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PERSPECTIVE

Leveraging premalignant biology for immune-based cancer prevention

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Prevention is an essential component of cancer eradication. Next-generation sequencing of cancer genomes and epigenomes has defined large numbers of driver mutations and molecular subgroups, leading to therapeutic advances. By comparison, there is a relative paucity of such knowledge in premalignant neoplasia, which inherently limits the potential to develop precision prevention strategies. Studies on the interplay between germ-line and somatic events have elucidated genetic processes underlying premalignant progression and preventive targets. Emerging data hint at the immune system's ability to intercept premalignancy and prevent cancer. Genetically engineered mouse models have identified mechanisms by which genetic drivers and other somatic alterations recruit inflammatory cells and induce changes in normal cells to create and interact with the premalignant tumor microenvironment to promote oncogenesis and immune evasion. These studies are currently limited to only a few lesion types and patients. In this Perspective, we advocate a large-scale collaborative effort to systematically map the biology of premalignancy and the surrounding cellular response. By bringing together scientists from diverse disciplines (e.g., biochemistry, omics, and computational biology; microbiology, immunology, and medical genetics; engineering, imaging, and synthetic chemistry; and implementation science), we can drive a concerted effort focused on cancer vaccines to reprogram the immune response to prevent, detect, and reject premalignancy. Lynch syndrome, clonal hematopoiesis, and cervical intraepithelial neoplasia which also serve as models for inherited syndromes, blood, and viral premalignancies, are ideal scenarios in which to launch this initiative.

premalignancy | biology | vaccines | cancer prevention | immune oncology

Cancer development is a complex process influenced by inherited variation in germ-line DNA and acquired somatic alterations. The stepwise accumulation of genetic changes leads to oncogenic transformation (1), and also co-opts neighboring normal cells (e.g., neuronal and vascular) to support tumor development and progression (1, 2). The immune system recognizes transformed cells, and avoiding immune elimination is now an accepted hallmark of cancer (2). Large-scale somatic sequencing initiatives, such as The Cancer Genome Atlas (TCGA), in parallel with large genome-wide association studies (GWAS) of germ-line variants have analyzed an increasing array of cancers (3). However, there remain some notable lacunae in our understanding of the biology of premalignancy and cancer development, including the roles of the immune system. Although cancers are increasingly being defined by alterations in genetic, epigenetic, and signaling networks, premalignant lesions [with few exceptions (4, 5)] are still largely identified only through morphological criteria.

In this Perspective, we discuss the influence and interactions of omic and cellular [e.g., tumor microenvironment (TME) and microbiome] events on the development and progression of premalignancy

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(1, 2, 4, 6). There is an unprecedented opportunity in single-cell next-generation sequencing (NGS), computational biology, high-throughput functional screens, and preclinical models (7, 8) to achieve an integrated understanding of premalignant biology and cancer risk to drive immune-based prevention.

Colorectal Adenoma-Carcinoma Model

Even though the seminal multistep genetic model of human carcinogenesis was defined in the colorectal adenoma-carcinoma sequence nearly three decades ago (9), it is unfortunate that NGS of only 25 sporadic colorectal adenomas have been reported to date (10, 11). This number contrasts radically with the plethora of genomic information (12–14) generated by major initiatives at multiple levels for colorectal carcinomas (CRC). Most reported molecular analyses of colorectal adenomas have interrogated only a limited number of genes or restricted-region assessments of copy number, rendering a narrow view of the biology of premalignancy. New technologies, including human organoids with CRISPR/Cas9-based gene editing, are being applied to this model (15). NGS studies of minute tissue specimens with isolated reports of small numbers of premalignant lesion types, such as Barrett's esophagus (16), ductal and lobular carcinoma in situ (DCIS, LCIS) (4, 17), serous tubal intraepithelial carcinoma (18), pancreatic intraepithelial neoplasia (PanIN) (19), monoclonal gammopathy of unknown significance (MGUS) (20), and high-count monoclonal B-cell lymphocytosis (MBL) (21), with colorectal adenomas being the most salient example.

Knowledge on the genomic annotation of intestinal carcinogenesis has come mainly from the study of premalignant lesions in hereditary CRC syndromes, which are thought to recapitulate the two major pathways of genomic instability. Familial adenomatous polyposis (FAP), caused by germ-line adenomatous polyposis coli (*APC*) mutations, is a molecular model for 85% of sporadic CRC characterized by Wnt alterations and chromosomal instability (22). Recent whole-exome sequencing characterized the genomic landscape of early adenoma tissue in FAP, which confirmed and extended the proposed "Big Bang" theory of CRC development (11), identifying >200 somatic hits in 25 adenomas, clonal selection, and a mutational load similar to that of stage I CRC (23). An estimated 25% of the mutational load (all passenger mutations) was present in adjacent, apparently normal mucosa (field effect). This study (23) and others (24) in FAP have provided a catalog of the somatic variation cooperating with *APC* in early colorectal carcinogenesis and indicated that a substantial proportion of the genomic variation present in CRCs is acquired in the earliest at-risk tissues. Understanding FAP biology has led to breakthrough combinatorial chemoprevention for this devastating syndrome (25).

Lynch syndrome (LS), caused by germ-line defects in the DNA mismatch repair (MMR) system, is a model for 15% of sporadic CRCs characterized by microsatellite instability (MSI). The absence of proficient MMR surveying DNA for these errors generates an exponential accumulation of frameshift (FS) mutations at microsatellite tracts, thus increasing mutation rate by several orders of magnitude and accelerating oncogenesis (26) (see *LS as a Model for Hypermutability and Immune-Based Prevention*, below). Chromosomal instability, defective DNA repair, and APOBEC (apolipoprotein B mRNA-editing enzyme; discussed in *Expanding the Scope of Immune Prevention to BRCA1/2 and APOBEC-Associated Neoplasia*) are major drivers of oncogenesis and clonal diversity/heterogeneity in other hereditary and sporadic cancers (27). The role of the microbiome in CRC risk is discussed below.

Germ-Line–Somatic Landscape

Colorectal neoplasia also provides examples of germ-line effects on somatic events and phenotype. The location and mechanism (point mutation versus deletion) of germ-line *APC* inactivation in FAP determines the somatic second hit in *APC* and amount of β-catenin optimal to promote intestinal carcinogenesis ("'just right' model of *APC*") (28). Germ-line 5′ *APC* mutations in FAP affect interactions with wild-type APC, allowing residual Wnt activity (29). Germ-line biallelic *MUTYH* causes G:T transversions due to base excision repair defects (24). Further, the *APC* I1307K (c.3920T > A) polymorphism, linked to CRC risk (30), generates a hypermutable, mononucleotide repeat (A8) that impairs replication fidelity, forming a mutational hotspot facilitating biallelic inactivation of *APC*.

Germ-line mutations of the transcription factor *GATA2* confer monocytopenia, atypical mycobacterial infections, and a propensity to develop preleukemia (myelodysplastic syndrome; MDS) or acute myeloid leukemia (AML). *GATA2* mutation carriers that develop myeloid malignancies harbor somatic *ASXL1* mutations (and monosomy 7) at rates much greater than expected by chance, suggesting a functional or epistatic interaction between these events in myeloid-lineage cells (31). Other examples of hereditary mutated transcription factors that predispose to hematologic neoplasia include mutations in *CEBPA*, *RUNX1*, *ETV6*, and *PAX5* (32). The culprit germ-line variants are typically heterozygous and may have dominant-negative activity against the remaining germ-line allele. Cooperating somatic mutations, often including mutation of the remaining wild-type allele, are clearly required and can be identified during periods of clonally skewed hematopoiesis that precede transformation (32).

The development of myeloproliferative neoplasms (MPN) can involve a *JAK2* haplotype (termed 46/1) that is highly associated with the acquisition of a somatic *JAK2* mutation in MPN patients. Strikingly, the somatic *JAK2* mutation associated with the 46/1 haplotype occurs on the *cis* (vs. *trans*) allele more often than predicted by chance, suggesting a local interaction (33). However, this mutational predisposition effect is not limited to the nearby *JAK2* gene. Patients with mutations of another MPN gene, *MPL*, are also more likely to carry the 46/1 variant (34).

Integrated analysis of germ-line and somatic variants is also beginning to inform precision prevention. Large-scale sequencing of over 4,000 tumors (12 cancer types) from the TCGA found rare germline truncations in 114 cancer-susceptibility-associated genes, ranging in frequency from 4% (AML) to 19% (ovarian cancer) (35). Of the 1% of lung cancer patients with somatic *EGFR* T790M resistance mutations at diagnosis, most actually carry germ-line *EGFR* T790M mutations. These families appear to have a different biology of lung neoplasia (slow-growing lung nodules) and so may be good candidates for lung cancer screening and precision chemoprevention with T790M inhibitors (36). Finally, repurposed NGS of "control" blood from large TCGA and GWAS cohorts identified clonal hematopoiesis as a new premalignant state, characterized by age-related myeloid malignancy driver mutations (mostly in *DNMT3A*, *TET2*, and *ASXL1*) (37, 38). The vast majority of these individuals harbored a single-driver mutation. Patients with idiopathic cytopenias of undetermined significance (ICUS) were noted to have higher rates (~40%) of clonal hematopoiesis and possibly transformation to MDS/AML (39, 40). Once germ-line–somatic relationships have been mapped, an atlas of shared and distinct oncogenic events can be analyzed for targetability.

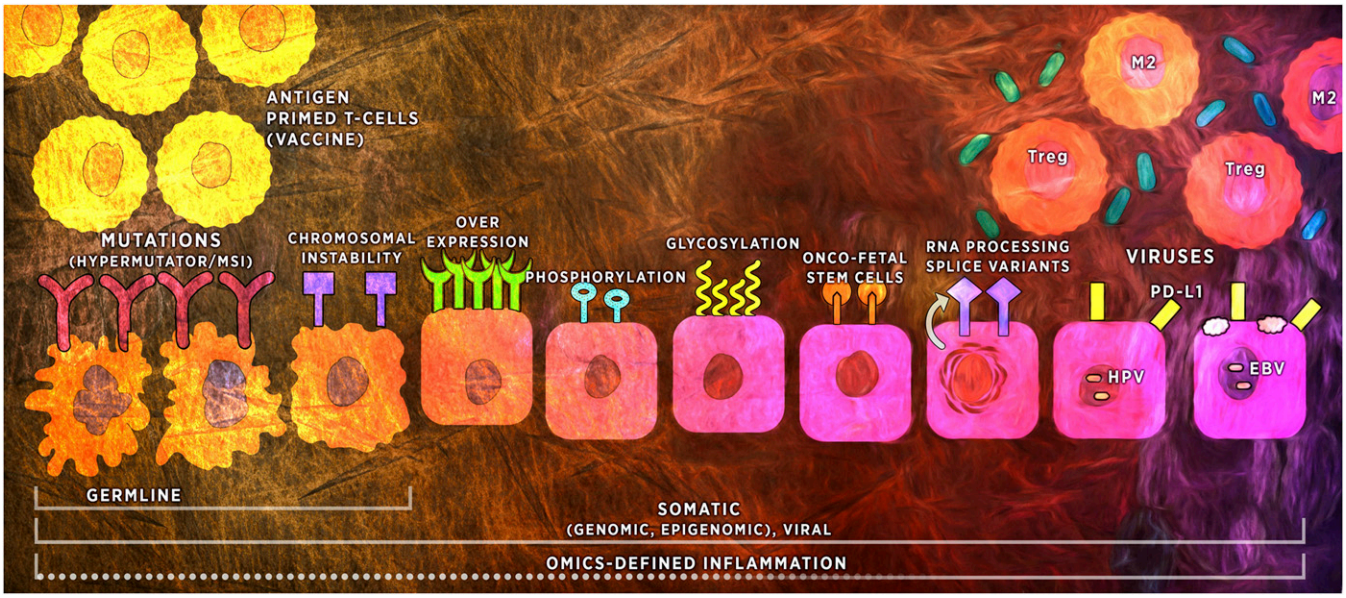

Fig. 1. The immunogenic repertoire of premalignancy. The horizontal lines at the bottom represent the layers of factors that can stimulate immunity, among them germ-line and somatic alterations and their complex dynamic interplay with the inflammatory TME (*Upper Right*). The upper half of the figure depicts the progressively immunosuppressive TME from left to right. The epithelial cells (middle row) illustrate two pathways of genomic instability on the left (irregular cell borders)—MSI and chromosomal instability—which can be inherited or acquired (see *Colorectal Adenoma-Carcinoma Model*). Inherited and acquired MSI-H lesions are highly immunogenic. The somatic cell alterations in the middle include complex posttranslational modifications (e.g., glycosylation), onco-fetal, and splice variants, important parts of the immunogenic repertoire, but their order in terms of cancer risk or immunogenicity is unclear. The cells on the far right middle row are virally infected cells, which have similar TME issues as the nonviral premalignancies. Vaccine-primed T-cells (*Upper Left*), capable of generating type I Th and $CD8^+$ cells, could overcome early TME changes to eradicate cells in the transformation process.

Harnessing the Immune System for Cancer Prevention

There exists a fascinating duality regarding the immune system's role in oncogenesis, the depths of which remain incompletely understood (41, 42). It is well known that inappropriate immune responses (as seen in chronic inflammatory conditions) are strongly associated with high risks of developing cancer (e.g., CRC in ulcerative colitis; 43). Immunosurveillance/immunity, however, is thought to be a critical mechanism for inhibiting cancer development and progression, as evidenced by the success of immune checkpoint inhibitors [e.g., programmed cell death protein 1 (PD-1) antibodies], which have been a game-changer for a number of patients, producing deep and durable clinical responses in a variety of malignancies, particularly high-mutational burden cancers (13, 44, 45). In parallel, the incredible efficacy of human papillomavirus (HPV) vaccines has shown the great promise for using the immune system for cancer prevention (46).

Vaccines to prevent cervical intraepithelial neoplasia (CIN) are standard practice (46) and work best when given to healthy individuals before they are exposed to HPV, so as to induce neutralizing antibodies against viral proteins while the cervical tissue is normal, without an immunosuppressive TME. Viral E6 and E7 proteins are well-understood oncogenic drivers, and CIN is relatively indolent and directly accessible by routine screening. Therapeutic vaccine studies targeting E6/E7 antigens in CIN2/3, including results from single-cell T-cell receptor sequencing, suggest that inducing efficient trafficking of functional effector T-cells to the epithelial disease site is critical to eliminate both the disease and virus (47). HPV E6 induces APOBEC3B, which in turn mutates chromosomal DNA and most likely contributes to precancer development (48). HPV also induces tumor-associated stromal fibroblasts (49), and E6 inactivates p53, which induces PD-L1 and cervical Tregs, causing immune evasion (47). HPV16 integration into the *PD-L1* 3′UTR enhances PD-L1 expression (50).

Mouse data have clearly shown that tumors can escape immune recognition by losing their antigenicity in a process termed "immunoediting" (51, 52). Furthermore, knockout mice lacking an adaptive immune system have dramatically increased rates of tumor (e.g., intestinal adenoma and adenocarcinoma) formation compared with wild-type mice (53). In humans, severe combined immunodeficiency (SCID) is similarly characterized by fundamental defects in adaptive immunity, although the associated risk of cancer (mostly lymphomas) is modest, possibly because SCID is almost universally fatal by age 2 (without stem cell transplant) as a result of infections (54). Other forms of inherited immunodeficiency—such as common variable immunodeficiency, X-linked hyper-IgM syndrome, Bloom syndrome, and ataxia telangiectasia—have been linked to increased risks of cancers, predominantly lymphomas but also a wide spectrum of solid malignancies, MDS and AML (54). Similar findings were reported in acquired immunosuppression, including people with HIV/AIDS and solid organ transplant recipients (55). The mechanisms underlying such cancer risks in immunodeficient patients are not well understood, given the complex and overlapping functions/components of innate and adaptive immunity, which may partially compensate for specific immune defects. Such gaps in knowledge further indicate the need to fully map the biology of premalignancy.

The Premalignant Antigenic Repertoire and Microenvironment. The premalignant antigenic repertoire/vaccine targets can include driver mutations and nonmutated self-proteins that are expressed at abnormal levels. It is unknown what determines immunogenicity, although it is more complex than simply the category of antigen (Fig. 1). Posttranslational modifications, such as

glycosylation, can have complex, poorly understood effects on immune response (56) and evasion (57). Tumor-specific mutant epitopes (called neoantigens) may be important factors for understanding and determining the specificity of an immunotherapy (58). This theory was confirmed experimentally in mice using genomics and bioinformatics to predict those cancer-specific mutations that function as neoantigens and demonstrate their effective use in cancer vaccines (59). Of note, vaccines against immunogenic tumor mutations in mice can be as effective as immune checkpoint blockade (60). This approach has led to considerable interest in the cancer epitope (mutation) landscape and has supported the potential to generate novel immunogenic neoepitopes. In CRC, whole-exome sequencing has been implemented to computationally predict the neoantigenic repertoire from archival specimens (61). A large-scale initiative, including high-throughput mass spectroscopy and single-cell proteomics (7), and rigorous clinical characterization and follow-up will be essential to define immunogenicity of premalignant antigens.

The first prevention example of a cancer vaccine targeting a driver mutation in premalignancy involved *Kras* in a pancreas genetically engineered mouse model (GEMM). *Kras* mutations are the earliest genetic drivers in human pancreas neoplasia, present in both early- and late-stage PanINs. Early *Kras*-mutated neoplastic cells secrete cytokines (e.g., IL-6), VEGF, and GM-CSF, which recruit Tregs, myeloid-derived suppressor cells (MDSCs), adipocytes and neutrophils, macrophage PI3Kγ, and chemokines (e.g., CXCL13), which recruit B-cells leading to a progressively immunosuppressive TME and immune escape (42, 62, 63). *Kras-p53*-Cre pancreatic GEMM were immunized with a *Listeria* vector encoded with the $Kras^{G12D}$ mutation and were found to generate $CD8^+$ T-cells specific for the *Kras* mutation (64). *Kras* vaccine combined with cyclophosphamide Treg depletion significantly slowed the progression of early (but not late) PanIN, compared with control mice. These and other mouse-model data show the potential of driver mutation-specific vaccination to prevent premalignant progression (56) and underscore immune evasion mechanisms. Serious concerns with checkpoint inhibitors in the prevention setting include potentially severe immune adverse effects and a dearth of long-term safety data. Modulators of ten-eleven translocation (TET) proteins (65) and other epigenetic regulators (e.g., deliverable forms of miRNAs) and metformin given during vaccination could reprogram early immunosuppressive cell populations. Metformin (a safe FDA-approved oral diabetes agent) can increase $CD8^+$ cells, reduce T-cell exhaustion, reprogram macrophages and stellate cells (to reduce desmoplasia) in pancreatic neoplasia (66), and can alter T-cell metabolism to generate long-lived immune memory (67, 68).

The influence of the premalignant TME is also well illustrated in DCIS: integrated DNA- and RNA-seq of high-grade DCIS identified high rates of p53 pathway inactivation and a molecular subclass of lesions characterized by a highly proliferative, basal-like phenotype with genomic signatures of activated Treg cells and checkpoint complexes indicative of a tumor-associated immunosuppressive phenotype (4). Suppressed immunity (e.g., high Tregs and $CD8^+$ HLA-DR-neg T-cells) correlated with progression from normal to DCIS to invasive ductal carcinoma. PD-$L1^+$ tumor-infiltrating lymphocytes are most prominent in triple-negative DCIS and microinvasive cancer (69).

There is increasing evidence that somatically mutated MDS cells can alter their TME to provide a clonal growth advantage. Examples include activation of inflammatory molecules, s100a8 and s100a9, and induction of *TP53* in mouse models. Similarly, MDS cells with various types of somatic mutations can activate inflammasome-mediated pathways that increase MDSC bone marrow number (70). Alteration to stromal cells may promote clonal hematopoiesis (e.g., mice carrying a *Dicer1* deletion in osteoblasts developed clonally derived leukemias) (71).

The presence of a robust adaptive T-cell immune response, evident either in tumor or peripheral blood from patients with certain cancers, has been associated with improved survival (72). Antibody response to vaccines is also important and can enhance T-cell immunity (73). Naturally occurring cytotoxic T-cell responses to tumor antigens can be detected in one-third of healthy people without cancer (74). Precancer-specific natural immune surveillance also exists and can prevent the development of cancer (56, 75). For example, MGUS patients can mount a T-cell immune response to SOX2, a transcription factor critical for self-renewal in stem cells, which is associated with reduced risk of progression to multiple myeloma (MM), supporting the potential for a vaccine to boost SOX2-specific immunity (76).

Host–microbiome interactions are important in premalignancy, adding to TME complexity (6). Studies in GEMMs have found that *APC* loss disrupts the intestinal epithelial barrier, facilitating invasion of microbes and microbial nucleic acids that activate adenoma-associated macrophages to produce IL-23, which then stimulates IL-17 production by T-cells, accelerating adenoma development and progression (77). Bacterial translocation can activate Toll-like receptors that can up-regulate other inflammatory elements. These barrier defects drive innate immunosuppressive TME, and adenoma proliferation (e.g., *F. nucleatum* in the TME can inhibit NK-cell cytotoxicity producing bacteria-dependent immune evasion) (43, 78). Metagenome study found different taxa in adenomas compared to carcinomas and healthy controls (79). Gut microbiota may explain the provocative link between MMR-deficiency and CRC (80). The interplay between the microbiome, virome, autophagy, inflammatory bowel disease, GWAS, and the immune system is also under active study in CRC development and prevention (81, 82).

LS as a Model for Hypermutability and Immune-Based Prevention. Somatic hypermutation can arise through diverse mechanisms. As described above, MSI is a form of hypermutability in which DNA MMR defects lead to genome-wide accumulation of FS mutations within predictable nucleotide repeat loci (microsatellites). MSI-related FS mutations drive tumorigenesis by occurring within microsatellite loci that lead to inactivation of tumor suppressors enriched for genes functionally involved in immune regulation (e.g., *TGFBR2* and *BAX*) in both CRCs and adenomas (83). When they occur in coding regions, such mutations generate FS-mutation-derived peptides (FSP), which function as highly immunogenic neoantigens and cause specific $CD8^+$ T-cell responses and neoplastic infiltrates. The "hotspot" nature of these MSI-related FS mutations leads to FSPs with predictable sequences, suggesting that multivalent vaccine development targeting specific, expected T-cell epitopes may be an effective prevention strategy for MSI-induced neoplasia (83). The feasibility of this approach was demonstrated by using a panel of FSPs expected to be generated by MSI-induced FS mutations at a specific hotspot locus within *MSH3*; engineered $CD8^+$ T-cells from a healthy volunteer specifically targeted these FSP-lysed, HLA-matched, high-level MSI (MSI-H) CRC cell lines (84).

MSI may represent a unique form of hypermutability, expressing high amounts of neoantigens, which up-regulate inhibitory molecules (e.g., PD-L1) to counterbalance the infiltrating immune cells; this is distinct from overall mutational load, in that it renders tumors very susceptible to immune-based destruction. There has been major

progress in using PD-1 inhibitors to treat advanced cancers with MSI-H and MMR deficiency (MMR-D), such as those that arise in LS or sporadic MSI-H CRC (44). LS patients are at very high risk of CRC and endometrial cancers, and recent data suggest that this classic LS-cancer spectrum is wider than traditionally appreciated.

The recognition in healthy (screened) LS carriers of MSI-H/MMR-D in preinvasive, normal-appearing tissues and circulating FSP-specific T-cells (85) suggests that immune surveillance mechanisms may help reduce MSI-H tumor development. Histologically normal but MSI-H/MMR-D intestinal crypt foci in LS carriers harbor MSI-related FS mutations, which may be a key source of these FSP-specific T-cells (86). There are conflicting data as to whether the size of LS adenomas correlates with MSI-H/MMR-D, although this may reflect technical aspects of MSI and MMR testing rather than actual adenoma biology (87). NGS can likely address this limitation, because mutational burden appears to be a reliable surrogate for MSI-H status (8).

In LS-associated MSI-H CRCs and adenomas, immune evasion can occur by MHC I loss as a result of β2-microglobulin mutations (a mechanism distinct from sporadic MSI-H CRCs) (88). Additionally, there is evidence of an immune-suppressive TME (increased density of $FOXP3^+$ Tregs) in normal mucosa adjacent (but not distant) to CRC in LS patients with wild-type β2-microglobulin (89).

Children who are homozygous for germ-line LS mutations have biallelic MMR deficiency (BMMR-D) (90) and present a compelling scenario for vaccine-prevention. BMMR-D confers a devastating phenotype of pediatric lymphomas, brain tumors, and intestinal cancers (91). In stark contrast to other pediatric cancers, which classically display few somatic mutations (1), BMMR-D–associated cancers have an "ultrahypermutated" phenotype (90) because of acquisition of somatic FS mutations in the proof-reading domains of the DNA polymerases *POLE* or *POLD1* and have mutational loads exceeding those in adult MSI-H CRC. BMMR-D cancers may be particularly responsive to PD-1 inhibitors (92).

Expanding the Scope of Immune Prevention to BRCA1/2- and APOBEC-Associated Neoplasia. LS represents an ideal proof-of-principle for using immune-based prevention, relevant to other hereditary cancers. This is particularly important as NGS data continue to expand the spectrum of cancers linked to various germ-line mutations, including *BRCA1/2* (93). Germ-line *BRCA1/2* mutations induce defects in homologous recombination (HR)-based DNA repair and confer markedly increased risks of cancers of the breast, ovaries/fallopian tubes, pancreas, prostate, and melanoma, although NGS germ-line testing suggest that they may also be linked to cancers more classically LS-associated (CRC and endometrial cancers) (93). Somatic mutational patterns found in HR deficient *BRCA1/2*-associated breast cancers and *BRCA2*-mutated prostate cancers demonstrate predictable "signatures" of somatic mutations (94, 95), suggesting the plausibility of creating vaccines to target specific hotspot neoantigens. Similarly, *BRCA1/2*-associated ovarian cancers (96) have been shown to exhibit an increased effector lymphocytic reaction (which likely first develop in serous tubal intraepithelial carcinoma) (97) and high numbers of immunogenic mutations.

A growing array of data are examining the role that loss of wild-type *BRCA1/2* function plays in the development and progression of breast, fallopian tube, pancreatic, and prostatic premalignant lesions from individuals with germ-line *BRCA1/2* mutations, including data suggesting that *BRCA1* haploinsufficiency promotes genomic instability in nonneoplastic breast epithelium before loss of the wild-type allele (98). Nonneoplastic breast epithelium from *BRCA1* mutation carriers have gene-expression profiles similar to luminal progenitor cells (which differs from the basal features of most *BRCA1* breast cancers) (99). Further efforts toward characterizing *BRCA1/2*-associated premalignancy are vital to developing preventive strategies for these high-risk patients. Poly ADP ribose polymerase (PARP) inhibitors, compelling precision therapy of certain *BRCA1/2*-associated cancers, have been shown to delay mammary tumor development in *BRCA1*-deficient mice (100). Exciting data suggest that the RANKL/RANK pathway has an integral role in breast oncogenesis in germ-line *BRCA1* mutation carriers. Interference with this pathway produced significant preventive activity, including pharmacologic RANK-ligand inhibition (e.g., denosumab) in *BRCA1*-mutant breast organoids and *Brca1*-deficient and mutation-driven mouse models (101, 102). Denosumab is an FDA-approved agent for bone loss with an established safety profile and could be repurposed for prevention trials for healthy mutation carriers.

NGS and biochemical characterization have identified key roles of APOBEC3 (A3) enzymes in inducing a hypermutated phenotype as part of innate immunity. A3 induction is a critical early event in HPV-related neoplasia (see above). A3 can be induced by IFN-α, IFN-γ, and other inflammatory cytokines (103), although the induction mechanism (104) in nonviral cancers is unclear and the timing varies by site and etiology (27, 103). A3A and A3B have intrinsic preference for deaminating cytosine residues in TCA and TCG trinucleotide contexts, and it is thus assumed that A3B-mediated neoplasia will be characterized by A3B-catalyzed mutational hotspots (e.g., generating *PIK3CA* driver mutations at helical domain hotspots E542K and E545K) (27) that could be used as part of a vaccine. A common germ-line *APOBEC3A/3B* chimeric deletion polymorphism (ΔA3B) has been associated with risk of breast, liver, and certain other cancers (105–108). Paradoxically, this ΔA3B deletion leads to increased A3A activity as a result of increased stability of the chimeric *APOBEC3A/B* mRNA (109). This increased A3A activity is thought to underlie the associated modest breast cancer risk, because cancers associated with these ΔA3B polymorphisms have A3 mutation signatures (distinct from those seen in HR-deficient or MMR-D breast cancers) that correlate with germ-line copy number (105) and seemingly higher penetrance of hypermutability and immune activation (106, 108). Study of the regulation of APOBEC3 in neoplasia will be critical, including ADAR1 oncogenic effects linking RNA editing to an innate inflammatory TME and potential suppression of hypermutation and immunity (110). Furthermore, the ΔA3B polymorphism is highly prevalent in certain populations (37% East Asians, 58% Native Americans, >90% Pacific Islanders) (111), suggesting that vaccines targeting A3-related neoantigens could have an important public health impact for preventing both ΔA3B- and viral-associated cancers (27, 103, 107, 109), the former possibly providing a roadmap to investigate preventive approaches for other germ-line polymorphisms linked to cancer risk. Increasing evidence from GWAS suggest a substantial germ-line effect in adult "sporadic" tumors (112), and suggest that most loci identified in cancer patients are present in the precursor (e.g., DCIS) and can influence chemoprevention (113, 114).

Summary and Next Steps

A new national investment in cancer—driven by the Vice President's Cancer Moonshot Initiative that includes the NIH, academia, Food and Drug Administration, private foundations, philanthropic partners, and industry—includes prevention and cancer vaccines

(115, cancer.gov/research/key-initiatives/moonshot-cancer-initiative/blue-ribbon-panel/blue-ribbon-panel-report-2016.pdf). A large fund infusion from The American Recovery and Reinvestment Act of 2009 advanced the TCGA from a small pilot program of three cancer types to the tremendous resource it has become (116). A similar opportunity exists in the realm of premalignant biology: a new prospective initiative in this setting could leverage and expand TCGA, GWAS, and related model infrastructures for systematic specimen collection, processing, storage, analyses to bioinformatics and data sharing (116, 117).

This initiative will require collaborations across diverse disciplines. For example, the oncogenic mechanisms of *IDH* mutations [discovered through broad sequencing (118)] remained unclear until modern metabolomic profiling (119) detected the novel "oncometabolite" 2-hydroxyglutarate, which inhibits TETs and other enzymes that are important in certain premalignancies (120), thereby identifying a completely novel method of oncogenesis and turning a genetic discovery into a drug and vaccine target. There is also a need for: (*i*) better preclinical models, e.g., CRISPR/Cas9 engineered immunosuppressive mouse strains (121), immune organoids (122), and new model organisms (e.g., zebrafish) providing insight into early premalignant biology, and the role of epigenetic reprogramming in transformation (123), (*ii*) discern site-specific patterns and timing of driver mutations and genomic instability in neoplastic progression (124) and specific acquired mutations predictive of immune resistance in premalignancy (88) and cancer (125), (*iii*) imaging immune responses (e.g., NK- and T-cell subtype trafficking) and TME composition to optimize priming and boosting regimens (126), and (*iv*) new single cell and computational methods to understand the increasingly complex cellular (e.g., adipocyte, myocyte interplay) compartment and tissue microenvironment (e.g., aging fibroblast effects on adaptive immunity) from which the malignancy arises (7, 127, 128).

The development of effective prevention will not be easy (1), but the potential public health benefits are extensive as can be illustrated by the case of cervical cancer, for which screening and HPV vaccination offer the potential to eradicate this disease, whereas recent progress in treating advanced disease included 2- to 3-mo improvements in median survival (129). Cancer vaccines have been studied extensively in thousands of people for many decades and have a very favorable safety profile setting the stage for prevention testing (56). HPV vaccine research supporting one- or two-dose regimens may apply to other cancer prevention vaccines and would greatly improve costs and adherence (46).

The initial phase of this initiative should include LS, clonal hematopoiesis/ICUS, and CIN—an inherited syndrome, blood, and viral premalignancy, respectively—which also serve as models for related disorders. The rationale for cancer-prevention vaccines in healthy LS carriers is particularly compelling: early immune surveillance, reduced MHC loss, predictable FSP patterns, high cancer risk, and young, immunocompetent probands who require serial cancer screening (85). Potential vaccine benefit could extend to LS-associated cancers beyond just CRC, because MSI-H has been found in a wide spectrum of preinvasive LS neoplasia (e.g., 130). This approach would also facilitate vaccine-based prevention for sporadic MSI-H carcinogenesis, which is implicated in subsets of many cancers (131). Outside of the colorectum and stomach, however, little is known about MSI in sporadic premalignancy. Sporadic MSI-H CRCs that arise from sessile serrated adenomas demonstrate FS mutations at the same hotspot microsatellite loci as in LS CRC. Although LS awareness is increasing the use of universal tumor testing of CRC (and now endometrial) specimens for MSI-H/MMR-D (132) and access to NGS germ-line testing, implementation is a major challenge. The estimated prevalence of LS in the general United States population is 1:280 (1.1 million). Colonoscopies are highly effective at reducing CRC risk in LS patients, but strategies for preventing other LS-associated cancers are limited (132). Existing infrastructure includes the international Colon Cancer Family Registry (133). Next steps could include web-based patient recruitment, successfully developed for other related hereditary cancer efforts (e.g., the PROMPT registry, promptstudy.info) and statewide LS registries. These registries would facilitate rapid, large-scale, systematic collection of data and tissue samples from LS and serve as a model that could be expanded to other inherited cancers, such as pancreatic cancer risk/precursors (e.g., identified by germline CDKN2A mutations) to drive vaccine prevention (134) and early detection (135). GWAS (and other) modifiers of high-penetrance mutation effects on risk, biology, precursors, and sites are also needed (136).

The timing is ideal to include clonal hematopoiesis in the initial phase of this initiative (37–40). First, it is important to leverage existing efforts of the National MDS Study (https://thenationalmdsstudy.net/), which will now include a longitudinal biobanking cohort of 500 patients with ICUS. Similarly, the MDS/AML CONNECT Registry sponsored by Celgene will follow 200 ICUS patients over time. Second, there is an opportunity to partner with the Leukemia and Lymphoma Society, patient advocacy groups, and commercial hematopathology laboratories to rapidly identify thousands of potential patients for focused longitudinal studies. Third, innovative prevention, including immune approaches, which have shown promise in MDS and AML, need to be developed for patients at highest risk of malignant transformation (to minimize over diagnosis). Patients with clonal hematopoiesis can harbor small clones for long periods of time (39, 40), and provocatively can account for "therapy-related" MDS/AML (137). Drugs targeting the inflammasome and innate immune responses implicated in remodeling the microenvironment to favor clonal expansion and vaccines against clonal antigens (138) are potential approaches. Analogous approaches can be adopted for MGUS and MBL. Solid-tumor incidence is three- to fourfold higher in MBL and CLL patients vs. healthy controls, likely due to defects in immune surveillance, which could dampen cancer vaccine response (139). Lenalidomide is in clinical trial to improve vaccine response in MBL via its beneficial T-cell effects (NCT02309515). Focusing on premalignancies of the blood has several advantages, including the ease of repeatedly acquiring neoplastic cells to study their clonal evolution over time, and although slightly more invasive, repeated access to the bone marrow to study changes in the cellular microenvironment is also safe and feasible within the scope of research study. Furthermore, study of MPNs (120) provide the only direct data that somatic mutation order (*JAK2* and *TET2*) can greatly influence disease features.

Finally, expanding the development of vaccines for HPV-related neoplasia is a major global need (46). CIN provides an invaluable model for developing these vaccines, for example: targeting E6/7 and/or A3B (to prevent other HPV-related cancers), including routine screening for longitudinal follow-up; and nonviral vaccines, which share premalignant biology (e.g., p53, A3B), T-cell trafficking, TME features (e.g., PD-L1, Tregs), and mechanisms of immune evasion. Epstein–Barr virus vaccine development has been more challenging than HPV in part because of complex virion surface and viral antigen expression patterns (115, 140). Analogous to the TCGA pan-cancer analyses (117), it will be important to combine premalignancy omic

and immune TME data from multiple sites, etiologies, and types to understand molecular alterations, timing, and interactions to target common and distinct events that drive oncogenesis across different lesions. The central theme of this initiative, to elucidate premalignant biology, requires collaborations across diverse disciplines, and leveraging other related initiatives, including the Global Human Vaccines Project, which brings tremendous expertise from infectious diseases and immunology to immune oncology, focused on decoding immune response, evasion, and immunogenicity (141).

Prevention research has produced encouraging results (25, 135, 142–144), in some cases possibly due to previously unrecognized immune effects (5, 67, 145–147). To move this field from isolated examples of progress to near elimination of all cancers will take a radically different focus and approach to premalignant disease and cancer prevention. For example, an imperative of cancer vaccines is the induction of long-term memory T-cell responses (68), overcoming a major limitation of chemoprevention. Fulfilling this vision will require a concerted effort across different initiatives and disciplines, the defining theme of the concept of Convergence Research (www.convergencerevolution.net/2016-report). We will need large-scale, systematic, integrated NGS with multiple omics and immuno-informatic platforms and clinically annotated longitudinal follow-up to lay the foundation of an effective framework for more precise early detection (e.g., liquid biopsy) and prevention and to develop cancer vaccines that reprogram the immune response at the earliest stages to durably reject tumor development. Providing adequate resources and developing multidisciplinary teams of expert prevention-focused scientists is the roadmap to success.

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