Whole-Exome Sequencing in Cancer Chemoprevention: Tracking Mutational Trajectories in High-Risk Populations

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Abstract

Whole-exome sequencing (WES) has revolutionized understanding of cancer chemoprevention by enabling comprehensive characterization of mutational landscapes in high-risk populations and monitoring of clonal evolution during prevention interventions. This review synthesizes current knowledge on WES applications in cancer prevention, emphasizing tracking of mutational trajectories from normal epithelium through dysplasia to invasive carcinoma. We examine discovery of driver mutations and mutational burden as chemoprevention biomarkers, computational approaches for inferring genetic progression (PhylogicNDT), and longitudinal monitoring of clonal evolution using circulating cell-free DNA in prevention trials. Particular emphasis is placed on oral squamous cell carcinoma (OSCC) and oral epithelial dysplasia (OED) as model systems where WES has identified key early driver mutations (TP53, NOTCH1, PIK3CA, CDKN2A) enabling molecular stratification of malignant transformation risk and personalization of prevention strategies. The review discusses universal cancer screening with WES identifying occult cancer predisposition syndromes, prospectively validated genetic progression models (gMART) predicting dysplasia-to-carcinoma risk, and emerging single-cell sequencing approaches revealing subclonal heterogeneity driving prevention failures. Integration of WES with spatial transcriptomics and single-cell RNA-seq provides unprecedented mechanistic insight into how dysplastic lesions acquire transformative mutations, enabling identification of optimal intervention windows during clonal evolution. Clinical applications including window-of-opportunity trials, real-time ctDNA monitoring of chemopreventive efficacy, and evolution-informed adaptive treatment strategies are discussed. We address critical challenges including tumor heterogeneity interpretation, distinguishing driver from passenger mutations in premalignant lesions, clinical translation of discovery findings, and cost-effectiveness of universal WES screening in prevention cohorts.

Keywords: whole-exome sequencing, cancer chemoprevention, mutational trajectories, clonal evolution, driver mutations, oral dysplasia, ctDNA, genetic progression, high-risk populations

1. Introduction

The fundamental challenge in cancer chemoprevention involves identifying high-risk individuals most likely to benefit from preventive interventions before invasive cancer develops, and monitoring whether preventive strategies successfully arrest or reverse progression through precancerous states[1][2]. Traditional approaches relying on clinical examination and histopathological grading provide limited prognostic accuracy: among patients with oral epithelial dysplasia (OED), malignant transformation rates vary from 0% to 36.4% despite identical histologic grades, indicating substantial biological heterogeneity masked by morphologic assessment[1][3]

Whole-exome sequencing technology now enables comprehensive characterization of the mutational landscape in high-risk tissues and circulating compartments, fundamentally transforming prevention strategies[4][5]. WES captures somatic mutations affecting ~20,000 protein-coding genes, providing mechanistic insight into which specific genetic alterations drive premalignant progression and which individuals harbor mutations conferring highest transformation risk[4][5]. Furthermore, serial WES monitoring through circulating cell-free DNA (cfDNA) enables real-time assessment of whether chemopreventive agents successfully arrest clonal evolution or inadvertently select for resistant clones[6][7].

This comprehensive approach addresses critical prevention medicine gaps: (1) discovering which specific molecular alterations predict progression risk, (2) identifying optimal intervention windows during clonal evolution before transformation becomes inevitable, (3) detecting occult cancer predisposition syndromes enabling hereditary cancer prevention, and (4) monitoring treatment response at molecular level without waiting for clinical endpoint maturation[1][4][5][6][7].

This review synthesizes current knowledge on WES applications in cancer prevention, emphasizing mutational trajectory tracking from normalcy through dysplasia to carcinoma, genetic progression modeling predicting individual risk, and evolutionary monitoring of prevention efficacy.

2. Whole-Exome Sequencing Discovery of Oral Cancer Driver Mutations

2.1 Mutational Landscape of Oral Epithelial Dysplasia

Whole-exome sequencing of oral epithelial dysplasia lesions identified a complex mutational signature distinct from both normal epithelium and invasive oral squamous cell carcinoma (OSCC), providing molecular stratification previously unavailable through histologic grading alone[8][9]. Analysis of 10 low- and high-grade OED samples from Brazilian and Chilean patients using WES revealed average single nucleotide variants (SNVs) ranging from significantly higher numbers in high-grade dysplasia (HGD) compared to low-grade dysplasia (LGD)[8]. This mutational burden increase with dysplasia grade suggests progressive genetic instability during malignant transformation[8].

Pathway analysis identified mutations predominantly affecting Wnt signaling and integrin pathways, both critical for epithelial integrity and cancer progression[8][9]. Thirteen genes harboring 15 functional variants were identified, including six novel variants representing splice-site mutations and frame-shift deletions with potential cancer-driving consequences[8]. Notably, genes mutated in OSCC (SHANK2, FARP1, MFC2L) were also altered in OED samples, establishing genetic continuity from dysplasia to malignancy[8].

High-impact mutations predicted to substantially affect amino acid structure were overrepresented in metabolic enzymes and cell adhesion proteins, suggesting dysplasia-specific selective pressures favoring metabolic remodeling and loss of epithelial constraints[8]. This genomic characterization establishes baseline understanding of mutational heterogeneity that individual prevention strategies must address[8][9]

2.2 Core Driver Mutations in Oral Cancer Progression

Targeted and whole-exome sequencing across diverse OSCC cohorts identified consistent driver gene mutations establishing stereotyped progression pathways[10][11][12][13]. TP53 (tumor suppressor p53) emerged as the most frequently mutated gene, with mutation rates ranging from 56-88% across different OSCC populations, with highest frequencies in tobacco and alcohol-associated cancers[10][11][12].

NOTCH1, a Notch pathway component, showed 37% mutation frequency in OSCC and demonstrated particular importance as an early driver mutation based on PhylogicNDT temporal analysis[11][13]. NOTCH1 mutations were identified in 25 of 67 OSCC cases and may represent initial events in clonal evolution, potentially preceding TP53 alterations[13].

PIK3CA (PI3K catalytic subunit) mutations occurred in 8-43% of OSCC depending on anatomic site and carcinogen exposure, with highest rates in oral tongue cancers[10]. CDKN2A (p16) mutations, affecting cell cycle checkpoint control, appeared in 4-24% of samples[10]. HRAS (Harvey rat sarcoma) activating mutations were identified in 2-21% of cases, typically in non-smoking/non-drinking populations, suggesting distinct etiologic pathways[10].

Mutational co-occurrence analysis revealed TP53 predominantly co-occurred with PIK3CA and CDKN2A, while HRAS co-occurred with PIK3CA, establishing non-random mutational networks reflecting pathway interactions[10]. This mutational architecture provides chemoprevention targets: blocking PI3K pathway may provide maximal benefit in TP53-mutant lesions where alternative growth signals become critical for survival[10].

2.3 Mutation Concordance Between Dysplasia and Carcinoma

A critical question involves whether mutations identified in dysplastic lesions persist in subsequent carcinomas or whether transformation involves acquisition of entirely new mutations[11][12]. WES analysis comparing primary tumors and associated metastases/recurrences showed 88-89% TP53 mutational concordance, indicating that dominant clone mutations established during dysplasia persist through malignant transformation[11]. This striking concordance suggests TP53 alterations occurring very early in progression and establishing the dominant clonal architecture[11].

Conversely, some subclonal mutations present in dysplasia disappear in carcinomas, indicating negative selection or replacement by advantageous clones during transformation[11]. This evolutionary dynamic demonstrates that dysplasia represents dynamic evolutionary landscape rather than static premalignant state, and chemoprevention must target multiple clonal populations rather than single dominant clone[11].

3. Genetic Progression Models: Predicting Individual Malignant Transformation Risk

3.1 Loss of Heterozygosity Analysis and gMART Model

Loss of heterozygosity (LOH) analysis examining chromosomal region losses (particularly 3p and 9p) emerged decades ago as a biomarker predicting dysplasia-to-carcinoma progression[14]. Prospective validation in 296 patients with oral dysplasia showed that 3p and 9p LOH increased progression risk >20-fold compared with retention of both regions, with 37% of high-risk (HR) patients progressing to carcinoma within 5 years versus only 6% of low-risk (LR) patients[14].

This early success led to development of the gMART model (genomic marker-based test) adding 4q and 17p LOH assessment, enabling more granular risk stratification[14]. The refined model stratified patients into three risk categories with markedly different progression rates: LR lesions showed 3.1% 5-year progression rate, intermediate-risk (IR) lesions 16.3%, and HR lesions 64.1%, establishing substantial predictive accuracy substantially exceeding histology alone[14].

The gMART model demonstrated clinical utility by identifying 52.1-fold increase in progression risk comparing high-risk (3p/4q/17p LOH) to low-risk (9p retention alone) profiles[14]. Critically, time-to-progression significantly shortened for HR cases, enabling prevention trials to use progression-to-severe dysplasia as earlier surrogate endpoint rather than waiting for invasive carcinoma development[14].

3.2 PhylogicNDT: Computational Inference of Genetic Progression Timing

Modern computational approaches enable reconstruction of genetic progression trajectories from single-timepoint WES data through detailed phylogenetic analysis of cancer cell fractions and mutation multiplicities[15][16]. PhylogicNDT integrates clonal deconvolution, phylogenetic reconstruction, and mutational timing algorithms to infer the sequence and timing of driver mutations occurring during cancer development[15][16].

Applied to HPV-negative HNSCC, PhylogicNDT recapitulated previously known early progression events (TP53, 3p/9p losses) and identified additional early drivers (NSD1, CASP8), establishing computational approach validity[15]. Extension to HPV-positive OSCC revealed previously unknown genetic progression: APOBEC mutational signatures (62% of mutations) and aging signatures (28% of mutations) revealed distinct mutational processes at different progression stages[15].

Critically, temporal mapping converted relative timing estimates to years before diagnosis using clock-like mutational signatures, revealing dysplasia emerges near mRT (mutational relative timing) ~0.25, carcinoma in situ at ~0.7, and invasive carcinoma at ~0.75 on a 0-1 scale[15]. This timeline indicates that dysplastic precursors exist for substantial periods before transformation (~0.25-0.75 of mutation burden evolution), providing extended intervention windows for chemoprevention[15].

3.3 Aneuploidy Timing and Genome Doubling Events

Whole-genome doubling (WGD) duplication of entire genome represents catastrophic genomic event dramatically increasing mutational load and genomic instability[15][17]. PhylogicNDT analysis identified WGD occurring mid-progression, with subsequent conversion to triploidy or higher ploidy in ~35% of tumors, and strongly associated with intratumoral genetic heterogeneity and shorter overall survival[15].

The temporal separation between initial driver mutations, WGD occurrence, and final aneuploidy events creates multiple intervention windows: early chemoprevention targeting pre-WGD clones might prevent genome doubling itself, while post-WGD interventions require targeting established polyploid populations with fundamentally altered selective pressures[15].

4. Single-Cell and Spatial Sequencing: Resolving Subclonal Heterogeneity

4.1 Single-Cell Whole-Genome Sequencing of Dysplastic Lesions

Single-cell whole-genome sequencing (scWGS) reveals subclonal heterogeneity invisible in bulk tissue WES, identifying multiple distinct clonal populations coexisting within dysplastic lesions[6][7][18]. Application of scWGS combined with spatial transcriptomics in oral premalignant lesions identified distinct cellular compositions and spatial organization at single-cell resolution[18].

Pairwise human oral mucosal biopsies from nine individuals containing very early-stage OSCC, adjacent moderate-to-severe dysplasia, and matched normal regions revealed marked cellular heterogeneity in dysplastic fields[18]. Transcriptomic profiling identified cell-type-specific gene expression patterns distinguishing dysplastic epithelial cells from stromal and immune populations, providing mechanistic insight into how dysplasia develops within complex tissue microenvironments[18].

This cellular resolution demonstrates critical limitation of bulk WES: aggregated mutations may mask critical subclonal differences where small populations carrying dangerous mutations escape detection, potentially explaining some prevention failures[18]. Single-cell approaches enable identification and targeting of such high-risk subpopulations[18].

4.2 Clonal Architecture Reconstruction from scWGS

Single-cell whole-genome sequencing enables reconstruction of clonal architecture by identifying clusters of cells sharing identical mutations and inferring clonal relationships through phylogenetic analysis of copy number variations across individual cells[6][7]. CloneSeq-SV methodology combines pseudobulk mutation calling from scWGS data with cfDNA tracking of clone-specific structural variants as endogenous barcodes, enabling longitudinal monitoring of individual clones during treatment and disease evolution[6][7].

Application to ovarian cancer demonstrated pre-existence of drug-resistant clones at diagnosis, establishing that chemotherapy-induced selection of resistance does not generate novel resistant mutations de novo but rather expands pre-existing subpopulations[6][7]. This critical finding transforms prevention strategy rationale: preventing early driver mutations is preferable to attempting eradication of established subclonal heterogeneity[6][7].

5. Circulating Cell-Free DNA Monitoring of Mutational Evolution

5.1 ctDNA as Real-Time Biomarker of Clonal Dynamics

Circulating tumor DNA (ctDNA) in blood enables non-invasive monitoring of tumor evolution and chemopreventive agent efficacy without serial tissue biopsies[6][7][19]. cfDNA hybrid capture deep sequencing identifies clone-specific structural variants (SVs) with dramatically lower error rates than single nucleotide variant (SNV) detection in ctDNA, enabling robust tracking of multiple clones over time[6].

Application of CloneSeq-SV to high-grade serous ovarian cancer patients demonstrated ctDNA clearance accompanying response to chemotherapy, with CA-125 correlation establishing molecular-clinical concordance[6][7]. Critically, ctDNA detection preceded clinical recurrence by 184 days in one patient, establishing ctDNA's superior sensitivity for early recurrence detection applicable to prevention trial monitoring[6][7].

5.2 Clonal Evolution Modeling During Prevention Interventions

Mathematical fitness modeling of clonal trajectories extracted from cfDNA measurements quantifies evolutionary properties of clonal populations under selective pressure from chemopreventive agents[6][7][20]. Populations showing positive selection (increasing frequencies of advantageous clones) require different management strategies than populations showing clonal stability or reduction in diversity[6][7][20].

Evolution-informed adaptive trial designs leverage real-time clonal monitoring to modify treatment strategies: populations showing clonal sweep toward drug-resistant phenotypes require immediate therapeutic adaptation, while populations showing sustained clonal diversity may benefit from continued current strategy[6][7].

6. Universal Cancer Predisposition Screening with WES

6.1 Broadening Cancer Screening Beyond NCCN Guidelines

The Tapestry clinical trial performed WES in 44,306 population participants evaluating cancer predisposition gene mutations for hereditary breast and ovarian cancer (HBOC) and Lynch syndrome[21][22]. Remarkably, 1.2% of participants (550 individuals) carried pathogenic mutations in established cancer predisposition genes, with 39.2% not qualifying for genetic testing under current NCCN guidelines[21][22].

Among 550 identified carriers, 52.1% were completely unaware of their cancer predisposition prior to study participation, establishing that current guideline-based testing strategies substantially underidentify at-risk individuals[21][22]. Among newly diagnosed carriers, 60% would have been ineligible for genetic testing per existing criteria, demonstrating systematic gaps in current screening algorithms[21][22].

Critically, racial and ethnic disparities in guideline-based testing were identified: 49% of minority-group carriers lacked NCCN-guideline indication compared with 32% of White carriers, suggesting systemic bias in current guidelines and need for universal population-level screening[21][22].

6.2 Feasibility and Cost-Effectiveness of Universal WES Screening

The Tapestry trial demonstrated technical feasibility of universal WES screening in large populations: saliva-based DNA collection, automated laboratory processing, and cloud-based result management enabled screening of >40,000 individuals within integrated health systems[21][22]. Declining WES costs ($200-500 per sample currently) combined with improved disease interception value of early identification suggests favorable cost-effectiveness compared with reactive screening of symptomatic individuals[21][22].

Future implementation may integrate WES into standard preventive care, enabling identification of all individuals with hereditary cancer predisposition enabling personalized prevention strategies matched to specific genetic risks[21][22].

7. Molecular Stratification and Personalized Prevention in Oral Dysplasia

7.1 TP53 Mutation Status as Prognostic Stratifier

TP53 mutations in dysplastic lesions represent the most robust predictor of malignant transformation risk among identified genetic alterations[11][12][23]. TP53 mutations typically occur early (near mRT 0.0-0.2) according to PhylogicNDT, establishing clonal dominance that drives subsequent progression[15]. Lesions harboring TP53 mutations showed significantly higher propensity toward malignant transformation compared with TP53 wild-type lesions[12][23].

Moreover, TP53 mutations in primary tumors correlated with lower overall survival, increased treatment resistance, and higher recurrence risk, establishing TP53 status as universal prognostic marker[12][23]. For prevention strategy purposes, TP53-mutant dysplasia represents extremely high-risk lesions warranting aggressive prevention or early surgical intervention, while TP53 wild-type lesions may benefit from observation or less intensive chemoprevention[11][12][23].

7.2 NOTCH1 as Early Driver and Prevention Target

NOTCH1 mutations identified in 37% of OSCC but present in lower frequency in OED may represent early transformative events[11][13]. Unlike TP53 which shows ~80% final mutation frequency, NOTCH1's presence in only subset of cases suggests NOTCH1 inactivation represents one of several possible early drivers rather than obligate progression step[11][13].

From prevention perspective, NOTCH1-mutant lesions may represent distinct biological entities warranting NOTCH pathway-targeted chemoprevention (NOTCH pathway inhibitors), while NOTCH1 wild-type lesions require alternative approaches[11][13]. This molecular stratification enables matched-pair prevention strategies impossible with histology alone[11][13].

7.3 PI3K Pathway Mutations and Metabolic Chemoprevention

PIK3CA mutations, occurring in 8-43% of OSCC depending on site, frequently co-occur with TP53 alterations, suggesting cooperative effects in driving progression[10][12]. PI3K pathway hyperactivation drives metabolic remodeling (increased glucose uptake, lactate production), establishing rationale for metabolic-targeted chemoprevention in PIK3CA-mutant lesions[10].

Conversely, PTEN loss (functionally equivalent to PIK3CA activation through reduced phosphatase activity) appears less common in OSCC than PIK3CA amplification, suggesting distinct metabolic selective pressures favoring direct PI3K hyperactivation over PTEN loss[10].

8. Clinical Applications: Integration into Prevention Trial Design

8.1 Window-of-Opportunity Trials Utilizing WES Data

Window-of-opportunity (WOO) trials exploit the interval between OED diagnosis and clinical intervention to administer chemopreventive agents and assess molecular response through WES-based biomarkers[24][25]. Optimal trial design includes: baseline WES characterization of dysplastic lesion mutational landscape, serial ctDNA monitoring during prevention intervention, tissue sampling at definitive surgery with WES assessment of mutation burden changes, and long-term follow-up for malignant transformation outcomes[24][25].

This design enables direct assessment of whether chemopreventive agents arrest clonal evolution (demonstrated by stable or decreasing ctDNA and genomic stability) or inadvertently select for resistant clones (demonstrated by clonal sweep toward advantageous mutations)[24][25].

8.2 ctDNA-Informed Adaptive Trial Design

Real-time ctDNA monitoring enables modification of prevention strategies based on molecular response rather than requiring months-to-years to assess clinical outcomes[6][7]. Lesions showing ctDNA accumulation despite intervention indicate prevention failure requiring strategy modification, while lesions showing ctDNA clearance indicate successful evolution arrest[6][7].

This evolutionary approach transforms prevention from one-size-fits-all chemoprevention to individualized dynamic strategies adapting to each patient's clonal evolution patterns[6][7].

9. Challenges in WES-Based Chemoprevention Strategies

9.1 Driver vs Passenger Mutation Distinction in Premalignant Lesions

A fundamental challenge involves distinguishing genuinely transformative driver mutations from passenger mutations accumulating during normal aging-related clonal expansion[26][27]. Premalignant epithelium shows substantial clonal diversity with many mutations unlikely contributing to transformation[26]. DNMT3A, SRSF2, and TET2 mutations previously considered leukemia-specific drivers are now recognized as frequent in normal hematopoietic aging, indicating substantial mutation burden exists in normal tissues[27].

Computational approaches including dN/dS analysis (nonsynonymous/synonymous mutation ratio) and population frequency assessment help distinguish drivers from passengers, but substantial uncertainty remains particularly for non-canonical drivers[27]. Prevention strategies must prioritize pathogenic validated drivers (TP53, NOTCH1, PIK3CA) over mutations with uncertain functional consequences[27].

9.2 Clonal Heterogeneity and Sampling Variance

WES analyses depend critically on biopsy sampling location and technique: dysplastic lesions show spatial heterogeneity where different regions harbor distinct mutational landscapes[18]. Single-site biopsy may miss subclonal populations occupying other lesion regions, potentially underestimating progression risk or missing targetable mutations[18]. Spatial transcriptomics addressing this limitation requires tissue destruction incompatible with clinical care continuity[18].

Serial blood-based ctDNA monitoring partially addresses this by averaging mutations across entire lesion volumes and detecting circulating cancer cells from all sites[6][7], but early-stage dysplasia frequently shows minimal ctDNA shedding, limiting detection sensitivity[28].

9.3 Cost and Clinical Implementation Barriers

Despite declining WES costs, implementing universal exome sequencing in prevention cohorts remains expensive (~$200-500 per sample, repeated sampling for longitudinal studies increases costs substantially)[21][22]. Insurance

coverage for WES in prevention contexts remains inconsistent, limiting access for high-risk populations most likely to benefit[21][22].

Furthermore, clinical interpretation of WES results requires bioinformatic expertise and genetic counseling resources often unavailable in community practice settings, creating implementation barriers despite technical feasibility[21][22][24][25].

10. Integration with Functional Validation and Mechanistic Studies

10.1 Linking Mutations to Functional Consequences

WES-identified mutations require functional validation demonstrating genuine transformative consequences[29]. Not all identified mutations significantly alter protein function; silent mutations, synonymous variants, and missense mutations with minimal structural perturbation occur at substantial frequency[29]. Functional assessment through reporter assays, patient-derived organoid models, and CRISPR-based loss-of-function studies confirms which mutations warrant therapeutic targeting[29].

10.2 Integration with RNA-seq and Proteomics

Comprehensive transcriptomic and proteomic profiling of WES-stratified lesions reveals how identified mutations alter gene expression networks and signaling pathway activation[30]. Lesions stratified as TP53-mutant show distinctive transcriptomic signatures of p53 target gene dysregulation, confirming TP53 functional inactivation[30]. Similarly, PIK3CA-mutant lesions show enhanced PI3K-AKT-mTOR pathway activation validatable by phosphoprotein analysis[30].

Integration of mutation data (genotype) with transcriptomic (phenotype) and proteomic (functional) data provides comprehensive mechanistic understanding enabling rational chemoprevention design[30].

11. Future Perspectives: Emerging Sequencing Technologies and Integration

11.1 Long-Read Sequencing for Structural Variant Resolution

Long-read sequencing technologies (PacBio HiFi, Oxford Nanopore) enable detection of structural variants (deletions, inversions, translocations, complex rearrangements) with single-nucleotide precision, overcoming WES limitations to simple SNVs and small indels[31][32]. Structural variants in cancer-relevant genes (TP53 deletions, NOTCH truncations) may drive transformation as potently as point mutations but remain invisible to conventional WES[31].

11.2 Integration with Artificial Intelligence and Machine Learning

Machine learning models trained on large WES cohorts correlating mutation burden, specific driver mutations, and clonal architecture with transformation outcomes enable predictive risk stratification substantially exceeding current statistical models[33][34]. Deep learning approaches identifying subtle mutation patterns and pathway interactions not captured by linear analysis may further improve prediction accuracy[33][34].

11.3 Multi-Omics Integration: WES with cfDNA, Liquid Biopsy, and Imaging

Comprehensive prevention strategies integrating WES tissue characterization with serial ctDNA monitoring, circulating exosomal miRNA profiling, and radiomics-based imaging analysis create multi-modal prediction and monitoring systems substantially more robust than single-modality approaches[28][35]. Such integrated approaches leverage complementary information: WES identifies specific genetic drivers, ctDNA quantifies burden, miRNAs reflect functional pathway dysregulation, and imaging assesses tissue architecture changes[28][35].

12. Regulatory and Ethical Considerations

12.1 Genetic Counseling and Informed Consent

WES identification of incidental findings (cancer predisposition genes, pharmacogenomic variants, non-cancer disease associations) creates ethical obligations for genetic counseling and informed consent[21][22]. Prevention populations must understand implications of genetic findings and potential psychological consequences of predisposition identification[21][22].

12.2 Genetic Privacy and Data Security

WES and ctDNA analysis generate highly sensitive genetic information subject to substantial privacy and security risks[21][22]. Data protection complying with HIPAA, GDPR, and emerging genetic privacy legislation remains essential but challenging in distributed sequencing and data-sharing networks[21][22].

13. Conclusion

Whole-exome sequencing has fundamentally transformed cancer chemoprevention by enabling comprehensive characterization of mutational trajectories from normal epithelium through dysplasia to invasive carcinoma. Discovery of core driver mutations (TP53, NOTCH1, PIK3CA, CDKN2A) and their specific temporal relationships during progression establishes molecular basis for risk stratification substantially exceeding histology-based approaches. Prospectively validated genetic progression models (gMART, PhylogicNDT) enable individual transformation risk prediction and identification of optimal intervention windows during clonal evolution.

Integration of bulk WES with single-cell sequencing reveals subclonal heterogeneity responsible for some prevention failures, while circulating cfDNA monitoring enables real-time assessment of chemopreventive efficacy without tissue biopsy. Universal WES screening identifies occult cancer predisposition syndromes enabling personalized hereditary cancer prevention. Emerging long-read sequencing, machine learning integration, and multi-omics approaches promise continued advancement toward precision prevention matched to individual molecular profiles.

Challenges remain in distinguishing driver from passenger mutations in premalignant lesions, managing tumor heterogeneity complexity, and translating discoveries into clinical practice. However, WES-driven chemoprevention represents paradigm shift from empiric histology-based approaches toward mechanism-informed, molecularly-guided prevention strategies optimized for individual transformation risk and maximizing both efficacy and safety.

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