these patients [79]. However, MC abundance was inversely correlated with OS in uterine corpus endometrial carcinoma patients, suggesting that MCs might exhibit diverse functions across different cancer types [80]. In humanized melanoma mouse model study, imatinib (a c-Kit receptor inhibitor that would be predicted to deplete MCs) together with anti-PD1 Ab therapy led to improved and complete tumor regression compared to either treatment alone [81]. Taken together, further studies aimed at revealing the phenotypic variation, architecture, and spatial distribution of MCs will be necessary to understand and validate how, and in which phenotypes, MCs might play reactive antitumorigenic or protumorigenic roles (Figure 3).

### The role of the microbiome in regulating reactive archetypes

Mouse studies have demonstrated associations between intestinal bacterial taxa and responses to ICB [82,83]. This appears to be related to class I archetype biology in at least one example in which healthy human fecal microbiota was transplanted (FMT) into germ-free mice, leading to expanded CD8<sup>+</sup>IFN- $\gamma$ <sup>+</sup> T cells and enhanced ICB-mediated antitumor immunity relative to controls [82]. By contrast, in cDC1-depleted mice (*Batf3*<sup>-/-</sup>), this FMT treatment failed to induce colonic expansion of CD8<sup>+</sup>IFN- $\gamma$ <sup>+</sup> T cells and did not inhibit tumor growth, suggesting that cDC1s are essential for CTL priming and the accumulation of intratumoral CD8<sup>+</sup>IFN- $\gamma$ <sup>+</sup> T cells in a context in which the presence of intestinal microbiota was relevant [82] (Figure 3).

Microbiome influences on class II reactive immune archetypes are less well studied. In one example, NSCLC patients demonstrated increased CD4<sup>+</sup> T<sub>H</sub>1 reactivity in the blood in the presence of *Akkermansia muciniphila* bacteria; this correlated with improved clinical outcome when comparing ICB-responsive versus nonresponsive patients [83]. This also suggested that CD4<sup>+</sup> T<sub>H</sub>1 reactivity may have been relevant in that setting. Moreover, CD4<sup>+</sup>/Foxp3<sup>+</sup> T<sub>reg</sub> ratios in tumor tissues and tumor clearance were increased when *Akkermansia muciniphila* was administered together with an anti-PD-1 Ab cotreatment in MCA-205 fibrosarcoma tumor-bearing mice; again, this suggested that a class II-dependent archetype may have been engaged. However, the exact cDC phenotypes causing T cell expansion and antitumor immunity in this study remain unknown, although IL-12 production was presumably implicated [83].

Taken together, we argue that the microbiota can influence key components of class I and class II reactive immune archetypes during ICB treatments. Nevertheless, it is unclear whether the microbiota changes observed in these studies involved interactions with all known (or presumed) cellular players, or simply represented a catalyst to steer key immune cells towards enabling antitumor immunity, or even ICB responsiveness.

### Spatial distribution of the TME: implications for reactive archetypes

Immune cells often form anatomical substructures – cellular neighborhoods (CNs) in which particular cell types are spatially colocalized and synergistically attuned to one another. Technologies are only beginning to home in on how the spatial organization of the CN can define reactive archetypes. Much of the current scientific focus in immuno-oncology is placed on identifying larger structures resembling LNs that comprise clearly defined B cell and T cell zones – namely **tertiary lymphoid structures (TLSs)**, which may be important for antitumor immunity. However, one caveat of the

### Clinician's corner

The success of ICB therapies in some human cancers depends greatly on whether such therapies target available and/or specific immune cell populations for antitumor immunity. These reactive cell populations could collectively be called 'reactive immune archetypes'.

Recent studies of the TME have identified a collection of these crucial immune cell types and conditions (beyond T cells) that might help to explain why some patients respond to ICB therapy that is ostensibly aimed at modulating T cell functions.

Healthcare providers might potentially treat cancer patients more optimally by measuring these key reactive archetypes. Prescreening for these reactive archetypes (e.g., tissue biopsies, blood, lymph nodes) might help to improve drug selection choices.

Ongoing studies of these cell archetypes and their origins might provide new strategies to elicit a positive ICB response.

TLS focus is that the presence of such large structures may obscure the identification of small cellular alliances that constitute the smaller archetypal collections defined previously. For example, in one study, 35 colorectal cancer patients were classified based on whether their tumors contained TLSs at the tumor-invasive front versus containing undefined and interspersed immune and tumor cells within the TME [84]. Those tumors with TLSs at the tumor-invasive front demonstrated higher OS following ICB compared to an interspersed TME spatial phenotype [84]. In a second study that focused only on OS without ICB in triple-negative breast cancer, these ordered TLS immune structures containing cDC2s, CD4<sup>+</sup> T cells, and T<sub>reg</sub> cells were also found near tumor-invasive fronts and correlated with OS [85].

Beyond the large TLSs, the spatial organization of small regions of tumors and the spatial cues that drive cells to one another are relatively poorly explored. Exceptions include studies on NK, cDC1, and CD8<sup>+</sup> T cells in tumors, which have shown that such cell populations associate together as result of specific chemokine networks [13,42,66] (Figure 4, left). As noted previously, PGE<sub>2</sub> [42] and  $\beta$ -catenin signaling [66,69] can modulate cDC1 frequency, respectively via chemokine production or directly. When considering various forms of a class II reactive archetype, we have little understanding of how T<sub>reg</sub> cells localize together with cDC2s, and indeed how this is coupled or uncoupled to the colocalization of cDC2s with CD4<sup>+</sup> T cells overall (Figure 4, right). Improved understanding of these variations and their potential influence on antitumor activities may better inform approaches to achieving improved ICB responsiveness [5,86,87]. Overall, multiple cell types and interactions are probably missing, each of which supports an aspect of the sequential biology which ultimately assembles component cell types and licenses T cell-mediated tumor cell killing. This certainly merits further attention.

Investigation of the spatial landscape of the tumor immune archetypes is much needed, and we anticipate that this could dramatically help to predict ICB responsiveness from bench to bedside. In the future, a combination of new and evolving technologies in multiplexed imaging [85,88] and single-cell spatial transcriptomics [89,90] could also help to propel these investigations.

## Concluding remarks

We argue here that ICB responsiveness comes in at least two 'flavors' – class I and class II reactive immunity archetypes – and numerous lines of evidence suggest that the component parts of these archetypes are already solidly prognostic. We posit that the combination of these features can help to better predict ICB responsiveness. A more comprehensive understanding of reactive archetypes also harbors the potential for adapting a preclinical screening platform to improve tumor cure rates, most likely coupled to additional approaches (see Outstanding questions). Some limitations need to be taken into consideration for modeling reactive immune archetypes. Although we have focused our attention on specific immune cell subsets and signaling pathways, it is undeniable that other cell types are likely involved and may comprise so far undiscovered dominant or reactive archetypes. For instance, MCs can produce Flt3L [78] as a substitute for NK cells, and therefore sustain cDC1 survival in particular tumors. We posit that the combined identification of cellular states and interactions within the spatial architecture of the TME is a translationally relevant endeavor and may constitute an important step towards advancing precision medicine. To that end, future studies characterizing the relationships between immune and non-immune cells that can influence these reactive archetypes, as well as the signaling pathways that stimulate and maintain such interactions and responses, might help the development of new therapeutic strategies to treat patients at all clinical stages of malignancy.

## Outstanding questions

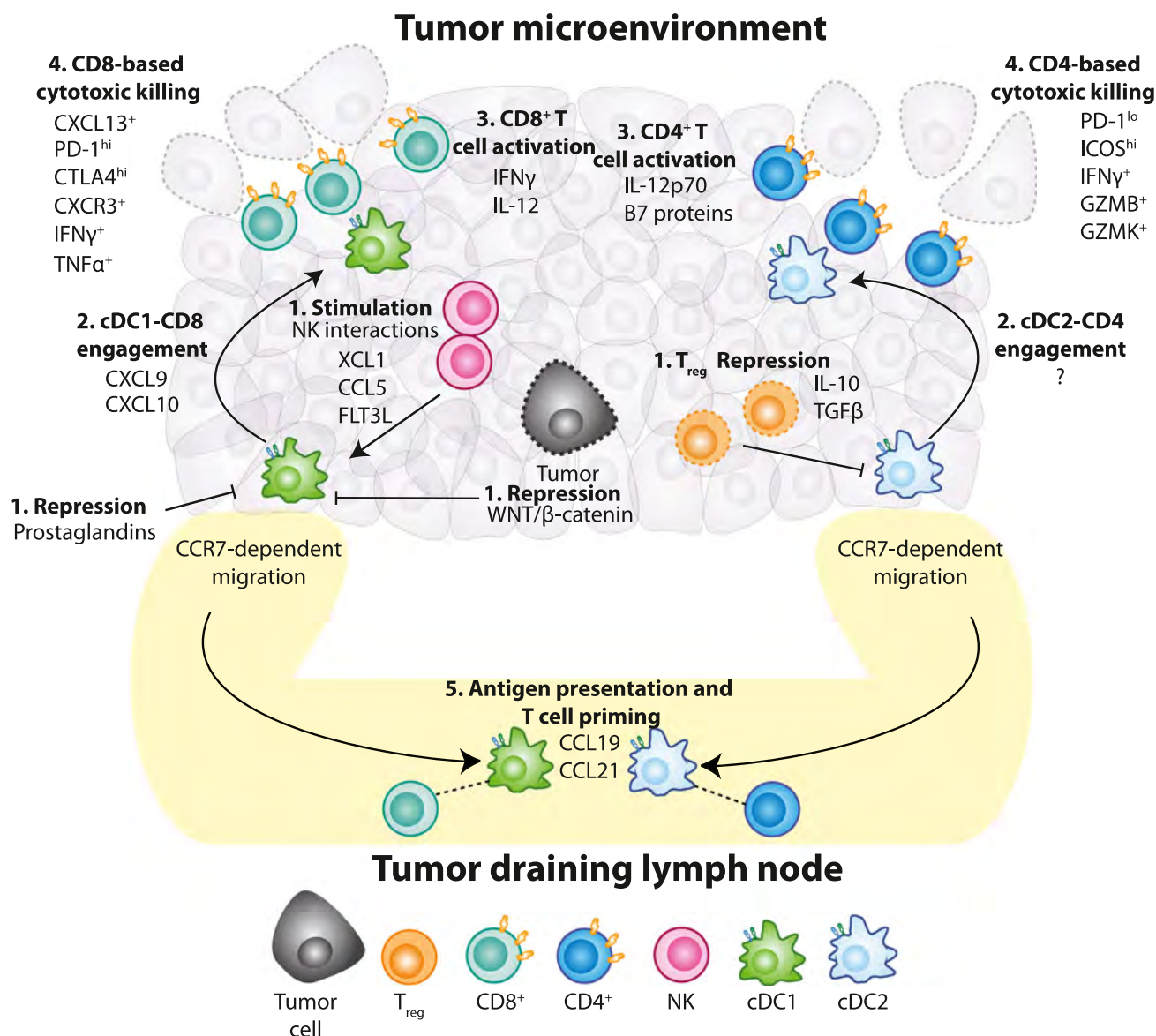
Can reactive archetypes be screened before ICB therapy to improve efficacy, possibly from blood?

What are the relationships between immune and non-immune cells within the TME, in terms of influencing ICB responsiveness and the abundance of reactive archetypes?

Are there additional reactive archetypes that can be characterized during ICB?

Can reactive archetypes that are nonexistent in the TME be constructed to elicit ICB responsiveness? If so, how?

What biochemical pathways and cellular interactions might be targeted to induce reactive archetypes within tumors?



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**Figure 4. Crucial spatial distribution of immune system components in the tumor microenvironment (TME).** The origin and spatial localization of immune cells comprising an archetype may be important in helping to predict ICB responsiveness and inform other aspects of target discovery. Under class I and class II archetype framing, previous studies have elucidated (1) conditions for type 1 conventional dendritic cells (cDC1s) to accumulate and function in the TME, which include the absence of WNT/ $\beta$ -catenin signaling or prostaglandins, the presence of natural killer (NK)-mediated functions (XCL1, CCL5, FLT3L), or the loss of regulatory T (T<sub>reg</sub>) cells for cDC2s; (2) cDC1 expression of CXCL9 mediates CD8<sup>+</sup> T cell recruitment or unknown cues that recruit CD4<sup>+</sup> T cells towards cDC2s; (3) cDC1s stimulate CD8<sup>+</sup> T cells, and cDC2s stimulate CD4<sup>+</sup> T cells, to support cytotoxicity [5,13,42,44]; and (4) T cell phenotypes (PD-1 and CTLA-4 high versus low expression, ICOS, granzymes, IFN- $\gamma$ , TNF- $\alpha$ , CXCL13, CXCR3) have been used to predict better overall survival and ICB responsiveness in some cancer patients [13,33,34,48,49,67,101]. Finally, (5) cDC1s and cDC2s can egress from the TME into the tumor-draining lymph nodes under the influence of CCR7 ligands which engage CD8<sup>+</sup> and CD4<sup>+</sup> T cells, respectively (dashed lines). The tumor-draining lymph node is further organized by chemoattractants such as CCL19/21 to facilitate antigen presentation and T cell priming.

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## Declaration of interests

M.F.K. is a founder and stockholder in Pionyr Immunotherapeutics, Foundry Innovations, and is a board member and stockholder in Deciduous Therapeutics.

## Resources

<sup>i</sup><https://www.clinicaltrials.gov/ct2/show/NCT00094653>

<sup>ii</sup><https://www.clinicaltrials.gov/ct2/show/NCT01844505>

<sup>iii</sup><https://www.clinicaltrials.gov/ct2/show/NCT01295827>

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