Evolution of Cancer, Adaptive Immunity, and Immunotherapy

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Abstract: The first clinical trials to investigate the efficacy of immunotherapy in cancer were problematic because of issues related to product availability, cost, and purity. Moreover, these factors could have contributed to the modest efficacy of these agents. The ability to clone specific genes coupled with the development of recombinant DNA technology removed some major barriers such that only 20 years later, approval of the first engineered monoclonal antibody (mAb) for clinical use occurred with practice-changing implications. Subsequent to rituximab, more than 30 additional mAbs have indications for a number of hematologic malignancies and solid tumors. Indeed, the application of adaptive immunity is now an integral component of therapy for many cancers. This paper delves into the complex science of immunology by investigating how the term evolution is applicable to tumorigenesis, the adaptive immune response, and cancer therapy.

Keywords: Adaptive immunity, $CD4^+$ T cells, $CD8^+$ T cells, CTLA-4, Darwinism, Evolution, Immune-related adverse events, Immunotherapy, Passive immunity, PD-1.

INTRODUCTION

Cancer is arguably the most feared of all health-related diagnoses, a reality darkened by numeric figures devoid of humanistic qualities. Indeed, cancer statistics contribute to this abject sense of misfortune. For instance, among the United States populace alone the incidence of new invasive cancers is projected to be 1.76 million* in 2019 [1]. The asterisk is noteworthy because this figure does not include non-melanoma skin cancers as well as *in-situ* melanoma and breast cancers, a total when combined exceeds 4 million additional cases. And though smaller in number, estimates of disease-related mortality adds another tangible layer to cancer's existential burden.

These doleful single-year projections can, nonetheless be counterbalanced with some optimism. Most notably, cancer-related deaths in women and men decreased by approximately 27% over a 25-year period beginning in 1991. Declines in type-specific cancer mortality can be attributed to multiple factors including changes in life-style (i.e., smoking cessation), early detection (i.e., breast, colorectal, and prostate cancer screenings), and improvements in treatment (i.e., novel targets identified and targeted agents developed). While material achievements appear modest, numerous aspects of oncology continue to evolve because of an urgency to bring further clarity to the malevolent tumorigenic process.

The impetus for undertaking this review is to demonstrate the applicability of the term evolution, even to a relatively short period of time during which substantial changes occurred in understanding tumor biology, treating cancers, and surviving the disease. Initially, several oncologic principles refresh and regard, in stepwise fashion, a historic, though contentious, evolutionary theory. The paper continues with a brief discussion of the clinical impact of immunotherapy followed by a critical analysis of the complex biology of immune-related adverse events (ir-AEs); the latter is the primary focus of this manuscript. Of note, while the review of published data provided scientific rigor, the review also exposed areas of uncertainty, some of which beckoned intellectual curiosity and consideration. One instance in particular, the authors provide proof-of-concept insight to bolster their opinion regarding the potential pathogenicity of passive immunity in the fetus.

PARADIGM

One of the major paradigm shifts in oncology relates to the approach taken towards cancer. Formerly, the pathway was characteristically more art than science. As such, the fundamental underpinnings involved detection, treatment, and outcome (cure if possible). Deft strokes have since altered the oncologic landscape. Now deemed more imperative, the current ideology is to achieve a better understanding of cancer (at the molecular level), target appropriate tumor drivers with the intent to achieve cure or chronic disease status; and lastly, perhaps uncover effective preventive strategies.

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PRINCIPLE

A paper published 20 years ago "simplified" cancer by advocating that six biological features were inherent in all malignant tumors [2]. Eleven years later four additional characteristics were recognized [3]. The significance associated with acquisition of these traits during a multi-step process belies several important putative principles and tumor tells. First, while the diagnosis of a pre-neoplastic lesion justifies closer monitoring the finding does not mean that the development of cancer is imminent. Second, the transition from normoplasia to neoplasia is associated with multiple genetic aberrations though not all of which have roles in the tumorigenic process. Furthermore, tumorigenesis is "driven" by *amplifying* gain of function oncogenes and/or *silencing* loss of function suppressor genes. Third, contrary to programmed death encoded into the genetic framework of fully differentiated normal cells, neoplastic cells in the oncogenic pathway not only evade apoptotic signals but also exhibit unlimited growth potential. And fourth, the "neo" in neoplasia presents a dialectic dilemma between self and non-self. That the 10 characteristics described by Hanahan and Weinberg may be unique to cancer indicate that changes, many of which may not visible to the naked eye, have occurred over time and yet, neoplastic cells have the ability to evade immune recognition and destruction.

PARADOX

Literally, transmutation from normality to abnormality may represent the quintessential biological paradox of chaos and conformity whereby *derangements* in the genome bring a sense of *order* to the neoplastic process; and wherefore vulnerability devolves to invincibility. Figuratively, the evolution of cancer appears to have Darwinian vibes, wherein genomic instability engenders *natural selection* and *formative retention* of traits that enhance the likelihood of tumor cell survival and growth. That some other traits are manifest external to the tumor cell strongly suggests "survival of the fittest" is also dependent on the tumor micro-*environment* [4].

Another paradox involves genes and their translated proteins. While essential for regulating cell growth and death, they may also be a liability to the cell if mutations or other deleterious alterations occur. For example, expression of the human epidermal growth factor receptor-related 2 (HER2) protein in mammary epithelial cells suggests a role in the normal development of breast tissue. However, amplification of the gene and/or overexpression of the protein is widely accepted to be a major driver of one, in particular, breast cancer subtype. Demonstrable support for this assertion is the efficacy of a number of pharmacologic products that target the receptor.

CONSTRUCTS

The tumorigenic pathway from initiation to detection can be time-intensive due to variations in tumor doubling time. However, the use of kinetic principles is an effective way to characterize and construct cogent models of tumor growth.

Biological

The evolution of cancer involves at least five phases, changes that are histologically distinct but infrequently observed prior to a pathologic diagnosis. It is now widely accepted that progression to the malignant state results from interactions of a multitude of factors both intrinsic and extrinsic to the nascent cell. A surreal construct of a human genomic kaleidoscope will one day enable scientists to preview distinctive changes in genetic patterns of the impending development of cancer.

Kinetic

Mathematically simplistic, realistically deceptive. To whit, 30 doublings of a single cancer cell result in over

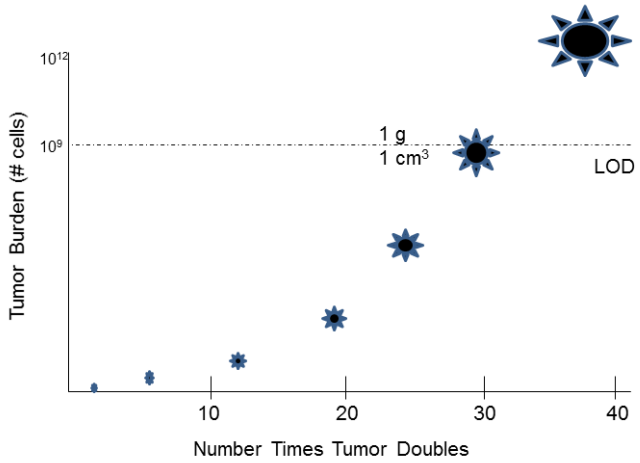

Figure 1: Tumor kinetics. Tumor growth is not linear. Slow early on because of the tumor's dependency on established vasculature as a source of oxygen, nutrients, and by-product removal. This is followed by a period of rapid growth as the initial necessity to co-op local resources diminishes, in part, because of angiogenesis and space. Depending on tumor doubling time, 30 doublings result in a clinically detectable mass, though not all tumors are detected at an early stage.

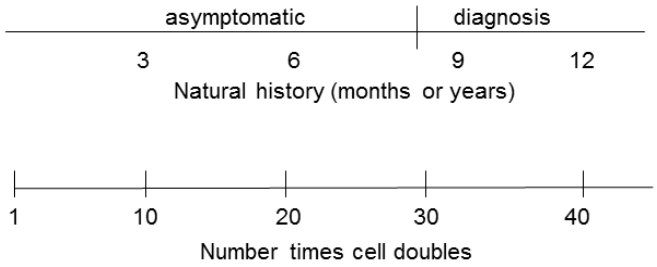

Figure 2: Clinical implications of tumor kinetics. Tumors exhibit variable doubling times. For example, a "slow" growing tumor with a doubling time of 100 days may be detectable approximately 8 years after initiation (i.e., 100 days x 30 doublings = 3,000 days or ~8 years). In contrast, an "aggressive" tumor with a doubling time of 8 days would be clinically detectable less than 8 months from initiation (i.e., 8 days x 30 doublings = 240 days or ~8 months).

a billion tumor cells, coalescence of which is a mass, 1 cm^3 in volume and 1 gram in weight. Unimpeded, 10 further doublings terminate in a mass weighing over 1000 grams (Figure **1**), a tumor burden believed to be incompatible with life. The time-relevance of this model relates to tumor detectability and cancer-related mortality. Clinically, the ramifications of this numeric construct are a bit more sobering. Because tumors do not have identical doubling times, cancers may be present but undetectable months to years after tumor initiation (Figure **2**).

CANCER TREATMENT

Cancer therapy, too, has evolved. Where once the toxicities associated with chemotherapy were so noxious that if not for the life-threatening implication of cancer in humans many agents would not be approved for clinical use. However, substantial advances in supportive care have improved treatment tolerability, patient quality of life, and even cancer survival [5,6].

Notwithstanding the prior, and current, importance of surgery, radiation, chemotherapy, endocrine therapy, and supportive care, the introduction of targeted and adaptive immune therapies represents a major inflection point in the overall management of patients with cancer. Indeed, the development and clinical application of novel agents is an offshoot of a growing understanding of the molecular basis by which cancer cells adapt, survive, and proliferate. Biologically, symbiosis is an integral part of evolution, even one as fractured as the oncogenic process. Symbiotic nonetheless, a close and usually prolonged association exists early on between cells destined to evolve via divergent pathways.

The sections that follow focus only on adaptive immunotherapy and its impact on clinical outcomes. And though maintenance of immune surveillance and response requires the involvement of multiple innate components, the primary effectors of adaptive immunity are the T-cells and antibodies.

T Cell Repertoire

Several biological myths perverted the desire to harness and engage the immune system as cancer therapy. First, the immune system does not recognize most tumor cells. Initial debunking of this belief was the finding of tumor-infiltrating lymphocytes in a number of solid tumors [7-10]. Despite the presence of immune components, this misconception endured because of the demonstration that only two solid tumors achieved modest benefit from early immunotherapy prototypes. And interestingly, rather than a direct effect on tumor cells the most important antitumor mechanism of both interleukin-2 and interferon-α appears to be dependent on recruitment of cytotoxic $CD8^+$ T cells [11-13].

Second, immune responses are inducible only by unique tumor antigens. The limited activity of non-specific stimuli such as bacillus Calmette Guerin (BCG), *Corynebacterium parvum*, and muraramyl dipeptide appeared to strengthen this myth. However, confounding this notion were the disappointing results of studies involving inoculation of intact, or fragments of, viable or killed cancer cells into animal models. These findings suggested that tumor-specific antigens were not universally expressed and therefore, not all tumors (or tumor cells) were immunogenic. The premise of tumor heterogeneity not only provides ample support for this belief but also conjures preferential immune responses. That the basis for selective tumor destruction relates to grade or degree of immunogenicity has two consequences. The first is obvious; selection of highly immunogenic tumor cells for destruction leaves, unimpaired, a population enriched with less immunogenic variants. Related to the latter, the second consequence is a biological

paradox; lower immunogenicity portends greater lethality.

Third, "self" origin is the major mechanism of immune protection. Whereas the concept of self is one of the most discriminating tenets in immunology, cancer poses a discerning challenge to this biological dogma. At the crux of this matter is how can the dual maxims of tumor antigens (especially if they are not unique) and effector specificity be reconciled. Even though the answer is unclear, one postulate asserts that *neo*-antigens are products of genes, altered or amplified by the neoplastic process [14]. However, expression of newly translated "non-self" proteins, in and of itself, may be insufficient to induce clonal T cell responses. Instead (and by way of an overly simplified explanation), these novel proteins are surreptitiously "processed" by major histocompatibility complex (MHC) class 1 molecules and then "presented" to $CD8^+$ T cells. A second (co-stimulatory) signal and cytokines such as IL-12 are required to fully activate effector cytotoxic T cells [15]. On the other hand, proteins in untransformed cells may not evade immune recognition simply because of "selfness" but rather expression at levels too low to be immunogenic. Accordingly, the latter suggests that even "normal" proteins could become immunogens when amplified by malignant cells [14].

Fourth, the phenomenon of T cell exhaustion induces expression of inhibitory receptors, a protective mechanism against immune encroachment [16]. Despite this finding, the concept that self, alone, provides an intangible safeguard against immune retort is no longer an impervious barrier to challenge. Instead of self as a defense, flashes of self-determination promote offensive posturing at the activation and effector stages of the immune response as primed T cells also express these same receptors [17]. Hence, T cells can be deactivated via high-affinity binding of cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) to the co-stimulatory ligand on the same antigen-presenting cell. In addition, tumor cell expression of the ligand for programmed cell death receptor (PD-1) expressed on T cells blocks activity at the effector stage (Figure **3**) [18,19].

Whether re-invigorating "exhausted" T cells or re-establishing cell-mediated effector function, targeted inhibition of inhibitory CTLA-4 and PD-1 displays a spectrum of antitumor effects broader than the confines of the "immunogenic" tumors. Nonetheless, the impact of these engineered inhibitory antibodies has been particularly successful in melanoma and kidney cancer, two tumors ostensibly resistant to traditional chemotherapy.

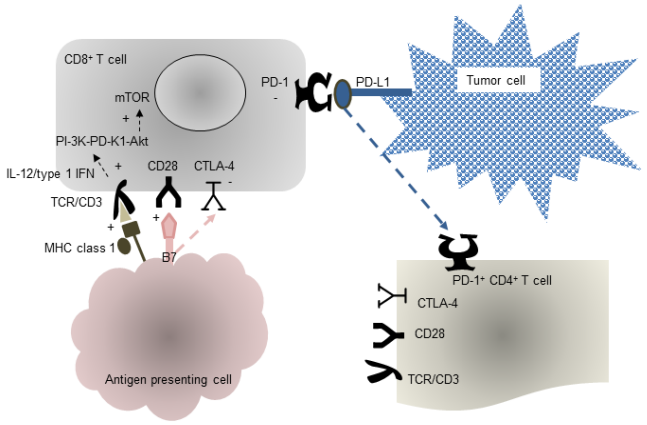

Figure 3: Stimulatory and inhibitory signaling pathways in T cells. (+) Stimulation: Antigen-primed T cells require additional costimulatory signals including the CD28/B7 interaction and inflammatory cytokines IL-12 and type 1 interferon (IFN). Stimulation of the T cell receptor (TCR), CD28, and cytokine receptors activates the phosphatidylinositol3 kinase (PI-3K)- phosphoinositide-dependent kinase 1 (PIDK1)-Akt pathway which stimulate downstream signals required for glucose metabolism. mTOR is a conserved sensor that regulates cell growth, proliferation, and metabolism. (-) Inhibition: PD-1 and CTLA-4 represent "immune checkpoints" as they counteract activating signals. These inhibitory receptors are upregulated during immune activation and response resulting in decreased (or loss of) T cell proliferation and function. Exhaustion of T cells can be both beneficial (i.e., limits autoimmune pathology) and potentially detrimental (diminishes immune-mediated antitumor response). However, the ultimate effect differs among T cell subsets (see text).

Efficacy and Toxicity-Checkpoint Inhibitors

On-Target Effects

Statistics do not always reveal the whole story. For example, one publication estimates that melanoma will account for approximately 5.5% of all new cancer diagnoses [1]. However, if basal and squamous cell carcinomas were included in the overall total, melanoma would comprise only 2% of new case diagnoses. In addition, melanoma is, by far, the most lethal of the skin cancers, which belies the 5- and 10-year survival rates of 91% and 89%, respectively. And even though the median overall survival (prior to 2010) was only 17 months for those with metastatic disease, earlier forms of immunotherapy could produce durable long-term responses (with a high toxicity profile) in a small number of patients [20].

The advent of the checkpoint inhibitors, ipilimumab and nivolumab, improved the duration of overall

survival (with better patient tolerance) in those with advanced melanoma to 20 months and 37 months, respectively [21]. And because of their "on-target" anticancer effects the applicability of these agents has now been extended to include several other solid tumors including lung, head and neck, colon and rectum, liver, and breast as well as one hematologic malignancy; and the list is likely to grow. Nonetheless, it important to emphasize that not all patients achieve responses with these agents; and even among responders, relapses and tumor progression are frequent. In its simplest form, this particular observation infers that the CTLA-4 and PD-1 signaling pathways are only two means by which tumor cells evade immune destruction. Indeed, additional, non-redundant physiologic mechanisms including inhibitory cytokines (e.g., interleukin-10, transforming growth factor-β) and cells of the adaptive (e.g., T regulatory and B regulatory) and innate (e.g., myeloid-derived suppressor) immune system are just a few that have been identified [22-25].

Off-Target Effects

Cast in a disparaging light because of their repressive effects on cell-mediated tumor destruction, CTLA-4 and PD-1 are also physiologically protective against autoimmune pathology. Because of the latter function, inhibition of these inhibitory receptors may induce numerous "off-target" ir-AEs. Although not included in this paper, an excellent overview of ir-AEs including systems involved, incidence, manifestations, and management is easily accessible [26]. Most adverse events are mild and reversible; some, however, are severe and irreversible, which may preclude further use of these agents. Moreover, inherent in all of these adverse events is the implication that cytotoxic $CD8^+$ T cells mediate these autoimmune reactions. This belief, however rational, may be a tacit misconception. Because of this intriguing probability, the authors opted to take a different approach by exploring the complex biological mechanisms underlying these events rather than elaborating on the clinical features of the ir-AEs which have already been reviewed numerous times.

While the list of toxicities is extensive, a number of peculiarities are still apparent. First, the relative incidence of ir-AEs is higher with CTLA-4 blockade. Results of clinical trials indicated that the overall frequency (i.e., 1% - 5%) of toxic effects involving the pituitary [27,28] and liver were higher with ipilimumab therapy compared to inhibitors of the PD-1 pathway [29]. Even more notable was the higher incidence and relative risk of dermatologic [30,31] and gastrointestinal systems toxicities [32,33]. An understanding of CTLA-4 provides a framework to reconstruct the biological mechanisms of the latter two system-specific adverse events.

T cells express several co-receptors of which the most frequently mentioned are CD28 and CD152 (CTLA-4). While both naïve and activated $CD8^+$ T cells express CD28, expression of CTLA-4 is restricted primarily to activated T cells. Although both share the same ligands, signaling through their respective complexes results in antagonistic effects on $CD8^+$ T cell activity; CD28 activates, CTLA-4 dampens. Taken together, the reason for their expression patterns and signaling effects may be teleological since the primary function of adaptive immune cells is protection, initially against non-self (i.e., foreign antigens), then subsequently, self (autoimmunity).

Of note, CTLA-4 expression is not restricted to cytotoxic $CD8^+$ T cells as the receptor is also present on all subsets of helper $CD4^+$ T cells. One subset of particular interest, regulatory T (T_{reg}) cells, may play a key role in the adverse reactions in skin and intestine. Because of the bacterial load found in the colon and skin (highest among any microbial habitat) [34,35], the presence of helper T cells, enriched with a population of T_{reg} cells, is not only a biological phenomenon but may also represent an evolutionary process whereby highly specialized cells are embedded to maintain immune vigilance and tolerance.

Mentioned previously was the tempering effect of CTLA-4 on T cell responses [36]. However, and perhaps not obvious initially, signals mediated through this receptor have opposing effects depending on T cell subset. Whereas CTLA-4 *represses* $CD8^+$ T cell activity, receptor-mediated signaling *enhances* $CD4^+$ T cell regulatory or suppressor function [37]. The suppressive effect of the latter is a biological version of yin and yang whereby protection is binary; one against autoantigen [38], the other against the antitumor effect. Although conflicting data have been published, the relevance and role of CTLA-4 and T_{reg} cells in these two "protective" outcomes may be inherent in the correlation some investigators have found between incident ir-AEs and improved tumor outcomes [39-42]. Based on the functional role of CTLA-4 on $CD8^+$ and $CD4^+$ T cells, a direct correlation is quite apparent. On the other hand, an explanation for the discordance between occurrence of adverse events and anti-tumor efficacy may relate to blockade of the receptor on

effector Th1 and Th17 subsets which effectively suppresses secretion of multiple pro-inflammatory cytokines [43,44]. In addition, and most interestingly, the relatively higher incidence of ir-AEs observed with CTLA-4 inhibition may be related, indirectly, to PD-1. Recent findings by Goods and colleagues found two unique subsets of tumor-derived effector $CD4^+$ T cells. Whereas the $PD\text{-}1^+$ cells exhibited diminished proliferative capacity, the $PD\text{-}1^-$ cells demonstrated greater metabolic and immune reactivity [45]. Notably, restoration of proliferation could not be achieved even in the presence of anti-PD-1 antibody suggesting that $PD\text{-}1^+$ helper T cells were irreversibly dysfunctional.

Second, activated cytotoxic $CD8^+$ T cells are the primary mediators of the antitumor effect but not the ir-AEs. Although the outcomes appear to be discordant, target-specificities are biologically (and immunologically) reasonably accurate. Recall that during T cell maturation, immature thymocytes undergo an exquisite de-selection process, whereby, approximately 98% of T cells that react to self-MHC molecules die. This biological revelation, however, gives some credibility to the apparent discrepancy regarding the antitumor and adverse effects. For example, while tumor cells are of "self" origin, generation of neo-antigens during tumorigenesis stimulates binding of MHC class I molecules to "altered self" antigens [46]. These MHC:peptide complexes serve as the initial signal for T cell (from the pool that had survived the selection process) activation. After integrating two additional signals, the "altered antigen"-specific $CD8^+$ T cells undergo clonal expansion, which ultimately leads to tumor cell kill.

Conversely, positive selection, inherently, *should* promote T cell tolerance and hence, avoidance of autoimmune pathology. However, evidence for the less than complete absolution of the activated cytotoxic effector cells in autoimmunity derives from the alleged role of $CD8^+$ T cells in rheumatoid arthritis, Type 1 diabetes, and multiple sclerosis [47-49]. Moreover, the concern for cells that express MHC class I molecules as potential targets for cytotoxic damage comes with two important caveats. One, the presence of MHC class I, in the absence of co-stimulatory, molecules in the target cells would not generate a cytotoxic T cell response. And two, discerning the relevant self-antigen; here, autoimmune diseases have one thing in common, the native immunogen, in nearly all cases, is unknown.

Although the intent was not to minimize the role of $CD8^+$T cells, a stronger case implicating helper $CD4^+$ T cells as the primary mediators of the ir-AEs is proffered. Helper T (Th) cells consist of at least nine distinct subsets, all progenies of one common progenitor [50,51]. Although subset destiny depends on a number of factors including type of antigen, cytokine milieu, and genetics [52], evidence suggests the involvement of more than one subset in autoimmunity. Further characterization of the subsets strengthens this assertion. Th1 cells not only secrete pro-inflammatory cytokines such as IL-2, IFN-γ, and lymphotoxin-α but also are potent macrophage activators. Indeed, the Th1 subset appears to be the primary "effector" of autoimmune diseases and tissue inflammation [53,54]. Th1 cells are also involved in cell-mediated immune reactions typified as delayed-type hypersensitivity; [55] and interestingly, all of the ir-AEs are delayed phenomena, some occurring months after discontinuing drug therapy [56]. In contrast, Th2 and Th3 are helper "suppressor" subsets, in part, because they secrete anti-inflammatory cytokines (i.e., IL-4, IL-5, and TGFβ). Furthermore, both Th2 and Th3 subsets have the ability to counter-modulate Th1-mediated inflammation [57,58]. Two additional important subsets with immune regulatory capabilities are also part of the helper $CD4^+$ immune repertoire. One, labeled Type 1 regulatory (Tr1) cell releases IL-10, a potent anti-inflammatory cytokine [59]. What is most impressive about Tr1 cells is their ability to dampen established immune reactions mediated by Th1. And mentioned previously, T_{reg} cells, a uniquely different subset, appear to be extremely important. Regarding their unique quality, these cells do not undergo clonal expansion in response to mitogenic stimulation, a feature characterized as anergy [60]. This finding, however, should not be construed that the cells are functionally inert. Phenotypically, T_{reg} cells are identified by surface expression of CD25 (α-chain of the IL-2 receptor) and Foxp3 (forkhead transcription factor). T_{reg} cells also comprise a relatively small fraction of the pool of circulating $CD4^+$ T cells. Nevertheless, animal models have demonstrated the importance of this subset, particularly when expression of the two surface markers is intact. Sakaguchi and colleagues reported that IL-2Rα gene knockout led to the development of several autoimmune diseases involving the thyroid, colon and pancreas [61]. Despite these findings, the expression of lineage-defining Foxp3 is functionally more important. For example, studies in mice and humans have shown that loss or mutation of the *Foxp3* gene not only disrupts development of T_{reg} cells but also results in lethal autoimmune syndromes [62,63]. In addition, aberrant expression of this transcription factor

enabled de-selection of T_{reg} cells to other T cell lineages capable of causing autoimmune pathology [64]. Still another study indicated that expression of Foxp3 results in restoration of the regulatory role of $CD25^-$ T_{reg} cells [65]. T_{reg} cells are unusual in one other aspect, constitutive expression of CTLA-4 regardless of activation status [66]. This trait may have evolved as part of the critical role this T helper subset has in order to enhance its protective effect. Indeed, an elegant study demonstrated that CTLA-4 in T_{reg} cells is required for stringent immune vigilance [38].

The data regarding T cell immunology provide compelling evidence to support the assertion that $CD4^+$ T cells are the principal instigators of ir-AEs. Some of the most provocative evidence comes from studies in mice and humans. For example, the *CTLA-4* gene was cloned initially from $CD8^+$ T cells in mice [67]. However, contrary to an earlier report [42] studies in humans revealed significantly higher expression of the inhibitory receptor in $CD4^+$ T cells compared to the cytotoxic effector T cells [68]. The significance of this finding may relate to the receptor's repressive effect on T cells in general, and $CD4^+$ T cells specifically. "Bio-Logically", deletion of *CTLA-4* on $CD8^+$ T cells should, but unexpectedly does not, promote autoimmune-mediated cytotoxic effector T cell activity [69]. Instead, Gattinoni and colleagues demonstrated that autoimmune manifestations became apparent only by knocking down *CTLA-4* on *both* cytotoxic and helper T cells subsets. The major inference is that $CD4^+$ T cells are critical for the induction of autoimmune pathology though this notion does not completely exclude a role for $CD8^+$ T cells. Besides autoimmunity, the presence of helper T cells influences $CD8^+$ T cell function in one other important way. In addition to pro-inflammatory interleukin-12 (IL-12), the importance of T cell growth factor (IL-2) in $CD8^+$ T cell terminal differentiation is now well established [70] However, well-defined *in-vitro* experiments with activated $CD8^+$ T cells demonstrated that cells cultured in medium containing high concentrations of IL-2 exhibited greater functionality compared to cells grown in medium with low IL-2 concentrations. The superior killing effect observed correlated with higher levels of perforin and granzymeB [71]. Notably, the principal source of IL-2 is activated $CD4^+$ T cells; lower amounts of the cytokine derive from activated $CD8^+$ T cells [72]. These findings are particularly relevant because CTLA-4 exerts a restrictive effect on $CD4^+$ T cell production of IL-2. Hence, receptor blockade is likely to have a significant influence on cytotoxic T cell-mediated anti-tumor effect [73,74]. As it relates to CTLA-4, these studies strongly suggest that receptor expression on $CD4^+$ T cells is functionally more important than its presence on $CD8^+$ T cells. As such, the allusion proscribed by others that ir-AEs result from "global activation of T cells or immunity enhancement" appears to be incorrect. Moreover, anti-CTLA-4 antibody may not globally activate all helper T cell subsets. Indeed, if blockade of CTLA-4 resulted in activation of T_{reg} cells, then a more potent anti-inflammatory, protective response would be expected. However, de-activation of constitutively expressed CTLA-4 on T_{reg} cells actually promoted the development of experimentally-induced Type 1 diabetes [38].

Third, most of the ir-AEs are generally mild inflammatory reactions. The explanation for this particular aspect is a paradox of not being overly complex yet at the same time being deceptively complicated. Simple, because inflammatory cytokines mediate most, if not all, ir-AEs. Moreover, several redundancies characterize these mediators. First, several different types of cells may be the source of the same cytokine. For example, TNF-α is released by immune (i.e., monocytes, macrophages, neutrophils, and lymphocytes) and non-immune (i.e., endothelial cells, smooth muscle and adipocytes) cells. Second, a single cytokine may target several different cell types. IL-6, for instance, generates three different helper subsets, Th17, Th22, and Tfh (T follicular helper), from naïve $CD4^+$ T cell. Third, redundancy is also reflected in their functional activities. Animal models indicate marked synergism between IL-1β, TNFα, and IL-6 in the pathogenesis of inflammatory disorders such as osteoarthritis [75]. Fourth, some of the cytokines can induce its own production and secretion; autocrine signaling reportedly occurs with IL-1β in joint cells [76]. Complicated, because some of the underlying risk factors (i.e., age, gender, genomic) that may contribute to the pathogenesis of ir-AEs are not alterable. Confusing also, because the autoantigens recognized by reactive T cells are either speculative or unknown. Furthermore, the primary T cell class involved in the adverse events remains immunologically challenging because of the duality of evidence. On one hand, expression of MHC class 1 molecules in nearly all cells supports the role of $CD8^+$ T cells in tissue damage; on the other hand, damaged self-tissue can be a source of peptides for presentation with class II molecules to $CD4^+$ T cells. As previously discussed, data support the latter.

Regardless of the breadth of potential tissues targeted, one striking characteristic is the relatively low-

grade toxicities observed, which adds yet another layer of complexity to these events. Muted and often self-limiting, the reason may relate to the dynamic interactions and *balance* of cells present and the role of pro- and anti-inflammatory cytokines as well as their downstream effects. Indeed, differentiation of an uncommitted helper T cell into subsets is conceptually evolutionary for host survival. Helper Th1 and Th2 cells illustrate the significance of this notion. Activation of the former leads to cell-mediated immunity against pathogens and autoimmunity. Hence, the dual effects of protection and destruction are downstream of a cascade promoted by the release of pro-inflammatory cytokines such as IL-2, IFN-γ, and TNF-β, with subsequent release of reactive oxygen species and nitric oxide by activated macrophages. However, the production of anti-inflammatory IL-4 and IL-5, downregulation of macrophages by Th2 cells and the presence of T_{reg} cells functionally mitigate tissue damage [54]. This over-simplified explanation is, nonetheless, plausible in two respects. First, autoimmune disorders *initially* driven by Th1 cells; and second, attenuation of tissue damage by Th2 cells, which predominate *after* initial reactivity. Inherent also in this dichotomized model is the belief that inappropriate differentiation or insufficient development of suppressor subsets to downregulate the inflammatory reactions could account for higher-grade toxic reactions.

Even more intriguing is the role of $CD8^+$ T cells may have in both inducing *and* minimizing the severity of immune reactions. Initiation; even though Th1 cells, NK cells, and macrophages are the primary sources of INF-γ and TNF-α, activated $CD8^+$ T cells also release these pro-inflammatory cytokines. Mitigation; less appreciated is the regulatory role effector $CD8^+$ T cells have in preventing excessive tissue damage. Results from three different groups of investigators demonstrated that activated cytotoxic T cells also release IL-10, one of the most potent inhibitors of cytokine production [77-79]. Of more interest were findings from a simulated model of virus-induced infection in the lung. At the height of the immune response, the primary source of IL-10 was $CD8^+$ T cells; once the infection was under control, $IL10^+$ $CD4^+$ T cells replaced the $IL\text{-}10^+$ $CD8^+$ T cells. Even more astonishing was the finding that the IL-10-producing $CD8^+$ T cells did not represent a distinct lineage but rather a transitive state of effector T cell differentiation to accomplish *both* protection against infection and excessive tissue injury.

Engineered Immunoglobulins (Igs)

Some of the most meaningful improvements in cancer survival outcomes are due to the clinical application of therapeutic antibodies. For example, the discovery of the protein product of the *neu* oncogene (erbB2/HER2) led to the development of trastuzumab which significantly altered the poor prognosis associated with HER2 amplified or overexpressed breast cancer. In addition, successful targeting of CD20 with rituximab has had a major impact on the treatment of several B cell malignancies as well as some autoimmune antibody-mediated disorders. And as mentioned earlier, the checkpoint inhibitors have already been given breakthrough status.

By definition, acquisition of short-term immunity by transfer of antibodies sourced from human, animal or recombinant DNA technology is passive immunity. One of many anomalies in biology, this type of immunity is, however, hardly “passive”. Mechanistically, engineered antibodies do more than merely bind to and neutralize their specific targets. In actuality, consummate lethality of the antigen-antibody complex derives from other effectors such as complement, macrophages, NK cells, and even effector $CD8^+$ T cells. In simple fashion, recruitment occurs via binding of Ig Fc fragment to effector-specific cell surface Fc receptors. For example, engagement of the complement system is as complex as it is unique. In the classical pathway, activation requires an “association” reaction between the C_H2 domain of the Ig and C1q, the first component of the complement cascade. This initial interaction is, however, much more involved. Three-dimensional images indicate that C1q contains six pseudopod-like structures, each having A, B, and C peptide chains as a triple helix [80]. This multivalent configuration facilitates high-affinity binding of Fc to only Ig:peptide complexes, subsequently inducing the cascading enzymatic reactions. Not discussed here is an alternative pathway for complement activation, one that does not require the presence of antibody.

Efficacy and Toxicity-mAbs

Despite their clinical effectiveness, the monoclonal antibody (mAb) represents a therapeutic paradox. Enabled with a highly sensitive ability to discriminate, these manufactured agents are also promiscuous in effect. In essence, uncoupling target specificity and tissue selectivity results in a diminution of the therapeutic index.

On-Target Effects

Currently, there are more than 30 approved mAbs on the market for clinical use as cancer therapies with varying degrees of applicability. One, trastuzumab, is arguably the most notable advancement in the treatment of HER2-positive breast cancer. Briefly, separate clinical trials of trastuzumab in patients with advanced, locally-advanced, and early disease demonstrated statistically, and clinically, significant improvement in tumor outcomes [81-84]. Substantial advances in the management of both indolent and aggressive subtypes of non-Hodgkins lymphoma have been reported with the addition of rituximab to traditional chemotherapy [85-87]. Smaller numbers of reports indicated therapeutic benefits in non-cancer disorders also [88,89].

Off-Target Effects

Although benefits outweigh risks, the mAbs are associated with potentially severe toxic reactions. For example, the absolute risk of heart failure with trastuzumab therapy is approximately 2.5% (range, 0-4%); [90] when used in conjunction with anthracyclines, the risk is nearly 30% [91]. Furthermore, this does not appear to be a wayward adverse effect because HER2 expression is detectable in a number of organs, including the myocardium [92]. A similar “on target” yet “off-target” adverse effect occurs with rituximab. Since all B cells express CD20, infectious risk increases as the duration of B-cell depletion is frequently longer than six months; while reductions of memory B cells may last up to two years [93,94]. Fortunately, B cell ablation does not occur because the CD20 molecule is absent on early B cell progenitors [95].

Passive Immunity Risk

Despite the severity of some adverse effects, results from numerous clinical trials demonstrate the relative tolerability of the mAbs. In contrast, there are only few indicators of toxic reactions occurring during fetal exposure. The latter finding is not surprising, in large part because of their specific targets and biological effects. For example, the antiangiogenic properties of bevacizumab and rituxumab’s reductive effect on B cell ontogeny suggest the possibility of adverse effects on the developing fetus. In addition, the use of cetuximab should be contraindicated in pregnant women because blockade of HER1 (EGFR, epidermal growth factor receptor) signaling has been shown to be lethal in neonatal mice [96]. While clinical application *during* pregnancy is discouraged, a correlative issue is whether fetal toxicity can manifest *after* mAb treatment is completed. Here, the authors make a learned effort to resolve this legitimate question by focusing on the “black box” warning of potential cardiac toxicity with trastuzumab.

The likelihood of detriment to the fetus is dependent on a number of critical factors including: 1) age-related HER2-positive breast cancer, 2) patient survivorship, 3) fertility after systemic therapy, 4) acquisition of passive immunity, and 5) the role of HER2 in cardiac morphogenesis. First, nearly one-fifth of the estimated 270,000 new cases of breast cancer diagnosed in 2019 will happen in premenopausal women [97]. In fact, breast cancer is the most common cancer diagnosed in women under 40 years old. Of major concern, however, is the finding that compared to postmenopausal women, younger patients have significantly worse breast cancer outcomes, including mortality, even among those with early-stage disease [98].

While there are many factors, genomic profiling revealed higher rates of HER2 overexpression among women <45 years of age suggesting tumor biology contributes to the poorer prognosis [99,100]. Second, survivorship has, nevertheless, improved significantly for patients with HER2-positive breast cancer, in large part, because of a better understanding of the biology of the disease [101]. One of the most important findings during the past three decades was the discovery of the proto-oncogene that encodes the HER2 receptor tyrosine kinase [102].

Normally expressed on mammary epithelial cells, overexpression of the receptor occurs in approximately 15% of all new breast cancer diagnoses [103]. The importance of HER2-positivity relates to the prognostic implications of gene amplification, the predictive value of receptor overexpression, the relevance of incorporating *HER2* with other genes to identify patients who are unlikely to benefit from systemic adjuvant chemotherapy, [104] and the development of trastuzumab [105]. Third, because of the previous findings, patients in their reproductive years who desire to have children should receive counseling regarding options that can increase or improve post-treatment fertility. Indeed, data indicate that young breast cancer survivors have a of 5% - 27% probability of a successful *in-vitro* fertility-assisted live birth [106]. Fourth, the maternal-fetal bond in the form of passive immunity is another immunologic paradox. While beneficial for fetal/neonatal survival, compelling evidence exists also for detriment related to maternal

antibody-transferred disorders. For example, a causal relationship has been established between maternal-derived anti-Ro/SSA antibodies and neonatal lupus erythematosus (NLE) [107]. Furthermore, resolution of the cutaneous manifestations parallels decreasing levels of the antinuclear antibody [108]. Passive immunity also appears to be responsible for a number of other medical problems in the newborn such as thromboembolic episodes, hypothyroidism, hemolysis, and thrombocytopenia [109-112]. Even more intriguing is laboratory evidence of autism spectrum disorder (ASD) in children exposed, *in utero*, to maternal antibodies [113,114].

Two of the most unappreciated characteristics in immunology is the fact that acquisition of passive immunity requires an active process; and that IgG is the only class of antibody that undergoes placental transfer [115]. With regard to the former, active transfer of maternal IgG is accomplished via a unique receptor, homologous to MHC class I molecules [116]. Derived from neonatal rat intestine and identified as FcRN, the carrier protein was firmly established in a study comparing IgG variants with and without affinity for the receptor [117]. Furthermore, class selectivity is likely evolutionary based on the antibody's protective role and its predominance in serum. It is also worth emphasizing that IgG has four subclasses with a transfer preference of IgG1>IgG4>IgG3>IgG2 primarily because of receptor affinity [118]. However, mere binding of antibody to FcRn oversimplifies the complexity of the process. Although a detailed description is beyond the scope of this paper, acquisition of passive immunity involves diffusion of antibody into the syncytiotrophoblast, internalization into endocytic vesicles, acidic ph-dependent formation of IgG-FcRn complexes, alkaline ph-dependent dissociation of bound IgG, and finally relegation of the antibody to the fetus. Transfer of humoral immunity begins as early as the first trimester; and notably, the entire IgG repertoire at term pregnancy is usually 130% of maternal antibody levels [119].

In contrast to natural humoral elements, trastuzumab is a "humanized" antibody of the IgG class and G1 subclass (IgG1). Transfected into this molecule are the exact nucleotides of the complementarity-determining regions (CDRs) of a mouse gene into a human Ig gene. The resulting construct retains epitope-specificity of the murine CDR for the HER2 protein as well as effector functions of the human Fc fragment. In addition, engineered trastuzumab contains an alanine for asparagine substitution at residue 434 (N434A) in the hinge region of Fc resulting in a variant with a 3.4-fold improvement in FcRn binding affinity compared to wild-type trastuzumab [120]. Beyond enhanced receptor binding, this minimalist alteration has profound pharmacokinetic implications including prolongation of half-life and protection from degradation [121,122]. Furthermore, FcRn is not only present in uterine syncytiotrophoblasts but expression occurs in luminal cells of the intestine and glomerulus, endothelial cells, monocytes, macrophages and dendritic cells. The relevance of this biological finding is that the reported half-life of trastuzumab does not account for antibody that may be sequestered and subsequently recycled into the circulation [123-125]. Although the embodiment of a naturally occurring antibody, this "designer" molecule is a drug engineered with unnatural high-performance characteristics. Fifth, the surprisingly high incidence of cardiac toxicity especially when given concomitantly with an anthracycline suggested the HER2-signaling pathway had a functional role in cardiac dynamics [126]. Even though murine models showed an association between mutations of *HER* family genes and impairment of cardiac morphogenesis and mid-gestational death, [127,128] only theoretical explanations for the cardiotoxic reaction of anti-HER2 therapy were initially proposed [129]. Furthermore, the role of (and signaling through) the receptor could be physiologically distinct in the myocardium of the adult and fetus [130]. For example, in adults HER2 is localized to transverse (t)-tubules of ventricular cardiomyocytes thereby facilitating calcium ion exchange and regulation of excitation-contraction coupling [131]. On the other hand, *HER2*-knockout mice fail to develop myocardial trabeculae, which governs blood flow in the embryonic heart tube and the ventricular conduction system as well as cardiac contractility [132].

Further preclinical data indicated that expression of HER2 in the endocardial lining occurred by embryonic day 10 (E10) though earlier expression of the receptor cannot be completely dismissed [133]. In addition, the *HER2*$^{-/-}$ genotype is lethal by embryonic day 11 (E11). Post-mortem examination revealed complete absence of ventricular trabeculae, which was, at least partially, responsible for the early deaths. Comparatively, derivation of the heart from the mesoderm in humans becomes evident during the third week of embryogenesis, a period of time that corresponds to E7 of the mouse. Extrapolation of the mice data further, it is conceivable that HER2 is also expressed though the extent to which receptor signaling contributes to

myocardial development has not been elucidated. What is known, however, is that formation of the ventricular loop is followed by arteriogenesis around day 25 in humans; [134] and shortly thereafter, the formation of the myocardium [135].

Extending beyond mythological lore, these biological constructs provide a strong and rational probability that even after cessation of therapy, blockade of the HER2-signaling pathway *in utero* by trastuzumab could result in severe deficits of cardiac morphogenesis and function in the fetus.

Detrimental effects of passive immunity on the fetus and newborn are relatively uncommon, frequently evanescent, and usually not lethal. The notion that severe fetal or neonatal abnormalities mediated by therapeutic anticancer antibodies, even after completion of therapy, is plausible. As cancer outcomes continue to improve, it can be anticipated that fertility will continue to be an important issue among women of child-bearing age.

CONCLUSION

The term evolution preferentially leans toward Darwinism, a process millions of years in the making from which the origin of beliefs regarding natural selection and development confers distinct survival advantages. A parallel construct in oncology relates to engagement of natural immune components and development of humanized or fully human products that improve cancer survivorship; this too, represents evolution, but occurring over a much shorter timeframe.

Rather than expanding on the profound therapeutic impact of immunotherapy in oncology (which has been done so frequently), the authors chose to focus on dissecting the complex biology of the ir-AEs, an effort that proved to be quite challenging. As such, even gaining uniform agreement with the proffered scientific explanations may be as difficult as achieving full acceptance of the theory of evolution. The latter is, perhaps, the most accurate of all author assessments. Nonetheless, what is certain is that cancer treatment continues to evolve; what is uncertain are the next steps in this evolutionary process.

CONFLICT OF INTEREST STATEMENT

The authors have no financial relationship or other conflicts of interest with industry, specifically related to any product referred to in, or could be inferred from, the contents of this paper.

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